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Droga do długowieczności - Efekty oddziaływania resweratrolu i nanodiamentów na aktywność sirtuin u *Acheta domesticus*

The Way to longevity - Effects of resveratrol and nanodiamonds on sirtuin activity in *Acheta domesticus*

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Rozprawa na stopień doktora
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Spis treści

STRESZCZENIE

W ostatnich latach nastąpił dynamiczny rozwój badań nad starzeniem się organizmów oraz rosnące zainteresowanie związkami bioaktywnymi i nanomateriałami w kontekście mechanizmów regulujących długowieczność. Szczególną rolę przypisuje się sirtuinom, enzymom zaangażowanym w regulację procesów metabolicznych, odpowiedzi na stres oraz utrzymanie homeostazy komórkowej. Pomimo licznych badań prowadzonych na modelach ssaczych i innych wybranych organizmach modelowych, wiedza dotycząca funkcjonowania sirtuin u owadów pozostaje ograniczona. Ponadto, interakcje pomiędzy związkami takimi jak resweratrol (RV), który jest uznawany za modulator aktywności sirtuin, a nanomateriałami, w tym nanodiamentami (NDs), są nadal słabo poznane, szczególnie w kontekście ich wpływu na procesy starzenia.

Pierwszy etap niniejszej pracy obejmował analizę przeglądową literatury, której celem było usystematyzowanie wiedzy dotyczącej roli sirtuin w regulacji procesów starzenia oraz podsumowanie informacji w temacie potencjalnych mechanizmów ich modulacji przez związki bioaktywne. Zidentyfikowano istotne luki badawcze, również w odniesieniu do organizmów innych niż klasyczne modele laboratoryjne, w tym owadów. Wyniki przeglądu uzasadniły podjęcie badań eksperymentalnych ukierunkowanych na ocenę wpływu RV, NDs oraz kompleksu na aktywność sirtuin oraz parametry związane z długowiecznością u modelowego organizmu - *Acheta domesticus.*

Etap II obejmował badania wstępne, których celem była ocena zależności pomiędzy aktywnością sirtuin a selekcją pod kątem długowieczności oraz odpowiedzią organizmu na ich potencjalne modulatory w postaci RV oraz NDs u *A. domesticus*. Uzyskane wyniki wykazały, że linia selekcjonowana pod kątem długowieczności (D) wykazywała istotnie wyższą aktywność sirtuin w porównaniu do linii dzikiej (H), co wskazało na przypuszczalne powiązanie tych enzymów z procesami regulującymi przeżywalność u badanych owadów. Wskazano również, że owady selekcjonowane pod kątem długowieczności (D) wskazują odmienną odpowiedź na modulatory względem linii dzikiej (H). Jednocześnie potwierdzono, że zarówno resweratrol, jak i nanodiamenty mogą wpływać na procesy związane z przeżywalnością i regulacją enzymatyczną, obejmującą sirtuiny (SIRT1, SIRT6) oraz enzymy oksydacyjne (SOD, katalaza), jednak ich działanie ma charakter złożony.

Etap III obejmował analizę wpływu RV oraz NDs podawanych oddzielnie na przeżywalność, aktywność sirtuin, stres oksydacyjny oraz uszkodzenia DNA. RV i NDs

wykazywały głównie krótkotrwałe, zależne od kontekstu działanie, bez trwałego wpływu na proces starzenia. W linii H, NDs zwiększały medianę długości życia, a RV wpływał głównie na maksymalną długość życia osobników, natomiast w linii D nie obserwowano istotnych zmian w zakresie tych parametrów, co wskazuje na większą stabilność tej linii owadów. W zakresie stresu oksydacyjnego i aktywności sirtuin nie stwierdzono spójnych trendów, a obserwowane zmiany były przejściowe i zależne od stadium rozwojowego. Aktywność SIRT1 i SIRT6 nie ulegała trwałej modulacji, przy czym SIRT6 nasilała aktywność wraz z wiekiem, niezależnie od zastosowanych czynników. Wpływ na uszkodzenia DNA był niespójny, a w niektórych przypadkach RV zwiększał poziom markerów uszkodzeń, co sugeruje jego dwojakie działanie. Ogólnie odpowiedź organizmu była silnie zależna od linii i etapu rozwoju.

Etap IV obejmował ocenę wpływu jednoczesnego podawania RV i NDs (RV+NDs). Wyniki wskazały na brak jednoznacznego efektu wydłużenia życia, gdyż w linii H nie obserwowano zmian, a w linii D jedynie tendencję do wzrostu długości maksymalnej. Parametry stresu oksydacyjnego pozostawały stabilne i wykazywały jedynie przejściowe zmiany po zastosowaniu kompleksu (RV+NDs). Najwyraźniejszy efekt dotyczył wzrostu całkowitej aktywności sirtuin w linii D, bez trwałych zmian w SIRT1 i SIRT6. Markery uszkodzeń DNA ulegały przejściowemu zwiększeniu (zwłaszcza w linii H), co wskazywało na aktywację mechanizmów naprawczych, a nie kumulację uszkodzeń.

Uzyskane wyniki pozwalają stwierdzić, że zarówno RV, jak i NDs, jak również kompleks, mogą modulować procesy związane ze starzeniem, jednak mechanizmy tych oddziaływań są złożone i zależne od wielu czynników biologicznych i środowiskowych. Praca podkreśla istotną rolę dalszych badań nad bezpieczeństwem stosowania nanomateriałów oraz ich interakcji ze związkami bioaktywnymi, a także wskazuje na potrzebę rozszerzenia badań na różnorodne modele biologiczne. *Acheta domesticus* jako organizm o krótkim cyklu życiowym i dostępnych liniach selekcyjnych, stanowi wartościowy model do dalszych badań mechanizmów starzenia oraz oceny efektów działania nowych czynników biologicznie aktywnych.

ABSTRACT

In recent years, there has been a dynamic development of research on organismal ageing, as well as a growing interest in bioactive compounds and nanomaterials in the context of mechanisms regulating longevity. Importance is attributed to sirtuins, enzymes involved in the regulation of metabolic processes, stress response, and the maintenance of cellular homeostasis. Despite numerous studies conducted on mammalian models and other selected model organisms, knowledge regarding the functioning of sirtuins in insects remains limited. Moreover, interactions between compounds such as resveratrol (RV), which is recognized as a modulator of sirtuin activity, and nanomaterials, including nanodiamonds (NDs), are still poorly understood, particularly in the context of their impact on ageing processes.

The first stage of this work included a literature review aimed at systematizing knowledge on the role of sirtuins in the regulation of ageing processes and summarizing information on the potential mechanisms of their modulation by bioactive compounds. Significant research gaps were identified, also regarding organisms other than classical laboratory models, including insects. The results of the review justified undertaking experimental studies focused on evaluating the effects of RV, NDs, and their combination on sirtuin activity and parameters related to longevity in a model organism - *Acheta domesticus*.

Stage II included preliminary studies, the aim of which was to assess the relationship between sirtuin activity, selection for longevity, and the organism's response to their potential modulators in the form of RV and NDs in *A. domesticus*. The obtained results showed that the longevity-selected strain (D) exhibited significantly higher sirtuin activity compared to the wild-type strain (H), which indicated a possible association of these enzymes with processes regulating survival in the studied insects. It was also indicated that insects selected for longevity (D strain) show a different response to modulators compared to the wild-type strain (H). At the same time, it was confirmed that both resveratrol and nanodiamonds may affect processes related to survival and enzymatic regulation, including sirtuins (SIRT1, SIRT6) and oxidative enzymes (SOD, catalase); however, their action is complex.

Stage III included the analysis of the effects of RV and NDs administered separately on survival, sirtuin activity, oxidative stress, and DNA damage. RV and NDs showed mainly short-term, context-dependent effects, without a sustained impact on the

ageing process. In the H strain, NDs increased the median lifespan, while RV mainly affected the maximum lifespan of individuals, whereas in the D strain no significant changes were observed in these parameters, which indicates greater stability of this strain. In the case of oxidative stress and sirtuin activity, no consistent trends were found, and the observed changes were transient and dependent on the developmental stage. The activity of SIRT1 and SIRT6 was not subject to sustained modulation, while SIRT6 increased its activity with age, independently of the applied factors. The effect on DNA damage was inconsistent, and in some cases, RV increased the level of damage markers, which suggests its dual action. Overall, the organism's response was strongly dependent on the strain and developmental stage.

Stage IV included the assessment of the effects of simultaneous administration of RV and NDs (RV+NDs). The results indicated a lack of a clear life-extending effect, as no changes were observed in the H strain, and in the D strain only a tendency towards an increase in maximum lifespan was observed. Oxidative stress parameters remained stable and showed only transient changes after the application of the combination (RV+NDs). The most pronounced effect concerned an increase in total sirtuin activity in the D strain, without sustained changes in SIRT1 and SIRT6. DNA damage markers showed transient increases (especially in the H strain), which indicated activation of repair mechanisms rather than accumulation of damage.

The obtained results allow the conclusion that both RV and NDs, as well as their combination, may modulate processes related to ageing; however, the mechanisms of these interactions are complex and dependent on many biological and environmental factors. The work highlights the important role of further research on the safety of nanomaterials and their interactions with bioactive compounds and also indicates the need to extend research to diverse biological models. *Acheta domesticus*, as an organism with a short life cycle and available selected strains, constitutes a valuable model for further studies on ageing mechanisms and the evaluation of the effects of new biologically active factors.

LISTA PUBLIKACJI POWIĄZANA Z ROZPRAWĄ DOKTORSKĄ

1. **Ziętara P**, Dziewięcka M, Augustyniak M. *Why Is Longevity Still a Scientific Mystery? Sirtuins-Past, Present and Future*. International Journal of Molecular Sciences. 2023; 24(1), 728. https://doi.org/10.3390/ijms24010728.

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2. **Ziętara P,** Flasz B, Augustyniak M. *Does Selection for Longevity in Acheta domesticus Involve Sirtuin Activity Modulation and Differential Response to Activators (Resveratrol and Nanodiamonds)?* International Journal of Molecular Sciences. 2024, 25(2), 1329. https://doi.org/10.3390/ijms25021329.

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3. **Ziętara-Krzyk P**, Flasz B, Augustyniak M. *Effects of resveratrol and nanodiamonds on sirtuin activity, oxidative stress and DNA damage in Acheta domesticus*. Biochemical and Biophysical Research Communications. 2026, 816, 153698. https://doi.org/10.1016/j.bbrc.2026.153698.

   IF: 2.2; MNiSW: 100

4. **Ziętara-Krzyk P**, Flasz B, Augustyniak M. *Evaluation of Molecular Responses and Longevity Markers in Acheta domesticus Following Combined Resveratrol and Nanodiamond Exposure*. International Journal of Molecular Sciences. 2026, 27(6), 2786. https://doi.org/10.3390/ijms27062786.

   IF: 4.9; MNiSW: 140

WPROWADZENIE

Długowieczność od dekad stanowi podmiot intrygujących i fundamentalnych dylematów i pytań ludzkości. Poszukiwanie sposobów zachowania młodości oraz wydłużenia życia towarzyszy człowiekowi od wieków, rozpoczynając od mitologicznych koncepcji eliksiru młodości, po współczesne, wysoko wyspecjalizowane badania molekularne. Zagadnienie ma z pewnością charakter wyraźnie interdyscyplinarny, łącząc refleksję filozoficzną nad naturą przemijania z rygorem metodologicznym nauk eksperymentalnych (Mahdihassan, 1976; Nicoletti i in., 2025). Niemniej jednak dynamiczny postęp medycyny, biologii molekularnej oraz biotechnologii sprawił, że zagadnienie „wydłużania życia" przestało być jedynie obszarem spekulacji czy teoretycznych rozważań. Stało się obszarem systematycznych badań i empirycznie weryfikowalnych analiz. W związku z tym współczesna nauka koncentruje się nie tylko na opisie fenomenu „starzenia", lecz podejmuje próby rozszyfrowania mechanizmów molekuralnych i komórkowych leżących u jego podstaw, dążąc do zrozumienia procesów regulujących tempo i jakość starzenia organizmu (Łopuszańska-Dawid i in., 2021; Pasupuleti, 2024; Vincent, 2009).

Zainteresowanie długowiecznością wzmacniają obserwacje demograficzne i epidemiologiczne. Szczególne miejsce zajmują populacje określane mianem „Niebieskich sfer" (ang. Blue Zones), zidentyfikowane m.in. w regionach takich jak Okinawa (Japonia), Sardynia (Włochy), Ikaria (Grecja) czy Nicoya (Kostaryka). Obszary te charakteryzuje ponadprzeciętny odsetek osób dożywających wieku stu lat oraz relatywnie niska zapadalność na choroby cywilizacyjne w postaci chorób układu sercowo-naczyniowego, zaburzeń metabolicznych czy chorób neurodegeneracyjnych. Obserwacje te sugerują, że na długość życia wpływa złożona interakcja czynników genetycznych, środowiskowych i stylu życia (Candal-Pedreira i in., 2025; Pieroni i in., 2023; Poulain i in., 2021; Settembrino, 2025). Szeroko pojęta „długowieczność" nie wydaje się zatem efektem pojedynczego czynnika lub „eliksiru", lecz konsekwencją wielopoziomowych procesów biologicznych i metabolicznych. W związku z tym powstało dotychczas wiele koncepcji wyjaśniających proces starzenia, rozpoczynając od teorii akumulacji uszkodzeń molekularnych, poprzez koncepcje stresu oksydacyjnego, aż do podejścia podkreślającego znaczenie regulacji metabolicznej i sygnalizacji komórkowej (Gladyshev i in., 2021; Keller i in., 2021; Liguori i in., 2018; Long i in., 2014; Maldonado i in., 2023; Wordsworth i in., 2022). Niezależnie od poznanych elementów składowych procesów starzenia, kluczowe pytania pozostają otwarte: czy

potencjał długowieczności jest zapisany przede wszystkim w naszym kodzie genetycznym, czy też w modulowanych mechanizmach komórkowych zależnych od diety i środowiska?

W ostatnich latach uwagę poświęca się sirtuinom uczestniczącym m.in w regulacji metabolizmu, procesach naprawczych DNA oraz modulacji procesów zapalnych. Wykazano, że ich aktywność może być modulowana przez czynniki środowiskowe, w tym składniki diety, co czyni je potencjalnymi ogniwami łączącymi styl życia z regulacją procesów starzenia. Ich aktywność może być regulowana zarówno na poziomie dostępności kofaktora NAD+, jak również poprzez działanie związków bioaktywnych (Dali-Youcef i in., 2007; Ziętara i in., 2022). Wśród czynników zwiększających ich aktywność wskazuje się na resweratrol (RV), który ze względu na swoje właściwości przeciwutleniające oraz zdolność modulowania szlaków metabolicznych, jest szeroko badany w kontekście procesów starzenia i długowieczności. Jednocześnie zwraca się uwagę na ograniczenia związane z jego niską biodostępnością oraz krótkim czasem utrzymywania się w organizmie, co może istotnie wpływać na efektywność jego działania (Kopeć i in., 2011; Vesely i in., 2021; Walle, 2011; Ziętara i in., 2022). W odpowiedzi na te ograniczenia rozwijane są strategie wykorzystujące nanomateriały jako nośniki związków bioaktywnych. Wśród nich możemy wyszczególnić nanodiamenty (NDs), charakteryzujące się wysoką biokompatybilnością, stabilnością chemiczną oraz zdolnością do adsorpcji i transportu cząsteczek biologicznie aktywnych. Ich potencjał jako systemów dostarczania substancji czynnych, w tym związków zwiększających aktywność sirtuin, otwiera nowe możliwości modulowania procesów komórkowych związanych ze starzeniem (Pedroso-Santana i in., 2018; J.-X. Qin i in., 2021; Xu & Chow, 2023).

W powyższym kontekście analiza mechanizmów prowadzących do długowieczności poprzez ocenę wpływu RV i NDs na aktywność sirtuin u *Acheta domesticus* może wskazać kierunki dalszych badań nad modulowaniem długości życia. Badania prowadzone w obrębie jednego gatunku mają szczególne znaczenie, ponieważ w przeciwieństwie do populacji ludzkich, wykorzystanie modeli zwierzęcych opartych na ściśle zdefiniowanych liniach hodowlanych pozwala na ograniczenie zmienności biologicznej. W związku z tym analiza dwóch kontrolowanych linii jednego gatunku *A. domesticus*, różniących się historią życia, umożliwia szybszą i precyzyjną identyfikację zależności molekularnych niż porównania prowadzone między

zróżnicowanymi populacjami ludzkimi, co czyni ten model szczególnie użytecznym w badaniach nad molekularnymi podstawami długowieczności czy też starzenia.

Przedmiot badań

Sirtuiny jako enzymy długowieczności

Sirtuiny stanowią wysoce konserwowaną ewolucyjnie rodzinę białek enzymatycznych, które odgrywają kluczową rolę w regulacji procesów związanych ze starzeniem organizmów. Ze względu na ich zdolność do modulowania szlaków metabolicznych, odpowiedzi na stres komórkowy oraz utrzymania integralności genomu, określane są one często mianem „enzymów długowieczności" (Park i in., 2013; Taborsky i in., 2022; Y. Wang i in., 2019). Związek sirtuin z procesami starzenia jest dodatkowo wspierany przez fakt, że ich aktywność może ulegać obniżeniu wraz z wiekiem, co sugeruje ich udział w mechanizmach degeneracyjnych organizmu (Grootaert & Bennett, 2022; Taborsky i in., 2022). Jednocześnie badania wskazują, że sirtuiny mogą stanowić jeden z brakujących elementów w zrozumieniu biologii długowieczności (Ziętara i in., 2022).

Charakterystyka molekularna i funkcjonalna sirtuin

Sirtuiny należą do klasy III deacetylaz histonowych (HDAC) zależnych od NAD+, co oznacza, że ich aktywność jest bezpośrednio powiązana ze stanem energetycznym komórki. Domena katalityczna sirtuin zbudowana jest średnio z 258 reszt aminokwasowych; najkrótsza należy do SIRT6 (239 aa), natomiast najdłuższa do SIRT2 (275 aa) (Ziętara i in., 2022). U ssaków zidentyfikowano siedem homologów (SIRT1-SIRT7), które różnią się lokalizacją komórkową oraz funkcją biologiczną (Tabela 1). Poszczególne izoformy sirtuin wykazują również odmienne powinowactwo do substratów oraz różnice w aktywności enzymatycznej, co dodatkowo różnicuje ich funkcje biologiczne. Co istotne, sirtuiny uczestniczą w mechanizmach ochronnych, ograniczając uszkodzenia DNA, procesy zapalne oraz powstawanie nowotworów (Anderson & Maes, 2021; Dali-Youcef i in., 2007; Giblin & Lombard, 2016).

Tabela 1. Charakterystyka sirtuin (SIRT1-SIRT7). Na podstawie: (Ziętara i in., 2022)

Sirtuina	Główna lokalizacja	Kluczowe funkcje biologiczne
SIRT1	Jądro komórkowe (także cytoplazma)	Regulacja transkrypcji, deacetylacja histonów i białek niehistonowych; kontrola metabolizmu, stresu oksydacyjnego i zapalenia; działanie antyapoptotyczne; interakcja z p53 i FOXO.
SIRT2	Głównie cytoplazma (może migrować)	Regulacja cyklu komórkowego, deacetylacja α-tubuliny; udział w odpowiedzi na stres oksydacyjny (FOXO, Nrf2); funkcje epigenetyczne.
SIRT3	Mitochondria (także jądro/cytoplazma)	Regulacja metabolizmu mitochondrialnego, redukcja ROS, ochrona układu sercowo-naczyniowego; wpływ na termogenezę i homeostazę energetyczną.
SIRT4	Mitochondria	Regulacja metabolizmu (m.in. insuliny i kwasów tłuszczowych), kontrola cyklu komórkowego, udział w homeostazie ATP.
SIRT5	Mitochondria	Regulacja cyklu Krebsa i metabolizmu lipidów; zdolność do usuwania grup malonylowych i sukcynylowych; wpływ na oddychanie mitochondrialne.
SIRT6	Jądro (także cytoplazma)	Stabilizacja genomu, naprawa DNA, regulacja metabolizmu glukozy i lipidów; silny związek z procesem starzenia.
SIRT7	Jąderko	Regulacja transkrypcji rDNA, biogeneza rybosomów, odpowiedź na stres; wpływ na proliferację komórek.

Aktywność enzymatyczna i regulacja

Aktywność poszczególnych izoform sirtuin jest modulowana przez interakcje z białkami regulatorowymi oraz lokalizację subkomórkową (Ziętara i in., 2022). Sirtuiny katalizują reakcję deacetylacji reszt lizynowych w białkach substratowych (Sanders i in., 2010). Proces ten rozpoczyna się od związania acetylowanej lizyny oraz NAD^+ w centrum aktywnym enzymu, co prowadzi do powstania kompleksu trójskładnikowego. Następnie

dochodzi do rozerwania wiązania N-glikozydowego w cząsteczce NAD^+ i uwolnienia nikotynamidu (NAM), czemu towarzyszy utworzenie reaktywnego kompleksu pośredniego. W kolejnych etapach następuje przegrupowanie strukturalne prowadzące do powstania bicyklicznego kompleksu pośredniego, a następnie jego hydroliza, której wynikiem jest zdeacetylowane białko oraz 2'-O-acetylo-ADP-ryboza (OAADPr) jako produkt uboczny (Imai & Guarente, 2014; Sauve, 2010). Ponadto, mechanizm katalityczny sirtuin może obejmować reakcję wymiany zasadowej (ang. base exchange pathway), w której nikotynamid reaguje z kompleksem pośrednim, prowadząc do odtworzenia NAD^+ i sprzężonego hamowania aktywności enzymu (Guan i in., 2014; Imai & Guarente, 2014; N. Zhang i in., 2025). Kluczowym regulatorem aktywności enzymatycznej jest również stosunek NAD^+ do NADH, który odzwierciedla stan redoks komórki, gdzie zazwyczaj wysokie stężenie NAD^+ sprzyja aktywacji sirtuin, natomiast jego obniżenie prowadzi do zahamowania aktywności enzymatycznej (Haigis & Sinclair, 2010; Sauve, 2010). Dodatkowo nikotynamid, będący produktem reakcji, pełni funkcję inhibitora zwrotnego poprzez mechanizm reakcji wymiany zasadowej (Imai & Guarente, 2014; Rymarchyk i in., 2021). Regulacja aktywności sirtuin zachodzi również na poziomie modyfikacji potranslacyjnych, takich jak fosforylacja, acetylacja czy sumoilacja, mogących wpływać na konformację enzymu oraz jego powinowactwo do substratów (Haigis & Sinclair, 2010; Rymarchyk i in., 2021).

Modulatory aktywności sirtuin - aktywatory i inhibitory

Badania nad modulatorami sirtuin zyskały znaczenie w kontekście ich potencjalnych zastosowań terapeutycznych oraz wykorzystania jako narzędzi do analizy funkcji biologicznych poszczególnych izoform. W ostatnich latach wykazano zdolność modulowania funkcji sirtuin poprzez związki małocząsteczkowe, zdolne do bezpośredniego lub pośredniego wpływu na ich aktywność katalityczną. Związki te mogą działać jako aktywatory lub inhibitory (Tabela 2), oddziałując na centrum aktywne enzymu, stabilizując kompleks enzym-substrat bądź wpływając na dynamikę konformacyjną białka (Bursch i in., 2024; Dai i in., 2018). Jednocześnie różnorodność mechanizmów działania tych związków oraz ich zróżnicowana specyficzność wobec poszczególnych sirtuin wskazują na złożoność regulacji tej grupy enzymów i podkreślają konieczność dalszych badań w tym zakresie (Bursch i in., 2024; Fiorentino i in., 2025; Hu i in., 2014).

Tabela 2. Wybrane aktywatory oraz inhibitory sirtuin. Na podstawie: (Ziętara i in., 2022)

Związek	Typ działania	Pochodzenie	Opis działania
Fisetyna	Aktywator	Naturalny	Podobna do resweratrolu; działanie przeciwstarzeniowe i przeciwutleniające
Resweratrol	Aktywator (głównie SIRT1)	Naturalny	Zwiększa aktywność SIRT1, działa przeciwutleniająco, neuro- i kardioprotekcyjnie; niska biodostępność ogranicza zastosowanie kliniczne
Kwercetyna	Aktywator (SIRT1)	Naturalny	Wzmacnia aktywność SIRT1, działa antyoksydacyjnie i przeciwnowotworowo
Triclosan	Aktywator (SIRT1, SIRT3)	Syntetyczny	Może zwiększać ekspresję sirtuin, ale jednocześnie wykazuje toksyczność i zaburzenia metaboliczne
Honokiol	Aktywator (SIRT1, SIRT3)	Naturalny	Poprawia funkcje mitochondrialne, działa neuroprotekcyjnie i przeciwnowotworowo
Polydatyna	Aktywator (SIRT1, SIRT3)	Naturalny	Efekt neuroprotekcyjny, przeciwzapalny i antyoksydacyjny
Oroksylina A	Aktywator (SIRT3)	Naturalny	Działanie kardioprotekcyjne i ochrona mitochondriów
SRT1720, SRT1460, SRT2183	Aktywatory (SIRT1)	Syntetyczne	Naśladują działanie resweratrolu; potencjalne zastosowanie terapeutyczne (badania kliniczne)
Nikotynamid	Inhibitor	Naturalny	Produkt reakcji sirtuin; działa jako inhibitor sprzężenia zwrotnego

EX-527	Inhibitor (głównie SIRT1)	Syntetyczny	Silny, selektywny inhibitor SIRT1; stosowany w badaniach nad nowotworami i chorobami metabolicznymi
Cambinol	Inhibitor (SIRT1, SIRT2)	Syntetyczny	Wykazuje działanie przeciwnowotworowe poprzez modulację acetylacji białek
Sirtinol	Inhibitor (SIRT1, SIRT2)	Syntetyczny	Niespecyficzny inhibitor; może wpływać na procesy nowotworowe i agregację płytek
Suramina	Inhibitor (SIRT1, SIRT5)	Syntetyczny	Brak selektywności ogranicza zastosowanie
Arekolina	Inhibitor (SIRT1-3)	Naturalny	Silniejszy inhibitor niż nikotynamid w niektórych modelach
TW-37	Inhibitor (SIRT5)	Syntetyczny	Słabe hamowanie aktywności SIRT5
Thiadiazole (np. ST29)	Inhibitory (SIRT2)	Syntetyczne	Potencjalne leki przeciwnowotworowe

Sirtuiny a owady - aktualny stan wiedzy

Pomimo intensywnego rozwoju badań nad sirtuinami w ostatnich dekadach, aktualny stan wiedzy pozostaje wyraźnie nierównomierny w zależności od badanej grupy organizmów (Ziętara-Krzyk i in., 2026a, 2026b). Podczas gdy u kręgowców dobrze scharakteryzowano rolę siedmiu izoform (SIRT1-SIRT7), ich lokalizację oraz funkcje biologiczne (Naseer i in., 2021; Ziętara i in., 2022), w przypadku owadów dane te pozostają fragmentaryczne. Dostępnych dane w większości pochodzą z badań prowadzonych na modelach ssaczych (*Mus musculus, Rattus norvegicus*) oraz wybranych organizmach modelowych, takich jak *Drosophila melanogaster* czy *Caenorhabditis* elegans (Burnett i in., 2011; Knop i in., 2025; Shukla & Kolthur-Seetharam, 2022; Tissenbaum & Guarente, 2001). Niemniej jednak eksperymenty na *D. melanogaster* pozwoliły wskazać uczestnictwo genu dSir2 w regulacji szlaków sygnałowych

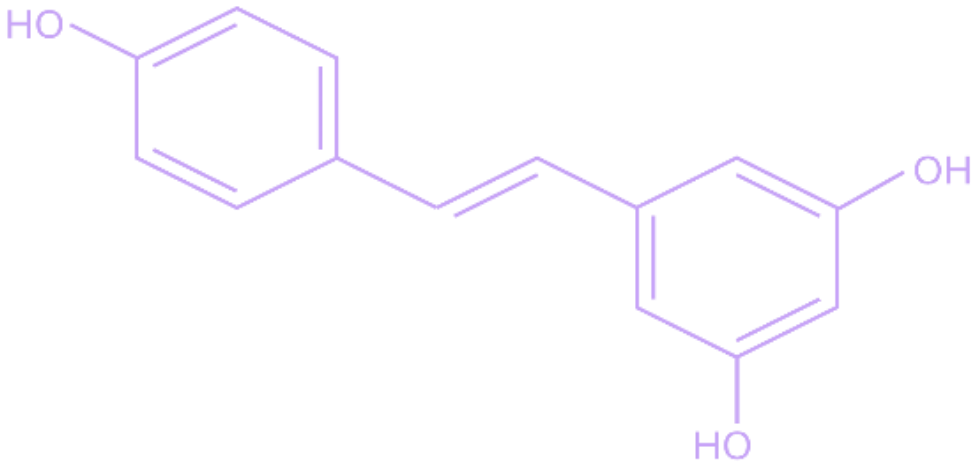
związanych z insuliną/IGF oraz czynnikiem transkrypcyjnym FOXO, wskazując znaczenie w adaptacji do warunków ograniczonej dostępności składników odżywczych (Frankel i in., 2011; Rogina & Helfand, 2004). W dodatku opisano, że białko SIRT6 może spowalniać rozwój larwalny i ma potencjalny wpływ na fizjologię osobników dorosłych (Shukla & Kolthur-Seetharam, 2022), a nadekspresja SIRT2 w mięśniach i neuronach przywraca zdolność wysiłkową u tego gatunku z dysfunkcją mitochondrialną (Damschroder i in., 2022). Jednocześnie podejście to ogranicza możliwość pełnego zrozumienia ewolucyjnej zmienności funkcji tych białek oraz ich znaczenia w innych grupach organizmów. W szczególności zdaje się brakować kompleksowych badań obejmujących jednocześnie aktywność enzymatyczną sirtuin, ich ekspresję oraz powiązania z kluczowymi procesami fizjologicznymi związanymi ze starzeniem. Istotnym problemem jest również niewystarczające poznanie funkcjonalnego zróżnicowania poszczególnych izoform sirtuin u owadów. W przypadku *Acheta domesticus* brak jest systematycznych badań dotyczących aktywności sirtuin oraz ich roli w regulacji procesów starzenia (Ziętara i in., 2022; Ziętara-Krzyk i in., 2026a, 2026b). Jest to istotne ograniczenie, ponieważ owady stanowią grupę o dużej różnorodności biologicznej i wykazują zróżnicowane strategie życiowe, co czyni je potencjalnie wartościowym modelem do badań nad długowiecznością.

Resweratrol jako modulator aktywności sirtuin

Resweratrol (RV; 3,5,4’-trihydroksystilben) jest naturalnym polifenolowym związkiem roślinnym należącym do grupy stilbenów, występującym m.in. w skórkach winogron (*Vitis vinifera*), czerwonym winie, orzeszkach ziemnych (*Rachis hypogaea*) oraz niektórych gatunkach roślin wytwarzających go jako fitoaleksynę w odpowiedzi na stres środowiskowy. Jest szeroko opisywany jako modulator aktywności sirtuin szczególnie SIRT1 zarówno w badaniach in vitro, jak i in vivo (H. Qin i in., 2021; Rogina & Tissenbaum, 2024; L.-X. Zhang i in., 2021).

Obecnie wiadomo, że RV wiąże się z domeną katalityczną SIRT1, stabilizując jej konformację i zwiększając powinowactwo do substratów. Może działać allosterycznie, ułatwiając interakcję enzym-substrat oraz zwiększając efektywność deacetylacji (Grzeczka & Kordowitzki, 2022). Ponadto uważa się, że nasila aktywację AMPK oraz modulację stosunku NAD+/NADH, pośrednio wzmacniając aktywność sirtuin (Matuszyński i in., 2025). Jego działanie niemniej jednak może być również zależne od

dawki (Mansouri i in., 2025). Jednakże w badaniach in vitro na rekombinowanym ludzkim SIRT3, RV nie aktywował SIRT3, lecz hamował jej aktywność enzymatyczną, zmniejszając zdolność tego białka do deacetylacji substratów. Równocześnie w tym samym badaniu autorzy wskazali, że na SIRT5 oddziałuje jako aktywator (Gertz i in., 2012). W przypadku SIRT2 wykazano, że RV może modulować jego aktywność oraz wpływać na regulację cyklu komórkowego i stabilność mikrotubul, jednak uzyskane wyniki są niejednoznaczne i wskazują zarówno na efekty aktywujące, jak i hamujące, zależne od modelu badawczego (Kilic Eren i in., 2015; Pan i in., 2017). Natomiast w badaniach przeprowadzonych w modelu in vitro na ludzkich liniach komórkowych wykazano, że resweratrol zwiększał ekspresję SIRT6 i wykorzystywał go do hamowania retrotranspozycji LINE-1 (L1), ograniczając niestabilność genomu, przy czym efekt ten był zależny od szlaków PPARα i MAPK i nie obejmował udziału SIRT3 (Okudaira i in., 2022). Należy jednak podkreślić, że wpływ RV na sirtuiny jest silnie zależny od rodzaju komórek, dostępności NAD^+ oraz zastosowanego substratu, co sugeruje, że jego działanie niekoniecznie ma charakter uniwersalnego, lecz selektywny (Grabowska i in., 2017; Price i in., 2012; Ziętara-Krzyk i in., 2026a). W dodatku część efektów przypisywanych bezpośredniej aktywacji sirtuin przez RV może wynikać z mechanizmów pośrednich, takich jak aktywacja kinazy AMPK, wzrost poziomu NAD^+ lub modulacji metabolizmu komórkowego, co wtórnie wpływa na aktywność enzymatyczną tych białek (Grabowska i in., 2017; Price i in., 2012).



Rycina 1. Resweratrol. Źródło: opracowanie własne.

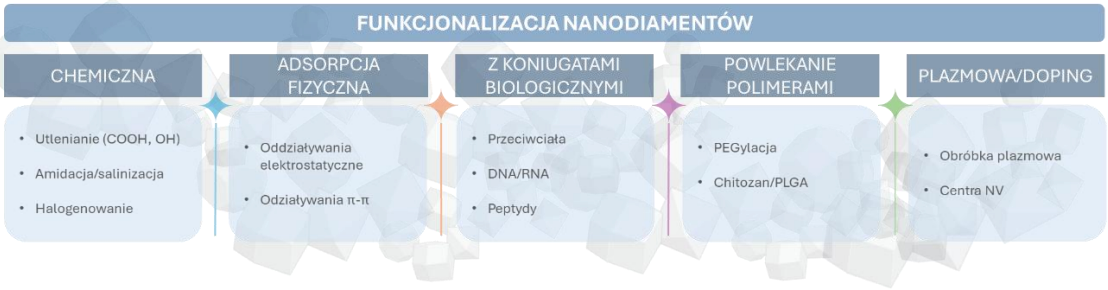
Nanodiamenty w systemach dostarczania substancji aktywnych

Nanodiamenty (NDs; ang. nanodiamonds) to nanocząstki węgla o strukturze krystalicznej identycznej jak w diamencie (sieć sp^3). Najczęściej przyjmują rozmiar od 2 do 10 nm (detonacyjne ND), chociaż mogą występować także w większych formach (~ 100 nm). Wykazują zdolność do tworzenia agregatów, stosunkowo wysoką stabilność chemiczną oraz mechaniczną, a na ich powierzchni występują liczne grupy funkcyjne (-COOH, -OH, $-NH_2$) z dużą powierzchnią właściwa (do kilkuset m^2/g) (Alexander & Leong, 2024; Mochalin i in., 2012). Mogą zwiększać stabilność i biodostępność wybranych cząsteczek, a także umożliwiać ich bardziej ukierunkowane dostarczanie do komórek lub tkanek, dzięki czemu mogą pełnić funkcję nośników substancji biologicznie aktywnych, w tym leków i związków o potencjale przeciwstarzeniowym (Ziętara-Krzyk i in., 2026a, 2026b).

NDs wykazują niską cytotoksyczność w szerokim zakresie stężeń, jednakże efekt biologiczny jest zależny od dawki, wielkości i modyfikacji powierzchni, a przy wysokich stężeniach mogą powodować stres oksydacyjny lub agregację komórkową (Chauhan i in., 2020). W badaniach nad *A. domesticus* wskazywano, że NDs mogą wykazywać przewlekłą toksyczność, prowadząc do zaburzeń rozwoju, obniżenia masy ciała oraz spadku zdolności reprodukcyjnych (Karpeta-Kaczmarek i in., 2018). W stężeniach 20 oraz 200 mg/g wykazano, że mimo braku zmian histologicznych w tkankach, nanodiamenty indukują uszkodzenia DNA, co wskazuje na ich potencjalną genotoksyczność zależną od dawki (Karpeta-Kaczmarek i in., 2016). W dodatku wskazywano również, że pomimo tego, iż mogą wykazywać niską toksyczność ostrą przy niskich stężeniach, to ich długotrwała wielopokoleniowa ekspozycja może prowadzić do subtelnych zmian fizjologicznych i adaptacyjnych (Augustyniak i in., 2022).

Niezależnie od wywoływanych efektów, jak zostało wspomniane, ich unikalne właściwości fizykochemiczne sprawiają, że są atrakcyjnymi nośnikami substancji aktywnych w nowoczesnych systemach dostarczania leków. Wykazują zdolność do kontrolowanego wiązania i uwalniania substancji aktywnych, dlatego coraz częściej wykorzystuje się funkcjonalizację NDs (Rycina 2) polegającą na chemicznej modyfikacji ich powierzchni w celu nadania im określonych właściwości fizykochemicznych i biologicznych. Ten etap stanowi przygotowanie ich do zastosowań biomedycznych, ponieważ surowe cząstki wykazują tendencję do agregacji oraz ograniczoną rozpuszczalność w środowiskach wodnych. Najczęściej stosowane metody obejmują utlenianie powierzchni (wprowadzanie grup karboksylowych i hydroksylowych), reakcje

kowalencyjne (np. amidacja), a także modyfikacje polimerowe, takie jak PEGylacja. Funkcjonalizacja umożliwia zwiększenie stabilności koloidalnej, poprawę biokompatybilności oraz przyłączanie cząsteczek bioaktywnych, w tym leków, peptydów czy ligandów celujących (Barzegar Amiri Olia i in., 2021; Costa i in., 2024; Jariwala i in., 2020; López De Arriba i in., 2025; Ray i in., 2006; Ryu i in., 2016).



Rycina 2. Wybrane metody funkcjonalizacji nanodiamentów. Na podstawie: (Barzegar Amiri Olia i in., 2021; Costa i in., 2024; Jariwala i in., 2020; López De Arriba i in., 2025; Ray i in., 2006).

W praktyce NDs znajdują zastosowanie w systemach dostarczania leków, szczególnie w terapii chorób nowotworowych, gdzie wykazano ich zdolność do wiązania i transportu leków cytotoksycznych, takich jak doksorubicyna (Cui i in., 2023; Jović i in., 2026). Kompleksy ND-lek umożliwiają zwiększenie retencji substancji w komórkach nowotworowych oraz ograniczenie jej toksyczności ogólnoustrojowej, m.in. poprzez stopniowe uwalnianie i ochronę przed mechanizmami oporności na leki (Cui i in., 2023; Mochalin i in., 2012; Priyadarshni i in., 2024; J.-X. Qin i in., 2021). Ponadto nanodiamenty są badane jako nośniki kwasów nukleinowych (np. siRNA, DNA), co otwiera możliwości ich zastosowania w terapii genowej, dzięki niskiej immunogenności i zdolności do penetracji komórek (Alexander & Leong, 2025; Solarska-Ściuk & Kleszczyńska, 2019). W kontekście związków naturalnych, takich jak resweratrol, NDs przypuszczalnie mogą poprawiać ich stabilność i biodostępność, co jest szczególnie istotne ze względu na ograniczenia farmakokinetyczne tych cząsteczek (Ziętara-Krzyk i in., 2026b). Należy jednak podkreślić, że ich zastosowanie w systemach biomedycznych wymaga każdorazowo szczegółowej oceny bezpieczeństwa, uwzględniającej zarówno właściwości fizykochemiczne nanocząstek, jak i biologiczną ekspozycję.

Acheta domesticus jako model badawczy

Świerszcz domowy (łac. *Acheta domesticus*) (Rycina 3) należący do rzędu prostoskrzydłych (Orthoptera), jest wykorzystywany jako organizm modelowy w badaniach biologicznych, fizjologicznych oraz toksykologicznych. Gatunek ten przechodzi metamorfozę niezupełną (hemimetabolia), obejmującą stadia jaja, larwy, oraz osobnika dorosłego (imago), bez stadium poczwarki. Rozwój postembrionalny przebiega poprzez serię kolejnych linień (zwykle 8-10), w trakcie których larwy stopniowo nabywają cechy osobników dorosłych, w tym rozwinięte skrzydła i dojrzałość płciową (Horch i in., 2017; Kaufman i in., 1989). Cykl życiowy *A. domesticus* w warunkach laboratoryjnych jest stosunkowo krótki i wynosi zazwyczaj od 2 do 3 miesięcy, co umożliwia prowadzenie badań wielopokoleniowych w relatywnie krótkim czasie. Charakteryzuje się dobrze rozwiniętym układem nerwowym oraz wyraźnie wyodrębnionymi strukturami sensorycznymi, co umożliwia prowadzenie badań nad percepcją bodźców, zachowaniem oraz regulacją procesów fizjologicznych. Dodatkowo jego rozwój, kontrolowany przez hormony juwenilne, stanowi wygodny model do analizy mechanizmów regulujących linienie, wzrost oraz dojrzewanie płciowe (Flasz i in., 2020; Horch i in., 2017; Kaufman i in., 1989).

Rycina 3. *Acheta domesticus*, stadium teneralne. Źródło: opracowanie własne

W badaniach wykazano przydatność *Acheta domesticus* w analizie procesów fizjologicznych związanych ze stresem oksydacyjnym, metabolizmem oraz starzeniem. Istotną zaletą tego gatunku jako organizmu modelowego jest jego stosunkowo duży rozmiar ciała w porównaniu z innymi modelami bezkręgowymi, co ułatwia manipulację eksperymentalną. Prace prowadzone przez zespół Uniwersytetu Śląskiego w Katowicach wskazują, że gatunek ten stanowi wartościowy model w badaniach nad oddziaływaniem nanomateriałów, w tym NDs oraz tlenku grafenu, zarówno w ujęciu krótkoterminowym, jak i wielopokoleniowym (Augustyniak i in., 2022, 2023; Flasz i in., 2021; Karpeta-Kaczmarek i in., 2018; Ziętara i in., 2024; Ziętara-Krzyk i in., 2026a, 2026b).

Wybór *Acheta domesticus* jako modelu badawczego w niniejszej pracy nie był przypadkowy i wynikał z unikalnych możliwości, jakie oferuje ten gatunek w badaniach nad procesami starzenia. W hodowli prowadzonej na Uniwersytecie Śląskim w Katowicach od blisko 30 lat utrzymywana jest linia selekcjonowana pod kątem długowieczności (linia D), stanowiąca cenny materiał do badań porównawczych. Zestawienie tej linii z linią dziką (H) umożliwia analizę różnic w obrębie tego samego gatunku, przy ograniczeniu zmienności międzygatunkowej. Relatywnie krótki cykl życiowy *A. domesticus* pozwala na analizę przeżywalności oraz efektów działania badanych związków w stosunkowo krótkim czasie, co jest istotną przewagą w badaniach eksperymentalnych i ocenie wpływu czynników biologicznie aktywnych, takich jak resweratrol czy nanomateriały, na procesy związane z długowiecznością.

Cel i zakres badań

Proces starzenia jest złożonym zjawiskiem regulowanym przez współdziałanie mechanizmów molekularnych, w tym odpowiedzi na stres oksydacyjny, stabilności genomu oraz aktywności białek regulatorowych, takich jak sirtuiny. Niemniej jednak dotychczasowe badania nad potencjalnymi modulatorami tych procesów nie zawsze jednoznacznie wskazują, czy oraz w jaki sposób związki bioaktywne i nanomateriały mogą w sposób trwały wpływać na mechanizmy starzenia, szczególnie w kontekście różnic między badanymi liniami organizmów. W związku z tym podjęto badania mające na celu kompleksową ocenę roli sirtuin, stresu oksydacyjnego oraz uszkodzeń DNA w regulacji długowieczności u *Acheta domesticus*, z uwzględnieniem zarówno czynników środowiskowych, jak i różnic wynikających z selekcji w kierunku długowieczności (linia H vs linia D).

Cele i zadania

Celem niniejszej pracy było określenie, jaki efekt wywoła zastosowanie RV oraz NDs, oddzielnie oraz w kombinacji, na regulację procesów metabolicznych u *Acheta domesticus*, ze szczególnym uwzględnieniem aktywności sirtuin oraz wybranych parametrów fizjologicznych i komórkowych.

ETAP I *- artykuł przeglądowy (Why Is Longevity Still a Scientific Mystery? Sirtuins-Past, Present and Future*)

1) Analiza literatury dotyczącej roli sirtuin w procesie starzenia oraz ich potencjalnych modulatorów
   a) uporządkowanie aktualnego stanu wiedzy dotyczącego funkcji sirtuin w regulacji procesów komórkowych,
   b) identyfikacja potencjalnych czynników modulujących ich aktywność (w tym resweratrolu),
   c) wskazanie luk badawczych uzasadniających podjęcie badań eksperymentalnych.

ETAP II *- badania wstępne (Does Selection for Longevity in Acheta domesticus Involve Sirtuin Activity Modulation and Differential Response to Activators (Resveratrol and Nanodiamonds)?)*

2) Wstępna ocena aktywności sirtuin u *Acheta domesticus*
   a) określenie poziomu całkowitej aktywności sirtuin (ogólnej aktywności oraz sirtuiny 1) w badanych liniach (H i D),
   b) identyfikacja różnic między linią H a D.
3) Ocena wpływu resweratrolu i nanodiamentów na aktywność sirtuin (ujęcie ogólne)
   a) określenie czy zastosowanie RV i NDs wpływa na całkowitą aktywność sirtuin,
   b) wstępna analiza zmian aktywności w czasie (różne punkty pomiarowe).
4) Ocena wpływu badanych czynników na przeżywalność
   a) porównanie przeżywalności osobników spożywających RV i NDs w pokarmie.

ETAP III *- działanie pojedynczych czynników (RV i NDs)* (*Effects of resveratrol and nanodiamonds on sirtuin activity, oxidative stress and DNA damage in Acheta domesticus*)

5) Charakterystyka aktywności wybranych sirtuin (SIRT1 i SIRT6)
   a) określenie ogólnej aktywności sirtuin oraz SIRT1 i SIRT6 w liniach H i D,

b) analiza zmian zależnych od badanego stadium rozwojowego,

6) Ocena wpływu resweratrolu i nanodiamentów na aktywność sirtuin
   a) określenie zmian aktywności SIRT1 i SIRT6 po ekspozycji na RV i NDs,
   b) analiza zależności od czasu i stadium rozwojowego.
7) Ocena wpływu RV i NDs na wybrane parametry biochemiczne i komórkowe
   a) analiza enzymów antyoksydacyjnych (SOD, CAT) i peroksydacji lipidów (LPO),
   b) ocena markerów odpowiedzi komórkowej (pATM, γH2A.X, DSB).
8) Ocena wpływu badanych czynników na parametry życiowe
   a) analiza przeżywalności i długości życia w liniach H i D.

***ETAP IV** - Ocena połączonego oddziaływania nanodiamentów i resweratrolu (RV+NDs)* (*Evaluation of Molecular Responses and Longevity Markers in Acheta domesticus Following Combined Resveratrol and Nanodiamond Exposure*)

9) Ocena wpływu kombinacji resweratrolu i nanodiamentów na aktywność sirtuin
   a) określenie zmian aktywności SIRT1 i SIRT6 po zastosowaniu RV+NDs,
   b) porównanie efektu kombinacji z działaniem pojedynczych czynników.
10) Ocena wpływu RV+NDs na parametry biochemiczne i komórkowe
   a) analiza zmian w poziomie enzymów antyoksydacyjnych oraz markerów odpowiedzi komórkowej,
   b) określenie charakteru odpowiedzi organizmu na kombinację czynników.
11) Ocena wpływu RV+NDs na parametry życiowe
   a) analiza przeżywalności i długości życia po zastosowaniu kombinacji.
12) Ocena charakteru działania kombinacji RV i NDs
   a) określenie czy działanie ma charakter addytywny, synergistyczny czy odmienny względem pojedynczych czynników.

***ETAP PRZEKROJOWY** (łączący wszystkie prace)*

13) Analiza zależności między aktywnością sirtuin a wybranymi parametrami komórkowymi
   a) ocena współzależności między aktywnością sirtuin a wskaźnikami stresu oksydacyjnego i odpowiedzi komórkowej,
   b) analiza tych zależności w obu liniach oraz na różnych etapach rozwoju.

Hipotezy badawcze

Sformułowano następujące hipotezy badawcze:

H1.0 Nie istnieje zależność pomiędzy aktywnością sirtuin a długością życia *Acheta domesticus*. W obu badanych liniach (dzikiej - H oraz selekcjonowanej pod kątem długowieczności - D) aktywność analizowanych sirtuin jest podobna lub nie różni się istotnie.

***Uzasadnienie**: Pomimo tego, iż sirtuiny są uznawane za enzymy długowieczności powiązane z procesami starzenia, w przypadku badanych linii A. domesticus mogą one nie odgrywać bezpośredniej roli w wydłużeniu życia. Linia długowieczna została uzyskana w drodze selekcji poprzez opóźnienie reprodukcji, a nie poprzez cechy bezpośrednio związane z aktywnością sirtuin. Aktywność badanych sirtuin nie uległa jednoznacznej i trwałej zmianie w następstwie długotrwałej selekcji, co może wskazywać, że za zwiększoną długość życia odpowiadają również inne mechanizmy, np. związane z metabolizmem.*

H1.1 Aktywność wybranych klas sirtuin różni się pomiędzy osobnikami linii dzikiej (H) a długowieczną linią selekcjonowaną (D) świerszcza domowego.

***Uzasadnienie**: Proces selekcji pod kątem długowieczności A. domesticus może prowadzić do zmian w regulacji procesów fizjologicznych, w tym szlaków związanych z aktywnością sirtuin. Różnice te mogą odzwierciedlać odmienne strategie funkcjonowania organizmu, związane z alokacją energii, metabolizmem oraz odpowiedzią na stres komórkowy.*

H1.2 Aktywność wybranych klas sirtuin jest wyższa u osobników linii selekcjonowanej w kierunku długowieczności (D) w porównaniu z linią dziką (H).

***Uzasadnienie:** Obecne badania sugerują powiązanie sirtuin z procesami sprzyjającymi wydłużeniu życia, w tym regulacją metabolizmu, odpowiedzią na stres oraz utrzymaniem homeostazy komórkowej. Wielopokoleniowa selekcja w kierunku długowieczności doprowadziła do utrwalenia wyższej aktywności tych enzymów w linii D jako elementu mechanizmów wspierających funkcjonowanie organizmu w warunkach wydłużonego życia.*

H2.0 Resweratrol nie wpływa istotnie na aktywność sirtuin ani na długość życia *Acheta domesticus* w żadnej z badanych linii (H lub D).

Uzasadnienie: *Chociaż resweratrol jest opisywany jako aktywator sirtuin w wielu modelach biologicznych, jego działanie nie ma charakteru uniwersalnego i zależy od gatunku, warunków ekspozycji oraz stanu fizjologicznego organizmu. W przypadku owadów o przeobrażeniu niezupełnym efekty jego działania mogą być ograniczone lub niewidoczne.*

H2.1 Resweratrol wpływa na aktywność sirtuin oraz parametry związane z długością życia *A. domesticus*, przy czym efekt ten jest zależny od linii oraz badanego stadium rozwojowego.

Uzasadnienie*: Działanie resweratrolu ma charakter zależny od dawki, czasu ekspozycji oraz kontekstu biologicznego. Możliwe jest zarówno zwiększenie, jak i brak zmian w aktywności sirtuin oraz parametrach życiowych, a odpowiedź organizmu może różnić się między linią dziką a selekcjonowaną, jak również pomiędzy poszczególnymi etapami rozwoju*.

H3.0 Zastosowanie nanodiamentów w połączeniu z resweratrolem nie wpływa na jego działanie biologiczne, a obserwowane efekty są porównywalne do działania resweratrolu stosowanego oddzielnie.

Uzasadnienie*: Jednoczesna obecność nanodiamentów i resweratrolu nie musi prowadzić do ich funkcjonalnej interakcji na poziomie organizmu. Nanodiamenty, mimo potencjalnej roli nośnika, mogą nie wpływać istotnie na sposób oddziaływania resweratrolu na komórki, a jego efekt biologiczny może pozostać niezmieniony. Możliwe jest również, że organizm nie wykazuje wrażliwości na zastosowaną formę kombinacji, co skutkuje odpowiedzią porównywalną do działania pojedynczego czynnika.*

H3.1 Zastosowanie nanodiamentów w połączeniu z resweratrolem modyfikuje odpowiedź biologiczną organizmu w porównaniu do działania pojedynczych czynników.

Uzasadnienie*: Nanodiamenty mogą wpływać na biodostępność, stabilność oraz czas działania resweratrolu, co może prowadzić do zmiany jego oddziaływania na komórki i organizm, w tym na aktywność sirtuin oraz badane parametry fizjologiczne.*

H3.2 Jednoczesne działanie nanodiamentów i resweratrolu może wywoływać efekty niekorzystne dla *A. domesticus*.
***Uzasadnienie**: Interakcje pomiędzy nanomateriałami a związkami bioaktywnymi mogą prowadzić do nieprzewidywalnych efektów biologicznych, w tym zaburzeń homeostazy komórkowej, nasilenia odpowiedzi stresowej lub efektów toksycznych.*

H4.0 Resweratrol, nanodiamenty oraz ich kombinacja nie wpływają istotnie na wybrane parametry komórkowe, a zmiany aktywności sirtuin nie są powiązane ze zmianami tych parametrów.
***Uzasadnienie**: Parametry komórkowe, takie jak poziom stresu oksydacyjnego czy wskaźniki odpowiedzi komórkowej, podlegają silnej regulacji homeostatycznej i mogą pozostawać stabilne mimo zmian w innych procesach biologicznych.*

H4.1 Resweratrol, nanodiamenty oraz ich kombinacja wpływają na wybrane parametry komórkowe, a zmiany te mogą współwystępować ze zmianami aktywności sirtuin.
***Uzasadnienie**: Zarówno resweratrol, jak i nanodiamenty mogą oddziaływać na stan komórki, w tym na odpowiedź na stres oksydacyjny, co może prowadzić do powiązanych zmian w aktywności enzymatycznej i parametrach komórkowych.*

H4.2 Resweratrol, nanodiamenty oraz ich kombinacja wpływają na badane parametry komórkowe, jednakże zmiany te zachodzą niezależnie od aktywności sirtuin.
***Uzasadnienie**: Resweratrol oraz nanodiamenty mogą oddziaływać na komórki poprzez różne mechanizmy, niekoniecznie związane bezpośrednio z aktywnością sirtuin. Związki te mogą wpływać na równowagę oksydacyjną, odpowiedź komórkową oraz poziom uszkodzeń komórkowych poprzez inne szlaki regulacyjne. W konsekwencji możliwe jest wystąpienie zmian w parametrach komórkowych bez jednoznacznych zmian w aktywności sirtuin*.

Metodologia badań

W celu realizacji założonych celów badawczych oraz weryfikacji postawionych hipotez, prace podzielono na cztery etapy, z których trzy miały charakter eksperymentalny, natomiast pierwszy stanowił analizę przeglądową Badania zostały przeprowadzone na Uniwersytecie Śląskim w Katowicach, na Wydziale Nauk Przyrodniczych w Instytucie

Biologii, Biotechnologii i Ochrony Środowiska. Analizy TEM, SEM oraz EDS przeprowadzono przez Sieć Badawczą Łukasiewicz - PORT Polski Ośrodek Rozwoju Technologii we Wrocławiu.

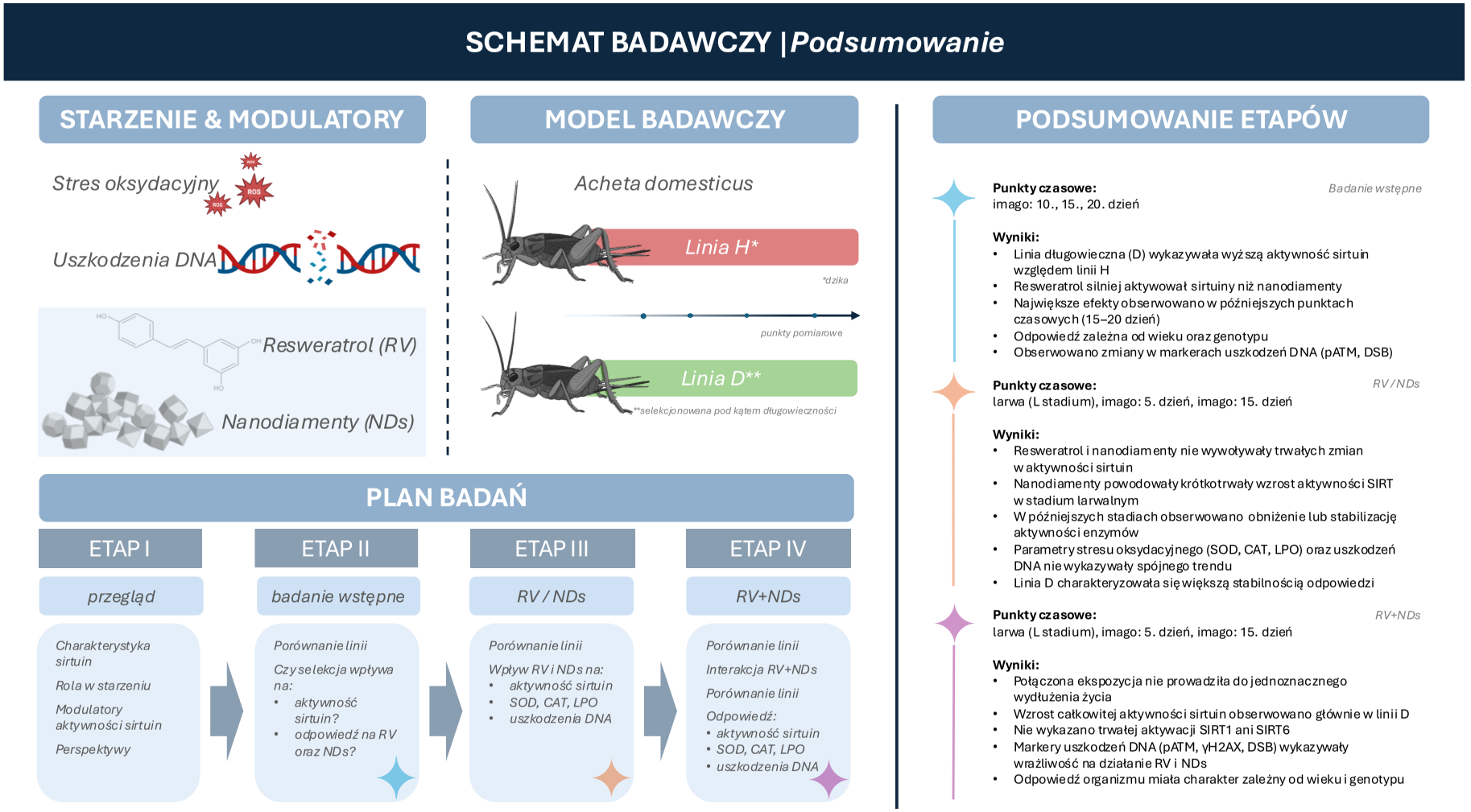
W kolejnych etapach zastosowano podział na grupy badawcze obejmujące: grupę kontrolną (C), grupę kontrolną z dodatkiem etanolu (C+E), grupę z resweratrolem (RV), grupę z nanodiamentami (NDs) oraz grupę poddaną jednoczesnej ekspozycji na oba czynniki (RV+NDs). Liczebność osobników w poszczególnych grupach była utrzymywana na poziomie umożliwiającym przeprowadzenie analiz statystycznych, natomiast w analizach biochemicznych stosowano powtórzenia biologiczne w liczbie n = 5. Materiał badawczy stanowiło jelito świerszcza domowego. Szczegółowy podział grup badawczych oraz ich przyporządkowanie do poszczególnych etapów eksperymentalnych przedstawiono w Tabela 3 oraz przedstawione na Rycina 4. Hodowla owadów była prowadzona w sposób ciągły, aż do naturalnej śmierci ostatniego osobnika w każdej z grup eksperymentalnych, z osobnymi insektariami do analizy przeżywalności. Metodologia została szczegółowo opisana w manuskryptach załączonych w niniejszej pracy a jej podsumowanie zestawiono w Tabela 3.

Tabela 3. Zestawienie metodologiczne badań. Źródło: opracowanie własne

Parametr	Etap badań	Zastosowane testy/metodologia
Przygotowanie pokarmu	Etap II, III	• RV: 23 mg/kg pokarmu + rozpuszczenie RV w etanolu (~0,01%); • NDs: 0,2 mg/kg pokarmu; • dodatek do pokarmu, suszenie.
	Etap IV	• RV: 23 mg/kg pokarmu + rozpuszczenie RV w etanolu (~0,01%) z NDs: 0,2 mg/kg pokarmu, sonifikacja; • dodatek do pokarmu, suszenie,
Prowadzenie hodowli	Etap II	• Kontrolowane warunki hodowlane (±28°C, 12:12 L:D); • randomizacja osobników do grup (C, RV, NDs).

	Etap III	• Kontrolowane warunki hodowlane (±28°C, 12:12 L:D); • randomizacja osobników do grup (C, C+E, RV, NDs).
	Etap IV	• Kontrolowane warunki hodowlane (±28°C, 12:12 L:D); • randomizacja osobników do grup (C, C+E, RV+NDs).
Analiza przeżywalności	Etap II, III, IV	• Analiza przeżywalności: Analiza Kaplan-Meier, test log-rank.
Parametry stresu oksydacyjnego	Etap II, III, IV	• SOD Activity Assay Kit (Merck); • Catalase Activity Kit (Thermo Fisher); • Lipid Peroxidation Assay Kit (Abbexa); • pomiar absorbancji (TECAN Infinite M200); • oznaczanie stężenia białka (Bradford).
Izolacja białek jądrowych	Etap II, III, IV	• Nuclear Extraction Kit (Abcam); • oznaczanie stężenia białka (Bradford).
Oznaczenie aktywności sirtuin	Etap II	• Universal SIRT Activity Assay Kit (Abcam); • SIRT1 Activity Assay Kit (Abcam); • pomiar fluorescencji (HITACHI F-7000). • oznaczanie stężenia białka (Bradford)
	Etap III, IV	• Universal SIRT Activity Assay Kit (Abcam); • SIRT1 Activity Assay Kit (Abcam); • SIRT6 Activity Assay Kit (Abcam); • pomiar fluorescencji (HITACHI F-7000). • oznaczanie stężenia białka (Bradford).
Analizy fizykochemiczne	Etap II	• DLS - dynamiczne rozpraszanie światła • Potencjał zeta; • AFM - mikroskopia sił atomowych.

	Etap III	• TEM - Transmisyjna mikroskopia elektronowa • EDS - spektroskopia rentgenowska z dyspersją energii
	Etap IV	• HR-TEM / TEM (mikroskopia elektronowa); • EDS; • DLS; • UV-Vis spektroskopia; • Zeta potencjał; • Spektroskopia Ramana.
Ocena uszkodzeń DNA	Etap II, III, IV	• Przygotowanie zawiesiny komórek jelita po homogenizacji w PBS; • Muse® Multi-Color DNA Damage Kit (ATM Ser1981-PE, γH2A.X PECy5); • analiza cytometryczna (Guava® Muse®).
Analiza danych	Etap II, III, IV	• Analiza statystyczna danych; • Testy parametryczne i nieparametryczne; • Analiza przeżywalności; • Modele wieloczynnikowe.



Rycina 4. Schemat badań. Źródło: opracowanie własne

38:9459734368

Kluczowe wyniki i ich interpretacja

Etap I - przegląd literaturowy

Punkt wyjścia cyklu badawczego stanowił kompleksowy przegląd literatury poświęcony sirtuinom jako enzymom regulującym procesy starzenia (Ziętara i in., 2024). Równocześnie stanowił wprowadzenie do problematyki starzenia i powiązanych procesów komórkowych. Na tym etapie podsumowano i opisano na podstawie dostępnych danych, rodzinę siedmiu izoform sirtuin (SIRT1 - SIRT7) zlokalizowanych odpowiednio w jądrze komórkowym, cytozolu i mitochondriach. Wskazano, że każda z izoform pełni odmienną rolę biologiczną: SIRT1 i SIRT6, zaliczane do białek jądrowych, są najszerzej wiązane z regulacją długości życia w modelach zwierzęcych, podczas gdy SIRT3-SIRT5, chroniące mitochondria, biorą udział w kontroli metabolizmu oksydacyjnego i neutralizacji ROS. Przegląd ujawnił istotną lukę poznawczą, ponieważ o ile sirtuiny zdają się być intensywnie badane u kręgowców i u *Drosophila melanogaster*, o tyle dane dotyczące ich aktywności u owadów z przeobrażeniem niezupełnym, takich jak *Acheta domesticus,* pozostają szczątkowe. Ponadto zostało podkreślone, że niskie powinowactwo biologiczne i krótki czas retencji RV stanowią zasadniczy problem ograniczający jego potencjalne działanie wydłużające życie, co uzasadnia poszukiwanie systemów nanodelivery (pol. dostarczania substancji aktywnych w oparciu o nanostruktury), zdolnych do poprawy biodostępności tego związku. Chociaż osiągnięcia nanobiotechnologii otwierają możliwości projektowania nowych cząsteczek i nośników mogących modulować aktywność sirtuin, wiele z tych rozwiązań pozostaje jeszcze na poziomie koncepcji. Zaznaczono również, że przed przystąpieniem do badań *in vitro* i *in vivo* konieczne jest szczegółowe poznanie struktury modulatorów oraz ich potencjalnej reaktywności wobec sirtuin, również z wykorzystaniem analiz *in silico* (Ziętara i in., 2024).

Etap II - Badania wstępne

Pierwszym krokiem po sformułowaniu hipotez badawczych było ustalenie, czy sirtuiny są aktywne u *A. domestics* oraz jak poziom tej aktywności różnicuje się w obrębie badanych linii (H vs D) tego gatunku. Badania nad szeroko pojętą „długowiecznością" wymagają podejścia interdyscyplinarnego i porównawczego, gdyż wspólne cechy różnych organizmów mogą stanowić klucz do poznania mechanizmów leżących

u podstaw wydłużania życia (Gabryel & Duszkiewicz, 2021; Grządziel & Goździalska, 2022; Zyriax & Windler, 2023). W niniejszym badaniu wykazano, że na tym etapie przeżywalność świerszczy była zarówno zależna od linii, jak i zastosowanych czynników. Wyniki analizy przeżywalności zasugerowały, że selekcja w kierunku długowieczności prowadzi do odmiennego profilu aktywności SIRT, gdyż wykazano istotne różnice między liniami, a aktywność sirtuin wykazywała tendencję do wyższych wartości w szczepie D, zależną od stadium rozwojowego. Zaobserwowane zjawisko może być związane z rodzajem zastosowanej selekcji, opartej na opóźnieniu reprodukcji, która mogła sprzyjać zmianom prowadzącym do wydłużenia przeżycia. Warto podkreślić, że aktywność SIRT1 w układach biologicznych jest silnie powiązana z deacetylacją kluczowych substratów, takich jak histony oraz czynniki transkrypcyjne p53, NF-κB i FOXO (Sivakumar i in., 2022; Zessin i in., 2023; Ziętara i in., 2022). Ponadto wskazano, że SIRT1 uczestniczy w regulacji procesów zależnych od acetylacji lizyny również poza klasycznymi układami modelowymi (Xuan i in., 2017). Może to sugerować zaangażowanie SIRT1 w szlaki regulacyjne również u *A. domesticus,* co dało przesłanki do kolejnych eksperymentów i wykonanie badań w innych punktach czasowych, w tym w stadium larwalnym.

Wykazano również, że zarówno RV, jak i NDs wpływają na aktywność sirtuin oraz przeżywalność organizmów, jednak efekt ten był zależny od czasu oraz typu czynnika. RV wykazywał punktowe działanie stymulujące, zależne od czasu i stadium rozwojowego, szczególnie w późniejszych punktach czasowych, natomiast NDs wykazywały zmienny, często przejściowy efekt, zależny od wieku i linii. Przejściowy wzrost aktywności sirtuin w odpowiedzi na NDs może wskazywać na adaptacyjny charakter odpowiedzi komórkowej, opisywany również w kontekście działania nanocząstek, co jest spójne z doniesieniami o hormetycznym działaniu niskich stężeń nanocząstek, w tym nanodiamentów, na komórkowe mechanizmy obronne i ekspresję SIRT1 (Mytych i in., 2016). Efekt ten był zależny od wieku, przy czym 15. dzień życia imago w linii D wykazywał zwiększoną wrażliwość w wybranych parametrach na RV i NDs. Stymulujące działanie RV na aktywność SIRT1 w szczepie H wpisuje się w jego rolę jako modulatora tych enzymów, jednak obserwowany efekt był zależny od czasu i nie miał jednoznacznego charakteru (Bhullar & Hubbard, 2015; Ungurianu i in., 2023). Efekty wydłużenia życia przez RV obserwowano również w modelach owadzich, choć ich charakter był zróżnicowany (J. Song i in., 2021; Yu & Li, 2012).

Analiza uszkodzeń DNA wykazała, że aktywacja ATM i H2A.X w badanych grupach może sygnalizować uruchomienie mechanizmów naprawczych, co jest spójne z bezpośrednim związkiem między SIRT1 a naprawą DNA opisanym przez Penga i wsp. (Peng i in., 2015). Uzyskane wyniki sugerują relatywnie bezpieczny profil działania NDs w zastosowanej dawce, co jest zgodne z wcześniejszymi obserwacjami (Augustyniak i in., 2023; Bilal i in., 2021).

Etap III - Eksperyment I (RV / NDs podawane oddzielnie)

Wyniki tego etapu badań skłaniają do refleksji w jakim stopniu biologiczna zmienność wewnątrzgatunkowa, która obejmuje różnice w tempie starzenia, odporności na stres oksydacyjny i mechanizmach naprawy DNA może modyfikować odpowiedź na czynniki modulujące aktywność sirtuin. Wskazano, że odpowiedź ta mogła być zależna od stadium rozwojowego, linii hodowlanej, a obserwowane zmiany nie miał charakteru jednoznacznego ani trwałego. Może to wynikać z faktu, że aktywność sirtuin jest precyzyjnie regulowana i ujawnia wyraźniejsze różnice dopiero w określonych warunkach fizjologicznych, co może sugerować, że ich aktywność istotnie zmienia się dopiero w warunkach intensywnego stresu lub poważnych uszkodzeń DNA, czyli warunki, których najprawdopodobniej nie zostały wywołane przez zastosowane stężenia RV i NDs (Imai & Guarente, 2014; Ziętara-Krzyk i in., 2026a). Obserwacje te są spójne z wcześniejszymi doniesieniami, wskazującymi na zależność aktywności sirtuin od poziomu stresu oksydacyjnego, gdzie łagodny stres oksydacyjny może działać stymulująco na sirtuiny jako mechanizm ochronny, natomiast intensywne uszkodzenia prowadzą do dysfunkcji tych enzymów (Santos i in., 2016; Webster i in., 2012).

Szczególnie interesująca była obserwacja dotycząca SIRT6, gdzie wzrost jej aktywności postępował z wiekiem niezależnie od linii i stosowanych substancji. To sugeruje związek tego enzymu ze zmianami towarzyszącymi starzeniu. Jest to zgodne z jego opisywaną rolą w utrzymaniu stabilności genomu u starzejących się organizmów w badaniach na myszach z deficytem tego enzymu (Mao i in., 2011; Mostoslavsky i in., 2006). Podobnie u *Drosophila melanogaster*, gdzie nadekspresja dSirt6 wydłużała życie i wzmacniała odporność na stres oksydacyjny (Taylor i in., 2022). Stabilność aktywności SIRT1 w czasie, przy braku wyraźnego związku z wiekiem, jest z kolei spójna z obserwacjami w modelach owadzich, gdzie zmiany aktywności tego enzymu zależały bardziej od warunków zewnętrznych niż od stanu fizjologicznego (Banerjee i in., 2012).

Badania na *C. elegans* i *Drosophila* wskazują ponadto, że sam wzrost aktywności sirtuin nie przekłada się automatycznie na wydłużenie życia bez towarzyszących mu zmian metabolicznych (Burnett i in., 2011). Uzyskane wyniki zdają się potwierdzać tę interpretację. Brak spójnego i jednoznacznego profilu zmian aktywności SIRT1 i SIRT6 pomimo wieloletniej selekcji na długowieczność skłania do wniosku, że ich udział w mechanizmach wydłużonego życia może być ograniczony w modelu opartym na opóźnieniu reprodukcji (Banerjee i in., 2012; Flasz i in., 2021). Takie ujęcie może uzupełniać teorię „disposable soma" (pol. teoria jednorazowego ciała lub teoria jednorazowej somy), sugerując, że nie wszystkie molekularne mechanizmy utrzymania soma są modyfikowane w toku selekcji ewolucyjnej lub hodowlanej (Kirkwood & Holliday, 1979).

Efekt RV na aktywność sirtuin obserwowano jedynie w wybranych grupach, co pozostaje zgodne z doniesieniami z innych modeli owadzich, gdzie wpływ RV na długość życia był silnie zależny od kompozycji diety i płci (Bass i in., 2007). Efekt RV jest zależny zarówno od dawki, jak i czasu suplementacji, co może tłumaczyć jego niejednolite działanie w długoterminowych zastosowaniach (Mansouri i in., 2025). Interpretację utrudniają też opisane w literaturze właściwości RV w postaci działania pro- i antyoksydacyjnego zależnego od warunków ekspozycji (Salehi i in., 2018; L.-X. Zhang i in., 2021). Wzrost aktywności sirtuin w larwach eksponowanych na NDs był natomiast interpretowany jako krótkotrwała odpowiedź na łagodny stres indukowany nanocząstkami (Mytych i in., 2016), analogiczna do efektów opisanych przez Augustyniak i wsp. (Augustyniak i in., 2023), którzy wykazali, że reakcje na NDs są zależne od linii i wieku i nie zawsze tworzą spójny wzorzec.

Selektywne korelacje między aktywnością SOD a SIRT1 (korelacja dodatnia) i SIRT6 (korelacja ujemna) oraz między całkowitą aktywnością SIRT a γH2A.X (korelacja ujemna) sugerowały częściowe połączenie aktywności sirtuin z obroną antyoksydacyjną i sygnalizacją uszkodzeń DNA, ale bez jednolitego wzorca obejmującego wszystkie badane parametry. Wskazuje to, że ewentualne działanie modulacyjne RV i NDs odbywa się raczej poprzez regulację sygnałową niż całkowitą zmianę aktywności enzymatycznej (Ziętara-Krzyk i in., 2026a).

Etap IV - Eksperyment II (RV + NDs podawane łącznie)

Połączenie resweratrolu z nanodiamentami wynikało z dwóch przesłanek. Po pierwsze, resweratrol cechuje się niską biodostępnością i krótkim czasem utrzymywania się w organizmie, co ogranicza jego potencjał biologiczny (Peñalva i in., 2018; Salla i in., 2024; Yang i in., 2020). Po drugie, oba czynniki stosowane oddzielnie wywołują odmienne, a niekiedy nawet przeciwne efekty, co sugeruje, że ich jednoczesne zastosowanie może prowadzić do uzyskania jakościowo innej odpowiedzi niż prosta suma ich działania (Ziętara i in., 2024; Ziętara-Krzyk i in., 2026a).

W kontekście interpretacji wyników biologicznych zasadne wydało się potwierdzenie fizykochemicznej interakcji RV z powierzchnią NDs. W tym celu zastosowano szereg niezależnych technik analitycznych: TEM, EDS, DLS, pomiar potencjału zeta, spektroskopię UV-Vis i Ramana. Zmiana morfologii agregatów w kierunku bardziej zwartych struktur, obniżenie potencjału zeta przy zachowanej średnicy hydrodynamicznej oraz obecność charakterystycznych pasm w widmie Ramana, interpretowanych jako możliwe ślady adsorpcji RV na powierzchni rdzenia diamentowego, wskazują na występowanie interakcji między RV a NDs w zastosowanych warunkach eksperymentalnych (Afandi i in., 2018; Bogdanov i in., 2021; Chen & Jensen, 2023; Ferrari & Robertson, 2004; Korepanov i in., 2017; Liang i in., 2021).

Brak wyraźnego wpływu RV+NDs na przeżywalność w szczepie H jest zgodny z obserwacjami wskazującymi, że oddziaływanie resweratrolu na długość życia zależy od genotypu, warunków środowiskowych oraz wieku organizmu (Staats i in., 2018; C. Wang i in., 2013). Doniesienia dotyczące układów nanonośnikowych wskazują, że choć mogą one poprawiać biodostępność związków bioaktywnych, efekty in vivo nie zawsze przekładają się na zmiany przeżywalności, lecz częściej manifestują się na poziomie procesów molekularnych (Kermanizadeh i in., 2018; Peñalva i in., 2018; Y. Song i in., 2024). W przypadku szczepu D zaobserwowano natomiast różnice w przebiegu krzywych przeżycia, w tym wydłużenie maksymalnej długości życia w grupie RV+NDs (przy braku istotnych zmian mediany przeżycia), co może sugerować zależność odpowiedzi od uwarunkowań genetycznych organizmu (Burgess, 2011).

Trwały wzrost całkowitej aktywności sirtuin w szczepie D, nieobserwowany przy RV i NDs stosowanych oddzielnie (Ziętara-Krzyk i in., 2026a), wskazuje na to, że jednoczesne podanie obu substancji może prowadzić do odmiennej regulacji aktywności

sirtuin, a nie do prostego wzmocnienia efektów każdej z nich. Efekt ten nie był jednak związany z jednoznaczną aktywacją SIRT1 ani SIRT6, co może sugerować udział innych izoform sirtuin. Biorąc pod uwagę, że całkowita aktywność SIRT odzwierciedla łączny udział różnych izoform oraz zależy od dostępności NAD^+, obserwowany wzrost może wynikać z pośredniej regulacji aktywności całej rodziny sirtuin, a nie selektywnej aktywacji pojedynczych enzymów. Cytoplazmatyczne i mitochondrialne sirtuiny (SIRT2, SIRT3, SIRT4 i SIRT5) uczestniczą w komórkowej odpowiedzi na stres i regulacji metabolicznej (Krekora i in., 2025; Sarikhani i in., 2018), a w modelach bezkręgowców mitochondrialne sirtuiny biorą udział w regulacji oksydacyjnego metabolizmu i usuwaniu ROS (Lombard i in., 2011; L. Zhao i in., 2020).

Aktywacja markerów odpowiedzi na uszkodzenia DNA (pATM, γH2A.X i DSBs), szczególnie widoczna we wczesnej dorosłości szczepu D, można interpretować w kontekście opisywanych w literaturze powiązań między szlakiem DDR a regulacją sirtuin. Wykazano, że aktywacja ATM może wpływać na aktywność SIRT1, a sirtuiny, takie jak SIRT6 i SIRT7, uczestniczą w regulacji odpowiedzi na uszkodzenia DNA (Dobbin i in., 2013; Rizzo i in., 2017; Tang i in., 2019). Jednak w przeprowadzonym badaniu nie stwierdzono istotnych korelacji między aktywnością sirtuin a markerami uszkodzeń DNA, co wskazuje, że obserwowane zmiany mogą zachodzić niezależnie od siebie. Brak narastania tych efektów w kolejnych punktach pomiarowych sugeruje ponadto, że odpowiedź ta ma charakter przejściowy lub adaptacyjny, a nie progresywny (Ziętara-Krzyk i in., 2026a).

Odnośnie do SIRT6, Kanfi i wsp. wykazali, że jej aktywność u myszy zależy od kontekstu fizjologicznego i wieku, a nadekspresja nie prowadzi do liniowych efektów wydłużania życia (Kanfi i in., 2012). Zhao i Wu potwierdzili tę zależność w modelu chondrocytów, gdzie aktywność SIRT6 zmieniała się wraz z postępem starzenia (H. Zhao & Wu, 2025). W modelu *Drosophila melanogaster* wykazano natomiast, że nadekspresja dSirt6 może wydłużać życie i zwiększać odporność na stres oksydacyjny (Taylor i in., 2022). W tym kontekście brak wyraźnych zmian aktywności SIRT6 w stadium larwalnym i we wczesnej dorosłości w przeprowadzonych badaniach może wskazywać na zależność funkcji tego enzymu od etapu rozwoju lub warunków fizjologicznych.

Podsumowanie

Przeprowadzone badania pozwoliły na kompleksową ocenę roli sirtuin oraz ich modulacji przez resweratrol, nanodiamenty oraz ich kombinację w regulacji procesów starzenia u *Acheta domesticus*. Uzyskane wyniki nie potwierdzają w pełni założenia, że aktywność sirtuin stanowi kluczowy czynnik determinujący długość życia w tym modelu. Pomimo obserwowanych różnic pomiędzy linią dziką (H) a linią selekcjonowaną pod kątem długowieczności (D), nie miały one charakteru jednoznacznego ani trwałego, co wskazuje, że zwiększona długość życia nie wynika bezpośrednio z podwyższonej aktywności badanych izoform (SIRT1 i SIRT6). Tym samym hipoteza zakładająca brak istotnej zależności pomiędzy aktywnością sirtuin a długowiecznością u *A. domesticus* z linii selekcjonowanej ($H_{1.0}$) może być przyjęta.

Wykazano, że zarówno resweratrol, jak i nanodiamenty stosowane oddzielnie wpływają na badane parametry biologiczne, jednak ich działanie ma charakter zależny od przynależności do linii oraz stadium rozwojowego. Obserwowane zmiany w aktywności sirtuin, parametrach stresu oksydacyjnego oraz markerach uszkodzeń DNA często były istotne statystycznie i często współwystępowały, jednak ich kierunek i intensywność nie były stałe. Wskazuje to, że odpowiedź organizmu ma charakter dynamiczny i kontekstowy, a nie liniowy i przewidywalny. W tym ujęciu odrzucono hipotezę $H_{2.0}$, natomiast hipoteza $H_{2.1}$ znalazła potwierdzenie.

Istotnym elementem pracy była ocena wpływu NDs oraz ich jednoczesnego zastosowania z RV. Uzyskane wyniki wskazują, że kombinacja RV i NDs nie prowadzi do prostego sumowania efektów obserwowanych dla pojedynczych czynników, lecz generuje jakościowo odmienną odpowiedź biologiczną. Szczególnie widoczne było to na poziomie odpowiedzi molekularnej, gdzie zmiany miały charakter zależny od wieku oraz uwarunkowań wewnętrznych organizmu. Tym samym odrzucono hipotezę o braku wpływu NDs na działanie RV ($H_{3.0}$), potwierdzając jednocześnie, że ich obecność modyfikuje odpowiedź biologiczną ($H_{3.1}$). Obserwowane zmiany w markerach uszkodzeń DNA wskazują również na potencjalne obciążenie komórkowe, jednak brak ich narastania w czasie sugeruje raczej uruchomienie mechanizmów adaptacyjnych niż jednoznacznie toksyczny efekt ich działania ($H_{3.2}$).

Analiza parametrów komórkowych wykazała ponadto, że zmiany w stresie oksydacyjnym, aktywności enzymów antyoksydacyjnych, markerach uszkodzeń DNA oraz aktywnością sirtuin wykazują istotne współzależności, jednak relacje te nie mają

charakteru jednoznacznego ani uniwersalnego. Relacje te zmieniały się w zależności od linii i stadium rozwojowego, co wskazuje na ich dynamiczny charakter ($H_{4.2}$).

Choć droga do długowieczności pozostaje nadal nie w pełni poznana, uzyskane wyniki pozwalają lepiej uchwycić jej przebieg i obrać właściwy kierunek. Nie prowadzą one jeszcze do jednoznacznych stwierdzeń, lecz wyznaczają kolejne punkty na tej drodze, wskazując kierunek dalszych badań i zawężając możliwe scenariusze interpretacyjne. Jednocześnie wyniki niniejszej pracy podkreślają istotną potrzebę badań nad bezpieczeństwem stosowania związków bioaktywnych oraz nanomateriałów, zarówno w ujęciu pojedynczym, jak i w formie ich kombinacji. Efekty takich interakcji nie mogą być przewidywane na podstawie działania pojedynczych czynników. Uzyskane wyniki stanowią wkład w rozwój badań nad mechanizmami starzenia oraz bezpieczeństwem stosowania nanomateriałów w układach biologicznych. Równocześnie wskazują na zasadność rozszerzenia badań na inne modele biologiczne.

Bibliografia

Afandi, A., Howkins, A., Boyd, I. W., & Jackman, R. B. (2018). Nanodiamonds for device applications: An investigation of the properties of boron-doped detonation nanodiamonds. *Scientific Reports*, *8*(1), 3270. https://doi.org/10.1038/s41598-018-21670-w

Alexander, E., & Leong, K. W. (2024). Nanodiamonds in biomedical research: Therapeutic applications and beyond. *PNAS Nexus*, *3*(5). https://doi.org/10.1093/pnasnexus/pgae198

Alexander, E., & Leong, K. W. (2025). Toxicity and biodistribution comparison of functionalized nanodiamonds, quantum dot nanocarbons and gold nanoparticles. *Frontiers in Nanotechnology*, *7*. https://doi.org/10.3389/fnano.2025.1512622

Anderson, G., & Maes, M. (2021). Sirtuins, mitochondria, and the melatonergic pathway in Alzheimer's disease. W *Sirtuin Biology in Medicine* (s. 117–135). Elsevier. https://doi.org/10.1016/B978-0-12-814118-2.00015-X

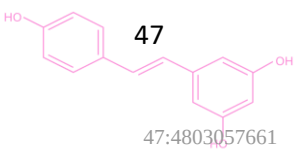
Augustyniak, M., Ajay, A. K., Kędziorski, A., Tarnawska, M., Rost-Roszkowska, M., Flasz, B., Babczyńska, A., Mazur, B., Rozpędek, K., Alian, R. S., Skowronek, M., Świerczek, E., Wiśniewska, K., & Ziętara, P. (2023). Survival, growth and digestive functions after exposure to nanodiamonds - Transgenerational effects beyond contact time in house cricket strains. *Chemosphere*, *349*, 140809. https://doi.org/10.1016/j.chemosphere.2023.140809

Augustyniak, M., Babczyńska, A., Dziewięcka, M., Flasz, B., Karpeta-Kaczmarek, J., Kędziorski, A., Mazur, B., Rozpędek, K., Seyed Alian, R., Skowronek, M., Świerczek, E., Świętek, A., Tarnawska, M., Wiśniewska, K., & Ziętara, P. (2022). Does age pay off? Effects of three-generational experiments of nanodiamond exposure and withdrawal in wild and longevity-selected model animals. *Chemosphere*, *303*, 135129. https://doi.org/https://doi.org/10.1016/j.chemosphere.2022.135129

Banerjee, K. Kr., Ayyub, C., Ali, S. Z., Mandot, V., Prasad, N. G., & Kolthur-Seetharam, U. (2012). dSir2 in the Adult Fat Body, but Not in Muscles, Regulates Life Span in a Diet-Dependent Manner. *Cell Reports*, *2*(6), 1485–1491. https://doi.org/10.1016/j.celrep.2012.11.013

Barzegar Amiri Olia, M., Donnelly, P. S., Hollenberg, L. C. L., Mulvaney, P., & Simpson, D. A. (2021). Advances in the Surface Functionalization of Nanodiamonds for Biological Applications: A Review. *ACS Applied Nano Materials*, *4*(10), 9985–10005. https://doi.org/10.1021/acsanm.1c02698

Bass, T. M., Weinkove, D., Houthoofd, K., Gems, D., & Partridge, L. (2007). Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans. *Mechanisms of Ageing and Development*, *128*(10), 546–552. https://doi.org/10.1016/j.mad.2007.07.007



Bhullar, K. S., & Hubbard, B. P. (2015). Lifespan and healthspan extension by resveratrol. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1852*(6), 1209–1218. https://doi.org/10.1016/j.bbadis.2015.01.012

Bilal, M., Cheng, H., González-González, R. B., Parra-Saldívar, R., & Iqbal, H. M. N. (2021). Bio-applications and biotechnological applications of nanodiamonds. *Journal of Materials Research and Technology*, *15*, 6175–6189. https://doi.org/10.1016/j.jmrt.2021.11.037

Bogdanov, D., Bogdanov, A., Plotnikov, V., Makarov, S., Yelisseyev, A., & Chepurov, A. (2021). Core growth of detonation nanodiamonds under high-pressure annealing. *RSC Advances*, *11*(21), 12961–12970. https://doi.org/10.1039/D1RA00270H

Burgess, D. J. (2011). The dangers lurking in the genetic background. *Nature Reviews Genetics*, *12*(11), 742–742. https://doi.org/10.1038/nrg3089

Burnett, C., Valentini, S., Cabreiro, F., Goss, M., Somogyvári, M., Piper, M. D., Hoddinott, M., Sutphin, G. L., Leko, V., McElwee, J. J., Vazquez-Manrique, R. P., Orfila, A.-M., Ackerman, D., Au, C., Vinti, G., Riesen, M., Howard, K., Neri, C., Bedalov, A., … Gems, D. (2011). Absence of effects of Sir2 overexpression on lifespan in C. elegans and Drosophila. *Nature*, *477*(7365), 482–485. https://doi.org/10.1038/nature10296

Bursch, K. L., Goetz, C. J., & Smith, B. C. (2024). Current Trends in Sirtuin Activator and Inhibitor Development. *Molecules*, *29*(5), 1185. https://doi.org/10.3390/molecules29051185

Candal-Pedreira, C., Martín-Gisbert, L., Teijeiro, A., García, G., Ruano-Ravina, A., & Pérez-Ríos, M. (2025). Blue Zones, an Analysis of Existing Evidence through a Scoping Review. *aging and disease*. https://doi.org/10.14336/AD.2025.0461

Chauhan, S., Jain, N., & Nagaich, U. (2020). Nanodiamonds with powerful ability for drug delivery and biomedical applications: Recent updates on in vivo study and patents. *Journal of Pharmaceutical Analysis*, *10*(1), 1–12. https://doi.org/10.1016/j.jpha.2019.09.003

Chen, R., & Jensen, L. (2023). Interpreting chemical enhancements of surface-enhanced Raman scattering. *Chemical Physics Reviews*, *4*(2). https://doi.org/10.1063/5.0138501

Costa, B. N. L., Camarneiro, F., Marote, A., Barbosa, C., Vedor, C., Tomé, D., Costa, F. J., Dias, M. S., Correia, J., Pires, J., Chícharo, A., Almeida, R. D., Salgado, A., & Nieder, J. B. (2024). Functionalized Nanodiamonds for Targeted Neuronal Electromagnetic Signal Detection. *ACS Applied Materials & Interfaces*, *16*(44), 60828–60841. https://doi.org/10.1021/acsami.4c12462

Cui, J., Hu, B., Fu, Y., Xu, Z., & Li, Y. (2023). pH-Sensitive nanodiamond co-delivery of retinal and doxorubicin boosts breast cancer chemotherapy. *RSC Advances*, *13*(39), 27403–27414. https://doi.org/10.1039/D3RA03907B

Dai, H., Sinclair, D. A., Ellis, J. L., & Steegborn, C. (2018). Sirtuin activators and inhibitors: Promises, achievements, and challenges. *Pharmacology & Therapeutics*, *188*, 140–154. https://doi.org/10.1016/j.pharmthera.2018.03.004

Dali-Youcef, N., Lagouge, M., Froelich, S., Koehl, C., Schoonjans, K., & Auwerx, J. (2007). Sirtuins: The 'magnificent seven', function, metabolism and longevity. *Annals of Medicine*, *39*(5), 335–345. https://doi.org/10.1080/07853890701408194

Damschroder, D., Zapata-Pérez, R., Richardson, K., Vaz, F. M., Houtkooper, R. H., & Wessells, R. (2022). Stimulating the sir2–spargel axis rescues exercise capacity and mitochondrial respiration in a Drosophila model of Barth syndrome. *Disease Models & Mechanisms*, *15*(10). https://doi.org/10.1242/dmm.049279

Dobbin, M. M., Madabhushi, R., Pan, L., Chen, Y., Kim, D., Gao, J., Ahanonu, B., Pao, P.-C., Qiu, Y., Zhao, Y., & Tsai, L.-H. (2013). SIRT1 collaborates with ATM and HDAC1 to maintain genomic stability in neurons. *Nature Neuroscience*, *16*(8), 1008–1015. https://doi.org/10.1038/nn.3460

Ferrari, A. C., & Robertson, J. (2004). Raman spectroscopy of amorphous, nanostructured, diamond–like carbon, and nanodiamond. *Philosophical Transactions of the Royal Society of London. Series A: Mathematical, Physical and Engineering Sciences*, *362*(1824), 2477–2512. https://doi.org/10.1098/rsta.2004.1452

Fiorentino, F., Fabbrizi, E., Mai, A., & Rotili, D. (2025). Activation and inhibition of sirtuins: From bench to bedside. *Medicinal Research Reviews*, *45*(2), 484–560. https://doi.org/10.1002/med.22076

Flasz, B., Dziewięcka, M., Kędziorski, A., Tarnawska, M., Augustyniak, J., & Augustyniak, M. (2021). Multigenerational selection towards longevity changes the protective role of vitamin C against graphene oxide-induced oxidative stress in house crickets. *Environmental Pollution*, *290*, 117996. https://doi.org/10.1016/j.envpol.2021.117996

Flasz, B., Dziewięcka, M., Kędziorski, A., Tarnawska, M., & Augustyniak, M. (2020). Vitellogenin expression, DNA damage, health status of cells and catalase activity in Acheta domesticus selected according to their longevity after graphene oxide treatment. *Science of The Total Environment*, *737*, 140274. https://doi.org/https://doi.org/10.1016/j.scitotenv.2020.140274

Frankel, S., Ziafazeli, T., & Rogina, B. (2011). dSir2 and longevity in Drosophila. *Experimental Gerontology*, *46*(5), 391–396. https://doi.org/10.1016/j.exger.2010.08.007

Gabryel, B., & Duszkiewicz, R. (2021). Sestryny jako modulatory procesów starzenia i chorób związanych z wiekiem*. *Postępy Higieny i Medycyny Doświadczalnej*, *75*(1), 437–447. https://doi.org/10.5604/01.3001.0014.9471

Gertz, M., Nguyen, G. T. T., Fischer, F., Suenkel, B., Schlicker, C., Fränzel, B., Tomaschewski, J., Aladini, F., Becker, C., Wolters, D., & Steegborn, C. (2012). A

Molecular Mechanism for Direct Sirtuin Activation by Resveratrol. *PLoS ONE*, *7*(11), e49761. https://doi.org/10.1371/journal.pone.0049761

Giblin, W., & Lombard, D. B. (2016). Sirtuins, Healthspan, and Longevity in Mammals. W *Handbook of the Biology of Aging* (s. 83–132). Elsevier. https://doi.org/10.1016/B978-0-12-411596-5.00003-4

Gladyshev, V. N., Kritchevsky, S. B., Clarke, S. G., Cuervo, A. M., Fiehn, O., de Magalhães, J. P., Mau, T., Maes, M., Moritz, R. L., Niedernhofer, L. J., Van Schaftingen, E., Tranah, G. J., Walsh, K., Yura, Y., Zhang, B., & Cummings, S. R. (2021). Molecular damage in aging. *Nature Aging*, *1*(12), 1096–1106. https://doi.org/10.1038/s43587-021-00150-3

Grabowska, W., Sikora, E., & Bielak-Zmijewska, A. (2017). Sirtuins, a promising target in slowing down the ageing process. *Biogerontology*, *18*(4), 447–476. https://doi.org/10.1007/s10522-017-9685-9

Grootaert, M. O. J., & Bennett, M. R. (2022). Sirtuins in atherosclerosis: guardians of healthspan and therapeutic targets. *Nature Reviews Cardiology*, *19*(10), 668–683. https://doi.org/10.1038/s41569-022-00685-x

Grządziel, P., & Goździalska, A. (2022). Etiology and possibilities of slowing down skin aging processes. *Aesthetic Cosmetology and Medicine*, *11*(1), 3–10. https://doi.org/10.52336/acm.2022.001

Grzeczka, A., & Kordowitzki, P. (2022). Resveratrol and SIRT1: Antiaging Cornerstones for Oocytes? *Nutrients*, *14*(23), 5101. https://doi.org/10.3390/nu14235101

Guan, X., Lin, P., Knoll, E., & Chakrabarti, R. (2014). Mechanism of Inhibition of the Human Sirtuin Enzyme SIRT3 by Nicotinamide: Computational and Experimental Studies. *PLoS ONE*, *9*(9), e107729. https://doi.org/10.1371/journal.pone.0107729

Haigis, M. C., & Sinclair, D. A. (2010). Mammalian Sirtuins: Biological Insights and Disease Relevance. *Annual Review of Pathology: Mechanisms of Disease*, *5*(1), 253–295. https://doi.org/10.1146/annurev.pathol.4.110807.092250

Horch, H. W., Mito, T., Popadić, A., Ohuchi, H., & Noji, S. (2017). *The Cricket as a Model Organism*. Springer Japan. https://doi.org/10.1007/978-4-431-56478-2

Hu, J., Jing, H., & Lin, H. (2014). Sirtuin Inhibitors as Anticancer Agents. *Future Medicinal Chemistry*, *6*(8), 945–966. https://doi.org/10.4155/fmc.14.44

Imai, S., & Guarente, L. (2014). NAD+ and sirtuins in aging and disease. *Trends in Cell Biology*, *24*(8), 464–471. https://doi.org/10.1016/j.tcb.2014.04.002

Jariwala, D. H., Patel, D., & Wairkar, S. (2020). Surface functionalization of nanodiamonds for biomedical applications. *Materials Science and Engineering: C*, *113*, 110996. https://doi.org/10.1016/j.msec.2020.110996

Jović, D., Jović, B., Borišev, I., Bogdanović, V., & Djordjevic, A. (2026). Spectroscopic Insights into Nanodiamond–Doxorubicin Interactions in Drug Delivery Systems

for Potential Cancer Treatment: "What Is Essential Is Invisible to the Eye". *Pharmaceutics*, *18*(4), 438. https://doi.org/10.3390/pharmaceutics18040438

Kanfi, Y., Naiman, S., Amir, G., Peshti, V., Zinman, G., Nahum, L., Bar-Joseph, Z., & Cohen, H. Y. (2012). The sirtuin SIRT6 regulates lifespan in male mice. *Nature*, *483*(7388), 218–221. https://doi.org/10.1038/nature10815

Karpeta-Kaczmarek, J., Dziewięcka, M., Augustyniak, M., & Rost-Roszkowska, M. (2016). Effects of short-term exposure of Acheta domesticus to nanodiamonds in food: DNA damage but no histological alteration in tissues. *Carbon*, *110*, 458–468. https://doi.org/10.1016/j.carbon.2016.09.053

Karpeta-Kaczmarek, J., Kędziorski, A., Augustyniak-Jabłokow, M. A., Dziewięcka, M., & Augustyniak, M. (2018). Chronic toxicity of nanodiamonds can disturb development and reproduction of Acheta domesticus L. *Environmental Research*, *166*, 602–609. https://doi.org/10.1016/j.envres.2018.05.027

Kaufman, M. G., Klug, M. J., & Merritt, R. W. (1989). Growth and food utilization parameters of germ-free house crickets, Acheta domesticus. *Journal of Insect Physiology*, *35*(12), 957–967. https://doi.org/10.1016/0022-1910(89)90019-X

Keller, A., Temple, T., Sayanjali, B., & Mihaylova, M. M. (2021). Metabolic Regulation of Stem Cells in Aging. *Current Stem Cell Reports*, *7*(2), 72–84. https://doi.org/10.1007/s40778-021-00186-6

Kermanizadeh, A., Powell, L. G., Stone, V., & Møller, P. (2018). Nanodelivery systems and stabilized solid-drug nanoparticles for orally administered medicine: current landscape. *International Journal of Nanomedicine*, *Volume 13*, 7575–7605. https://doi.org/10.2147/IJN.S177418

Kilic Eren, M., Kilincli, A., & Eren, Ö. (2015). Resveratrol Induced Premature Senescence Is Associated with DNA Damage Mediated SIRT1 and SIRT2 Down-Regulation. *PLOS ONE*, *10*(4), e0124837. https://doi.org/10.1371/journal.pone.0124837

Kirkwood, T. B. L., & Holliday, R. (1979). The evolution of ageing and longevity. *Proceedings of the Royal Society of London. Series B. Biological Sciences*, *205*(1161), 531–546. https://doi.org/10.1098/rspb.1979.0083

Knop, M., Treitz, C., Bettendorf, S., Bossen, J., von Frieling, J., Doms, S., Saboukh, A., Bruchhaus, I., Kühnlein, R. P., Baines, J. F., Tholey, A., & Roeder, T. (2025). Mitochondrial sirtuin 4 shapes the intestinal microbiota of Drosophila by controlling lysozyme expression. *Animal Microbiome*, *7*(1), 63. https://doi.org/10.1186/s42523-025-00431-x

Kopeć, A., Piątkowska, E., Leszczyńska, T., & Bieżanowska-Kopeć, R. (2011). Prozdrowotne właściwości resweratrolu. *ŻYWNOŚĆ. Nauka. Technologia. Jakość*, *5*(78), 5–15.

Korepanov, V. I., Hamaguchi, H., Osawa, E., Ermolenkov, V., Lednev, I. K., Etzold, B. J. M., Levinson, O., Zousman, B., Epperla, C. P., & Chang, H.-C. (2017). Carbon

structure in nanodiamonds elucidated from Raman spectroscopy. *Carbon*, *121*, 322–329. https://doi.org/10.1016/j.carbon.2017.06.012

Krekora, J., Pawlowska, E., Derwich, M., Drożdż, J., & Blasiak, J. (2025). Oxidative Stress, Mitochondrial Homeostasis, and Sirtuins in Atrial Fibrillation. *International Journal of Molecular Sciences*, *27*(1), 175. https://doi.org/10.3390/ijms27010175

Liang, X., Li, N., Zhang, R., Yin, P., Zhang, C., Yang, N., Liang, K., & Kong, B. (2021). Carbon-based SERS biosensor: from substrate design to sensing and bioapplication. *NPG Asia Materials*, *13*(1), 8. https://doi.org/10.1038/s41427-020-00278-5

Liguori, I., Russo, G., Curcio, F., Bulli, G., Aran, L., Della-Morte, D., Gargiulo, G., Testa, G., Cacciatore, F., Bonaduce, D., & Abete, P. (2018). Oxidative stress, aging, and diseases. *Clinical Interventions in Aging*, *Volume 13*, 757–772. https://doi.org/10.2147/CIA.S158513

Lombard, D. B., Tishkoff, D. X., & Bao, J. (2011). *Mitochondrial Sirtuins in the Regulation of Mitochondrial Activity and Metabolic Adaptation* (s. 163–188). https://doi.org/10.1007/978-3-642-21631-2_8

Long, Y. C., Tan, T. M. C., Takao, I., & Tang, B. L. (2014). The biochemistry and cell biology of aging: metabolic regulation through mitochondrial signaling. *American Journal of Physiology-Endocrinology and Metabolism*, *306*(6), E581–E591. https://doi.org/10.1152/ajpendo.00665.2013

López De Arriba, L. V., Kharissova, O. V., Nikolaev, A. L., & Kharisov, B. I. (2025). Multimodal functionalization of nanodiamonds for advanced biomedical and optoelectronic applications. *Nano-Structures & Nano-Objects*, *44*, 101576. https://doi.org/10.1016/j.nanoso.2025.101576

Łopuszańska-Dawid, M., Kopiczko, A., Charzewska, J., & Piłsudskiego, A. W. F. J. (2021). *Antropologia w obliczu starzejacego sie społeczeństwa: biologiczne przejawy procesów starzenia sie*. Akademia Wychowania Fizycznego Józefa Piłsudskiego w Warszawie.

Mahdihassan, S. (1976). Early Terms for Elixir Hitherto Unrecognized in Greek Alchemy. *Ambix*, *23*(3), 129–133. https://doi.org/10.1179/amb.1976.23.3.129

Maldonado, E., Morales-Pison, S., Urbina, F., & Solari, A. (2023). Aging Hallmarks and the Role of Oxidative Stress. *Antioxidants*, *12*(3), 651. https://doi.org/10.3390/antiox12030651

Mansouri, F., Feliziani, G., Bordoni, L., & Gabbianelli, R. (2025). Impact of Resveratrol Supplementation on Human Sirtuin 1: A Grading of Recommendations Assessment, Development and Evaluation–Assessed Systematic Review and Dose-Response Meta-Analysis of Randomized Controlled Trials. *Journal of the Academy of Nutrition and Dietetics*, *125*(9), 1299-1314.e1. https://doi.org/10.1016/j.jand.2025.03.011

Mao, Z., Hine, C., Tian, X., Van Meter, M., Au, M., Vaidya, A., Seluanov, A., & Gorbunova, V. (2011). SIRT6 Promotes DNA Repair Under Stress by Activating PARP1. *Science*, *332*(6036), 1443–1446. https://doi.org/10.1126/science.1202723

Matuszyński, W., Matuszyńska, M., Nowicka, P., Jezierska, K., Kalinowska, W., & Gural, M. (2025). Sirtuins as multifunctional regulators: Role in the pathogenesis of metabolic, inflammatory and neurodegenerative diseases and the effect of physical activity on their activity. *Quality in Sport*, *42*, 60514. https://doi.org/10.12775/QS.2025.42.60514

Mochalin, V. N., Shenderova, O., Ho, D., & Gogotsi, Y. (2012). The properties and applications of nanodiamonds. *Nature Nanotechnology*, *7*(1), 11–23. https://doi.org/10.1038/nnano.2011.209

Mostoslavsky, R., Chua, K. F., Lombard, D. B., Pang, W. W., Fischer, M. R., Gellon, L., Liu, P., Mostoslavsky, G., Franco, S., Murphy, M. M., Mills, K. D., Patel, P., Hsu, J. T., Hong, A. L., Ford, E., Cheng, H.-L., Kennedy, C., Nunez, N., Bronson, R., … Alt, F. W. (2006). Genomic Instability and Aging-like Phenotype in the Absence of Mammalian SIRT6. *Cell*, *124*(2), 315–329. https://doi.org/10.1016/j.cell.2005.11.044

Mytych, J., Wnuk, M., & Rattan, S. I. S. (2016). Low doses of nanodiamonds and silica nanoparticles have beneficial hormetic effects in normal human skin fibroblasts in culture. *Chemosphere*, *148*, 307–315. https://doi.org/10.1016/j.chemosphere.2016.01.045

Naseer, A., Mir, S. S., Takacs-Vellai, K., & Nazir, A. (2021). Sirtuins and Autophagy in Age-Associated Neurodegenerative Diseases: Lessons from the C. elegans Model. *International Journal of Molecular Sciences*, *22*(22), 12263. https://doi.org/10.3390/ijms222212263

Nicoletti, G. R. Pietro, Mangano, K., Nicoletti, F., & Cavalli, E. (2025). From Elixirs to Geroscience: A Historical and Molecular Perspective on Anti-Aging Medicine. *Molecules*, *30*(24), 4728. https://doi.org/10.3390/molecules30244728

Okudaira, N., Ishizaka, Y., & Tamamori-Adachi, M. (2022). Resveratrol blocks retrotransposition of LINE-1 through PPAR α and sirtuin-6. *Scientific Reports*, *12*(1), 7772. https://doi.org/10.1038/s41598-022-11761-0

Pan, Y., Zhang, H., Zheng, Y., Zhou, J., Yuan, J., Yu, Y., & Wang, J. (2017). Resveratrol Exerts Antioxidant Effects by Activating SIRT2 To Deacetylate Prx1. *Biochemistry*, *56*(48), 6325–6328. https://doi.org/10.1021/acs.biochem.7b00859

Park, S., Mori, R., & Shimokawa, I. (2013). Do sirtuins promote mammalian longevity?: A Critical review on its relevance to the longevity effect induced by calorie restriction. *Molecules and Cells*, *35*(6), 474–480. https://doi.org/10.1007/s10059-013-0130-x

Pasupuleti, M. K. (2024). *Beyond Aging: Bioprinting and Biotechnology at the Frontier of Life Extension*. National Education Services. https://doi.org/10.62311/nesx/rb978-81-982172-8-8

Pedroso-Santana, S., Fleitas-Salazar, N., Sarabia-Sainz, A., Silva-Campa, E., Burgara-Estrella, A., Angulo-Molina, A., Melendrez, R., Pedroza-Montero, M., & Riera, R. (2018). A nanodiamond-fluorescein conjugate for cell studies. *Advances in Natural Sciences: Nanoscience and Nanotechnology*, *9*(1), 015013. https://doi.org/10.1088/2043-6254/aaa6f2

Peñalva, R., Morales, J., González-Navarro, C. J., Larrañeta, E., Quincoces, G., Peñuelas, I., & Irache, J. M. (2018). Increased Oral Bioavailability of Resveratrol by Its Encapsulation in Casein Nanoparticles. *International Journal of Molecular Sciences*, *19*(9), 2816. https://doi.org/10.3390/ijms19092816

Peng, L., Yuan, Z., Li, Y., Ling, H., Izumi, V., Fang, B., Fukasawa, K., Koomen, J., Chen, J., & Seto, E. (2015). Ubiquitinated Sirtuin 1 (SIRT1) Function Is Modulated during DNA Damage-induced Cell Death and Survival. *Journal of Biological Chemistry*, *290*(14), 8904–8912. https://doi.org/10.1074/jbc.M114.612796

Pieroni, A., Morini, G., Piochi, M., Sulaiman, N., Kalle, R., Haq, S. M., Devecchi, A., Franceschini, C., Zocchi, D. M., Migliavada, R., Prakofjewa, J., Sartori, M., Krigas, N., Ahmad, M., Torri, L., & Sõukand, R. (2023). Bitter Is Better: Wild Greens Used in the Blue Zone of Ikaria, Greece. *Nutrients*, *15*(14), 3242. https://doi.org/10.3390/nu15143242

Poulain, M., Herm, A., Errigo, A., Chrysohoou, C., Legrand, R., Passarino, G., Stazi, M. A., Voutekatis, K. G., Gonos, E. S., Franceschi, C., & Pes, G. M. (2021). Specific features of the oldest old from the Longevity Blue Zones in Ikaria and Sardinia. *Mechanisms of Ageing and Development*, *198*, 111543. https://doi.org/10.1016/j.mad.2021.111543

Price, N. L., Gomes, A. P., Ling, A. J. Y., Duarte, F. V., Martin-Montalvo, A., North, B. J., Agarwal, B., Ye, L., Ramadori, G., Teodoro, J. S., Hubbard, B. P., Varela, A. T., Davis, J. G., Varamini, B., Hafner, A., Moaddel, R., Rolo, A. P., Coppari, R., Palmeira, C. M., … Sinclair, D. A. (2012). SIRT1 Is Required for AMPK Activation and the Beneficial Effects of Resveratrol on Mitochondrial Function. *Cell Metabolism*, *15*(5), 675–690. https://doi.org/10.1016/j.cmet.2012.04.003

Priyadarshni, N., Singh, R., & Mishra, M. K. (2024). Nanodiamonds: Next generation nano-theranostics for cancer therapy. *Cancer Letters*, *587*, 216710. https://doi.org/10.1016/j.canlet.2024.216710

Qin, H., Zhang, H., Zhang, X., Zhang, S., Zhu, S., & Wang, H. (2021). Resveratrol attenuates radiation enteritis through the SIRT1/FOXO3a and PI3K/AKT signaling pathways. *Biochemical and Biophysical Research Communications*, *554*, 199–205. https://doi.org/10.1016/j.bbrc.2021.03.122

Qin, J.-X., Yang, X.-G., Lv, C.-F., Li, Y.-Z., Liu, K.-K., Zang, J.-H., Yang, X., Dong, L., & Shan, C.-X. (2021). Nanodiamonds: Synthesis, properties, and applications in nanomedicine. *Materials & Design*, *210*, 110091. https://doi.org/10.1016/j.matdes.2021.110091

Ray, M. A., Shenderova, O., Hook, W., Martin, A., Grishko, V., Tyler, T., Cunningham, G. B., & McGuire, G. (2006). Cold plasma functionalization of nanodiamond particles. *Diamond and Related Materials*, *15*(11–12), 1809–1812. https://doi.org/10.1016/j.diamond.2006.06.003

Rizzo, A., Iachettini, S., Salvati, E., Zizza, P., Maresca, C., D'Angelo, C., Benarroch-Popivker, D., Capolupo, A., del Gaudio, F., Cosconati, S., Di Maro, S., Merlino, F., Novellino, E., Amoreo, C. A., Mottolese, M., Sperduti, I., Gilson, E., & Biroccio, A. (2017). SIRT6 interacts with TRF2 and promotes its degradation in response to DNA damage. *Nucleic Acids Research*, *45*(4), 1820–1834. https://doi.org/10.1093/nar/gkw1202

Rogina, B., & Helfand, S. L. (2004). Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proceedings of the National Academy of Sciences*, *101*(45), 15998–16003. https://doi.org/10.1073/pnas.0404184101

Rogina, B., & Tissenbaum, H. A. (2024). SIRT1, resveratrol and aging. *Frontiers in Genetics*, *15*. https://doi.org/10.3389/fgene.2024.1393181

Rymarchyk, S., Kang, W., & Cen, Y. (2021). Substrate-Dependent Sensitivity of SIRT1 to Nicotinamide Inhibition. *Biomolecules*, *11*(2), 312. https://doi.org/10.3390/biom11020312

Ryu, T.-K., Kang, R.-H., Jeong, K.-Y., Jun, D.-R., Koh, J.-M., Kim, D., Bae, S. K., & Choi, S.-W. (2016). Bone-targeted delivery of nanodiamond-based drug carriers conjugated with alendronate for potential osteoporosis treatment. *Journal of Controlled Release*, *232*, 152–160. https://doi.org/10.1016/j.jconrel.2016.04.025

Salehi, B., Mishra, A., Nigam, M., Sener, B., Kilic, M., Sharifi-Rad, M., Fokou, P., Martins, N., & Sharifi-Rad, J. (2018). Resveratrol: A Double-Edged Sword in Health Benefits. *Biomedicines*, *6*(3), 91. https://doi.org/10.3390/biomedicines6030091

Salla, M., Karaki, N., El Kaderi, B., Ayoub, A. J., Younes, S., Abou Chahla, M. N., Baksh, S., & El Khatib, S. (2024). Enhancing the Bioavailability of Resveratrol: Combine It, Derivatize It, or Encapsulate It? *Pharmaceutics*, *16*(4), 569. https://doi.org/10.3390/pharmaceutics16040569

Sanders, B. D., Jackson, B., & Marmorstein, R. (2010). Structural basis for sirtuin function: What we know and what we don't. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1804*(8), 1604–1616. https://doi.org/10.1016/j.bbapap.2009.09.009

Santos, L., Escande, C., & Denicola, A. (2016). Potential Modulation of Sirtuins by Oxidative Stress. *Oxidative Medicine and Cellular Longevity*, *2016*(1). https://doi.org/10.1155/2016/9831825

Sarikhani, M., Mishra, S., Desingu, P. A., Kotyada, C., Wolfgeher, D., Gupta, M. P., Singh, M., & Sundaresan, N. R. (2018). SIRT2 regulates oxidative stress-induced cell death through deacetylation of c-Jun NH2-terminal kinase. *Cell Death & Differentiation*, *25*(9), 1638–1656. https://doi.org/10.1038/s41418-018-0069-8

Sauve, A. A. (2010). Sirtuin chemical mechanisms. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1804*(8), 1591–1603. https://doi.org/10.1016/j.bbapap.2010.01.021

Settembrino, A. (2025). Music as Additional Attribute to Healthy Longevity in Nicoya, Costa Rica Blue Zone. *Music Therapy Perspectives*, *43*(2). https://doi.org/10.1093/mtp/miaf010

Shukla, N., & Kolthur-Seetharam, U. (2022). Drosophila Sirtuin 6 mediates developmental diet-dependent programming of adult physiology and survival. *Aging Cell*, *21*(3). https://doi.org/10.1111/acel.13576

Sivakumar, K. K., Stanley, J. A., Behlen, J. C., Wuri, L., Dutta, S., Wu, J., Arosh, J. A., & Banu, S. K. (2022). Inhibition of Sirtuin-1 hyperacetylates p53 and abrogates Sirtuin-1-p53 interaction in Cr(VI)-induced apoptosis in the ovary. *Reproductive Toxicology*, *109*, 121–134. https://doi.org/10.1016/j.reprotox.2022.03.007

Solarska-Ściuk, K., & Kleszczyńska, H. (2019). Possibilities of nanodiamonds application – biological and medical aspects. *Acta Poloniae Pharmaceutica - Drug Research*, *76*(5), 779–796. https://doi.org/10.32383/appdr/109501

Song, J., Liu, L., Hao, K., Mao, S., Tang, Y., Tong, X., & Dai, F. (2021). Resveratrol elongates the lifespan and improves antioxidant activity in the silkworm Bombyx mori. *Journal of Pharmaceutical Analysis*, *11*(3), 374–382. https://doi.org/10.1016/j.jpha.2020.06.005

Song, Y., Zhang, J., Zhu, L., Zhang, H., Wu, G., & Liu, T. (2024). Recent advances in nanodelivery systems of resveratrol and their biomedical and food applications: a review. *Food & Function*, *15*(17), 8629–8643. https://doi.org/10.1039/D3FO03892K

Staats, S., Wagner, A., Kowalewski, B., Rieck, F., Soukup, S., Kulling, S., & Rimbach, G. (2018). Dietary Resveratrol Does Not Affect Life Span, Body Composition, Stress Response, and Longevity-Related Gene Expression in Drosophila melanogaster. *International Journal of Molecular Sciences*, *19*(1), 223. https://doi.org/10.3390/ijms19010223

Taborsky, B., Kuijper, B., Fawcett, T. W., English, S., Leimar, O., McNamara, J. M., & Ruuskanen, S. (2022). An evolutionary perspective on stress responses, damage and repair. *Hormones and Behavior*, *142*, 105180. https://doi.org/10.1016/j.yhbeh.2022.105180

Tang, M., Li, Z., Zhang, C., Lu, X., Tu, B., Cao, Z., Li, Y., Chen, Y., Jiang, L., Wang, H., Wang, L., Wang, J., Liu, B., Xu, X., Wang, H., & Zhu, W.-G. (2019). SIRT7-mediated ATM deacetylation is essential for its deactivation and DNA damage repair. *Science Advances*, *5*(3). https://doi.org/10.1126/sciadv.aav1118

Taylor, J. R., Wood, J. G., Mizerak, E., Hinthorn, S., Liu, J., Finn, M., Gordon, S., Zingas, L., Chang, C., Klein, M. A., Denu, J. M., Gorbunova, V., Seluanov, A., Boeke, J. D., Sedivy, J. M., & Helfand, S. L. (2022). Sirt6 regulates lifespan in

Drosophila melanogaster. *Proceedings of the National Academy of Sciences*, *119*(5). https://doi.org/10.1073/pnas.2111176119

Tissenbaum, H. A., & Guarente, L. (2001). Increased dosage of a sir-2 gene extends lifespan in Caenorhabditis elegans. *Nature*, *410*(6825), 227–230. https://doi.org/10.1038/35065638

Ungurianu, A., Zanfirescu, A., & Margină, D. (2023). Sirtuins, resveratrol and the intertwining cellular pathways connecting them. *Ageing Research Reviews*, *88*, 101936. https://doi.org/10.1016/j.arr.2023.101936

Vesely, O., Baldovska, S., & Kolesarova, A. (2021). Enhancing Bioavailability of Nutraceutically Used Resveratrol and Other Stilbenoids. *Nutrients*, *13*(9), 3095. https://doi.org/10.3390/nu13093095

Vincent, J. A. (2009). Ageing, Anti-ageing, and Anti-anti-ageing: Who are the Progressives in the Debate on the Future of Human Biological Ageing? *Medicine Studies*, *1*(3), 197–208. https://doi.org/10.1007/s12376-009-0016-6

Walle, T. (2011). Bioavailability of resveratrol. *Annals of the New York Academy of Sciences*, *1215*(1), 9–15. https://doi.org/10.1111/j.1749-6632.2010.05842.x

Wang, C., Wheeler, C. T., Alberico, T., Sun, X., Seeberger, J., Laslo, M., Spangler, E., Kern, B., de Cabo, R., & Zou, S. (2013). The effect of resveratrol on lifespan depends on both gender and dietary nutrient composition in Drosophila melanogaster. *AGE*, *35*(1), 69–81. https://doi.org/10.1007/s11357-011-9332-3

Wang, Y., He, J., Liao, M., Hu, M., Li, W., Ouyang, H., Wang, X., Ye, T., Zhang, Y., & Ouyang, L. (2019). An overview of Sirtuins as potential therapeutic target: Structure, function and modulators. *European Journal of Medicinal Chemistry*, *161*, 48–77. https://doi.org/10.1016/j.ejmech.2018.10.028

Webster, B. R., Lu, Z., Sack, M. N., & Scott, I. (2012). The role of sirtuins in modulating redox stressors. *Free Radical Biology and Medicine*, *52*(2), 281–290. https://doi.org/10.1016/j.freeradbiomed.2011.10.484

Wordsworth, J., O' Keefe, H., Clark, P., & Shanley, D. (2022). The damage-independent evolution of ageing by selective destruction. *Mechanisms of Ageing and Development*, *207*, 111709. https://doi.org/10.1016/j.mad.2022.111709

Xu, J., & Chow, E. K.-H. (2023). Biomedical applications of nanodiamonds: From drug-delivery to diagnostics. *SLAS Technology*, *28*(4), 214–222. https://doi.org/10.1016/j.slast.2023.03.007

Xuan, W., Yao, A., & Schultz, P. G. (2017). Genetically Encoded Fluorescent Probe for Detecting Sirtuins in Living Cells. *Journal of the American Chemical Society*, *139*(36), 12350–12353. https://doi.org/10.1021/jacs.7b05725

Yang, B., Dong, Y., Wang, F., & Zhang, Y. (2020). Nanoformulations to Enhance the Bioavailability and Physiological Functions of Polyphenols. *Molecules*, *25*(20), 4613. https://doi.org/10.3390/molecules25204613

Yu, X., & Li, G. (2012). Effects of resveratrol on longevity, cognitive ability and aging-related histological markers in the annual fish Nothobranchius guentheri. *Experimental Gerontology*, *47*(12), 940–949. https://doi.org/10.1016/j.exger.2012.08.009

Zessin, M., Meleshin, M., Hilscher, S., Schiene-Fischer, C., Barinka, C., Jung, M., & Schutkowski, M. (2023). Continuous Fluorescent Sirtuin Activity Assay Based on Fatty Acylated Lysines. *International Journal of Molecular Sciences*, *24*(8), 7416. https://doi.org/10.3390/ijms24087416

Zhang, L.-X., Li, C.-X., Kakar, M. U., Khan, M. S., Wu, P.-F., Amir, R. M., Dai, D.-F., Naveed, M., Li, Q.-Y., Saeed, M., Shen, J.-Q., Rajput, S. A., & Li, J.-H. (2021). Resveratrol (RV): A pharmacological review and call for further research. *Biomedicine & Pharmacotherapy*, *143*, 112164. https://doi.org/10.1016/j.biopha.2021.112164

Zhang, N., Pow, K. C., Chen, L., & Hao, Q. (2025). Structural basis of SIRT2 pre-catalysis NAD^+ binding dynamics and mechanism. *RSC Chemical Biology*, *6*(11), 1749–1758. https://doi.org/10.1039/D5CB00169B

Zhao, H., & Wu, W. (2025). The Influence of Sirtuin 6 on Chondrocyte Senescence in Osteoarthritis Under Aging: Focusing on Mitochondrial Dysfunction and Oxidative Stress. *Antioxidants*, *14*(10), 1228. https://doi.org/10.3390/antiox14101228

Zhao, L., Cao, J., Hu, K., He, X., Yun, D., Tong, T., & Han, L. (2020). Sirtuins and their Biological Relevance in Aging and Age-Related Diseases. *Aging and disease*, *11*(4), 927. https://doi.org/10.14336/AD.2019.0820

Ziętara, P., Dziewięcka, M., & Augustyniak, M. (2022). Why Is Longevity Still a Scientific Mystery? Sirtuins—Past, Present and Future. *International Journal of Molecular Sciences*, *24*(1), 728. https://doi.org/10.3390/ijms24010728

Ziętara, P., Flasz, B., & Augustyniak, M. (2024). Does Selection for Longevity in Acheta domesticus Involve Sirtuin Activity Modulation and Differential Response to Activators (Resveratrol and Nanodiamonds)? *International Journal of Molecular Sciences*, *25*(2), 1329. https://doi.org/10.3390/ijms25021329

Ziętara-Krzyk, P., Flasz, B., & Augustyniak, M. (2026a). Effects of resveratrol and nanodiamonds on sirtuin activity, oxidative stress and DNA damage in Acheta domesticus. *Biochemical and Biophysical Research Communications*, *816*, 153698. https://doi.org/10.1016/j.bbrc.2026.153698

Ziętara-Krzyk, P., Flasz, B., & Augustyniak, M. (2026b). Evaluation of Molecular Responses and Longevity Markers in Acheta domesticus Following Combined Resveratrol and Nanodiamond Exposure. *International Journal of Molecular Sciences*, *27*(6), 2786. https://doi.org/10.3390/ijms27062786

Zyriax, B.-C., & Windler, E. (2023). Lifestyle changes to prevent cardio- and cerebrovascular disease at midlife: A systematic review. *Maturitas*, *167*, 60–65. https://doi.org/10.1016/j.maturitas.2022.09.003

Manuskrypt I: Why Is Longevity Still a Scientific Mystery? Sirtuins - Past, Present and Future

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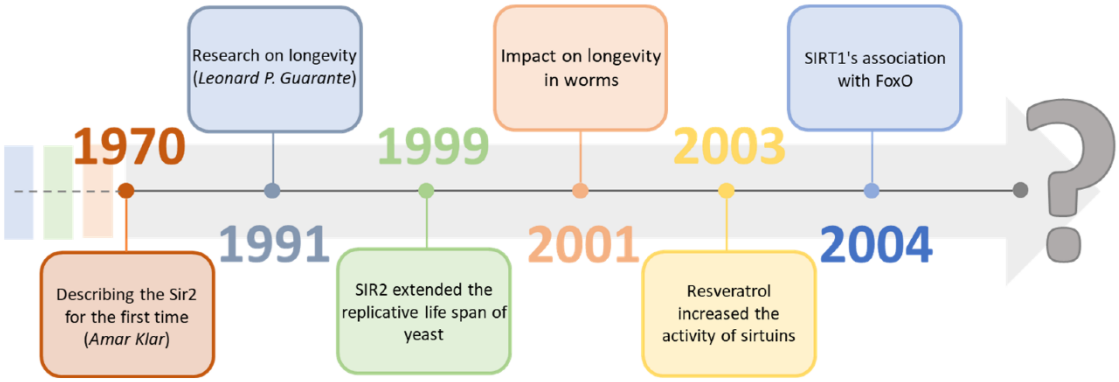
Graphical abstract: Timeline of sirtuin history



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Review

Why Is Longevity Still a Scientific Mystery? Sirtuins—Past, Present and Future

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Abstract: The sirtuin system consists of seven highly conserved regulatory enzymes responsible for metabolism, antioxidant protection, and cell cycle regulation. The great interest in sirtuins is associated with the potential impact on life extension. This article summarizes the latest research on the activity of sirtuins and their role in the aging process. The effects of compounds that modulate the activity of sirtuins were discussed, and in numerous studies, their effectiveness was demonstrated. Attention was paid to the role of a caloric restriction and the risks associated with the influence of careless sirtuin modulation on the organism. It has been shown that low modulators' bioavailability/ retention time is a crucial problem for optimal regulation of the studied pathways. Therefore, a detailed understanding of the modulator structure and potential reactivity with sirtuins in silico studies should precede in vitro and in vivo experiments. The latest achievements in nanobiotechnology make it possible to create promising molecules, but many of them remain in the sphere of plans and concepts. It seems that solving the mystery of longevity will have to wait for new scientific discoveries.

Keywords: sirtuins; longevity; modulators

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1. Introduction

Sirtuins are a group of proteins regulating many physiological functions and life processes. They have become an essential factor in research focused on aging processes as the deletion in the Sir-2 gene, which is responsible for the expression of sirtuins, results in a reduced lifespan of *Saccharomyces cerevisiae* [1]. With high probability, they may be one of the many missing elements on the way to longevity, significantly since the activity of individual sirtuins in cells characteristically decreases with age [2,3]. Geneticist Amar Klar first described sirtuins in the 1970s by discovering the Sir-2 (Yeast Silent Information Regulators II, SIR2) gene in *Saccharomyces cerevisiae* cells. Still, re-search indicating their potential impact on longevity was carried out about 20 years later, in 1991, by Leonard P. Guarante [4,5]. In 1999, by adding SIR2 to the DNA, it was possible to extend the replication life of yeast compared to the wild line. It was also confirmed two years later, extending the life cycle of worms. In 2003, David Sinclair showed that the activity of sirtuins could be modified by other substances, for example, resveratrol. A year later, they showed the connection of SIRT1 with FOXO proteins and proved their neuroprotective effect [6–8]. In the following years, there was further re-search on sirtuins, compounds modulating their activity, bioavailability, and their connection with metabolic functions (Figure 1). The focus was on classifying them and analyzing the mechanisms of their impact on various organisms, which, according to many scientists, may be crucial in determining them as regulators of aging [9–11].

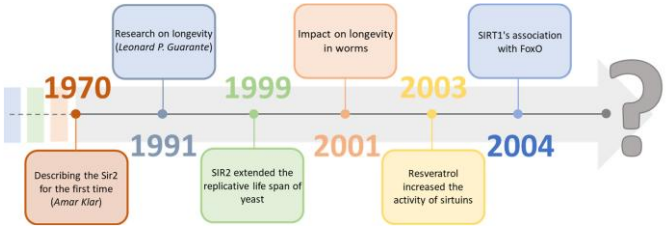


Figure 1. Timeline of sirtuin history [4–11].

2. General Characteristics of Sirtuins: Classification, Structure, and Localization

Sirtuins are NAD+ cofactor-dependent histone deacetylases (class III–HDAC). Seven mammalian sirtuins have been identified so far (SIRT1 to SIRT7) [4,12]. The catalytic domain of the NAD+ sirtuins contains 258 amino acid residues on average (the shortest is attributed to SIRT6 of 239 amino acid residues, while the longest is to SIRT2–275 amino acid residues composing) [13,14]. They occur in both bacteria and complex organisms [15] and take part in many processes regulating biological functions (cell cycle, cellular metabolism, genome homeostasis) [4,16–18]. It is considered that sirtuins can prevent DNA damage, the formation of cancer cells, and inflammatory reactions. They play a crucial role in the antioxidant defense [19,20]. In the human body, sirtuins are present in many different organs. From the level of the liver and kidneys, they can be involved in reducing stress and inflammatory reactions [21,22] because they have a unique role in the production of cytokines [18]. Assigning individual types of sirtuins to organisms is difficult since data on their presence in specific species are unclear or incomplete and constantly updated. Bioinformatics analysis and spatial visualization of the sirtuin particle show that a sirtuin's N- and C-terminal regions may be unique within a species. Thus, the catalytic activity and dynamics may differ in unrelated species [23,24]. The current classification of sirtuins depends on the sirtuin location in the cell and is still updated through new research. Three main localization sites have been determined: cytoplasm, mitochondrion, and cell nuclei (Figure 2) [18].

Nuclear sirtuins are histone-modifying deacetylases and are responsible for gene expression. These include SIRT1, SIRT6, and SIRT7, but there are reports of SIRT2 and SIRT3 that can migrate between organelles. Their mission is to determine in the cell when aging will begin [18,25], and probably have cytoprotective activity [26]. In turn, mitochondrial sirtuins are also called imported mitochondrial sirtuins (mtSIRT) with an N-terminal sequence [27]. Mitochondrial sirtuins include: SIRT3, SIRT4 and SIRT5. Regulation of their function occurs in post-translational modification of substrate proteins through deacetylation, demalonylation, lipoamidation, and poly ADP-ribosylation [12]. They can modify and regulate oxidative stress in cells and reduce reactive oxygen species directly in the organism [28,29]. Mitochondrial sirtuins reduced expression may contribute to the disturbance of the body's homeostasis, ultimately leading to the formation of pathologies and diseases related to the aging process, mainly affecting organs such as skeletal muscles, liver, pancreas, heart, or bones [25,30,31]. Cytoplasmic sirtuins coordinate the processes associated with cell apoptosis and DNA repair [32]. Sirtuin 2, deacetylating α-tubulin, is mainly located in the cytosol [33]. Sirtuin 1, on the other hand, is most found in the cell nucleus but can migrate and be present in the cytoplasm [34,35]. It regulates the activity of proteins responsible for chromatin structure, holds the cell cycle, and maintains homeostasis [36]. Explicit determination and understanding of the biological role of sirtuins are difficult due to their function in many biochemical pathways and the lack of inhibitors directed against specific types of sirtuins. Current studies are based on presumptions, defining newly discovered compounds as potential or promising activators [37,38].

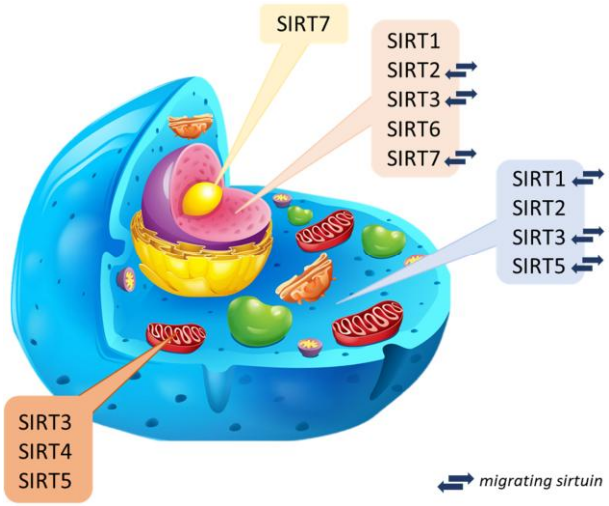


Figure 2. Intracellular localization of mammalian sirtuins [2,34,39–43].

2.1. Sirtuin 1 (SIRT1)

Sirtuin 1 is the human homolog to Sir2, derived from *Saccharomyces cerevisiae* (yeast) [39]. It is localized in the cell nucleus, but numerous reports in the literature about its occurrence in the cytoplasm [4]. It is the best-tested and well-known sirtuin so far [40]. SIRT1 affects insulin secretion from pancreatic beta cells, oxidation, fatty acid synthase induction, transcriptional activity, and deacetylation of histones and non-histone proteins [41–43]. It may inhibit inflammation, produce pro-inflammatory cytokines, have anti-apoptotic effects, and increase resistance to cellular stress. Moreover, it inhibits the p53 protein [44,45] and helps to maintain organelles integrity (peroxisomes and mitochondria) [46].

2.2. Sirtuin 2 (SIRT2)

Sirtuin 2 plays an essential role in oxidative stress protection, reducing its intensity through the deacetylation of FOXO and Nrf2 transcription factor [17,47]. It interacts with the protein p53, histone H4 lysine 16, and α-tubulin [48] and acts as a mitotic checkpoint protein that prevents the formation of hyperploid cells in early metaphase [49]. SIRT2 is also an epigenetic regulator because its dysregulation affects tumor progression (its expression is reduced in reproductive, urinary, or digestive systems cancer) [50,51]. Park et al. (2021) showed that SIRT2 has functions in lipid metabolism in mice, activating hepatocytes and hepatic stellate cells [52]. Furthermore, it is associated with cell proliferation and neurological disorders [53]. Jeong and Cho (2017) state that SIRT2 can regulate neuronal differentiation through the deacetylation of α-tubulin but the mechanisms of this process are not clarified [54].

2.3. Sirtuin 3 (SIRT3)

The primary location of sirtuin 3 is the mitochondria. However, it can migrate to the cell nucleus or cytoplasm [55]. It regulates lysine acetylation in mitochondria [56]. In humans, it affects the stabilization of heterochromatin and cytosol [25,57]. Some studies indicate that it appears in the mitochondrion only due to cellular stress, moving there from the cell nucleus [58]. Sirtuin 3 activates the mitochondrial genes PGC-1a and UCP-1, which suggests its essential role in thermogenesis [59]. It is associated with the longevity

gene in men [60] and may prevent bone loss in mice by regulating E11/ gp38 [61]. According to Liu et al. (2022), SIRT3 activation might reduce the anti-inflammatory effect by weakening NF-κB signaling [62]. SIRT3 is a kind of mitochondrial protector that can prevent cardiovascular diseases because it participates in their metabolism and dynamics by regulating the acetylation of mitochondrial proteins [63]. Its connection to cardiovascular diseases was also demonstrated by Liu et al. (2021) in studies on the development and regression of atherosclerosis. SIRT3 participating in acyl modifications maintains mitochondrial homeostasis and prevents vasculitis. However, there is scarce information about its use as a therapeutic against atherosclerotic lesions, which suggests the need for further research [64].

2.4. Sirtuin 4 (SIRT4)

Jaiswal et al. (2022) reported that SIRT4 is localized only in the mitochondrial matrix [57]. It is known that due to its location, it does not participate directly in DNA repair, but it shows strong enzymatic activity against oxidative stress generated during cellular respiration. It can protect the genome indirectly by lowering glutamate dehydrogenase activity and arresting the cell cycle. It participates in mitochondrial ATP homeostasis and, with other sirtuins, in stem cell differentiation [57]. Aside from its mitochondrial role, further enzymatic activity is puzzling.

Studies indicate its function in the oxidation of fatty acids in the muscles and liver and regulating insulin secretion. It probably also plays the role of a mitochondrial tumor suppressor [65]. SIRT4 is little known and described in vertebrates [66,67]. He et al. (2022) suggested that it may inhibit doxorubicin (DOX)-induced cardiotoxicity by regulating the AKT/ mTOR/ autophagy pathway [68] or prevent the release of pro-inflammatory cytokines IL-1β, TNF-α and IL-6 [69].

2.5. Sirtuin 5 (SIRT5)

SIRT5 is classified as mitochondrial and regulates metabolic pathways such as the Krebs and fatty acid cycles. Probably SIRT5 polymorphisms result in shortened human lifespan [28]. Sirtuin 5 affects brown adipose tissue activity as it can regulate mitochondrial respiration and protein kinase activity after treatment with the sirtuin inhibitor MC3482 [70]. It can catalyze deglutarylation, demalonylation, and desuccinylation reactions while exhibiting low deacetylation activity [37,71]. Jung et al. (2022) showed the influence of SIRT5 on mitochondrial respiration, and the TNF-α factor induces its expression, contributing to the delaying of the aging process [72].

2.6. Sirtuin 6 (SIRT6)

Sirtuin 6 has broad molecular functions and is a critical factor in the aging process due to its increased activity in the cell [73]. It is most often found in the cell nucleus. However, there are many reports of its occurrence in the cytosol [25]. It is related to glucose and lipid metabolism in the liver and pancreas via FoxO1 [74]. Research conducted on SIRT6 a few years earlier indicated its genetic instability, and some studies even proved that it caused premature aging processes. SIRT62/ 2 mice die prematurely and exhibit severe defects such as lymphopenia, loss of subcutaneous fat, reduced bone mineral density, and impaired glucose homeostasis. These are similar pathologies to those occurring in seniors [75]. Then, it was shown how its overexpression affects the extension of the life of mice [3]. According to Carreno et al. (2020), age-related diseases may lead to oxidative DNA damage. Sirtuin 6 may prevent these processes while stabilizing the genome and serving as a diagnostic biomarker [76,77]. SIRT6 has become one of the leading research subjects, thus diverting attention from SIRT 1 due to its more significant therapeutic potential, especially in neurodegenerative diseases [2].

2.7. Sirtuin 7 (SIRT7)

It is the only sirtuin located in the nucleolus, is involved in stress response and ribosome biogenesis, and is the least described among the sirtuins [78]. Sirtuin 7 interacts with RNA polymerase (Pol I) and histones to regulate rDNA transcription (transcript elongation). Its inhibition induces a decrease in transcription, stops cell proliferation, and initiates apoptosis [79]. Presumably, it may also inhibit cell apoptosis under stress conditions (e.g., hypoxia) [78]. According to Li et al. (2022), sirtuin 7 may inhibit the NF-κB signaling pathway, activated after entering an external factor into the body, resulting in the inhibition of inflammation caused by inflammation lipopolysaccharides [80]. SIRT7 activity is associated with the pathology of the cardiovascular system in the form of heart failure, atherosclerotic changes, and myocardial hypertrophy, thus is considered a potential therapeutic target against cardiovascular and kidney diseases [81,82]. There have also been reports that it may affect mitochondrial activity [83].

2.8. Sirtuin 8—Myth or Fact?

There are few reports on the discovery of SIRT8 expression. Sun et al. reported its activity in thyroid cancer, which was later contradicted by classifying this sirtuin as SIRT7 [84]. Other studies on the *Locusta migratoria* genome showed that this species has a higher amount of enzymes affecting the modification of histones compared to other insects and indicated the presence of a gene similar to SIRT3 in mammals (regulation of mitochondrial fatty acid oxidation). It was proposed to create a new subfamily for SIR2-Sirt8, but from the experiment until 2022, no new classification was recorded due to the lack of subsequent independent analyses [85].

2.9. Sirtuins and Invertebrates

Sirtuins are mainly described in vertebrates, where mice (*Mus musculus*) are a significant research object. So far, it has not been possible to identify sirtuins in invertebrates fully, and the information, sometimes contradictory, relates only to selected species. It was found that insect sirtuins are significantly different from mammalian ones. Research conducted on *Caenorhabditis elegans* confirmed the presence of the SIR2 (Sir-2.1) ortholog, which increases the viability of individuals by almost 50%. It depends on the transcription factor Daf-16 and is regulated in the insulin/ IGF-1 pathway [86]. Other results concern *Drosophila melanogaster*. It has been described that the dSir2 gene does not play a significant role in the viability of individuals but is only a regulator of heterochromatin formation. Individuals with a mutation in the dSir-2 gene are not characterized by a shorter life cycle like it is in worms [87]. Shukla et al. (2022) proved that the SIRT6 protein in *D. melanogaster* can potentially slow down larval development [88]. Other studies on this species have shown that SIRT4 has a regulatory effect on oxidative metabolism and may promote longevity. Overexpression of SIRT2 in muscles and neurons enables restoration of exercise capacity in *Drosophila* with mitochondrial dysfunction [89–92]. Wang et al. (2021) identified in *Ruditapes philippinarum* five types of sirtuins: SIRT1, SIRT2, SIRT4, SIRT6, and SIRT7, the expression of which depends on the developmental stage of the individual and environmental conditions. They play a role in restoring homeostasis after exposure of individuals to air [15]. Zhang et al. (2022) showed that in *Bombyx mori* (silkworm), SIRT2 and SIRT5 could enhance antiviral immunity, and the SIRT5 inhibitor (suramin) promotes nucleopolyhedrovirus replication [93]. Three years earlier, in 2019, Brent et al. reported inhibition of arbovirus genetic replication in adult flies treated with SIRT1 and SIRT2 inhibitors, which may contribute to a more efficient fight against diseases transmitted by forceps and mosquitoes (yellow fever, dengue fever, West Nile fever) [94]. In turn, May et al. (2021) presume that sirtuins play a role in regulating heat shock in *Mytilus californianus* (Conrad). However, determining the role of specific sirtuins in regulating this process requires more precise and targeted research [95].

3. Enzymatic Activity

Due to the complicated mechanism and many correlations, the enzymatic functions of sirtuins have not yet been summarized. Existing studies on sirtuin activity across species are inconsistent. The mechanism of stimulation and inhibition of specific sirtuins is still not fully understood, which makes it challenging to create the activators that must be matched to a particular sirtuin/ substrate pair. It is known that sirtuins, as class III HDACs require the NAD+ cofactor to catalyze the deacetylation of substrate proteins. The reaction proceeds to produce a deacetylated protein product in the form of nicotinamide and O-acetyl-ADP-ribose, which is a functional difference compared to class I and class II HDACs [96].

Modulating repair pathways for which sirtuins are responsible occurs through the deacetylation of repair factors, which changes the availability and cohesion of chromatin [58]. For example, SIRT1 directly affects histone deacetylation by structural remodeling chromatin and silencing the transcription process [58]. According to Sun et al. (2022), inhibition of sirtuin 1 may additionally regulate the deacetylation reaction in mitochondria, although many targets of SIRT1 action have primarily been identified as nuclear [97]. Sirtuins serve as a catalyst in lysine deacetylation reactions that require NAD+ as a co-substrate [98]. Sirtuins, as NAD+-dependent deacetylases, can remove various acyl groups from acylated lysines in histones and non-histone proteins. They are involved in the activity of transferases. The catalytic mechanisms of sirtuins are not fully known and described. However, studies indicate their catalytic potential in the case of ADP-ribose transfer to the acceptor medium [99]. Moreover, sirtuins are activated during malnutrition. In a caloric restriction/ starvation, all sirtuin isoforms are activated (Figure 3) due to the increased activity of the electron transport chain (respiratory chain), which leads to an increase in the ratio of NAD+ to NADH [100].

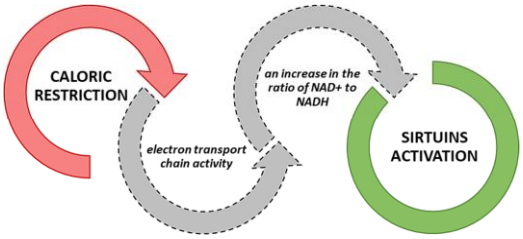


Figure 3. Mechanism of sirtuin activation during caloric restriction [100].

3.1. The Sirtuin Activity in the Light of Exercise Training and Caloric Restriction

Proper metabolism and homeostasis, various environmental factors, physical activity, and nutrients of consumed food are only selected components affecting the organism's condition. Both nutrition and exercise training are thought to delay the aging process in many species indirectly. Studies in rats have shown that swimming training inhibits inflammatory signaling and Fas-dependent apoptotic pathways in the hippocampus of the aging brain. In the group of physically active animals, signaling associated with longevity (AMPK/ SIRT1/ PGC-1α) increased significantly, as did the IGF1/ PI3K/ Akt pathway (brain survival) [101]. Sellitto et al. (2022) recognized exercise training as a natural activator of SIRT1 in humans. A higher level of SIRT1 mRNA characterized middle-distance runners compared to people leading a sedentary lifestyle. Interestingly, taking dietary supplements such as vitamin C, vitamin E, and mineral salts during training hindered the activation of SIRT1 by physical exercise [102]. Physical activity stimulates the expression of SIRT1 and SIRT3 in skeletal muscles by regulating oxidative metabolism (MnSOD, FOXO3a), mitochondrial biogenesis, and enhancing ATP production [103]. Edget et al. (2016) explained that single workouts do not cause genetic expression of SIRT3, to increase the amount of

protein, effort must be a repeated stimulus [104]. In addition, the expression of sirtuins in skeletal muscle depends on the type of exercise. Increased AMPK phosphorylation via high-intensity interval training activates SIRT1 increasing PGC-1α. In turn, regular aerobic exercise in people leading a sedentary lifestyle stimulates SIRT3, increases the activity of COX, β-hydroxyacyl-CoA dehydrogenase and contributes to the increase of NAMPT, allowing the synthesis of the sirtuin substrate–NAD+ [105,106].

The first information about the caloric restriction and its positive impact on an organism date back to the 20th century. Crucial was caloric restriction without malnutrition [107]. Maldonado et al. (2021) described calorie restriction as the most effective method of delaying the onset of many age-related diseases. Short-term caloric restriction after switching from standard nutrition in mice contributes to increased expression of all seven sirtuin proteins [108]. Furthermore, caloric restriction reduces telomere erosion in various mouse tissues (leukocytes, lungs, kidneys, muscles) observed with overexpression of telomerase reverse transcriptase (TERT) [109]. It has been established that increasing the activity of SIRT1 in combination with other sirtuins stabilizes telomeres and alleviates telomere-related disorders [110]. Increased expression of SIRT1 was also observed in mice switched to a 20% reduced calorie diet after an initial overfeeding period. Caloric restriction reduced hepatic steatosis, decreased superoxide anion levels, and increased catalase and superoxide dismutase protein expression [111]. In addition, it was proved that the energy supply at the level of 70% of the demand increased the expression of SIRT3 in the liver, muscles, and adipose tissue of obese rats [112]. Being overweight significantly contributes to the formation of acute inflammation and oxidative stress in the organism [113]. SIRT2 is essential for the protective effect of caloric restriction against high-fat diet-induced cancer [114]. SIRT1 and SIRT2 expression also increase after reducing the proportion of simple sugars in the diet. Their expression is reduced in type 1 and 2 diabetes [115]. In turn, a high-fructose diet causes high levels of glycation products that inhibit SIRT1 expression and impair muscle performance in mice [116]. A caloric restriction also reduces the adverse effects of excessively accumulated fatty tissue. Opstad et al. (2022) confirmed a strong correlation between sirtuins, obesity, and caloric restriction. They researched obese patients who underwent bariatric surgery. The gastric bypass procedure resulted in a significant decrease in the concentration of sirtuin 1 in the plasma of the studied patients, where triglycerides and CRP were determined as the independent variable for the level of SIRT1. Along with the weight loss, the concentration of SIRT1 in patients decreased, which indicates a significant reduction of inflammation and oxidative stress [117]. Savastano et al. (2015) also pointed to the relationship of SIRT4 with obesity. The reduced level in the blood serum in patients suggests that increasing its activity through the diet may positively reduce the accumulation of adipose tissue [118]. It is assumed that SIRT4 and SIRT1 control insulin secretion in opposite directions. Therefore, research on their effect on type 1 and type 2 diabetes is continued [119]. Introducing a specialized diet with caloric restriction and exercise training as a therapeutic measure requires a very individual approach. Both diet and sport activate hormesis. The dose–response relationship for hormetines is not linear, and individual variability has been found in the Nrf2 nuclear pathway in human studies. Individual variability can be observed in response to dietary hormetins (bioactive phytochemicals, peptides, polysaccharides) and exercise of varying intensity [120] For example, reactive oxygen species, reactive nitrogen species, and reactive lipid species derived from polyunsaturated fatty acids (PUFA) are formed during low-intensity training [121]. There are also interactions between enzymes involved in cytokine regulation. Increased production of antioxidants can cause reductive stress, and some antioxidants have a pro-oxidative effect, reducing the response to physical effort. According to the "mitohormesis" hypothesis, reactive oxygen species (ROS) should be at low levels to produce beneficial effects as signaling molecules that promote viability. Caloric restriction and physical effort become a stress for the cell, which, well-balanced, will cause an appropriate increase in ROS and activation of Nrf2, improving the redox balance [103,122]. To obtain the desired effects, factors such as gender, lifestyle, and general condition of the individual must be considered [107].

3.2. FOXO Proteins and P53 Protein

Forkhead box proteins present in mitochondria may interact with sirtuins and affect the processes responsible for aging [123]. The proteins belonging to the FoxO family (forkhead boxO) are transcription factors that play a significant role in the organism at many levels. These include four molecules: FOXO1 (FKHR), FOXO3A (FKHRL1), FOXO4 (AFX1) and FOXO6. Invertebrates have one FOXO gene, while mammals have four: FOXO1, FOXO3, FOXO4, and FOXO6 [124]. Since they are expressed in almost every cell of the body, and many genes encoding FOX proteins have been identified, their biological significance is constantly being studied and supplemented with new information. A characteristic feature of FOX proteins is a specific sequence of 80 to 100 amino acids forming a forkhead box motif, which can bind to DNA [125].

FoxO proteins regulate cell profiling and differentiation and promote programmed cell death [126]. They are involved in response to oxidative stress and are engaged in glucose and lipid metabolism. The activity of FoxO proteins is regulated by post-translational modifications such as phosphorylation, acetylation, and glycosylation. Therefore, changes in the affinity of FOXO to DNA and modification of the transcriptional activity of the FoxO function are involved not only in the pathogenesis of civilization diseases (diabetes, cancer) but can also contribute to the life-extending of organisms by activating longevity genes responsible for the antioxidant's synthesis or HSP chaperones [127–129]. Longevity-regulating genes include the Forkhead transcription factor FOXO and the NAD-dependent histone deacetylase, silent information regulator 2 (Sir2). Thus, sirtuins may regulate the activity of FOXO factors through deacetylation and involvement in DNA damage repair. Studies have shown that, in a sirtuin-dependent manner, a chronic stressor can prolong the life of *S. cerevisiae* and *C. elegans*, and deacetylation of the transcription factor FOXO1a by sirtuin 1 increases the transcription of gluconeogenesis genes and activates transcription factors responsible for the action of insulin genes [130,131]. SIRT1 also reverses H2O2-induced FOXO4 acetylation by inducing the protein GADD45 (DNA damage-induced growth arrest and α) [132], which is involved in cell cycle arrest and DNA repair [133]. In addition, it has been proven that Sir2 can increase the lifespan of individuals by inhibiting homologous recombination of ribosomal DNA [1,126]. Oxidative stress, which reduces the efficiency of cellular repair processes, is managed by deacetylation of FOXO3a via SIRT2 [134], SIRT1 and proper regulation of FOXO1, FOXO3, FOXO4, and p53 proteins [131]. Deacetylation of the p53 protein reduces p53-induced apoptosis, and increased p53 expression is responsible for the symptoms of premature aging in mice [135]. Therefore, the interaction of SIRT1, FOXO, and p53 seems to be of interest in the organism's survival. Mammalian SIRT1 may bind to FOXO4 and increase its activity by catalyzing acetylation in a NAD-dependent manner. In response to oxidative stress, FOXO accumulates in the nucleus and induces the expression of appropriate genes, such as MnSOD or SOD2. FOXO4 activity can be enhanced by resveratrol or suppressed by a SIRT1 inhibitor, such as nicotinamide. Mechanisms of interaction between sirtuins and forkhead box proteins are complicated. Thus, they are constantly studied in various experimental models [132,136–139]. Krishnamoorthy and Vilwanathan (2020) have linked SIRT6 to tumor suppression. Researchers believe that the p53/ p21 complex mediates the inhibition of lung cancer in humans with simultaneous suppression of sirtuin 6, indicating the need for further research [140]. The p53 protein has tumor suppressor features and can promote aging and cell death [141]. It regulates antioxidant processes by promoting oxidative enzymes and may be associated with cellular metabolism [142]. Kim et al. (2022) showed a link between SIRT1 and the promotion of the p53/ p21 complex by lowering acetylation at lysine residues in the C-terminal lysine residues of the p53 protein [141]. Igase et al. (2020) also proved that SIRT1 could inhibit its tumor suppressor functions [143] and affect the production of cytokines in an organism [144].

4. Modulators of Sirtuin Activity

Due to the participation of sirtuins in the aging process, determining which compounds have the most effective effect on the modulation of their activity is an urgent issue [145]. The mutually antagonistic mechanisms of sirtuins initiated by proper modulators can prevent many different diseases and thus become a valuable therapeutic agent. Information on sirtuin activators and inhibitors is still unclear, which has changed and even become mutually exclusive over the years. The action mechanism of these compounds is vague, and the different strengths directed against other members of the sirtuin family show that they do not target only one conserved enzyme [146], making their classification difficult. For example, suramin, initially considered a synthetic inhibitor of SIRT1, has also shown inhibition of SIRT5 through virtual screening data. Due to its lack of specificity and chemical multifunctionality, it is not considered for further research [146,147]. Sirtuins engage in many metabolic pathways, so changing their activity may affect one area positively but another negatively. For example, inhibiting sirtuin activity by sirtinol causes platelet aggregation and causes thrombocytopenia, but at the same time may be beneficial in cancer therapy [148]. Sirtinol, similarly to suramin, acts non-specifically by inhibiting SIRT2 and, to a lesser extent, SIRT1 [149].

Studies on the use of sirtuin activators focus on resveratrol (RV), which is found as a flavonoid in food and beverages [150–152]. Quercetin and fisetin, as natural polyphenols structurally similar to RV, also have many health benefits and the ability to activate SIRT1. Unfortunately, all of them have been shown to have low bioavailability, which is insufficient for clinical use. Moreover, their metabolites may be pharmacologically active [76,153,154]. In recent years, (until 2022), there has been a noticeable increase in interest in synthetic activators and inhibitors of sirtuins that can support natural modulators in action. Several effective synthetic SIRT1 activators have been developed that mimic the health benefits of resveratrol (SRT1720, SRT1460, SRT2183) and are in clinical trials in combination with other drugs [153].

Gozelle et al. (2022) used compounds based on 5-benzyl-1,3,4-thiadiazole-2-carboxamide, obtaining effective sirtuin 2 inhibitors-ST29 and ST30, which may also potentially support the treatment of cancer [154]. Additionally, Laaroussi et al. (2022) indicated indole analogs EX-527 as cytotoxic to cancer cells and many times more efficient than nicotinamide and other mentioned inhibitors [146,148,155]. Table 1 summarizes the latest sirtuin modulators with effects on selected model species.

Table 1. Summary of studies (from 2017 to 2022) on sirtuin activators and inhibitors (1–7).

Sirtuin	Organism	Modulator	Effect	Reference
ACTIVATORS				
Sirtuin 1	Human and Drosophila melanogaster	ginsenosides (*Panax ginseng* Meyer extract)	- promoting mitochondrial biosynthesis; - modification of the SIRT1 pathway, neuro- and cardioprotective effects;	[156]
	Mammalian cells (*Mus musculus*)	resveratrol	- increased SIRT1 and AMPK activity, improved cardiac function in mice; - reduced expression of parasitic cells;	[157]
		cucurbitacin E glucoside (CuE)	- inhibition of the NF-kB/ NLRP3 signaling pathway; - enhancing the SIRT1/ Nrf2/ HO-1 pathway, suppressing oxidative stress in liver cells in mice;	[158]
		quercetin	- SIRT1 increasing activity and PKM2 inhibition;	[159]

Table 1. *Cont.*

Sirtuin	Organism	Modulator	Effect	Reference
ACTIVATORS				
	Rattus norvegicus	resveratrol	- reducing oxidative stress and reducing the level of cardiac biomarkers; - activation of SIRT1 and protection of heart cells by reducing hyperlipidemia;	[160]
	Humans	1,4-dihydropyridine (DHP)	- activation of SIRT1 in the cells of the nucleus pulposus, protection in osteoarthritis of the spine;	[161]
Sirtuin 2	*Drosophila melanogaster*	food nitrites	- SIRT1 and 2 activators, regulation of metabolism;	[162]
	Human (HepG2 cells)	resveratrol	- SIRT2 activation through the Prx1 factor, antioxidant effect in the aging process;	[163]
Sirtuin 3	*Rattus rattus*	theacrine (1,3,7,9-tetramethyluric acid)	- non-toxic effect, reduction of liver fibrosis and fatty liver in rats by SIRT3 activating;	[164]
	Mus musculus	dexmedetomidine	- upregulation of SIRT3, reduction of mitochondrial damage caused by ischemic kidney injury;	[165]
	Human	oroxylin A	- cardioprotective effect by increasing the activity of SIRT3 in myocardial cells while preventing mitochondrial dysfunction in cardiac myocytes;	[166]
		Retama monosperma (L.) Boiss extract	- upregulation of SIRT3 in human HaCaT cells, treatment of skin scarring.	[167]
Sirtuin 4	*Mus musculus*	resveratrol	- upregulation of SIRT4 in cells (prolonged exposure), protective effect against excitotoxicity.	[168]
Sirtuin 5	*Mus musculus*	dioscin	- downregulation Of Mir-145-5p expression with upregulation of SIRT5 activity; - reduction of oxidative stress;	[169]
		resveratrol	- recovery of cellular metabolism after subarachnoid hemorrhage.	[170]
Sirtuin 6	*Mus musculus*	glicyryzine	- anti-aging effect, upregulating SIRT6 in 24-month-old mice with cranial osteolysis;	[171]
		UBCS039	- SIRT6 overexpression, treatment of lung injury in mice by promoting M2 bone marrow-derived macrophage;	[172]
		Gami-Yukmijihwang-Tang extract	- regulation of SIRT6 pathways, prevention of histone H3 lysine 56 acetylations.	[173]
Sirtuin 7	*Bombyx mori*	resveratrol	- upregulation of SIRT7; - activation of the SIRT7-FoxO-GST pathway;	[174]

Table 1. *Cont.*

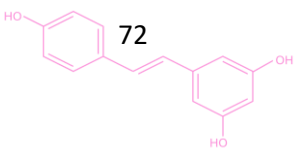
Sirtuin	Organism	Modulator	Effect	Reference
		INHIBITORS		
Sirtuin 1	Sprague Dawley rats	EX-527	- EX-527 mediates in the p53-SIRT1 pathway, regulation of germ cell apoptosis Cr(VI)-induced;	[43]
	Rattus rattus		- decrease of sirt1 activity, memory impairment in rats, oxidative stress as the response to prior quercetin stimulation;	[175]
			- SIRT1 may be linked to morphine addiction in a rat model, and its inhibition could be a potential therapeutic strategy;	[176]
	Bone marrow cells-*Mus musculus*	sirtinol	- inhibiting the activity of sirtuin 1 enhances the effects of astaxanthin, antioxidant and anti-inflammatory effects;	[177]
	Anser domesticus	nicotinamide	- inhibition of SIRT1 activity, increased cell proliferation in primary hepatocytes, and involvement in the FoxO1 process.	[178]
Sirtuin 1&2	hepatocellular carcinoma cells	EX-527 & cambiol	- SIRT1 and 2 inhibitors increased the effectiveness of sorafenib treatment targeting HCC cells; - reduction of MRP3 and BCRP expression;	[179]
	colorectal carcinoma HCT 116 (CCL-247) cell line	BZD9L1	- SIRT1 nad SIRT2 inhibitor, anti-cancer activity through the p53 pathway;	[180]
Sirtuin 1–3	murine Neur-oblastoma cell line	arekoline (*Areca catechu* extract)	- higher ability to inhibit the activity of sirtuins 1–3 than nicotinamide;	[181]
Sirtuin 2	MCF-7 human breast cancer cell line	thiadiazole derivatives	- new potential SIRT2 inhibitors marked as ST29 and ST30. ST29 showed antipro-liferative activity, and ST30 was cytotoxic;	[154]
Sirtuin 3	mass spectrometry of SIRT3	ampicillin trihydrate	- SIRT3 inhibitor, no additional information;	[37]
Sirtuin 4	Human	nicotinamide	- SIRT4 inhibition by homologous modeling and docking of NAD+;	[182]
Sirtuin 5	fluorogenic peptide substrates and human sirtuins	TW-37	- slight inhibition of SIRT5 activity;	[183]
	molecular docking	4-((4-(4-acetamido-phenyl)thiazol-2-yl) amino)-2-hydroxy-benzoic acid	- strong inhibition of SIRT5 activity, enhanced stability of inhibitors;	[184]
Sirtuin 6	articular chond-rocytes from human	EX-527	- downregulation of SIRT6 with oxidative stress formation;	[185]

4.1. Resveratrol—An Antidote for Aging?

Resveratrol (RSV) (3,5,4-trihydroksystylben) (Table 2) is a natural chemical compound of plant origin (phytoalexin). It is most found in the skin and seeds of *Vitis vinifera* grapes. It occurs in the form of two geometric isomers -cis and -trans, where only the trans isomer shows biological activity. In red wine, the concentration of resveratrol ranges from 0.1 to 15 mg/ L and is 3–10 times higher than in white wine [186]. Resveratrol has a broad, positive physiological effect, but it remains in cells for a limited time. Therefore, its effect may not be sufficient [187]. Due to its nephroprotective effect in diabetes patients, it may reduce the risk of its occurrence. RV is toxic when an inappropriate therapeutic dose is used [188]. It is noted that resveratrol may inhibit L1-RTP, which in turn is dependent on SIRT1, SIRT6, and SIRT7. An increase in SIRT6 was observed after the use of resveratrol as a therapeutic agent [189]. RV in mice increases LSK ex vivo and reduces T cell proliferation in vivo and ex vivo [18]. In addition, its neuroprotective effect is constantly being assessed. Recent research shows that it mediates multiple molecular pathways related to aging and central nervous system function. It contributes to beneficial epigenetic changes that last for generations. It prevents cognitive impairment and cell neurodegeneration primarily due to its potent antioxidant properties [190]. In addition to scavenging free radicals, it modulates synergistic pathways responsible for anti-inflammatory and anti-apoptotic effects [191]. Demonstrated reduced neuroinflammatory stress with decreased pro-inflammatory cytokines (TNF-α) in rats. Moreover, after experimental central nervous system injury, RSV can ameliorate functional deficits when administered on an ad hoc basis [192]. Studies also indicate the inhibitory effect of resveratrol on the activity of SIRT1, SIRT3, and SIRT5, depending on the substrate used [37]. Chou et al. (2022) reported that treatment with resveratrol as a SIRT1 activator might attenuate kidney aging and inhibit oxidative stress [193]. In mice with traumatic brain injury, it increased SIRT1 expression, which activated p38 MAPK phosphorylation and improved their neurological function due to neuronal loss [194]. Yang et al. (2021) noted that resveratrol in mice increased the expression of SIRT1 and induced DNA repair by regulating NBS1 [195].

4.2. Polydatin—An Improved Resveratrol?

Polydatin (PD) (Table 2), the glycosidic form of resveratrol isolated from *Polygonum cuspidatum* is considered more effective because its concentration in plants can be up to 15 times higher than resveratrol. It is found in wine, grapes, and peanuts [84,196] in two forms, cis- and trans-. Trans-polydatin is attractive due to its anti-inflammatory and antioxidant properties [197]. It has a protective effect against numerous diseases. Like RV, it is promising in treating many diseases of the nervous system due to its neuroprotective effects (Alzheimer's disease, Parkinson's disease, brain, and spinal cord injuries, strokes). The polydatin mechanism works through several pathways Nrf2/ Keap1/ ARE, PI3K/ Akt, ERK/ MAPK, TLR/ NF-κB/ TNF-α/ ILs or Bax/ Bcl-2/ caspases Oral administration of polydatin reduces malondialdehyde (MDA) production and increases antioxidant activity (SOD, CAT) [198]. Tong et al. 2020 showed an effective reduction in cognitive impairment caused by chemotherapy. Doxorubicin-induced stress was reduced by activating the NF-κB pathway and reducing apoptosis [199]. Bao et al. (2022) recognized polydatin as an effective agent in liver damage by NF-κB signaling [200]. Jin et al. (2022) showed that polydatin could increase body weight in mice while reducing insulin resistance [201]. It is considered a potential therapeutic agent in case of oxidative stress and radiation-induced lung damage (SIRT 3 activation) [202,203]. Zhang et al. (2017), based on their research and associations with SIRT3 expression, suggested its use as a drug against diabetic cardiomyopathy [204]. Moreover, by upregulating SIRT1 expression, PD may prevent mitochondrial dysfunction and stimulate IRT mRNA. Unfortunately, polydatin show pharmacological disadvantages such as low solubility, low plasma concentration, or rapid chemical degradation, which makes therapeutic applications difficult [205].



4.3. Honokiol—An Effective Antioxidant

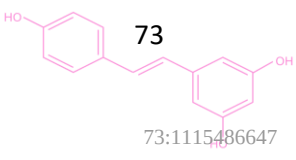
Honokiol (HKL) (Table 2) is a polyphenol extracted from the bark and leaves of magnolia (*Magnolia officinalis*). Classified as an antioxidant and is traditionally used in treating inflammatory diseases [206]. Recent studies indicate its effectiveness against SARS-CoV-2 by inhibiting furin activity and blocking the function of S-protein (spike) [207]. Due to the ability to cross the blood–brain barrier and the blood-cerebrospinal fluid barrier is characterized by significant bioavailability. Studies on a mouse model by Zhou et al. (2022) report its effectiveness in the treatment of neurodegenerative diseases and the ability to improve mitochondrial function and antioxidant properties. It exerts therapeutic effects in several neurological diseases, including amyotrophic lateral sclerosis (ALS), in both in vitro and in vivo models. It improves the viability of NSC-34 motoneuron cells, characterized by mutated G93A SOD1 proteins, and the morphology of mitochondria in SOD1-G93A cells. This effect has also been shown in the spinal cord. Moreover, honokiol extended the lifespan of SOD1-G93A transgenic mice and improved their motor function [208]. As a GABA modulator and CB1 agonist, it is being considered for treating mood disorders. In the treatment of depression and anxiety disorders, it has a selective anxiolytic effect [209]. By SIRT1 expression, HKL modulates endoplasmic reticulum stress and promotes cell viability [208,210]. HKL applied to lung cancer cells destabilizes Hif-1a, induces apoptosis and arrests the G1 phase. Its anticancer properties are regulated by the SIRT3/ Hif-1a pathway [211]. Activating SIRT3 alleviates cardiac cell impairment [212]. Li et al. (2016) showed its effectiveness in increasing the activity of SIRT3 in the smooth muscles of the mouse liver and reducing its steatosis [213]. Moreover, the use of honokiol in older mice stopped bone loss, and the effectiveness was dose-dependent [61].

4.4. Triclosan-Synthetic Sirtuin Activator

Triclosan (TCS) (5-chloro-2-(2,4-dichlorophenoxy)phenol (Table 2) is a synthetic chemical compound with broad bacteriostatic (in higher concentrations also bactericidal), antifungal, and antiviral properties. Due to its properties, it is often used in cosmetics, and it was overused during the COVID-19 pandemic, which raised concerns about environmental safety because it shows high bioavailability through the skin and oral administration [214,215]. Excessive exposure to triclosan may contribute to thyroid homeostasis disorders, metabolic disorders, cardiotoxicity, and increased cancer risk [215–217]. It has been proven that triclosan in rodents can impair the immune response and contribute to liver diseases. At environmental concentrations, it modulates the transcriptional response of Nrf2, SIRT1, and SIRT2 in the livers of *Gambusia affinis* fish, negatively affecting their antioxidant system [218]. Szychowski et al. (2022) showed that short and long triclosan exposure increases the expression of SIRT1 and SIRT3 in neurons of the neural cortex of mice, which initially increased the level of neurosteroids in the cells, and then, despite further stimulation of the aryl hydrocarbon receptor (Ahr), caused their significant decrease [217,218]. So far, there is little information in the literature about triclosan and its effect on sirtuins, which requires further research to explain its action mechanism.

4.5. Cambinol–SIRT 1 and SIRT 2 Inhibitor

Cambinol ((5-((2-hydroxynaphthalen-1-yl)methyl)-6-phenyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one) (Table 2) is a β-naphthol derivative with an anticancer effect, defined as a sirtuin 1 and 2 inhibitor [219,220] because it inhibits NAD-dependant deacetylases to reduce cell survival under stress. It has anti-inflammatory effects and is involved in the immune response and metabolic control [221]. Chowdhury et al. (2020) identified cambinol as cytotoxic against cancer cells in vitro [222]. Its inhibition of sirtuins 1 and 2 have been shown to increase p53, FOXO3a, and Ku70 acetylation; however, its effect's complete mechanism is unknown [223]. Its inhibitory effect is related to the simultaneous inhibition of histone 4 and NAD+ [224]. There are papers on more effective inhibitors than cambinol and better selectivity for SIRT 1 and 2, but further studies in this area are required [225].



4.6. EX-527—A Precursor of Modern Sirtuin Inhibitors

EX-527 (6-Chloro-2,3,4,9-tetrahydro-1H-Carbazole-1-carboxamide) (Table 2) as an indole compound is a matrix for synthesizing sirtuin inhibitor analogs. It is mainly used in studies of physiological and cancer cells [149,155]. Nikseresht et al. (2019) showed the relationship of EX-527 microinjections with the reduction of cerebral ischemic infarcts and proved that by inhibiting SIRT1, they act as an inhibitor of necroptosis in an animal model [226]. According to Kundu et al. (2020), it protects the organism against the effects of a high-fat diet (diabetic nephropathy), as it contributes to lowering blood glucose levels and reducing SIRT1 activity while increasing SIRT 3 action in the kidneys [227]. In addition, inhibiting SIRT1 also increases renal allograft survival in mice, which may be related to T cells [228]. It inhibits the activity of SIRT1 100-fold more strongly than SIRT2 and SIRT3, which is presumably associated with NAD+ as a colligand in the binding of EX-527 in cells [149]. Kumari et al. (2015) pointed out that it may cause thrombocytopenia as a side effect in research on anticancer therapy, suggesting while sirtuins are involved in platelet aging and the general aging processes [148].

Table 2. Characteristics of selected sirtuin modulators.

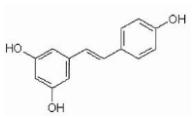
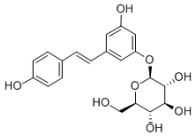
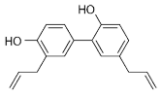
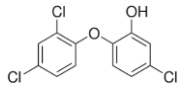
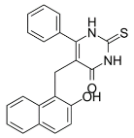
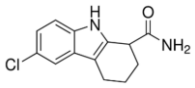
Modulator	Chemical Formula	Effect	Characteristic	Reference
Resveratrol		activator	- biological activity in neurogenic diseases; - affects the CNS; - neuroprotective effect; - antiapoptotic effect; - anti-inflammatory effect; - modulates the action of the antioxidant system; - reduces nicotine genotoxicity in *D. melanogaster* by activating sirtuins.	[187,229]
Polydatin		activator	- liver protection and anti-apoptosis; - neuroprotector in chemiotherapy - anti-cancer and anti-inflammatory effects; - inhibition of oxidative stress and removal of ROS; - prevents diseases of the cardiovascular and nervous systems.	[84,199,204]
Honokiol		activator	- regulates the process of acetylation of mitochondrial proteins through SIRT; - therapeutic agent against obesity by activating SIRT3 in cells.	[206,208,213]
Triclosan		activator	- antiviral and bacteriostatic effect; - widely used in industrial products; - toxic to the environment; - induces the expression of SIRT1 and SIRT3 in mouse neuronal cells; - modulates the expression of SIRT1 and SIRT2 in Gambusia affinis liver.	[230–232]
Cambinol		inhibitor	- inhibition of SIRT1 and 2 activity; - inhibition of immune responses and inflammatory reactions; - reduces the activity of NF-κB, which is indirectly dependent on sirtuins.	[219–221,233]

Table 2. *Cont.*

Modulator	Chemical Formula	Effect	Characteristic	Reference
EX-527		inhibitor	- inhibitor of SIRT 1 and 2, cytotoxic effect in tumor cells; - protective effect in diabetic nephropathy; - reduces blood sugar in a rat model; - inhibitor of necroptosis in animal model (by inhibiting SIRT1)	[155,226,227]

5. Diseases and Sirtuin-Based Therapies

The multidirectional functions of sirtuins on biological processes interest the scientific community and the pharmaceutical industry [187]. Because sirtuins play a key role in life processes, they are considered potential participants in modern therapies. Apart from prolonging life, they can also "extend the period of healthy life" (Table 3) [234]. Therefore, the sirtuin diet, "SIRTfood", based on caloric restrictions, promoting sports, and consuming products rich in sirtuin activators, e.g., resveratrol, is gaining popularity [235]. Abnormal lipid, sugar, or protein economy functioning are common metabolic disorders. Sirtuins affect the maintenance of proper metabolism and may promote the condition of the heart. Cao et al. (2022) point to SIRT3 agonists as promising therapeutics against cardiovascular diseases [63]. In addition, SIRT 1, 2, 6, and 7 reduce myocardial hypertrophy, and SIRT7 may prevent cardiomyopathy [236]. Zaganjor et al. (2021) researched sirtuin 4 and its link to branched-chain amino acid (BCAA) catabolism to treat abnormal amino acid metabolism. Its reduced expression in the adipose tissue of mice has been shown, which is probably a consequence of its ability to increase BCAA catabolism and promote the activity of PPARγ, which regulates glucose metabolism and fatty acid storage in the body [237]. It has been proven that the cellular metabolism of sirtuins is correlated with, e.g., the pathophysiology of bone and joint diseases [238]. Estrogen receptor (ER) deacetylation by SIRT6 in proosteoclasts prevents bone loss and shows promise for the treatment of osteoporosis in elderly or postmenopausal patients [239]. In addition, NAD+, as a metabolite consumed by sirtuins, plays an essential role in pain regulation and peripheral neuropathic pain [240]. He et al. (2022) demonstrated that nuclear and mitochondrial sirtuins might have a neuroprotective/ regulatory character in Parkinson's (PD), Alzheimer's (AD), and Huntington's disease [12]. Govindarajulu et al. (2021) showed that AD is associated with aberrant SIRT3 expression [241]. SIRT1 was also shown to be attenuated in the cerebral cortex of AD patients with severe cognitive impairment. The phenomenon is typically associated with the accumulation of amyloid-β and father tau [242]. In addition, it has been observed with signs of low SIRT6 growth, which is related to telomere destabilization. AD is considered the leading cause of senile dementia worldwide, and additional research may contribute to therapeutic benefits [243]. In neurodegenerative processes, apart from oxidative stress, enzymes of histone deacetylase (HDAC), α-synuclein, and autophagy are involved. Parkinson's disease progresses with age, and the proper function and biological activity of mitochondrial proteins are attributed to SIRT3, SIRT4, and SIRT5 (mitochondrial sirtuins). Mitochondrial sirtuin activators positively affect the stability of the respiratory chain and oxidative stress in neurons. However, clinical trials are insufficient to resolve their complex function in favor of future PD therapies [12,244]. Interestingly, SIRT1 inhibition also gives good results in treating HIV infection or fragile X syndrome [245,246]. As mentioned, sirtuins are involved in metabolic control and epigenetic modification [25]. Moreover, they are significantly associated with diabetic neuropathy and cardiomyopathy [28,247]. Research by Hu et al. (2021) indicated that SIRT1 is a significant regulator responsible for body carbohydrate metabolism disorders [248]. Sirtuins are also responsible for women's gynecological health because they regulate ovarian function on many levels. They are responsible for the secretion of steroid hormones and maintain normal reproductive functions [249]. Overexpression of SIRT7 with simultaneous repression of SIRT1,

SIRT2, SIRT4, and SIRT5 promotes uterine tumor growth [250]. On the other hand, SIRT7 deficiency in the cytoplasm of oocytes and granulosa cells contributes to the inhibition of estrogen synthesis, cell proliferation, and reproductive function [251]. SIRT2 is also responsible for steroid hormone homeostasis and follicle maturation by regulating estrogen and testosterone secretion with its specific inhibitors [252]. In addition, Zhang et al. (2022) suggested a link between SIRT3 activity and polycystic ovary syndrome, as it may regulate glucose metabolism, which plays an essential role in signaling granulosa cells and oocytes. SIRT3 deficiency causes defects in the cellular insulin signaling pathway and contributes to impaired oocyte production [253]. Some sirtuin inhibitors (SIRT1), such as nicotinamide, have the potential to be used in cancer therapy. Sirtuin inhibition leads to an increase in p53-dependent apoptosis and a reduction in tumor cell proliferation [254]. Studies also show the involvement of sirtuins in cellular processes responsible for inflammatory responses, indicating that SIRT1 may modify immune responses in reaction to microbes, reducing the production of pro-inflammatory cytokines in human cells [255,256].

Table 3. Characteristics of selected diseases associated with sirtuins.

Sirtuin	Disease	Model	Effect	Reference
Sirtuin 1	Non-alcoholic fatty liver disease (NAFLD)	C57BL/ 6 mice	- bariatric surgery with sleeve gastrectomy in mice contributed to upregulating the NRK1/ NAD+/ SIRT1 pathway, alleviating NAFLD;	[257]
	Coronary heart disease (CHD)	Rat cell lines	- inhibition of miR-323-3p or overexpression of SIRT1 resulted in a reduction of cardiac muscle fiber disorder with simultaneous inhibition of vascular endothelial cell apoptosis;	[258]
	Traumatic brain injury (TBI)	Male rats	- increasing SIRT1 expression by regulating PGC-1α expression (upregulated in TBI) may reduce neuronal damage and inhibit microglial activity;	[259]
	Parkinson's disease	Male C57BL/ 6JNarl mice	- activation of SIRT1 with SRT1720, reduced cytotoxicity in PD with simultaneous down-regulation of PGC-1α level;	[260]
Sirtuin 2	Non-alcoholic fatty liver disease (NAFLD)	Mice	- reducing the amount of SIRT2 in the liver may contribute to hepatocyte damage. It decreased the expression of GLUT2, FATP2 and FAPT5;	[52]
	Psoriasis	Mice and Human embryonic kidney 93T cell line	- reduced amount of SIRT2 contributes to the pathogenesis of psoriasis development through the effect of sirtuin on the function of Th17 cells, which trigger an abnormal proliferation of KCs;	[261]

Table 3. *Cont.*

Sirtuin	Disease	Model	Effect	Reference
Sirtuin 3	inflammatory responses elicited by lipopolysaccharide	Bovine mammary epithelial cells	- presumably, increased expression of SIRT3 is involved in the regulation and amelioration of inflammatory responses as it affects oxidative stress while suppressing the PGC1α-NFκB pathway responsible for the inflammatory response;	[262]
	Chronic Constriction Injury–Induced Neuropathic Pain	Mice	- SIRT3 overexpression resulted in neuropathic pain relief by inhibiting CaMKIIα phosphorylation;	[263]
	Polycystic ovary syndrome (PCOS)	Sprague-Dawley rats	- increased expression of SIRT3 because of the use of Bu-Shen-Tian-Jing Formula (BSTJF) reduced the pathogenesis of polycystic ovary syndrome with concurrent regulation of p38 MAPK and PI3K/ AKT signaling pathways;	[264]
Sirtuin 4	Doxorubicin-Induced Cardiotoxicity (DIC)	Male C57BL/ 6 mice	- SIRT4 overexpression activated the Akt/ mTOR signaling pathway, thanks to which it alleviated DIC with simultaneous improvement of cardiac function;	[68]
	Diabetic nephropathy		- SIRT4 activated by FOXM1, inhibits the NFκB pathway alleviated kidney damage.	[265]
Sirtuin 5	hepatic ischemia and reperfusion injury	Male C57BL/ 6J mice	- increasing the level of SIRT5 causes attenuation of liver damage through the mechanism of inhibition of oxidative stress, raising the level of IHH2 and SOD1;	[266]
	Pancreatic Cancer	PDAC cell lines, genetically engineered mice models, and human tissue samples	- activation of SIRT5 with MC3138, showed anticancer effects in human cells by regulating glutamine/ glutathione metabolism.	[267]
Sirtuin 6	type 1 diabetes	C57BL/ 6 mice	- SIRT6 regulates FoxO1 in renal proximal tubules by addects renal glucose reabsorption and gluconeogenesis in type 1 diabetes;	[74]
	neuroinflammation and ischemic brain injury	Cell culture from C57BL/ 6 mice and human blood cells	- activation of SIRT6 with MDL-811 has been shown to have neuroprotective and ameliorating effects on neuroinflammation, which is linked to the SIRT6/ EZH2/ FOXC1 signaling pathway.	[268]

Table 3. *Cont.*

Sirtuin	Disease	Model	Effect	Reference
Sirtuin 7	Chronic kidney disease (CKD) with hypertensive	Mice	- in hypertensive SIRT7 alleviates kidney damage (kidney ferroptosis, pathological fibrogenesis, renal dysfunction) by facilitating the KLF15/ Nrf2 signaling;	[82]
	colorectal cancer	Human CRC cell lines HCT-8, HCT-116, DLD1, SW620 and HT15	- increased expression of SIRT7 by GRIM-19, affects the SIRT7/ PCAF/ MDM2 axis, increasing p53 stabilization.	[269]

6. Sirtuins as an Elixir of Youth?

Aging irreversibly stops somatic cell division and deteriorates viability [270]. It can be a process of various extents and manifests itself in neurodegradation, bone decalcification, chronic inflammatory response, DNA instability, and cancer [271]. With age, various irreversible changes occur in an organism, which may significantly weaken the immune system [18]. The search for an effective "elixir of youth" has been going on since antiquity and attempts to understand the complex mechanisms of aging continue. Sirtuins are products of the Sir-2 gene, which along with the p66shc, ink4a, FOXO, and daf-2 genes, is referred to as the longevity gene [272]. Sirtuins have dual metabolic activity (deacetylation and ADP-ribosylation) coupled with NAD+ hydrolysis. As mentioned earlier, sirtuins regulate many biological processes due to their high substrate specificity and impact on numerous factors. Sirtuins have been shown to play a role in DNA repair, transcription processes, post-translational modification of proteins, maintaining chromosome stability, cell proliferation, apoptosis and cell cycle regulation, control of metabolic processes, gene silencing, and many others. Therefore, they are responsible for the hormonal and metabolic balance and proper work of the nervous, muscular, cardiovascular, digestive, and immune systems [19,273]. They have the potential to counteract the aging process by modulating the response of cells to stress, changing their pattern, and restoring tissue homeostasis responsible for phenotypic aging [6]. Several studies have linked SIRT1, SIRT6, and SIRT7 to life extension in rodents and humans [18,73,274,275]. Most often, studies of sirtuins in terms of longevity concern SIRT1 and SIRT6 due to the correlation of their expression with lifespan in mice, although it is difficult to clearly define which of the sirtuins significantly increases lifespan in vertebrates [276]. Sirtuin 6 has also been evaluated to prevent dendritic cell immune aging [277]. Sirtuins might potentially protect the kidneys, maintaining homeostasis of podocytes damaged with age. However, this theory has not been fully confirmed [17]. Mitochondrial sirtuins may play an essential role in preventing aging, especially in the context of the "mitochondrial theory of aging" and oxidative stress [278]. An indirect anti-aging agent is ergothioneine, which through its antioxidant properties, eliminates mitochondrial oxidative stress, increases the expression of SIRT1 and SIRT6, and reduces hyperglycemia [279]. Reprogramming cells towards self-rejuvenation seems to solve the mystery of longevity and a sought-after element of the philosopher's stone. However, theories regarding the usefulness of sirtuins as longevity enzymes are not unambiguous. Studies also point to the potential dangers of manipulating sirtuin activity via modulating compounds (e.g., STACs) [280]. Excessive, uncontrolled expression of sirtuins can lead to dysregulation of an organism, promote epithelial-mesenchymal transition and cancer, and cause therapeutic resistance. SIRT genes are upregulated in many types of cancer, presumably due to the dysregulation of positive and negative feedback loops [40,280].

7. The Darkside of Sirtuins

The neuroprotective and life-prolonging function of sirtuins is the main direction of research. Until 2022, a few published studies pointed to the effects of the threat caused by modifying the activity of sirtuins and interfering with their mechanisms of action. Preliminary research often leads to erroneous conclusions. Palmer and Vaccarezza (2020) showed that current research on sirtuins has focused on the benefits rather than the potential side effects [280]. Liberale et al. (2020) demonstrated in studies on transgenic mice that SIRT5 might promote venous thrombosis and silencing its expression may be a potential therapeutic in this disease [281]. It has also been proven that overexpression of SIRT1 may cause memory deficit in mice and has no neuroprotective effect [282].

Moreover, SIRT1 is a crucial stimulator of proliferation and angiogenesis. Therefore, its increased expression in women leads to the development of endometriosis and ovarian cancer [283]. He et al. (2022) also confirmed the stimulating effect of SIRT5 on cell proliferation and breast cancer metastasis. Increased expression of SIRT5 is probably related to aerobic glycolysis and the activity of HK2 and PKM2 enzymes in promoting liver and breast cancer [284]. An imprecise and quick application of sirtuins in therapies can cause long-term and unforeseen consequences. Sirtuins involve numerous mechanisms in multicellular organisms, many of which have not yet been described or are based only on general information. To find safe therapeutic solutions, it is necessary to study the mechanisms of action of sirtuins in many experimental models, seeing a holistic effect on the species' physiology. The phenomenon of hormesis should also be considered [128,285]. Visioli et al. (2022) noted the strategies scientists use to stop the aging process. They recommend optimal dosing of activator substances before clinical trials on humans, as the results obtained from animal models are often inadequate or difficult to interpret [286].

8. Looking into the Future—Are We Ready for Longevity?

The future of sirtuins as longevity enzymes remains unclear and difficult to predict. It seems that the topic of compounds that modulate their effects to prolong life and treat diseases remains in the realm of science fiction. Many studies describe their great therapeutic potential, extinguished by many uncertainties and suggestions for further research. For example, sirtuins may simultaneously act as promoters and suppressors in neoplastic diseases. However, these and other conflicting data make the study of sirtuins even more fascinating. There are undoubtedly more reports about new sirtuin modulators and related mechanisms. We know that sirtuin activators and inhibitors are imperfect and require laboratory "discipline", but the achievements outline the direction to follow to get the way of longevity. Science has an excellent database that gives hope that sirtuins will be included in medicine (Table 3) and used in future anti-cancer strategies [267], thrombotic disease therapies [287], treatment of nephrogenic diabetes [74] or suppressing inflammatory responses [262]. Perhaps, in some time, the next stage will be clinical trials and the widespread availability of drugs. However, caution should be exercised with as many substances because various processes may be associated with sirtuins and alter known and developed strategies. The desire for a healthy and long life promotes overexposure to sirtuin modulators and may paradoxically lead to homeostasis disorders and long-term, unforeseen consequences. Therefore, it is a priority to understand the mechanisms of action of sirtuins and develop their safe modifications. Many scientists believe these processes are too complex to plan our future with sirtuins today.

9. Conclusions

Sir2 proteins are involved in a wide range of biological processes and have potential effects in the treatment of lifestyle diseases. The scientists' curiosity is stimulated by the connection of sirtuins with the length of life. We are certainly at the beginning of understanding the phenomenon of these proteins. Every year new pieces of information are obtained, but there is still a long way to create the perfect anti-aging panacea. Undoubtedly, focusing on sirtuin modulators and understanding the mechanism of their action is

currently the biggest challenge. A big step into the future will be the systematic search for compounds with a similar structure to known modulators. Research in silico as a modern strategy in science gives a chance for a holistic view of data obtained by scientists around the world. We believe molecular modeling tools can select the best candidates with enormous potential. Next, work on increasing the bioavailability and retention time of sirtuin modulators should be considered. In the case of some compounds of high biological importance, satisfactory results are obtained by designing structures of the active substance-nanoparticle type. However, biological aging is a complex process affected by many factors, such as environment, species, and individual characteristics of the population. Therefore, scientists will undoubtedly invest a lot of invention, creativity, and effort before solving the mystery of aging and manipulating this process by selected factors.

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Abbreviations

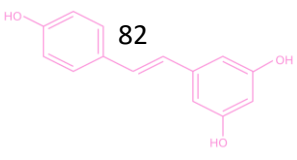
AD	Alzheimer's disease
Ahr	aryl hydrocarbon receptor
Akt	Type of serine/ threonine protein kinase
AMPK	AMP-activated protein kinase
ATP	Adenosine triphosphate
BAX	Bcl-2 Associated X-protein
BCAA	branched-chain amino acid
Bcl-2	B-cell lymphoma 2
CAT	Catalase
CaMKIIα	Calcium/ calmodulin-dependent protein kinase type II subunit alpha
CB1	Cannabinoid type 1 receptor
CHD	Coronary heart disease
CKD	Chronic kidney disease
COX	Cyclooxygenase
CRC cell lines	Colorectal cancer cell lines
CRP	C Reactive Protein
CuE	cucurbitacin E glucoside
daf-2	abnormal dauer formation protein 2
DHP	1,4-dihydropyridine
DIC	Doxorubicin-Induced Cardiotoxicity
DNA	deoxyribonucleic acid
DOX	doxorubicin
dSir2	*Drosophila* Sir2
E11/ gp38	transmembrane glycoprotein
ERK	Extracellular signal-regulated kinase
EX-527	6-Chloro-2,3,4,9-tetrahydro-1H-Carbazole-1-carboxamide
EZH2	Enhancer of zeste homolog 2
FATP2	Fatty acid transport protein 2
FATP5	Fatty acid transport protein 5
FKHR	Forkhead protein

FKHRL1	Transcription factor
FOXC1	Forkhead Box C1
FOXM1	Forkhead Box M1
FOXO	Forkhead Box O
FoxO	Transcription factor
FOXO1	Forkhead Box protein O1(FKHR)
FOXO3A	Forkhead Box protein O3 (FKHRL1)
FOXO4	Forkhead Box Protein O4 (AFX1)
FOXO6	Forkhead Box Protein O6
SOD1-G93A	Transgene
GABA	Gamma-aminobutyric acid
GADD45	The Growth Arrest and DNA Damage
GLUT2	Glucose transporter 2
H2O2	Hydrogen peroxide
HDAC	Histone deacetylases
Hif-1a	Hypoxia-inducible factor 1
HIV	Human immunodeficiency virus
HK2	Hexokinase 2
HKL	Honokiol
HSP	Heat-shock proteins
IGF-1	Insulin-like Growth Factor 1
IL-1β	Interleukin-1β
IL-6	Interleukin 6
IRT	Immunoreactive Trypsinogen
Keap1	Kelch-like ECH-associated protein 1
KLF15	Krüppel-like factor 15
LSK	Lin-Sca1(+) c-kit(+)
MAPK	mitogen-activated protein kinase
MDL-811	Chemical drug, SIRT6 activator
MDM2	Mouse double minute 2 homolog
miR-323-3p	microRNA-323-3p
MnSOD	manganese superoxide dismutase
mtSIRT	mitochondrial sirtuins
NAD+	nicotinamide adenine dinucleotide (oxidized form)
NADH	nicotinamide adenine dinucleotide (reduced form)
NAFLD	Non-alcoholic fatty liver disease
NAMPT	nicotinamide phosphoribosyltransferase
NBS1	nibrin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	nuclear factor erythroid 2-related factor 2
NRK1	Nicotinamide riboside kinase
NSC-34	Neuroblastoma hybrid cell line
p21	cyclin-dependent kinase inhibitor 1
p53	cellular tumor antigen p53
PCAF	P300/ CBP-associated factor
PCOS	Polycystic ovary syndrome
PD	polydatin (reservatrol-3-β-mono-D-glucoside)
PDAC	pancreatic ductal adenocarcinoma
PGC-1 α	Peroxisome proliferator–activated receptor gamma coactivator-1 alpha
PI3K	Phosphoinositide 3-kinases
PKM2	pyruvate kinase isozymes M1/ M2
Pol	DNA polymerase I
Prx1	peroxiredoxin PRX1
PUFA	polyunsaturated fatty acids
RSV	resveratrol
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2
SIR2	NAD-dependent histone deacetylase
Sir-2	Yeast Silent Information Regulators II, SIR2

SIRT	sirtuin
SOD	superoxide dismutase
SOD2	superoxide dismutase 2
STACs	Sirtuin-activating compounds
TBI	Traumatic brain injury
TCS	triclosan
Teacrine	1,3,7,9-tetramethyluric acid
TERT	telomerase reverse transcriptase
Th17 cells	T helper 17 cells
TLRS	Toll-like receptors
TNF-α	tumor necrosis factor α
UCP-1	Uncoupling protein 1

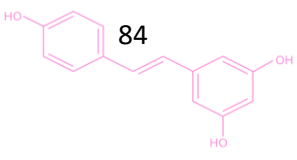
References

1. Kaeberlein, M.; McVey, M.; Guarente, L. The SIR2/ 3/ 4 Complex and SIR2 Alone Promote Longevity in Saccharomyces Cerevisiae by Two Different Mechanisms. *Genes Dev.* **1999**, *13*, 2570–2580. [CrossRef]
2. Grootaert, M.O.J.; Bennett, M.R. Sirtuins in Atherosclerosis: Guardians of Healthspan and Therapeutic Targets. *Nat. Rev. Cardiol.* **2022**, *19*, 668–683. [CrossRef]
3. Taborsky, B.; Kuijper, B.; Fawcett, T.W.; English, S.; Leimar, O.; McNamara, J.M.; Ruuskanen, S. An Evolutionary Perspective on Stress Responses, Damage and Repair. *Horm. Behav.* **2022**, *142*, 105180. [CrossRef]
4. Naseer, A.; Mir, S.S.; Takacs-Vellai, K.; Nazir, A. Sirtuins and Autophagy in Age-Associated Neurodegenerative Diseases: Lessons from the C. Elegans Model. *Int. J. Mol. Sci.* **2021**, *22*, 12263. [CrossRef]
5. Wang, Y.; He, J.; Liao, M.; Hu, M.; Li, W.; Ouyang, H.; Wang, X.; Ye, T.; Zhang, Y.; Ouyang, L. An Overview of Sirtuins as Potential Therapeutic Target: Structure, Function and Modulators. *Eur. J. Med. Chem.* **2019**, *161*, 48–77. [CrossRef]
6. Haigis, M.C.; Sinclair, D.A. Mammalian Sirtuins: Biological Insights and Disease Relevance. *Annu. Rev. Pathol. Mech. Dis.* **2010**, *5*, 253–295. [CrossRef]
7. Couzin-Frankel, J. Aging Genes: The Sirtuin Story Unravels. *Science* **2011**, *334*, 1194–1198. [CrossRef]
8. Dang, W. The Controversial World of Sirtuins. *Drug Discov. Today Technol.* **2014**, *12*, e9–e17. [CrossRef]
9. Gold, D.A.; Sinclair, D.A. Sirtuin Evolution at the Dawn of Animal Life. *Mol. Biol. Evol.* **2022**, *39*, msac192. [CrossRef]
10. Costantini, S.; Sharma, A.; Raucci, R.; Costantini, M.; Autiero, I.; Colonna, G. Genealogy of an Ancient Protein Family: The Sirtuins, a Family of Disordered Members. *BMC Evol. Biol.* **2013**, *13*, 60. [CrossRef]
11. Curry, A.M.; White, D.S.; Donu, D.; Cen, Y. Human Sirtuin Regulators: The "Success" Stories. *Front. Physiol.* **2021**, *12*, 1853. [CrossRef]
12. He, L.; Wang, J.; Yang, Y.; Li, J.; Tu, H. Mitochondrial Sirtuins in Parkinson's Disease. *Neurochem. Res.* **2022**, *47*, 1491–1502. [CrossRef]
13. Mei, Z.; Zhang, X.; Yi, J.; Huang, J.; He, J.; Tao, Y. Sirtuins in Metabolism, DNA Repair and Cancer. *J. Exp. Clin. Cancer Res.* **2016**, *35*, 182. [CrossRef]
14. Zhang, F.; Wang, S.; Gan, L.; Vosler, P.S.; Gao, Y.; Zigmond, M.J.; Chen, J. Protective Effects and Mechanisms of Sirtuins in the Nervous System. *Prog. Neurobiol.* **2011**, *95*, 373–395. [CrossRef]
15. Wang, J.; Zhang, H.; Xu, S.; Liu, Z.; Yang, L.; Fang, L.; Huo, Z.; Yan, X. Characterization of the SIRT Genes and Their Distinct Expression Patterns in Manila Clams (Ruditapes Philippinarum) Exposed to Air. *Meta Gene* **2021**, *28*, 100892. [CrossRef]
16. Denu, R.A.; Hematti, P. Sirtuins and Stem Cell Maintenance, Proliferation, and Differentiation. In *Sirtuin Biology in Medicine*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 175–190.
17. Liu, T.; Yang, L.; Mao, H.; Ma, F.; Wang, Y.; Li, S.; Li, P.; Zhan, Y. Sirtuins as Novel Pharmacological Targets in Podocyte Injury and Related Glomerular Diseases. *Biomed. Pharmacother.* **2022**, *155*, 113620. [CrossRef]
18. Gámez-García, A.; Vazquez, B.N. Nuclear Sirtuins and the Aging of the Immune System. *Genes* **2021**, *12*, 1856. [CrossRef]
19. Watroba, M.; Szukiewicz, D. Sirtuins at the Service of Healthy Longevity. *Front. Physiol.* **2021**, *12*, 724506. [CrossRef]
20. Chang, H.-C.; Guarente, L. SIRT1 and Other Sirtuins in Metabolism. *Trends Endocrinol. Metab.* **2014**, *25*, 138–145. [CrossRef]
21. Priya, S.H.; Kedari, G.S.R.; Naidu, M.P. Higher Serum Sirtuin 1 Levels and GA Heterozygote of SIRT1 Gene Polymorphism Rs10823108 Serve as Independent Risk Factor for Diabetic Nephropathy in Women. *Hum. Gene* **2022**, *34*, 201084. [CrossRef]
22. Alves-Fernandes, D.K.; Jasiulionis, M.G. DNA Damage, Sirtuins, and Epigenetic Marks. In *Epigenetics and DNA Damage*; Elsevier: Amsterdam, The Netherlands, 2022; pp. 87–108.
23. Lagunas-Rangel, F.A. Bioinformatic Analysis of SIRT7 Sequence and Structure. *J. Biomol. Struct. Dyn.* **2022**, 1–11. [CrossRef] [PubMed]
24. Hałasa, M.; Bartuzi, D.; Cieślak, D.; Kaczor, A.A.; Miziak, P.; Stepulak, A.; Matosiuk, D. Role of N-Terminus in Function and Dynamics of Sirtuin 7: An in Silico Study. *J. Biomol. Struct. Dyn.* **2020**, *38*, 1283–1291. [CrossRef] [PubMed]
25. Ji, Z.; Liu, G.-H.; Qu, J. Mitochondrial Sirtuins, Metabolism, and Aging. *J. Genet. Genom.* **2022**, *49*, 287–298. [CrossRef] [PubMed]
26. Mazumder, S.; Barman, M.; Bandyopadhyay, U.; Bindu, S. Sirtuins as Endogenous Regulators of Lung Fibrosis: A Current Perspective. *Life Sci.* **2020**, *258*, 118201. [CrossRef] [PubMed]



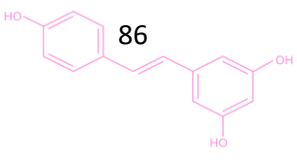
27. Hussain, M.Z.; Haris, M.S.; Khan, M.S.; Mahjabeen, I. Role of Mitochondrial Sirtuins in Rheumatoid Arthritis. *Biochem. Biophys. Res. Commun.* **2021**, *584*, 60–65. [CrossRef]
28. Mahjabeen, I.; Rizwan, M.; Fareen, G.; Waqar Ahmed, M.; Farooq Khan, A.; Akhtar Kayani, M. Mitochondrial Sirtuins Genetic Variations and Gastric Cancer Risk: Evidence from Retrospective Observational Study. *Gene* **2022**, *807*, 145951. [CrossRef] [PubMed]
29. Bai, L.; Yang, Z.-X.; Ma, P.-F.; Liu, J.-S.; Wang, D.-S.; Yu, H.-C. Overexpression of SLC25A51 Promotes Hepatocellular Carcinoma Progression by Driving Aerobic Glycolysis through Activation of SIRT5. *Free Radic. Biol. Med.* **2022**, *182*, 11–22. [CrossRef]
30. van de Ven, R.A.H.; Santos, D.; Haigis, M.C. Mitochondrial Sirtuins and Molecular Mechanisms of Aging. *Trends Mol. Med.* **2017**, *23*, 320–331. [CrossRef]
31. Tang, X.; Chen, X.-F.; Chen, H.-Z.; Liu, D.-P. Mitochondrial Sirtuins in Cardiometabolic Diseases. *Clin. Sci.* **2017**, *131*, 2063–2078. [CrossRef]
32. Anderson, G.; Maes, M. Sirtuins, Mitochondria, and the Melatonergic Pathway in Alzheimer's Disease. In *Sirtuin Biology in Medicine*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 117–135.
33. Olmos, Y.; Brosens, J.J.; Lam, E.W.-F. Interplay between SIRT Proteins and Tumour Suppressor Transcription Factors in Chemotherapeutic Resistance of Cancer. *Drug Resist. Updates* **2011**, *14*, 35–44. [CrossRef]
34. Bai, W.; Zhang, X. Nucleus or Cytoplasm? The Mysterious Case of SIRT1's Subcellular Localization. *Cell Cycle* **2016**, *15*, 3337–3338. [CrossRef] [PubMed]
35. Yanagisawa, S.; Baker, J.R.; Vuppusetty, C.; Koga, T.; Colley, T.; Fenwick, P.; Donnelly, L.E.; Barnes, P.J.; Ito, K. The Dynamic Shuttling of SIRT1 between Cytoplasm and Nuclei in Bronchial Epithelial Cells by Single and Repeated Cigarette Smoke Exposure. *PLoS ONE* **2018**, *13*, e0193921. [CrossRef] [PubMed]
36. Kupis, W.; Pałyga, J.; Tomal, E.; Niewiadomska, E. The Role of Sirtuins in Cellular Homeostasis. *J. Physiol. Biochem.* **2016**, *72*, 371–380. [CrossRef] [PubMed]
37. Loharch, S.; Chhabra, S.; Kumar, A.; Swarup, S.; Parkesh, R. Discovery and Characterization of Small Molecule SIRT3-Specific Inhibitors as Revealed by Mass Spectrometry. *Bioorg. Chem.* **2021**, *110*, 104768. [CrossRef]
38. Ramarao, S.; Pothireddy, M.; Venkateshwarlu, R.; Moturu, K.M.V.R.; Siddaiah, V.; Kapavarapu, R.; Dandela, R.; Pal, M. Sonochemical Synthesis and In Silico Evaluation of Imidazo[1,2-*a*]Pyridine Derivatives as Potential Inhibitors of Sirtuins. *Polycycl. Aromat. Compd.* **2022**, 1–20. [CrossRef]
39. Smith, J.S. Human Sir2 and the 'Silencing' of P53 Activity. *Trends Cell Biol.* **2002**, *12*, 404–406. [CrossRef]
40. Selepe, M.A.; Kunyane, P.; Seboletswe, P.; Nair, S.; Cele, N.; Engelbrecht, M.; Joubert, D.F.; Vandevoorde, C.; Singh, P.; Sonopo, M.S. Synthesis and Evaluation of Benzoylbenzofurans and Isoflavone Derivatives as Sirtuin 1 Inhibitors with Antiproliferative Effects on Cancer Cells. *Bioorg. Chem.* **2022**, *128*, 106101. [CrossRef]
41. Najafi, M.; Nikpayam, O.; Tavakoli-Rouzbehani, O.M.; Papi, S.; Bioky, A.A.; Ahmadiani, E.S.; Sohrab, G. A Comprehensive Insight into the Potential Effects of Resveratrol Supplementation on SIRT-1: A Systematic Review. *Diabetes Metab. Syndr. Clin. Res. Rev.* **2021**, *15*, 102224. [CrossRef]
42. Li, J.; Yang, C.; Ran, J.; Yu, C.; Yin, L.; Li, Z.; Liu, Y. The Age-Dependent Variations for Fatty Acid Composition and Sensory Quality of Chicken Meat and Associations between Gene Expression Patterns and Meat Quality. *Livest. Sci.* **2021**, *254*, 104736. [CrossRef]
43. Sivakumar, K.K.; Stanley, J.A.; Behlen, J.C.; Wuri, L.; Dutta, S.; Wu, J.; Arosh, J.A.; Banu, S.K. Inhibition of Sirtuin-1 Hyperacetylates P53 and Abrogates Sirtuin-1-P53 Interaction in Cr(VI)-Induced Apoptosis in the Ovary. *Reprod. Toxicol.* **2022**, *109*, 121–134. [CrossRef]
44. Zhao, J.; Tan, Y.; Feng, Z.; Zhou, Y.; Wang, F.; Zhou, G.; Yan, J.; Nie, X. Catalpol Attenuates Polycystic Ovarian Syndrome by Regulating Sirtuin 1 Mediated NF-KB Signaling Pathway. *Reprod. Biol.* **2022**, *22*, 100671. [CrossRef] [PubMed]
45. Charążka, B.; Siejka, A. Correlations between Serum Sirtuin Levels and Cardiovascular Risk Factors in Women with Polycystic Ovary Syndrome. *Adv. Med. Sci.* **2022**, *67*, 123–128. [CrossRef]
46. Packer, M. Mutual Antagonism of Hypoxia-Inducible Factor Isoforms in Cardiac, Vascular, and Renal Disorders. *JACC Basic Transl. Sci.* **2020**, *5*, 961–968. [CrossRef] [PubMed]
47. Eldridge, M.J.G.; Pereira, J.M.; Impens, F.; Hamon, M.A. Active Nuclear Import of the Deacetylase Sirtuin-2 Is Controlled by Its C-Terminus and Importins. *Sci. Rep.* **2020**, *10*, 2034. [CrossRef]
48. Hoffmann, G.; Breitenbücher, F.; Schuler, M.; Ehrenhofer-Murray, A.E. A Novel Sirtuin 2 (SIRT2) Inhibitor with P53-Dependent Pro-Apoptotic Activity in Non-Small Cell Lung Cancer. *J. Biol. Chem.* **2014**, *289*, 5208–5216. [CrossRef] [PubMed]
49. Inoue, T.; Hiratsuka, M.; Osaki, M.; Yamada, H.; Kishimoto, I.; Yamaguchi, S.; Nakano, S.; Katoh, M.; Ito, H.; Oshimura, M. SIRT2, a Tubulin Deacetylase, Acts to Block the Entry to Chromosome Condensation in Response to Mitotic Stress. *Oncogene* **2007**, *26*, 945–957. [CrossRef]
50. Wang, H.-L.; Ma, X.; Guan, X.-Y.; Song, C.; Li, G.-B.; Yu, Y.-M.; Yang, L.-L. Potential Synthetic Lethality for Breast Cancer: A Selective Sirtuin 2 Inhibitor Combined with a Multiple Kinase Inhibitor Sorafenib. *Pharmacol. Res.* **2022**, *177*, 106050. [CrossRef]
51. Head, P.E.; Zhang, H.; Bastien, A.J.; Koyen, A.E.; Withers, A.E.; Daddacha, W.B.; Cheng, X.; Yu, D.S. Sirtuin 2 Mutations in Human Cancers Impair Its Function in Genome Maintenance. *J. Biol. Chem.* **2017**, *292*, 9919–9931. [CrossRef]
52. Park, S.; Chung, M.-J.; Son, J.-Y.; Yun, H.H.; Park, J.-M.; Yim, J.-H.; Jung, S.-J.; Lee, S.-H.; Jeong, K.-S. The Role of Sirtuin 2 in Sustaining Functional Integrity of the Liver. *Life Sci.* **2021**, *285*, 119997. [CrossRef]

53. Chen, X.; Lu, W.; Wu, D. Sirtuin 2 (SIRT2): Confusing Roles in the Pathophysiology of Neurological Disorders. *Front. Neurosci.* **2021**, *15*, 518. [CrossRef]
54. Jeong, S.-G.; Cho, G.-W. The Tubulin Deacetylase Sirtuin-2 Regulates Neuronal Differentiation through the ERK/ CREB Signaling Pathway. *Biochem. Biophys. Res. Commun.* **2017**, *482*, 182–187. [CrossRef]
55. Chojdak-Łukasiewicz, J.; Bizoń, A.; Waliszewska-Prosół, M.; Piwowar, A.; Budrewicz, S.; Pokryszko-Dragan, A. Role of Sirtuins in Physiology and Diseases of the Central Nervous System. *Biomedicines* **2022**, *10*, 2434. [CrossRef]
56. Boardman, N.T.; Migally, B.; Pileggi, C.; Parmar, G.S.; Xuan, J.Y.; Menzies, K.; Harper, M.-E. Glutaredoxin-2 and Sirtuin-3 Deficiencies Impair Cardiac Mitochondrial Energetics but Their Effects Are Not Additive. *Biochim. Biophys. Acta (BBA)—Mol. Basis Dis.* **2021**, *1867*, 165982. [CrossRef]
57. Jaiswal, A.; Xudong, Z.; Zhenyu, J.; Saretzki, G. Mitochondrial Sirtuins in Stem Cells and Cancer. *FEBS J.* **2022**, *289*, 3393–3415. [CrossRef]
58. Lagunas-Rangel, F.A. Current Role of Mammalian Sirtuins in DNA Repair. *DNA Repair* **2019**, *80*, 85–92. [CrossRef]
59. Shi, T.; Wang, F.; Stieren, E.; Tong, Q. SIRT3, a Mitochondrial Sirtuin Deacetylase, Regulates Mitochondrial Function and Thermogenesis in Brown Adipocytes. *J. Biol. Chem.* **2005**, *280*, 13560–13567. [CrossRef]
60. Bellizzi, D.; Dato, S.; Cavalcante, P.; Covello, G.; di Cianni, F.; Passarino, G.; Rose, G.; de Benedictis, G. Characterization of a Bidirectional Promoter Shared between Two Human Genes Related to Aging: SIRT3 and PSMD13. *Genomics* **2007**, *89*, 143–150. [CrossRef]
61. Li, Q.; Wang, R.; Zhang, Z.; Wang, H.; Lu, X.; Zhang, J; Kong, A.P.-S.; Tian, X.Y.; Chan, H.-F.; Chung, A.C.-K.; et al. Sirt3 Mediates the Benefits of Exercise on Bone in Aged Mice. *Cell Death Differ.* **2022**. [CrossRef]
62. Liu, L.; Lu, H.; Loor, J.J.; Aboragah, A.; Du, X.; He, J; Peng, T.; Su, J; Wang, Z.; Liu, G.; et al. Sirtuin 3 Inhibits Nuclear Factor-KB Signaling Activated by a Fatty Acid Challenge in Bovine Mammary Epithelial Cells. *J. Dairy Sci.* **2021**, *104*, 12871–12880. [CrossRef]
63. Cao, M.; Zhao, Q.; Sun, X.; Qian, H.; Lyu, S; Chen, R.; Xia, H.; Yuan, W. Sirtuin 3: Emerging Therapeutic Target for Cardiovascular Diseases. *Free Radic. Biol. Med.* **2022**, *180*, 63–74. [CrossRef]
64. Liu, Y.; Shen, X.; Pang, M.; Sun, Z.; Qian, Y.; Xue, W.; Wang, Z.; Li, L. Role of Histone Deacetylase Sirt3 in the Development and Regression of Atherosclerosis. *Life Sci.* **2021**, *272*, 119178. [CrossRef] [PubMed]
65. Min, Z.; Gao, J.; Yu, Y. The Roles of Mitochondrial SIRT4 in Cellular Metabolism. *Front. Endocrinol.* **2019**, *9*, 783. [CrossRef] [PubMed]
66. Li, Y.; Zhou, Y.; Wang, F.; Chen, X.; Wang, C.; Wang, J.; Liu, T.; Li, Y.; He, B. SIRT4 Is the Last Puzzle of Mitochondrial Sirtuins. *Bioorg. Med. Chem.* **2018**, *26*, 3861–3865. [CrossRef] [PubMed]
67. Li, S.; Zheng, W. Mammalian Sirtuins SIRT4 and SIRT7. *Prog. Mol. Biol. Transl. Sci.* **2018**, *154*, 147–168.
68. He, L.; Wang, J.; Yang, Y.; Zou, P.; Xia, Z.; Li, J. SIRT4 Suppresses Doxorubicin-Induced Cardiotoxicity by Regulating the AKT/ MTOR/ Autophagy Pathway. *Toxicology* **2022**, *469*, 153119. [CrossRef]
69. Chen, Y.; Wang, H.; Luo, G.; Dai, X. SIRT4 Inhibits Cigarette Smoke Extracts-Induced Mononuclear Cell Adhesion to Human Pulmonary Microvascular Endothelial Cells via Regulating NF-KB Activity. *Toxicol. Lett.* **2014**, *226*, 320–327. [CrossRef]
70. Molinari, F.; Feraco, A.; Mirabilii, S.; Saladini, S.; Sansone, L.; Vernucci, E.; Tomaselli, G.; Marzolla, V.; Rotili, D.; Russo, M.A.; et al. SIRT5 Inhibition Induces Brown Fat-Like Phenotype in 3T3-L1 Preadipocytes. *Cells* **2021**, *10*, 1126. [CrossRef]
71. Zhou, Y.; Zhang, H.; He, B.; Du, J; Lin, H.; Cerione, R.A.; Hao, Q. The Bicyclic Intermediate Structure Provides Insights into the Desuccinylation Mechanism of Human Sirtuin 5 (SIRT5). *J. Biol. Chem.* **2012**, *287*, 28307–28314. [CrossRef]
72. Jung, Y.H.; Chae, C.W.; Chang, H.S.; Choi, G.E.; Lee, H.J; Han, H.J. Silencing SIRT5 Induces the Senescence of UCB-MSCs Exposed to TNF-α by Reduction of Fatty Acid β-Oxidation and Anti-Oxidation. *Free Radic. Biol. Med.* **2022**, *192*, 1–12. [CrossRef]
73. Korotkov, A.; Seluanov, A.; Gorbunova, V. Sirtuin 6: Linking Longevity with Genome and Epigenome Stability. *Trends Cell Biol.* **2021**, *31*, 994–1006. [CrossRef]
74. Bian, C.; Zhang, R.; Wang, Y.; Li, J; Song, Y.; Guo, D.; Gao, J; Ren, H. Sirtuin 6 Affects Glucose Reabsorption and Gluconeogenesis in Type 1 Diabetes via FoxO1. *Mol. Cell. Endocrinol.* **2022**, *547*, 111597. [CrossRef] [PubMed]
75. Mostoslavsky, R.; Chua, K.F.; Lombard, D.B.; Pang, W.W.; Fischer, M.R.; Gellon, L.; Liu, P.; Mostoslavsky, G.; Franco, S.; Murphy, M.M.; et al. Genomic Instability and Aging-like Phenotype in the Absence of Mammalian SIRT6. *Cell* **2006**, *124*, 315–329. [CrossRef] [PubMed]
76. Carreño, M.; Bresque, M.; Machado, M.R.; Santos, L.; Durán, R.; Vitturi, D.A.; Escande, C.; Denicola, A. Nitro-Fatty Acids as Activators of HSIRT6 Deacetylase Activity. *J. Biol. Chem.* **2020**, *295*, 18355–18366. [CrossRef] [PubMed]
77. Wang, S.; Zhang, Z.; Gao, Q. Transfer of MicroRNA-25 by Colorectal Cancer Cell-Derived Extracellular Vesicles Facilitates Colorectal Cancer Development and Metastasis. *Mol. Ther. Nucleic Acids* **2021**, *23*, 552–564. [CrossRef] [PubMed]
78. Li, X.; Liu, J; Lu, L.; Huang, T.; Hou, W.; Wang, F.; Yu, L.; Wu, F.; Qi, J; Chen, X.; et al. Sirt7 Associates with ELK1 to Participate in Hyperglycemia Memory and Diabetic Nephropathy via Modulation of DAPK3 Expression and Endothelial Inflammation. *Transl. Res.* **2022**, *247*, 99–116. [CrossRef] [PubMed]
79. Grummt, I.; Pikaard, C.S. Epigenetic Silencing of RNA Polymerase I Transcription. *Nat. Rev. Mol. Cell Biol.* **2003**, *4*, 641–649. [CrossRef]
80. Li, G.; Tang, X.; Zhang, S.; Deng, Z.; Wang, B.; Shi, W.; Xie, H.; Liu, B.; Li, J. Aging-Conferred SIRT7 Decline Inhibits Rosacea-Like Skin Inflammation by Modulating Toll-Like Receptor 2-NF-KB Signaling. *J. Investig. Dermatol.* **2022**, *142*, 2580–2590.e6. [CrossRef]



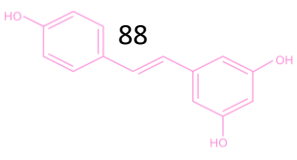
81. Li, X.-T.; Zhang, Y.-P.; Zhang, M.-W.; Zhang, Z.-Z.; Zhong, J.-C. Sirtuin 7 Serves as a Promising Therapeutic Target for Cardiorenal Diseases. *Eur. J. Pharmacol.* **2022**, *925*, 174977. [CrossRef]
82. Li, X.-T.; Song, J.-W.; Zhang, Z.-Z.; Zhang, M.-W.; Liang, L.-R.; Miao, R.; Liu, Y.; Chen, Y.-H.; Liu, X.-Y.; Zhong, J.-C. Sirtuin 7 Mitigates Renal Ferroptosis, Fibrosis and Injury in Hypertensive Mice by Facilitating the KLF15/ Nrf2 Signaling. *Free Radic. Biol. Med.* **2022**, *193*, 459–473. [CrossRef]
83. Hajishengallis, G.; Li, X.; Chavakis, T. Immunometabolic Control of Hematopoiesis. *Mol. Aspects Med.* **2021**, *77*, 100923. [CrossRef]
84. Sun, Z.; Wang, X.; Xu, Z. SIRT1 Provides New Pharmacological Targets for Polydatin through Its Role as a Metabolic Sensor. *Biomed. Pharmacother.* **2021**, *139*, 111549. [CrossRef] [PubMed]
85. Guo, S.; Jiang, F.; Yang, P.; Liu, Q.; Wang, X.; Kang, L. Characteristics and Expression Patterns of Histone-Modifying Enzyme Systems in the Migratory Locust. *Insect Biochem. Mol. Biol.* **2016**, *76*, 18–28. [CrossRef] [PubMed]
86. Tissenbaum, H.A.; Guarente, L. Increased Dosage of a Sir-2 Gene Extends Lifespan in Caenorhabditis Elegans. *Nature* **2001**, *410*, 227–230. [CrossRef] [PubMed]
87. Newman, B.L.; Lundblad, J.R.; Chen, Y.; Smolik, S.M. A Drosophila Homologue of Sir2 Modifies Position-Effect Variegation but Does Not Affect Life Span. *Genetics* **2002**, *162*, 1675–1685. [CrossRef]
88. Shukla, N.; Kolthur-Seetharam, U. Drosophila Sirtuin 6 Mediates Developmental Diet-dependent Programming of Adult Physiology and Survival. *Aging Cell* **2022**, *21*, e13576. [CrossRef]
89. Sejour, R.; Sanguino, R.A.; Mikolajczak, M.; Ahmadi, W.; Villa-Cuesta, E. Sirt4 Modulates Oxidative Metabolism and Sensitivity to Rapamycin through Species-Dependent Phenotypes in Drosophila MtDNA Haplotypes. *G3 Genes| Genomes| Genetics* **2020**, *10*, 1599–1612. [CrossRef]
90. Carneiro Dutra, H.L.; Deehan, M.A.; Frydman, H. Wolbachia and Sirtuin-4 Interaction Is Associated with Alterations in Host Glucose Metabolism and Bacterial Titer. *PLoS Pathog.* **2020**, *16*, e1008996. [CrossRef]
91. Wood, J.G.; Schwer, B.; Wickremesinghe, P.C.; Hartnett, D.A.; Burhenn, L.; Garcia, M.; Li, M.; Verdin, E.; Helfand, S.L. Sirt4 Is a Mitochondrial Regulator of Metabolism and Lifespan in Drosophila Melanogaster. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 1564–1569. [CrossRef]
92. Damschroder, D.; Zapata-Pérez, R.; Richardson, K.; Vaz, F.M.; Houtkooper, R.H.; Wessells, R. Stimulating the Sir2–Spargel Axis Rescues Exercise Capacity and Mitochondrial Respiration in a Drosophila Model of Barth Syndrome. *Dis. Model Mech.* **2022**, *15*, dmm049279. [CrossRef]
93. Zhang, M.; Fei, S.; Xia, J.; Wang, Y.; Wu, H.; Li, X.; Guo, Y.; Swevers, L.; Sun, J.; Feng, M. Sirt5 Inhibits BmNPV Replication by Promoting a Relish-Mediated Antiviral Pathway in Bombyx Mori. *Front. Immunol.* **2022**, *13*, 2259. [CrossRef]
94. Hackett, B.A.; Dittmar, M.; Segrist, E.; Pittenger, N.; To, J.; Griesman, T.; Gordesky-Gold, B.; Schultz, D.C.; Cherry, S. Sirtuin Inhibitors Are Broadly Antiviral against Arboviruses. *mBio* **2019**, *10*, e01446-19. [CrossRef] [PubMed]
95. May, M.A.; Feezell, M.K.; Gonzalez, S.J.; Vasquez, M.C.; Todgham, A.E.; Tomanek, L. Sirtuin-Dependent Recovery from Aerial Heat Shock: The Effects of Food Ration, Thermal History, and Sirtuin Inhibition on Clearance Rates and Valve Gape Activity of the California Mussel, Mytilus Californianus (Conrad). *J. Exp. Mar. Biol. Ecol.* **2021**, *536*, 151510. [CrossRef]
96. Shah, S.M.A.; Taju, S.W.; Dlamini, B.B.; Ou, Y.-Y. DeepSIRT: A Deep Neural Network for Identification of Sirtuin Targets and Their Subcellular Localizations. *Comput. Biol. Chem.* **2021**, *93*, 107514. [CrossRef] [PubMed]
97. Sun, Y.; Yang, Y.-M.; Hu, Y.-Y.; Ouyang, L.; Sun, Z.-H.; Yin, X.-F.; Li, N.; He, Q.-Y.; Wang, Y. Inhibition of Nuclear Deacetylase Sirtuin-1 Induces Mitochondrial Acetylation and Calcium Overload Leading to Cell Death. *Redox. Biol.* **2022**, *53*, 102334. [CrossRef] [PubMed]
98. Seifert, T.; Malo, M.; Kokkola, T.; Stéen, E.J.L.; Meinander, K.; Wallén, E.A.A.; Jarho, E.M.; Luthman, K. A Scaffold Replacement Approach towards New Sirtuin 2 Inhibitors. *Bioorg. Med. Chem.* **2020**, *28*, 115231. [CrossRef]
99. Zähringer, S.; Rumpf, T.; Melesina, J.; Lang, A.E.; Aktories, K.; Sippl, W.; Jung, M.; Wagner, G.K. Defined Stereoisomers of 2"-Amino NAD+ and Their Activity against Human Sirtuins and a Bacterial (ADP-Ribosyl) Transferase. *Bioorg. Med. Chem.* **2022**, *68*, 116875. [CrossRef]
100. Frydzińska, Z.; Owczarek, A.; Winiarska, K. Sirtuiny i Ich Rola w Regulacji Metabolizmu. *Postepy Biochem.* **2019**, *65*, 31–40. [CrossRef]
101. Lin, J.-Y.; Kuo, W.-W.; Baskaran, R.; Kuo, C.-H.; Chen, Y.-A.; Chen, W.S.-T.; Ho, T.-J.; Day, C.H.; Mahalakshmi, B.; Huang, C.-Y. Swimming Exercise Stimulates IGF1/ PI3K/ Akt and AMPK/ SIRT1/ PGC1α Survival Signaling to Suppress Apoptosis and Inflammation in Aging Hippocampus. *Aging* **2020**, *12*, 6852–6864. [CrossRef]
102. Sellitto, C.; Corbi, G.; Stefanelli, B.; Manzo, V.; Trucillo, M.; Charlier, B.; Mensitieri, F.; Izzo, V.; Lucariello, A.; Perna, A.; et al. Antioxidant Supplementation Hinders the Role of Exercise Training as a Natural Activator of SIRT1. *Nutrients* **2022**, *14*, 2092. [CrossRef]
103. Vargas-Ortiz, K.; Pérez-Vázquez, V.; Macías-Cervantes, M.H. Exercise and Sirtuins: A Way to Mitochondrial Health in Skeletal Muscle. *Int. J. Mol. Sci.* **2019**, *20*, 2717. [CrossRef]
104. Edgett, B.A.; Hughes, M.C.; Matusiak, J.B.L.; Perry, C.G.R.; Simpson, C.A.; Gurd, B.J. SIRT3 Gene Expression but Not SIRT3 Subcellular Localization Is Altered in Response to Fasting and Exercise in Human Skeletal Muscle. *Exp. Physiol.* **2016**, *101*, 1101–1113. [CrossRef] [PubMed]

105. Little, J.P.; Safdar, A.; Wilkin, G.P.; Tarnopolsky, M.A.; Gibala, M.J. A Practical Model of Low-Volume High-Intensity Interval Training Induces Mitochondrial Biogenesis in Human Skeletal Muscle: Potential Mechanisms. *J. Physiol.* **2010**, *588*, 1011–1022. [CrossRef] [PubMed]
106. Radak, Z.; Bori, Z.; Koltai, E.; Fatouros, I.G.; Jamurtas, A.Z.; Douroudos, I.I.; Terzis, G.; Nikolaidis, M.G.; Chatzinikolaou, A.; Sovatzidis, A.; et al. Age-Dependent Changes in 8-Oxoguanine-DNA Glycosylase Activity Are Modulated by Adaptive Responses to Physical Exercise in Human Skeletal Muscle. *Free Radic. Biol. Med.* **2011**, *51*, 417–423. [CrossRef]
107. Wu, Q.; Gao, Z.-J.; Yu, X.; Wang, P. Dietary Regulation in Health and Disease. *Signal Transduct. Target Ther.* **2022**, *7*, 252. [CrossRef] [PubMed]
108. Maldonado, M.; Chen, J.; Duan, H.; Huang, T.; Jiang, G.; Zhong, Y. High Calorie Diet Background Alters the Expression of Sirtuins in the Testes of Mice under Caloric Restriction. *Transl. Med. Aging* **2021**, *5*, 10–16. [CrossRef]
109. Vera, E.; Bernardes de Jesus, B.; Foronda, M.; Flores, J.M.; Blasco, M.A. Telomerase Reverse Transcriptase Synergizes with Calorie Restriction to Increase Health Span and Extend Mouse Longevity. *PLoS ONE* **2013**, *8*, e53760. [CrossRef]
110. Amano, H.; Chaudhury, A.; Rodriguez-Aguayo, C.; Lu, L.; Akhanov, V.; Catic, A.; Popov, Y.V.; Verdin, E.; Johnson, H.; Stossi, F.; et al. Telomere Dysfunction Induces Sirtuin Repression That Drives Telomere-Dependent Disease. *Cell Metab.* **2019**, *29*, 1274–1290.e9. [CrossRef]
111. Yzydorczyk, C.; Li, N.; Rigal, E.; Chehade, H.; Mosig, D.; Armengaud, J.B.; Rolle, T.; Krishnasamy, A.; Orozco, E.; Siddeek, B.; et al. Calorie Restriction in Adulthood Reduces Hepatic Disorders Induced by Transient Postnatal Overfeeding in Mice. *Nutrients* **2019**, *11*, 2796. [CrossRef]
112. Tauriainen, E.; Luostarinen, M.; Martonen, E.; Finckenberg, P.; Kovalainen, M.; Huotari, A.; Herzig, K.-H.; Lecklin, A.; Mervaala, E. Distinct Effects of Calorie Restriction and Resveratrol on Diet-Induced Obesity and Fatty Liver Formation. *J. Nutr. Metab.* **2011**, *2011*, 1–10. [CrossRef]
113. Heindel, J.J.; Howard, S.; Agay-Shay, K.; Arrebola, J.P.; Audouze, K.; Babin, P.J.; Barouki, R.; Bansal, A.; Blanc, E.; Cave, M.C.; et al. Obesity II: Establishing Causal Links between Chemical Exposures and Obesity. *Biochem. Pharmacol.* **2022**, *199*, 115015. [CrossRef]
114. Ahmed, M.A.; O'Callaghan, C.; Chang, E.D.; Jiang, H.; Vassilopoulos, A. Context-Dependent Roles for SIRT2 and SIRT3 in Tumor Development Upon Calorie Restriction or High Fat Diet. *Front. Oncol.* **2020**, *9*, 1462. [CrossRef] [PubMed]
115. Zhou, L.; Xu, D.; Sha, W.; Shen, L.; Lu, G.; Yin, X.; Wang, M. High Glucose Induces Renal Tubular Epithelial Injury via Sirt1/ NF-KappaB/ MicroR-29/ Keap1 Signal Pathway. *J. Transl. Med.* **2015**, *13*, 352. [CrossRef] [PubMed]
116. Mastrocola, R.; Nigro, D.; Chiazza, F.; Medana, C.; Dal Bello, F.; Boccuzzi, G.; Collino, M.; Aragno, M. Fructose-Derived Advanced Glycation End-Products Drive Lipogenesis and Skeletal Muscle Reprogramming via SREBP-1c Dysregulation in Mice. *Free Radic. Biol. Med.* **2016**, *91*, 224–235. [CrossRef] [PubMed]
117. Opstad, T.B.; Farup, P.G.; Rootwelt, H.; Aaseth, J.O. Changes in Circulating Sirtuin 1 after Bariatric Surgery. *Nutr. Metab. Cardiovasc. Dis.* **2022**, *32*, 2858–2864. [CrossRef] [PubMed]
118. Savastano, S.; di Somma, C.; Colao, A.; Barrea, L.; Orio, F.; Finelli, C.; Pasanisi, F.; Contaldo, F.; Tarantino, G. Preliminary Data on the Relationship between Circulating Levels of Sirtuin 4, Anthropometric and Metabolic Parameters in Obese Subjects According to Growth Hormone/ Insulin-like Growth Factor-1 Status. *Growth Horm. IGF Res.* **2015**, *25*, 28–33. [CrossRef]
119. Dali-Youcef, N.; Lagouge, M.; Froelich, S.; Koehl, C.; Schoonjans, K.; Auwerx, J. Sirtuins: The 'Magnificent Seven', Function, Metabolism and Longevity. *Ann. Med.* **2007**, *39*, 335–345. [CrossRef]
120. Peluso, I. Diet and Exercise in Lifestyle Medicine: The Hormetic Effects of Bioactive Compounds on Human Health. *Curr. Opin. Toxicol.* **2022**, *30*, 100342. [CrossRef]
121. Abrescia, P.; Treppiccione, L.; Rossi, M.; Bergamo, P. Modulatory Role of Dietary Polyunsaturated Fatty Acids in Nrf2-Mediated Redox Homeostasis. *Prog. Lipid Res.* **2020**, *80*, 101066. [CrossRef]
122. Tapia, P.C. Sublethal Mitochondrial Stress with an Attendant Stoichiometric Augmentation of Reactive Oxygen Species May Precipitate Many of the Beneficial Alterations in Cellular Physiology Produced by Caloric Restriction, Intermittent Fasting, Exercise and Dietary Phytonutrients: "Mitohormesis" for Health and Vitality. *Med. Hypotheses* **2006**, *66*, 832–843. [CrossRef]
123. Jerome, M.S.; Kuthethur, R.; Kabekkodu, S.P.; Chakrabarty, S. Regulation of Mitochondrial Function by Forkhead Transcription Factors. *Biochimie* **2022**, *198*, 96–108. [CrossRef]
124. Martins, R.; Lithgow, G.J.; Link, W. Long Live FOXO: Unraveling the Role of FOXO Proteins in Aging and Longevity. *Aging Cell* **2016**, *15*, 196–207. [CrossRef] [PubMed]
125. Lam, E.W.-F.; Brosens, J.J.; Gomes, A.R.; Koo, C.-Y. Forkhead Box Proteins: Tuning Forks for Transcriptional Harmony. *Nat. Rev. Cancer* **2013**, *13*, 482–495. [CrossRef]
126. Daitoku, H.; Hatta, M.; Matsuzaki, H.; Aratani, S.; Ohshima, T.; Miyagishi, M.; Nakajima, T.; Fukamizu, A. Silent Information Regulator 2 Potentiates Foxo1-Mediated Transcription through Its Deacetylase Activity. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 10042–10047. [CrossRef] [PubMed]
127. Zhao, Y.; Sun, H.; Lu, J.; Li, X.; Chen, X.; Tao, D.; Huang, W.; Huang, B. Lifespan Extension and Elevated Hsp Gene Expression in Drosophila Caused by Histone Deacetylase Inhibitors. *J. Exp. Biol.* **2005**, *208*, 697–705. [CrossRef]
128. Merksamer, P.I.; Liu, Y.; He, W.; Hirschey, M.D.; Chen, D.; Verdin, E. The Sirtuins, Oxidative Stress and Aging: An Emerging Link. *Aging* **2013**, *5*, 144–150. [CrossRef] [PubMed]
129. Kim, H.; Skurk, C.; Maatz, H.; Shiojima, I.; Ivashchenko, Y.; Yoon, S.; Park, Y.; Walsh, K. Akt/ FOXO3a Signaling Modulates the Endothelial Stress Response through Regulation of Heat Shock Protein 70 Expression. *FASEB J.* **2005**, *19*, 1042–1044. [CrossRef]



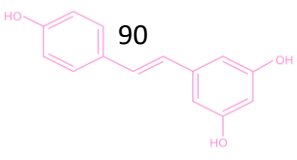
130. Greiss, S.; Hall, J.; Ahmed, S.; Gartner, A.C. Elegans SIR-2.1 Translocation Is Linked to a Proapoptotic Pathway Parallel to Cep-1 / P53 during DNA Damage-Induced Apoptosis. *Genes Dev.* **2008**, *22*, 2831–2842. [CrossRef]
131. Giannakou, M.E.; Partridge, L. The Interaction between FOXO and SIRT1: Tipping the Balance towards Survival. *Trends Cell Biol.* **2004**, *14*, 408–412. [CrossRef]
132. Kobayashi, Y.; Furukawa-Hibi, Y.; Chen, C.; Horio, Y.; Isobe, K.; Ikeda, K.; Motoyama, N. SIRT1 Is Critical Regulator of FOXO-Mediated Transcription in Response to Oxidative Stress. *Int. J. Mol. Med.* **2005**, *16*, 237–243. [CrossRef]
133. E Tamura, R.; F de Vasconcellos, J.; Sarkar, D.; A Libermann, T.; B Fisher, P.; F Zerbini, L. GADD45 Proteins: Central Players in Tumorigenesis. *Curr. Mol. Med.* **2012**, *12*, 634–651. [CrossRef]
134. Wang, F.; Nguyen, M.; Qin, F.X.-F.; Tong, Q. SIRT2 Deacetylates FOXO3a in Response to Oxidative Stress and Caloric Restriction. *Aging Cell* **2007**, *6*, 505–514. [CrossRef] [PubMed]
135. Tyner, S.D.; Venkatachalam, S.; Choi, J.; Jones, S.; Ghebranious, N.; Igelmann, H.; Lu, X.; Soron, G.; Cooper, B.; Brayton, C.; et al. P53 Mutant Mice That Display Early Ageing-Associated Phenotypes. *Nature* **2002**, *415*, 45–53. [CrossRef] [PubMed]
136. Xia, N.; Strand, S.; Schlufter, F.; Siuda, D.; Reifenberg, G.; Kleinert, H.; Förstermann, U.; Li, H. Role of SIRT1 and FOXO Factors in ENOS Transcriptional Activation by Resveratrol. *Nitric Oxide* **2013**, *32*, 29–35. [CrossRef]
137. Brunet, A.; Sweeney, L.B.; Sturgill, J.F.; Chua, K.F.; Greer, P.L.; Lin, Y.; Tran, H.; Ross, S.E.; Mostoslavsky, R.; Cohen, H.Y.; et al. Stress-Dependent Regulation of FOXO Transcription Factors by the SIRT1 Deacetylase. *Science* **2004**, *303*, 2011–2015. [CrossRef]
138. Kume, S.; Haneda, M.; Kanasaki, K.; Sugimoto, T.; Araki, S.; Isono, M.; Isshiki, K.; Uzu, T.; Kashiwagi, A.; Koya, D. Silent Information Regulator 2 (SIRT1) Attenuates Oxidative Stress-Induced Mesangial Cell Apoptosis via P53 Deacetylation. *Free Radic. Biol. Med.* **2006**, *40*, 2175–2182. [CrossRef] [PubMed]
139. van der Horst, A.; Tertoolen, L.G.J.; de Vries-Smits, L.M.M.; Frye, R.A.; Medema, R.H.; Burgering, B.M.T. FOXO4 Is Acetylated upon Peroxide Stress and Deacetylated by the Longevity Protein HSir2. *J. Biol. Chem.* **2004**, *279*, 28873–28879. [CrossRef] [PubMed]
140. Krishnamoorthy, V.; Vilwanathan, R. Silencing Sirtuin 6 Induces Cell Cycle Arrest and Apoptosis in Non-Small Cell Lung Cancer Cell Lines. *Genomics* **2020**, *112*, 3703–3712. [CrossRef]
141. Kim, U.; Kim, K.S.; Park, J.-K.; Um, H.-D. Hyperacetylation of the C-Terminal Domain of P53 Inhibits the Formation of the P53/ P21 Complex. *Biochem. Biophys. Res. Commun.* **2022**, *635*, 52–56. [CrossRef]
142. Chang, H.W.; Lee, M.; Lee, Y.S.; Kim, S.H.; Lee, J.C.; Park, J.J.; Nam, H.Y.; Kim, M.R.; Han, M.W.; Kim, S.W.; et al. P53-Dependent Glutamine Usage Determines Susceptibility to Oxidative Stress in Radioresistant Head and Neck Cancer Cells. *Cell Signal* **2021**, *77*, 109820. [CrossRef]
143. Igase, M.; Fujiki, N.; Shibutani, S.; Sakai, H.; Noguchi, S.; Nemoto, Y.; Mizuno, T. Tenovin-6 Induces the SIRT-Independent Cell Growth Suppression and Blocks Autophagy Flux in Canine Hemangiosarcoma Cell Lines. *Exp. Cell Res.* **2020**, *388*, 111810. [CrossRef]
144. Bordoni, V.; Tartaglia, E.; Sacchi, A.; Fimia, G.M.; Cimini, E.; Casetti, R.; Notari, S.; Grassi, G.; Marchioni, L.; Bibas, M.; et al. The Unbalanced P53/ SIRT1 Axis May Impact Lymphocyte Homeostasis in COVID-19 Patients. *Int. J. Infect. Dis.* **2021**, *105*, 49–53. [CrossRef]
145. Wang, P.; Chen, D.; An, J.; Lin, S.; Liu, T.; Li, Y.; Chen, L.; He, B. Development of a Single-Step Fluorogenic Sirtuin Assay and Its Applications for High-Throughput Screening. *Org. Biomol. Chem.* **2022**, *20*, 1243–1252. [CrossRef] [PubMed]
146. Sanders, B.D.; Jackson, B.; Marmorstein, R. Structural Basis for Sirtuin Function: What We Know and What We Don't. *Biochim. Biophys. Acta (BBA)—Proteins Proteom.* **2010**, *1804*, 1604–1616. [CrossRef] [PubMed]
147. Yang, F.; Su, H.; Deng, J.; Mou, L.; Wang, H.; Li, R.; Dai, Q.-Q.; Yan, Y.-H.; Qian, S.; Wang, Z.; et al. Discovery of New Human Sirtuin 5 Inhibitors by Mimicking Glutaryl-Lysine Substrates. *Eur. J. Med. Chem.* **2021**, *225*, 113803. [CrossRef]
148. Kumari, S.; Chaurasia, S.N.; Nayak, M.K.; Mallick, R.L.; Dash, D. Sirtuin Inhibition Induces Apoptosis-like Changes in Platelets and Thrombocytopenia. *J. Biol. Chem.* **2015**, *290*, 12290–12299. [CrossRef]
149. Gertz, M.; Fischer, F.; Nguyen, G.T.T.; Lakshminarasimhan, M.; Schutkowski, M.; Weyand, M.; Steegborn, C. Ex-527 Inhibits Sirtuins by Exploiting Their Unique NAD^+ -Dependent Deacetylation Mechanism. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E2772–E2781. [CrossRef]
150. Qin, H.; Zhang, H.; Zhang, X.; Zhang, S.; Zhu, S.; Wang, H. Resveratrol Attenuates Radiation Enteritis through the SIRT1/ FOXO3a and PI3K/ AKT Signaling Pathways. *Biochem. Biophys. Res. Commun.* **2021**, *554*, 199–205. [CrossRef] [PubMed]
151. Ye, S.; Fang, L.; Xie, S.; Hu, Y.; Chen, S.; Amin, N.; Fang, M.; Hu, Z. Resveratrol Alleviates Postpartum Depression-like Behavior by Activating Autophagy via SIRT1 and Inhibiting AKT/ MTOR Pathway. *Behav. Brain Res.* **2022**, *438*, 114208. [CrossRef]
152. Yu, D.; Zhao, X.-Y.; Meng, Q.-P.; Teng, D.; Deng, K.; Lin, N. Resveratrol Activates the SIRT1/ PGC-1 Pathway in Mice to Improve Synaptic-Related Cognitive Impairment after TBI. *Brain Res.* **2022**, *1796*, 148109. [CrossRef]
153. Alcaín, F.J.; Villalba, J.M. Sirtuin Activators. *Expert Opin. Ther. Pat.* **2009**, *19*, 403–414. [CrossRef]
154. Gozelle, M.; Kaya, S.G.; Aksel, A.B.; Ozkan, E.; Bakar-Ates, F.; Ozkan, Y.; Eren, G. Hit Evaluation Results in 5-Benzyl-1,3,4-Thiadiazole-2-Carboxamide Based SIRT2-Selective Inhibitor with Improved Affinity and Selectivity. *Bioorg. Chem.* **2022**, *123*, 105746. [CrossRef] [PubMed]
155. Laaroussi, H.; Ding, Y.; Teng, Y.; Deschamps, P.; Vidal, M.; Yu, P.; Broussy, S. Synthesis of Indole Inhibitors of Silent Information Regulator 1 (SIRT1), and Their Evaluation as Cytotoxic Agents. *Eur. J. Med. Chem.* **2020**, *202*, 112561. [CrossRef] [PubMed]

156. Huang, Q.; Lou, T.; Lu, J.; Wang, M.; Chen, X.; Xue, L.; Tang, X.; Qi, W.; Zhang, Z.; Su, H.; et al. Major Ginsenosides from Panax Ginseng Promote Aerobic Cellular Respiration and SIRT1-Mediated Mitochondrial Biosynthesis in Cardiomyocytes and Neurons. *J. Ginseng Res.* **2022**, *46*, 759–770. [CrossRef] [PubMed]
157. Rodriguez, M.E.; Tekiel, V.; Campo, V.A. In Vitro Evaluation of Resveratrol as a Potential Pre-Exposure Prophylactic Drug against Trypanosoma Cruzi Infection. *Int. J. Parasitol. Drugs Drug Resist.* **2022**, *20*, 54–64. [CrossRef] [PubMed]
158. Mohamed, G.A.; Ibrahim, S.R.M.; El-Agamy, D.S.; Elsaed, W.M.; Sirwi, A.; Asfour, H.Z.; Koshak, A.E.; Elhady, S.S. Cucurbitacin E Glucoside Alleviates Concanavalin A-Induced Hepatitis through Enhancing SIRT1/ Nrf2/ HO-1 and Inhibiting NF-kB/ NLRP3 Signaling Pathways. *J. Ethnopharmacol.* **2022**, *292*, 115223. [CrossRef]
159. Chen, L.; Song, C.; Zhang, Y.; Li, Y.; Zhao, Y.; Lin, F.; Han, D.; Dai, M.; Li, W.; Pan, P. Quercetin Protects against LPS-Induced Lung Injury in Mice via SIRT1-Mediated Suppression of PKM2 Nuclear Accumulation. *Eur. J. Pharmacol.* **2022**, *936*, 175352. [CrossRef]
160. Ibrahim, K.A.; Abdelgaid, H.A.; Eleyan, M.; Mohamed, R.A.; Gamil, N.M. Resveratrol Alleviates Cardiac Apoptosis Following Exposure to Fenitrothion by Modulating the Sirtuin1/ c-Jun N-Terminal Kinases/ P53 Pathway through pro-Oxidant and Inflammatory Response Improvements: In Vivo and in Silico Studies. *Life Sci.* **2022**, *290*, 120265. [CrossRef]
161. Song, Y.; Wang, Z.; Liu, L.; Zhang, S.; Zhang, H.; Qian, Y. 1,4-Dihydropyridine (DHP) Suppresses against Oxidative Stress in Nucleus Pulposus via Activating Sirtuin-1. *Biomed. Pharmacother.* **2020**, *121*, 109592. [CrossRef]
162. Moretti, C.H.; Schiffer, T.A.; Montenegro, M.F.; Larsen, F.J.; Tsarouhas, V.; Carlström, M.; Samakovlis, C.; Weitzberg, E.; Lundberg, J.O. Dietary Nitrite Extends Lifespan and Prevents Age-Related Locomotor Decline in the Fruit Fly. *Free Radic. Biol. Med.* **2020**, *160*, 860–870. [CrossRef]
163. Pan, Y.; Zhang, H.; Zheng, Y.; Zhou, J.; Yuan, J.; Yu, Y.; Wang, J. Resveratrol Exerts Antioxidant Effects by Activating SIRT2 To Deacetylate Prx1. *Biochemistry* **2017**, *56*, 6325–6328. [CrossRef]
164. Lv, X.-T.; Wang, R.-H.; Liu, X.-T.; Ye, Y.-J.; Liu, X.-Y.; Qiao, J.-D.; Wang, G.-E. Theacrine Ameliorates Experimental Liver Fibrosis in Rats by Lowering Cholesterol Storage via Activation of the Sirtuin 3-Farnesoid X Receptor Signaling Pathway. *Chem. Biol. Interact.* **2022**, *364*, 110051. [CrossRef] [PubMed]
165. Si, Y.; Bao, H.; Han, L.; Chen, L.; Zeng, L.; Jing, L.; Xing, Y.; Geng, Y. Dexmedetomidine Attenuation of Renal Ischaemia-Reperfusion Injury Requires Sirtuin 3 Activation. *Br. J. Anaesth.* **2018**, *121*, 1260–1271. [CrossRef] [PubMed]
166. Saldaña, N.T.; de Jesús García Rivas, G.; Montemayor, L.L.E. Oroxylin A Reduces Angiotensin II- Induced Hypertrophy and Mitochondrial Dysfunction by Activating Sirtuin 3 in Cardiac Myocytes. *J. Mol. Cell. Cardiol.* **2017**, *112*, 148. [CrossRef]
167. Zefzoufi, M.; Fdil, R.; Bouamama, H.; Gadhi, C.; Katakura, Y.; Mouzdahir, A.; Sraidi, K. Effect of Extracts and Isolated Compounds Derived from *Retama Monosperma* (L.) Boiss. on Anti-Aging Gene Expression in Human Keratinocytes and Antioxidant Activity. *J. Ethnopharmacol.* **2021**, *280*, 114451. [CrossRef]
168. Wei, W.; Hu, P.; Qin, M.; Chen, G.; Wang, F.; Yao, S.; Jin, M.; Xie, Z.; Zhang, X. SIRT4 Is Highly Expressed in Retinal Müller Glial Cells. *Front. Neurosci.* **2022**, 16. [CrossRef]
169. Li, Y.; Gao, M.; Yin, L.-H.; Xu, L.-N.; Qi, Y.; Sun, P.; Peng, J.-Y. Dioscin Ameliorates Methotrexate-Induced Liver and Kidney Damages via Adjusting MiRNA-145-5p-Mediated Oxidative Stress. *Free Radic. Biol. Med.* **2021**, *169*, 99–109. [CrossRef]
170. Xiao, Z.-P.; Lv, T.; Hou, P.-P.; Manaenko, A.; Liu, Y.; Jin, Y.; Gao, L.; Jia, F.; Tian, Y.; Li, P.; et al. Sirtuin 5-Mediated Lysine Desuccinylation Protects Mitochondrial Metabolism Following Subarachnoid Hemorrhage in Mice. *Stroke* **2021**, *52*, 4043–4053. [CrossRef]
171. Yamada, C.; Ho, A.; Akkaoui, J.; Garcia, C.; Duarte, C.; Movila, A. Glycyrrhizin Mitigates Inflammatory Bone Loss and Promotes Expression of Senescence-Protective Sirtuins in an Aging Mouse Model of Periprosthetic Osteolysis. *Biomed. Pharmacother.* **2021**, *138*, 111503. [CrossRef]
172. Wang, Q.-L.; Yang, L.; Liu, Z.-L.; Peng, Y.; Gao, M.; Deng, L.-T.; Liu, X.; Xing, W. Sirtuin 6 Regulates Macrophage Polarization to Alleviate Sepsis-Induced Acute Respiratory Distress Syndrome via Dual Mechanisms Dependent on and Independent of Autophagy. *Cytotherapy* **2022**, *24*, 149–160. [CrossRef]
173. Kang, J.-Y.; Lee, J.-S.; Seol, I.-C.; Kim, Y.-S.; Park, M.S.; Yoo, H.-R. Pharmacological Effects of Gami-Yukmijihwang-Tang on the Lipopolysaccharide-Induced Hippocampus Oxidation and Inflammation via Regulation of Sirt6. *Pharmaceuticals* **2022**, *15*, 293. [CrossRef]
174. Song, J.; Liu, L.; Hao, K.; Mao, S.; Tang, Y.; Tong, X.; Dai, F. Resveratrol Elongates the Lifespan and Improves Antioxidant Activity in the Silkworm Bombyx Mori. *J. Pharm. Anal.* **2021**, *11*, 374–382. [CrossRef] [PubMed]
175. Alshammari, G.M.; Al-Qahtani, W.H.; Alshuniaber, M.A.; Yagoub, A.E.A.; Al-Khalifah, A.S.; Al-Harbi, L.N.; Alhussain, M.H.; AlSedairy, S.A.; Yahya, M.A. Quercetin Improves the Impairment in Memory Function and Attenuates Hippocampal Damage in Cadmium Chloride-Intoxicated Male Rats by Suppressing Acetylcholinesterase and Concomitant Activation of SIRT1 Signaling. *J. Funct. Foods* **2021**, *86*, 104675. [CrossRef]
176. Wei, L.; Liu, B.; Yao, Z.; Yuan, T.; Wang, C.; Zhang, R.; Wang, Q.; Zhao, B. Sirtuin 1 Inhibitor EX527 Suppresses Morphine-Induced Behavioral Sensitization. *Neurosci. Lett.* **2021**, *744*, 135599. [CrossRef] [PubMed]
177. Kang, H.; Lee, Y.; Bae, M.; Park, Y.-K.; Lee, J.-Y. Astaxanthin Inhibits Alcohol-Induced Inflammation and Oxidative Stress in Macrophages in a Sirtuin 1-Dependent Manner. *J. Nutr. Biochem.* **2020**, *85*, 108477. [CrossRef] [PubMed]
178. Wei, R.; Han, C.; He, F.; Xiong, X.; Ye, F.; Liu, H.; Li, L.; Xu, H.; Wei, S.; Zeng, X. Role of Forkhead Box Protein O1 and Insulin on Cell Proliferation Mediated by Sirtuin 1 in Goose Primary Hepatocytes. *J. Appl. Poult. Res.* **2021**, *30*, 100144. [CrossRef]



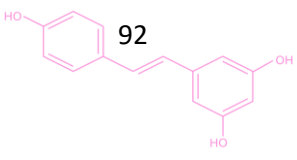
179. Ceballos, M.P.; Angel, A.; Delprato, C.B.; Livore, V.I.; Ferretti, A.C.; Lucci, A.; Comanzo, C.G.; de Luján Alvarez, M.; Quiroga, A.D.; Mottino, A.D.; et al. Sirtuin 1 and 2 Inhibitors Enhance the Inhibitory Effect of Sorafenib in Hepatocellular Carcinoma Cells. *Eur. J. Pharmacol.* **2021**, *892*, 173736. [CrossRef]
180. Tan, Y.J.; Lee, Y.T.; Mancera, R.L.; Oon, C.E. BZD9L1 Sirtuin Inhibitor: Identification of Key Molecular Targets and Their Biological Functions in HCT 116 Colorectal Cancer Cells. *Life Sci.* **2021**, *284*, 119747. [CrossRef]
181. Hirata, Y.; Nishino, H.; Sasaki, T.; Nagaoka, Y.; Uesato, S.; Taniguchi, M. Sirtuin Inhibition and Neurite Outgrowth Effect as New Biological Activities for Areca Catechu Nut Alkaloids. *Phytomedicine Plus* **2022**, *2*, 100294. [CrossRef]
182. Kato, Y.; Kihara, H.; Fukui, K.; Kojima, M. A Ternary Complex Model of Sirtuin4-NAD+-Glutamate Dehydrogenase. *Comput. Biol. Chem.* **2018**, *74*, 94–104. [CrossRef]
183. Yang, L.-L.; Wang, H.-L.; Yan, Y.-H.; Liu, S.; Yu, Z.-J.; Huang, M.-Y.; Luo, Y.; Zheng, X.; Yu, Y.; Li, G.-B. Sensitive Fluorogenic Substrates for Sirtuin Deacylase Inhibitor Discovery. *Eur. J. Med. Chem.* **2020**, *192*, 112201. [CrossRef]
184. Liu, Y.; Debnath, B.; Kumar, S.; Lombard, D.B.; Neamati, N. Identification of 2-Hydroxybenzoic Acid Derivatives as Selective SIRT5 Inhibitors. *Eur. J. Med. Chem.* **2022**, *241*, 114623. [CrossRef] [PubMed]
185. Collins, J.A.; Kapustina, M.; Bolduc, J.A.; Pike, J.F.W.; Diekman, B.O.; Mix, K.; Chubinskaya, S.; Eroglu, E.; Michel, T.; Poole, L.B.; et al. Sirtuin 6 (SIRT6) Regulates Redox Homeostasis and Signaling Events in Human Articular Chondrocytes. *Free Radic. Biol. Med.* **2021**, *166*, 90–103. [CrossRef]
186. Chudzińska, M.; Rogowicz, D.; Wołowiec, Ł.; Banach, J.; Sielski, S.; Bujak, R.; Sinkiewicz, A.; Grześk, G. Resveratrol and Cardiovascular System—The Unfulfilled Hopes. *Ir. J. Med. Sci.* **2021**, *190*, 981–986. [CrossRef] [PubMed]
187. Fonseca-Santos, B.; Chorilli, M. The Uses of Resveratrol for Neurological Diseases Treatment and Insights for Nanotechnology Based-Drug Delivery Systems. *Int. J. Pharm.* **2020**, *589*, 119832. [CrossRef]
188. Li, K.-X.; Ji, M.-J.; Sun, H.-J. An Updated Pharmacological Insight of Resveratrol in the Treatment of Diabetic Nephropathy. *Gene* **2021**, *780*, 145532. [CrossRef]
189. Okudaira, N.; Ishizaka, Y.; Tamamori-Adachi, M. Resveratrol Blocks Retrotransposition of LINE-1 through PPARα and Sirtuin-6. *Sci. Rep.* **2022**, *12*, 7772. [CrossRef]
190. Griñán-Ferré, C.; Bellver-Sanchis, A.; Izquierdo, V.; Corpas, R.; Roig-Soriano, J.; Chillón, M.; Andres-Lacueva, C.; Somogyvári, M.; Sőti, C.; Sanfeliu, C.; et al. The Pleiotropic Neuroprotective Effects of Resveratrol in Cognitive Decline and Alzheimer's Disease Pathology: From Antioxidant to Epigenetic Therapy. *Ageing Res. Rev.* **2021**, *67*, 101271. [CrossRef]
191. Miguel, C.A.; Noya-Riobó, M.V.; Mazzone, G.L.; Villar, M.J.; Coronel, M.F. Antioxidant, Anti-Inflammatory and Neuroprotective Actions of Resveratrol after Experimental Nervous System Insults. Special Focus on the Molecular Mechanisms Involved. *Neurochem. Int.* **2021**, *150*, 105188. [CrossRef] [PubMed]
192. Kinra, M.; Ranadive, N.; Mudgal, J.; Zhang, Y.; Govindula, A.; Anoopkumar-Dukie, S.; Davey, A.K.; Grant, G.D.; Nampoothiri, M.; Arora, D. Putative Involvement of Sirtuin Modulators in LPS-Induced Sickness Behaviour in Mice. *Metab. Brain Dis.* **2022**, *37*, 1969–1976. [CrossRef]
193. Chou, X.; Li, X.; Min, Z.; Ding, F.; Ma, K.; Shen, Y.; Sun, D.; Wu, Q. Sirtuin-1 Attenuates Cadmium-Induced Renal Cell Senescence through P53 Deacetylation. *Ecotoxicol. Environ. Saf.* **2022**, *245*, 114098. [CrossRef]
194. Zhao, X.-Y.; Yu, D.; Shi, X.; Hou, S.; Teng, D. Resveratrol Reduces P38 Mitogen-Activated Protein Kinase Phosphorylation by Activating Sirtuin 1 to Alleviate Cognitive Dysfunction after Traumatic Brain Injury in Mice. *Neuroreport* **2022**, *33*, 463–469. [CrossRef] [PubMed]
195. Yang, Y.; Liu, C.; Yang, J.; Yuan, F.; Cheng, R.; Chen, R.; Shen, Y.; Huang, L. Impairment of Sirtuin 1-Mediated DNA Repair Is Involved in Bisphenol A-Induced Aggravation of Macrophage Inflammation and Atherosclerosis. *Chemosphere* **2021**, *265*, 128997. [CrossRef] [PubMed]
196. Zada, N.S.; Belduz, A.O.; Güler, H.I.; Sahinkaya, M.; Khan, S.I.; Saba, M.; Bektas, K.I.; Kara, Y.; Kolaylı, S.; Badshah, M.; et al. Cloning, Biochemical Characterization and Molecular Docking of Novel Thermostable β-Glucosidase BglA9 from Anoxybacillus Ayderensis A9 and Its Application in de-Glycosylation of Polydatin. *Int. J. Biol. Macromol.* **2021**, *193*, 1898–1909. [CrossRef] [PubMed]
197. Sunsong, R.; Du, T.; Etim, I.; Zhang, Y.; Liang, D.; Gao, S. Development of a Novel UPLC-MS/MS Method for the Simultaneously Quantification of Polydatin and Resveratrol in Plasma: Application to a Pharmacokinetic Study in Rats. *J. Chromatogr. B* **2021**, *1185*, 123000. [CrossRef] [PubMed]
198. Fakhri, S.; Gravandi, M.M.; Abdian, S.; Akkol, E.K.; Farzaei, M.H.; Sobarzo-Sánchez, E. The Neuroprotective Role of Polydatin: Neuropharmacological Mechanisms, Molecular Targets, Therapeutic Potentials, and Clinical Perspective. *Molecules* **2021**, *26*, 5985. [CrossRef]
199. Tong, Y.; Wang, K.; Sheng, S.; Cui, J. Polydatin Ameliorates Chemotherapy-Induced Cognitive Impairment (Chemobrain) by Inhibiting Oxidative Stress, Inflammatory Response, and Apoptosis in Rats. *Biosci. Biotechnol. Biochem.* **2020**, *84*, 1201–1210. [CrossRef]
200. Bao, H.-L.; Chen, C.-Z.; Ren, C.-Z.; Sun, K.-Y.; Liu, H.; Song, S.-H.; Fu, Z.-R. Polydatin Ameliorates Hepatic Ischemia-Reperfusion Injury by Modulating Macrophage Polarization. *Hepatobiliary Pancreat. Dis. Int.* **2022**. [CrossRef]
201. Jin, W.; Fan, M.; Zhang, Y.; Zhang, Q.; Jing, C.; Jiang, R.; Piao, C.; Sun, L. Polydatin Prevents Lipotoxicity-Induced Dysfunction in Pancreatic β-Cells by Inhibiting Endoplasmic Reticulum Stress and Excessive Autophagy. *Phytomedicine* **2022**, *106*, 154410. [CrossRef]

202. Zhang, H.; Chen, Y.; Pei, Z.; Gao, H.; Shi, W.; Sun, M.; Xu, Q.; Zhao, J.; Meng, W.; Xiao, K. Protective Effects of Polydatin against Sulfur Mustard-Induced Hepatic Injury. *Toxicol. Appl. Pharmacol.* **2019**, *367*, 1–11. [CrossRef]
203. Cao, K.; Lei, X.; Liu, H.; Zhao, H.; Guo, J.; Chen, Y.; Xu, Y.; Cheng, Y.; Liu, C.; Cui, J.; et al. Polydatin Alleviated Radiation-Induced Lung Injury through Activation of Sirt3 and Inhibition of Epithelial-Mesenchymal Transition. *J. Cell. Mol. Med.* **2017**, *21*, 3264–3276. [CrossRef]
204. Zhang, M.; Wang, S.; Cheng, Z.; Xiong, Z.; Lv, J.; Yang, Z.; Li, T.; Jiang, S.; Gu, J.; Sun, D.; et al. Polydatin Ameliorates Diabetic Cardiomyopathy via Sirt3 Activation. *Biochem. Biophys. Res. Commun.* **2017**, *493*, 1280–1287. [CrossRef] [PubMed]
205. Ahmad, P.; Alvi, S.S.; Iqbal, D.; Khan, M.S. Insights into Pharmacological Mechanisms of Polydatin in Targeting Risk Factors-Mediated Atherosclerosis. *Life Sci.* **2020**, *254*, 117756. [CrossRef] [PubMed]
206. Chen, F.; Zhang, H.; Zhao, N.; Du, E.; Jin, F.; Fan, Q.; Guo, W.; Huang, S.; Wei, J. Effects of Magnolol and Honokiol Blend on Performance, Egg Quality, Hepatic Lipid Metabolism, and Intestinal Morphology of Hens at Late Laying Cycle. *Animal* **2022**, *16*, 100532. [CrossRef] [PubMed]
207. Tanikawa, T.; Hayashi, T.; Suzuki, R.; Kitamura, M.; Inoue, Y. Inhibitory Effect of Honokiol on Furin-like Activity and SARS-CoV-2 Infection. *J. Tradit. Complement. Med.* **2022**, *12*, 69–72. [CrossRef]
208. Zhou, Y.; Tang, J.; Lan, J.; Zhang, Y.; Wang, H.; Chen, Q.; Kang, Y.; Sun, Y.; Feng, X.; Wu, L.; et al. Honokiol Alleviated Neurodegeneration by Reducing Oxidative Stress and Improving Mitochondrial Function in Mutant SOD1 Cellular and Mouse Models of Amyotrophic Lateral Sclerosis. *Acta Pharm. Sin. B* **2022**. [CrossRef]
209. Borgonetti, V.; Governa, P.; Manetti, F.; Miraldi, E.; Biagi, M.; Galeotti, N. A Honokiol-Enriched Magnolia Officinalis Rehder & E.H. Wilson. Bark Extract Possesses Anxiolytic-like Activity with Neuroprotective Effect through the Modulation of CB1 Receptor. *J. Pharm. Pharmacol.* **2021**, *73*, 1161–1168. [CrossRef]
210. Khatoon, F.; Ali, S.; Kumar, V.; Elasbali, A.M.; Alhassan, H.H.; Alharethi, S.H.; Islam, A.; Hassan, M.I. Pharmacological Features, Health Benefits and Clinical Implications of Honokiol. *J. Biomol. Struct. Dyn.* **2022**, 1–23. [CrossRef]
211. Luo, L.-X.; Li, Y.; Liu, Z.-Q.; Fan, X.-X.; Duan, F.-G.; Li, R.-Z.; Yao, X.-J.; Leung, E.L.-H.; Liu, L. Honokiol Induces Apoptosis, G1 Arrest, and Autophagy in KRAS Mutant Lung Cancer Cells. *Front. Pharmacol.* **2017**, *8*, 199. [CrossRef]
212. Storder, J.; Renard, P.; Arnould, T. Update on the Role of Sirtuin 3 in Cell Differentiation: A Major Metabolic Target That Can Be Pharmacologically Controlled. *Biochem. Pharmacol.* **2019**, *169*, 113621. [CrossRef]
213. Li, Y.H.; Choi, D.H.; Lee, E.H.; Seo, S.R.; Lee, S.; Cho, E.-H. Sirtuin 3 (SIRT3) Regulates α-Smooth Muscle Actin (α-SMA) Production through the Succinate Dehydrogenase-G Protein-Coupled Receptor 91 (GPR91) Pathway in Hepatic Stellate Cells. *J. Biol. Chem.* **2016**, *291*, 10277–10292. [CrossRef]
214. Tresch, S. Strategies and Future Trends to Identify the Mode of Action of Phytotoxic Compounds. *Plant Sci.* **2013**, *212*, 60–71. [CrossRef] [PubMed]
215. Milanović, M.; Đurić, L.; Milošević, N.; Milić, N. Comprehensive Insight into Triclosan—From Widespread Occurrence to Health Outcomes. *Environ. Sci. Pollut. Res.* **2021**, 1–22. [CrossRef] [PubMed]
216. Guo, J.; Ito, S.; Nguyen, H.T.; Yamamoto, K.; Tanoue, R.; Kunisue, T.; Iwata, H. Effects of Prenatal Exposure to Triclosan on the Liver Transcriptome in Chicken Embryos. *Toxicol. Appl. Pharmacol.* **2018**, *347*, 23–32. [CrossRef] [PubMed]
217. Szychowski, K.A.; Skóra, B.; Wójtowicz, A.K. Involvement of Sirtuins (Sirt1 and Sirt3) and Aryl Hydrocarbon Receptor (AhR) in the Effects of Triclosan (TCS) on Production of Neurosteroids in Primary Mouse Cortical Neurons Cultures. *Pestic. Biochem. Physiol.* **2022**, *184*, 105131. [CrossRef]
218. Bao, S.; He, C.; Ku, P.; Xie, M.; Lin, J.; Lu, S.; Nie, X. Effects of Triclosan on the RedoximiRs/ Sirtuin/ Nrf2/ ARE Signaling Pathway in Mosquitofish (Gambusia Affinis). *Aquat. Toxicol.* **2021**, *230*, 105679. [CrossRef]
219. Dykes, S.S.; Friday, E.; Pruitt, K.; Cardelli, J.A. The Histone Deacetylase Inhibitor Cambinol Prevents Acidic PHe-Induced Anterograde Lysosome Trafficking Independently of Sirtuin Activity. *Biochem. Biophys. Rep.* **2015**, *3*, 83–93. [CrossRef]
220. Botta, L.; Filippi, S.; Bizzarri, B.M.; Meschini, R.; Caputo, M.; Proietti-De-Santis, L.; Iside, C.; Nebbioso, A.; Gualandi, G.; Saladino, R. Oxidative Nucleophilic Substitution Selectively Produces Cambinol Derivatives with Antiproliferative Activity on Bladder Cancer Cell Lines. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 78–82. [CrossRef]
221. Lugrin, J.; Ciarlo, E.; Santos, A.; Grandmaison, G.; dos Santos, I.; le Roy, D.; Roger, T. The Sirtuin Inhibitor Cambinol Impairs MAPK Signaling, Inhibits Inflammatory and Innate Immune Responses and Protects from Septic Shock. *Biochim. Biophys. Acta (BBA)—Mol. Cell Res.* **2013**, *1833*, 1498–1510. [CrossRef]
222. Chowdhury, S.; Sripathy, S.; Webster, A.A.; Park, A.; Lao, U.; Hsu, J.H.; Loe, T.; Bedalov, A.; Simon, J.A. Discovery of Selective SIRT2 Inhibitors as Therapeutic Agents in B-Cell Lymphoma and Other Malignancies. *Molecules* **2020**, *25*, 455. [CrossRef]
223. Wawruszak, A.; Luszczki, J.; Okon, E.; Czerwonka, A.; Stepulak, A. Antagonistic Pharmacological Interaction between Sirtuin Inhibitor Cambinol and Paclitaxel in Triple-Negative Breast Cancer Cell Lines: An Isobolographic Analysis. *Int. J. Mol. Sci.* **2022**, *23*, 6458. [CrossRef]
224. Hong, J.Y.; Lin, H. Sirtuin Modulators in Cellular and Animal Models of Human Diseases. *Front. Pharmacol.* **2021**, *12*, 735044. [CrossRef] [PubMed]
225. Khalil, N.A.; Ahmed, E.M.; Zaher, A.F.; El-Zoghbi, M.S.; Sobh, E.A. Synthesis of Certain Benzothieno[3,2-d]Pyrimidine Derivatives as a Selective SIRT2 Inhibitors. *Eur. J. Med. Chem.* **2020**, *187*, 111926. [CrossRef]
226. Nikseresht, S.; Khodagholi, F.; Ahmadiani, A. Protective Effects of Ex-527 on Cerebral Ischemia–Reperfusion Injury through Necroptosis Signaling Pathway Attenuation. *J. Cell. Physiol.* **2019**, *234*, 1816–1826. [CrossRef] [PubMed]



227. Kundu, A.; Richa, S.; Dey, P.; Kim, K.S.; Son, J.Y.; Kim, H.R.; Lee, S.-Y.; Lee, B.-H.; Lee, K.Y.; Kacew, S.; et al. Protective Effect of EX-527 against High-Fat Diet-Induced Diabetic Nephropathy in Zucker Rats. *Toxicol. Appl. Pharmacol.* **2020**, *390*, 114899. [CrossRef] [PubMed]
228. Levine, M.H.; Wang, Z.; Xiao, H.; Jiao, J; Wang, L.; Bhatti, T.R.; Hancock, W.W.; Beier, U.H. Targeting Sirtuin-1 Prolongs Murine Renal Allograft Survival and Function. *Kidney Int.* **2016**, *89*, 1016–1026. [CrossRef]
229. Velázquez-Ulloa, N.A.; Heres-Pulido, M.E.; Santos-Cruz, L.F.; Durán-Díaz, A.; Castañeda-Partida, L.; Browning, A.; Carmona-Alvarado, C.; Estrada-Guzmán, J.C.; Ferderer, G.; Garfias, M.; et al. Complex Interactions between Nicotine and Resveratrol in the Drosophila Melanogaster Wing Spot Test. *Heliyon* **2022**, *8*, e09744. [CrossRef]
230. Li, R.; Zhan, W.; Ren, J.; Zhang, F.; Huang, X.; Ma, Y. Temporal Trends in Risk of Bisphenol A, Benzophenone-3 and Triclosan Exposure among U.S. Children and Adolescents Aged 6–19 Years: Findings from the National Health and Nutrition Examination Survey 2005–2016. *Environ. Res.* **2023**, *216*, 114474. [CrossRef]
231. Li, S.; Feng, S.; van Schepdael, A.; Wang, X. Hollow Fiber Membrane-Protected Amino/ Hydroxyl Bifunctional Microporous Organic Network Fiber for Solid-Phase Microextraction of Bisphenols A, F, S, and Triclosan in Breast Milk and Infant Formula. *Food Chem.* **2022**, *390*, 133217. [CrossRef]
232. Beroukhim, G.; Kayani, J.; Taylor, H.S.; Pal, L. Implications of Triclosan for Female Fertility: Results from the National Health and Nutrition Examination Survey, 2013–2016. *F S Rep.* **2022**, *3*, 204–210. [CrossRef]
233. Fernandes, C.A.; Fievez, L.; Neyrinck, A.M.; Delzenne, N.M.; Bureau, F.; Vanbever, R. Sirtuin Inhibition Attenuates the Production of Inflammatory Cytokines in Lipopolysaccharide-Stimulated Macrophages. *Biochem. Biophys. Res. Commun.* **2012**, *420*, 857–861. [CrossRef]
234. Morris, B.J. Sirtuins and Aging. In *Sirtuin Biology in Medicine*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 49–77.
235. Goggins, A.; Matten, G. *The Sirtfood Diet*; Simon and Schuster: New York, NY, USA, 2017; ISBN 1501163779 or 9781501163777.
236. He, Y.; Huang, W.; Zhang, C.; Chen, L.; Xu, R.; Li, N.; Wang, F.; Han, L.; Yang, M.; Zhang, D. Energy Metabolism Disorders and Potential Therapeutic Drugs in Heart Failure. *Acta Pharm. Sin. B* **2021**, *11*, 1098–1116. [CrossRef] [PubMed]
237. Zaganjor, E.; Yoon, H.; Spinelli, J.B.; Nunn, E.R.; Laurent, G.; Keskinidis, P.; Sivaloganathan, S.; Joshi, S.; Notarangelo, G.; Mulei, S.; et al. SIRT4 Is an Early Regulator of Branched-Chain Amino Acid Catabolism That Promotes Adipogenesis. *Cell Rep.* **2021**, *36*, 109345. [CrossRef] [PubMed]
238. Yudoh, K.; Yui, N.; Terauchi, K.; Kobayashi, H.; Kumai, T.; Somemura, S. Sirtuins in Bone and Cartilage Biology. In *Sirtuin Biology in Medicine*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 341–351.
239. Moon, Y.J.; Zhang, Z.; Bang, I.H.; Kwon, O.K.; Yoon, S.-J.; Kim, J.R.; Lee, S.; Bae, E.J.; Park, B.-H. Sirtuin 6 in Preosteoclasts Suppresses Age- and Estrogen Deficiency-Related Bone Loss by Stabilizing Estrogen Receptor α. *Cell Death Differ.* **2019**, *26*, 2358–2370. [CrossRef]
240. Dai, Y.; Lin, J.; Ren, J.; Zhu, B.; Wu, C.; Yu, L. NAD+ Metabolism in Peripheral Neuropathic Pain. *Neurochem. Int.* **2022**, *161*, 105435. [CrossRef]
241. Govindarajulu, M.; Ramesh, S.; Neel, L.; Fabbrini, M.; Buabeid, M.; Fujihashi, A.; Dwyer, D.; Lynd, T.; Shah, K.; Mohanakumar, K.P.; et al. Nutraceutical Based SIRT3 Activators as Therapeutic Targets in Alzheimer's Disease. *Neurochem. Int.* **2021**, *144*, 104958. [CrossRef] [PubMed]
242. Julien, C.; Tremblay, C.; Émond, V.; Lebbadi, M.; Salem, N.; Bennett, D.A.; Calon, F. Sirtuin 1 Reduction Parallels the Accumulation of Tau in Alzheimer Disease. *J. Neuropathol. Exp. Neurol.* **2009**, *68*, 48–58. [CrossRef]
243. Mohamad Nasir, N.F.; Zainuddin, A.; Shamsuddin, S. Emerging Roles of Sirtuin 6 in Alzheimer's Disease. *J. Mol. Neurosci.* **2018**, *64*, 157–161. [CrossRef]
244. Kumar, V.; Kundu, S.; Singh, A.; Singh, S. Understanding the Role of Histone Deacetylase and Their Inhibitors in Neurodegenerative Disorders: Current Targets and Future Perspective. *Curr. Neuropharmacol.* **2022**, *20*, 158–178. [CrossRef]
245. Yu, Z.; Fan, D.; Gui, B.; Shi, L.; Xuan, C.; Shan, L.; Wang, Q.; Shang, Y.; Wang, Y. Neurodegeneration-Associated TDP-43 Interacts with Fragile X Mental Retardation Protein (FMRP)/ Staufen (STAU1) and Regulates SIRT1 Expression in Neuronal Cells. *J. Biol. Chem.* **2012**, *287*, 22560–22572. [CrossRef]
246. Wang, X.; Liu, R.; Zhang, W.; Hyink, D.P.; Das, G.C.; Das, B.; Li, Z.; Wang, A.; Yuan, W.; Klotman, P.E.; et al. Role of SIRT1 in HIV-Associated Kidney Disease. *Am. J. Physiol.-Ren. Physiol.* **2020**, *319*, F335–F344. [CrossRef]
247. Palomer, X.; Aguilar-Recarte, D.; García, R.; Nistal, J.F.; Vázquez-Carrera, M. Sirtuins: To Be or Not To Be in Diabetic Cardiomyopathy. *Trends Mol. Med.* **2021**, *27*, 554–571. [CrossRef] [PubMed]
248. Hu, J.; Pan, L.; Li, Y.; Zou, X.; Liu, B.; Jiang, B.; Zhang, C. Deacetylation-Activated Construction of Single Quantum Dot-Based Nanosensor for Sirtuin 1 Assay. *Talanta* **2021**, *224*, 121918. [CrossRef] [PubMed]
249. Kratz, E.M.; Kokot, I.; Dymicka-Piekarska, V.; Piwowar, A. Sirtuins—The New Important Players in Women's Gynecological Health. *Antioxidants* **2021**, *10*, 84. [CrossRef] [PubMed]
250. Bartosch, C.; Monteiro-Reis, S.; Almeida-Rios, D.; Vieira, R.; Castro, A.; Moutinho, M.; Rodrigues, M.; Graça, I.; Lopes, J.M.; Jerónimo, C. Assessing Sirtuin Expression in Endometrial Carcinoma and Non-Neoplastic Endometrium. *Oncotarget* **2016**, *7*, 1144–1154. [CrossRef] [PubMed]
251. Gao, S.; Yang, L.; Yang, X.; Xiong, Y.; Fu, W.; Li, J.; Yin, S. Sirtuin 7 Is Essential for the Survival and Synthesis of Oestrogen in Yak (Bos Grunniens) Cumulus Granulosa Cells. *Reprod. Domest. Anim.* **2022**, 1–10. [CrossRef]

252. Xu, D.; He, H.; Jiang, X.; Hua, R.; Chen, H.; Yang, L.; Cheng, J.; Duan, J.; Li, Q. SIRT2 Plays a Novel Role on Progesterone, Estradiol and Testosterone Synthesis via PPARs/ LXRα Pathways in Bovine Ovarian Granular Cells. *J. Steroid Biochem. Mol. Biol.* **2019**, *185*, 27–38. [CrossRef]
253. Zhang, Q.; Ren, J.; Wang, F.; Pan, M.; Cui, L.; Li, M.; Qu, F. Mitochondrial and Glucose Metabolic Dysfunctions in Granulosa Cells Induce Impaired Oocytes of Polycystic Ovary Syndrome through Sirtuin 3. *Free Radic. Biol. Med.* **2022**, *187*, 1–16. [CrossRef]
254. Porcu, M.; Chiarugi, A. The Emerging Therapeutic Potential of Sirtuin-Interacting Drugs: From Cell Death to Lifespan Extension. *Trends Pharmacol. Sci.* **2005**, *26*, 94–103. [CrossRef]
255. Fernando, K.K.M.; Wijayasinghe, Y.S. Sirtuins as Potential Therapeutic Targets for Mitigating Neuroinflammation Associated With Alzheimer's Disease. *Front. Cell. Neurosci.* **2021**, *15*, 372. [CrossRef]
256. Mourits, V.P.; Helder, L.S.; Matzaraki, V.; Koeken, V.A.C.M.; Groh, L.; de Bree, L.C.J.; Moorlag, S.J.C.F.M.; van der Heijden, C.D.C.C.; Keating, S.T.; van Puffelen, J.H.; et al. The Role of Sirtuin 1 on the Induction of Trained Immunity. *Cell. Immunol.* **2021**, *366*, 104393. [CrossRef]
257. Hua, R.; Wang, G.-Z.; Shen, Q.-W.; Yang, Y.-P.; Wang, M.; Wu, M.; Shao, Y.-K.; He, M.; Zang, Y.; Yao, Q.-Y.; et al. Sleeve Gastrectomy Ameliorated High-Fat Diet (HFD)-Induced Non-Alcoholic Fatty Liver Disease and Upregulated the Nicotinamide Adenine Dinucleotide +/ Sirtuin-1 Pathway in Mice. *Asian J. Surg.* **2021**, *44*, 213–220. [CrossRef] [PubMed]
258. Du, S.; Shen, S.; Ding, S.; Wang, L. Suppression of MicroRNA-323-3p Restrains Vascular Endothelial Cell Apoptosis via Promoting Sirtuin-1 Expression in Coronary Heart Disease. *Life Sci.* **2021**, *270*, 119065. [CrossRef] [PubMed]
259. Chen, X.; Wei, G.; Li, D.; Fan, Y.; Zeng, Y.; Qian, Z.; Jia, Z.; Tang, Y.; Shi, Y.; Wu, H.; et al. Sirtuin 1 Alleviates Microglia-Induced Inflammation by Modulating the PGC-1α/ Nrf2 Pathway after Traumatic Brain Injury in Male Rats. *Brain Res. Bull.* **2022**, *185*, 28–38. [CrossRef]
260. Chao, C.-C.; Huang, C.-L.; Cheng, J.-J.; Chiou, C.-T.; Lee, I.-J.; Yang, Y.-C.; Hsu, T.-H.; Yei, C.-E.; Lin, P.-Y.; Chen, J.-J.; et al. SRT1720 as an SIRT1 Activator for Alleviating Paraquat-Induced Models of Parkinson's Disease. *Redox Biol.* **2022**, *58*, 102534. [CrossRef]
261. Hao, L.; Park, J.; Jang, H.-Y.; Bae, E.J.; Park, B.-H. Inhibiting Protein Kinase Activity of Pyruvate Kinase M2 by SIRT2 Deacetylase Attenuates Psoriasis. *J. Investig. Dermatol.* **2021**, *141*, 355–363.e6. [CrossRef] [PubMed]
262. Liu, L.; Wang, B.; Yang, W.; Jiang, Q.; Loor, J.J.; Ouyang, L.; Tang, H.; Chang, R.; Peng, T.; Xu, C. Sirtuin 3 Relieves Inflammatory Responses Elicited by Lipopolysaccharide via the PGC1α-NFκB Pathway in Bovine Mammary Epithelial Cells. *J. Dairy Sci.* **2022**. [CrossRef] [PubMed]
263. Zhang, Y.; Lin, C.; Yang, Q.; Wang, Y.; Zhao, W.; Li, L.; Ren, X.; Zhao, J.; Zang, W.; Cao, J. Spinal Sirtuin 3 Contributes to Electroacupuncture Analgesia in Mice with Chronic Constriction Injury–Induced Neuropathic Pain. *Neuromodulation Technol. Neural Interface* **2022**. [CrossRef]
264. Zhang, Q.; Ren, J.; Wang, F.; Li, M.; Pan, M.; Zhang, H.; Qu, F. Chinese Herbal Medicine Alleviates the Pathogenesis of Polycystic Ovary Syndrome by Improving Oxidative Stress and Glucose Metabolism via Mitochondrial Sirtuin 3 Signaling. *Phytomedicine* **2022**, 154556. [CrossRef]
265. Xu, X.; Zhang, L.; Hua, F.; Zhang, C.; Zhang, C.; Mi, X.; Qin, N.; Wang, J.; Zhu, A.; Qin, Z.; et al. FOXM1-Activated SIRT4 Inhibits NF-KB Signaling and NLRP3 Inflammasome to Alleviate Kidney Injury and Podocyte Pyroptosis in Diabetic Nephropathy. *Exp. Cell Res.* **2021**, *408*, 112863. [CrossRef]
266. Zheng, D.; Zeng, Q.; He, D.; He, Y.; Yang, J. SIRT5 Alleviates Hepatic Ischemia and Reperfusion Injury by Diminishing Oxidative Stress and Inflammation via Elevating SOD1 and IDH2 Expression. *Exp. Cell Res.* **2022**, *419*, 113319. [CrossRef]
267. Hu, T.; Shukla, S.K.; Vernucci, E.; He, C.; Wang, D.; King, R.J.; Jha, K.; Siddhanta, K.; Mullen, N.J.; Attri, K.S.; et al. Metabolic Rewiring by Loss of Sirt5 Promotes Kras-Induced Pancreatic Cancer Progression. *Gastroenterology* **2021**, *161*, 1584–1600. [CrossRef] [PubMed]
268. He, T.; Shang, J.; Gao, C.; Guan, X.; Chen, Y.; Zhu, L.; Zhang, L.; Zhang, C.; Zhang, J.; Pang, T. A Novel SIRT6 Activator Ameliorates Neuroinflammation and Ischemic Brain Injury via EZH2/ FOXC1 Axis. *Acta Pharm. Sin. B* **2021**, *11*, 708–726. [CrossRef] [PubMed]
269. Wang, D.; Wei, X.; Chen, X.; Wang, Q.; Zhang, J.; Kalvakolanu, D.V.; Guo, B.; Zhang, L. GRIM-19 Inhibits Proliferation and Induces Apoptosis in a P53-Dependent Manner in Colorectal Cancer Cells through the SIRT7/ PCAF/ MDM2 Axis. *Exp. Cell Res.* **2021**, *407*, 112799. [CrossRef]
270. Yang, K.-E.; Jang, H.-J.; Hwang, I.-H.; Hong, E.M.; Lee, M.-G.; Lee, S.; Jang, I.-S.; Choi, J.-S. Stereoisomer-Specific Ginsenoside 20(S)-Rg3 Reverses Replicative Senescence of Human Diploid Fibroblasts via Akt-MTOR-Sirtuin Signaling. *J. Ginseng Res.* **2020**, *44*, 341–349. [CrossRef] [PubMed]
271. Okada, N.; Oshima, K.; Iwasaki, Y.; Maruko, A.; Matsumura, K.; Iioka, E.; Vu, T.-D.; Fujitsuka, N.; Nishi, A.; Sugiyama, A.; et al. Intron Retention as a New Pre-Symptomatic Marker of Aging and Its Recovery to the Normal State by a Traditional Japanese Multi-Herbal Medicine. *Gene* **2021**, *794*, 145752. [CrossRef] [PubMed]
272. Morris, B.J.; Willcox, B.J.; Donlon, T.A. Genetic and Epigenetic Regulation of Human Aging and Longevity. *Biochim. Biophys. Acta (BBA)—Mol. Basis Dis.* **2019**, *1865*, 1718–1744. [CrossRef] [PubMed]
273. Wang, H.; Diao, D.; Shi, Z.; Zhu, X.; Gao, Y.; Gao, S.; Liu, X.; Wu, Y.; Rudolph, K.L.; Liu, G.; et al. SIRT6 Controls Hematopoietic Stem Cell Homeostasis through Epigenetic Regulation of Wnt Signaling. *Cell Stem Cell* **2016**, *18*, 495–507. [CrossRef]
274. Hadar, A.; Milanesi, E.; Walczak, M.; Puzianowska-Kuźnicka, M.; Kuźnicki, J.; Squassina, A.; Niola, P.; Chillotti, C.; Attems, J.; Gozes, I.; et al. SIRT1, MiR-132 and MiR-212 Link Human Longevity to Alzheimer's Disease. *Sci. Rep.* **2018**, *8*, 8465. [CrossRef]



275. Donlon, T.A.; Morris, B.J.; Chen, R.; Masaki, K.H.; Allsopp, R.C.; Willcox, D.C.; Tiirikainen, M.; Willcox, B.J. Analysis of Polymorphisms in 59 Potential Candidate Genes for Association With Human Longevity. *J. Gerontol. Ser. A* **2018**, *73*, 1459–1464. [CrossRef]
276. Giblin, W.; Lombard, D.B. Sirtuins, Healthspan, and Longevity in Mammals. In *Handbook of the Biology of Aging*; Elsevier: Amsterdam, The Netherlands, 2016; pp. 83–132.
277. Lasigliè, D. Sirtuins and the Prevention of Immunosenescence. In *Vitamins and Hormones*; Academic Press: Cambridge, MA, USA, 2021; pp. 221–264.
278. Apparao, Y.; Phan, C.W.; Kuppusamy, U.R.; Sabaratnam, V. Ergothioneine and Its Prospects as an Anti-Ageing Compound. *Exp. Gerontol.* **2022**, 111982. [CrossRef]
279. D'Onofrio, N.; Servillo, L.; Giovane, A.; Casale, R.; Vitiello, M.; Marfella, R.; Paolisso, G.; Balestrieri, M.L. Ergothioneine Oxidation in the Protection against High-Glucose Induced Endothelial Senescence: Involvement of SIRT1 and SIRT6. *Free Radic. Biol. Med.* **2016**, *96*, 211–222. [CrossRef] [PubMed]
280. Palmer, R.D.; Vaccarezza, M. Nicotinamide Adenine Dinucleotide and the Sirtuins Caution: Pro-cancer Functions. *Aging Med.* **2021**, *4*, 337–344. [CrossRef] [PubMed]
281. Liberale, L.; Akhmedov, A.; Vlachogiannis, N.I.; Bonetti, N.; Nageswaran, V.; Miranda, M.X.; Montecucco, F.; Beer, J.; Lüscher, T.F.; Stamatelopoulos, K.; et al. Sirtuin 5 Promotes Arterial Thrombosis through Endothelial Plasminogen Activator Inhibitor-1. *Atherosclerosis* **2020**, *315*, e78–e79. [CrossRef]
282. Kakefuda, K.; Fujita, Y.; Oyagi, A.; Hyakkoku, K.; Kojima, T.; Umemura, K.; Tsuruma, K.; Shimazawa, M.; Ito, M.; Nozawa, Y.; et al. Sirtuin 1 Overexpression Mice Show a Reference Memory Deficit, but Not Neuroprotection. *Biochem. Biophys. Res. Commun.* **2009**, *387*, 784–788. [CrossRef] [PubMed]
283. Teasley, H.E.; Beesley, A.; Kim, T.H.; Risinger, J.; Young, S.L.; Jeong, J.-W.; Schammel, D.P.; Lessey, B.A.; Elder, J.W.; Puls, L. Differential Expression of KRAS and SIRT1 in Ovarian Cancers with and Without Endometriosis. *Reprod. Sci.* **2020**, *27*, 145–151. [CrossRef]
284. He, S.; Jia, Q.; Zhou, L.; Wang, Z.; Li, M. SIRT5 Is Involved in the Proliferation and Metastasis of Breast Cancer by Promoting Aerobic Glycolysis. *Pathol. Res. Pract.* **2022**, *235*, 153943. [CrossRef]
285. Pande, S.; Raisuddin, S. The Underexplored Dimensions of Nutritional Hormesis. *Curr. Nutr. Rep.* **2022**, *11*, 386–394. [CrossRef]
286. Visioli, F.; Ingram, A.; Beckman, J.S.; Magnusson, K.R.; Hagen, T.M. Strategies to Protect against Age-Related Mitochondrial Decay: Do Natural Products and Their Derivatives Help? *Free Radic. Biol. Med.* **2022**, *178*, 330–346. [CrossRef]
287. Gaul, D.S.; Calatayud, N.; Pahla, J.; Bonetti, N.R.; Wang, Y.-J.; Weber, J.; Ambrosini, S.; Liberale, L.; Costantino, S.; Mohammed, S.A.; et al. Endothelial SIRT6 Deficiency Promotes Arterial Thrombosis in Mice. *J. Mol. Cell. Cardiol.* **2023**, *174*, 56–62. [CrossRef]

OŚWIADCZENIA WSPÓŁAUTORÓW

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Oświadczam, że mój wkład w przygotowanie publikacji:

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stanowiącego część mojej rozprawy doktorskiej obejmował opracowanie koncepcji pracy, przeprowadzenie analizy literatury, udział w interpretacji danych, przygotowanie wizualizacji, opracowanie pierwotnej wersji manuskryptu oraz jego redakcję i korektę przed publikacją, jak również współudział w korespondencji z redakcją i recenzentami.

Patrycja Ziętara-Krzyk
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Prof. dr hab. Maria Augustyniak

Katowice, 27.04.2026

Dr Marta Dziewięcka

OŚWIADCZENIE

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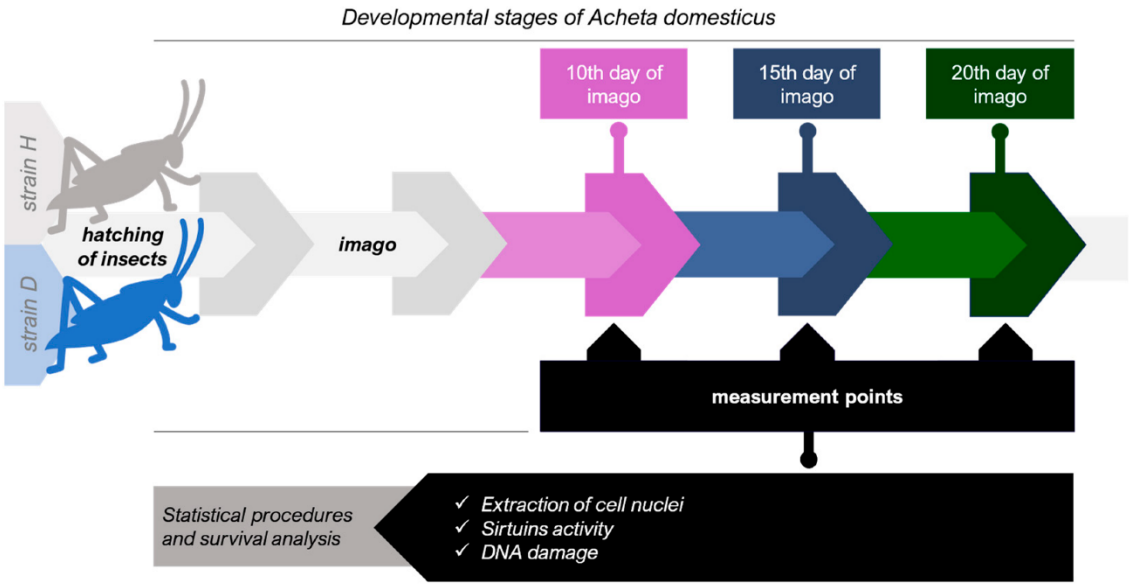
Prof. dr hab. Maria Augustyniak

Manuskrypt II: Does Selection for Longevity in *Acheta domesticus* Involve Sirtuin Activity Modulation and Differential Response to Activators (Resveratrol and Nanodiamonds)?

Authors: Patrycja Ziętara, Barbara Flasz, Maria Augustyniak

Graphical abstract: Experimental design



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Does Selection for Longevity in *Acheta domesticus* Involve Sirtuin Activity Modulation and Differential Response to Activators (Resveratrol and Nanodiamonds)?

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Abstract: Sirtuins, often called "longevity enzymes", are pivotal in genome protection and DNA repair processes, offering insights into aging and longevity. This study delves into the potential impact of resveratrol (RV) and nanodiamonds (NDs) on sirtuin activity, focusing on two strains of house crickets (*Acheta domesticus*): the wild-type and long-lived strains. The general sirtuin activity was measured using colorimetric assays, while fluorescence assays assessed SIRT1 activity. Additionally, a DNA damage test and a Kaplan–Meier survival analysis were carried out. Experimental groups were fed diets containing either NDs or RV. Notably, the long-lived strain exhibited significantly higher sirtuin activity compared to the wild-type strain. Interestingly, this heightened sirtuin activity persisted even after exposure to RVs and NDs. These findings indicate that RV and NDs can potentially enhance sirtuin activity in house crickets, with a notable impact on the long-lived strain. This research sheds light on the intriguing potential of RV and NDs as sirtuin activators in house crickets. It might be a milestone for future investigations into sirtuin activity and its potential implications for longevity within the same species, laying the groundwork for broader applications in aging and lifespan extension research.

Keywords: sirtuins; longevity; resveratrol; nanodiamonds; *Acheta domesticus*

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1. Introduction

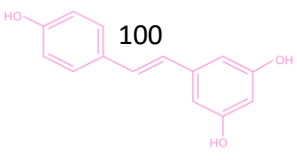
Aging represents a multifaceted phenomenon discussed at various levels—populations, individuals, organs, cells, and molecules. This intricate process arises from external environmental factors and internal genetic influences [1]. Numerous theories highlight pivotal elements that expedite the aging process, including factors such as UV radiation, cellular oxidative stress, genetic mutations, underlying diseases, and even deficiencies in essential nutrients [2,3]. The subjects of anti-aging and longevity have perpetually intrigued the scientific community, spanning several decades of dedicated research. The extensive body of literature encounters numerous reports detailing novel particles, pharmaceutical compounds, bioactive substances, and even specific genes that offer valuable insights into the intricate mechanisms underlying lifespan extension. However, it is imperative to recognize that this field remains relatively underexplored and is the subject of ongoing research discourse. Consequently, it becomes imperative to search for general relationships and factors that hold the potential to unravel the complex puzzle of longevity, further advancing our comprehension of this intriguing phenomenon.

Considering recent studies, sirtuins (SIRT) emerge as significant players and can be regarded as a pivotal milestone in investigating aging biology. These signaling proteins constitute a noteworthy group closely linked with metabolic disorders. Thanks to their unique capacity for downregulating aging-associated genes, they are often perceived as "longevity enzymes" [4–6]. However, due to the complicated mechanism, the enzymatic functions of sirtuins have not yet been fully defined [7,8], but as far as we know, they

are also involved in regulating the level of reactive oxygen species (ROS) by modulating the expression of antioxidant and detoxification enzymes [9,10]. Sirtuins are categorized from SIRT1 to SIRT7, and SIRT1 is currently (since 2023) recognized as the most extensively described, well-known, and thoroughly studied member of the sirtuin family. It plays a significant role in both developing and preventing neurological disorders. SIRT1 activates the AMPK pathway, the mTOR pathway, and the PPAR-γ pathway, which affects the secretion of adipokines and steroid hormones and modulates glucose and lipid metabolism [11–14]. Contemporary research has unveiled three primary sites of sirtuin localization: the cytoplasm, nucleus, and mitochondria. These distinct cellular compartments serve as key arenas where sirtuins exert diverse biological functions [4,15,16]. Sirtuins seem to be relatively widely described, but the complexity of the subject requires going beyond the framework and tackling many research issues. Information on sirtuin activity in cricket (a model species of incomplete metamorphosis) is so far unavailable, and current studies on invertebrates are limited to a few species like *Drosophila melanogaster* (predominantly), *Caenorhabditis elegans*, and *Ruditapes philippinarum* [4,17–20].

Numerous compounds have also been identified as potential activators of SIRT, but studying their mechanisms of action and discovering or modifying new activators presents challenges. Notably, these activators must be finely tailored to specific sirtuin–substrate pairings, introducing complexity to the research endeavors. Furthermore, selecting activator substances for sirtuins that adhere to stringent criteria, including safety, wide availability, non-toxicity, and high bioavailability, is imperative [4]. Research findings have proved that resveratrol (RV; 3, 5, 4′-trihydroxystilbene) acts as a sirtuin activator with specificity toward substrates. Moreover, RV can potentially influence an individual's survival time [21–23]. In the appropriate concentration, it can extend the life span of *Saccharomyces cerevisiae* by up to 70% [24]. The retention time of RV in the cells depends on various factors, including dose, method of administration, metabolism, and enzyme activity in an organism. A study by Walle et al. [25] reported a half-life of RV in humans of approximately 9 h following a single oral dose of 25 mg RV, with an absorption capacity of 70%. After oral administration, Iannitti et al. [26] indicated that its plasma half-life in the human body ranges from 2 to 4 h. Regarding the specific formulation studied, Resv@MHD contains about 30% RV and 70% magnesium dihydroxide. This composition creates two types of microparticles: one of pure RV and the other of a complex with magnesium. This formulation demonstrates a different plasma profile compared to pure RV, including an earlier peak plasma concentration (25 min) and greater bioavailability. Additionally, a reported half-life of 80 min was observed with a dosage of 180 mg of pure RV [25,26].

Nanoparticles have become the subject of many studies, and scientists are looking for new ways to apply them, e.g., in medicine, biology, physics, or engineering [27]. Nanodiamonds (NDs) have garnered significant attention in diverse biomedical applications owing to their exceptional properties, including their diminutive size, expansive surface area, and compatibility with biological systems. These remarkable nanomaterials have undergone extensive investigation for purposes including drug delivery, tissue engineering, and bioimaging, among numerous other potential applications within biomedicine. Additionally, they have been shown to have low toxicity, making them a potential alternative to other nanomaterials that may have harmful effects. Overall, using NDs in biomedicine is a rapidly growing field with exciting potential for future advancements [28–30]. Some nanoparticles have demonstrated the capacity to augment the stability and bioavailability of diverse therapeutic agents, thereby facilitating precision-targeted and controlled drug delivery. Additionally, nanoparticle utilization holds the potential to diminish the requisite drug dosage, mitigating potential adverse effects and enhancing patient adherence to treatment regimens. While further investigations are imperative to comprehensively assess the safety and efficacy of nanoparticles in sirtuin modulation, they represent a promising avenue for developing innovative therapies targeting aging and age-related ailments and diseases. Nonetheless, it is noteworthy that nanoparticles may exhibit a limited cellular



residence period, potentially limiting their effective interaction and complete realization of beneficial effects.

The main goal of this study was to characterize sirtuin proteins in the model insect species *Acheta domesticus* within two distinct strains, the wild-type (H) and the unique long-lived (D), and subsequently assess the impact of RV and NDs on their activity, considering selected physiological parameters, including those associated with lifespan extension. The following hypotheses were tested:

H 1. *The SIRT selected for analysis can significantly differ between individuals from the wild strain (H) and the long-lived strain (D). It is more likely that individuals from the D strain exhibit higher activity in specific SIRT classes (all or part of them). However, the reverse relationship is also possible.*

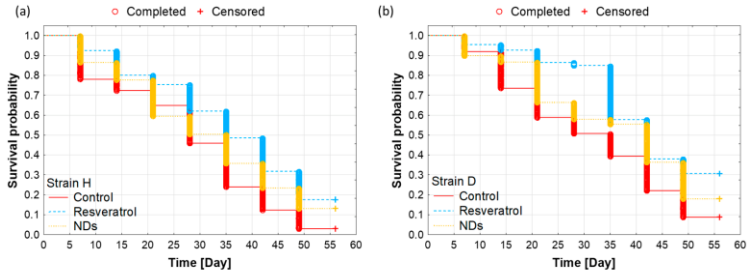
H 2. *There is a correlation between sirtuin activity, RV, or ND exposure and the lifespan of A. domesticus. Possible scenarios must involve not only the prolongation but also the shortening of the lifespan of individuals. RV may result in more noticeable effects for the D strain.*

H 3. *There is a correlation between cell DNA damage and exposure to additional factors (NDs or RV). Such an effect may occur independently of changes in sirtuin activity. However, a positive correlation between disrupted sirtuin activity, impaired lifespan, and increased cellular damage/adverse effects is more likely.*

2. Results

2.1. Survival Analysis

Survival probabilities (Kaplan–Meier method) allowed for the illustration of the difference in survival between the studied groups (H and D strains). Individuals consuming food with RV exhibited the highest survival probability in both strains compared to the control and NDs groups. However, during a specific observation period, the survival rate in the NDs group in both strains was also higher than that in the control group (Figure 1a,b).



(a')

Log-rank test	
Groups (strain H)	p value
Control & Resveratrol	<0.00001
Control & NDs	p = 0.00118
Resveratrol & NDs	p = 0.00600

(b')

Log-rank test	
Groups (strain D)	p value
Control & Resveratrol	<0.00001
Control & NDs	p = 0.00007
Resveratrol & NDs	p = 0.00115

(c')

Log-rank test	
Groups (strain H & D)	p value
Control H & D	p = 0.00238
Resveratrol H & D	p = 0.00011
NDs H & D	p = 0.00115

Figure 1. Kaplan–Meier survival analysis: (**a**) for the wild-type strain (H) over 56 days; (**b**) for the long-lived strain (D) over 56 days. (**a'**,**b'**) Log-rank test results comparing survival distributions within the respective strain. Experimental groups: control-free food; resveratrol group (23 mg/ kg in food); and nanodiamonds group (0.2 mg/ kg in food). (**c'**) Comparison of the same study groups between strains H and D (STATISTICA® 13, TIBCO Software Inc., Palo Alto, CA, USA).

The Log-rank test was conducted to assess the significance of survival differences. Similar patterns were observed in both strains after the application of RV or NDs, leading to a significant increase in the survival probability of exposed crickets compared to the

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control group. Importantly, RV exhibited a more pronounced effect than NDs (Figure 1a',b'). Additionally, when comparing groups from both strains, a significantly higher survival probability was observed in long-lived insects than in wild-type ones (Figure 1c').

2.2. Sirtuin Activity in *Acheta domesticus*

The Brown–Forsythe test indicated homogeneity of variance in the groups (Figure 2a). Therefore, the results were analyzed using ANOVA. Results of the Factorial ANOVA (Figure 2b) indicate a significant main effect of all factors ('Strain', 'Group', and 'Time') as well as most of the interactions among them regarding the total sirtuin activity in the house cricket. The nonsignificant 'Strain' × 'Group' and 'Group' × 'Time' interactions indicate a similar pattern of SIRT activity in both strains after RV or ND treatment, which is similar over time.

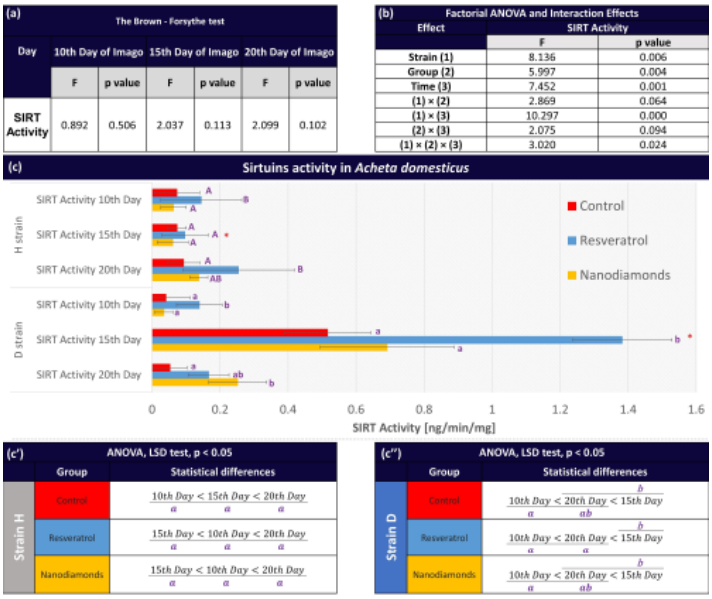

Figure 2. (**a**) Results of the Brown–Forsythe test for SIRT activity within days. (**b**) Results of a factorial ANOVA assessing the effects of strain, group, time, and their interactions on SIRT in *Acheta domesticus*. (**c**) The activity of sirtuins was expressed as mean ± SD (ANOVA, $p < 0.05$). Capital letters denote homogenous groups within the H strain and the same measurement day. Lower-case letters denote homogenous groups within the D strain and the same measurement day; "*" Differences between strains (H and D). (**c'**,**c''**) Within-group comparisons of SIRT activity across different measurement days (10th, 15th, and 20th of the imago stage) for H (**c'**) and D (**c''**) *A. domesticus*. Statistical differences between the days within each group are denoted by distinct lower-case letters. Groups that share the same letter are not significantly different (ANOVA, LSD test, $p < 0.05$).

On the sirtuins activity of *Acheta domesticus* in the H strain (Figure 2), it was observed that the control group maintained similar sirtuin activity values at all examined time points. RV stimulated SIRT activity in strain H, while NDs did not show this property. RV SIRT stimulation was most robust on day 20.

The results regarding sirtuin activity in the D strain (Figure 2) appear to correlate with the observed higher survival rates in this strain. Notably, the highest sirtuin activity values

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are achieved on the 15th day of observation in each tested group (control, RV, and NDs) compared to the 10th and 20th days. Interestingly, on day 20, stronger and more significant stimulation compared to the control was caused by NDs and not RV. Sirtuin activity on the 15th day remains higher than on the 10th and 20th days within treated groups (Figure 2c"), and the stimulation caused by RV was significantly higher than in the H strain at the same time point.

The results for the RV- and ND-treated groups were compared with the control group (depicted by the red line) in two strains (H and D). These measurements were taken over time. The presented data represent an averaged sample (Figure 3). Differences in the dynamics of the reaction were observed, dependent on the strain and the time and type of applied factor. These observations were confirmed by examining the interaction effects in a generalized linear model (GLM) (Figure 3a), indicating that time, strain, and group are substantial factors influencing the observed results.

(a) Interaction effects in GLMs

Effect	10th Day of Imago		15th Day od Imago		20th Day of Imago	
	F	p value	F	p value	F	p value
Time	55.193	0.000000	11.6655	0.000747	16.083	0.000081
Strain	849.620	0.000000	347.0845	0.000000	355.299	0.000000
Group	879.454	0.000000	128.6500	0.000000	557.684	0.000000

Figure 3. (**a**) Interaction effects in GLMs. (**b1**–**b3**) Mean percent of SIRT 1 fluorescence in *Acheta domesticus* over 60 min within time points of measurements (10th, 15th, and 20th day of imago stage, respectively) in H strain. (**c1**–**c3**) Mean percent of SIRT 1 fluorescence in *A. domesticus* over 60 min within time points of measurements (10th, 15th, and 20th day of imago stage, respectively) in D strain. (**b**,**c**) Control was presented as 100% (red line), and the results for the RV or ND-treated groups were recalculated accordingly.

Figure 3(b1–c3) shows that in strain H, the RV group on the 10th day exhibits a variable response of SIRT1, which is weaker than in the D strain. For the NDs group (10th day), the initial course of the reaction is similar in both strains; however, NDs exhibit slight stimulating effects from around 30 min after the start of the reaction. On the 15th day, we observe that the reaction to RV is similar in both strains (H and D), while NDs in strain H seem to show a greater effect than in strain D. On day twenty in the H strain, the response to RV and ND has the same dynamics, presenting an initial intense stimulation that declines

over time. In the D group, the changes were not as spectacular as in the H strain, although RV stimulation is still evident.

When analyzing results concerning the interaction effect for sirtuin 1, a graphical interpretation of expected marginal means was carried out in the tested groups, focusing on the H strain (Figure 4(a1–a3)) and the D strain (Figure 4(b1–b3)).

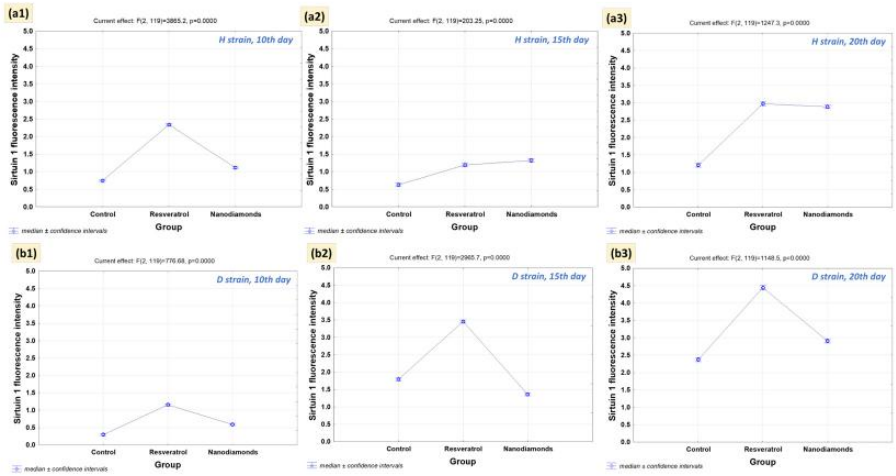

Figure 4. Graphical interpretation of the interaction effect for sirtuin 1. Expected marginal means in tested groups at three time points (10th, 15th, and 20th days of the imago stage). (**a1**–**a3**) H strain and (**b1**–**b3**) D strain. Vertical bars represent 0.95 confidence intervals (GLM, STATISTICA®13, TIBCO Software Inc., Palo Alto, CA, USA).

A peak was observed in the H strain on the 10th day of the experiment (Figure 4(a1)), indicating a difference between the experimental groups. The control group exhibited a lower response than those treated with RV and NDs. On the 15th day (Figure 4(a2)), the marginal means for the RV and NDs groups began to converge, and by the 20th day, values for these groups increased, while the control group remained relatively stable.

A significant peak was also observed in the D strain on the 10th day (Figure 4(b1)), indicating a difference between the groups, like the H strain. The RV group achieved the highest marginal mean. On the 15th day (Figure 4(b2)), differences between groups (C, RV, and NDs) became more pronounced, especially for the RV group, which showed a significant increase in the marginal mean compared to the control group. On the 20th day, the marginal means for the control and RV groups indicated an increase compared to the levels observed on the 10th and 15th days.

To provide additional information regarding SIRT1 fluorescence, data were visualized using Relative Fluorescence Units (RFUs) (Figure 5). In the H strain, the control on the 10th day achieves a lower Relative Fluorescence Unit (RFU) than the control on the 10th day in the D strain, suggesting differences in the reaction dynamics between the strains. On the 10th day, in the RV and NDs groups in the H strain, initiation of the fast reaction was earlier than in control, with higher RFU values observed for the RV group. In the RV groups on the 15th and 20th days, the reaction onset appeared to be delayed compared to the 10th day, but NDs demonstrated a similar temporal pattern in these instances.

In contrast to these observations, in the D strain, the reaction started most rapidly in the RV-treated group, while in the ND-treated group, the dynamics of changes seemed to be similar to the control group.

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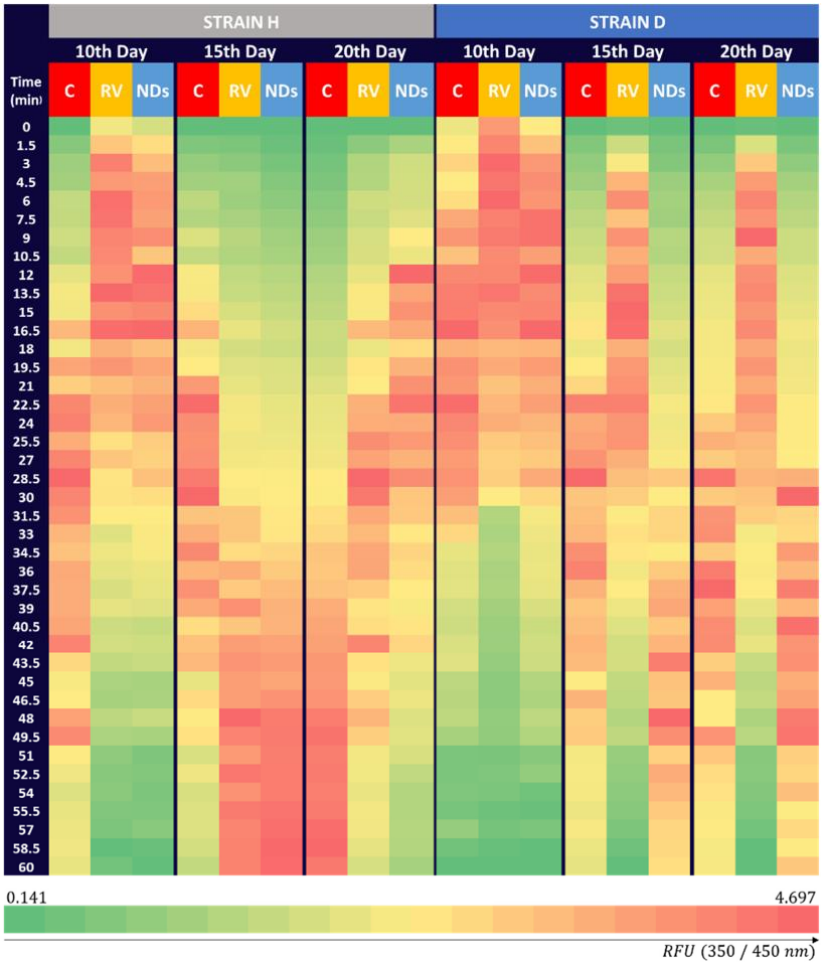

Figure 5. Mean RFU (Ex = 350 nm, Em = 450 nm) SIRT 1 fluorescence in *Acheta domesticus* over 60 min in two strains (H and D) and groups tested (control—C; resveratrol—RV; nanodiamond—NDs) at three time points (10th, 15th, and 20th day of imago stage).

2.3. DNA Damage

The results of the Brown–Forsythe test (Figure 6a) indicate no statistically significant differences in variances among groups for pATM, DSB, and pH2A.X on various days of the life cycle. The *p*-values for each parameter exceed the standard significance level of 0.05 on all three days (10th day, 15th day, and 20th day), suggesting homogeneity of variances across the studied groups.

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(a) The Brown - Forsythe test

Day	10th Day of Imago		15th Day of Imago		20th Day of Imago	
	F	p value	F	p value	F	p value
pATM	0.575	0.718	1.629	0.192	2.008	0.115
DSB	0.460	0.802	1.537	0.216	0.756	0.590
pH2A.X	0.455	0.805	2.416	0.070	1.791	0.153

(b) Factorial ANOVA and Interaction Effects

Effect	pATM		DSB		pH2A.X	
	F	p value	F	p value	F	p value
Strain (1)	2.430	0.124	0.777	0.381	0.113	0.738
Group (2)	0.006	0.995	13.241	0.000	4.193	0.019
Time (3)	0.771	0.466	95.556	0.000	2.718	0.073
(1) × (2)	0.867	0.425	12.976	0.000	5.183	0.008
(1) × (3)	3.202	0.047	0.061	0.941	6.481	0.003
(2) × (3)	1.956	0.111	5.977	0.000	0.567	0.687
(1) × (2) × (3)	2.066	0.094	5.283	0.001	1.612	0.181

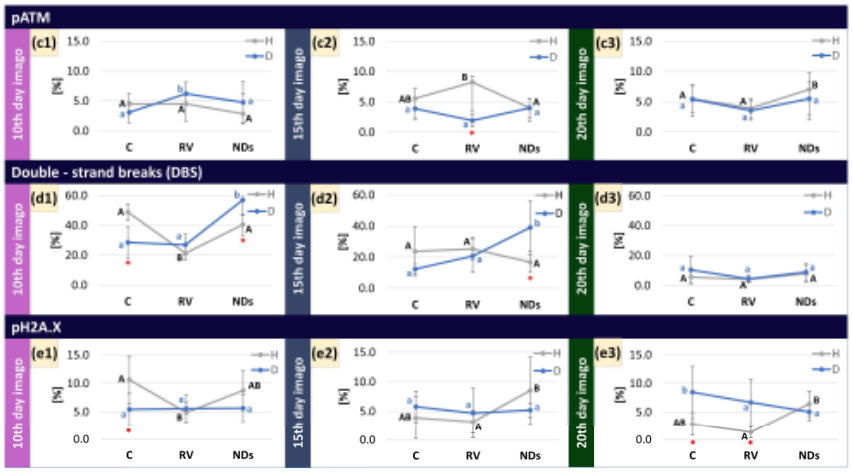

Figure 6. (**a**) Results of the Brown–Forsythe test. (**b**) Results of a factorial ANOVA assessing the effects of strain, group, and time, and their interactions, on SIRT in *Acheta domesticus*. The activity of sirtuins is expressed as mean ± SD (ANOVA, $p < 0.05$). (**c1**–**c3**) ATM activated cells (pATM) measured on 10th, 15th, and 20th time points, respectively; (**d1**–**d3**) double-strand breaks (DSBs) measured on 10th, 15th, and 20th time points, respectively; and (**e1**–**e3**) histone H2A.X-activated cells (pH2A.X) measured by Muse® Multi-Color DNA Damage on 10th, 15th, and 20th time points, respectively. (**c**–**e**) Mean ± SD are shown in the charts. The same capital letters denote homogenous groups within the H strain; the same lower-case letters denote homogenous groups within the D strain; "*" means differences between groups within different strains (ANOVA, LSD test, $p < 0.05$).

The main effect of 'Strain' was not significant for the parameters pATM, DSB, and pH2A.X, indicating a similar range of values for these parameters in both strains of crickets. The 'Group' main effect was significant for DSB and pH2A.X, while the 'Time' main effect was significant only for DSB (Figure 6b). The interaction effects of 'Strain' × 'Time' differed significantly for a percentage of ATM-activated cells. For DSB, only the interaction of 'Strain' × 'Time' does not significantly impact. The interaction between 'Strain' × 'Group' and 'Strain' × 'Time' significantly affects pH2A.X.

The percentage of cells with activated ATM kinase (Figure 6) in the control groups did not differ between strains at any tested time point. RV caused a significant increase in pATM only in insects from the D strain on day 10 of adult life. However, when comparing the response to RV in both strains, a significantly higher level of pATM was observed in insects from the H strain compared to the D strain, but only on day 15 of adult life. Moreover, NDs increased the pATM level, but only in insects from the H strain on day 20 of adult life.

In the control group of strain H, the percentage of double-strand breaks (DSBs) (Figure 6) exhibited a decreasing trend over time at the examined points, mirroring similar observations in strain D insects. Additionally, on the 10th day of RV administration, a reduction in the value of DSB was noted. In the group consuming food with NDs, where initially the DSB levels were close to 40% for H and D strains on the 20th day, a decrease in these values occurred, reaching approximately 15% for both strains.

The percentage of H2A.X-activated cells (Figure 6) in the control group of strain H exhibited a twofold higher result compared to the control group of strain D on the 10th day. The administration of RV in the group consuming this compound significantly reduced the percentage of H2A.X-activated cells in strain H, similar to double-strand breaks (DSBs) (Strain H, 10th day). Moreover, the pH2A.X levels in the group with NDs in strain H showed only a slight decrease on the 20th day.

3. Discussion

3.1. Is the Life History of A. domesticus Significant for Sirtuin Activity?

Longevity, which is still a subject of open discussion, requires ongoing findings in interdisciplinary research as it represents a stage in the quest to identify common traits among different organisms and (typically) attempt to extrapolate these findings to humans [1–3]. Therefore, investigating the same species, even with distinctions such as different populations or, as in the case of crickets, various strains, emerges as a crucial aspect in unraveling the complexities of sirtuin activity. Commencing with survival analysis, a critical area of study in biology, we explore the factors influencing organism longevity. This investigation delves into survival data, unveiling the mechanisms that impact extended lifespan as our long-lived crickets indeed live longer and respond differently to SIRT stimulators.

The long-lived selected strain, as opposed to the H- strain, resulted from systematic efforts to obtain organisms with an extended lifespan, which has significant implications for understanding the impact of RV and NDs on survival. It is important to note that the decision to use this strain was non-random; rather, it was based on prior research and experiments carried out by our team [29,31]. The results of our study indicate that the selection for longevity is associated with a distinct pattern of SIRT activity in both strains. The main 'Strain' effect was significant for SIRT activity, which was higher in the long-lived strain (Figure 2b). Thus, the life history of this strain, including selection with the delay in reproduction, likely induced profound changes that support longevity at the level of regulatory proteins. Additional analysis of SIRT1 activity concerning its substrates, such as histones and transcription factors (e.g., p53, NF-κB, and FOXO), may help elucidate the observed changes [4,32,33]. Based on the information provided by Xuan et al. (2017), the fluorescence studies involving SIRT1 with the EGFP-K85AcK probe illuminate its role in enhancing fluorescence in *E. coli* ΔcobB, particularly in response to the acetylated lysine modification (HibK) at position K85 [34]. This would also indicate the involvement of SIRT1 in regulatory pathways in *Acheta domesticus*.

The observation that two distinct strains of *Acheta domesticus* (H and D) exhibit differences in survival underscores evolutionary adaptations in lifespan within a single species. Similar observations are also apparent in human populations' lifespans. This indicates that specific genetic, environmental, or other factors in a population may impact organism longevity. In 2011, scientists identified long-lived populations, with the Nepalese being highlighted as one such group [35]. In recent years, it has been pointed out that the mortality rate for this country has decreased and the average lifespan in society has increased [36].

The research also points towards the polymorphism of SIRT3, which is more prevalent in long-lived populations [23].

Moreover, the differences in responses to RV or NDs among strains (H or D) underscore the complexity of genotype and environment interactions on the impact of bioactive substances or other factors in organisms, such as environment or even health status, as in humans [37]. This is particularly relevant given that May and Tomanek (2024) indicated in their research that environmental conditions in *Mytilus californianus* were one of the factors influencing total sirtuin activity [38]. The increase in sirtuin activity in response to NDs may suggest their potential beneficial impact on the regulatory ability of sirtuins, especially given that NDs are considered highly biocompatible [30]. However, observations suggest that RV may also induce different effects depending on the strain of *A. domesticus*.

The results regarding the total sirtuin activity prompted us to conduct a further analysis, including the study of SIRT1 fluorescence (Figure 3). The diverse reactions observed in different strains suggest different distinct dynamics in the stimulation of SIRT1. This variability may be attributed to factors such as age, specific stages of analysis, and the unique characteristics of each cricket strain, highlighting the intricate nature of RV or NDs' impact on SIRT1 activity at the cellular level. In our previous studies, we observed variable responses in crickets at the level of oxidative stress, apoptosis, and DNA damage after exposure to graphene oxide [39]. Similarly, as in 2021, we indicated that prolonged exposure to NDs and differences between strains become noticeable after multigenerational exposure, whereas in the D strain, mobilization of the organism and activation of defense mechanisms were observed. This could be one of the factors influencing diverse cellular responses [29].

The decrease in double-strand breaks (DSBs) over time, especially in the D strain across all groups, suggests a potential reparative effect or reduced induction of DNA damage. Considering that DSBs are known to be cytotoxic, the observed reduction in their occurrence due to the tested substances implies potential therapeutic effects. However, the unexpected increase in DSBs after RV treatment in the H strain on day 15 indicates a dual role of RV in modulating DNA damage, possibly influenced by strain-dependent factors [40].

3.2. Is There a Correlation between the Age of Acheta domesticus and Sirtuin Activity?

The increase in SIRT enzyme activity does not always correlate with lifespan, although the Kaplan–Meier analysis demonstrates an association between increased activity of these enzymes and extended life. While heightened enzyme activity may contribute to favorable effects, excessive elevation could have adverse consequences [4,41]. In the context of our obtained results, the cellular parameters appear consistent because these suggest that the treatments have a time-dependent effect on the strains, with the NDs treatment showing a particularly dynamic response in the D strain. The differences between days could be due to the progression of the treatment effects or the adaptation of the strains to the treatments. An intriguing aspect is that on the 15th and 20th days in the D strain, RV appears to have a stimulating effect on SIRT1 activity, significantly expediting the initiation of the reaction. That is why the D strain appears to be more reactive to the treatments, compared to the H strain for strain D, and the 15th day appears to be the optimal window of sensitivity to stimulators in the form of RV and NDs.

However, the impact of RV and NDs on SIRT1 activity may not be only contingent upon the reaction initiation time (Figure 5) but also on specific regulatory mechanisms, varying between the analyzed strains (H or D) and, as previously mentioned, measurement days. The actions of these substances can exhibit diversity and dynamics, leading to disparate effects at different stages of the experiment. As the organism ages, metabolic changes may impact sirtuin activity [23]. Given their involvement in DNA damage repair, the quantity and types of damage in genetic material may increase with aging, necessitating greater sirtuin activity in repair processes. These assumptions appear to be supported by

Peng et al. (2015), which indicated a direct association between SIRT1 and DNA damage repair [42].

In our research, the observed fluctuations in pATM activity in the H strain, especially the significant increase on day 15 after RV treatment, suggest a dynamic response to the substance over time. This contrasts with the D strain, where the highest pATM activity was recorded in the RV group on day 10, indicating potential strain-dependent differences in the temporal dynamics of the response to DNA damage. This phenomenon indicates that the ATM kinase plays a crucial role in the early stages of the cellular response to double-strand breaks (DSBs) in DNA. This kinase performs a key function by phosphorylating the H2AX protein, a significant step in the cellular response to DNA damage. ATM is a major regulator of DNA repair mechanisms, particularly in situations involving severe DNA damage, such as double-strand breaks. This phenomenon is critical to understanding how cells respond to such damage and initiate repair processes [40].

The D strain increases H2A.X activity in the control group, indicating potential accumulation of DNA damage with the insect's age. Notably, the ND group in the D strain maintains relatively stable H2A.X activity, aligning with the RV group on days 10 and 15. This consistency maintenance raises questions about the potential protective role of NDs against age-related DNA damage in the D strain. However, in our recent studies, we suggested that NDs could stabilize DNA by interacting with chromatin, forming a compact structure, and potentially influencing chromatin-stabilizing proteins. This may incidentally limit the amount of DNA damage [43].

In addition, it covered a selected period of life (10, 15, and 20 days of imago), which may not be crucial for sirtuins in *A. domesticus*. For this reason, it is also necessary to check the activity in other stages.

3.3. Does the Resveratrol and Nanodiamonds Influence Sirtuin Activity?

Discussions regarding the safety of using NDs in various scientific fields (e.g., biology, medicine, genetics, and chemistry) are ongoing [44,45]. The direct correlation has no clear-cut pieces of evidence or studies suggesting that NDs possess such an ability. Nevertheless, research into the use of NDs as drug carriers or other substances for therapeutic purposes is underway. Such applications may aid in treating various ailments and improving health, potentially influencing the lifespan of individuals affected by specific diseases [46–48]. RV is known for its potential health benefits that may influence longevity [4]. It can be perceived as a general activator of sirtuins, influencing the regulation of metabolic processes and the organism's aging [49,50]. Current studies have confirmed this. However, crickets from the NDs group also exhibited an increased percentage of survival probabilities, which suggests that responses to NDs are not random.

In our previous research, we discussed the complex response of *A. domesticus*, whereby graphene oxide exposure involves intricate adaptive mechanisms. The study underscores the importance of considering multigenerational effects and the potential trade-offs between short-term adaptations and long-term impacts on survivability and genomic stability [51]. Notably, Mytych et al. (2016) reported that low concentrations of nanoparticles, including NDs, may lead to increased expression of SIRT1 while simultaneously activating cellular stress response mechanisms [45]. That is why the presence in the diet or activation during the strain selection process may lead to increased sirtuin activity because the observed differences in the dynamics of sirtuin 1 activity were dependent on the strain, time, and the type of applied factor. These findings were confirmed by examining interaction effects in GLMs, highlighting the substantial influence of time, strain, and group on the observed results. Moreover, among the studies carried out by other researchers, varying effects on lifespan extension through RV have been demonstrated. For example, in studies on *Nothobranchius guentheri*, RV not only delayed aging but also provided protection against neurodegeneration [52]. Conversely, Song et al. (2021), researching *Bombyx mori*, demonstrated an activation of the SIRT7-FoxO-GST signaling pathway and an extension of lifespan following treatment with RV. Their studies also conducted a lifespan assay, indicating a

connection between RV and longevity [53]. Current research provides a wider insight into the interaction mechanism, not only of RV but also NDs, with sirtuins in *Acheta domesticus*.

Nevertheless, on the 10th day, stimulation of SIRT1 activity was observed under the influence of NDs and RV in both strains (H and D). This suggests that the impact of NDs and RV on SIRT1 regulation is more complex and may vary at different stages of the organism's response to their presence. Such complex interactions between RV and NDs may be related to interactions with other regulatory factors in organisms, such as longevity-associated genes, metabolic pathways, or stress factors [29,48]. These significant differences in responses to RV and NDs between the H and D strains were also observed in the analysis of their survival. As mentioned earlier, these distinct responses are suspected to arise from genetic, molecular, and regulatory aspects of the cells. However, it is important to note that nuclear extracts can identify the general presence of SIRT1, SIRT6, and SIRT7 in the analyzed tissues.

The activation of histones (ATM and H2A.X) (Figure 6) in tested groups may indicate the initiation of repair mechanisms, as evidenced by the decreasing trend in double-strand breaks relative to the measurement points (10, 15, and 20 days) and the aging of individuals. Similar conclusions were drawn in our previous studies on NDs [29]. Furthermore, NDs at the applied dose appear not to induce a toxic effect, which aligns with recent studies emphasizing their relatively low toxicity and facile biodegradation, as highlighted by Bilal et al. (2021) [54]. A similar trend is observed for the group treated with RV. This also allows us to infer that RV may exhibit a potentially therapeutic effect, which could be linked to the activation of sirtuin or/ and a specific sirtuin.

In summary, these results suggest that RV and NDs exert a diverse and dynamic impact on the activity of SIRT1 in the examined strains. This, in turn, may influence various regulatory processes related to longevity, providing an important avenue for further research and a better understanding of these interactions. Research into possible interaction between SIRTs, NDs, and RV might unveil new insights into the metabolic processes governing organismal longevity. The findings from this study can be pivotal in guiding future experiments, not only on other animal models but also potentially in clinical settings. With appropriate ethical considerations and optimizations, this experimental model could be extended to vertebrates, paving the way for clinical trials exploring RV as an aging process inhibitor and marking a step toward translating laboratory findings into clinical applications. Further research is needed to fully understand these interactions and their potential implications for the health and longevity of organisms consuming RV and NDs.

4. Materials and Methods

4.1. Nanodiamonds

We employed NDs commercially known as Single-Digit NDs in the study, grade "SDND" 5 wt.% aq. suspension, obtained from PlasmaChem GmnH, Berlin, Germany. As reported by the manufacturer, these nanodiamonds were produced through chemical disintegration and are devoid of additives and milling impurities. The diamond crystallite size ranges from 3.5 to 5.2 nm, and the particle size, as determined by Dynamic Light Scattering (DLS), falls within the range of 5 to 15 nm. These NDs underwent characterization as described in the article [55]. In the research carried out by the team in 2022 [29], a comprehensive analysis revealed the gentle aggregation of NDs within the suspension. However, a negative and relatively low zeta potential (− 15.6 mV, 25 ° C) proved good ND stability in the suspension. The NDs were also visualized using AFM (Agilent 5500 Atomic Force Microscopy, Agilent Technologies, CA, USA), confirming that the size of the NDs is consistent with the scale declared by the manufacturer, with results ranging from 2.8 to 5.5 nm (Figure 7). A detailed characterization can be found in the studies by Augustyniak et al. (2023), where the same material was employed for the research [43].

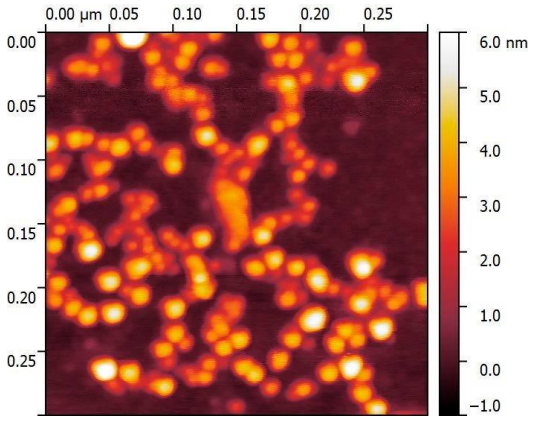

Figure 7. AFM of nanodiamonds analysis (Agilent 5500 Atomic Force Microscopy).

4.2. Resveratrol

The experiments employed RV (CAS 501-36-0; molecular weight: 228.24 g/ mol) sourced from Pol-Aura®. As indicated by the manufacturer, this compound has a natural origin. The Certificate of Analysis (COA) confirms the compound's high purity, as evidenced by its ≥ 98% content according to high-performance liquid chromatography (HPLC) analysis.

4.3. Experimental Design

4.3.1. Characteristics of *Acheta domesticus*

The insect species used in our research was *Acheta domesticus*, a member of the family *Gryllidae* within the order Orthoptera. This species, commonly known as the house cricket, is widely distributed and frequently encountered in human habitats worldwide. It undergoes several developmental stages in its life cycle, starting as an egg, progressing through multiple nymphal stages resembling miniature adult forms without fully developed reproductive organs, and culminating in the adult stage (IMAGO) with fully developed wings and reproductive capabilities. The lifespan is around 3–4 months. Due to its distinctive attributes and widespread presence, house cricket has garnered significant interest from researchers across diverse fields. It is widely used in toxicological, physiological, and genetic research and is considered a model organism because it is a relatively easy organism to cultivate [56]. We have been cultivating this species for nearly 30 years [29], providing us with extensive knowledge and experience. In the present study, we utilized two strains of house crickets: the wild-type (designated as H) and the long-lived (designated as D). The long-lived strain was obtained through selection lasting many years, which involved postponing reproduction.

The long-lived strain is characterized not only by extended longevity but also by a delayed onset of reproduction compared to the wild-type strain upon reaching the imago stage. Due to its high reproductive capacity, the house cricket allows enough individuals for research purposes in a short time [51,57].

4.3.2. Experimental Model

All experiments were carried out within tightly controlled environmental conditions, including a temperature of 28.8 ± 1 °C, a photoperiod of 12 h of light and 12 h of darkness (L:D 12:12), and a humidity level of 43.80 ± 6.72%. Insects were fed standardly by providing finely ground "Rabbit Fattening Feed KDT, UNIPASZ; granulate form" [ingredients: wheat bran, sunflower seed cake, sunflower seed meal, wheat, beet molasses, triticale, calcium

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carbonate, calcium-magnesium carbonate, sodium chloride, family oils and fats, sodium salts of organic acids, herbal extracts]. This feed was subsequently blended with either RV or NDs in appropriate proportions to achieve the desired concentrations of RV or NDs in food. After blending, the mixture was dried, sterilized, and stored in containers until use.

Subsequently, freshly hatched insects from both strains H and D were categorized into three distinct groups: the control group (receiving food without additives), the RV group (RV in concentration 23 mg kg^{-1} in food), and the NDs group (NDs in concentration 0.2 mg kg^{-1}). Each group was consistently fed with their respective diets from hatching throughout the experiment, which lasted for 56 days. After the insects reached the imago stage, five individuals were randomly selected from each experimental group at three time points: 10, 15, and 20 days (Figure 8). To prepare insect tissues, individuals were first anesthetized on ice before measurements were taken. As we have previously detailed in our prior articles [29,51,57], cell suspensions derived from the house cricket intestine were prepared in 0.1 M PBS (pH 7.4) and homogenized using the Minilys homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France).

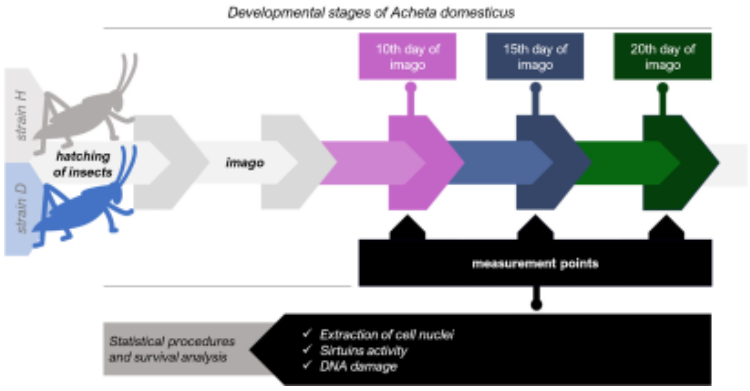

Figure 8. Experimental Design.

4.4. Biochemical Analysis

4.4.1. Extraction of Cell Nuclei and SIRT Activity

Before commencing the procedure for detecting sirtuin activity in *A. domesticus* tissue, nuclear extracts were prepared using the Nuclear Extraction Kit (Abcam, Cambridge, UK, ab113474), recommended by the manufacturer of the Universal SIRT Activity Assay Kit (Colorimetric) (Abcam, Cambridge, UK, ab156915) test. The Nuclear Extraction Kit employs a 1000× Protease Inhibitor Cocktail, 10X Pre-Extraction Buffer, DTT Solution (1000×), and EN E2 (Extraction Buffer) to rapidly isolate abundant nuclear extracts within a relatively short assay time of approximately 1 h. This was used for *A. domesticus* intestine tissues, and then the extracted nuclear components were promptly employed for experimentation.

Activity Assay Kit (colorimetric) (Abcam, Cambridge, UK, ab156915) measured the overall SIRT enzyme activity in the previously prepared nuclear extracts. The kit incorporates a deacetylated histone standard, simplifying the quantitative evaluation of the activity of SIRT enzymes. Samples were measured using a UV–vis spectrometer (TECAN Infinite M200, Tecan Austria GmbH, Grödig, Austria).

The intensity of the SIRT1 reaction was assessed in nuclear extracts obtained using the SIRT1 Activity Assay Kit (fluorometric) (Abcam, ab156065). Measurements were performed using a Fluorescence Spectrometer Plate Reader (HITACHI F-7000, Hitachi, Ltd., Tokyo, Japan) over a 60 min duration. This assay facilitated the detection of SIRT1 activity.

4.4.2. DNA Damage

The research kit employed, Muse® Multi-Color DNA Damage Kit (Luminex Corporation, TX, USA, Part Number: MCH200107), enabled the detection of ATM and H2A.X protein activation, which is integral in the ATM-dependent signaling pathway associated with DNA damage response. ATM kinase activation and H2A.X phosphorylation are key indicators of cellular responses to DNA damage, especially in the context of double-strand break repair. The test provides percentages encompassing cells without DNA damage (negative cells), ATM-activated cells, H2A.X-activated cells, and cells with double-strand DNA damage (dual activation of both ATM and H2A.X).

The method, relying on the simultaneous use of two antibodies conjugated with fluorochromes, enabled the analysis of the phosphorylation status of ATM (Ser1981) and Histone H2A.X. This approach consequently allowed for the examination of signaling pathways associated with DNA damage repair. The following steps of the testing procedure were conducted in accordance with the manufacturer's recommendations. Cells were prepared, fixed, permeabilized, and then incubated with an antibody cocktail specific for ATM and H2A.X. Following incubation, the cells were washed, resuspended in assay buffer, and analyzed using the Muse® Cell Analyzer to quantify DNA damage indicators.

4.5. Statistical Procedures

The non-parametric statistical test, Kaplan–Meier survival analysis, was employed to assess survival rates in the experimental groups. Survival curves depicting the probability of survival across various time intervals were graphically presented. Subsequently, the Log-Rank Test (Chi-squared, $p < 0.05$), particularly valuable for evaluating differences in survival, was carried out.

SIRT activity and DNA damage across three time points (10th, 15th, and 20th days of the imago stage of *A. domesticus*) were measured five 5 replicates per group (control, RV-, and ND-treated). The Levene and Brown–Forsythe tests were used to check differences in the homogeneity of variance between the studied groups. To identify differences between experimental groups and strains, an ANOVA (LSD test, $p < 0.05$) was employed using STATISTICA®13 software (TIBCO Software Inc., Palo Alto, CA, USA). Univariate factorial ANOVA and interaction effects were also performed for total SIRT activity and DNA damage using sigma-constrained parameterization. GLM analysis with consideration of the interaction effect for SIRT1 was performed.

5. Conclusions

Our study not only represents a significant step in understanding the impact of RV and NDs on organisms, including *A. domesticus*, but also focuses on the sirtuin activity within the same species across two different strains. One of these strains was deliberately selected for longevity, while the other remained wild-type.

The results of our study indicate a diverse impact of RV and NDs on SIRT1 activity, depending on the strain and the life stage of the organisms. The reduction in SIRT1 activity in the D strain may be associated with regulatory mechanisms linked to longevity. Simultaneously, the observation of SIRT1 activity- and stimulation-specific conditions (depending on age, strain, or time) suggests that the influence of these substances may be more complex and dynamic.

It is worth emphasizing that there are potential therapeutic implications arising from these observations. The action of RV may prove beneficial to the health of organisms. However, due to the complexity of the impact of these substances on organisms, further studies at different life stages are necessary for a better understanding of these relationships and their potential implications for extending lifespans. Our research opens new perspectives in the study of the effects of RV and NDs on aging processes and organism health, constituting an important field of research in the biology of aging.

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Abbreviations

AFM	Atomic Force Microscopy
AMPK	AMP-activated protein kinase
ATM	Ataxia Telangiectasia Mutated
COA	The Certificate of Analysis
D strain	Long-lived strain of *Acheta domesticus*
DLS	Dynamic Light Scattering
DSBs	Double-strand nreaks
DTT	Dithiothreitol
EGFP-K85AcK	Name of the novel genetically encoded fluorescent probe
ENE2	Extraction Buffer
FOXO	Forkhead box class O
FoxO-GST	Forkhead box O Glutathione S
GLM	Generalized linear model
H strain	Wild-type strain of *Acheta domesticus*
H2A.X	H2A histone family member X
HibK	Type of histone mark
HPLC	High-performance liquid chromatography
mTOR	The mammalian target of rapamycin
ND	Nanodiamond
NDs	Nanodiamonds
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
p53	Cellular tumor antigen p53
PBS	Phosphate buffered saline
PPAR-γ	Peroxisome proliferator activated receptor gamma
RFU	Relative Fluorescence Unit
ROS	Reactive Oxygen Species
RV	Resveratrol (3, 5, 4′trihydroxystilbene)

References

1. Grządziel, P.; Goździalska, A. Etiology and Possibilities of Slowing down Skin Aging Processes. *Aesthetic Cosmetol. Med.* **2022**, *11*, 3–10. [CrossRef]
2. Gabryel, B.; Duszkiewicz, R. Sestryny Jako Modulatory Procesów Starzenia i Chorób Związanych z Wiekiem. *Postęp. Hig. I Med. Doświadczalnej* **2021**, *75*, 437–447. [CrossRef]
3. Zyriax, B.-C.; Windler, E. Lifestyle Changes to Prevent Cardio- and Cerebrovascular Disease at Midlife: A Systematic Review. *Maturitas* **2023**, *167*, 60–65. [CrossRef] [PubMed]
4. Ziętara, P.; Dziewięcka, M.; Augustyniak, M. Why Is Longevity Still a Scientific Mystery? Sirtuins-Past, Present and Future. *Int. J. Mol. Sci.* **2022**, *24*, 728. [CrossRef] [PubMed]

5. Ji, Z.; Liu, G.-H.; Qu, J. Mitochondrial Sirtuins, Metabolism, and Aging. *J. Genet. Genom.* **2022**, *49*, 287–298. [CrossRef]
6. Frydzińska, Z.; Owczarek, A.; Winiarska, K. Sirtuiny i Ich Rola w Regulacji Metabolizmu. *Postępy Biochem.* **2019**, *65*, 31–40. [CrossRef] [PubMed]
7. Siedlecka, K.; Bogusławski, W. Sirtuiny—Enzymy Długowieczności? *Gerontol. Pol.* **2005**, *13*, 147–152.
8. O'Callaghan, C.; Vassilopoulos, A. Sirtuins at the Crossroads of Stemness, Aging, and Cancer. *Aging Cell* **2017**, *16*, 1208–1218. [CrossRef]
9. Zullo, A.; Guida, R.; Sciarrillo, R.; Mancini, F.P. Redox Homeostasis in Cardiovascular Disease: The Role of Mitochondrial Sirtuins. *Front. Endocrinol.* **2022**, *13*, 858330. [CrossRef]
10. Zhu, N.; Xu, M.-H.; Li, Y. Bioactive Oligopeptides from Ginseng (*Panax ginseng* Meyer) Suppress Oxidative Stress-Induced Senescence in Fibroblasts via NAD+/ SIRT1/ PGC-1α Signaling Pathway. *Nutrients* **2022**, *14*, 5289. [CrossRef]
11. Yao, Z.; Zhang, X.; Zhen, Y.; He, X.-Y.; Zhao, S.; Li, X.-F.; Yang, B.; Gao, F.; Guo, F.-Y.; Fu, L.; et al. A Novel Small-Molecule Activator of Sirtuin-1 Induces Autophagic Cell Death/ Mitophagy as a Potential Therapeutic Strategy in Glioblastoma. *Cell Death Dis.* **2018**, *9*, 767. [CrossRef] [PubMed]
12. Zu, Y.; Liu, L.; Lee, M.Y.K.; Xu, C.; Liang, Y.; Man, R.Y.; Vanhoutte, P.M.; Wang, Y. SIRT1 Promotes Proliferation and Prevents Senescence Through Targeting LKB1 in Primary Porcine Aortic Endothelial Cells. *Circ. Res.* **2010**, *106*, 1384–1393. [CrossRef] [PubMed]
13. Zhu, S.-L.; Wang, M.-L.; He, Y.-T.; Guo, S.-W.; Li, T.-T.; Peng, W.-J.; Luo, D. Capsaicin Ameliorates Intermittent High Glucose-Mediated Endothelial Senescence via the TRPV1/ SIRT1 Pathway. *Phytomedicine* **2022**, *100*, 154081. [CrossRef] [PubMed]
14. Moraes, D.S.; Moreira, D.C.; Andrade, J.M.O.; Santos, S.H.S. Sirtuins, Brain and Cognition: A Review of Resveratrol Effects. *IBRO Rep.* **2020**, *9*, 46–51. [CrossRef]
15. Zhou, L.; Pinho, R.; Gu, Y.; Radak, Z. The Role of SIRT3 in Exercise and Aging. *Cells* **2022**, *11*, 2596. [CrossRef] [PubMed]
16. Morigi, M.; Perico, L.; Benigni, A. Sirtuins in Renal Health and Disease. *J. Am. Soc. Nephrol.* **2018**, *29*, 1799–1809. [CrossRef] [PubMed]
17. Wood, J.G.; Schwer, B.; Wickremesinghe, P.C.; Hartnett, D.A.; Burhenn, L.; Garcia, M.; Li, M.; Verdin, E.; Helfand, S.L. Sirt4 Is a Mitochondrial Regulator of Metabolism and Lifespan in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 1564–1569. [CrossRef]
18. Tissenbaum, H.A.; Guarente, L. Increased Dosage of a Sir-2 Gene Extends Lifespan in Caenorhabditis Elegans. *Nature* **2001**, *410*, 227–230. [CrossRef]
19. Shukla, N.; Kolthur-Seetharam, U. Drosophila Sirtuin 6 Mediates Developmental Diet-dependent Programming of Adult Physiology and Survival. *Aging Cell* **2022**, *21*, e13576. [CrossRef]
20. Wang, J.; Zhang, H.; Xu, S.; Liu, Z.; Yang, L.; Fang, L.; Huo, Z.; Yan, X. Characterization of the SIRT Genes and Their Distinct Expression Patterns in Manila Clams (*Ruditapes philippinarum*) Exposed to Air. *Meta Gene* **2021**, *28*, 100892. [CrossRef]
21. Damschroder, D.; Zapata-Pérez, R.; Richardson, K.; Vaz, F.M.; Houtkooper, R.H.; Wessells, R. Stimulating the Sir2–Spargel Axis Rescues Exercise Capacity and Mitochondrial Respiration in a Drosophila Model of Barth Syndrome. *Dis. Models Mech.* **2022**, *15*, dmm049279. [CrossRef] [PubMed]
22. Watroba, M.; Szukiewicz, D. Sirtuins at the Service of Healthy Longevity. *Front. Physiol.* **2021**, *12*, 724506. [CrossRef]
23. Grabowska, W.; Sikora, E.; Bielak-Zmijewska, A. Sirtuins, a Promising Target in Slowing down the Ageing Process. *Biogerontology* **2017**, *18*, 447–476. [CrossRef] [PubMed]
24. Wierman, M.B.; Smith, J.S. Yeast Sirtuins and the Regulation of Aging. *FEMS Yeast Res.* **2014**, *14*, 73–88. [CrossRef]
25. Iannitti, R.G.; Floridi, A.; Lazzarini, A.; Tantucci, A.; Russo, R.; Ragonese, F.; Monarca, L.; Caglioti, C.; Spogli, R.; Leonardi, L.; et al. Resveratrol Supported on Magnesium DiHydroxide (Resv@MDH) Represents an Oral Formulation of Resveratrol With Better Gastric Absorption and Bioavailability Respect to Pure Resveratrol. *Front. Nutr.* **2020**, *7*, 570047. [CrossRef] [PubMed]
26. Walle, T.; Hsieh, F.; DeLegge, M.H.; Oatis, J.E.; Walle, U.K. High Absorption but Very Low Bioavailability of Oral Resveratrol in Humans. *Drug Metab. Dispos.* **2004**, *32*, 1377–1382. [CrossRef] [PubMed]
27. Bayda, S.; Adeel, M.; Tuccinardi, T.; Cordani, M.; Rizzolio, F. The History of Nanoscience and Nanotechnology: From Chemical–Physical Applications to Nanomedicine. *Molecules* **2019**, *25*, 112. [CrossRef]
28. Wanek, T.; Mairinger, S.; Raabe, M.; Alam, M.N.A.; Filip, T.; Stanek, J.; Winter, G.; Xu, L.; Laube, C.; Weil, T.; et al. Synthesis, Radiolabeling, and Preclinical in Vivo Evaluation of 68Ga-Radiolabelled Nanodiamonds. *Nucl. Med. Biol.* **2023**, *116–117*, 108310. [CrossRef]
29. Augustyniak, M.; Babczyńska, A.; Dziewięcka, M.; Flasz, B.; Karpeta-Kaczmarek, J.; Kędziorski, A.; Mazur, B.; Rozpędek, K.; Seyed Alian, R.; Skowronek, M.; et al. Does Age Pay off? Effects of Three-Generational Experiments of Nanodiamond Exposure and Withdrawal in Wild and Longevity-Selected Model Animals. *Chemosphere* **2022**, *303*, 135129. [CrossRef]
30. Zupančič, D.; Veranič, P. Nanodiamonds as Possible Tools for Improved Management of Bladder Cancer and Bacterial Cystitis. *Int. J. Mol. Sci.* **2022**, *23*, 8183. [CrossRef]
31. Karpeta-Kaczmarek, J.; Kędziorski, A.; Augustyniak-Jabłokow, M.A.; Dziewięcka, M.; Augustyniak, M. Chronic Toxicity of Nanodiamonds Can Disturb Development and Reproduction of *Acheta domesticus* L. *Environ. Res.* **2018**, *166*, 602–609. [CrossRef] [PubMed]

32. Sivakumar, K.K.; Stanley, J.A.; Behlen, J.C.; Wuri, L.; Dutta, S.; Wu, J.; Arosh, J.A.; Banu, S.K. Inhibition of Sirtuin-1 Hyperacetylates P53 and Abrogates Sirtuin-1-P53 Interaction in Cr(VI)-Induced Apoptosis in the Ovary. *Reprod. Toxicol.* **2022**, *109*, 121–134. [CrossRef] [PubMed]
33. Zessin, M.; Meleshin, M.; Hilscher, S.; Schiene-Fischer, C.; Barinka, C.; Jung, M.; Schutkowski, M. Continuous Fluorescent Sirtuin Activity Assay Based on Fatty Acylated Lysines. *Int. J. Mol. Sci.* **2023**, *24*, 7416. [CrossRef] [PubMed]
34. Xuan, W.; Yao, A.; Schultz, P.G. Genetically Encoded Fluorescent Probe for Detecting Sirtuins in Living Cells. *J. Am. Chem. Soc.* **2017**, *139*, 12350–12353. [CrossRef] [PubMed]
35. Parker, S.; Pant, B. Longevity in Nepal: Health, Policy and Service Provision Challenges. *Int. J. Soc. Syst. Sci.* **2011**, *3*, 333. [CrossRef]
36. Ghimire, S.; Baral, B.K.; Karmacharya, I.; Callahan, K.; Mishra, S.R. Life Satisfaction among Elderly Patients in Nepal: Associations with Nutritional and Mental Well-Being. *Health Qual Life Outcomes* **2018**, *16*, 118. [CrossRef]
37. Chen, L.-K.; Iijima, K.; Shimada, H.; Arai, H. Community Re-Designs for Healthy Longevity: Japan and Taiwan Examples. *Arch. Gerontol. Geriatr.* **2023**, *104*, 104875. [CrossRef]
38. May, M.A.; Tomanek, L. Uncovering the Roles of Sirtuin Activity and Food Availability during the Onset of the Heat Shock Response in the California Mussel (*Mytilus californianus*): Implications for Antioxidative Stress Responses. *Comp. Biochem. Physiol. Part B Biochem. Mol. Biol.* **2024**, *269*, 110902. [CrossRef]
39. Karpeta-Kaczmarek, J.; Dziewięcka, M.; Augustyniak, M.; Rost-Roszkowska, M. Effects of Short-Term Exposure of *Acheta domesticus* to Nanodiamonds in Food: DNA Damage but No Histological Alteration in Tissues. *Carbon* **2016**, *110*, 458–468. [CrossRef]
40. Xie, D.; Huang, Q.; Zhou, P. Drug Discovery Targeting Post-Translational Modifications in Response to DNA Damages Induced by Space Radiation. *Int. J. Mol. Sci.* **2023**, *24*, 7656. [CrossRef]
41. Mahjabeen, I.; Rizwan, M.; Fareen, G.; Waqar Ahmed, M.; Farooq Khan, A.; Akhtar Kayani, M. Mitochondrial Sirtuins Genetic Variations and Gastric Cancer Risk: Evidence from Retrospective Observational Study. *Gene* **2022**, *807*, 145951. [CrossRef] [PubMed]
42. Peng, L.; Yuan, Z.; Li, Y.; Ling, H.; Izumi, V.; Fang, B.; Fukasawa, K.; Koomen, J.; Chen, J.; Seto, E. Ubiquitinated Sirtuin 1 (SIRT1) Function Is Modulated during DNA Damage-Induced Cell Death and Survival. *J. Biol. Chem.* **2015**, *290*, 8904–8912. [CrossRef] [PubMed]
43. Augustyniak, M.; Ajay, A.K.; Kędziorski, A.; Tarnawska, M.; Rost-Roszkowska, M.; Flasz, B.; Babczyńska, A.; Mazur, B.; Rozpędek, K.; Alian, R.S.; et al. Survival, Growth and Digestive Functions after Exposure to Nanodiamonds—Transgenerational Effects beyond Contact Time in House Cricket Strains. *Chemosphere* **2023**, *349*, 140809. [CrossRef] [PubMed]
44. Xu, J.; Chow, E.K.-H. Biomedical Applications of Nanodiamonds: From Drug-Delivery to Diagnostics. *SLAS Technol.* **2023**, *28*, 214–222. [CrossRef] [PubMed]
45. Mytych, J.; Wnuk, M.; Rattan, S.I.S. Low Doses of Nanodiamonds and Silica Nanoparticles Have Beneficial Hormetic Effects in Normal Human Skin Fibroblasts in Culture. *Chemosphere* **2016**, *148*, 307–315. [CrossRef] [PubMed]
46. Sun, B.; Wang, W.; Sain, M. Carbonaceous Nanocomposites for Biomedical Applications as High-Drug Loading Nanocarriers for Sustained Delivery: A Review. *J. Compos. Sci.* **2022**, *6*, 379. [CrossRef]
47. Selvam, R.; Gandhi, A.; Hung, S.-C.; Karmenyan, A.V.; Perevedentseva, E.; Yeh, W.-C.; Wu, S.-Y.; Chang, H.-H.; Cheng, C.-L. Multifunctional Ferromagnetic Nanodiamond for Dual-Mode Fluorescence Imaging and Magnetic Drug Targeting. *Diam. Relat. Mater.* **2023**, *139*, 110398. [CrossRef]
48. Ryu, T.-K.; Kang, R.-H.; Jeong, K.-Y.; Jun, D.-R.; Koh, J.-M.; Kim, D.; Bae, S.K.; Choi, S.-W. Bone-Targeted Delivery of Nanodiamond-Based Drug Carriers Conjugated with Alendronate for Potential Osteoporosis Treatment. *J. Control. Release* **2016**, *232*, 152–160. [CrossRef]
49. Bhullar, K.S.; Hubbard, B.P. Lifespan and Healthspan Extension by Resveratrol. *Biochim. Biophys. Acta (BBA) Mol. Basis Dis.* **2015**, *1852*, 1209–1218. [CrossRef]
50. Ungurianu, A.; Zanfirescu, A.; Margină, D. Sirtuins, Resveratrol and the Intertwining Cellular Pathways Connecting Them. *Ageing Res. Rev.* **2023**, *88*, 101936. [CrossRef]
51. Dziewięcka, M.; Flasz, B.; Rost-Roszkowska, M.; Kędziorski, A.; Kochanowicz, A.; Augustyniak, M. Graphene Oxide as a New Anthropogenic Stress Factor-Multigenerational Study at the Molecular, Cellular, Individual and Population Level of *Acheta domesticus*. *J. Hazard. Mater.* **2020**, *396*, 122775. [CrossRef]
52. Yu, X.; Li, G. Effects of Resveratrol on Longevity, Cognitive Ability and Aging-Related Histological Markers in the Annual Fish *Nothobranchius guentheri*. *Exp. Gerontol.* **2012**, *47*, 940–949. [CrossRef]
53. Song, J.; Liu, L.; Hao, K.; Mao, S.; Tang, Y.; Tong, X.; Dai, F. Resveratrol Elongates the Lifespan and Improves Antioxidant Activity in the Silkworm Bombyx Mori. *J. Pharm. Anal.* **2021**, *11*, 374–382. [CrossRef]
54. Bilal, M.; Cheng, H.; González-González, R.B.; Parra-Saldívar, R.; Iqbal, H.M.N. Bio-Applications and Biotechnological Applications of Nanodiamonds. *J. Mater. Res. Technol.* **2021**, *15*, 6175–6189. [CrossRef]
55. Karpeta-Kaczmarek, J.; Dziewięcka, M.; Augustyniak, M.; Rost-Roszkowska, M.; Pawlyta, M. Oxidative Stress and Genotoxic Effects of Diamond Nanoparticles. *Environ. Res.* **2016**, *148*, 264–272. [CrossRef]

56. Horch, H.W.; Mito, T.; Popadić, A.; Ohuchi, H.; Noji, S. *The Cricket as a Model Organism*; Springer: Tokyo, Japan, 2017; ISBN 978-4-431-56476-8.
57. Flasz, B.; Dziewięcka, M.; Kędziorski, A.; Tarnawska, M.; Augustyniak, M. Vitellogenin Expression, DNA Damage, Health Status of Cells and Catalase Activity in *Acheta domesticus* Selected According to Their Longevity after Graphene Oxide Treatment. *Sci. Total Environ.* **2020**, *737*, 140274. [CrossRef]

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Oświadczam, że mój wkład w przygotowanie publikacji:

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stanowiącego część mojej rozprawy doktorskiej obejmował opracowanie koncepcji badań, przygotowanie i optymalizację metodologii, przeprowadzenie eksperymentów, gromadzenie i analizę danych, interpretację wyników, przygotowanie wizualizacji oraz opracowanie wraz z redakcją manuskryptu, jak również współudział w korespondencji z recenzentami.

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stanowiącej część rozprawy doktorskiej Patrycji Ziętara-Krzyk obejmował pomoc w realizacji badań eksperymentalnych, analizie wyników oraz w przygotowaniu manuskryptu.

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stanowiącej część rozprawy doktorskiej Patrycji Ziętara-Krzyk obejmował pomoc w opracowaniu koncepcji badawczej, nadzór nad realizacją badań, interpretacji wyników oraz redakcji manuskryptu.

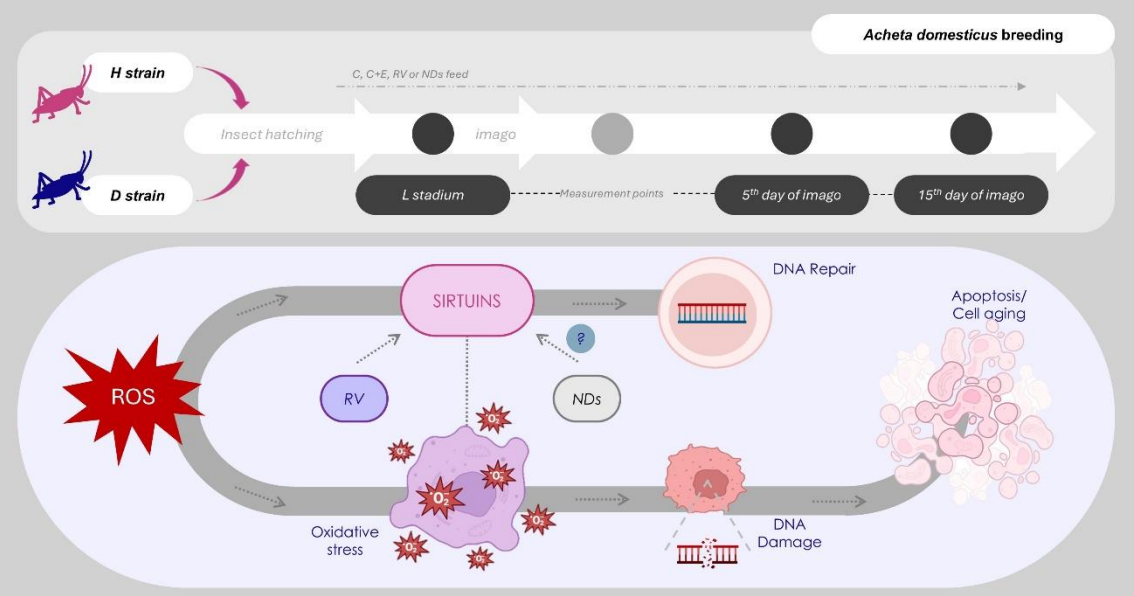
.....

Prof. dr hab. Maria Augustyniak

Manuskrypt III: Effects of resveratrol and nanodiamonds on sirtuin activity, oxidative stress and DNA damage in *Acheta domesticus*

Authors: Patrycja Ziętara-Krzyk, Barbara Flasz, Maria Augustyniak

Graphical abstract



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Effects of resveratrol and nanodiamonds on sirtuin activity, oxidative stress and DNA damage in *Acheta domesticus*

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ABSTRACT

The quest for an elixir of longevity has long inspired research into molecular mechanisms that govern aging. The present study investigated the effects of resveratrol (RV) and nanodiamonds (NDs) on sirtuin activity, oxidative stress, and DNA damage in two *strains of Acheta domesticus*: wild-type (H) and longevity-selected (D). Analyses of total SIRT and SIRT1, SIRT6 activities, antioxidant markers (CAT, SOD, LPO), and DNA damage indicators (pATM, γH2A.X, DSBs) revealed strain- and stage-dependent variations without a consistent pattern, suggesting long-term modulation of lifespan or sirtuin activity. RV and NDs induced only transient and adaptive effects, including a short-term increase in sirtuin activity exposed to NDs. The long-lived strain displayed stability in sirtuin response and survival, indicating that longevity-regulating mechanisms are genetically conserved and resistant to external modulation. These findings offer new insights into the limited and context-dependent influence of nanomaterials and bioactive compounds on aging-related molecular pathways.

1. Introduction

Sirtuins are enzymes that play a key role in regulating cell aging and protecting against oxidative stress, which is why they are often referred to as "longevity enzymes" [1–3]. They are NAD-dependent deacetylases that regulate the activity of FOXO transcription factors, promoting antioxidant gene expression and supporting the cellular stress response [4]. Their ability to influence metabolism, DNA repair, and response to cellular damage makes them the subject of intense research into potentially extending life and improving health in later years [1]. One of their activators is Resveratrol (RV), a naturally occurring phenolic molecule with anti-inflammatory, immunomodulatory, and antioxidant qualities. RV may be found mainly in grape skins, especially dark varieties and red wine. Studies have shown that it is effective in the prevention and management of various diseases, including cancer, cardiovascular conditions, and neurodegenerative disorders. It has gained attention for its potential role in anti-aging and lifespan extension [5,6].

Nanodiamonds (NDs), in turn, are diamond nanoparticles that can vary significantly in size depending on the production method. While detonation NDs are typically smaller, often ranging from 4 to 100 nm in diameter, other synthesis methods can yield larger particles [7]. In biomedicine, NDs are studied for their potential to deliver drugs and other therapeutic agents directly to target cells with high precision [8]. Their recognized biocompatibility and ability to penetrate cellular membranes without causing significant toxicity to make them promising candidates for cancer treatment, among other medical applications [9]. Given the ability of both RV and NDs to influence cellular processes related to aging and disease prevention, their effects are believed to involve the modulation of oxidative stress [10,11].

Oxidative stress, caused by elevated levels of reactive oxygen species (ROS) and a decline in antioxidant defences, plays a significant role in the aging process by contributing to cellular damage [12]. Moreover, it is associated with many chronic diseases, including cancer, cardiovascular disease, neurodegenerative diseases (e.g., Alzheimer's disease), diabetes, and age-related diseases [13]. Several well-established markers are used for checking oxidative stress, some of which play a role in systems that help mitigate damage and maintain cellular homeostasis [14,15]. Superoxide dismutase (SOD) is an essential antioxidant enzyme that protects cells from oxidative stress by converting harmful superoxide radicals ($O_2\bullet$) into less reactive hydrogen peroxide (H_2O_2) [16]. High SOD activity may help prevent or delay the development of these diseases by minimizing oxidative damage [17]. Catalase (CAT) degrades H_2O_2 into water and oxygen [14]. With age, catalase activity decreases, increasing the risk of cellular degeneration and aging [18]. Lipid peroxidation (LPO) is a valuable marker for monitoring

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oxidative stress and is a key biochemical process in which free radicals induce the oxidative degradation of lipids, leading to cellular dysfunction. Elevated LPO levels are intricately linked to the accumulation of oxidative damage in cellular structures, which accelerates aging and affects longevity [15,19]. Since sirtuins influence the expression of the antioxidant system, CAT, SOD, and LPO can be used as indicators of the effectiveness of sirtuin activation [20].

This study used two unique *Acheta domesticus* strains: a wild strain (H) and a long-lived strain (D), providing a valuable opportunity to track the interplay between sirtuin activity, oxidative stress, and antioxidant defenses. Preliminary data on sirtuin activity in *Acheta domesticus*, a model for incomplete metamorphosis, are documented [21]. However, they demand further research. Although RV is widely studied in the context of aging and sirtuin activation, the impact of NDs on these processes remains unclear, making it crucial to assess their role and safety in longevity-related mechanisms. Given the growing biomedical significance of NDs, evaluating the potential risks associated with their use is essential to ensure safe application in future therapeutic strategies, particularly in potential synergism with RV. A thorough understanding of how NDs independently modulate sirtuin activity is necessary to harness their future potential in conjunction with RV and enhance the effectiveness of therapy. Therefore, the main goal was to investigate the relationship between sirtuin activity (SIRT1, SIRT6), oxidative stress regulation, lifespan modulation in exposure to RV and NDs, and potential modifications of sirtuin activity. The following hypotheses were tested:

H1. The expression and activity of specific sirtuins, particularly SIRT1 and SIRT6, may significantly vary between individuals from the wild H and long-lived strain D. It is likely that the D strain exhibits higher activity in these sirtuins, contributing to enhanced cellular maintenance and longevity.

H2. A relationship exists between sirtuin activity (especially SIRT1 and SIRT6), exposure to RV or NDs, and the lifespan of *Acheta domesticus*. These factors may either extend or reduce longevity. Resveratrol, known for its SIRT1-activating and antioxidant properties, may promote lifespan extension, while NDs could have the opposite effect. Selection for longevity (D strain) may influence this response.

2. Material and methods

2.1. Nanodiamonds

In our study, we used Single-Digit NDs, grade 'SDND', 5 wt% aqueous suspension (PlasmaChem GmbH, Berlin, Germany). The TEM image (Fig. 1A) shows nanodiamonds with clearly visible lattice fringes, indicating a high degree of crystallinity. The regular atomic arrangement corresponds to the planes of the diamond structure. Morphology reveals the formation of a continuous nanoscale film (TEM, FIB/SEM Helios NanoLab 450 H P, Thermo Fisher Scientific, USA). Fig. 1B shows agglomerated NDs composed of nearly spherical particles (5-10 nm), which is consistent with the manufacturer's specification indicating a particle size of 5–15 nm. The observed morphology shows a uniform distribution of nanosized crystallites forming a porous, chain-like structure (TEM, TECNAI G2 X-TWIN, Thermo Fisher Scientific, USA). The EDS analysis (Fig. 1A, .Fig. 1C) of the representative sample revealed that the material consists mainly of carbon (94.2 wt%) with small amounts of oxygen (4.0 wt%) and traces of silicon (1.3 wt%), phosphorus (0.2 wt%), sulfur (0.15 wt%), and chlorine (0.05 wt%). The copper peaks observed in the EDS spectrum originate from the TEM grid and are not part of the sample composition (EDS, FIB/SEM HELIOS NANOLAB 450 H P, Thermo Fisher Scientific, USA).

2.2. Resveratrol

Natural RV (molecular weight: 228.24 g/mol) from Pol-Aura® Zabrze, Poland (catalog number: PA-03-2549-P, CAS number: 501-36-0) was used. The provided specifications by the Certificate of Analysis

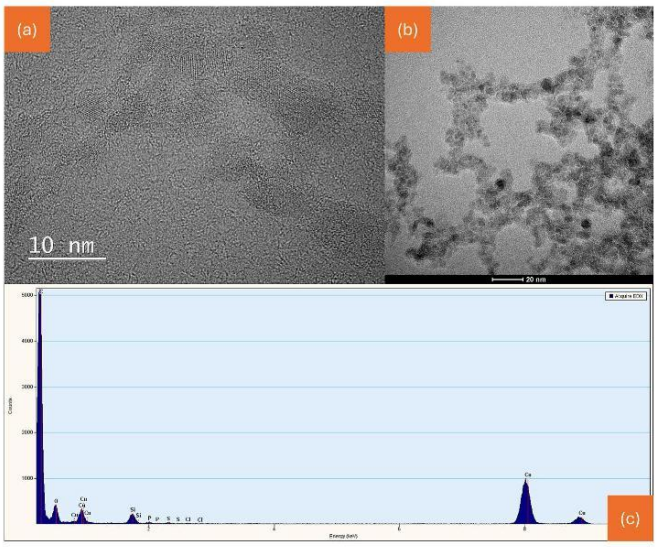

Fig. 1. (A) TEM image (TITAN3 G2 60-300, Thermo Fisher Scientific, USA), (B) TEM micrograph (TECNAI G2 X-TWIN, Thermo Fisher Scientific, USA), and (C) EDS spectrum (FIB/SEM HELIOS NANOLAB 450 H P, Thermo Fisher Scientific, USA) of nanodiamonds (Single-Digit Nanodiamonds, 50 mg/mL, PlasmaChem GmbH, Germany).

(COA) include a heavy metal content of no more than 10 ppm: arsenic (As) content of up to 1 ppm, lead (Pb) content not exceeding two ppm, cadmium (Cd) content limited to 1 ppm, and mercury (Hg) content of no more than 0.5 ppm. The plate count does not exceed 1000 CFU/g, while the yeast and mold count remains below 100 CFU/g. The purity, as determined by HPLC, is at least 98.0%.

2.3. Experimental design

2.3.1. Characteristics of Acheta domesticus

The house cricket (*Acheta domesticus*) is an orthopteran insect commonly used in biological research due to its well-documented life cycle and ease of breeding [22]. This species undergoes incomplete metamorphosis, developing through nymphal stages before adulthood [23]. Typically, they have a lifespan of about 3 to 4 months, which provides a condensed timeframe for studying life cycle processes across multiple generations. This relatively short lifespan, coupled with their regenerative abilities and well-characterized developmental stages, makes them an ideal model organism for investigating aging, longevity, and various aspects of developmental biology [24–27]. The two strains of house crickets developed at the University of Silesia in Katowice exhibit distinct ontogenetic trajectories. The long-lived strain (D) undergoes an extended developmental period and demonstrates increased life expectancy compared to the wild type (H) [25,27].

2.3.2. Experimental Model

The experiments were carried out under controlled conditions, with a rearing humidity level of 43.80 ± 6.72%, a temperature of 28 ± 1 °C, and a photoperiod of 12 h of light and 12 h of darkness (L:D 12:12). These conditions correspond to commonly used laboratory rearing parameters for *Acheta domesticus* and support the species' circadian rhythm, which is characterized by higher activity during the dark phase [24]. The insects were fed a finely ground version of Rabbit Fattening Feed KDT, UNIPASZ; granulate form with the following composition per kilogram: 17% crude protein, 14.5% fiber, 3.50% fat, and 7.50% ash. The mineral content consisted of 0.95% Ca, 0.19% Na, 0.50% P, 85 mg Zn, 25 mg Fe, and approximately 100 mg of other trace elements. The feed also contained various antioxidants and additives, including vitamins (A, C, E, and B complex) and amino acids (methionine, L-lysine, and L-threonine). Before use, the feed was finely ground and prepared with either RV or NDs at the proper concentrations and ratios. The mixture was then dried to obtain a uniform dry mass. NDs were added as an aqueous suspension. Before incorporation into the feed, the suspension was sonicated to ensure homogeneous dispersion and reduce nanoparticle aggregation. The prepared feed was stored in airtight containers at RT to prevent contamination.

The next stage involved preparing the experimental groups. Newly hatched insects from strains H and D were divided into four groups within each strain: control (C), control with ethanol (C + E), the RV group, and the NDs group. Resveratrol was dissolved in ethanol (ethanol concentration ~0.01%) before incorporation into the feed, and the resulting solution was mixed with the ground feed to obtain the final concentration of 23 mg kg^{-1}. To control for potential solvent effects, the C + E group received feed containing the same amount of ethanol as used for the resveratrol preparation (final ethanol concentration ~0.01%). The NDs group received feed supplemented with nanodiamonds at a concentration of 0.2 mg kg^{-1}. The insects were consistently fed these diets throughout the 19-week experiment. Crickets were selected for analysis at the larval stage before the imago stage and subsequently at the 5th and 15th days after adulthood. Tissues were prepared following the methodology established in previous experiments [21]. A suspension of *A. domesticus* intestinal cells was prepared in 0.1 M PBS buffer (pH 7.4) and homogenized using a Minilys homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France). All biochemical measurements were performed using five biological replicates per treatment group within each strain (n = 5).

2.4. Biochemical analysis

2.4.1. Antioxidant marker measurements

The Lipid Peroxidation (LPO) Assay Kit (Abbexa Ltd, Cambridge, UK, abx 096010) was employed to evaluate oxidative lipid damage. This assay quantifies lipid peroxidation by measuring the production of lipid peroxides, which react with a specific substrate to form a coloured compound. The color intensity correlates with the extent of lipid peroxidation, indicating the sample's oxidative stress level. Tissue homogenates were centrifuged at 8000×*g* for 10 min at 4 °C, and the obtained supernatants were used for further analysis according to the manufacturer's protocol.

The catalase activity in biological samples was measured using the Catalase Colorimetric Activity Kit (Thermo Fisher Scientific, CA, USA, EIACATC). Prior to analysis, the homogenates were washed with PBS and centrifuged at 10,000×*g*. The resulting pellet was then resuspended in the assay buffer provided with the kit and processed according to the manufacturer's instruction.

The Superoxide Dismutase (SOD) Activity Assay Kit (Merck KGaA, Darmstadt, Germany, CS0009) was used to determine SOD activity, with the results expressed as either percent inhibition or enzymatic units based on a standard curve. The assessment procedure followed the manufacturer's protocol, using the UV–Vis spectrometer (TECAN Infinite M200, Tecan Austria GmbH, Grödig, Austria). The homogenates were centrifuged at 12,000×*g* for 5 min at 4 °C in PBS, and the obtained supernatants were used for the assay according to the manufacturer's protocol.

2.4.2. DNA damage

The Muse® Multi-Color DNA Damage Kit (Luminex Corporation, TX, USA, Part Number: MCH200107) was used to detect DNA damage by enabling simultaneous ATM and H2A.X activation analysis. This kit utilizes two directly conjugated, phospho-specific antibodies: ATM (Ser 1981)-PE and Histone H2A.X PECy5, to precisely measure their phosphorylation states. The ATM–H2AX DNA damage response pathway is highly conserved across eukaryotes. In insects, the histone variant H2Av acts as a functional homolog of mammalian H2AX and undergoes phosphorylation in response to DNA double-strand breaks, allowing detection of DNA damage signaling using antibodies recognizing phosphorylated H2AX [28,29]. After homogenization (Experimental Model section), the cell suspension was filtered through an 80 μm nylon cell strainer to remove tissue debris and obtain a uniform single-cell suspension suitable for flow cytometric analysis. The testing procedure followed the manufacturer's guidelines, involving cell preparation, fixation, permeabilization, and incubation with a specific ATM and H2A.X antibody cocktail. After incubation, cells were washed and suspended in an assay buffer and analysed using the Guava® Muse® cell analyser (Luminex Corporation, TX, USA) to quantify DNA damage markers.

2.4.3. Cell nuclei extraction and SIRT activity measurements

Nuclear extracts were prepared from intestinal cell suspensions obtained after tissue homogenization in PBS as described above. Following the manufacturer's instructions, the Nuclear Extraction Kit (Abcam, Cambridge, UK, ab113474) was used to prepare nuclear extracts for sirtuin assays by lysing cells from intestinal tissues of *A. domesticus* and separating the nuclear fraction through a series of buffer-washing and centrifugation steps. This process ensures the isolation of intact nuclear proteins while minimizing contamination from cytoplasmic components. Subsequently, the protein content of the extracts was evaluated using the Bradford method (Bradford, 1976) and measured on the UV–Vis spectrometer (TECAN Infinite M200, Tecan Austria GmbH, Grödig, Austria).

The Universal SIRT Activity Assay Kit (Colorimetric) (Abcam, Cambridge, UK, ab156915) was used to measure total sirtuin activity in nuclear extracts. During the reaction, the SIRT enzyme deacetylates a specific substrate in the presence of NAD+, resulting in the production

of a deacetylated product. The UV–Vis spectrometer (TECAN Infinite M200, Tecan Austria GmbH, Grödig, Austria) was used for colorimetric detection.

The measurements of specific sirtuins were performed using the SIRT1 Activity Assay Kit (fluorometric) (Abcam, Cambridge, UK, ab156065) and SIRT6 Activity Assay Kit (fluorometric) (Abcam, Cambridge, UK, ab156068) on the Fluorescence Spectrometer Plate Reader (HITACHI F-7000, Hitachi, Ltd., Tokyo, Japan). These assays measure NAD^+-dependent deacetylase activity using acetylated peptide substrates and therefore assess enzymatic activity in nuclear extracts rather than species-specific antibody recognition. To quantify SIRT1 and SIRT6 activity, those assays utilize a fluorescent substrate deacetylated by the SIRT1/SIRT6 enzyme, releasing a fluorophore and emitting a fluorescent signal. Measure the change in fluorescence intensity, which is proportional to the SIRT1/SIRT6 enzymatic activity, enabling precise and sensitive measurement of SIRT1/SIRT6 activity in nuclear extracts.

2.5. Data visualization

Several libraries were used in Python (version 3.10.11) for data processing and visualization. Matplotlib served as the primary tool for creating static and interactive visualizations. Pandas, a data manipulation library, was used to manage structured data, while NumPy, known for its efficient numerical computations, was utilized to support statistical operations and enhance data processing. Boxplots were generated using Pandas and NumPy, allowing a detailed representation of data distribution and variability. Each boxplot displays the distribution of data, where the box extends from the first quartile (Q1) to the third quartile (Q3), with a line inside the box representing the median (Q2). The whiskers extend to 1.5 times the interquartile range (IQR) from Q1 and Q3, showing the range of the data excluding outliers. Heatmaps were created with Pandas and Seaborn, which provided an intuitive way to visualize correlations and intensity patterns within the data. The heatmap was prepared based on the mean values of LPO after removing outliers using the Interquartile Range (IQR) method. The Jupyter Notebook (Project Jupyter, California, USA) was used to prepare and execute the scripts within a Windows 11 (Microsoft, Washington, USA) environment.

2.6. Statistical procedures

The normality of distribution was assessed, followed by a univariate factorial ANOVA to evaluate the effects of experimental factors on the measured variables. Depending on the homogeneity of variances, proper post-hoc tests were applied: for datasets with equal variances, the LSD test ($p < 0.05$) was performed using STATISTICA® 13 software (TIBCO Software Inc., Palo Alto, CA, USA). A PERMANOVA (Permutational Multivariate Analysis of Variance; $p < 0.05$) was conducted using Python's Permanova function from the scikit-bio package for non-parametric multivariate comparisons. To assess whether observed differences were due to actual group separation rather than dispersion, PERMDISP (Permutational Analysis of Multivariate Dispersions) was additionally performed. Group differences were considered reliable only when PERMANOVA indicated significance ($p < 0.05$), and PERMDISP was not significant ($p \geq 0.05$), suggesting that differences in dispersion did not confound group separation. All biochemical measurements (LPO, CAT, SOD, % inhibition, total sirtuin activity, SIRT1, SIRT6, pATM, γH2A.X, and DSBs) were performed using five biological replicates per treatment group ($n = 5$ per group: C, C + E, RV, NDs).

3. Results

3.1. Survival rate

Both strains exhibited comparable median lifespan (28 days). However, the D strain showed a tendency toward extended maximum lifespan relative to the H strain (119 vs. 98 days).

In the H strain, supplementation with NDs resulted in a clear increase in median lifespan (42 days), corresponding to a 50% increase relative to the control, while the maximum lifespan increased slightly (105 days). In contrast, resveratrol (RV) did not alter median lifespan but slightly increased the maximum lifespan (112 days). The C + E group exhibited a reduced median lifespan (14 days), although the maximum lifespan remained unchanged relative to the control. Log-rank tests confirmed significant differences within the H strain, particularly between C vs NDs ($p = 0.003$), C + E vs RV ($p = 0.013$), and C + E vs NDs ($p \leq 0.001$).

In the D strain, none of the treatments produced statistically significant changes in survival relative to the control group. Median lifespan remained constant across groups (28 days), while maximum lifespan varied slightly (105–126 days), with the highest value observed in the NDs group. Between-strain comparisons revealed significant differences in survival distributions for the control ($p = 0.047$), C + E ($p = 0.014$), and RV groups ($p = 0.049$), whereas no difference was observed for the NDs treatment ($p = 0.547$).

3.2. Antioxidant markers in Acheta domesticus

3.2.1. LPO

In strain H, no significant differences were observed between the control group (C) and the C + E group at the L stadium. However, the LPO level in the C + E group was significantly higher than in the RV group (Fig. 3 a1). In the time-course analysis, lipid peroxidation levels in groups C, C + E, and NDs were not statistically significant. In contrast, a general increase in LPO was recorded on the 5th Day of adult life compared to the L stadium in the group receiving RV (Supplementary Fig. 2A). On the 5th Day of adult life, the C + E group showed a significantly higher LPO level than groups C, RV, and NDs (Fig. 3 a2). On the 15th Day of adult life, no significant differences between groups were found (Fig. 3 a3).

In strain D, at the L stadium, a tendency to increase in LPO level was observed in the NDs group compared to groups C, C + E, and RV; however, the differences between groups were not statistically significant (Fig. 3 a4). In strain D, at the 5th Day stadium, a significant increase in lipid peroxidation was found in the NDs group compared to C + E (Fig. 3 a5). Similarly to strain H (Fig. 3 a3), no significant differences between groups were observed on the 15th Day of adult life (Fig. 3 a6). No significant time-dependent changes were recorded within the same groups (Supplementary Fig. 2A).

In the context of between-strain comparisons, lipid peroxidation levels were significantly higher in strain H in the C + E group compared to strain D only at the 5th Day stadium (Fig. 3 a2, a4).

3.2.2. Catalase

Catalase (CAT) activity showed significant variability depending on the strain, developmental stage, and the applied compound. At the L stadium in strain H, the control group (C) showed significantly higher CAT activity than the C + E, RV, and NDs groups, which showed comparable activity levels (Fig. 4 a1).

Time-course analysis revealed that in the control group (C), CAT activity in the L stadium of strain H was higher than at later time points; however, significant differences appeared only between the 15th and 5th Day (Supplementary Fig. 2B). In the C + E group, activity was significantly lower in the L stadium compared to the 15th Day. In contrast, in the RV group, significantly lower enzyme activity was seen on the 15th Day of adult life compared to both the L stadium and the 5th Day. The NDs group showed a significant increase in catalase activity on the 15th Day of adult life relative to the L stadium (Supplementary Fig. 2B). On the 5th Day of adult life, the C group from strain H did not differ significantly from C + E, while the RV group showed significantly higher activity than C (Fig. 4 a2). An opposite trend was observed on the 15th Day of adult life, where RV significantly reduced CAT activity compared to C (Fig. 4 a3).

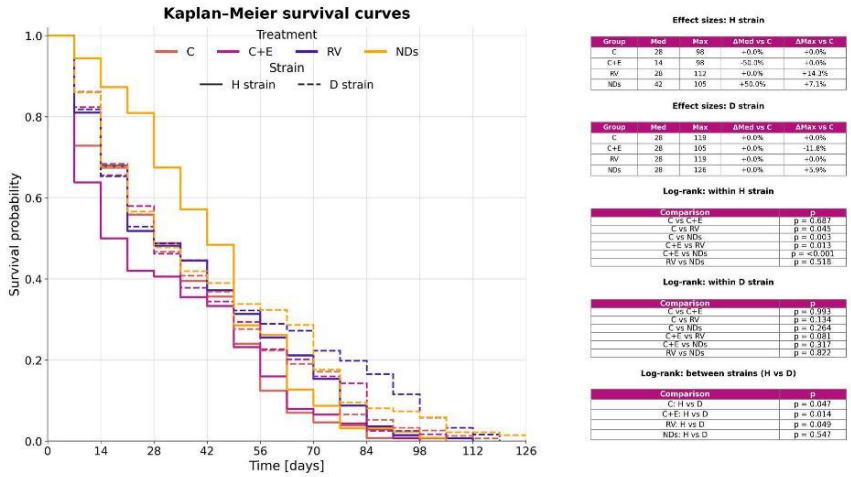

Fig. 2. Kaplan–Meier survival curves for the wild-type strain (H) and the long-lived strain (D). Individuals were maintained separately within each treatment group (C, C + E, RV, NDs), with approximately n ≈ 140 individuals per strain in each group. Median survival time and maximum observed lifespan are summarized for each group. Differences in survival distributions were assessed using the log-rank test within each strain, and between strains for corresponding groups (Python, lifelines package).

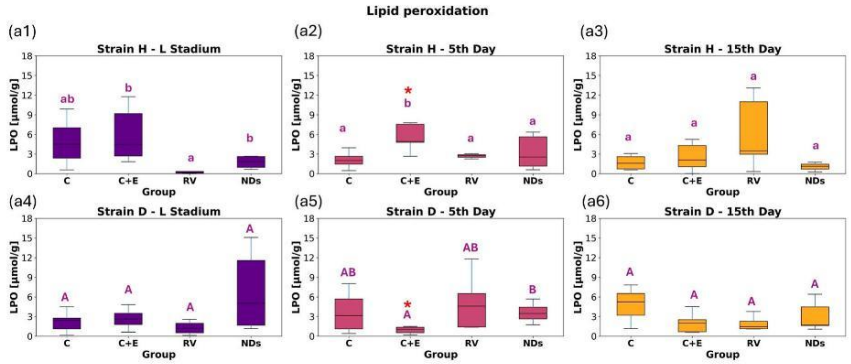

Fig. 3. Box plots show medians (horizontal line) and interquartile ranges (IQR; box spans Q1–Q3); whiskers extend to values within 1.5 × IQR from the quartiles. Data represent lipid peroxidation (LPO; µmol/g) in two *A. domesticus* strains (H and D) across four experimental groups (C, C + E, RV, NDs) at three time points: larval stage (a1, a4), 5th day (a2, a5), and 15th day of adulthood (a3, a6). Statistical analysis was performed using PERMANOVA ($p < 0.05$, corrected) and PERMDISP ($p \geq 0.05$) in Python (scikit-bio). Lowercase letters indicate significant differences between groups within strain H on the same day; uppercase letters indicate differences within strain D. Asterisks (*) denote significant differences between strains within the same group and time point.

In strain D, at all-time points, no significant differences in CAT activity were observed between groups C and C + E. At the L stadium and on the 5th Day of adult life, catalase activity in the NDs group was significantly higher than in the RV group (Fig. 4 a4, a5). On the 15th Day of adult life, enzyme activity in all groups was similar, with no significant differences (Fig. 4 a6). Time-course analysis for strain D showed stable CAT activity in groups C, C + E, and RV. Only the NDs group exhibited a significant decrease in activity on the 15th Day of adult life compared to the L stadium (Supplementary Fig. 2B).

Regarding between-strain comparisons, at the L stadium, CAT activity in the NDs group was significantly lower in strain H than in strain D (Fig. 4 a1, a4). On the 5th Day of adult life, higher catalase activity was found in the RV group of strain H compared to strain D (Fig. 4 a2, a5), while on the 15th Day of adult life, higher values were observed in the C group of strain H compared to strain D (Fig. 4 a3, a6).

3.2.3. *SOD activity*

At the L stadium of strain H, significantly higher SOD activity was recorded in the control group (C) compared to the C + E group (Fig. 5 a1). This represents the only seen effect of ethanol as a solvent relative to the control across all analysed time points and strains. Notably, SOD activity in the C group did not differ significantly from the values

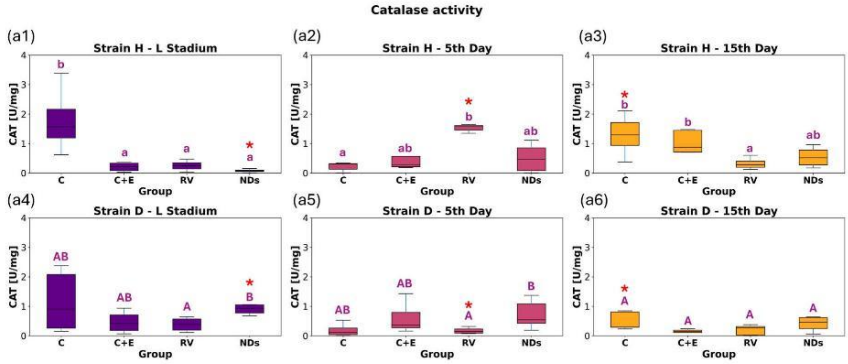

Fig. 4. Box plots showing medians and IQRs for catalase (CAT) activity in *Acheta domesticus* strains H and D across experimental groups and time points. Abbreviations and significance notations are shown in Fig. 3.

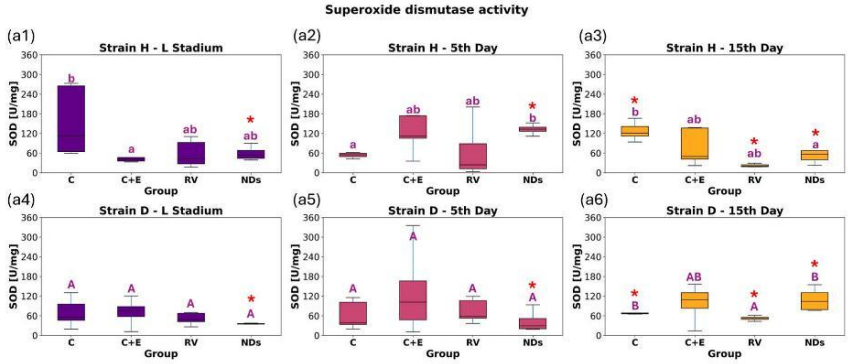

Fig. 5. Box plots showing medians and IQRs for superoxide dismutase (SOD) activity in *Acheta domesticus* strains H and D across experimental groups and time points. Abbreviations and significance notations are shown in Fig. 3.

obtained in the RV and NDs groups (Fig. 5 a1). Analyzing changes in SOD activity over time in strain H, a decrease in activity was observed in the control group on the 5th Day of adult life compared to the L stadium. In contrast, in the C + E group, the decrease in activity occurred only on the 15th Day of adult life. A similar trend to that observed in the C + E group was also found in the NDs group (Supplementary Fig. 2C). On the 5th Day of adult life of H strain insects, nanodiamond supplementation significantly increased SOD activity (Fig. 5 a2), while on the 15th Day of adult life, the opposite effect was recorded – a significant decrease in comparison to the control group (Fig. 5 a3).

In strain D, no significant changes in SOD activity were seen at either the L stadium or on the 5th Day of adult life (Fig. 5 a4, a5). The only exception was the RV group, in which a significant decrease in SOD activity was observed on the 15th Day of adult life compared to the control (Fig. 5 a6). Additionally, in L-strain insects, regardless of the group, enzyme activity did not change significantly between successive time points (Supplementary Fig. 2C).

When comparing both strains, at the L stadium and on the 5th Day of adult life, SOD activity in the NDs group was significantly higher in strain H than in strain D (Fig. 5 a1, a2, a4, a5). Moreover, on the 15th Day of adult life, in the control group, enzyme activity was higher in strain H compared to strain D, while in the RV and NDs groups, higher SOD activity was observed in strain D (Fig. 5 a3, a6).

3.2.4. *SOD inhibition*

In strain H at the L stadium, all groups (C, C + E, RV, NDs) showed comparable inhibition percentages, with no statistically significant differences observed between experimental groups (Fig. 6 a1). On the 5th Day of adult life (Fig. 6 a2), only the NDs group significantly increased superoxide reaction inhibition compared to the control group. Interestingly, RV reduced the % inhibition of the superoxide reaction, although the statistical significance of the median difference was not confirmed at this time point. On the 15th Day of adult life (Fig. 6 a3), the C group showed a significantly higher median inhibition compared to C + E and RV. RV also lowered the value of this parameter compared to the other experimental groups (C + E, NDs), while NDs significantly enhanced inhibition compared to RV (Fig. 6 a3). On time-dependent changes, in the C group of strain H, activity on the 15th Day of adult life was significantly higher than at the L stadium and on the 5th Day of adult life (Supplementary Fig. 2D). For the remaining groups (C + E, RV, and NDs) in strain H, no significant time-related changes in the percentage of inhibition were observed (Supplementary Fig. 2D).

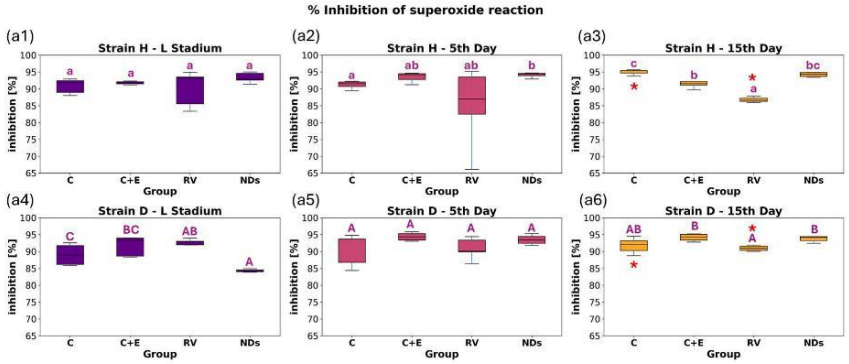

Fig. 6. Box plots showing medians and IQRs for percentage inhibition of the superoxide reaction in *Acheta domesticus* strains H and D across experimental groups and time points. Abbreviations and significance notations as in Fig. 3.

In strain D at the L stadium, NDs significantly reduced the % inhibition of the SOD-related reaction compared to the control, while RV induced the opposite effect (Fig. 6 a4). On the 5th Day of adult life, no significant differences were found between experimental groups (Fig. 6 a5). On the 15th Day of adult life, RV caused a substantial reduction in the analysed parameter compared to the C + E and NDs groups (Fig. 6 a6). Concerning time-course effects, in both control groups (C and C + E), differences across time points were not statistically significant (Supplementary Fig. 2D). RV administration resulted in a decrease, while NDs caused an increase in the % inhibition on the 5th Day of adult life compared to the L stadium. Moreover, on the 15th Day of adult life, NDs also significantly increased the % inhibition compared to the L stadium (Supplementary Fig. 2D).

Additionally, on the 15th Day of adult life, significant differences between strains were seen. In the control group, strain H showed significantly higher inhibition than strain D (Fig. 6 a3, a6). In contrast, in the RV group, the opposite trend was observed (Fig. 6 a3, a6).

3.3. Sirtuin activity in Acheta domesticus

3.3.1. Universal SIRT activity

Overall, sirtuin activity in both strains remains relatively low; however, there were some significant differences between the larval stadium and adult individuals (Supplementary Fig. 2E). Sirtuin activity tends to increase on the 5th Day of adult life compared to the larval stadium in the C and C + E groups (both strains) and the RV group (only in the D strain) (Fig. 7). On the 15th Day of adult life, SIRT activity was lower than that of the control groups, and the trend of low sirtuin activity in adult 15th day individuals persists across all experimental groups (Supplementary Fig. 2E). However, significant differences were noticed in groups C, C + E, and RV in H strain, as well as in the NDs group of strain D. When comparing strain H to strain D, there is a tendency toward slightly higher sirtuin activity in strain D, yet significant differences are observed only at the larval stadium (Fig. 7 a1, a4) as well as 5th Day stadium treated with RV (Fig. 7 a2, a5).

NDs induced SIRT activity compared to control at the larval stadium; however, this activity gradually declined in subsequent time points (Fig. 7 a1–a6). As expected, RV stimulated overall sirtuin activity in strain H, but a significant effect was detected only in young individuals (L stadium).

Interestingly, in contrast to strain H, in strain D (selected for longevity), RV did not cause significant changes in sirtuin activity in the L stadium and on the 5th Day of adult life of adult life. On the contrary, it led to a significant decrease in SIRT activity in the 15th Day group. NDs

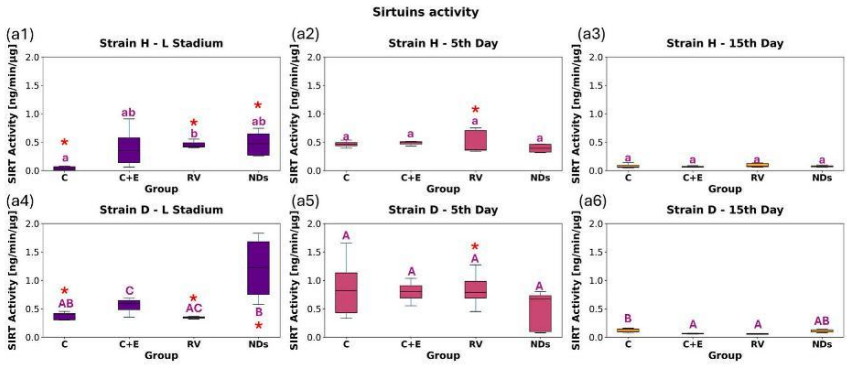

Fig. 7. Box plots showing medians and IQRs for sirtuin activity in *Acheta domesticus* strains H and D across experimental groups and time points. Abbreviations and significance notations are shown in Fig. 3.

did not significantly affect sirtuin activity in strain D. It is worth noting, however, that at the larval stadium, SIRT activity in the NDs group was high. Still, due to large data variability, significant differences were observed only between the RV and NDs groups (Fig. 7 a4).

3.3.2. SIRT 1

In the control group of strain H, the highest median SIRT1 activity was observed in adult individuals on the 15th Day of adult life of life (Supplementary Fig. 2F; Fig. 8 a1–a3). A different trend was noted in strain D, where SIRT1 activity was lowest in the oldest individuals, and the highest median was recorded in adults on the 5th Day of adult life (heatmap and Fig. 8 a4–a6). However, the differences between control groups across time points in both strains were not statistically significant (Supplementary Fig. 2F).

SIRT1 activity in both strains (H and D) in the C + E group (ethanol) did not differ significantly from the control group - regardless of insect age - indicating that ethanol as a solvent did not substantially affect this parameter (Fig. 8 a1–a6). Compared to the control, both RV and NDs led to a significant decrease in SIRT1 activity only in 5-day-old individuals of strain D (Fig. 8 a5). In other age stadia of strain D and across all age stadia in strain H, supplementation with RV or NDs did not induce statistically significant changes in SIRT1 activity compared to the control (Fig. 8 a1–a6).

The temporal dynamics of SIRT1 activity exhibited distinct trends, depending on the strain and the applied compound. In strain H, RV increased SIRT1 activity with age, from the L stadium to the 15th Day, with significantly higher activity observed on the 15th Day of adult life than in the larval stadium (Supplementary Fig. 2F). In contrast, groups receiving NDs showed an age-related decrease in SIRT1 activity in both strains. In both strains, the difference between the L stadium and the 15th Day was statistically significant (Supplementary Fig. 2F).

Additionally, only a few differences in SIRT1 activity were identified between strains. At the larval stadium, the median SIRT1 activity in the NDs group was significantly higher in strain D compared to strain H (Fig. 8 a1, a4). The opposite trend was observed on the 15th Day of adult life, where the median was higher in strain H than in strain D.

3.3.3. SIRT 6

SIRT6 activity was low at the L stadium and on the 5th Day of adult life, but increased markedly in adult insects from the 15th Day group. This pattern was observed in both strains – H and D. Importantly, the results for the 15th Day groups showed high variability, which was reflected in the statistical analysis outcomes. Despite noticeably higher medians in experimental groups on the 15th Day of adult life compared to the L stadium and 5th Day, these differences were not always statistically significant. The absence of significant differences was observed for the C + E and RV groups in strain H, as well as for all groups in strain D (Supplementary Fig. 2G).

Ethanol as a solvent also had little impact on SIRT6 activity, except for L stadium insects from strain D, where the median activity in the C + E group was significantly higher than in the control (Fig. 9 a4).

Administration of RV or NDs did not lead to statistically significant changes in SIRT6 activity, regardless of strain or developmental stadium (Fig. 9 a1–a6). Additionally, significantly higher enzyme activity was found in 15th Day adults from strain D exposed to NDs compared to the corresponding group from strain H (Fig. 9 a3, a6).

3.4. DNA damage

3.4.1. pATM

At the L stadium, in both strains, the %pATM levels were comparable across all experimental groups. In both strains, the control group (C) did not differ significantly from C + E, RV, or NDs (Fig. 10 a1). On the 5th Day of adult life, %pATM levels in the control group (C) were not significantly different from C + E. However, in strain H, %pATM levels were significantly higher in C compared to RV and NDs. Additionally, in strain H, the RV group showed lower levels than C + E (Fig. 10 a2). On the 15th Day of adult life, %pATM levels in the control group (C) of strain H were lower than in the RV group (Fig. 10 a3). Notably, the highest percentage of pATM-positive cells was recorded in the RV group, with the median significantly exceeding those of the other groups as well as earlier time points (L stadium and 5th Day).

In strain D, at the L stadium, the %pATM level in the RV group was significantly lower than in the NDs group (Fig. 10 a1). On the 5th Day of adult life, the C group did not differ significantly from the other groups; however, significant differences were observed between the remaining groups, with the highest %pATM levels found in the NDs group (Fig. 10 a2). On the 15th Day of adult life, similar to strain H, strain D showed significantly higher %pATM levels in the RV group than NDs (Fig. 10 a3). Regarding time-course analysis in strain D, the C group, median % pATM levels were significantly higher on the 15th Day of the adult life stadium compared to the L stadium and the 15th Day (Fig. 10 a3 vs. a1, a2).

In the between-strain comparison, at the L stadium, %pATM levels in the RV group were significantly higher in strain H than in strain D. On the 5th Day of adult life, significant inter-strain differences in %pATM were observed in the C + E group (lower in strain D) and in the NDs group (higher in strain D). On the 15th Day of adult life, %pATM levels

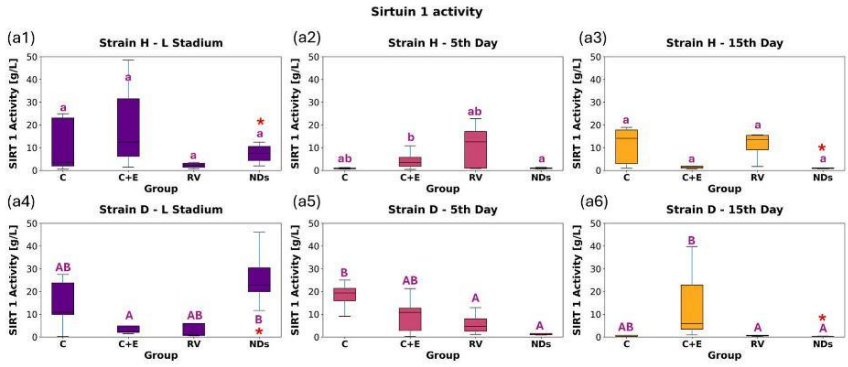

Fig. 8. Box plots showing medians and IQRs for sirtuin 1 activity in *Acheta domesticus* strains H and D across experimental groups and time points. Abbreviations and significance notations are shown in Fig. 3.

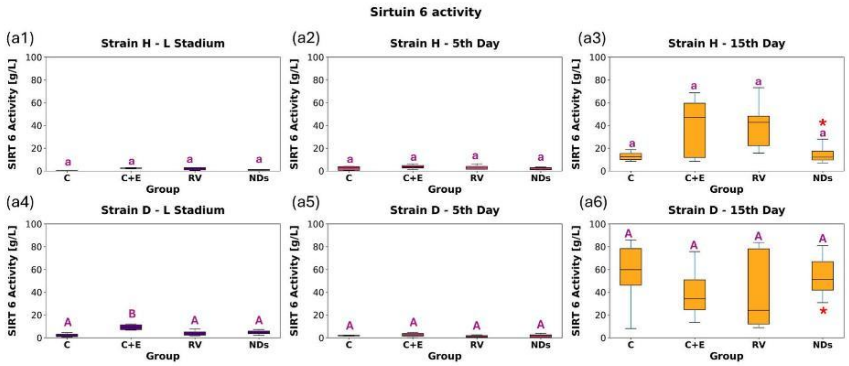

Fig. 9. Box plots showing medians and IQRs for sirtuin 6 activity in *Acheta domesticus* strains H and D across experimental groups and time points. Abbreviations and significance notations as in Fig. 3.

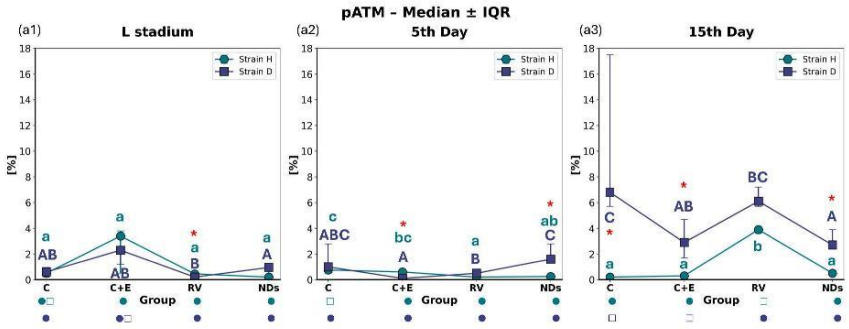

Fig. 10. Line plots show medians (horizontal line) and interquartile ranges (IQR; box spans Q1–Q3); whiskers extend to values within 1.5 × IQR from the quartiles. Data represent phosphorylated ATM kinase (pATM) levels in two *Acheta domesticus* strains (H and D), across four experimental groups (C, C + E, RV, NDs) at three time points: larval stage (a1), and the 5th (a2) and 15th (a3) day of adulthood. Statistical analysis was performed using PERMANOVA ($p < 0.05$, corrected) and PERMDISP ($p \geq 0.05$) (Python, scikit-bio). Lowercase letters indicate significant differences ($p < 0.05$) between groups within strain H; uppercase letters indicate differences within strain D for the same time point. Asterisks (*) indicate differences between strains within the same group and time point. Symbols under the x-axis (circles, squares, triangles) denote within-group comparisons across time; identical symbols indicate no significant change.

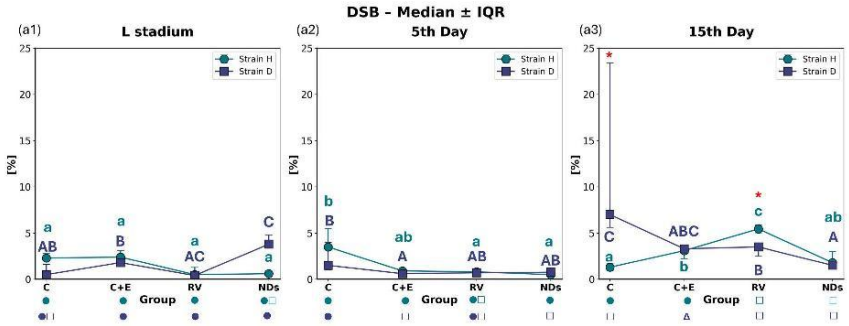

Fig. 11. Line plots showing medians and interquartile ranges (IQRs) of DNA double-strand breaks (DSBs) in *Acheta domesticus* strains H and D across three time points (larval stage, 5th and 15th day of adulthood) and four experimental groups. Abbreviations and statistical notations are as shown in Fig. 10.

in the C, C + E, and NDs groups were significantly higher in strain D compared to strain H (Fig. 10).

3.4.2. DSB

In strain H, at the L stadium, no significant differences were observed between the experimental groups Fig. 11 a1). On the 5th Day of adult life, the level of DSBs in the control group (C) was higher than in the remaining groups; however, statistically significant differences were found only when comparing the control to the RV and NDs groups (Fig. 11 a2). On the 15th Day of adult life, DSB levels in groups C, C + E, and NDs were lower than in the RV group, which showed the highest level of DNA damage (Fig. 11 a3). In the NDs group, a significant increase in %DSBs was also recorded, although this was explicitly compared between the 5th and 15th Days (Fig. 11 a2 vs. a3).

In strain D at the L stadium, the DSB level in the control group (C) was significantly lower than in the NDs group (Fig. 11a1). On the 5th Day of adult life, the control group showed higher DSB levels than the C + E group (Fig. 11 a2). On the 15th Day of adult life, no significant differences were found between groups C and C + E (Fig. 11 a3). However, the DSB level in the control group was significantly higher than in the RV group, which in turn showed a significant increase compared to the L stadium (Fig. 11 a3 vs. a1).

In the between-strain comparison, on the 15th Day of adult life, the DSB level in the control group was significantly higher in strain D than in strain H. An opposite trend was observed for the RV group, where strain H exhibited significantly higher DSB levels than strain D (Fig. 11).

3.4.3. H2A.X

In strain H, at the L stadium, γH2A.X levels were low across all experimental groups (Fig. 12 a1). Notably, in the NDs group, γH2A.X levels were significantly higher compared to the 5th Day (Fig. 12 a1, a2). On the 5th Day of adult life, a significant decrease in γH2A.X levels was observed in the NDs group relative to C + E, whereas no differences were found between C + E and the control group (Fig. 12 a2). On the 15th Day of adult life, γH2A.X levels remained similar across all groups, with no significant changes compared to earlier time points (Fig. 12 a3).

In strain D, a similar trend to that observed in strain H was noted - at the L stadium, γH2A.X levels were low in all groups (Fig. 12 a1). On the 5th Day of adult life, the control group (C) exhibited lower γH2A.X levels compared to C + E and RV, with the difference between C and C + E being statistically significant (Fig. 12 a2). On the 15th Day of adult life, γH2A.X levels in the NDs group were significantly lower than in the control group (Fig. 12 a3).

In the between-strain comparison at the 15th Day, γH2A.X levels were significantly higher in strain H than in strain D, both in the control and NDs groups (Fig. 12 a3).

4. Discussion

This study is a continuation of our previously published research [21] in which we analysed the effects of RV and NDs on SIRT1 activity in *Acheta domesticus* within two lines: the wild-type (H) and the longevity-selected (D) line. In that study, SIRT6 activity was not examined, and markers of oxidative stress (CAT, SOD, LPO) in larvae were omitted; the assessment was limited to selected time points in adult individuals. However, it was clearly observed that the response to the tested compounds was complex and dependent on age and line, suggesting the potential involvement of longevity-related regulatory mechanisms and the need for more in-depth analyses. The present study was therefore expanded to include earlier developmental stages (larvae), added measurements of SIRT6 activity, assessed oxidative stress markers, and conducted a detailed survival analysis under long-term exposure conditions. This approach not only allowed us to verify earlier conclusions but also to gain a better understanding of whether and how RV and NDs influence aging and longevity processes in a strain- and life stage-dependent manner.

4.1. Strain-specific differences in sirtuins

Variability and uniqueness are among the cornerstones of biology, but they also constitute prerequisites for evolution, adaptation, and even a challenge for experimental studies [30,31]. Even within a single species and often within a single population, organisms exhibit significant phenotypic and molecular differences, including variations in aging rate, stress resistance, and DNA repair efficiency [32,33]. In the context of aging, sirtuins are considered essential regulators that not only potentially modulate metabolism but also influence genome stability and responses to oxidative stress [1]. In recent years, particular attention has been paid to SIRT1 and SIRT6, whose activity in animal models has been associated with longevity and resistance to cellular damage [34–37]. Hypothesis H1 assumed that the expression and activity of selected sirtuins, particularly SIRT1 and SIRT6, differ significantly between the wild-type (H) and longevity-selected (D) strains, with higher activity in the D strain potentially supporting mechanisms that promote longevity. However, neither total sirtuin activity nor the activity of specific isoforms (SIRT1, SIRT6) showed consistent or significant differences between the H and D strains. Only at isolated time points or in specific experimental groups (e.g., universal sirtuin activity or SIRT1 in the larval stage under NDs treatment) (Figs. 7 and 8 a1 vs a4) were statistical differences observed, but these did not form a coherent pattern. Importantly, inter-strain differences were not restricted to a single developmental stage but occurred at different time points depending on the analysed parameter. Nevertheless, for several markers

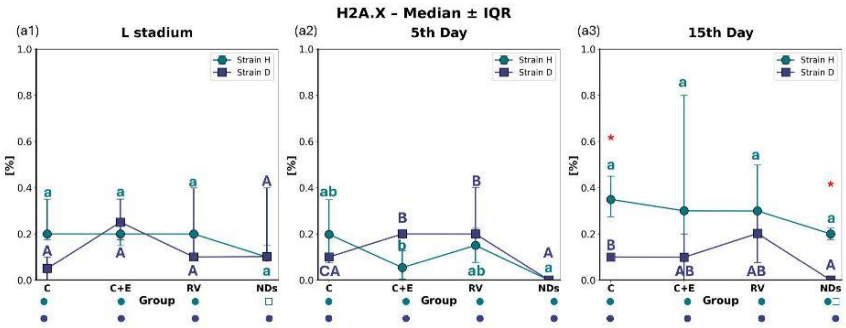

Fig. 12. Line plots showing medians and interquartile ranges (IQRs) of γH2AX levels in *Acheta domesticus* strains H and D across three time points (larval stage, 5th and 15th day of adulthood) and four experimental groups. Abbreviations and statistical notations are as shown in Fig. 10.

the earliest differences appeared already at the larval stage, suggesting that early developmental stages may be particularly sensitive to physiological modulation. This may be because sirtuin activity is strongly regulated post-translationally and via NAD^+ [38], meaning that their basal activity may be similar, while differences could emerge only under conditions of severe stress or DNA damage, which were not induced under our experimental conditions. It is worth noting that the levels of the oxidative stress markers tested (LPO, SOD, and CAT) generally did not indicate the presence of intense oxidative stress in either strain. The changes observed were occasional and limited, lacking a consistent temporal pattern or clear relation to supplementation (Figs. 3–6). This aligns with findings by Santos et al. [39] who reported that oxidative stress modulates sirtuin activity, mildly enhancing it as a protective mechanism, but leading to dysfunction and degradation under more severe stress conditions. Similarly, Webster et al. [40] demonstrated that sirtuins regulate oxidative stress by controlling redox systems. Importantly, all experimental groups received the same base feed containing identical levels of vitamins and antioxidants (A, C, E and B complex). Therefore, potential effects of these food components were consistent across treatments and did not influence the comparative assessment of RV and NDs.

Thus, it can be observed that both strains display comparable basal activity of total SIRT, SIRT1, and SIRT6. This suggests that the mechanisms involving these enzymes are strongly genetically conserved, and even long-term selection for longevity has not altered the baseline pattern of their activity. Because sirtuin isoforms may differ among taxa, the obtained results should be interpreted as relative changes in NAD^+-dependent deacetylase activity associated with SIRT1-and SIRT6-type enzymes rather than direct measurements of specific orthologous proteins in *Acheta domesticus*. At the same time, it should be noted that sirtuin activity is highly dependent on environmental conditions, for example, in *Drosophila*, dietary stress (protein deficiency) induces the expression of the mitochondrial sirtuin dSirt4 in enterocytes, affecting gut physiology and microbiota composition [41]. This highlights the context- and species-dependent variability in sirtuin responses, as well as the difficulty of unambiguously interpreting their activity.

In our study, an increase in SIRT6 activity was observed in older individuals, which is consistent with broader observations suggesting the role of this enzyme in maintaining genome stability and DNA repair in aging organisms, as shown in *SIRT6*-deficient mice [42]. Notably, this phenomenon occurred regardless of strain (Fig. 9 a3, a6), further supporting the idea that SIRT6 may be activated in response to age-related DNA damage and cellular stress, given its established role in DNA repair and regulation [43]. Similar conclusions were reached by Taylor et al. [44] in studies on *Drosophila melanogaster*, overexpression of dSirt6 led to lifespan extension, increased resistance to oxidative stress, and reduced protein synthesis rates.

It should also be emphasized that the *Acheta domesticus* used in this study was bred through selection for delayed reproduction [45], aligning with the disposable soma theory [46]. However, the results of this study suggest that sirtuin activity (both SIRT1 and SIRT6; Figs. 8 and 9) remained unchanged despite long-term selection, which may indicate that these enzymes are not directly involved in the mechanisms responsible for increased lifespan in this selection model. This may complement the disposable soma theory, suggesting that not all molecular mechanisms involved in soma maintenance are necessarily modified through evolutionary or breeding selection.

For comparison, SIRT1 activity did not show such a pronounced association with age, which is consistent with previous observations in insects. In studies on *Drosophila melanogaster*, SIRT1 activity remained relatively stable over time, with any observed changes being more dependent on environmental factors than on age itself [47]. Research on *C. elegans* and *Drosophila* has suggested that an increase in sirtuin activity alone does not necessarily translate directly into lifespan extension unless accompanied by other metabolic changes [48]. Our findings appear to support this observation, as selection for longevity did not alter the activity patterns of SIRT1 or SIRT6 (Figs. 8 and 9). This may indicate that other mechanisms, such as energy metabolism or autophagy, play a more critical role in longevity in *A. domesticus*.

4.2. The impact of RV and NDs on sirtuin activity and their potential significance for longevity

Another hypothesis we tested assumed that RV and NDs modify sirtuin activity, thereby influencing the potential lifespan of *A. domesticus*. We also hypothesized that organisms from the long-lived strain might respond differently to these compounds. Our results indicate that such an effect cannot be clearly confirmed. Importantly, the lifespan-related effects observed in some experimental groups were not accompanied by a consistent or sustained increase in sirtuin activity. This suggests that although sirtuin-related pathways may participate in the physiological responses to RV or NDs, they are unlikely to represent the sole mechanism underlying the observed survival patterns.

Researchers describe RV as a SIRT1 activator, acting, for example, through mechanisms associated with caloric restriction [49,50], but also via modulation of energy metabolism (PGC-1α, PPARα), enhancement of oxidative stress resistance (activation of FOXO and antioxidant genes), as well as through effects on genome stability and inflammatory control (p53, NF-κB) [51,52]. However, in our study, this effect was weak and limited to isolated groups (Fig. 8 a2, a4). Notably, the most visible response was observed in the wild-type strain (H) at the larval stage, whereas the longevity-selected strain (D) showed little or no response to RV supplementation. This may indicate that organisms selected for longevity possess more stable regulatory mechanisms that limit the responsiveness of sirtuin pathways to external modulators. Similar observations have been reported in insect models such as *Drosophila*, where the effect of RV was highly dependent on diet and sex, and its influence on lifespan was ambiguous or insignificant [53]. It can therefore be assumed that RV does not always act as a universal "longevity elixir," and its effects are strongly dependent on biological context. Mansouri [54] also reported that the impact of RV depends on both duration and dosage of treatment. Furthermore, our supplementary data (LPO, CAT, pATM, DSBs) did not confirm that RV protects against oxidative stress or DNA damage. In some cases (e.g., elevated pATM levels in the RV group on day 15; Fig. 10), RV was even associated with an increase in DNA damage. Similar bifunctional (pro- and antioxidant) effects of RV, depending on dose and exposure time, have been described in the literature [55,56].

It should be emphasized that supplementation was maintained throughout the insects' entire lifespan in this experiment, which may have led to physiological adaptation and attenuation of treatment effects at later life stages. Survival analysis indicates that the most pronounced differences between groups occurred during early and mid-life, while the survival curves converged at later ages (Fig. 2). In the wild-type strain (H), nanodiamonds primarily increased median lifespan, suggesting an improvement in early-life survival rather than a substantial extension of extreme longevity. In contrast, RV had a limited effect on median lifespan but showed a tendency to extend maximum lifespan. Interestingly, the C + E group exhibited a reduced median lifespan compared with the control, although the maximum lifespan remained similar. This pattern likely reflects a small shift in early survival events rather than a consistent biological effect, as other physiological and biochemical parameters did not differ between the C and C + E groups. In the strain D, neither RV nor NDs significantly altered survival patterns. Median lifespan remained similar across treatments, and only minor differences in maximum lifespan were observed. This relative stability suggests that organisms from the longevity-selected strain may be less responsive to external modulators, possibly due to genetic mechanisms already optimizing longevity-related physiological processes (Fig. 2). Although biochemical and molecular analyses were limited to early adult stages, these time points were selected to capture initial physiological responses to long-term exposure. Future studies

extending the analysis to later life stages could further clarify how these mechanisms relate to aging processes.

Correlation analysis (Supplementary Table 2) revealed several significant associations between selected biochemical and molecular parameters. In particular, SOD activity was positively correlated with SIRT1 and negatively correlated with SIRT6, while the percentage inhibition of the SOD-related reaction was positively correlated with total SIRT activity. In addition, total SIRT activity was negatively correlated with γH2A.X, and γH2A.X was positively correlated with pATM. However, these relationships were selective rather than uniform, indicating partial links between oxidative stress, sirtuin-related responses and DNA damage signaling, rather than one consistent pattern across all analysed parameters.

Our previous research indicated that the differences in survival and responses to RV and NDs between *Acheta domesticus* strains (H and D) may result from distinct molecular and regulatory mechanisms shaped by selection for longevity, and that the stimulation of SIRT1 activity by both compounds depended on time and treatment type [21]. Similarly [57], showed that RV supplementation at a dose of 500 μmol/L did not affect lifespan, body composition, locomotor activity, or expression of genes related to oxidative stress and longevity in *Drosophila melanogaster*. Other studies on *D. melanogaster* by Wang et al. [58] also demonstrated that the impact of RV on lifespan is highly dependent on diet composition and sex, with effects appearing only under specific nutritional conditions such as low-sugar and high-protein diets. These findings confirm the context dependency and limited persistence of RV's effects.

NDs, although increasingly investigated as potentially safe nanomaterials for nanomedicine, remain controversial [59]. In our study, no apparent effect of NDs on SIRT1 activity was observed, except on day 5 in the D strain, where SIRT1 activity (Fig. 8) was significantly lower than in the control. Nevertheless, the action of NDs is challenging to interpret and often depends on dose, developmental stage, and particle characteristics. Augustyniak et al. [27] showed that responses to NDs can vary between strains and change with age, and that observed effects do not always follow a consistent pattern. Other studies [60] demonstrated that low ND concentrations may act hormetically by inducing mild stress and activating cellular defense mechanisms or sirtuins. Our results suggest that the increase in general sirtuin activity observed in larvae exposed to NDs (Fig. 7) may reflect such a short-term adaptive response to mild stress, which in later developmental stages becomes compensated or neutralized by other mechanisms. The increase in SIRT6 activity (Fig. 9) observed in older individuals appeared to be independent of the compounds administered (NDs, RV) and likely represents a general aging-related physiological response.

In multigenerational studies with low ND concentrations (0.2 and 2 mg/kg), differences between strains were observed in CAT, TAC, apoptosis, and ROS levels [11]. These findings indicate that NDs may induce oxidative stress, but the effects depend strongly on dose, duration, and strain. In our experiment, the absence of clear changes in oxidative stress markers (LPO, CAT, SOD) may be due to the relatively low NDs dose, physiological adaptation during long-term exposure, or compensatory defense mechanisms. At this dosage, NDs appear to be relatively safe for chronic exposure, as Kaplan-Meier survival curves (Supplementary Fig. 2) and log-rank test results indicate that NDs did not show toxicity leading to reduced lifespan. In both strains (H and D), survival in the ND groups was comparable to that of the controls. In the H strain, it was even slightly higher during early life stages, suggesting a potential protective effect.

5. Conclusions

This study offers new insights into the regulation of sirtuins in *Acheta domesticus*, an insect species undergoing incomplete metamorphosis, thereby filling a knowledge gap in the understanding of aging mechanisms in hemimetabolous organisms. The analysis of SIRT1 and SIRT6 activity, as well as oxidative stress markers, in two genetic strains revealed that the response to RV and NDs supplementation depends on both life stage and strain but does not form a clear pattern that supports the hypothesis of a lasting impact on lifespan. Fascinating is the observation that the longevity-selected strain did not show noticeable changes in sirtuin activity or survival, which may suggest that longevity-regulating mechanisms are strongly genetically conserved and resistant to modulation by external factors such as RV or NDs.

Both RV and NDs, although often described as compounds with potential health-promoting properties, showed limited and highly context-dependent effects. The observed responses, particularly the transient increase in sirtuin activity in larvae exposed to NDs and minor changes in oxidative stress parameters, suggest adaptive rather than lasting effects. This interpretation is further supported by survival data, which showed no differences in long-term survival between the NDs-treated and control groups, suggesting low toxicity in this model but no evidence of a protective or life-extending effect.

The results of this study represent a step forward in understanding the biological basis of responses to environmental factors, while also highlighting the need for caution. In an era of increasing use of dietary supplements, pharmaceuticals, and bioactive compounds, it becomes essential not only to conduct experiments but also to provide precise characterization of the tested substances, their origin, and dosage. Many studies fail to specify whether the examined RV is derived from natural sources or chemical synthesis, which may be critical for interpreting results, assessing safety, and ensuring interdisciplinary responsibility. Our research integrates perspectives from developmental biology, physiology, and toxicology, which are necessary to understand the complexity of aging processes better and to evaluate the true potential and limitations of compounds increasingly discussed in the context of "elixirs of youth".

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CRediT authorship contribution statement

Patrycja Ziętara-Krzyk: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Software, Visualization, Writing – original draft, Writing – review & editing. **Barbara Flasz:** Formal analysis, Investigation, Methodology, Validation. **Maria Augustyniak:** Conceptualization, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2026.153698.

Abbreviations

ATM	Ataxia Telangiectasia Mutated
CAT	Catalase
CFU	colony-forming unit
D strain	Long-lived strain of *Acheta domesticus*
EAA	essential amino acids
FOXO	Proteins belonging to the forkhead box O family
H strain	Wild-type strain of *Acheta domesticus*
H2A.X	Histone H2A variant X
H_2O	water
H_2O_2	hydrogen peroxide
LPO	Lipid peroxidation
NAD+	Nicotinamide adenine dinucleotide oxidized
NDs	Nanodiamonds
O_2	Oxygen
PBS	Phosphate-buffered saline
ppm	Parts per million
ROS	Reactive oxygen species
RT	Room temperature
RV	Resveratrol
SIRT	Sirtuin
SOD	Superoxide Dismutase

Data availability

Raw data are provided on the RepOD database; DOI: https://doi.org/10.18150/ELZ4UR (https://repod.icm.edu.pl/loginpage.xhtml?redirectPage=%2Fdataset.xhtml%3FpersistentId%3Ddoi%3A10.18150%2FELZ4UR).

References

[1] P. Ziętara, M. Dziewięcka, M. Augustyniak, Why is longevity still a scientific mystery? Sirtuins-Past, present and future, Int. J. Mol. Sci. 24 (2022) 728, https://doi.org/10.3390/ijms24010728.

[2] M. Morigi, L. Perico, A. Benigni, Sirtuins in renal health and disease, J. Am. Soc. Nephrol. 29 (2018) 1799–1809, https://doi.org/10.1681/ASN.2017111218.

[3] J. Couzin-Frankel, Aging genes: the sirtuin story unravels, Science (1979) 334 (2011) 1194–1198, https://doi.org/10.1126/science.334.6060.1194.

[4] A. Brunet, L.B. Sweeney, J.F. Sturgill, K.F. Chua, P.L. Greer, Y. Lin, H. Tran, S. E. Ross, R. Mostoslavsky, H.Y. Cohen, L.S. Hu, H.-L. Cheng, M.P. Jedrychowski, S. P. Gygi, D.A. Sinclair, F.W. Alt, M.E. Greenberg, Stress-Dependent regulation of FOXO transcription factors by the SIRT1 deacetylase, Science 303 (1979) 2011–2015, https://doi.org/10.1126/science.1094637, 2004.

[5] J. Song, L. Liu, K. Hao, S. Mao, Y. Tang, X. Tong, F. Dai, Resveratrol elongates the lifespan and improves antioxidant activity in the silkworm Bombyx mori, J. Pharm. Anal. 11 (2021) 374–382, https://doi.org/10.1016/j.jpha.2020.06.005.

[6] M. Chudzińska, D. Rogowicz, Ł. Wołowiec, J. Banach, S. Sielski, R. Bujak, A. Sinkiewicz, G. Grześk, Resveratrol and cardiovascular system—the unfulfilled hopes, Ir. J. Med. Sci. 190 (1971) 981–986, https://doi.org/10.1007/s11845-020-02441-x, 2021.

[7] F. Pan, M. Khan, A.H. Ragab, E. Javed, H.A. Alsalmah, I. Khan, T. Lei, A. Hussain, A. Mohamed, A. Zada, M.Z. Ansari, Recent advances in the structure and biomedical applications of nanodiamonds and their future perspectives, Mater. Des. 233 (2023) 112179, https://doi.org/10.1016/j.matdes.2023.112179.

[8] J. Xu, E.K.-H. Chow, Biomedical applications of nanodiamonds: from drug-delivery to diagnostics, SLAS Technol 28 (2023) 214–222, https://doi.org/10.1016/j.slast.2023.03.007.

[9] Y. Li, X. Zhu, H. Zhang, Y. Lu, T. Zeng, H. Liu, T. Li, J. Wang, L. Tang, Nanodiamond in cancer theranostics, Nano TransMed 2 (2023) e9130019, https://doi.org/10.26599/NTM.2023.9130019.

[10] D.-D. Zhou, M. Luo, S.-Y. Huang, A. Saimaiti, A. Shang, R.-Y. Gan, H.-B. Li, Effects and mechanisms of resveratrol on aging and age-related diseases, Oxid. Med. Cell. Longev. 2021 (2021), https://doi.org/10.1155/2021/9932218.

[11] M. Augustyniak, A. Babczyńska, M. Dziewięcka, B. Flasz, J. Karpeta-Kaczmarek, A. Kędziorski, B. Mazur, K. Rozpędek, R. Seyed Alian, M. Skowronek, E. Świerczek, A. Świętek, M. Tarnawska, K. Wiśniewska, P. Ziętara, Does age pay off? Effects of three-generational experiments of nanodiamond exposure and withdrawal in wild and longevity-selected model animals, Chemosphere 303 (2022) 135129, https://doi.org/10.1016/j.chemosphere.2022.135129.

[12] E. Maldonado, S. Morales-Pison, F. Urbina, A. Solari, Aging hallmarks and the role of oxidative stress, Antioxidants 12 (2023) 651, https://doi.org/10.3390/antiox12030651.

[13] J. Yang, J. Luo, X. Tian, Y. Zhao, Y. Li, X. Wu, Progress in understanding oxidative stress, aging, and aging-related diseases, Antioxidants 13 (2024) 394, https://doi.org/10.3390/antiox13040394.

[14] S. Anwar, F. Alrumaihi, T. Sarwar, A.Y. Babiker, A.A. Khan, S.V. Prabhu, A. H. Rahmani, Exploring therapeutic potential of catalase: strategies in disease prevention and management, Biomolecules 14 (2024) 697, https://doi.org/10.3390/biom14060697.

[15] A. García-Sánchez, A.G. Miranda-Díaz, E.G. Cardona-Muñoz, The role of oxidative stress in physiopathology and pharmacological treatment with Pro- and antioxidant properties in chronic diseases, Oxid. Med. Cell. Longev. 2020 (2020) 1–16, https://doi.org/10.1155/2020/2082145.

[16] H. Younus, Therapeutic potentials of superoxide dismutase, Int. J. Health Sci. 12 (2018) 88–93.

[17] A.R. Mahmud, T.I. Ema, MohdF.-R. Siddiquee, A. Shahriar, H. Ahmed, Md Mosfeq-Ul-Hasan, N. Rahman, R. Islam, M.R. Uddin, MdF.R. Mizan, Natural flavonols: actions, mechanisms, and potential therapeutic utility for various diseases, Beni. Suef. Univ. J. Basic Appl. Sci. 12 (2023) 47, https://doi.org/10.1186/s43088-023-00387-4.

[18] E.E. Aslantas, H.D. Buzoglu, S.F. Muftuoglu, P. Atilla, S.P. Karapinar, Y. Aksoy, Effects of aging and inflammation on catalase activity in human dental pulp, Arch. Oral Biol. 141 (2022) 105482, https://doi.org/10.1016/j.archoralbio.2022.105482.

[19] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal, Oxid. Med. Cell. Longev. 2014 (2014) 1–31, https://doi.org/10.1155/2014/360438.

[20] Z. Pan, H. Dong, N. Huang, J. Fang, Oxidative stress and inflammation regulation of sirtuins: new insights into common oral diseases, Front. Physiol. 13 (2022), https://doi.org/10.3389/fphys.2022.953078.

[21] P. Ziętara, B. Flasz, M. Augustyniak, Does selection for longevity in Acheta domesticus involve Sirtuin activity modulation and differential response to activators (Resveratrol and Nanodiamonds)? Int. J. Mol. Sci. 25 (2024) 1329, https://doi.org/10.3390/ijms25021329.

[22] A.T. Dossey, B. Oppert, F.-C. Chu, M.D. Lorenzen, B. Scheffler, S. Simpson, S. Koren, J.S. Johnston, K. Kataoka, K. Ide, Genome and genetic engineering of the house cricket (Acheta domesticus): a resource for sustainable agriculture, Biomolecules 13 (2023) 589, https://doi.org/10.3390/biom13040589.

[23] J.W. Truman, L.M. Riddiford, The evolution of insect metamorphosis: a developmental and endocrine view, Phil. Trans. Biol. Sci. 374 (2019) 20190070, https://doi.org/10.1098/rstb.2019.0070.

[24] H.W. Horch, T. Mito, A. Popadić, H. Ohuchi, S. Noji, The Cricket as a Model Organism, Springer Japan, Tokyo, 2017, https://doi.org/10.1007/978-4-431-56478-2.

[25] B. Flasz, M. Dziewięcka, A.K. Ajay, M. Tarnawska, A. Babczyńska, A. Kędziorski, Ł. Napora-Rutkowski, P. Ziętara, E. Świerczek, M. Augustyniak, Age- and lifespan-dependent differences in GO caused DNA damage in Acheta domesticus, Int. J. Mol. Sci. 24 (2022) 290, https://doi.org/10.3390/ijms24010290.

[26] M. Dziewięcka, J. Karpeta-Kaczmarek, M. Augustyniak, Ł. Majchrzycki, M. A. Augustyniak-Jabłokow, Evaluation of in vivo graphene oxide toxicity for Acheta domesticus in relation to nanomaterial purity and time passed from the exposure, J. Hazard. Mater. 305 (2016) 30–40, https://doi.org/10.1016/j.jhazmat.2015.11.021.

[27] M. Augustyniak, A.K. Ajay, A. Kędziorski, M. Tarnawska, M. Rost-Roszkowska, B. Flasz, A. Babczyńska, B. Mazur, K. Rozpędek, R.S. Alian, M. Skowronek, E. Świerczek, K. Wiśniewska, P. Ziętara, Survival, growth and digestive functions after exposure to nanodiamonds - transgenerational effects beyond contact time in house cricket strains, Chemosphere 349 (2023) 140809, https://doi.org/10.1016/j.chemosphere.2023.140809.

[28] J.P. Madigan, DNA double-strand break-induced phosphorylation of Drosophila histone variant H2Av helps prevent radiation-induced apoptosis, Nucleic Acids Res. 30 (2002) 3698–3705, https://doi.org/10.1093/nar/gkf496.

[29] J.R. Simmons, R. An, B. Amankwaa, S. Zayac, J. Kemp, M. Labrador, Phosphorylated histone variant γH2Av is associated with chromatin insulators in Drosophila, PLoS Genet. 18 (2022) e1010396, https://doi.org/10.1371/journal.pgen.1010396.

[30] T. Badrick, Biological variation: understanding why it is so important? Pract. Lab. Med. 23 (2021) e00199 https://doi.org/10.1016/j.plabm.2020.e00199.

[31] D.J. Nicholson, Population thinking and the uniqueness of biological entities, Acta Biotheor. 73 (2025) 8, https://doi.org/10.1007/s10441-025-09498-0.

[32] C. López-Otín, M.A. Blasco, L. Partridge, M. Serrano, G. Kroemer, Hallmarks of aging: an expanding universe, Cell 186 (2023) 243–278, https://doi.org/10.1016/j.cell.2022.11.001.

[33] B. Schumacher, J. Pothof, J. Vijg, J.H.J. Hoeijmakers, The central role of DNA damage in the ageing process, Nature 592 (2021) 695–703, https://doi.org/10.1038/s41586-021-03307-7.

[34] M.H. Levine, Z. Wang, H. Xiao, J. Jiao, L. Wang, T.R. Bhatti, W.W. Hancock, U. H. Beier, Targeting Sirtuin-1 prolongs murine renal allograft survival and function, Kidney Int. 89 (2016) 1016–1026, https://doi.org/10.1016/j.kint.2015.12.051.

[35] V.P. Mourits, L.S. Helder, V. Matzaraki, V.A.C.M. Koeken, L. Groh, L.C.J. de Bree, S. J.C.F.M. Moorlag, C.D.C.C. van der Heijden, S.T. Keating, J.H. van Puffelen, M. Jaeger, L.A.B. Joosten, M.G. Netea, The role of sirtuin 1 on the induction of trained immunity, Cell. Immunol. 366 (2021) 104393, https://doi.org/10.1016/j.cellimm.2021.104393.

[36] H.-Y. Jang, D.H. Ha, S.-Y. Rah, D.-H. Lee, S.-M. Lee, B.-H. Park, Sirtuin 6 is a negative regulator of FcεRI signaling and anaphylactic responses, J. Allergy Clin. Immunol. 149 (2022) 156–167.e7, https://doi.org/10.1016/j.jaci.2021.05.012.

[37] N. Shukla, U. Kolthur-Seetharam, Drosophila Sirtuin 6 mediates developmental diet-dependent programming of adult physiology and survival, Aging Cell 21 (2022), https://doi.org/10.1111/acel.13576.

[38] S. Imai, L. Guarente, NAD+ and sirtuins in aging and disease, Trends Cell Biol. 24 (2014) 464–471, https://doi.org/10.1016/j.tcb.2014.04.002.

[39] L. Santos, C. Escande, A. Denicola, Potential modulation of sirtuins by oxidative stress, Oxid. Med. Cell. Longev. 2016 (2016), https://doi.org/10.1155/2016/9831825.

[40] B.R. Webster, Z. Lu, M.N. Sack, I. Scott, The role of sirtuins in modulating redox stressors, Free Radic. Biol. Med. 52 (2012) 281–290, https://doi.org/10.1016/j.freeradbiomed.2011.10.484.

[41] M. Knop, C. Treitz, S. Bettendorf, J. Bossen, J. von Frieling, S. Doms, A. Saboukh, I. Bruchhaus, R.P. Kühnlein, J.F. Baines, A. Tholey, T. Roeder, Mitochondrial sirtuin 4 shapes the intestinal microbiota of Drosophila by controlling lysozyme expression, Anim. Microbiome 7 (2025) 63, https://doi.org/10.1186/s42523-025-00431-x.

[42] R. Mostoslavsky, K.F. Chua, D.B. Lombard, W.W. Pang, M.R. Fischer, L. Gellon, P. Liu, G. Mostoslavsky, S. Franco, M.M. Murphy, K.D. Mills, P. Patel, J.T. Hsu, A. L. Hong, E. Ford, H.-L. Cheng, C. Kennedy, N. Nunez, R. Bronson, D. Frendewey, W. Auerbach, D. Valenzuela, M. Karow, M.O. Hottiger, S. Hursting, J.C. Barrett, L. Guarente, R. Mulligan, B. Demple, G.D. Yancopoulos, F.W. Alt, Genomic instability and aging-like phenotype in the absence of Mammalian SIRT6, Cell 124 (2006) 315–329, https://doi.org/10.1016/j.cell.2005.11.044.

[43] Z. Mao, C. Hine, X. Tian, M. Van Meter, M. Au, A. Vaidya, A. Seluanov, V. Gorbunova, SIRT6 promotes DNA repair under stress by activating PARP1, Science (1979) 332 (2011) 1443–1446, https://doi.org/10.1126/science.1202723.

[44] J.R. Taylor, J.G. Wood, E. Mizerak, S. Hinthorn, J. Liu, M. Finn, S. Gordon, L. Zingas, C. Chang, M.A. Klein, J.M. Denu, V. Gorbunova, A. Seluanov, J.D. Boeke, J.M. Sedivy, S.L. Helfand, Sirt6 regulates lifespan in *Drosophila melanogaster*, Proc. Natl. Acad. Sci. 119 (2022), https://doi.org/10.1073/pnas.2111176119.

[45] B. Flasz, M. Dziewięcka, A. Kędziorski, M. Tarnawska, J. Augustyniak, M. Augustyniak, Multigenerational selective towards longevity changes the protective role of vitamin C against graphene oxide-induced oxidative stress in house crickets, Environ. Pollut. 290 (2021) 117996, https://doi.org/10.1016/j.envpol.2021.117996.

[46] T.B.L. Kirkwood, R. Holliday, The evolution of ageing and longevity, Proc. R. Soc. Lond. B Biol. Sci. 205 (1979) 531–546, https://doi.org/10.1098/rspb.1979.0083.

[47] K.Kr Banerjee, C. Ayyub, S.Z. Ali, V. Mandot, N.G. Prasad, U. Kolthur-Seetharam, dSir2 in the adult fat body, but not in muscles, regulates life span in a diet-dependent manner, Cell Rep. 2 (2012) 1485–1491, https://doi.org/10.1016/j.celrep.2012.11.013.

[48] C. Burnett, S. Valentini, F. Cabreiro, M. Goss, M. Somogyvári, M.D. Piper, M. Hoddinott, G.L. Sutphin, V. Leko, J.J. McElwee, R.P. Vazquez-Manrique, A.-M. Orfila, D. Ackerman, C. Au, G. Vinti, M. Riesen, K. Howard, C. Neri, A. Bedalov, M. Kaeberlein, C. Söti, L. Partridge, D. Gems, Absence of effects of Sir 2 overexpression on lifespan in C. elegans and Drosophila, Nature 477 (2011) 482–485, https://doi.org/10.1038/nature10296.

[49] B. Rogina, H.A. Tissenbaum, SIRT1, resveratrol and aging, Front. Genet. 15 (2024), https://doi.org/10.3389/fgene.2024.1393181.

[50] K. Pallauf, I. Günther, G. Kühn, D. Chin, S. de Pascual-Teresa, G. Rimbach, The Potential of Resveratrol to Act as a caloric restriction mimetic appears to be limited: insights from studies in mice, Adv. Nutr. 12 (2021) 995–1005, https://doi.org/10.1093/advances/nmaa148.

[51] Y. Fu, Y. Wang, Y. Liu, C. Tang, J. Cai, G. Chen, Z. Dong, p53/sirtuin 1/NF-κB signaling axis in chronic inflammation and maladaptive kidney repair after Cisplatin nephrotoxicity, Front. Immunol. 13 (2022), https://doi.org/10.3389/fimmu.2022.925738.

[52] C. Kalliora, I.D. Kyriazis, S. Oka, M.J. Lieu, Y. Yue, E. Area-Gomez, C.J. Pol, Y. Tian, W. Mizushima, A. Chin, D. Scerbo, P.C. Schulze, M. Civelek, J. Sadoshima, M. Madesh, I.J. Goldberg, K. Drosatos, Dual PPARα/γ activation inhibitsSIRT1-PGC1α axis and causes cardiac dysfunction, JCI Insight 4 (2019), https://doi.org/10.1172/jci.insight.129556.

[53] T.M. Bass, D. Weinkove, K. Houthoofd, D. Gems, L. Partridge, Effects of resveratrol on lifespan in Drosophila melanogaster and Caenorhabditis elegans, Mech. Ageing Dev. 128 (2007) 546–552, https://doi.org/10.1016/j.mad.2007.07.007.

[54] F. Mansouri, G. Feliziani, L. Bordoni, R. Gabbianelli, Impact of resveratrol supplementation on Human Sirtuin 1: a grading of recommendations assessment, development and evaluation–assessed systematic review and dose-response meta-analysis of randomized controlled trials, Acad. Nutr. Diet. 125 (2025) 1299–1314. e1, https://doi.org/10.1016/j.jand.2025.03.011.

[55] L.-X. Zhang, C.-X. Li, M.U. Kakar, M.S. Khan, P.-F. Wu, R.M. Amir, D.-F. Dai, M. Naveed, Q.-Y. Li, M. Saeed, J.-Q. Shen, S.A. Rajput, J.-H. Li, Resveratrol (RV): a pharmacological review and call for further research, Biomed. Pharmacother. 143 (2021) 112164, https://doi.org/10.1016/j.biopha.2021.112164.

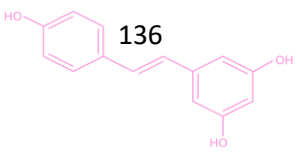
[56] B. Salehi, A.P. Mishra, M. Nigam, B. Sener, M. Kilic, M. Sharifi-Rad, P.V.T. Fokou, N. Martins, J. Sharifi-Rad, Resveratrol: a double-edged sword in health benefits, Biomedicines 6 (2018) 91, https://doi.org/10.3390/biomedicines6030091.

[57] S. Staats, A. Wagner, B. Kowalewski, F. Rieck, S. Soukup, S. Kulling, G. Rimbach, Dietary resveratrol does not affect life span, body composition, stress response, and longevity-related gene expression in Drosophila melanogaster, Int. J. Mol. Sci. 19 (2018) 223, https://doi.org/10.3390/ijms19010223.

[58] C. Wang, C.T. Wheeler, T. Alberico, X. Sun, J. Seeberger, M. Laslo, E. Spangler, B. Kern, R. de Cabo, S. Zou, The effect of resveratrol on lifespan depends on both gender and dietary nutrient composition in Drosophila melanogaster, Age (Omaha). 35 (2013) 69–81, https://doi.org/10.1007/s11357-011-9332-3.

[59] J.-X. Qin, X.-G. Yang, C.-F. Lv, Y.-Z. Li, K.-K. Liu, J.-H. Zang, X. Yang, L. Dong, C.-X. Shan, Nanodiamonds: synthesis, properties, and applications in nanomedicine, Mater. Des. 210 (2021) 110091, https://doi.org/10.1016/j.matdes.2021.110091.

[60] J. Mytych, M. Wnuk, S.I.S. Rattan, Low doses of nanodiamonds and silica nanoparticles have beneficial hormetic effects in normal human skin fibroblasts in culture, Chemosphere 148 (2016) 307–315, https://doi.org/10.1016/j.chemosphere.2016.01.045.



Supplementary materials

Supplementary Table 1. Results of Levene's test for homogeneity of variances ($p < 0.05$; STATISTICA®13, TIBCO Software Inc., Palo Alto, CA, USA)

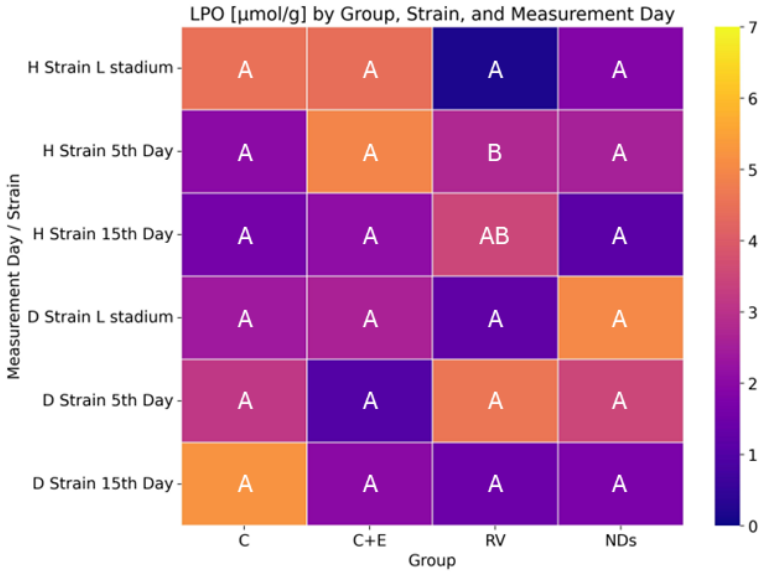
Levene's test							
Measurement day	L Stadium		5th Day		15th Day	
Value	F	p	F	p	F	p
SOD	11.751	0.000	3.252	0.011	5.125	0.000
% Inhibition of superoxide reaction	12.928	0.000	4.523	0.002	2.054	0.084
CAT	5.177	0.001	3.173	0.013	3.431	0.009
LPO	7.664	0.000	2.951	0.020	6.895	0.000
SIRT	14.192	0.000	4.366	0.002	4.452	0.002
SIRT 1	4.052	0.003	5.054	0.001	15.796	0.000
SIRT 6	2.603	0.037	1.467	0.219	3.105	0.014
DSB	3.130	0.016	9.001	0.000	20.995	0.000
H2A.X	5.295	0.001	11.895	0.000	7.213	0.000
pATM	5.562	0.000	9.910	0.000	25.079	0.000

Supplementary Table 2. Correlation matrix between biochemical and molecular parameters ($p < 0.05$; STATISTICA®13, TIBCO Software Inc., Palo Alto, CA, USA)

Variable	Correlations (The indicated correlation coefficients are significant at p < .05000.)									
	LPO	CAT	SOD	% INH SOD	SIRT Activity	SIRT 1	SIRT 6	DSB	H2AX	pATM
LPO	2.797	3.273	1.000	0.085	0.049	-0.145	0.150	-0.023	-0.038	0.162
CAT	0.546	0.547	0.085	1.000	-0.065	-0.099	-0.144	0.040	-0.033	0.039
SOD	81.572	42.185	0.049	-0.065	1.000	0.677	-0.375	-0.165	0.167	-0.077
% inhibition SOD	91.982	2.851	-0.145	-0.099	0.677	1.000	-0.303	-0.181	0.191	-0.048
SIRT Activity	0.356	0.366	0.150	-0.144	-0.375	-0.303	1.000	0.110	-0.440	-0.173
SIRT 1	6.966	10.344	-0.023	0.040	-0.165	-0.181	0.110	1.000	-0.280	-0.054
SIRT 6	16.167	23.420	-0.038	-0.033	0.167	0.191	-0.440	-0.280	1.000	0.268
DSB	2.735	5.424	0.162	0.039	-0.077	-0.048	-0.173	-0.054	0.268	1.000
H2AX	0.197	0.222	0.041	0.118	0.089	-0.111	-0.005	-0.057	0.215	-0.002
pATM	1.689	3.313	0.062	-0.084	-0.186	-0.063	-0.234	-0.033	0.369	0.904

Supplementary Fig. 1. Heatmaps of median values for oxidative stress and sirtuin activity markers across experimental conditions: strain (H or D), and groups—Control (C), Control with ethanol (C+E; ~0.01% ethanol in feed), RV group (23 mg/kg in feed), and NDs group (0.2 mg/kg in feed). Measurements were taken during the larval stage and on days 5 and 15 of the adult stage. A - Lipid peroxidation (LPO; µmol/g), B - Catalase activity (CAT; catalase units/mg), C - Superoxide dismutase activity (SOD; units/mg), D - % inhibition of superoxide reaction (SOD; %), E - Total sirtuin activity (mg/min/µg), F - SIRT1 activity (g/L), G - SIRT6 activity (g/L). Statistical analysis was performed using PERMANOVA ($p < 0.05$, corrected) and PERMDISP ($p \geq 0.05$) (Python, scikit-bio). Letters indicate statistically significant differences within the same strain and group across different time points.

A.



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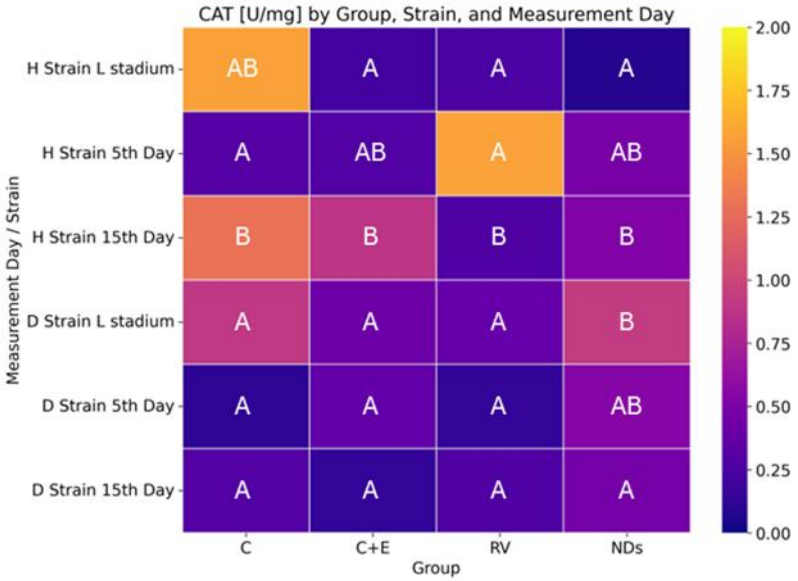

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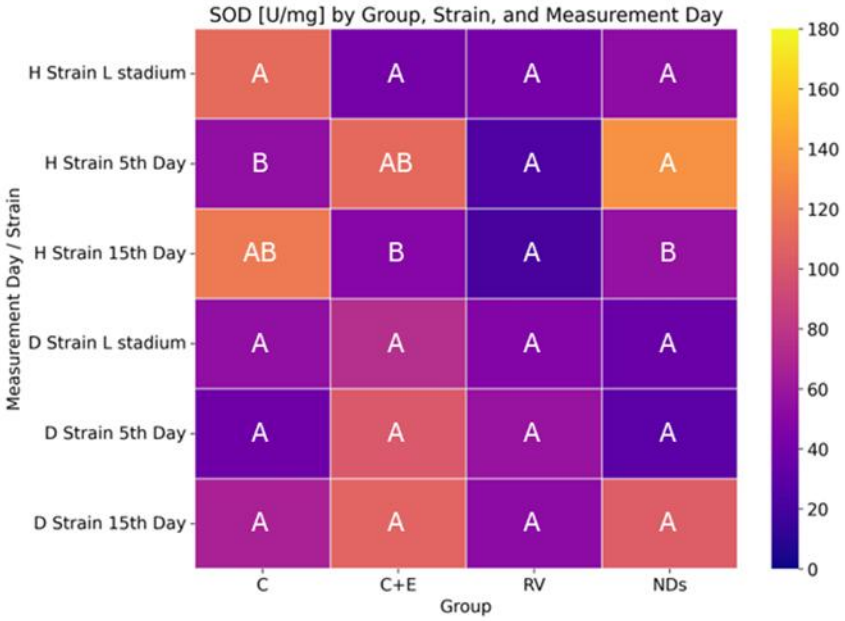

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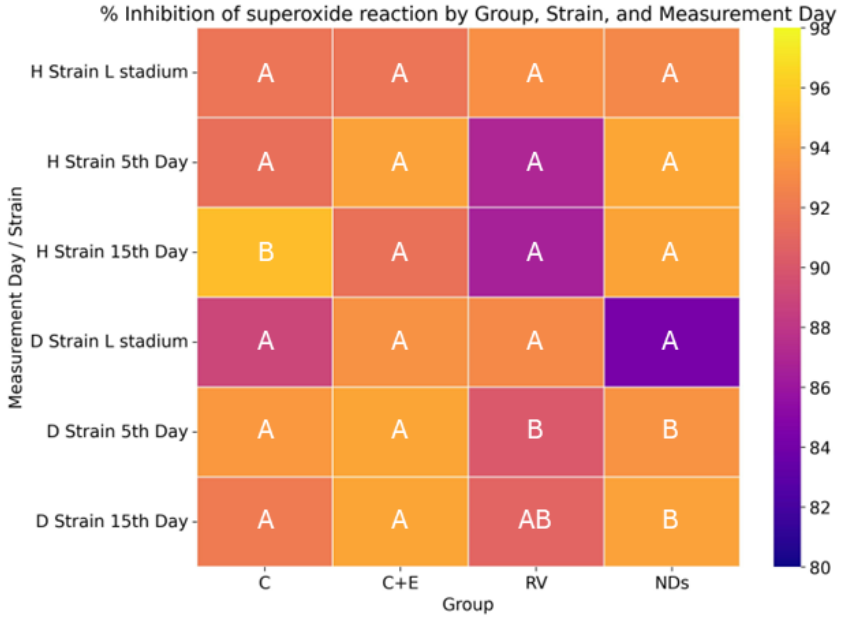

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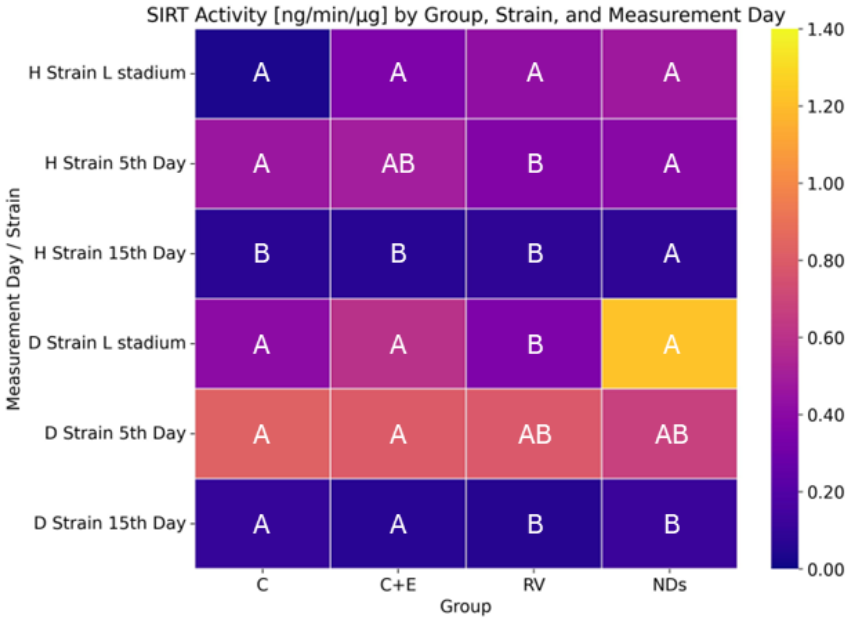

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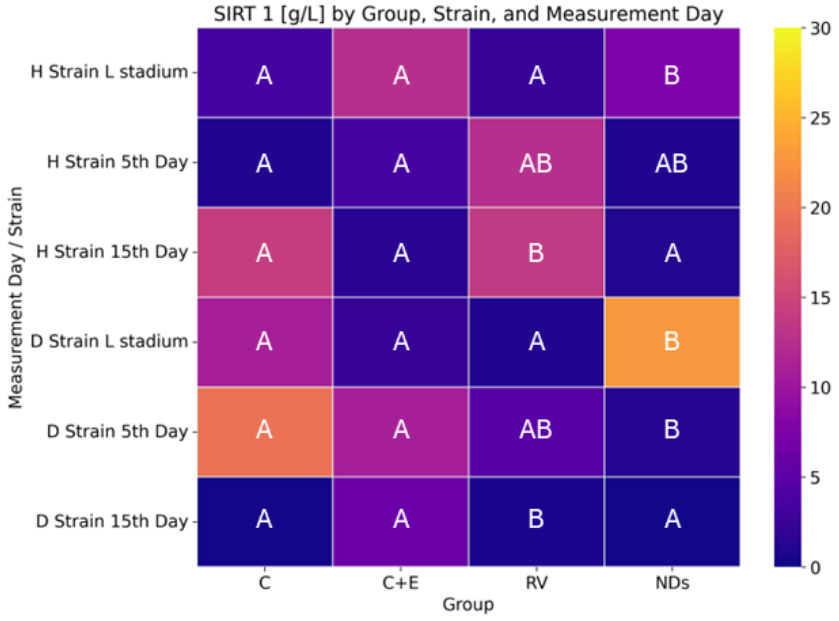

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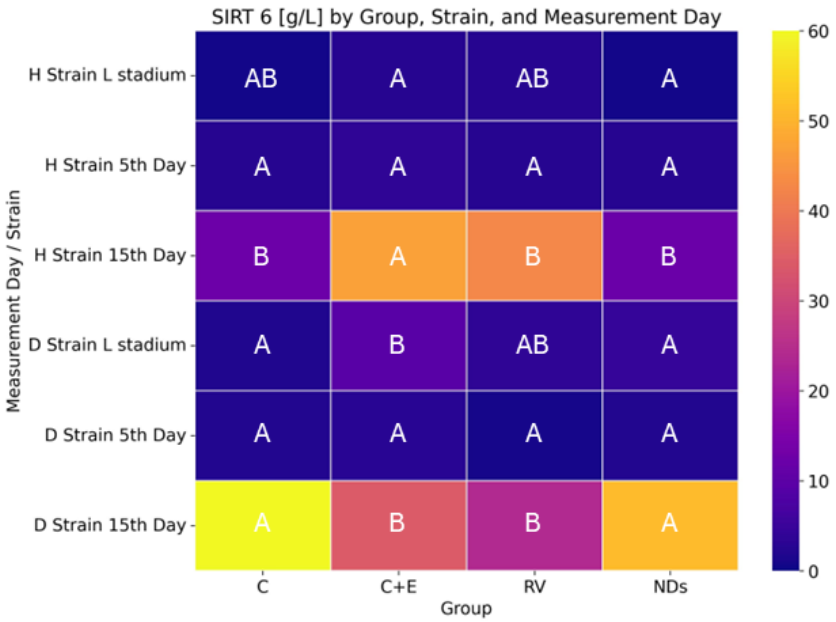
SIRT 6 [g/L] by Group, Strain, and Measurement Day
H Strain L stadium
H Strain 5th Day
H Strain 15th Day
D Strain L stadium
D Strain 5th Day
D Strain 15th Day
Measurement Day / Strain
AB A AB A
A A A A
B A B B
A B AB A
A A A A
A B B A
C
C+E
RV
NDs
Group
60
50
40
30
20
10
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OŚWIADCZENIA WSPÓŁAUTORÓW

Katowice, 27.04.2026

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OŚWIADCZENIE

Oświadczam, że mój wkład w przygotowanie publikacji:

Ziętara-Krzyk P, Flasz B, Augustyniak M. Effects of resveratrol and nanodiamonds on sirtuin activity, oxidative stress and DNA damage in Acheta domesticus. Biochemical and Biophysical Research Communications. 2026, 816, 153698. https://doi.org/10.1016/j.bbrc.2026.153698.

stanowiącego część mojej rozprawy doktorskiej obejmował opracowanie koncepcji badań, przygotowanie i realizację eksperymentów, opracowanie metod badawczych, gromadzenie i analizę danych, interpretację wyników, przygotowanie wizualizacji, opracowanie, redakcję i korektę manuskryptu, a także pełnienie roli autora korespondencyjnego, w tym prowadzenie korespondencji z redakcją i recenzentami.

Patrycja Ziętara-Krzyk
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stanowiącej część rozprawy doktorskiej Patrycji Ziętara-Krzyk obejmował pomoc w przeprowadzeniu eksperymentów, analizie wyników oraz w przygotowaniu manuskryptu.

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stanowiącej część rozprawy doktorskiej Patrycji Ziętara-Krzyk obejmował udział w opracowaniu koncepcji badań, nadzorze naukowym, interpretacji wyników oraz udział w redakcji manuskryptu.

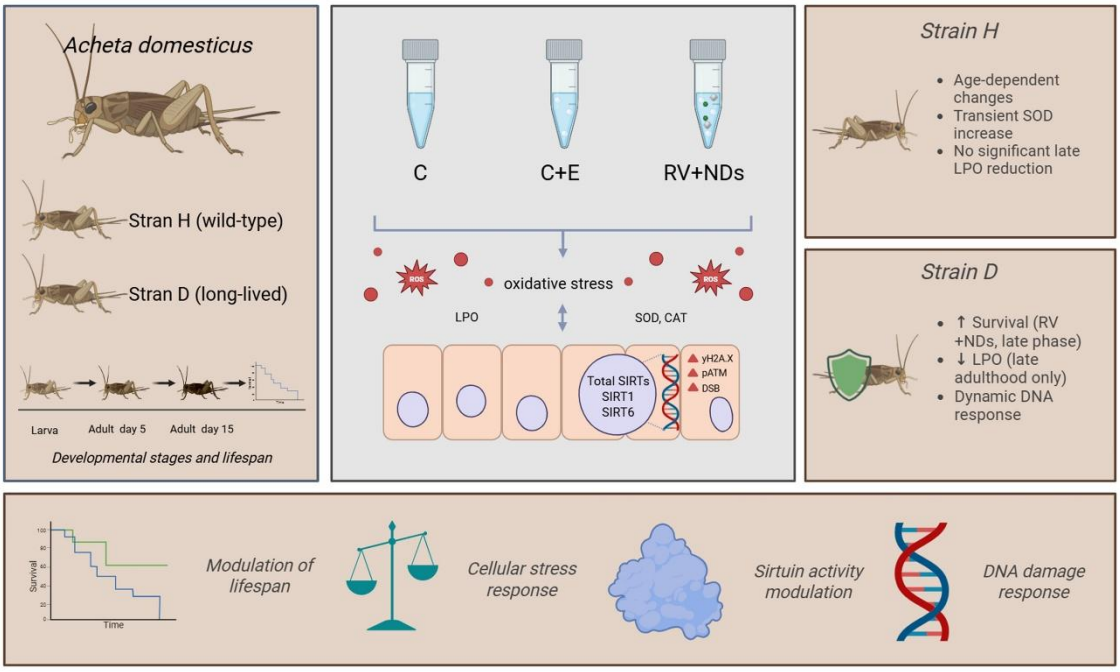
.....

Prof. dr hab. Maria Augustyniak

Manuskrypt IV: Evaluation of Molecular Responses and Longevity Markers in *Acheta domesticus* Following Combined Resveratrol and Nanodiamond Exposure

Authors: Patrycja Ziętara-Krzyk, Barbara Flasz, Maria Augustyniak

Graphical abstract



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Article

Evaluation of Molecular Responses and Longevity Markers in *Acheta domesticus* Following Combined Resveratrol and Nanodiamond Exposure

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Abstract

Sirtuins are conserved proteins regulating oxidative stress and lifespan. While they enhance cellular adaptability, the long-term biological consequences of combining bioactive compounds with nanomaterials remain poorly understood. This study examined the effects of combined resveratrol and nanodiamonds (RV+NDs) in two *Acheta domesticus* strains: wild-type (H) and longevity-selected (D). The impact was assessed across developmental stages, focusing on survival, total sirtuin activity, specific isoforms (SIRT1, SIRT6), oxidative stress, antioxidant enzymes, and DNA damage markers. RV+NDs exposure did not result in consistent lifespan extension or sustained oxidative stress. Molecular responses were strongly dictated by genetic background and age, as reflected by significant survival differences between strains H and D ($p < 0.001$) Notably, a persistent increase in total sirtuin activity (~60% ↑ across developmental stages) occurred exclusively in the longevity-selected strain, though no stable activation of SIRT1 or SIRT6 was detected. While classical redox parameters showed only transient changes, DNA damage response markers emerged as the most sensitive indicators of RV+NDs exposure. Overall, the findings demonstrate that RV+NDs treatment induces context-dependent, adaptive molecular responses. This highlights the critical role of genetic background and age in shaping ageing-related pathways, suggesting that nanodelivery systems do not produce universal effects across different genotypes.

Keywords: *Acheta domesticus*; resveratrol; nanodiamonds; sirtuins; longevity

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1. Introduction

The ageing process is closely linked to cellular defence mechanisms and oxidative stress [1]. It is an inherently interdisciplinary phenomenon that requires combining knowledge from molecular biology, physiology, biochemistry, and toxicology [2,3]. Its complexity results from the involvement of many different cellular factors that together determine the pace of ageing and the lifespan of an organism [1].

Attention from researchers has been drawn to sirtuins—enzymes often referred to as "longevity enzymes" [4]. They form a family of NAD^+-dependent deacetylases and ADP-ribosyltransferases that regulate numerous cellular processes, including genome stability, metabolism, stress response, and apoptosis [5,6]. In mammals, seven sirtuin isoforms (SIRT1-SIRT7) have been identified, which differ in their cellular localisation and biological roles. SIRT1, SIRT6, and SIRT7 are primarily nuclear proteins, SIRT2 is mainly found in the cytoplasm, whereas SIRT3–SIRT5 are predominantly localised in mitochondria [7,8].

Through NAD^+-dependent deacetylation or ADP-ribosylation, sirtuins regulate chromatin structure, DNA repair, mitochondrial metabolism, and stress responses, linking cellular energy status to ageing and longevity [8–10]. Studies in model organisms (mainly vertebrates: mice, rats) have shown that certain sirtuins—especially SIRT1 and SIRT6—may play a direct role in regulating lifespan [11–13]. Moreover, it has been demonstrated that the activity of these enzymes can be modulated by natural compounds such as resveratrol (RV), which exhibits health-promoting and anti-ageing properties [14,15]. One of the significant limitations of using RV is its low bioavailability and short retention time in the body [16]. In humans, the bioavailability of RV is considered very low, and its rapid metabolism significantly limits its potential biological effects [17,18]. Literature reports indicate that although nanodelivery systems can increase the bioavailability of bioactive compounds, their pharmacokinetic and biological consequences do not always translate into changes in lifespan and are more often reflected in the regulation of molecular processes rather than in global longevity indicators [19,20]. Nevertheless, the need for drug carrier systems that could enhance the effectiveness of this compound is emphasised [17,21]. Among nanomaterials used as drug delivery vehicles, nanodiamonds exhibit several advantageous properties, including high biocompatibility, large surface area for adsorption of bioactive compounds, chemical stability, and relatively low cytotoxicity [22,23]. Nanodiamond surface chemistry enables adsorption of polyphenols such as resveratrol via hydrogen bonding and π–π interactions, potentially influencing its stability and release [24,25]. In this context, nanodiamonds (NDs) appear to be a promising solution—biocompatible nanomaterials capable of binding and gradually releasing active substances [23,26,27]. Previous studies indicate that nanodiamonds show low toxicity and can interact with insect biological systems, including *Acheta domesticus*, without causing acute lethality [4,28]. However, the effects of administering NDs combined with resveratrol (RV+NDs) through food on insect organisms have not yet been investigated, either in terms of efficacy or preliminary safety. Insects, including *Drosophila melanogaster* and *Acheta domesticus* (the house cricket), are valuable models for studying the mechanisms of ageing and the response to pharmacological interventions due to the conservation of key pathways regulating oxidative stress and longevity-related processes [29]. Although *D. melanogaster* appears to be the most widely used insect model in ageing research, *A. domesticus* provides several complementary advantages, including larger body size facilitating biochemical analyses, clearly defined developmental stages, and genetically differentiated laboratory lines, including strains selected for extended lifespan [30–32]. Notably, *A. domesticus*, unlike *D. melanogaster*, undergoes incomplete metamorphosis, offering an opportunity to gain additional insights into insect ageing processes. In this context, *A. domesticus* emerges as a promising, yet still underutilised, invertebrate model. Previous studies have shown that both dietary factors and environmental conditions can significantly modulate survival and functional parameters in this species, highlighting its potential in research on the mechanisms of ageing [33,34]. In turn, reviews of nanodelivery systems for RV highlight that nanoformulations improve the solubility and potentially the bioavailability of RV, but in vivo evidence for their biological efficacy is still limited and requires further evaluation in the context of mechanisms of action [20]. While numerous nanodelivery systems for resveratrol have been investigated in mammalian and cellular models, their evaluation in insects remains very limited. Although sirtuins play an essential role in the ageing process, they are not the sole indicator of longevity-related changes. Oxidative stress, defined as an imbalance between the production of reactive oxygen species (ROS) and the organism's ability to neutralise them, is widely recognised as one of the key drivers of cellular ageing [3,35,36]. Therefore, the level of oxidative stress may serve as a sensitive marker of the potential biological effects induced by RV+NDs, providing a starting point for toxicological risk assessment.

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In this study, two strains of *A. domesticus* were used: a wild-type strain (H) and a longevity-selected strain (D). The latter constitutes a valuable model for studying ageing-related mechanisms, as selection for extended lifespan results in distinct physiological and metabolic traits associated with longevity. This approach allowed comparison of responses between organisms with different lifespan within the same species. In our previous research, we demonstrated that NDs and RV individually affect survival, sirtuin activity, oxidative stress parameters, and DNA damage markers in *A. domesticus* under corresponding experimental conditions. These findings provided the biological rationale for investigating whether their combined administration would result in additive, synergistic, or qualitatively distinct molecular responses [4,37]. Based on the above, the main research question is as follows: how does the combination of RV+NDs affect sirtuin activity in *Acheta domesticus*? The primary aim of this study was to determine the impact of this combination on overall sirtuin activity, with a particular focus on specific classes, such as SIRT1 and SIRT6, and to assess potential changes in oxidative stress levels and genome stability using complementary molecular markers, including antioxidant enzymes (SOD and CAT), lipid peroxidation, and DNA damage response indicators (pATM and γH2A.X). Accordingly, the following hypotheses were tested:

H_1. *The application of RV+NDs positively influences molecular processes in Acheta domesticus, leading to increased sirtuin activity, particularly SIRT1 and/or SIRT6. NDs may modulate the bioavailability and duration of RV action, thereby enhancing its effect and potentially leading to significant changes in sirtuin activity, especially in the longevity-selected insect strain.*

H_2. *Exposure of Acheta domesticus to RV+NDs leads to an increase in oxidative stress at the cellular level, regardless of observed changes in sirtuin activity (including SIRT1 and/or SIRT6). This effect may manifest as a disruption of antioxidant balance, indicating potential toxicity of this combination even in the presence of activated SIRT-related pathways.*

2. Results

2.1. This Survival Analysis in Acheta domesticus

In strain H, the survival curves of the individual experimental groups were largely overlapping, indicating highly similar survival patterns (Figure 1a). Overall survival in strain H was shorter compared with strain D. In contrast, strain D exhibited more pronounced divergence among survival curves across experimental groups (Figure 1b). A particularly critical period was observed between days 20 and 60, during which apparent differences in the rate of survival decline were evident among groups. During the late phase of the observation period, an extension of survival was noted, most prominently in the RV+NDs group (Figure 1b). Quantitative survival parameters derived from the Kaplan–Meier estimator indicated that in strain H, median survival was 14 days across all groups, with maximum lifespan ranging from 105 to 112 days. In strain D, median survival was 21 days in C (control) and RV+NDs (resveratrol with nanodiamonds), 14 days in C+E (control with ethanol), while the maximum lifespan reached 147 days in RV+NDs compared with 126 days in the control groups (Figure 1d′). The log-rank test identified a statistically significant difference in survival exclusively between the C+E and RV+NDs groups, and only within strain D (Figure 1b′).

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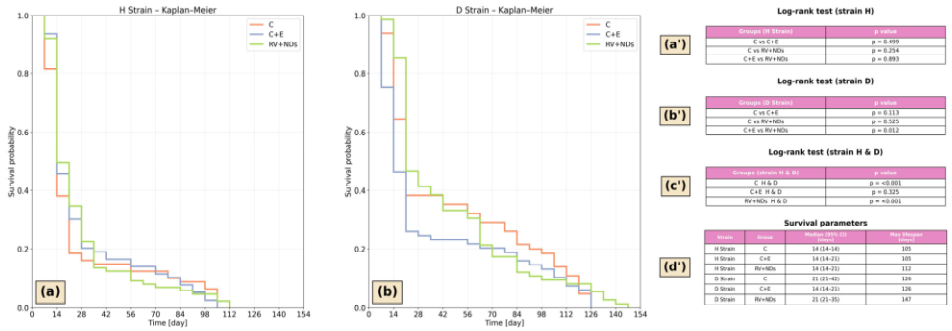

Figure 1. Kaplan–Meier survival analysis: (**a**) survival curves for the wild-type strain (H) monitored for up to 154 days; (**b**) survival curves for the long-lived strain (D) monitored for up to 154 days. The analysed groups included control (C), control with ethanol (C+E), and the combined resveratrol and nanodiamond treatment (RV+NDs). (**a′**,**b′**) Log-rank test results comparing survival distributions among experimental groups within each strain. (**c′**) Comparison of corresponding experimental groups between strains H and D (Python, lifelines package). (**d′**) Quantitative survival parameters derived from the Kaplan–Meier estimator implemented in Python (lifelines package), including median survival time (95% confidence intervals based on Greenwood's variance estimate) and maximum observed lifespan for each experimental group.

A direct comparison between strains H and D revealed highly significant differences ($p < 0.001$). In both the control group and the RV+NDs group, strain D exhibited significantly longer survival compared with strain H (Figure 1a,b).

2.2. Superoxide Dismutase (SOD)

In the larval stage of strain H, superoxide dismutase (SOD) activity in the RV+NDs group was significantly higher than in the control (C) group, but it did not differ significantly from the C+E group (Figure 2a). A similar pattern was observed on day 5 of adulthood, when SOD activity in the RV+NDs group was significantly higher than in both the C and C+E groups (Figure 2b). However, this trend of elevated SOD activity in the RV+NDs group was not maintained over time, as it was not reproduced in measurements performed on day 15 of adulthood (Figure 2c). Concurrently, within the RV+NDs group, a significant temporal decline in SOD activity was observed, with SOD activity on day 15 of adult life being significantly lower than during the larval stage and on day 5 of adulthood (Figure 2a–c).

In insects of strain D, significant differences in SOD activity were observed exclusively between time points, indicating that RV+NDs treatment did not affect SOD activity within a given age group of strain D. In the control group, SOD activity measured on day 5 was higher than that recorded on day 15. Similarly, in both the C+E and RV+NDs groups, SOD activity at the larval stage and on day 5 was higher than that observed on day 15 (Figure 2a,c).

Moreover, only on day 5 of adult life and exclusively in the control group, was SOD activity significantly higher in strain H than in strain D (Figure 2b).

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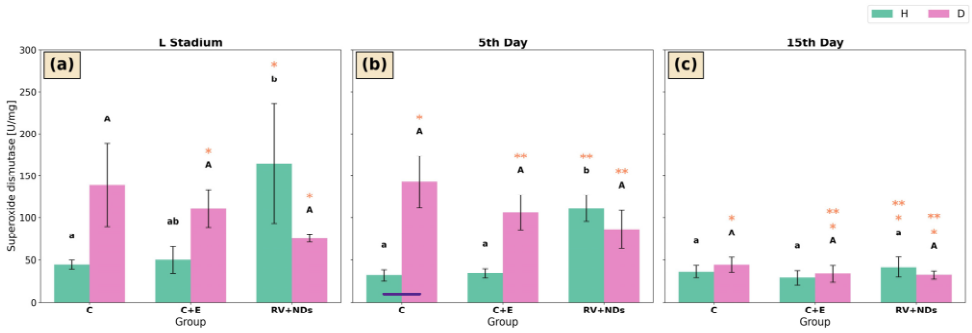

Figure 2. Bar charts presenting mean ± standard deviation of SOD activity (U/ mg) in two *Acheta domesticus* strains (H and D) across three experimental groups: control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Measurements were performed at three developmental time points: larval stage (**a**), 5th day of adulthood (**b**), and 15th day of adulthood (**c**). Statistical analysis was conducted using PERMANOVA ($p < 0.05$, corrected) and PERMDISP ($p \geq 0.05$) in Python (scikit-bio). Lowercase letters denote significant differences among experimental groups within the same developmental stage for strain H, whereas uppercase letters indicate corresponding differences for strain D. Groups sharing at least one common letter do not differ significantly ($p \geq 0.05$), whereas groups marked with different letters differ significantly ($p < 0.05$). Horizontal bars indicate significant differences between strains H and D within the same experimental group ($p < 0.05$). Asterisks indicate significant pairwise differences between developmental time points within the same experimental group; identical asterisks (e.g., (*)) mark the two time points that differ significantly from each other.

2.3. Percent Inhibition of SOD Reaction in Acheta domesticus

Across the analysed time points (larval stage, day 5, and day 15 of adult life), insects of strain H exhibited comparable levels of superoxide reaction inhibition across all experimental groups (C, C+E, and RV+NDs), with no statistically significant differences attributable to the applied treatments (Figure 3a–c).

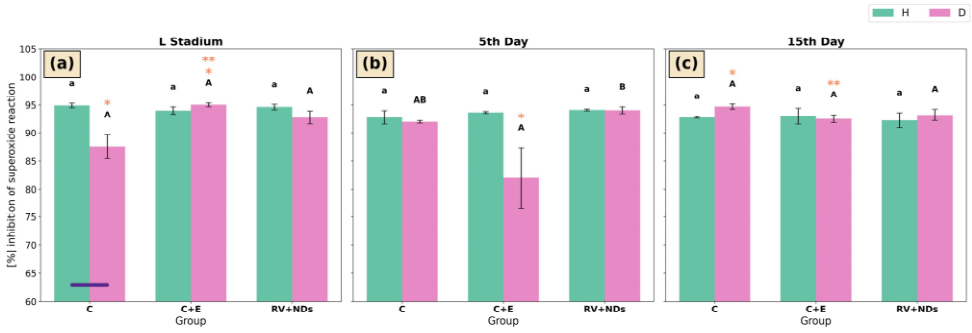

Figure 3. Bar charts presenting mean ± SD % inhibition of SOD reaction in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

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In strain D, significant intergroup differences were observed exclusively on day 5 of adult life. Specifically, the percentage of superoxide inhibition in the C+E group was significantly lower than in the RV+NDs group, whereas no difference was observed compared with the control group (Figure 3b). Temporal analysis revealed a significant increase in % inhibition in the control group of long-lived insects between the larval stage and day 15 of adulthood (Figure 3a,c). A distinct temporal pattern was observed in the C+E group, in which the highest level of superoxide reaction inhibition was recorded at the larval stage, a lower level at day 15, and the lowest level at day 5 of adult life (Figure 3a–c).

A significant difference between strains was detected only in the control group at the larval stage, where the percentage of superoxide reaction inhibition was significantly higher in strain H compared with strain D (Figure 3a).

2.4. Catalase (CAT)

In strain H, catalase (CAT) activity did not show statistically significant differences among the experimental groups (C, C+E, and RV+NDs) at any of the analysed time points (Figure 4a–c). However, significant temporal changes were observed in the C+E group, in which CAT activity on day 15 was significantly higher than on day 5 of adult life (Figure 4b,c). In strain D, a significant reduction in CAT activity was detected only on day 5 of adult life, where CAT activity in the C+E group was significantly lower than in the control (C) group (Figure 4b). In addition, within the control group (C), a significant temporal decline in CAT activity was observed, with CAT activity on day 5 being significantly higher than that recorded on day 15 of adult life (Figure 4b,c).

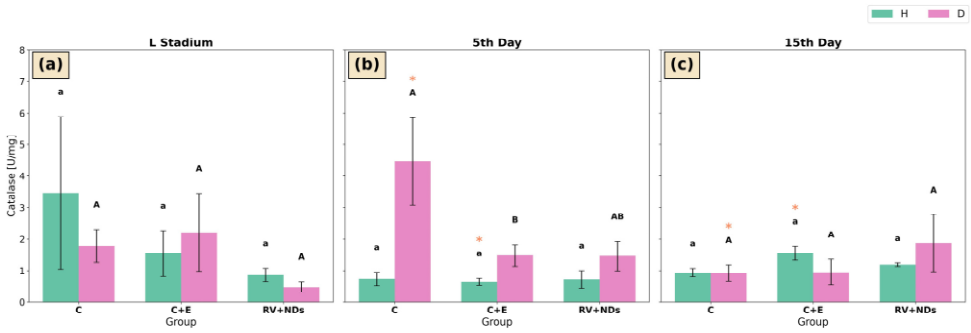

Figure 4. Bar charts presenting mean ± SD of catalase (CAT) activity in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

2.5. Lipid Peroxidation (LPO)

In strain H, the only significant difference in lipid peroxidation (LPO) levels was the lower LPO level observed in the C+E group at the larval stage compared with the C and RV+NDs groups (Figure 5a). No significant differences among experimental groups were detected on day 5 or day 15 of adult life (Figure 5b,c). However, within the C+E group of strain H, LPO levels on day 15 of adult life were significantly higher than those measured at the larval stage (Figure 5a,c).

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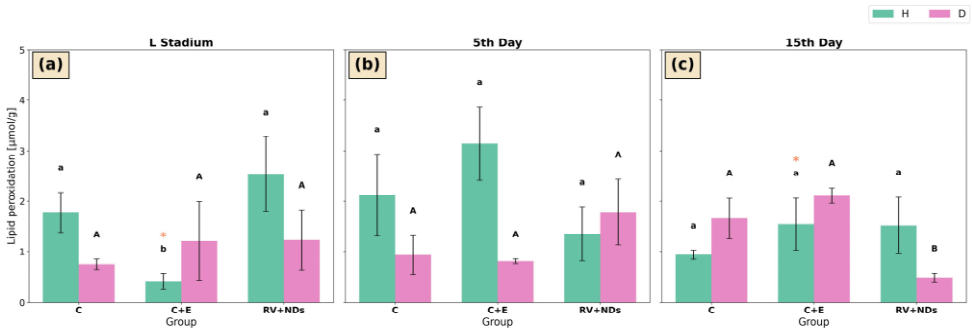

Figure 5. Bar charts presenting mean ± SD of lipid peroxidation (LPO) level in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

In strain D, LPO levels did not differ significantly among experimental groups at the larval stage or on day 5 of adult life. Changes were observed only on day 15 of adult life, when LPO levels in the RV+NDs group were significantly lower than those in the C and C+E groups (Figure 5c).

2.6. Sirtuins

2.6.1. Universal Sirtuin Activity

At the larval stage and on day 5 of adult life in strain H, no significant differences in sirtuin activity were detected among the experimental groups (C, C+E, and RV+NDs) (Figure 6a,b). On day 15 of adult life, a significant reduction in sirtuin activity was observed in the C+E group compared with the control group (Figure 6c). Concurrently, in strain H, sirtuin activity in the control group was significantly higher on day 15 than on day 5 of adult life (Figure 6b,c).

In strain D, the first significant intergroup difference was observed on day 5 of adult life, when sirtuin activity in the control group was significantly lower than in the RV+NDs group (Figure 6b). This pattern persisted on day 15 of adult life (Figure 6c). Moreover, temporal analysis revealed a significant increase in sirtuin activity in the C and C+E groups between the larval stage and day 5 of adult life (Figure 6a,b), whereas in the RV+NDs group, sirtuin activity on day 15 was significantly higher than at the larval stage (Figure 6a,c).

Numerous inter-strain differences were also observed. At the larval stage, sirtuin activity in strain H was significantly lower than in strain D in both the control and RV+NDs groups (Figure 6a). On day 15 of adult life, higher sirtuin activity was recorded in strain H than in strain D in the control group; in contrast, the opposite relationship was observed in the C+E group, where sirtuin activity was significantly higher in strain D than in strain H (Figure 6c).

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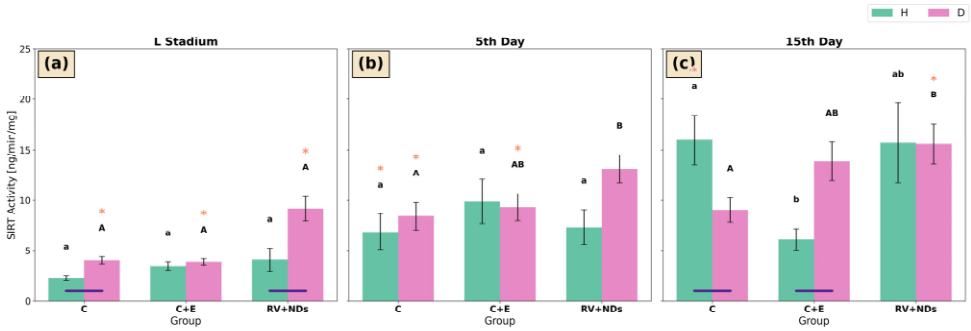

Figure 6. Bar charts presenting mean ± SD of sirtuin activity in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

2.6.2. Sirtuin 1 Activity

At the larval stage in strain H, sirtuin 1 activity in the control group was significantly higher than in the RV+NDs group (Figure 7a). In contrast, on day 5 of adult life, sirtuin 1 activity in the C+E group was significantly higher than in the RV+NDs group, while showing no significant difference relative to the control group (Figure 7b). On day 15 of adult life, sirtuin 1 activity in the control group was significantly higher than in both remaining experimental groups (C+E and RV+NDs) (Figure 7c). Temporal analysis revealed a significant decrease in sirtuin 1 activity in the control group on day 15 compared with the control group at the larval stage and on day 5 of adult life (Figure 7a–c).

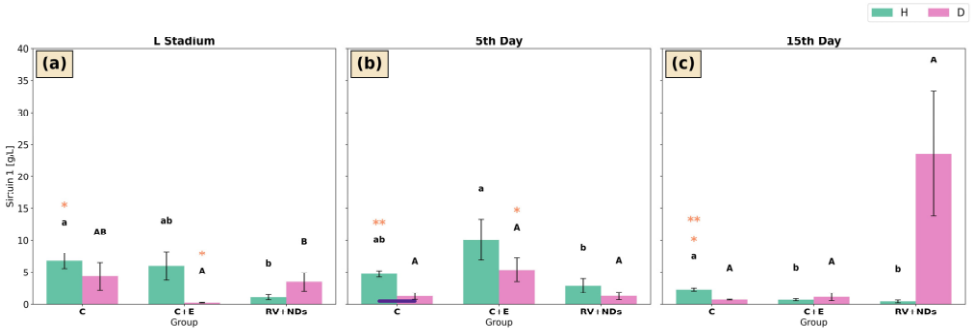

Figure 7. Bar charts presenting mean ± SD of Sirtuin 1 activity in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

In strain D, the only significant differences in sirtuin 1 activity were observed at the larval stage, where activity in the RV+NDs group was significantly higher than in the C+E

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group (Figure 7a). In addition, a significant temporal increase in sirtuin 1 activity between the larval stage and day 5 of adult life was detected in the C+E group (Figure 7a,b).

Differences between strains H and D were observed exclusively on day 5 of adult life in the control group, where sirtuin 1 activity was significantly higher in strain H compared with strain D (Figure 7b).

2.6.3. Sirtuin 6 Activity

In both strain H and strain D, no statistically significant differences were observed among the experimental groups (C, C+E, and RV+NDs) at any of the analysed time points (Figure 8a–c). Nevertheless, in strain H, a significant temporal increase in activity was recorded in the control group between the larval stage and day 5 of adult life (Figure 8a,b). In strain D, significant temporal changes were also observed in the control group, including a significant increase in activity between the larval stage and day 15 of adult life (Figure 8a,c) and between day 5 and day 15 of adult life (Figure 8b,c).

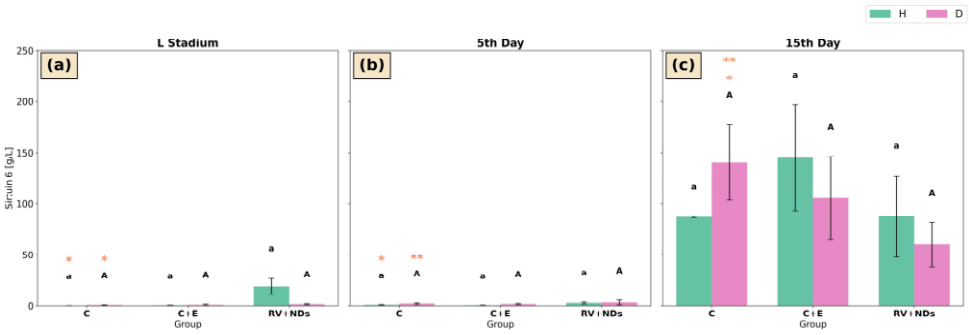

Figure 8. Bar charts presenting mean ± SD of Sirtuin 6 activity in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

2.7. DNA Damage

2.7.1. Double-Strand Break (DSB)

At the larval stage in strain H, the level of DNA double-strand breaks (DSBs) differed significantly among the experimental groups. The lowest percentage of DSBs was observed in the control group, whereas significantly higher values were detected in the C+E and RV+NDs groups (Figure 9a). Another significant intergroup difference in DSB levels in strain H was observed only on day 15 of adult life, when the highest level of DNA damage was detected in the RV+NDs group and the lowest in the control group (Figure 9c). In addition, temporal analysis revealed that at the larval stage, the percentage of DSBs in the control group was significantly lower than the values recorded on days 5 and 15 of adult life (Figure 9a–c).

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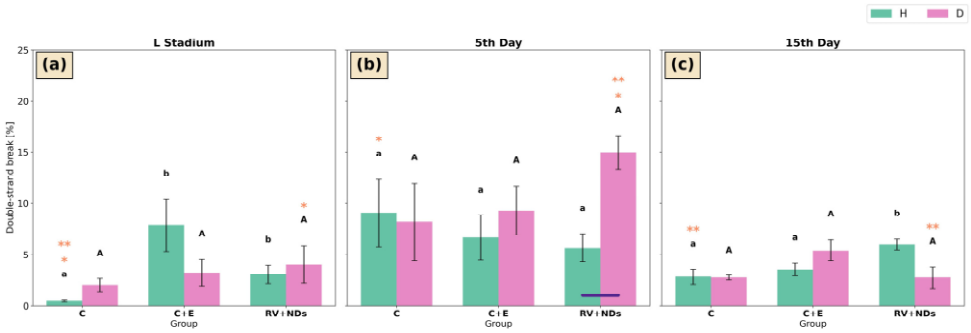

Figure 9. Bar charts presenting mean ± SD of double-strand break (DSB) in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

In strain D, no significant differences among the experimental groups were detected at any of the analysed time points (Figure 9a). Nevertheless, temporal analysis demonstrated a significant increase in DSB levels on day 5 of adult life compared with the larval stage in the RV+NDs group (Figure 9a,b), followed by a significant decrease on day 15 relative to day 5 of adult life (Figure 9b,c).

Comparison between strains revealed that, on day 5 of adult life, DSB levels were significantly lower in strain H than in strain D in the RV+NDs group (Figure 9b).

2.7.2. H2A.X

In strain H, no significant differences in H2A.X levels were detected among the experimental groups (C, C+E, and RV+NDs) at any of the analysed developmental stages (Figure 10a–c). Temporal analysis indicated a significantly higher level of H2A.X phosphorylation in the RV+NDs group on day 15 compared with individuals on day 5 of adult life (Figure 10b,c).

In strain D, significant intergroup differences were observed only on day 5 of adult life, with the highest H2A.X level detected in the RV+NDs group, which was significantly higher than that in the control group (Figure 10b). A significant increase in H2A.X levels on day 5 compared with the larval stage was also observed in the RV+NDs group (Figure 10a,b).

Comparison between strains H and D revealed that at the larval stage, H2A.X levels were significantly higher in strain D than in strain H in the C+E group (Figure 10a). On day 5 of adult life, H2A.X levels in the RV+NDs group were also significantly higher in strain D compared with strain H (Figure 10b).

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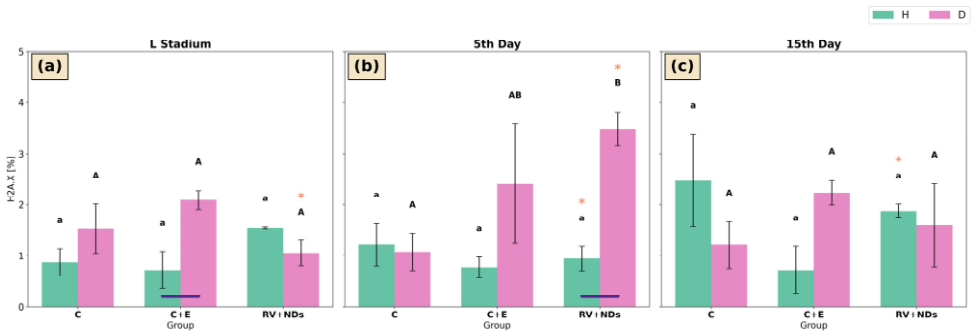

Figure 10. Bar charts presenting mean ± SD of H2A.X in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

2.7.3. pATM

In strain H, at the larval stage the highest pATM activity was recorded in the C+E group, where it was significantly higher than in the control group (Figure 11a). Subsequently, on day 15 of adult life, pATM levels exhibited significant intergroup variation, with the highest values observed in the RV+NDs group and the lowest in the control group (Figure 11c). Temporal analysis revealed a significant decrease in pATM levels in the C+E group on day 15 compared with the larval stage (Figure 11a,c) as well as in the control group, where pATM levels were significantly lower in 15-day-old insects than in 5-day-old individuals (Figure 11b,c).

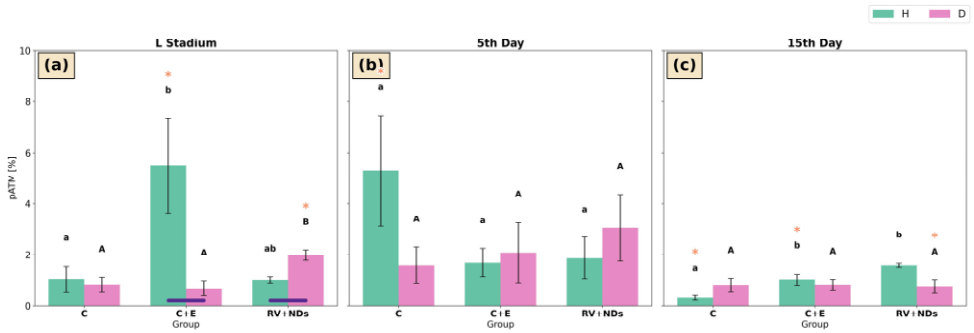

Figure 11. Bar charts presenting mean ± SD pATM in *Acheta domesticus* strains H and D across experimental treatments and measurement points: (**a**) larval stage, (**b**) 5th day of adulthood, and (**c**) 15th day of adulthood. Experimental groups were control (C), control with ethanol (C+E), and combined resveratrol and nanodiamond treatment (RV+NDs). Lowercase and uppercase letters denote significant differences for strains H and D, respectively; identical asterisks mark significantly different developmental time points within the same experimental group, as defined in Figure 2.

In strain D, a significant difference was observed only at the larval stage in the RV+NDs group, where pATM levels were increased relative to both the control and C+E groups

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(Figure 11a). Temporal analysis demonstrated a significant decrease in pATM levels on day 15 compared with the larval stage exclusively in the RV+NDs group (Figure 11a,c).

Comparison between strains H and D revealed significant differences only at the larval stage. pATM levels were significantly higher in strain H than in strain D in the C+E group (Figure 11a). In contrast, in the RV+NDs group at the same developmental stage, an opposite pattern was observed, with pATM levels being significantly lower in strain H compared with strain D (Figure 11a).

To further examine the potential relationship between sirtuin activity and DNA damage markers, Spearman's rank correlation analysis was performed separately for strains H and D. The obtained correlation coefficients between total sirtuin activity, Sirtuin 1, or Sirtuin 6 and DNA damage markers (pATM, H2A.X, and DSB) were weak and not statistically significant ($p \geq 0.05$) (Supplementary Figure S1).

3. Discussion

Previous studies on ageing in *Acheta domesticus* have focused primarily on describing age-related behavioural and functional changes, as well as on analyses of individual environmental or dietary factors. In contrast, the impact of combined interventions on molecular mechanisms associated with longevity mainly remains unexplored [33,38]. Consequently, an important step was to investigate the effects of individual bioactive factors that may influence broadly defined "longevity".

In our earlier studies, RV and NDs were analysed exclusively as independent treatments under analogous experimental conditions, which allowed for separate assessment of their effects on sirtuin activity, oxidative stress parameters, and markers of DNA damage. The present work extends this framework by examining whether their combined administration generates a distinct regulatory profile rather than a simple additive response [4,37]. The results indicated that both compounds elicited transient responses that were strongly dependent on age and strain, without a clear or sustained effect on lifespan or stable modulation of sirtuin activity. At the same time, the occasionally divergent directions of change induced by RV and NDs suggested that their simultaneous administration might influence the pattern of the analysed parameters. On this basis, two research hypotheses were formulated: first, that combined administration of RV and NDs (RV+NDs) may modulate molecular processes associated with longevity in *Acheta domesticus*, including sirtuin activity (SIRT1 and/ or SIRT6); second, exposure to RV+NDs may lead to an enhancement of oxidative stress at the cellular level, independently of changes in sirtuin activity. Given that NDs are considered potential modifiers of the bioavailability of bioactive compounds and as carriers [23,39], a subsequent stage of research was therefore designed to evaluate the effects of simultaneous RV and NDs administration on the analysed parameters.

3.1. Influence on Molecular Processes in Acheta domesticus

Verification of the hypotheses began with an analysis of survival, treated as an indicator of the long-term effects of the studied factors (Figure 1). The results showed that combined administration of RV and NDs did not significantly affect the lifespan of individuals from strain H (Figure 1a), whereas in strain D (Figure 1b), significant differences in the survival curves were observed. This outcome suggests that the effects of RV+NDs may differ across genetic backgrounds and do not necessarily reflect a simple additive effect of the two factors. It should also be noted that ethanol was used as a solvent for resveratrol preparation and was therefore included in the C+E group as an additional control. Although all feed variants were thoroughly dried prior to administration, which may have resulted in ethanol evaporation, the C+E group was intentionally retained to control for potential solvent-related effects. The inclusion of a vehicle control helps to distinguish the biological effects

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of the tested compound from potential responses associated with the solvent used for its preparation [40,41]. Importantly, no consistent differences between the C and C+E groups were observed across the analysed parameters, suggesting that the contribution of ethanol to the observed responses was likely limited. In this context, it is first worth referring to reports on resveratrol administered alone, which have demonstrated that its impact on lifespan is strongly dependent on genotype, environmental conditions, and age, and that the absence of an apparent pro-longevity effect is more the rule than the exception. For example, studies by Staats et al. showed that dietary RV did not significantly affect survival, oxidative stress, or the expression of longevity-related genes under standard conditions in *D. melanogaster* [42]. In contrast, Wang et al. demonstrated that the effect of RV on *D. melanogaster* lifespan was observed only under strictly defined dietary conditions, particularly those with altered nutrient composition, and that the response depended on sex and the protein-to-carbohydrate ratio. By contrast, no such effect was observed under standard, dietary restriction, or high-sugar low-protein diets [43].

Similarly, studies on nanodelivery systems have shown that improved bioavailability of bioactive compounds does not necessarily translate directly into changes in survival but may instead manifest at the level of molecular process regulation [44,45]. Although various types of nanomaterials, including polymeric, metallic, and lipid-based carriers, enhance the stability, solubility, and absorption of RV, their actual biological effectiveness remains dependent on the type of carrier and the application context and does not always result in unequivocal therapeutic effects in in vivo studies [17,46]. Moreover, pharmacokinetic studies have demonstrated that encapsulation of RV in nanoparticles significantly increases its pharmacokinetic bioavailability (AUC, $t_{1/2}$), without assuming a direct translation of this effect into improved survival [47]. Considering these findings, further analyses focused on molecular responses, including sirtuin activity, oxidative stress markers, and indicators of DNA damage. Recent studies also highlight that nanodiamonds exhibit favourable biological properties, including high biocompatibility and a large surface area enabling interaction with bioactive molecules, which supports their increasing exploration in various biomedical applications, particularly in drug delivery systems [48–50].

This analysis showed that simultaneous administration of RV+NDs resulted in a qualitatively different molecular response compared with that observed when each factor was applied separately. In contrast to previous studies in which RV and NDs induced numerous, often inconsistent changes in oxidative stress parameters and DNA damage markers [37], the RV+NDs combination did not cause a sustained increase in lipid peroxidation (Figure 5) or significant deregulation of SOD and CAT activity (Figures 2 and 4). The observed changes were transient and strongly dependent on age and strain. This is consistent with reports indicating that the in vivo action of RV may not involve a persistent reduction in reactive oxygen species levels but rather modulation of signalling pathways and stress responses in a manner dependent on dose, organismal age, and metabolic state [43,51]. From this perspective, the presence of NDs in the RV+NDs system may stabilise RV and modify its interaction kinetics, thereby limiting the variability of oxidative responses observed when the compound is administered in its free form [22,45,52]. Beyond acting as a carrier, NDs may exert a hormetic effect, where their presence induces a mild mechanical or chemical stress that may prime the antioxidant defence system of *A. domesticus*. This potentially explains the lack of sustained lipid peroxidation in the RV+NDs group (Figure 5). Furthermore, the large surface area of NDs, characterised by various functional groups, may actively scavenge free radicals or interact with cellular proteins, further modulating the redox state and limiting the variability of oxidative responses [53,54].

HR-TEM image (Figure 12) revealed well-defined lattice fringes forming a regular crystalline pattern typical for nanodiamond nanocrystallites. This interpretation is supported

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by TEM and EDS analyses, which demonstrated changes in the degree of aggregation and surface characteristics of NDs in the presence of RV (Figures 13 and 14). Similar ordered arrangements corresponding to the cubic crystal structure of diamond and parallel crystallographic planes have been widely described in HR-TEM studies of NDs [55,56]. UV–Vis spectra confirmed the characteristic absorption of RV and enabled estimation of the fraction of non-bound RV in the supernatant, allowing determination of the loading efficiency of RV on NDs (Supplementary Figures S2 and S3). Moreover, DLS and zeta potential analyses together provided additional evidence suggesting interactions between RV molecules and the NDs surface (Supplementary Figures S4 and S5). The Raman spectrum of NDs showed broad bands in the region of approximately 1350 and 1580–1600 cm^{-1}, which is characteristic of detonation nanodiamonds and is commonly attributed to D and G bands of surface-related sp^2 carbon surrounding the diamond core [57–60]. The absence of a sharp bulk-diamond peak at 1332 cm^{-1} should not be considered unexpected, since in very small NDs, particularly sub-10 nm particles, this band is often broadened, shifted, or poorly resolved due to phonon confinement and the complex core–shell structure of detonation NDs [57,58]. In the RV+NDs, attenuation of ND-related bands together with the appearance of a shifted feature in the high-wavenumber region may suggest modification of the nanodiamond surface environment following RV adsorption. Although Raman spectroscopy alone does not provide definitive proof of a stable complex, these changes, together with UV–Vis loading analysis and the reduction in zeta potential, are consistent with the formation of an RV associated with NDs based on surface interaction. The reduced Raman intensity may reflect adsorption-induced changes in Raman scattering efficiency related to modifications in molecular polarizability and the local electronic environment at the nanodiamond surface [61,62]. At the same time, it should be emphasised that for NDs, the size and degree of aggregation observed by electron microscopy do not necessarily directly correspond to particle behaviour in solution. The DLS method determines the hydrodynamic diameter, which includes the hydration layer and loosely associated particle clusters, often resulting in values substantially larger than the sizes observed by TEM [28,63,64]. These physicochemical analyses provide an initial characterisation of the RV+NDs and confirm the interaction between both components, including changes in aggregation behaviour and surface properties. More detailed characterisation of parameters such as encapsulation efficiency and release kinetics would require additional targeted experiments and therefore remains a direction for future investigation.

Additionally, a clear activation of DNA damage response markers (pATM, γH2A.X, and DSBs) was observed (Figures 9–11). The concurrent activation of DNA damage response markers such as pATM and γH2A.X suggests that this response may initiate DDR (DNA damage response) mechanisms, which may secondarily modulate total sirtuin activity without selectively activating individual isoforms [65–67]. Dobbin et al. demonstrated that ATM activation in response to DNA damage leads to secondary regulation of SIRT1 activity [68]. Furthermore, Tang et al. described a mechanism in which SIRT7 regulates ATM activity during the attenuation phase of the DNA damage response, indicating functional coupling between DDR pathways and sirtuin activity [69]. In turn, according to Song et al., SIRT6 may be activated in response to DNA damage, with its enzymatic activity depending on the nature and dynamics of the damage [70]. Rizzo et al. further showed that SIRT6 is involved in regulating the DNA damage response by influencing the stability of components that maintain genome integrity, particularly under conditions of replication stress [71]. These findings indicate that SIRT6's role in DDR is regulatory and context-dependent rather than sustained activation. Although no statistically significant correlations were detected, the concurrent activation of DNA damage response markers and increased total sirtuin activity in strain D suggests a potential indirect relationship between these processes. How-

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ever, this observation requires further investigation to clarify the underlying mechanisms. Importantly, in contrast to the effects observed following administration of RV or NDs alone, these changes did not show a tendency to accumulate with age [37], suggesting that the response induced by RV+NDs may reflect adaptive mechanisms rather than progressive damage. It should also be noted that the present analysis was limited to an early time window (up to day 15 of adult life). Therefore, potential long-term effects such as gradual ND accumulation and its possible influence on DNA repair processes cannot be excluded, especially considering that studies in mammalian models indicate that nanodiamonds may persist in tissues during prolonged exposure [72,73]. Accordingly, in the subsequent stage, an attempt was made to evaluate sirtuin activity as a potential mechanistic contributor to the observed changes.

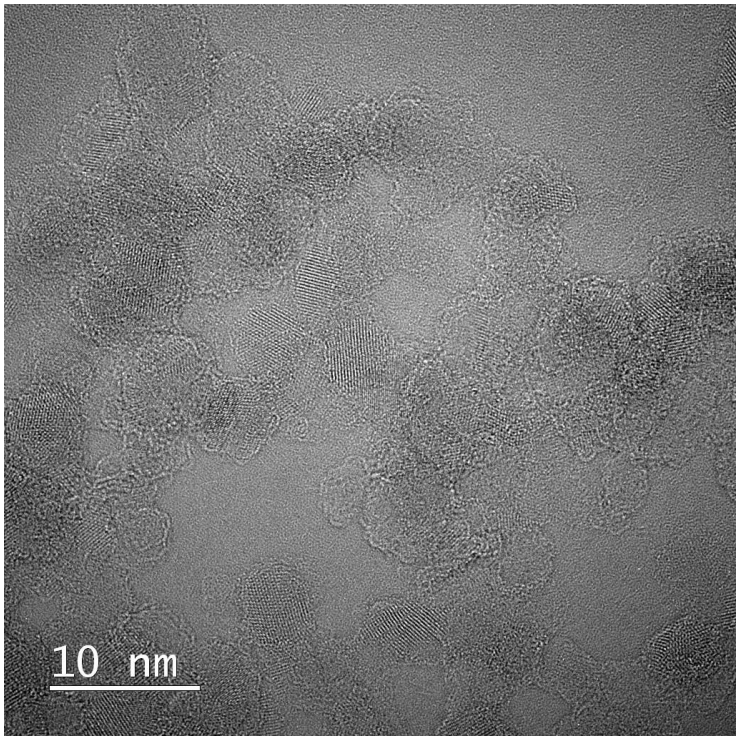

Figure 12. HR-TEM micrograph of nanodiamonds (NDs; Single-Digit Nanodiamonds, 50 mg mL^{-1}, PlasmaChem GmbH, Germany) showing lattice fringes characteristic of crystalline nanodiamond structures (TITAN Cubed, Thermo Fisher Scientific, USA).

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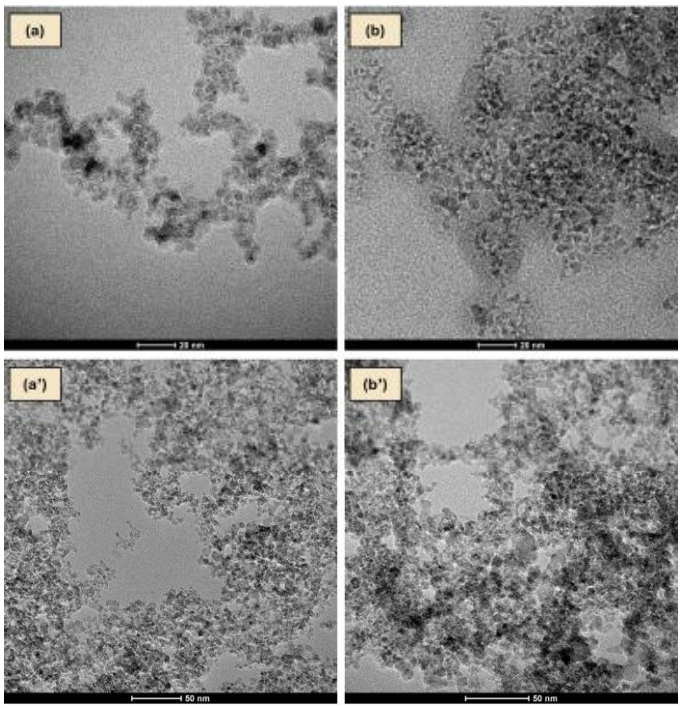

Figure 13. TEM micrographs of nanodiamonds (NDs; Single-Digit Nanodiamonds, 50 mg mL^{-1}, PlasmaChem GmbH, Germany) at scales (**a**) 20 nm and (**a**′) 50 nm, as well as RV+NDs (resveratrol with nanodiamonds) at scales (**b**) 20 nm and (**b**′) 50 nm (TECNAI G2 X-TWIN, Thermo Fisher Scientific, USA).

3.2. Observed Changes in Sirtuin Activity

The analysis of total sirtuin activity (Figure 6) revealed differential responses to combined RV+NDs administration in the examined *A. domesticus* strains (H and D). The absence of a pronounced response in strain H and the sustained sirtuin activation in strain D indicate that the effect of RV+NDs may depend on genetic background and manifest only under specific conditions. This suggests that selection for longevity is associated with altered sensitivity of regulatory systems controlling sirtuin activity and the molecular stress response, which may underlie strain D's susceptibility to the modulatory effects of RV+NDs. Notably, the literature has highlighted a significant influence of genetic background on the observed effects of factors modulating SIRT pathway activity, which may account for their manifestation in only a specific strain [74]. Importantly, the distinct nature of this response compared with the effects of RV or NDs administered individually indicates that the simultaneous application of both substances leads to altered sirtuin regulation rather than a simple amplification of the effects of each alone [37].

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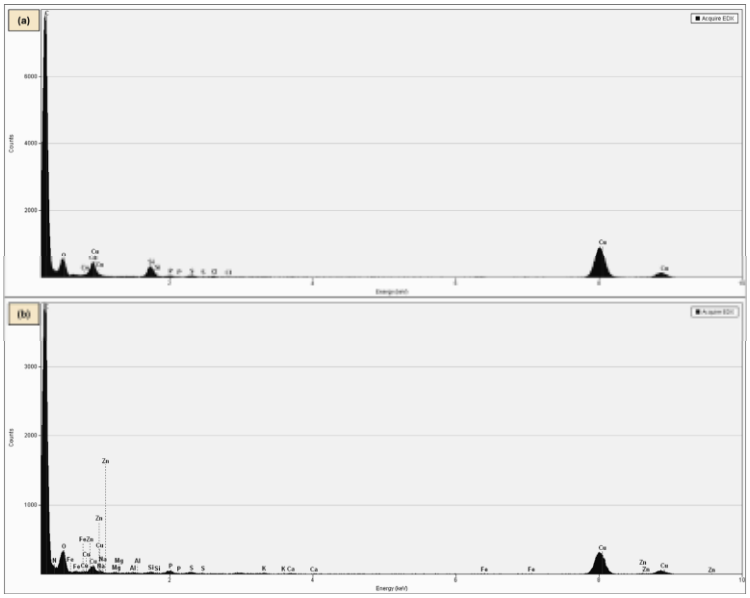

Figure 14. EDS spectrum of (**a**) NDs; (**b**) RV+NDs (SEM-FIB Helios NanoLab 450HP, Thermo Fisher Scientific, USA). Abbreviations provided in Figure 13.

The analysis of the examined sirtuin isoforms showed that the observed increase in total sirtuin activity was not associated with unequivocal activation of either SIRT1 or SIRT6 (Figures 7 and 8). The activities of both sirtuins showed only limited, stage-specific changes, which, in strain D, were not sustained over time. Moreover, SIRT6 activity remained essentially unchanged across experimental groups, and the observed differences were primarily attributable to individual age. This distribution of effects suggests that the RV+NDs combination does not selectively activate individual sirtuin isoforms but may instead modulate the activity of this enzyme family indirectly in a developmentally dependent manner. It should also be considered that total sirtuin activity reflects the combined contribution of multiple sirtuin isoforms, not exclusively SIRT1 or SIRT6. In addition to nuclear sirtuins, cytoplasmic and mitochondrial members of this family (e.g., SIRT2, SIRT3, SIRT4, and SIRT5) are involved in cellular stress responses and metabolic regulation [75,76]. In invertebrate models, mitochondrial sirtuins have been shown to participate in the regulation of oxidative metabolism and ROS detoxification, particularly under conditions of metabolic or oxidative stress [77,78]. For example, SIRT3-related pathways are associated with the regulation of mitochondrial antioxidant enzymes and metabolic adaptation, while SIRT2 has been linked to cytoskeletal dynamics and cellular stress responses [79,80]. Therefore, the observed increase in total sirtuin activity may reflect a broader activation of the sirtuin family rather than selective modulation of SIRT1 or SIRT6 alone.

In contrast to previous studies [37], the present results indicate that simultaneous administration of RV+NDs is associated with a distinct regulatory profile of SIRT1 and SIRT6. The observed effect may result from the cumulative regulation of multiple SIRTs or from changes in the availability of the NAD^+ cofactor, which constitutes a common

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determinant of the enzymatic activity of the entire sirtuin family, irrespective of selective activation of SIRT1 or SIRT6 [81].

Literature reviews on sirtuin activators emphasise that many compounds previously regarded as activators (including resveratrol) act through indirect mechanisms, often by modulating NAD^+ and AMPK levels rather than directly activating a specific enzymatic isoform [7,82,83]. However, SIRT1 activation is not always correlated with these factors or with metabolic or stress-related effects, as demonstrated in rat models [84]. The transient changes in SIRT1 activity observed here appear to be consistent with findings from mammalian models, in which SIRT1 expression and activity have been shown to vary in an age- and physiological state-dependent manner, often declining during later stages of life [85]. In insect models, the role of the SIRT1 ortholog (dSir2) in lifespan regulation has been shown to be highly context dependent. In *D. melanogaster*, alterations in dSir2 activity are not linearly correlated with lifespan and may reflect adaptive metabolic responses rather than sustained activation of longevity pathways [86]. At the same time, in *C. elegans* and *D. melanogaster*, when genetic background and appropriate controls were considered, dSir2 overexpression did not result in persistent lifespan extension, and previously reported effects were largely attributable to confounding factors unrelated to this sirtuin's activity [87].

Similarly, regarding SIRT6, Kanfi et al. demonstrated that SIRT6 activity and function in mice are dependent on organismal age and physiological context, and that its overexpression or activation does not always result in linear pro-longevity effects [88]. In turn, Zhao and Wu showed in a chondrocyte model that SIRT6 regulates mitochondrial number, ROS levels, and antioxidant enzymes, while SIRT6 activity itself changes with the progression of ageing [89]. In the *Drosophila melanogaster* model, it has been shown that the SIRT6 ortholog (dSirt6) regulates lifespan, and that its overexpression extends median survival and increases resistance to oxidative stress, whereas reduced expression leads to lifespan shortening. These findings underscore the biological importance of this isoform in insects and highlight the genetic and environmental dependence of SIRT6-related effects on organismal conditions [30]. The results obtained in the present study suggest that the lack of pronounced SIRT6 activity during the larval stage and early adult life (Figure 8a,b) indicates that this enzyme does not constitute a dominant component of molecular regulation at these stages, or that its function becomes apparent only in later phases of life or under different physiological conditions. This is particularly relevant given that, in *A. domesticus*, a gene homologous to mammalian SIRT6 has not yet been unequivocally described; nevertheless, the applied approach allows for a functional assessment of enzymatic activity attributed to this class of sirtuins. It should also be considered that, although *A. domesticus* represents a useful model organism, knowledge of the molecular basis of sirtuin regulation in this species remains limited, underscoring the need for further studies. The present study focused on molecular responses to RV+NDs exposure. Quantification of RV accumulation in insect tissues (e.g., using HPLC) would require dedicated analytical approaches and therefore remains an interesting direction for future studies addressing the biodistribution of RV delivered by nanodiamonds.

4. Materials and Methods

4.1. Nanodiamonds and Resveratrol

For the experiments, commercially available Single-Digit Nanodiamonds (NDs) were used in aqueous suspension form (50 mg mL^{-1}) as provided by the manufacturer (PlasmaChem GmbH, Berlin, Germany), who specifies the material as detonation nanodiamonds possessing a diamond crystalline structure. As a source of resveratrol (RV), natural RV with a molecular weight of 228.24 g mol^{-1} was used (Pol-Aura®, Zabrze, Poland; catalogue

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number: PA-03-2549-P; CAS number: 501-36-0). The RV+NDs dispersion was prepared by physical adsorption. The nanodiamond suspension was first sonicated to ensure homogeneous dispersion, after which the RV solution was added and the mixture was stirred using a magnetic stirrer and subsequently sonicated for 15 min to promote interaction between RV and the ND surface.

High-resolution transmission electron microscopy (HR-TEM) was used to visualise the structural features of NDs (TITAN Cubed, Thermo Fisher Scientific, Waltham, MA, USA). HR-TEM analysis enabled visualisation of nanoscale crystalline domains and characteristic lattice fringes typical for NDs particles (Figure 12).

The comparative analysis of TEM images (TECNAI G2 X-TWIN, Thermo Fisher Scientific, USA) revealed differences in ND aggregation. Nanodiamonds alone formed looser and more porous structures (Figure 13a,a′), whereas in the presence of RV, more compact agglomerates were observed (Figure 13b,b′). Similar differences in aggregate morphology were noted at both lower (50 nm) and higher (20 nm) magnification, confirming the reproducibility of the observed effect (Figure 13a vs. Figure 13a′; Figure 13b vs. Figure 13b′).

In turn, EDS analyses revealed clear differences between NDs and RV+NDs (Figure 14). Both samples were characterised by a dominant carbon content; however, in the presence of RV, a systematic decrease in the carbon fraction was observed—from 93.68 wt% for NDs to 85.17 wt% for RV+NDs (Figure 14a,b)—accompanied by an increased contribution of elements typical of organic compounds, particularly nitrogen (2.13 wt%) (2.13 wt%) (Figure 14b). These changes, together with the TEM-observed modification in the degree of ND aggregation (Figure 1), suggest that the presence of RV may influence the system's surface characteristics while preserving the carrier's carbon-based nature.

This interpretation is further supported by DLS and zeta potential (Figure 15) measurements, which indicate that the addition of RV does not induce NDs aggregation, as the hydrodynamic diameter remains comparable (~126 nm). At the same time, a decrease in zeta potential from approximately −49 mV to ~−34 mV is consistent with RV interaction with the ND surface and a slight reduction in surface charge while maintaining overall system stability (Litesizer 500, Anton Paar, Warsaw, Poland) (Supplementary Figure S5).

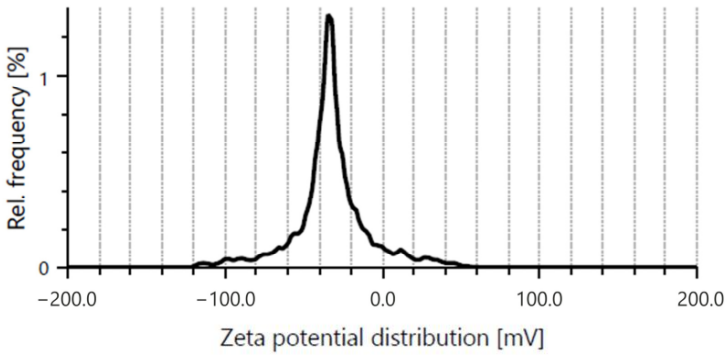

Figure 15. Zeta potential of RV+NDs dispersion in water (Litesizer 500, Anton Paar, Poland). Abbreviations provided in Figure 13.

UV–Vis spectroscopy was used to verify the absorbance characteristics of resveratrol (RV), nanodiamonds (NDs), and the RV+NDs dispersion in the wavelength range 240–900 nm. Measurements were performed using a UV–Vis spectrophotometer (TECAN Infinite M200, Tecan Austria GmbH, Grödig, Austria) with appropriate blank correction

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(H_2O or H_2O/ EtOH depending on the sample composition). The obtained spectra confirmed the characteristic absorption maximum of RV at 305 nm and allowed verification that NDs do not introduce interfering absorbance in the wavelengths used in subsequent colorimetric assays (Supplementary Figure S2).

To quantify the amount of non-bound RV in supernatants after centrifugation of RV-ND mixtures, a calibration curve was constructed based on absorbance measured at λ = 305 nm using standard RV solutions. Linear regression was applied to determine the relationship between RV concentration and absorbance (Supplementary Figure S3).

Additionally, Raman spectroscopy was used to assess the structural characteristics of nanodiamonds and the RV+NDs. Raman spectra of RV, NDs, and RV+NDs were recorded using a Raman spectrometer (WITec Alpha 300 R, WITec GmbH, Ulm, Germany) equipped with a 100× objective and a 600 g mm^{-1} grating. Spectra were acquired with multiple accumulations and integration times depending on the sample (Supplementary Figure S4). The interpretation was focused on the carbon-related spectral region, with particular attention to broad features observed in the ~1340–1390 cm^{-1} and ~1580–1620 cm^{-1} ranges, which are typically used to evaluate structural disorder and surface carbon species in detonation NDs.

4.2. Characteristics of *Acheta domesticus*

Acheta domesticus is a valuable model organism due to its short life cycle, typically living around four months from hatching, its high reproductive rate, and its accessibility for laboratory research [90,91]. At the University of Silesia in Katowice, we have maintained a breeding programme for this species for over 30 years, as reported in our previous publications [4,92,93]. The breeding includes a long-lived strain (D), selectively bred for longevity, and a wild-type strain (H). *Acheta domesticus* is easy to maintain in laboratory conditions and exhibits well-defined developmental stages, making it suitable for studies in physiology, ageing, and developmental biology. Additionally, its relatively large size compared to other insects facilitates experimental manipulations and tissue sampling [33,91].

4.3. Experimental Model

All experiments were performed under standardised environmental conditions. Crickets were reared at a stable temperature of 28 ± 1 °C, with relative humidity maintained at 43.80 ± 6.72%, and subjected to a controlled light–dark cycle of 12 h each (12:12 L:D). For feeding, the insects were provided with a commercially available rabbit feed (KTD, produced by UNIPASZ, Siemiatycze, Poland), which is routinely used to sustain laboratory cultures of *Acheta domesticus* at our facility. The diet is rich in readily absorbable nutrients, with approximately 55% of its carbohydrates derived from plants. It includes proteins, fibre, fats, as well as essential minerals (such as Ca, Na, P, Zn, and Fe), and it is supplemented with vitamins, amino acids, and antioxidants. In the treatment groups, the feed was supplemented with RV at 23 mg kg^{-1} and NDs at 0.2 mg kg^{-1}. The doses of RV and NDs were selected based on our previous studies conducted in the same *A. domesticus* strains under comparable experimental conditions, where both compounds were analysed separately [4,28]. Prior to feed supplementation, RV (pre-dissolved in ethanol) and NDs were co-dispersed in an aqueous solution. Before administration, all feed variants were thoroughly dried and stored in airtight containers to maintain their quality and stability. Crickets were randomly assigned to three experimental groups: Control (C), fed with the standard KTD diet; C+E, receiving feed containing ethanol (~0.01%) as a vehicle control for RV; and RV+NDs, receiving feed enriched with both RV and NDs. The experimental period spanned 22 weeks. Individuals were sampled at the larval stage and at two defined time points during adulthood: the 5th and 15th days. Intestinal tissues were dissected

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and processed according to previously established protocols using phosphate-buffered saline (PBS, 0.1 M, pH 7.4) [4,92]. Tissue homogenisation was performed using a Minilys homogeniser (Bertin Technologies, Montigny-le-Bretonneux, France) to obtain intestinal cell suspensions for further analyses.

4.4. Biochemical Analysis

4.4.1. Antioxidant Enzymes Measurements

Oxidative stress markers were evaluated using a set of colorimetric assays. Lipid peroxidation levels were measured with the Lipid Peroxidation (LPO) Assay Kit (Abbexa Ltd., Cambridge, UK, abx096010), which detects lipid hydroperoxides reacting with a chromogenic substrate to indicate membrane damage. Catalase activity, responsible for neutralising hydrogen peroxide, was assessed using the Catalase Colorimetric Activity Kit (Thermo Fisher Scientific, CA, USA, EIACATC) according to the manufacturer's protocol. The function of superoxide dismutase (SOD), crucial for converting superoxide radicals into less reactive species, was determined using the Superoxide Dismutase (SOD) Activity Assay Kit (Merck KGaA, Darmstadt, Germany, CS0009). Results were expressed as percent inhibition or enzymatic units based on a standard curve. Absorbance for all assays was measured using a TECAN Infinite M200 spectrophotometer (Tecan Austria GmbH, Grödig, Austria).

4.4.2. Cell Nuclei Extraction and SIRT Activity Measurements

Nuclear proteins were extracted from *Acheta domesticus* intestinal tissue using the Nuclear Extraction Kit (Abcam, Cambridge, UK, ab113474) according to the manufacturer's protocol. The procedure involved multiple buffer treatments and centrifugation steps, enabling the selective isolation of the nuclear fraction while minimising contamination from cytoplasmic components. Protein concentrations in the nuclear extracts were determined using the Bradford assay [94], and absorbance was measured with a UV–Vis spectrometer (TECAN Infinite M200, Tecan Austria GmbH, Grödig, Austria).

Sirtuin activity was assessed at three levels—total sirtuin activity, SIRT1, and SIRT6—each analysed using a specific assay. General sirtuin activity was measured using the Universal SIRT Activity Assay Kit (Colorimetric) (Abcam, Cambridge, UK, ab156915), which detects the deacetylation of a target substrate in the presence of NAD^+ through a colorimetric change, as recorded via spectrophotometry. For isoform-specific analyses, the SIRT1 Activity Assay Kit (fluorometric) (Abcam, Cambridge, UK, ab156065) and the SIRT6 Activity Assay Kit (fluorometric) (Abcam, Cambridge, UK, ab156068) were employed. These assays use fluorescent substrates that, upon enzymatic deacetylation, release a fluorophore. Fluorescence intensity was measured using a Fluorescence Spectrometer Plate Reader (HITACHI F-7000, Hitachi, Ltd., Tokyo, Japan), allowing for sensitive quantification of enzyme activity.

4.4.3. DNA Damage Measurements

DNA damage was assessed using the Muse® Multi-Colour DNA Damage Kit (Luminex Corporation, Austin, TX, USA, Part Number: MCH200107), which allows concurrent evaluation of ATM and H2A.X activation. The assay is based on phospho-specific, directly conjugated antibodies targeting ATM (Ser1981)-PE and Histone H2A.X PECy5, enabling detection of their phosphorylation status. Sample preparation and staining were performed according to the manufacturer's protocol, including fixation, permeabilisation, and antibody incubation. Following washing and resuspension in assay buffer, measurements were obtained using the Guava® Muse® cell analyser (Luminex Corporation, TX, USA).

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4.5. Data Visualisation and Statistical Procedures

Kaplan–Meier survival analysis was used to evaluate survival in the experimental groups, with survival probabilities over time presented as survival curves. The variance of the Kaplan–Meier survival estimator was calculated using Greenwood's formula. Differences between groups were assessed using the Log-Rank test (Chi-squared, $p < 0.05$). The analysis was conducted on the wild-type strain (H) and the long-lived strain (D) over 147 days. Individuals were maintained separately within each experimental group, with approximately $n \sim 80$ individuals per strain (Python, lifelines package).

Data visualisation and statistical analyses were performed using Python (version 3.13.2). Data processing was conducted with the following Python libraries: Pandas (version 2.2.3) for data management, NumPy (version 2.2.4) for numerical computations, and Matplotlib.pyplot (version 3.10.1) for graphical representation. Experimental datasets were imported from CSV files, and group labels and measurement timepoints were standardised to ensure clarity and consistency in subsequent analyses. Descriptive statistics, including means and standard errors of the mean (SEM), were calculated for each strain, treatment group, and time point. The results were visualised as grouped bar plots, enabling direct comparison across experimental conditions.

Preliminary statistical assumptions were verified using STATISTICA® 13 (TIBCO Software Inc., Palo Alto, CA, USA), where the data were tested for normality ($p < 0.05$) and homogeneity of variances using Levene's test. LPO, CAT, SOD, percentage inhibition, total sirtuin activity, SIRT1, SIRT6, pATM, γH2A.X, and DSBs were quantified using five independent biological replicates per treatment group ($n = 5$ per group: C, C+E, RV+NDs). Subsequent multivariate analyses were conducted in Python (version 3.13.2). Group-level differences were assessed using PERMANOVA (Permutational Multivariate Analysis of Variance) with 999 permutations, followed by PERMDISP to assess multivariate dispersion homogeneity. PERMANOVA revealed significant effects ($p < 0.05$), whereas PERMDISP was not significant ($p \geq 0.05$), confirming that observed differences were not driven by variance heterogeneity. Associations between sirtuin activity and DNA damage markers were assessed using Spearman's rank correlation (r_s; $p < 0.05$), calculated separately for strains H and D (SciPy, Python 3.13.2). These analyses were conducted using the Pandas, NumPy, SciPy, scikit-bio, and tqdm libraries. All pairwise comparisons were automated, and the resulting matrices were exported to Excel (Microsoft, Redmond, WA, USA) for further interpretation. The complete analytical workflow was implemented and executed in the Jupyter Notebook (version 4.3.6) environment (Project Jupyter, San Luis Obispo, CA, USA).

5. Conclusions

The overall results indicate that hypothesis H1 was only partially confirmed, and this effect was strictly dependent on the genetic background of the studied organism. The simultaneous administration of resveratrol and nanodiamonds (RV+NDs) led to a sustained increase in total sirtuin activity exclusively in the long-lived strain of *Acheta domesticus* (strain D), without clear selective activation of SIRT1 or SIRT6 and without a universal translation into lifespan extension. These findings indicate that modulation of sirtuin activity by RV+NDs is context-dependent and does not constitute a simple pro-longevity mechanism.

Hypothesis H2 was not unequivocally confirmed, as combined RV+NDs administration did not result in a persistent enhancement of oxidative stress. The observed changes in parameters were transient and age- and strain-dependent, indicating the absence of a cumulative pro-oxidative effect of this combination. Further studies are therefore required, particularly within a single species.

At the same time, it was demonstrated that the most sensitive indicators of the response to combined RV+NDs administration were markers of the DNA damage response and

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total sirtuin activity, whereas classical oxidative stress parameters as well as SIRT1 and SIRT6 activity exhibited greater stability and a stronger dependence on organismal age. This suggests that the RV+NDs combination induces a qualitatively distinct molecular response profile, involving activation of adaptive mechanisms rather than progressive cellular damage.

These findings also highlight the importance of considering genetic background and organismal age when evaluating nanodelivery strategies aimed at modulating ageing-related pathways. The results suggest that nanodiamond-based systems may alter molecular responses to bioactive compounds without necessarily producing universal longevity effects. Future studies should therefore address long-term exposure, biodistribution, and tissue accumulation of nanodiamonds, as well as the bioavailability and release kinetics of resveratrol delivered by nanocarriers.

In an era of growing interest in life-extending strategies and the search for factors modulating the rate of ageing, the present study highlights the need for cautious interpretation of the effects of long-term supplementation with substances considered potentially pro-longevity. Moreover, it indicates that even compounds with a presumed beneficial and safe action profile may, when used chronically, induce complex and context-dependent molecular responses whose biological consequences are not always unequivocally favourable. These studies constitute a valuable contribution to knowledge on the biological effects of combined application of bioactive compounds and nanocarriers in the insect model organism *Acheta domesticus*, for which the mechanisms regulating sirtuins and molecular stress responses remain poorly understood. Such an approach may lead to subtle yet biologically significant modulation of molecular processes without disrupting cellular homeostasis.

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Abbreviations

The following abbreviations are used in this manuscript:

AMPK	AMP-activated protein kinase
ATM	Ataxia telangiectasia mutated kinase
AUC	Area under the curve
CAT	Catalase
DDR	DNA damage response
DSBs	Double-strand breaks
EDS	Energy-dispersive X-ray spectroscopy
H	Wild-type strain
D	Longevity-selected strain
H2A.X (γH2A.X)	Phosphorylated histone H2A.X
L:D	Light–dark cycle
LPO	Lipid peroxidation
NAD^+	Nicotinamide adenine dinucleotide (oxidised form)
NDs	Nanodiamonds
PBS	Phosphate-buffered saline
PERMANOVA	Permutational multivariate analysis of variance
PERMDISP	Permutational analysis of multivariate dispersions
pATM	Phosphorylated ATM
ROS	Reactive oxygen species
RV	Resveratrol
SEM	Scanning electron microscopy
SEM (statistics)	Standard error of the mean
SIRT	Sirtuin
SIRT1	Sirtuin 1
SIRT6	Sirtuin 6
SOD	Superoxide dismutase
TEM	Transmission electron microscopy
wt%	Weight percent

References

1. Cabello-Verrugio, C.; Ruiz-Ortega, M.; Mosqueira, M.; Simon, F. Oxidative Stress in Disease and Aging: Mechanisms and Therapies. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 8786564. [CrossRef]
2. Malecki, K.M.C.; Andersen, J.K.; Geller, A.M.; Harry, G.J.; Jackson, C.L.; James, K.A.; Miller, G.W.; Ottinger, M.A. Integrating Environment and Aging Research: Opportunities for Synergy and Acceleration. *Front. Aging Neurosci.* **2022**, *14*, 824921. [CrossRef] [PubMed]
3. Yang, J.; Luo, J.; Tian, X.; Zhao, Y.; Li, Y.; Wu, X. Progress in Understanding Oxidative Stress, Aging, and Aging-Related Diseases. *Antioxidants* **2024**, *13*, 394. [CrossRef]
4. Ziętara, P.; Flasz, B.; Augustyniak, M. Does Selection for Longevity in Acheta Domesticus Involve Sirtuin Activity Modulation and Differential Response to Activators (Resveratrol and Nanodiamonds)? *Int. J. Mol. Sci.* **2024**, *25*, 1329. [CrossRef] [PubMed]
5. Frydzińska, Z.; Owczarek, A.; Winiarska, K. Sirtuiny i Ich Rola w Regulacji Metabolizmu. *Postepy Biochem.* **2019**, *65*, 31–40. [CrossRef] [PubMed]
6. Wu, Q.-J.; Zhang, T.-N.; Chen, H.-H.; Yu, X.-F.; Lv, J.-L.; Liu, Y.-Y.; Liu, Y.-S.; Zheng, G.; Zhao, J.-Q.; Wei, Y.-F.; et al. The Sirtuin Family in Health and Disease. *Signal Transduct. Target. Ther.* **2022**, *7*, 402. [CrossRef]
7. Ziętara, P.; Dziewięcka, M.; Augustyniak, M. Why Is Longevity Still a Scientific Mystery? Sirtuins—Past, Present and Future. *Int. J. Mol. Sci.* **2022**, *24*, 728. [CrossRef]
8. Ji, Z.; Liu, G.-H.; Qu, J. Mitochondrial Sirtuins, Metabolism, and Aging. *J. Genet. Genom.* **2022**, *49*, 287–298. [CrossRef]
9. Lagunas-Rangel, F.A. Current Role of Mammalian Sirtuins in DNA Repair. *DNA Repair* **2019**, *80*, 85–92. [CrossRef]
10. Mei, Z.; Zhang, X.; Yi, J.; Huang, J.; He, J.; Tao, Y. Sirtuins in Metabolism, DNA Repair and Cancer. *J. Exp. Clin. Cancer Res.* **2016**, *35*, 182. [CrossRef]

https:/ / doi.org/ 10.3390/ ijms27062786

11. Satoh, A.; Brace, C.S.; Rensing, N.; Cliften, P.; Wozniak, D.F.; Herzog, E.D.; Yamada, K.A.; Imai, S. Sirt1 Extends Life Span and Delays Aging in Mice through the Regulation of Nk2 Homeobox 1 in the DMH and LH. *Cell Metab.* **2013**, *18*, 416–430. [CrossRef] [PubMed]
12. Roichman, A.; Elhanati, S.; Aon, M.A.; Abramovich, I.; Di Francesco, A.; Shahar, Y.; Avivi, M.Y.; Shurgi, M.; Rubinstein, A.; Wiesner, Y.; et al. Restoration of Energy Homeostasis by SIRT6 Extends Healthy Lifespan. *Nat. Commun.* **2021**, *12*, 3208. [CrossRef]
13. Tian, X.; Firsanov, D.; Zhang, Z.; Cheng, Y.; Luo, L.; Tombline, G.; Tan, R.; Simon, M.; Henderson, S.; Steffan, J.; et al. SIRT6 Is Responsible for More Efficient DNA Double-Strand Break Repair in Long-Lived Species. *Cell* **2019**, *177*, 622–638.e22. [CrossRef]
14. Zhou, D.-D.; Luo, M.; Huang, S.-Y.; Saimaiti, A.; Shang, A.; Gan, R.-Y.; Li, H.-B. Effects and Mechanisms of Resveratrol on Aging and Age-Related Diseases. *Oxid. Med. Cell. Longev.* **2021**, *2021*, 9932218. [CrossRef]
15. Gertz, M.; Nguyen, G.T.T.; Fischer, F.; Suenkel, B.; Schlicker, C.; Fränzel, B.; Tomaschewski, J.; Aladini, F.; Becker, C.; Wolters, D.; et al. A Molecular Mechanism for Direct Sirtuin Activation by Resveratrol. *PLoS ONE* **2012**, *7*, e49761. [CrossRef]
16. Walle, T. Bioavailability of Resveratrol. *Ann. N. Y. Acad. Sci.* **2011**, *1215*, 9–15. [CrossRef] [PubMed]
17. Salla, M.; Karaki, N.; El Kaderi, B.; Ayoub, A.J.; Younes, S.; Abou Chahla, M.N.; Baksh, S.; El Khatib, S. Enhancing the Bioavailability of Resveratrol: Combine It, Derivatize It, or Encapsulate It? *Pharmaceutics* **2024**, *16*, 569. [CrossRef] [PubMed]
18. Ramírez-Garza, S.; Laveriano-Santos, E.; Marhuenda-Muñoz, M.; Storniolo, C.; Tresserra-Rimbau, A.; Vallverdú-Queralt, A.; Lamuela-Raventós, R. Health Effects of Resveratrol: Results from Human Intervention Trials. *Nutrients* **2018**, *10*, 1892. [CrossRef]
19. Vesely, O.; Baldovska, S.; Kolesarova, A. Enhancing Bioavailability of Nutraceutically Used Resveratrol and Other Stilbenoids. *Nutrients* **2021**, *13*, 3095. [CrossRef]
20. Santos, A.C.; Pereira, I.; Pereira-Silva, M.; Ferreira, L.; Caldas, M.; Collado-González, M.; Magalhães, M.; Figueiras, A.; Ribeiro, A.J.; Veiga, F. Nanotechnology-Based Formulations for Resveratrol Delivery: Effects on Resveratrol in Vivo Bioavailability and Bioactivity. *Colloids Surf. B Biointerfaces* **2019**, *180*, 127–140. [CrossRef]
21. Dikmetas, D.N.; Yenipazar, H.; Can Karaca, A. Recent Advances in Encapsulation of Resveratrol for Enhanced Delivery. *Food Chem.* **2024**, *460*, 140475. [CrossRef] [PubMed]
22. Qin, J.-X.; Yang, X.-G.; Lv, C.-F.; Li, Y.-Z.; Liu, K.-K.; Zang, J.-H.; Yang, X.; Dong, L.; Shan, C.-X. Nanodiamonds: Synthesis, Properties, and Applications in Nanomedicine. *Mater. Des.* **2021**, *210*, 110091. [CrossRef]
23. Xu, J.; Chow, E.K.-H. Biomedical Applications of Nanodiamonds: From Drug-Delivery to Diagnostics. *SLAS Technol.* **2023**, *28*, 214–222. [CrossRef]
24. Badea, I.; Kaur, R. Nanodiamonds as Novel Nanomaterials for Biomedical Applications: Drug Delivery and Imaging Systems. *Int. J. Nanomedicine* **2013**, *8*, 203–220. [CrossRef]
25. Mochalin, V.N.; Pentecost, A.; Li, X.-M.; Neitzel, I.; Nelson, M.; Wei, C.; He, T.; Guo, F.; Gogotsi, Y. Adsorption of Drugs on Nanodiamond: Toward Development of a Drug Delivery Platform. *Mol. Pharm.* **2013**, *10*, 3728–3735. [CrossRef]
26. Wang, X.; Sang, D.; Zou, L.; Ge, S.; Yao, Y.; Fan, J.; Wang, Q. Multiple Bioimaging Applications Based on the Excellent Properties of Nanodiamond: A Review. *Molecules* **2023**, *28*, 4063. [CrossRef]
27. Ryu, T.-K.; Kang, R.-H.; Jeong, K.-Y.; Jun, D.-R.; Koh, J.-M.; Kim, D.; Bae, S.K.; Choi, S.-W. Bone-Targeted Delivery of Nanodiamond-Based Drug Carriers Conjugated with Alendronate for Potential Osteoporosis Treatment. *J. Control. Release* **2016**, *232*, 152–160. [CrossRef]
28. Augustyniak, M.; Ajay, A.K.; Kędziorski, A.; Tarnawska, M.; Rost-Roszkowska, M.; Flasz, B.; Babczyńska, A.; Mazur, B.; Rozpędek, K.; Alian, R.S.; et al. Survival, Growth and Digestive Functions after Exposure to Nanodiamonds—Transgenerational Effects beyond Contact Time in House Cricket Strains. *Chemosphere* **2023**, *349*, 140809. [CrossRef]
29. Lee, S.-H.; Min, K.-J. Drosophila Melanogaster as a Model System in the Study of Pharmacological Interventions in Aging. *Transl. Med. Aging* **2019**, *3*, 98–103. [CrossRef]
30. Taylor, J.R.; Wood, J.G.; Mizerak, E.; Hinthorn, S.; Liu, J.; Finn, M.; Gordon, S.; Zingas, L.; Chang, C.; Klein, M.A.; et al. Sirt6 Regulates Lifespan in *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2111176119. [CrossRef] [PubMed]
31. Wood, J.G.; Schwer, B.; Wickremesinghe, P.C.; Hartnett, D.A.; Burhenn, L.; Garcia, M.; Li, M.; Verdin, E.; Helfand, S.L. Sirt4 Is a Mitochondrial Regulator of Metabolism and Lifespan in Drosophila Melanogaster. *Proc. Natl. Acad. Sci. USA* **2018**, *115*, 1564–1569. [CrossRef]
32. Augustyniak, M.; Babczyńska, A.; Dziewięcka, M.; Flasz, B.; Karpeta-Kaczmarek, J.; Kędziorski, A.; Mazur, B.; Rozpędek, K.; Seyed Alian, R.; Skowronek, M.; et al. Does Age Pay off? Effects of Three-Generational Experiments of Nanodiamond Exposure and Withdrawal in Wild and Longevity-Selected Model Animals. *Chemosphere* **2022**, *303*, 135129. [CrossRef]
33. Liao, G.Y.; Dai, S.; Bae, E.; Singh, S.; Klug, J.; Pettan-Brewer, C.; Ladiges, W. Morphological Features of the Domestic House Cricket (Acheta Domesticus) for Translational Aging Studies. *Geroscience* **2025**, *47*, 6487–6511. [CrossRef] [PubMed]
34. Liao, G.; Wezeman, J.; Ladiges, W. THE INSECT ACHETA DOMESTICUS IS A PROMISING MODEL FOR AGING AND AGING INTERVENTION STUDIES. *Innov. Aging* **2024**, *8*, 751. [CrossRef]
35. Aranda-Rivera, A.K.; Cruz-Gregorio, A.; Arancibia-Hernández, Y.L.; Hernández-Cruz, E.Y.; Pedraza-Chaverri, J. RONS and Oxidative Stress: An Overview of Basic Concepts. *Oxygen* **2022**, *2*, 437–478. [CrossRef]

https:/ / doi.org/ 10.3390/ ijms27062786

36. le Bourg, É. Oxidative Stress, Aging and Longevity in Drosophila Melanogaster. *FEBS Lett.* **2001**, *498*, 183–186. [CrossRef]
37. Zietara-Krzyk, P.; Flasz, B.; Augustyniak, M. Effects of resveratrol and nanodiamonds on sirtuin activity, oxidative stress and DNA damage in *Acheta domesticus*. *Biochem. Biophys. Res. Commun.* 2026, *Submitted*.
38. Liao, G.; Singh, S.; Dai, S.; Bae, E.; Ladiges, W. Behavioral and Cognitive Decline Are Observable in the Aging House Cricket (*Acheta domesticus*). *Innov. Aging* **2025**, *9*, igaf122.3330. [CrossRef]
39. Singh, A.; Maheshwari, S.; Yadav, J.P.; Kumar, R.; Verma, A.; Singh, S.; Prajapati, B.G. Bioactive Compound-Fortified Nanocarriers in the Management of Neurodegenerative Disease: A Review. *Chem. Biodivers.* **2025**, *22*, e202402018. [CrossRef]
40. Bisconti, M.; Grosjean, P.; Arcolia, V.; Simon, J.-F.; Hennebert, E. Influence of Two Widely Used Solvents, Ethanol and Dimethyl Sulfoxide, on Human Sperm Parameters. *Int. J. Mol. Sci.* **2022**, *24*, 505. [CrossRef]
41. Navarro-Cruz, A.R.; Cesar-Arteaga, I.; Juárez-Serrano, D.; Sánchez, R.Á.-S.; Cid-Pérez, T.S.; Vera-López, O.; Quezada-Figueroa, G.; Kammar-García, A.; Segura-Badilla, O. Evaluation of the Concentration of Ethanol as a Vehicle for the Administration of Resveratrol Measured Through Its Antioxidant Effect in the Hippocampus of Wistar Rats. *J. Nutr. Metab.* **2025**, *2025*, 6614635. [CrossRef]
42. Staats, S.; Wagner, A.; Kowalewski, B.; Rieck, F.; Soukup, S.; Kulling, S.; Rimbach, G. Dietary Resveratrol Does Not Affect Life Span, Body Composition, Stress Response, and Longevity-Related Gene Expression in Drosophila Melanogaster. *Int. J. Mol. Sci.* **2018**, *19*, 223. [CrossRef] [PubMed]
43. Wang, C.; Wheeler, C.T.; Alberico, T.; Sun, X.; Seeberger, J.; Laslo, M.; Spangler, E.; Kern, B.; de Cabo, R.; Zou, S. The Effect of Resveratrol on Lifespan Depends on Both Gender and Dietary Nutrient Composition in Drosophila Melanogaster. *Age* **2013**, *35*, 69–81. [CrossRef] [PubMed]
44. Kermanizadeh, A.; Powell, L.G.; Stone, V.; Møller, P. Nanodelivery Systems and Stabilized Solid-Drug Nanoparticles for Orally Administered Medicine: Current Landscape. *Int. J. Nanomed.* **2018**, *13*, 7575–7605. [CrossRef]
45. Song, Y.; Zhang, J.; Zhu, L.; Zhang, H.; Wu, G.; Liu, T. Recent Advances in Nanodelivery Systems of Resveratrol and Their Biomedical and Food Applications: A Review. *Food Funct.* **2024**, *15*, 8629–8643. [CrossRef]
46. Yang, B.; Dong, Y.; Wang, F.; Zhang, Y. Nanoformulations to Enhance the Bioavailability and Physiological Functions of Polyphenols. *Molecules* **2020**, *25*, 4613. [CrossRef]
47. Peñalva, R.; Morales, J.; González-Navarro, C.J.; Larrañeta, E.; Quincoces, G.; Peñuelas, I.; Irache, J.M. Increased Oral Bioavailability of Resveratrol by Its Encapsulation in Casein Nanoparticles. *Int. J. Mol. Sci.* **2018**, *19*, 2816. [CrossRef] [PubMed]
48. Priyadarshni, N.; Singh, R.; Mishra, M.K. Nanodiamonds: Next Generation Nano-Theranostics for Cancer Therapy. *Cancer Lett.* **2024**, *587*, 216710. [CrossRef]
49. Vo, D.; You, T.; Lin, Y.; Angela, S.; Trong-Nghia, L.; Hsiao, W. Toxicity Assessments of Nanodiamonds. In *Nanodiamonds in Analytical and Biological Sciences*; Wiley: Hoboken, NJ, USA, 2023; pp. 73–94.
50. Milcovich, G.; Jesus, C.F.; Esteves, M.I.M.; Calzaferri, F.; Antunes, F.E.; Di Rosa, L.; Baldassano, S.; Vasto, S.; Gazi, S.; Fernández-Yagüe, M.A.; et al. Advances in Nanomedicine: From Design and Formulation to Regenerative Application and Theranostics. *Nanoscale* **2026**, *18*, 1765–1802. [CrossRef]
51. Baur, J.A.; Sinclair, D.A. Therapeutic Potential of Resveratrol: The in Vivo Evidence. *Nat. Rev. Drug Discov.* **2006**, *5*, 493–506. [CrossRef]
52. Kashi, T.S.; Eskandarion, S.; Esfandyari-Manesh, M.; Marashi, S.M.; Samadi, N.; Fatemi, S.M.; Atyabi, F.; Eshraghi, S.; Dinarvand, R. Improved Drug Loading and Antibacterial Activity of Minocycline-Loaded PLGA Nanoparticles Prepared by Solid/ Oil/ Water Ion Pairing Method. *Int. J. Nanomed.* **2012**, *7*, 221–234. [CrossRef]
53. Iavicoli, I.; Leso, V.; Fontana, L.; Calabrese, E. Nanoparticle Exposure and Hormetic Dose–Responses: An Update. *Int. J. Mol. Sci.* **2018**, *19*, 805. [CrossRef]
54. Mytych, J.; Wnuk, M.; Rattan, S.I.S. Low Doses of Nanodiamonds and Silica Nanoparticles Have Beneficial Hormetic Effects in Normal Human Skin Fibroblasts in Culture. *Chemosphere* **2016**, *148*, 307–315. [CrossRef]
55. Németh, P.; Garvie, L.A.J.; Buseck, P.R. Twinning of Cubic Diamond Explains Reported Nanodiamond Polymorphs. *Sci. Rep.* **2015**, *5*, 18381. [CrossRef]
56. Karpeta-Kaczmarek, J.; Dziewięcka, M.; Augustyniak, M.; Rost-Roszkowska, M.; Pawlyta, M. Oxidative Stress and Genotoxic Effects of Diamond Nanoparticles. *Environ. Res.* **2016**, *148*, 264–272. [CrossRef]
57. Korepanov, V.I.; Hamaguchi, H.; Osawa, E.; Ermolenkov, V.; Lednev, I.K.; Etzold, B.J.M.; Levinson, O.; Zousman, B.; Epperla, C.P.; Chang, H.-C. Carbon Structure in Nanodiamonds Elucidated from Raman Spectroscopy. *Carbon* **2017**, *121*, 322–329. [CrossRef]
58. Afandi, A.; Howkins, A.; Boyd, I.W.; Jackman, R.B. Nanodiamonds for Device Applications: An Investigation of the Properties of Boron-Doped Detonation Nanodiamonds. *Sci. Rep.* **2018**, *8*, 3270. [CrossRef] [PubMed]
59. Ferrari, A.C.; Robertson, J. Raman Spectroscopy of Amorphous, Nanostructured, Diamond–like Carbon, and Nanodiamond. *Philos. Trans. R. Soc. Lond. Ser. A Math. Phys. Eng. Sci.* **2004**, *362*, 2477–2512. [CrossRef] [PubMed]
60. Bogdanov, D.; Bogdanov, A.; Plotnikov, V.; Makarov, S.; Yelisseyev, A.; Chepurov, A. Core Growth of Detonation Nanodiamonds under High-Pressure Annealing. *RSC Adv.* **2021**, *11*, 12961–12970. [CrossRef]

https:/ / doi.org/ 10.3390/ ijms27062786

61. Liang, X.; Li, N.; Zhang, R.; Yin, P.; Zhang, C.; Yang, N.; Liang, K.; Kong, B. Carbon-Based SERS Biosensor: From Substrate Design to Sensing and Bioapplication. *NPG Asia Mater.* **2021**, *13*, 8. [CrossRef]
62. Chen, R.; Jensen, L. Interpreting Chemical Enhancements of Surface-Enhanced Raman Scattering. *Chem. Phys. Rev.* **2023**, *4*, 021305. [CrossRef]
63. Dorfmüller, T. R. Pecora (Ed.): *Dynamic Light Scattering—Applications of Photon Correlation Spectroscopy*, Plenum Press, New York and London 1985. 420 Seiten, Preis: $59.90. *Berichte Bunsenges. Phys. Chem.* **1987**, *91*, 498–499. [CrossRef]
64. Pedroso-Santana, S.; Fleitas-Salazar, N.; Sarabia-Sainz, A.; Silva-Campa, E.; Burgara-Estrella, A.; Angulo-Molina, A.; Melendrez, R.; Pedroza-Montero, M.; Riera, R. A Nanodiamond-Fluorescein Conjugate for Cell Studies. *Adv. Nat. Sci. Nanosci. Nanotechnol.* **2018**, *9*, 015013. [CrossRef]
65. Gatz, S.A.; Keimling, M.; Baumann, C.; Dörk, T.; Debatin, K.-M.; Fulda, S.; Wiesmüller, L. Resveratrol Modulates DNA Double-Strand Break Repair Pathways in an ATM/ ATR–P53- and –Nbs1-Dependent Manner. *Carcinogenesis* **2008**, *29*, 519–527. [CrossRef]
66. Meng, F.; Qian, M.; Peng, B.; Peng, L.; Wang, X.; Zheng, K.; Liu, Z.; Tang, X.; Zhang, S.; Sun, S.; et al. Synergy between SIRT1 and SIRT6 Helps Recognize DNA Breaks and Potentiates the DNA Damage Response and Repair in Humans and Mice. *eLife* **2020**, *9*, e55828. [CrossRef]
67. Lee, J.-H.; Guo, Z.; Myler, L.R.; Zheng, S.; Paull, T.T. Direct Activation of ATM by Resveratrol under Oxidizing Conditions. *PLoS ONE* **2014**, *9*, e97969. [CrossRef]
68. Dobbin, M.M.; Madabhushi, R.; Pan, L.; Chen, Y.; Kim, D.; Gao, J.; Ahanonu, B.; Pao, P.-C.; Qiu, Y.; Zhao, Y.; et al. SIRT1 Collaborates with ATM and HDAC1 to Maintain Genomic Stability in Neurons. *Nat. Neurosci.* **2013**, *16*, 1008–1015. [CrossRef]
69. Tang, M.; Li, Z.; Zhang, C.; Lu, X.; Tu, B.; Cao, Z.; Li, Y.; Chen, Y.; Jiang, L.; Wang, H.; et al. SIRT7-Mediated ATM Deacetylation Is Essential for Its Deactivation and DNA Damage Repair. *Sci. Adv.* **2019**, *5*, eaav1118. [CrossRef] [PubMed]
70. Song, S.; Eckerle, S.; Onichtchouk, D.; Marrs, J.A.; Nitschke, R.; Driever, W. Pou5f1-Dependent EGF Expression Controls E-Cadherin Endocytosis, Cell Adhesion, and Zebrafish Epiboly Movements. *Dev. Cell* **2013**, *24*, 486–501. [CrossRef] [PubMed]
71. Rizzo, A.; Iachettini, S.; Salvati, E.; Zizza, P.; Maresca, C.; D'Angelo, C.; Benarroch-Popivker, D.; Capolupo, A.; del Gaudio, F.; Cosconati, S.; et al. SIRT6 Interacts with TRF2 and Promotes Its Degradation in Response to DNA Damage. *Nucleic Acids Res.* **2017**, *45*, 1820–1834. [CrossRef] [PubMed]
72. Alexander, E.; Leong, K.W. Toxicity and Biodistribution Comparison of Functionalized Nanodiamonds, Quantum Dot Nanocarbons and Gold Nanoparticles. *Front. Nanotechnol.* **2025**, *7*, 1512622. [CrossRef]
73. Rai, R.; Alwani, S.; Khan, B.; Viswas Solomon, R.; Vuong, S.; Krol, E.S.; Fonge, H.; Badea, I. Biodistribution of Nanodiamonds Is Determined by Surface Functionalization. *Diam. Relat. Mater.* **2023**, *137*, 110071. [CrossRef]
74. Burgess, D.J. The Dangers Lurking in the Genetic Background. *Nat. Rev. Genet.* **2011**, *12*, 742. [CrossRef]
75. Sarikhani, M.; Mishra, S.; Desingu, P.A.; Kotyada, C.; Wolfgeher, D.; Gupta, M.P.; Singh, M.; Sundaresan, N.R. SIRT2 Regulates Oxidative Stress-Induced Cell Death through Deacetylation of c-Jun NH2-Terminal Kinase. *Cell Death Differ.* **2018**, *25*, 1638–1656. [CrossRef] [PubMed]
76. Krekora, J.; Pawlowska, E.; Derwich, M.; Drożdż, J.; Blasiak, J. Oxidative Stress, Mitochondrial Homeostasis, and Sirtuins in Atrial Fibrillation. *Int. J. Mol. Sci.* **2025**, *27*, 175. [CrossRef] [PubMed]
77. Lombard, D.B.; Tishkoff, D.X.; Bao, J. Mitochondrial Sirtuins in the Regulation of Mitochondrial Activity and Metabolic Adaptation. In *Handbook of Experimental Pharmacology*; Springer: Berlin/ Heidelberg, Germany, 2011; pp. 163–188.
78. Zhao, L.; Cao, J.; Hu, K.; He, X.; Yun, D.; Tong, T.; Han, L. Sirtuins and Their Biological Relevance in Aging and Age-Related Diseases. *Aging Dis.* **2020**, *11*, 927. [CrossRef]
79. Rahman, M.; Nirala, N.K.; Singh, A.; Zhu, L.J.; Taguchi, K.; Bamba, T.; Fukusaki, E.; Shaw, L.M.; Lambright, D.G.; Acharya, J.K.; et al. Drosophila Sirt2/ Mammalian SIRT3 Deacetylates ATP Synthase β and Regulates Complex V Activity. *J. Cell Biol.* **2014**, *206*, 289–305. [CrossRef] [PubMed]
80. Trinh, D.; Al Halabi, L.; Brar, H.; Kametani, M.; Nash, J.E. The Role of SIRT3 in Homeostasis and Cellular Health. *Front. Cell. Neurosci.* **2024**, *18*, 1434459. [CrossRef]
81. Feldman, J.L.; Dittenhafer-Reed, K.E.; Denu, J.M. Sirtuin Catalysis and Regulation. *J. Biol. Chem.* **2012**, *287*, 42419–42427. [CrossRef]
82. Fullerton, M.D.; Steinberg, G.R. SIRT1 Takes a Backseat to AMPK in the Regulation of Insulin Sensitivity by Resveratrol. *Diabetes* **2010**, *59*, 551–553. [CrossRef]
83. Guan, G.; Chen, Y.; Dong, Y. Unraveling the AMPK-SIRT1-FOXO Pathway: The In-Depth Analysis and Breakthrough Prospects of Oxidative Stress-Induced Diseases. *Antioxidants* **2025**, *14*, 70. [CrossRef]
84. Wojnarová, L.; Kutinová Canová, N.; Farghali, H.; Kučera, T. Sirtuin 1 Modulation in Rat Model of Acetaminophen-Induced Hepatotoxicity. *Physiol. Res.* **2015**, *64*, S477–S487. [CrossRef]
85. Gong, H.; Pang, J.; Han, Y.; Dai, Y.; Dai, D.; Cai, J.; Zhang, T.-M. Age-Dependent Tissue Expression Patterns of Sirt1 in Senescence-Accelerated Mice. *Mol. Med. Rep.* **2014**, *10*, 3296–3302. [CrossRef]

https:/ / doi.org/ 10.3390/ ijms27062786

86. Rogina, B.; Helfand, S.L. Sir2 Mediates Longevity in the Fly through a Pathway Related to Calorie Restriction. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 15998–16003. [CrossRef] [PubMed]
87. Burnett, C.; Valentini, S.; Cabreiro, F.; Goss, M.; Somogyvári, M.; Piper, M.D.; Hoddinott, M.; Sutphin, G.L.; Leko, V.; McElwee, J.J.; et al. Absence of Effects of Sir2 Overexpression on Lifespan in C. Elegans and Drosophila. *Nature* **2011**, *477*, 482–485. [CrossRef]
88. Kanfi, Y.; Naiman, S.; Amir, G.; Peshti, V.; Zinman, G.; Nahum, L.; Bar-Joseph, Z.; Cohen, H.Y. The Sirtuin SIRT6 Regulates Lifespan in Male Mice. *Nature* **2012**, *483*, 218–221. [CrossRef] [PubMed]
89. Zhao, H.; Wu, W. The Influence of Sirtuin 6 on Chondrocyte Senescence in Osteoarthritis Under Aging: Focusing on Mitochondrial Dysfunction and Oxidative Stress. *Antioxidants* **2025**, *14*, 1228. [CrossRef]
90. Kaufman, M.G.; Klug, M.J.; Merritt, R.W. Growth and Food Utilization Parameters of Germ-Free House Crickets, Acheta Domesticus. *J. Insect Physiol.* **1989**, *35*, 957–967. [CrossRef]
91. Horch, H.W.; Mito, T.; Popadić, A.; Ohuchi, H.; Noji, S. *The Cricket as a Model Organism*; Springer: Tokyo, Japan, 2017; ISBN 978-4-431-56476-8.
92. Flasz, B.; Dziewięcka, M.; Ajay, A.K.; Tarnawska, M.; Babczyńska, A.; Kędziorski, A.; Napora-Rutkowski, Ł.; Ziętara, P.; Świerczek, E.; Augustyniak, M. Age- and Lifespan-Dependent Differences in GO Caused DNA Damage in Acheta Domesticus. *Int. J. Mol. Sci.* **2022**, *24*, 290. [CrossRef]
93. Flasz, B.; Dziewięcka, M.; Kędziorski, A.; Tarnawska, M.; Augustyniak, M. Multigenerational Graphene Oxide Intoxication Results in Reproduction Disorders at the Molecular Level of Vitellogenin Protein Expression in Acheta Domesticus. *Chemosphere* **2021**, *280*, 130772. [CrossRef]
94. Bradford, M.M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem.* **1976**, *72*, 248–254. [CrossRef] [PubMed]

https:/ / doi.org/ 10.3390/ ijms27062786

Supplementary Materials

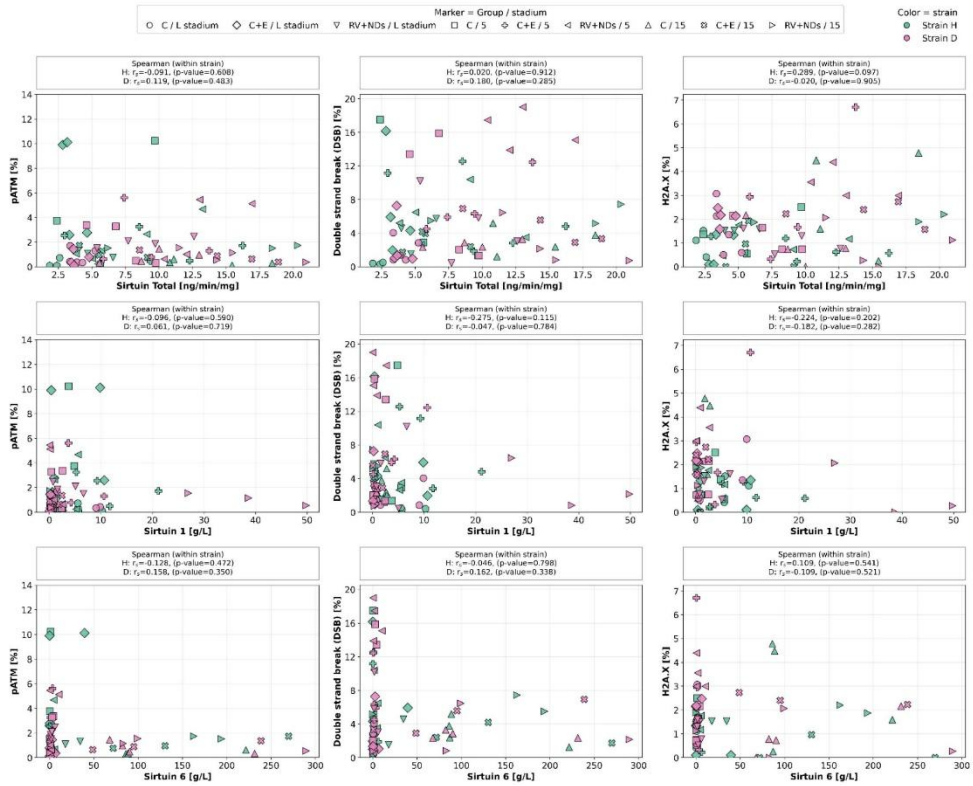

Figure S1. Spearman correlation analysis between sirtuin activity (Sirtuin total, Sirtuin 1, and Sirtuin 6) and DNA damage response markers (pATM, H2A.X, and DSB) in *Acheta domesticus*. Correlation coefficients (r_s) and corresponding p-values were calculated separately for the wild-type strain (H) and the long-lived strain (D). Each point represents an individual measurement from experimental groups (C, C+E, RV+NDs) across different developmental stages (L stadium, Day 5, Day 15). Statistical analyses and visualisation were performed in Python (version 3.13.2) using the Pandas, NumPy, SciPy, and Matplotlib libraries.

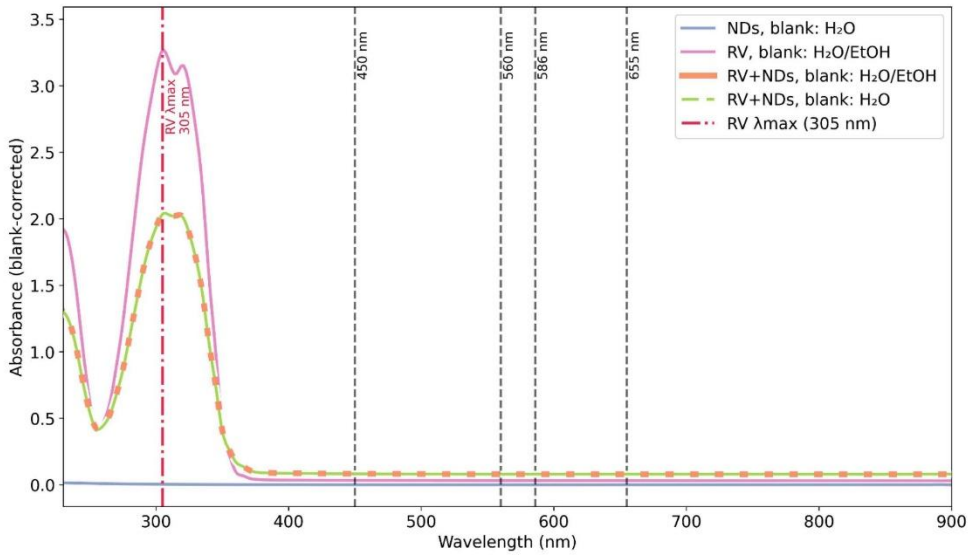

Figure S2. UV-Vis absorption spectra of nanodiamonds (NDs), resveratrol (RV), and the RV-NDs mixture after blank correction. Measurements were performed using a UV-Vis spectrometer (TECAN Infinite M200, Tecan Austria GmbH, Grödig, Austria) over the spectral range of 240-900 nm, with a 5 nm scanning interval. Spectra are shown for NDs (0.2 mg kg^{-1} feed) measured against a water blank (H_2O), RV (23 mg kg^{-1} feed) measured against a water-ethanol blank (H_2O/EtOH), and the RV+NDs mixture (23 mg kg^{-1} RV + 0.2 mg kg^{-1} NDs) corrected either with an H_2O/EtOH blank or with an H_2O blank. The dashed grey vertical lines indicate wavelengths used for spectrophotometric assays (450, 560, 586, and 655 nm). The red dashed line marks the maximum absorption wavelength of RV (λmax = 305 nm). Absorbance values represent mean spectra after blank subtraction. The plot was generated in Python (version 3.13.2; NumPy, Pandas, Matplotlib, SciPy).

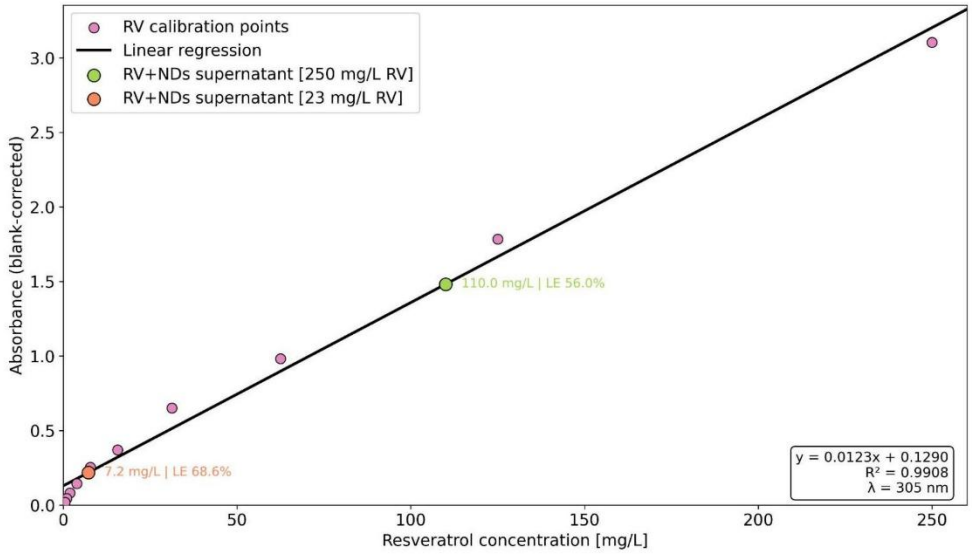

Figure S3. Calibration curve of resveratrol (RV) determined from UV-Vis absorbance at λ = 305 nm after blank correction. Standard solutions of RV were prepared in the concentration range of 0.49-250 mg L^{-1} and measured using a UV-Vis spectrometer (TECAN Infinite M200, Tecan Austria GmbH, Grödig, Austria). The linear regression equation obtained for the calibration curve was y = 0.0123x + 0.1290 (R^2 = 0.9908). The calculated concentrations of non-bound RV in the supernatants obtained after centrifugation of RV-NDs mixtures are indicated on the plot 250 mg L^{-1} RV with 2 mg kg^{-1} nanodiamonds (NDs) and 23 mg L^{-1} RV with 0.2 mg kg^{-1} NDs, respectively. Loading efficiency (LE) of RV on nanodiamonds (NDs) was determined based on the difference between the initial RV concentration and the concentration of free RV in the supernatant. The plot was generated in Python (version 3.13.2; NumPy, Matplotlib, SciPy).

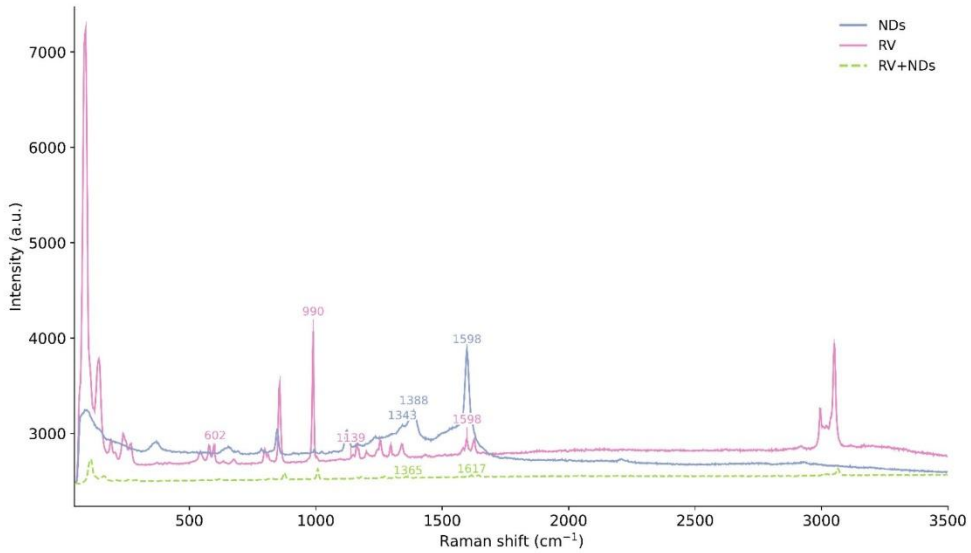

Figure S4. Raman spectra of resveratrol (RV), nanodiamonds (NDs), and the RV+NDs recorded using a Raman spectrometer (WITec Alpha 300 R, WITec GmbH, Ulm, Germany). Measurements were performed using a 100× objective (Zeiss LD EC Epiplan-Neofluar, NA 0.75) and a 600 g mm^{-1} grating. The excitation wavelength was 531.951 nm for RV and NDs, and 487.982 nm for the RV+NDs sample. Spectra were collected with 10 accumulations and integration times of 5 s for RV and 3 s for NDs and RV+NDs. The Rayleigh scattering region below 45 cm^{-1} was excluded from the spectra prior to visualization. The spectra were processed and plotted in Python (version 3.13.2; NumPy, Matplotlib, SciPy).

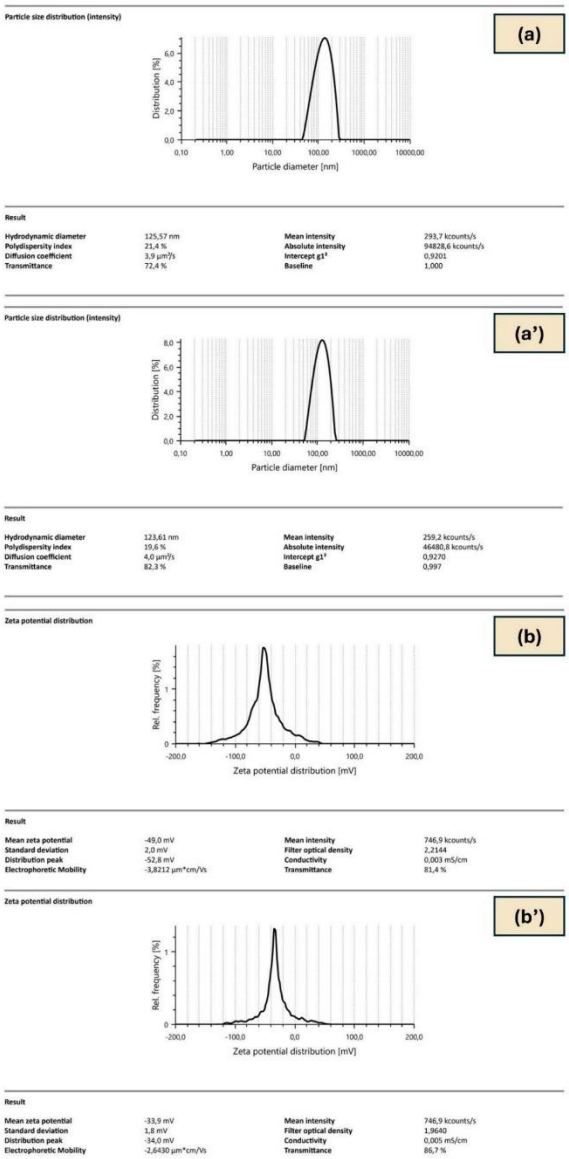

Figure S5. Particle size distribution and zeta potential of nanodiamonds (NDs) and the RV+NDs system measured by dynamic light scattering (DLS). (a) Hydrodynamic diameter distribution of NDs and (a′) RV+NDs dispersion. (b) Zeta potential distribution of NDs and (b′) RV+NDs (Litesizer 500, Anton Paar, Poland).

OŚWIADCZENIA WSPÓŁAUTORÓW

Katowice, 27.04.2026

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OŚWIADCZENIE

Oświadczam, że mój wkład w przygotowanie publikacji:

Ziętara-Krzyk P, Flasz B, Augustyniak M. Evaluation of Molecular Responses and Longevity Markers in Acheta domesticus Following Combined Resveratrol and Nanodiamond Exposure. International Journal of Molecular Sciences. 2026, 27(6), 2786. https://doi.org/10.3390/ijms27062786.

stanowiącego część mojej rozprawy doktorskiej obejmował opracowanie koncepcji badań, przygotowanie i optymalizację metodologii, przeprowadzenie eksperymentów, gromadzenie i analizę danych, interpretację wyników, przygotowanie wizualizacji oraz opracowanie, redakcję i korektę manuskryptu przed publikacją, jak również współudział w korespondencji z redakcją i recenzentami.

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stanowiącej część rozprawy doktorskiej Patrycji Ziętara-Krzyk obejmował pomoc w realizacji badań eksperymentalnych, analizie danych oraz w przygotowaniu manuskryptu.

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OŚWIADCZENIE

Oświadczam, że mój wkład w przygotowanie publikacji:

Ziętara-Krzyk P, Flasz B, Augustyniak M. Evaluation of Molecular Responses and Longevity Markers in Acheta domesticus Following Combined Resveratrol and Nanodiamond Exposure. International Journal of Molecular Sciences. 2026, 27(6), 2786. https://doi.org/10.3390/ijms27062786.

stanowiącej część rozprawy doktorskiej Patrycji Ziętara-Krzyk obejmował pomoc w opracowaniu koncepcji badawczej, nadzór nad projektem, pomoc w interpretacji wyników oraz redakcję manuskryptu.

.....

Prof. dr hab. Maria Augustyniak

INFORMACJE DODATKOWE

Wykaz pozostałych publikacji, konferencji, finansowanych badań i osiągnięć

Publikacje

1. Augustyniak M, Babczyńska A, Dziewięcka M, Flasz B, Karpeta-Kaczmarek J, Kędziorski A, Mazur B, Rozpędek K, Seyed Alian R, Skowronek M, Świerczek E, Świętek A, Tarnawska M, Wiśniewska K, **Ziętara P**. *Does age pay off? Effects of three-generational experiments of nanodiamond exposure and withdrawal in wild and longevity-selected model animals*. Chemosphere. 2022: 135129. doi: 10.1016/j.chemosphere.2022.135129.

   IF: 8.1; MNiSW: 140

2. Augustyniak M, Ajay AK, Kędziorski A, Tarnawska M, Rost-Roszkowska M, Flasz B, Babczyńska A, Mazur B, Rozpędek K, Seyed Alian R, Skowronek M, Świerczek E, Wiśniewska K, **Ziętara P**. *Survival, growth and digestive functions after exposure to nanodiamonds - Transgenerational effects beyond contact time in house cricket strains.* Chemosphere. 2024: 140809. doi: 10.1016/ j.chemosphere.2023.140809.

   IF: 8.1; MNiSW: 140

3. Flasz B, Dziewięcka M, Ajay A.K, Tarnawska M, Babczyńska A, Kędziorski A, Napora-Rutkowski Ł, **Ziętara P**, Świerczek E, Augustyniak M. *Age- and Lifespan-Dependent Differences in GO Caused DNA Damage in Acheta domesticus.* International Journal of Molecular Sciences. 2022 Dec 24;24(1):290. doi: 10.3390/ijms24010290.

   IF: 4.9; MNiSW: 140

4. Bereś PK, **Ziętara P**, Nakonieczny M, Kontowski Ł, Grzbiela M, Augustyniak M. *Cydalima perspectalis in Poland - 8 Years of Invasion against the Background of Three Other Invasive Species*. Diversity 2022, 14, 22. https://doi.org/10.3390/ d14010022.

   IF: 2.3; MNiSW: 70

Konferencje

1. **Ziętara-Krzyk P.** (2025). LA Tech Week - czynne uczestnictwo w wydarzeniach. Rubicon Bridge Ltd. Santa Monica, CA, USA, 13-19.10.2025.
2. Flasz B., **Ziętara-Krzyk P**, Babczyńska A, Tarnawska M, Kędziorski A, Napora-Rutkowski Ł., Augustyniak M. (2025). Multigenerational effects on DNA stability in organisms with different life strategies after carbon-based nanomaterials intoxication. NanoMed Europe, Barcelona, Spain, 27-30.05.2025. Book of Abstracts, pp. 134-135.
3. **Ziętara-Krzyk P**, Flasz B, Augustyniak M. (2025). Stressed out - Oxidative stress and sirtuin activity in *Acheta domesticus* treated with resveratrol or nanodiamonds. NanoMed Europe, Barcelona, Spain, 27-30.05.2025. Book of Abstracts, pp. 231-232.
4. **Ziętara-Krzyk P**. (2024-2025). Ogólnopolska Noc Biologów 2025. Organizator ds. komunikacji - przygotowanie korespondencji do patronów i sponsorów, opracowanie treści komunikatów do mediów społecznościowych, mediów tradycyjnych oraz serwisów UŚ i zewnętrznych. Katowice, 10.2024-01.2025.
5. **Ziętara-Krzyk P**. (2024). UNPA Expert Insights: AI or Humans: Who Owns Intellectual Property? Technology and Intellectual Property Group at Vorys, Sater, Seymour and Pease LLP, webinar online, 27.06.2024.
6. **Ziętara-Krzyk P**. (2023). Biostacje - warsztaty naukowe. 7. Śląski Festiwal Nauki KATOWICE, Katowice, 9-11.12.2023. Udział czynny - prowadzenie warsztatów.
7. **Ziętara-Krzyk P**. (2023). AI<>BA Conference. Silesian Museum, Katowice, Polska, 25.10.2023.
8. Flasz B, Tarnawska M, Babczyńska A, Dziewięcka M, **Ziętara P**, Napora-Rutkowski Ł, Kędziorski A, Augustyniak M. (2023). Multigenerational effects of graphene oxide nanoparticles on *Acheta domesticus* DNA stability. NanoToday Conference, konferencja międzynarodowa, San Diego, USA, 22-25.04.2023.
9. Flasz B, Dziewięcka M., Tarnawska M, Babczyńska A, Napora-Rutkowski Ł, **Ziętara P**, Kędziorski A, Augustyniak M. (2022). Different susceptibility to DNA damage caused by graphene oxide in two strains of model organism *Acheta domesticus*. International Forum on Research Excellence (IFoRE), konferencja międzynarodowa, Waszyngton, USA, 4-6.11.2022. Poster.

10. **Ziętara P.** (2022). VII Krajowa Konferencja Arachnologiczna. Uniwersytet Śląski w Katowicach. Konferencja krajowa, Katowice, Polska, 23.09.2022.
11. **Ziętara P.** (2022). Perspektywy Women in Tech Summit 2022. Konferencja ogólnopolska, Warszawa, Polska, 7-8.06.2022.
12. **Ziętara P**, Seyed Alian R, Dziewięcka M. (2022). Ocena toksyczności tlenku grafenu z różnych źródeł pochodzenia z użyciem standardowych biotestów. Konferencja naukowa NATURE 2022, Stowarzyszenie Młodych Naukowców, konferencja ogólnopolska, online, 21.05.2022. Poster.
13. Bereś, PK, **Ziętara P**, Nakonieczny M, Kontowski Ł, Grzbiela M, Augustyniak M. (2021). Eight years of *Cydalima perspectalis* in Poland - From the first finding to the status of invasive species. Proceedings of 1st International Electronic Conference on Biological Diversity, Ecology and Evolution session Invasive Species and Diversity. 16.03.2021. 68, 9 pp, DOI: doi.org/10.3390/BDEE2021-09474.
14. **Ziętara P.** (2020). Stanowisko pokazowe „Loża przyrodników". 4. Śląski Festiwal Nauki KATOWICE, Katowice, 26-17.01.2020.
15. **Ziętara P.** (2019). Stanowisko pokazowe „Stanowisko Pokazowe Interdyscyplinarnego Koła Naukowego PLANETA". 3. Śląski Festiwal Nauki KATOWICE, Katowice, 13-14.01.2019.

Projekty naukowe

Rodzaj projektu: *Preludium 22, NCN*

Tytuł: *Droga do długowieczności - Efekty oddziaływania resweratrolu lub/i nanodiamentów na aktywność sirtuin u Acheta domesticus*

Numer projektu: *2023/49/N/NZ7/01058*

Okres realizacji: *01.2024 - 01.2027*

Kierownik projektu: *Patrycja Ziętara-Krzyk*

Opiekun Naukowy: *prof. dr hab. Maria Augustyniak*

Kursy i szkolenia

1. 2022-2026 - Kursy programowania i technologii web/AI: podstawy programowania, data science, HTML5 (semantyczny markup), CSS3, Python, wprowadzenie do uczenia maszynowego i AI, LLM (Large Language Models).
2. 25.11-21.12.2019 - Warsztaty „Bezpieczeństwo radiologiczne" w ramach projektu Innovative Start, SPZOZ Zagłębiowskie Centrum Onkologii im. Sz. Starkiewicza w Dąbrowie Górniczej.
3. 13.12.2019 - „Funkcjonowanie oczyszczalni ścieków i gospodarowanie osadami ściekowymi".
4. W ramach projektu Innovative Start, Chorzowsko-Świętochłowickie Przedsiębiorstwo Wodociągów i Kanalizacji.
5. 4-13.12.2019 - Warsztaty „Bezpieczeństwo radiologiczne", projekt Innovative Start.
6. 08-09.06.2019 - Szkolenie w zakresie CITES w ramach projektu NEW, Śląski Ogród Zoologiczny w Chorzowie.
7. 19.12.2018 - Szkolenie „Profesjonalna prezentacja" w ramach projektu Innovative Start.

Nagrody i wyróżnienia

1. Laureatka konkursu na zwiększenie stypendium doktoranckiego (rok akademicki 2024/2025). Wyróżnienie przyznane w ramach programu Inicjatywy Doskonałości Badawczej (IDB).
2. Nagroda za najlepszy poster w kategorii Environmental Sciences (2022). International Forum on Research Excellence (IFoRE), International Conference, Washington, USA. Tytuł pracy: „Different susceptibility to DNA damage caused by graphene oxide in two strains of model organism *Acheta domesticus*". Nagroda przyznana zespołowi badawczemu; prezentująca: dr Barbara Flasz.
3. Nagroda publiczności - III miejsce (05.2022). Ogólnopolska Konferencja Naukowa NATURE Polish Scientific Conference 2022 za prezentację posteru pt. „Ocena toksyczności tlenku grafenu z różnych źródeł pochodzenia z użyciem standardowych biotestów".
4. Laureatka programu „Najlepszy Absolwent 2021", Uniwersytet Śląski w Katowicach (12.2021).

Funkcje, działalność organizacyjna i popularyzatorska

1. Członkostwo w zespole projektowym T4EU - Uniwersytet Śląski w Katowicach. Okres: 11.2023 r. - 10.2024 r.
2. Prowadzenie warsztatów w ramach Uniwersytetu Młodzieży UŚ (02.2023).
3. Przedstawiciel URSD podczas rozmów kwalifikacyjnych w ramach naborów do MŚSD.
4. Ambasador kierunku Biologia (2021/2022). Udział w kampanii wizerunkowej promującej studia na Uniwersytecie Śląskim.