12-17-2022

Resistance to Mild Cold Stress is Greater in Both Wild-Type and Long-Lived GHR-KO Female Mice.

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Recommended Citation

Fang, Yimin, Medina, David, Stockwell, Robert, McFadden, Samuel, Hascup, Erin R, Hascup, Kevin N and Bartke, Andrzej. "Resistance to Mild Cold Stress is Greater in Both Wild-Type and Long-Lived GHR-KO Female Mice.." *Geroscience* 45, No. 2 (Dec 2022): 1081-1093. doi:doi.org/10.1007/s11357-022-00706-0.

GeroScience
https://doi.org/10.1007/s11357-022-00706-0

ORIGINAL ARTICLE

Resistance to mild cold stress is greater in both wild-type and long-lived GHR-KO female mice

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Received: 8 September 2022 / Accepted: 30 November 2022

Abstract Adapting to stress, including cold environmental temperature (eT), is crucial for the survival of mammals, especially small rodents. Long-lived mutant mice have enhanced stress resistance against oxidative and non-oxidative challenges. However, much less is known about the response of those long-lived mice to cold stress. Growth hormone receptor knockout (GHR-KO) mice are long-lived with reduced growth hormone signaling. We wanted to test whether GHR-KO mice have enhanced resistance to cold stress. To examine the response of GHR-KO mice to cold eT, GHR-KO mice were housed at mild cold eT (16 °C) immediately following weaning. Longevity results showed that female GHR-KO and wild-type (WT) mice retained similar lifespan, while both male GHR-KO and WT mice had shortened lifespan compared to the mice housed at 23 °C eT. Female GHR-KO and WT mice housed at 16 °C had upregulated fibroblast growth factor 21 (FGF21), enhanced energy metabolism, reduced plasma triglycerides, and increased mRNA expression of some xenobiotic enzymes compared to females housed at 23 °C and male GHR-KO and WT mice housed under the same condition. In contrast, male GHR-KO and WT mice housed at 16 °C showed deleterious effects in parameters which might be associated with their shortened longevity compared to male GHR-KO and WT mice housed at 23 °C. Together, this study suggests that in response to mild cold stress, sex plays a pivotal role in the regulation of longevity, and female GHR-KO and WT mice are more resistant to this challenge than the males.

Keywords GHR-KO mice · Longevity · Cold stress · FGF21 · Energy metabolism

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Introduction

Genetic extension of longevity in laboratory mice is associated with enhanced stress resistance, including both cellular and whole-animal responses to a variety of oxidative and non-oxidative challenges [1]. Growth hormone receptor knockout (GHR-KO) mice are long-lived with reduced growth hormone

Published online: 17 December 2022

signaling [2, 3]. In vitro experiments with dermal fibroblasts isolated from GHR-KO mice showed that they have enhanced resistance to multiple forms of stress, including paraquat-induced oxidative stress, hydrogen peroxide, and ultraviolet light, compared to cells isolated from control wild-type (WT) mice [4]. However, it was reported by others that in vivo response of GHR-KO mice to paraquat challenge was sexually dimorphic, with WT males surviving longer than male GHR-KO mice and mean survival of GHR-KO females not differing from that of WT mice [5]. Moreover, the available reports indicate that the female long-lived mutant mice have enhanced oxidative stress resistance compared to the males [1].

There is little information about the response of these long-lived growth-hormone (GH)-resistant mice to the stress from alterations of ambient environmental temperature (eT). Our previous study showed that the longevity of female GHR-KO mice housed at thermoneutral eT (30 °C) was extended compared to male GHR-KO mice housed at the same condition [6]. While the longevity of male and female WT mice did not differ from those of the control littermates housed at 23 °C (unpublished data). The response of GHR-KO mice to cold stress remains to be elucidated. Adapting to cold stress is crucial for the survival of mammals, especially small rodents [7]; thus, we set up experiments to examine whether GHR-KO mice could be more resistant to cold stress than WT mice at eT 16 °C. To study the impact of GH signaling on responses to life-long cold stress, GHR-KO and WT mice were housed in mild cold eT (16 °C) starting immediately after weaning. This avoided combining the impact on the pups and the impact on the dam (such as altered milk production or maternal behavior). The results showed a sexually dimorphic response to mild cold stress associated with upregulated fibroblast growth factor 21 (FGF21), enhanced energy metabolism, reduced plasma triglycerides, and increased mRNA expression of some xenobiotic enzymes that may confer beneficial effects on longevity in female mice.

Methods

Mice maintenance and longevity study

All animal procedures were approved by the Institutional Animal Care and Use Committee of Southern Illinois University School of Medicine. Mice were group-housed (five animals per cage) under temperature and light-controlled conditions (23 °C/16 °C ± 1 °C, 12-h light/dark cycles) with ad libitum access to food (Chow 5001 with 23.4% protein, 5% fat, and 5.8% crude fiber; LabDiet PMI Feeds) and water.

Our breeding colony was developed by crossing male GHR-KO mice with 129 Ola/BALB/c background generated in Dr. John J. Kopchick's laboratory [2] with female WT mice derived from the crossing of C57BL/6 and C3H strains and thereafter breeding of the resulting animals in a closed colony without brother × sister mating. Thus, the animals have a heterogeneous genetic background.

Immediately postweaning (3 weeks of age), mice were group-housed according to sex and genotype (five animals/cage) and randomly assigned to either 23 °C or 16 °C eT. For the longevity study, animals remained at 23 °C or 16 °C eT until natural end of life. Animals that appeared near death were euthanized, and the date of euthanasia was considered the date of death. A separate cohort of group-housed mice was sacrificed after exposure to 23 °C or 16 °C eT for 8 months and used for tissue and plasma collection.

Core body temperature (Tb) was measured by rectal thermometry, as described by others [8], for mice housed at 23 °C or 16 °C eT at age of 7 months.

Indirect calorimetry

Indirect calorimetry was performed as previously described [9] using AccuScan Metabolic System (AccuScan Instruments) at age of seven and a half months. In this system, mice are housed individually in metabolic chambers at 23 °C or 16 °C eT with ad libitum access to food and water. After a 24-h acclimation period, gas samples were collected and analyzed every 10 min per mouse and averaged each hour for measurement of oxygen consumption (VO_2), energy expenditure (EE), and respiratory quotient (RQ).

Assessment of blood chemistry

Plasma was collected from isoflurane-anesthetized animals by cardiac puncture at sacrifice. The blood was mixed with EDTA, followed by centrifugation at 10,000 g for 15 min at 4 °C for plasma collection. As per the manufacturer's protocol, FGF21 was

measured with a Mouse FGF21 ELISA Kit (BioVendor R&D, Cat# RD291108200R), triglycerides (TG) with Pointe Scientific Triglycerides Liquid Reagents (Pointe Scientific, Cat# 23–666-410), a non-esterified fatty acids (NEFA) test (Roche, Ref# 11 383 175 001), testosterone with a Mouse Testosterone ELISA Kit (Crystal Chem, Cat# 80,552), corticosterone with a Corticosterone ELISA Kit (Cayman chemical, Cat# 501,320), and adrenocorticotropic hormone (ACTH) with a Adrenocorticotropic hormone Enzyme Immunoassay Kit (RayBiotech, Cat# EIA-ACTH).

RT–PCR

The mRNA expression was analyzed by quantitative RT-PCR as previously described [9] using the StepOne Real-Time PCR System (Thermo Fisher Scientific) and SYBR Green MasterMix (Applied Biosystems). RNA was extracted using RNeasy mini kits or RNeasy Lipid Tissue Mini Kits (Qiagen) following the manufacturer's instructions. Relative expression was calculated as previously described [10].

Statistical analysis

Differences between two groups were assessed with unpaired two-tailed Student's *t*-tests. The significance of area under the curve was analyzed using the two-way ANOVA. A log-rank test followed by a Tukey post-hoc analysis between animals at 23 °C and 16 °C eT was used for the longevity study. Maximum lifespan was estimated by calculating average longevity of the oldest surviving 20% of animals in each group and comparing them using conditional Student's *t*-tests between the group at 23 °C and the group at 16 °C. Significance was set at $p < 0.05$. Data are presented as means ± SEM. All statistical analyses and graphs were completed using Prism 9 (GraphPad Inc, La Jolla, CA, USA).

Results

The longevity of GHR-KO and WT mice was impacted in a sex-specific manner in response to housing under mild cold stress (16 °C eT)

To examine whether a mild reduction of eT could affect the longevity of GHR-KO and WT control littermates, the animals were transferred to 16 °C eT at 21 days of age (immediately postweaning). The data showed that the overall longevity of female GHR-KO and WT mice chronically housed at 16 °C was similar to genotype-matched females housed at 23 °C (Fig. 1a and b), despite some increased early life mortality of female GHR-KO mice. In contrast, the longevity of male GHR-KO (Fig. 1c) and WT (Fig. 1d) mice chronically housed at 16 °C was reduced compared to genotype-matched males housed at 23 °C. The longevity of male GHR-KO and WT mice chronically housed at 16 °C was shortened by 16% ($p \leq 0.0001$) and 11% ($p < 0.01$) (Table 1) respectively, compared to 23 °C housing conditions. The results indicate that in the examined range of eT, female GHR-KO and WT mice were resistant to mild cold stress compared to the males. As a result, the longevity of female GHR-KO and WT mice housed at 16 °C since weaning was similar to females housed at 23 °C. Male GHR-KO and WT mice were sensitive to mild cold stress, since their longevity was reduced compared to males housed at 23 °C.

Female mice chronically housed at 16 °C had higher levels of FGF21 and increased energy metabolism

To analyze parameters associated with the response of GHR-KO and WT mice to mild cold eT, another cohort was transferred to 16 °C immediately postweaning, and tissues and plasma were collected after 8 months. FGF21 is a stress hormone that is upregulated by cold exposure in mice [7], and a growing number of publications indicate the regulation of circulating FGF21 in response to cold in humans [11–13]. FGF21 is also a potent longevity factor that coordinates interactions between energy metabolism and stress responses [14]. To determine whether chronically housing at reduced eT could affect FGF21, the hepatic mRNA expression and circulating plasma levels of FGF21 were measured. The results showed that the induction of FGF21 by mild cold stress was also sex-dependent. After exposure to 16 °C for 8 months, the hepatic mRNA expression (Fig. 2a) and plasma concentration (Fig. 2b) of FGF21 were increased in both female genotypes, particularly in WT mice. No differences were observed in the males. It has been reported that increased FGF21 levels improved mitochondrial function by increasing the expression of peroxisome

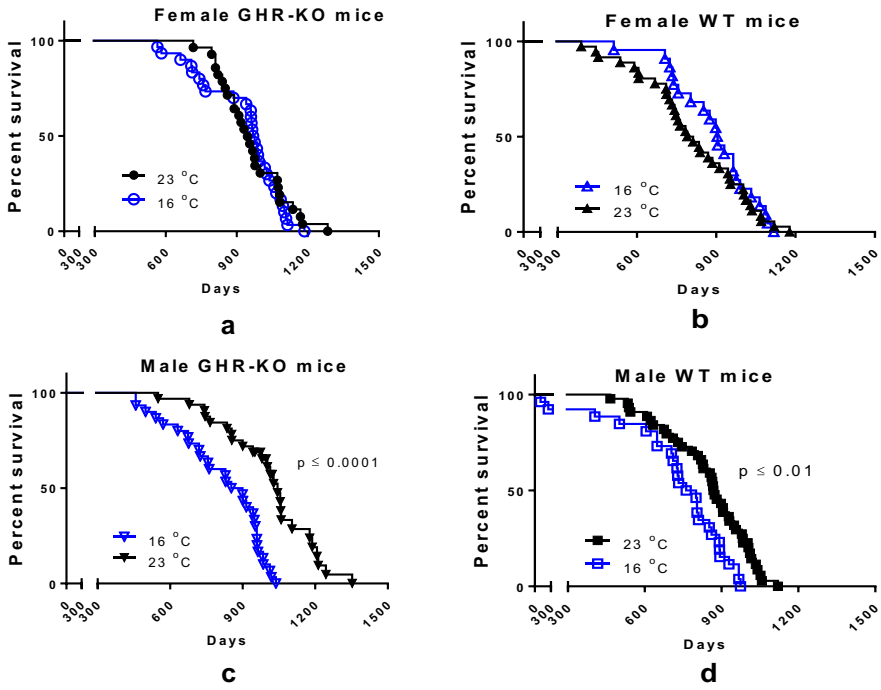


Fig. 1 The longevity of GHR-KO and WT mice had sexually dimorphic response to mild cold stress at 16 °C eT. **a** Survival plots for female GHR-KO mice at 16 °C eT ($n=30$) or 23 °C eT ($n=30$). **b** Survival plots for female WT mice at 16 °C eT ($n=22$) or 23 °C eT ($n=35$). **c** Survival plots for male GHR-KO mice at 16 °C eT ($n=31$) or 23 °C eT ($n=35$). **d** Survival plots for male WT mice at 16 °C eT ($n=26$) or 23 °C eT ($n=32$). The mice were group-housed (five animals/cage) at 23 °C or 16 °C eT immediately postweaning (3 weeks of age). For the longevity study, animals remained at 23 °C or 16 °C eT until natural end of life. *p*-values based on the log-rank test

Table 1 The analysis of longevity for GHRKO and WT mice at 16 ℃ and 23 ℃ eT

Mice	Treatment	*n*	Median
Female WT	16 ℃ eT	22	822 ± 153
	23 ℃ eT	35	812 ± 198
Female GHRKO	16 ℃ eT	30	945 ± 167
	23 ℃ eT	30	972 ± 134
Male WT	16 ℃ eT	26	781** ± 214
	23 ℃ eT	32	873 ± 163
Male GHRKO	16 ℃ eT	31	876**** ± 179
	23 ℃ eT	35	1044 ± 173

Data represented as mean ± SEM. *p* values for lifespan were calculated by a log-rank test followed by a Tukey post-hoc analysis of groups at 16 ℃ and 23 ℃ eT

** $p \leq 0.01$, **** $p \leq 0.0001$

proliferator-activated receptor-gamma coactivator (PGC)-1α [15–17], a major transcription factor that is strongly induced by cold exposure and plays a central role in the regulation of energy metabolism [18]. Thus, the mRNA expression of PGC-1α and energy metabolism was examined. The mRNA expression of PGC-1α in interscapular brown adipose tissue (BAT) was increased in female GHR-KO and WT mice chronically housed at 16 °C (Fig. 2c), which was consistent with the increased hepatic and plasma levels of FGF21. Cold exposure did not alter PGC-1α BAT gene expression in males, which was also consistent with their FGF21 levels.

Energy metabolic results showed that in both sexes of GHR-KO and WT mice chronically housed at 16 °C, oxygen consumption (VO_2) (Fig. 2d–g), energy expenditure (EE) (Fig. 2h–k), and respiratory quotient (RQ) (Fig. 2l–o) significantly increased compared to the mice housed at 23 °C. Additionally, there were some differences in energy metabolism between sexes. First, female WT mice chronically housed at 16 °C were the only animals to maintain a respiratory quotient diurnal rhythm that was similar to female WT mice housed at 23 °C (Fig. 2l). Second, there was a difference in

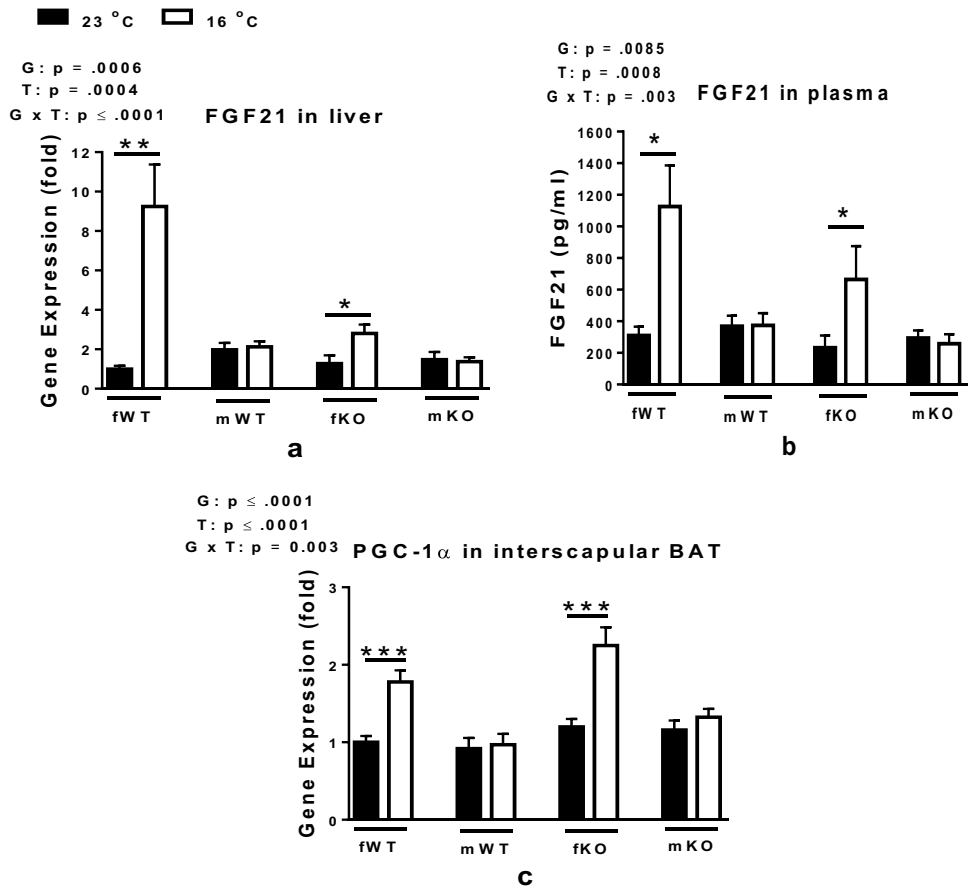


Fig. 2 Female mice chronically housed at 16 °C eT had increased levels of FGF21 and energy metabolism. Abbreviations: mWT, male WT mice; fWT, female WT mice; mKO, male GHR-KO mice; fKO, female GHR-KO mice. **a** mRNA levels of FGF21 in the liver. **b** Concentration of FGF21 in plasma. **c** mRNA levels of PGC-1α in interscapular BAT. Oxygen consumption (VO_2) in WT mice (**d** and **e**) or in GHR-KO mice (**f** and **g**). Energy expenditure (EE) in WT mice (**h** and **i**) or in GHR-KO mice (**j** and **k**). Respiratory Quotient (RQ) in WT mice (**l** and **m**) or in GHR-KO mice (**n** and **o**). **p** mRNA levels of UCP1 in interscapular BAT. Data are means ± SEM ($n = 8$–20). A two-way ANOVA was used to determine p-values for the categorial variables (G = genotype and T = eT) and their interaction ($G \times T$), which are shown for each bar graph. $^*p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$ based on a two-tailed Student's t-test

energy metabolism between GHR-KO females and GHR-KO males chronically housed at 16 °C. GHR-KO females had enhanced VO_2 (Fig. 2f and g) and EE (Fig. 2j and k), but similar RQ (Fig. 2n and o), compared to male GHR-KO mice housed under the same condition. The lower energy metabolism in male GHR-KO mice chronically housed at 16 °C may have been associated with lower mRNA expression of uncoupling protein 1 (UCP1), a key regulator of thermogenesis [19, 20], in their interscapular BAT (Fig. 2p). When chronically housed at 16 °C, the improved energy metabolism observed in female WT and GHR-KO mice could be driven by increased FGF21 and PGC-1α. These adaptations might provide some of the beneficial effects on the females compared to the males.

Beneficial alterations in lipid metabolism induced by housing GHR-KO and WT mice at reduced temperature were greater in females

Serum triglycerides (TG) were reported to be lower in transgenic mice overexpressing FGF21 [21]. Given that female GHR-KO and WT mice chronically housed at 16 °C had higher hepatic and plasma FGF21 levels (Fig. 2a and b), TG in plasma were

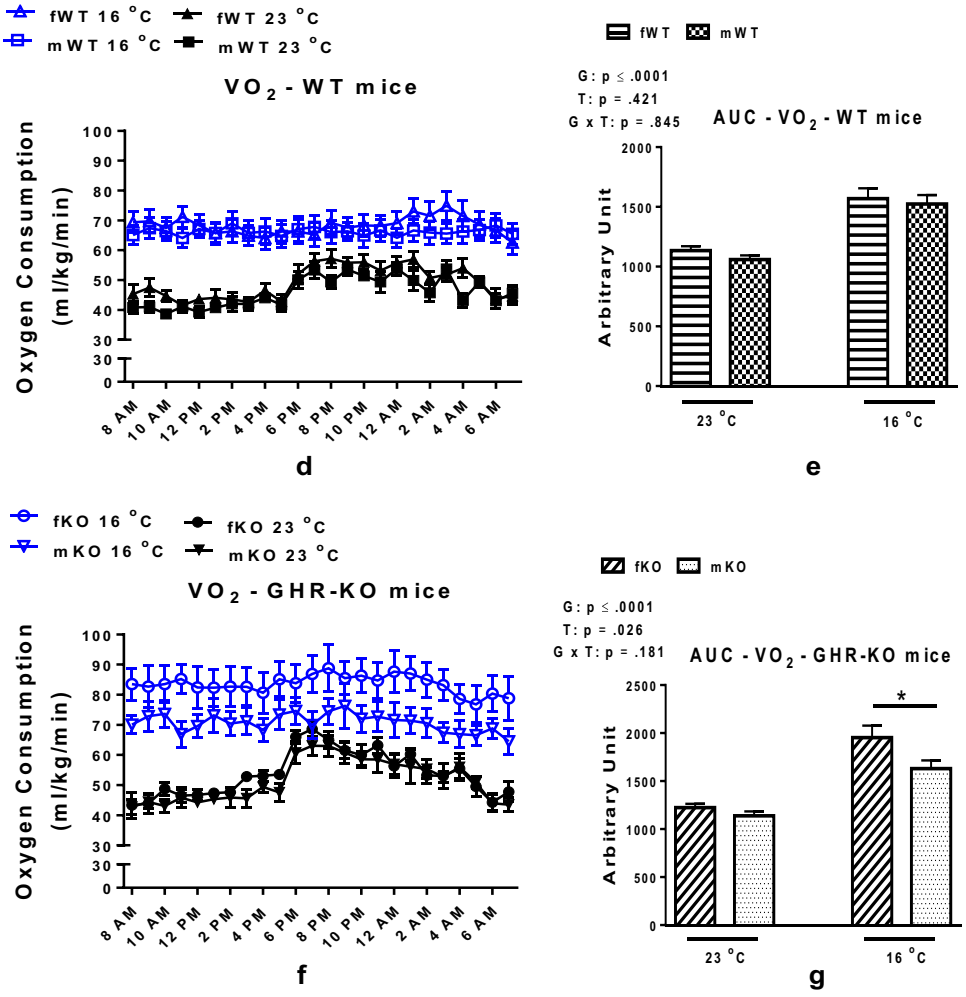
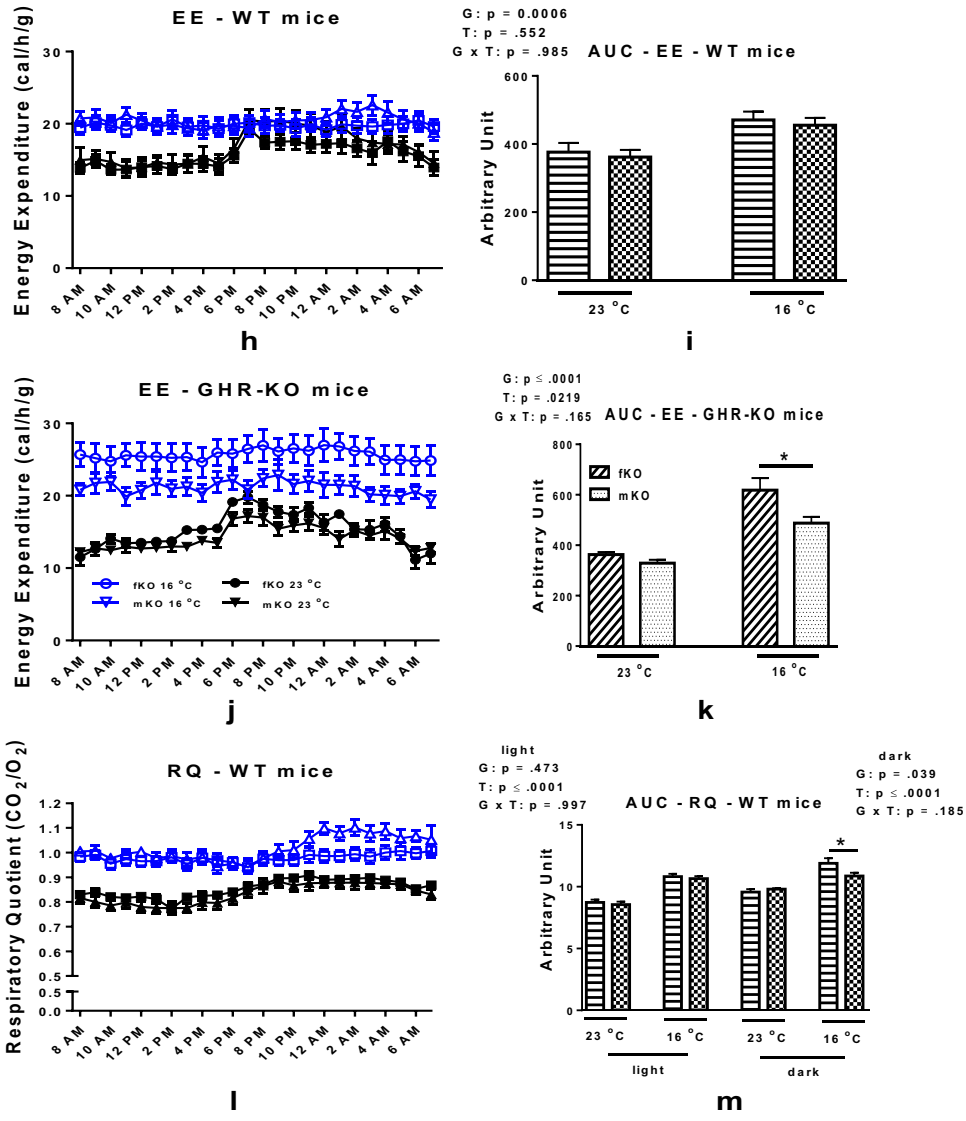


Fig. 2 (continued)

measured. The results revealed that plasma TG concentration was lower in female GHR-KO and WT mice chronically housed at 16 °C compared to genotype-matched females housed at 23 °C (Fig. 3a). These findings are consistent with elevated levels of FGF21 in females chronically housed at 16 °C (Fig. 2a and b). FGF21 decreases plasma TG levels via a dual mechanism that involves reducing plasma non-esterified fatty acids (NEFA), while concomitantly increasing CD36 (facilitates fatty acid uptake) and lipoprotein lipase (LPL) mediated-TG disposal [22]. The examination of these potential mechanisms showed no differences in plasma NEFA from the mice housed at different eT (Fig. 3b). Next, the mRNA expression of CD36 and LPL was measured in subcutaneous white adipose tissue (WAT), since this is a target organ of FGF21 action [22]. In subcutaneous WAT, the mRNA expression of LPL was higher in both female and male GHR-KO mice housed at 16 °C, but not in either sex of WT mice (Fig. 3c). Conversely, the mRNA expression of CD36 was reduced in both female and male WT mice, but not in either sex of GHR-KO mice (Fig. 3d) chronically housed at 16 °C. The NEFA, LPL, and CD36 levels suggest they might not associate with lower TG in female GHR-KO and WT mice chronically housed at 16 °C, even though these mice had elevated FGF21 (Fig. 2a and b). Nevertheless, a reduction in the levels of TG could be beneficial for the females under mild cold stress at 16 °C.



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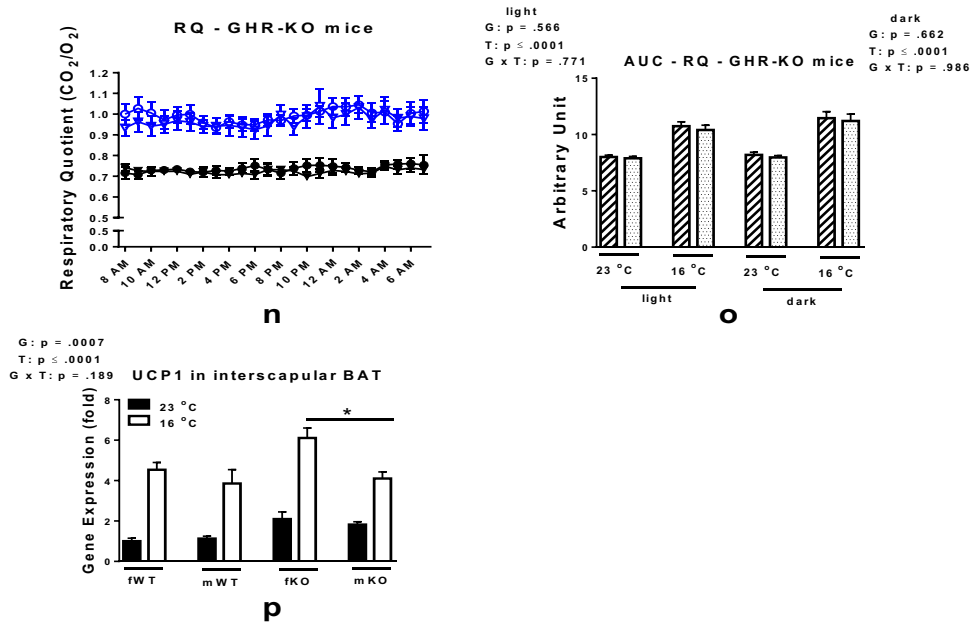
The impact of reduced eT on xenobiotic metabolism is greater in the females

Xenobiotic metabolism has been linked to the rate of aging. For example, the expression of xenobiotic metabolizing enzymes (XME) was increased in GH-deficient and GH-resistant long-lived mutant mice, including GHR-KO mice [23]. However, it was unknown whether chronically exposing GHR-KO mice to mild cold eT had any effects on XME. Thus, we measured hepatic cytochrome P450 family 4 (Cyp4) expression, since enzymes in the CYP4 family are involved in the metabolism of fatty acids, xenobiotics, therapeutic drugs, and signaling molecules [24]. The mRNA expression of Cyp4a10 and Cyp4a14 was elevated in female WT animals and in both sexes of GHR-KO mice housed at 16 °C (Fig. 3e and f). Cyp4 enzymes contribute to the ω-hydroxylation of fatty acids in the liver and kidney [25, 26]. During periods of cellular stress, the contribution of the ω-hydroxylation pathway to overall fatty acid oxidation is increased. The ω-oxidation of fatty acids can decrease the accumulation of free fatty acids and reduce the potential for lipotoxicity [25].



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However, no differences in plasma NEFA in the current study (Fig. 3b) suggest that elevated Cyp4a10 and Cyp4a14 expression did not affect the levels of free fatty acids in circulation and thus might not have an impact on the reduction of the potential for lipotoxicity. Alteration in these two Cyp4 enzymes could involve other functions [24], which warrants further investigation.

The mRNA expression of Flavin-containing monooxygenase 4 (Fmo4), one of the XME enzymes that is a conserved regulator of stress resistance and metabolism [27], was increased only in female WT mice chronically housed at 16 °C (Fig. 3g). This indicates that WT females have an additional mechanism to enhance resistance to mild cold stress. In general, female WT and female GHR-KO mice chronically housed at 16 °C had higher levels of XME than the mice housed at 23 °C (Fig. 3e–g), and that could provide protective roles under mild hypothermic condition.

Potentially detrimental effects on male mice chronically housed at mild cold eT

In mammals, lower core body temperature (Tb) is associated with increased longevity [28, 29], and increased secretion of FGF21 was reported to reduce Tb [14]. The measurement of the Tb in all groups of mice showed that male WT mice chronically housed at 16 °C had elevated Tb. The other groups of mice had similar Tb, regardless of housing conditions (Fig. 4a).

Body composition analysis revealed that both male GHR-KO and WT mice chronically housed at 16 °C had increased relative kidney (Fig. 4b) and liver (Fig. 4c) mass compared to genotype-matched males housed at 23 °C. Higher mass of the kidney or liver could be associated with higher circulating testosterone levels [30, 31]. Thus, we examined plasma testosterone levels. The results revealed that the concentration of plasma testosterone was significantly higher in male GHR-KO mice, and an increasing trend was observed in WT males chronically housed at 16 °C (Fig. 4d). The large variations in plasma testosterone levels in WT males may be attributed to fluctuations in plasma testosterone levels in mice [32]. To examine whether the mild cold temperature could also affect corticosterone and adrenocorticotropic hormone (ACTH) (hormones responding to environmental challenges), the concentration of corticosterone and ACTH in plasma was measured. Consistent with a previous report [33], plasma levels of corticosterone

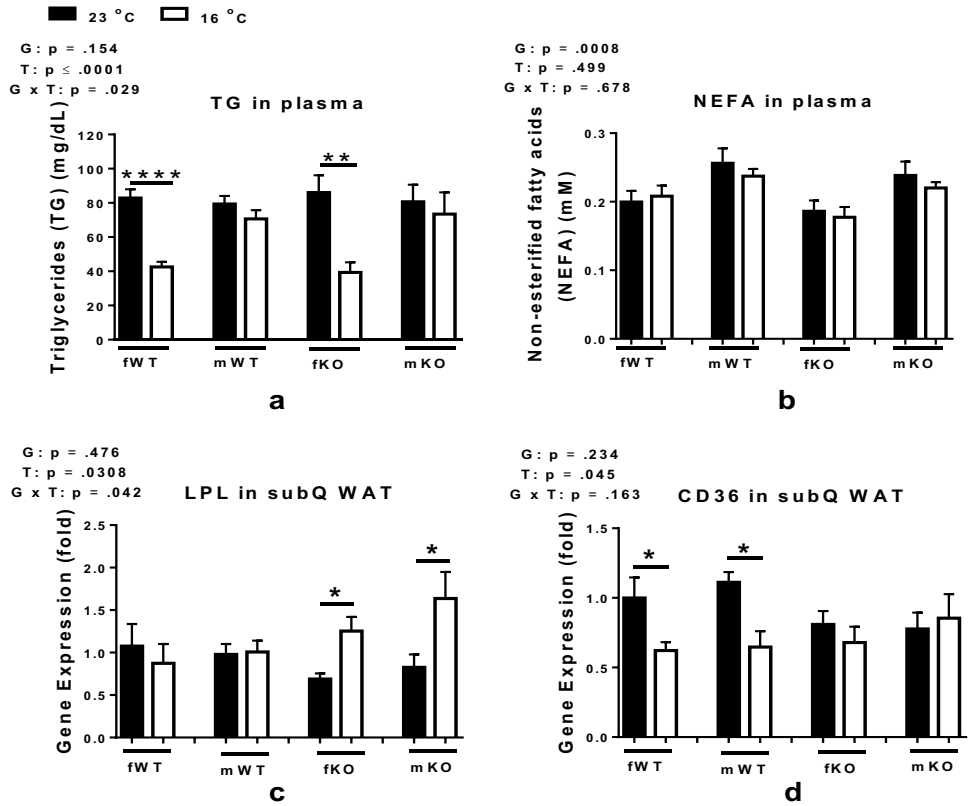
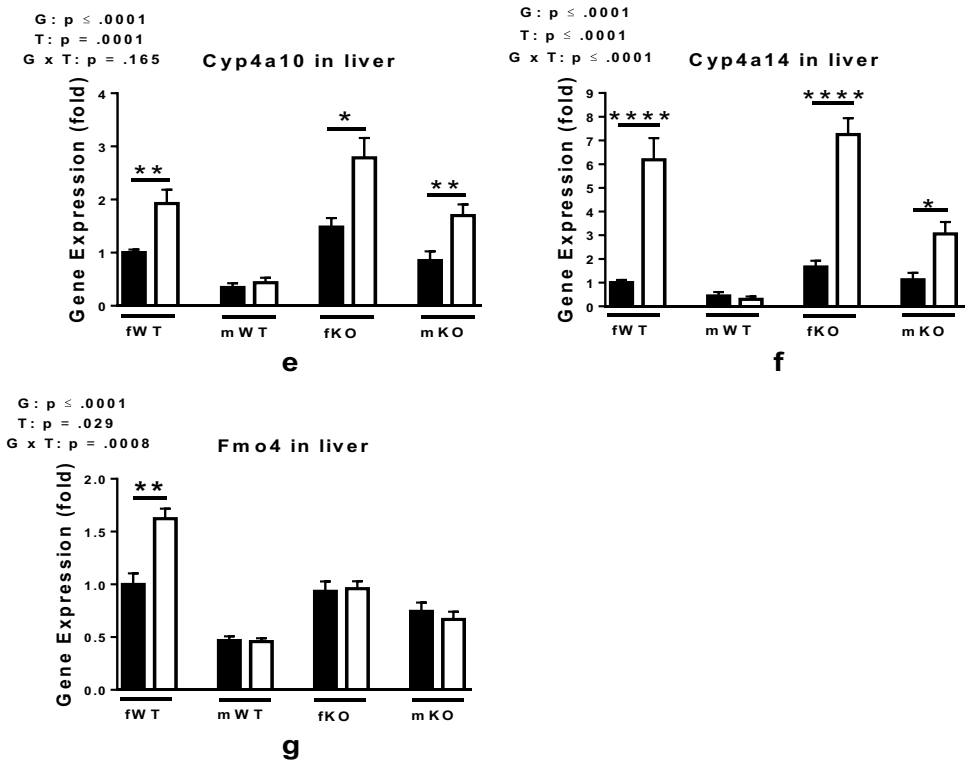


Fig. 3 Beneficial alterations induced by housing GHR-KO and WT mice at 16 °C eT were greater in females. Abbreviations: mWT, male WT mice; fWT, female WT mice; mKO, male GHR-KO mice; fKO, female GHR-KO mice. **a** Concentration of triglycerides (TG) in plasma. **b** Concentration of non-esterified fatty acids (NEFA) in plasma. **c** mRNA levels of lipoprotein lipase (LPL) in subcutaneous WAT. **d** mRNA levels of CD36 in subcutaneous WAT. **e** mRNA levels of cytochrome P450 family 4 (Cyp) 4a10 (Cyp4a10) in the liver. **f** mRNA levels of Cyp4a14 in the liver. **g** mRNA levels of Flavin-containing monooxygenase 4 (Fmo4) in the liver. Data are represented as means ± SEM ($n = 8–10$). A two-way ANOVA was used to determine p-values for the categorial variables (G = genotype and T = eT) and their interaction (G × T), which are shown for each graph. *$p \leq 0.05$, **$p \leq 0.01$, ****$p \leq 0.0001$ based on a two-tailed Student's t-test

in male GHR-KO mice were higher than in male WT mice housed at 23 °C (Fig. 4e). Moreover, at mild cold stress (16 °C), the plasma levels of corticosterone were further elevated in male GHR-KO mice chronically housed at 16 °C compared to male GHR-KO mice housed at 23 °C (Fig. 4e). The plasma concentration of corticosterone in both GHR-KO and WT female mice was higher than in the male mice housed at 23 °C, but mild cold stress did not affect plasma levels of corticosterone in females of either genotype (Fig. 4e). Interestingly, plasma levels of ACTH in male WT mice were higher than in female WT mice at both housing conditions, and mild cold stress led to the increased levels of ACTH only in male GHR-KO mice chronically housed at 16 °C (Fig. 4f). Together, the elevated Tb in male WT mice, increased testosterone in both male GHR-KO and WT mice and raised levels of corticosterone and ACTH in male GHR-KO mice, could negatively impact the longevity of male mice chronically housed at 16 °C.

Discussion

Based on the results from the current study, the response of GHR-KO and WT mice to mild cold stress showed sexual dimorphism with female GHR-KO and WT mice being more resistant to mild cold stress at eT of 16 °C. Although numerous phenotypic characteristics differ greatly between GHR-KO and



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WT mice [3, 34, 35], the responses to reduced eT examined in the present study were influenced more strongly by sex than by the genotype of the animals. Sexually dimorphic responses to the mild cold stress were consistent with the whole-animal response to paraquat challenge, in which the survival of GHR-KO and WT females was similar to control GHR-KO and WT females [5].

Some physiological and biochemical traits of GHR-KO and WT mice chronically housed at 16 °C were also altered in a sex-dependent manner. When GHR-KO and WT mice were chronically housed under mild cold eT condition, FGF21 was upregulated in females of both genotypes. It was reported by others that in female mice, estrogen (one of main female sex hormones) acting on hepatocytes increases FGF21 transcription and production, which promotes energy expenditure [36]. Thus, it is reasonable to speculate that in our study, female sex hormones could play a role in higher FGF21 production in female GHR-KO and WT mice than in the male mice under mild cold stress. Higher FGF21 levels in female mice may have accounted for the improvements in energy metabolism (Fig. 2d–o) and the reduction in TG concentration in plasma (Fig. 3a). Additionally, the increased mRNA expression of some XME genes in the female mice was also found when chronically housed at 16 °C (Fig. 3e–g). These alterations may have provided protective effects for the females under mild cold stress conditions.

In mammals, core body temperature (Tb) is inversely correlated with longevity, and higher Tb is associated with decreased longevity [28, 29]. The Tb response to increased levels of sex hormone has opposing effects depending upon the animal's sex [29]. For example, the reduced levels of testosterone resulted in reduced Tb in male C57Bl/6 mice, while the reduced levels of progesterone led to increased Tb in female C57Bl/6 mice [29]. In our study, male WT mice chronically housed at 16 °C had elevated testosterone and Tb (Fig. 4a), which could be a contributing factor for their reduced longevity. The increased testosterone, corticosterone, and ACTH levels in male GHR-KO mice chronically exposed to mild cold

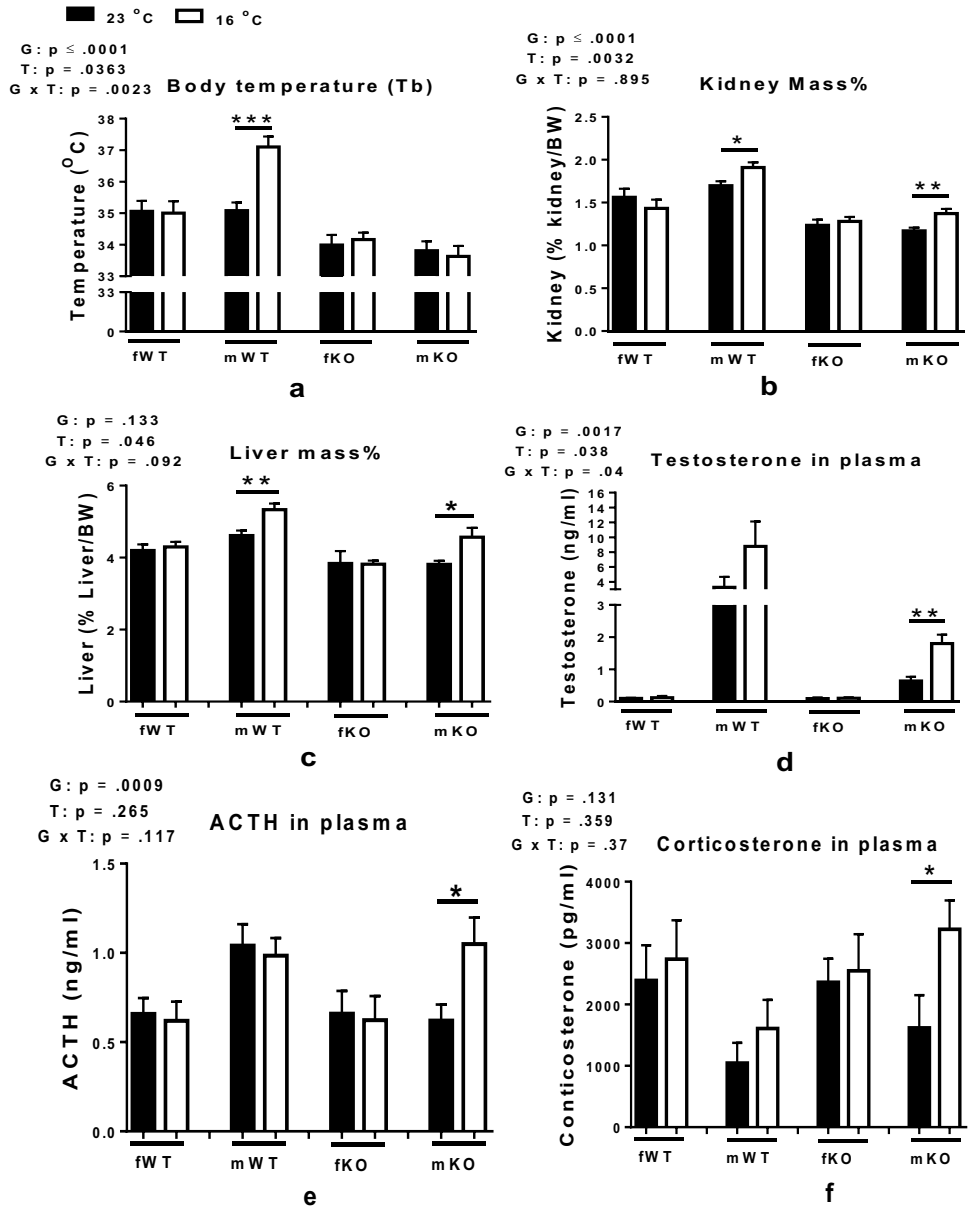

Fig. 4 Potentially detrimental effects on male mice chronically housed at mild cold eT. Abbreviations: mWT, male WT mice; fWT, female WT mice; mKO, male GHR-KO mice; fKO, female GHR-KO mice. **a** Body temperature (Tb) measured by rectal thermometer. **b** Percentage of kidney mass ((kidney mass/body weight)×100) at sacrifice. **c** Percentage of liver mass ((liver mass/body weight)×100) at sacrifice. **d** Concentration of testosterone in plasma. **e** Concentration of corticosterone in plasma. **f** Concentration of adrenocorticotropic hormone (ACTH) in plasma. Data are represented as means ± SEM ($n = 10$–20). A two-way ANOVA was used to determine p-values for the categorial variables (G = genotype and T = eT) and their interaction (G×T), which are shown for each graph. $*p \le 0.05$, $**p \le 0.01$, $***p \le 0.001$ based on a two-tailed Student's t-test

stress suggest a chronically elevated stress response that has negative impacts on overall health [37–39]. Together, these alterations might have negatively impacted the longevity of male mice chronically housed at 16 °C, which warrants further examination in future studies.

Our study suggests that under conditions of mild cold stress, sex plays a pivotal role in the regulation of longevity, and females are more resistant than males to this challenge. Based on these findings, future research will examine differences in gonadal hormones, sex chromosome genes, or additional metabolic factors. However, genotype, presumably acting via its effects on the strength of GH and insulin-like growth factor 1 signals, also influenced some of the responses to reduced eT. For example, changes in the expression of genes related to xenobiotic metabolism in male GHR-KO mice were absent in male WT (Fig. 3e and f), and elevated Tb detected in WT males was absent in male GHR-KO mice (Fig. 4a). Although both GHR-KO and WT female mice were more resistant to mild cold eT, as shown through elevated FGF21, they could use different mechanisms to adapt to these conditions. These combined effects induced by mild cold eT could cause the sexually dimorphic impact on lifespan.

Compared to global GHR-KO mice, adult-onset GHRKO (aGHRKO) and various tissue-specific GHR knockout mice also showed sexual dimorphism in some physiological and biochemical parameters. For instance, aGHRKO females, not males, have an increased maximal lifespan when compared to female controls [40]. The body weights of muscle GHR knockout (MuGHR-KO) mice were decreased in the male mice and increased in the female mice [41]. The males, not females, of liver-specific GHR (LiGHR-KO) knockout mice did not increase in relative fat mass percentage after 6 months of age when compared to their control males. Male LiGHR-KO mice had increased TG concentration and lipid accumulation in the liver compared to their control males [42]. In an adult-onset, hepatocyte-specific, GHR knockdown (aLivGHRkd) mouse, hepatic de novo lipogenesis (DNL) was enhanced in both males and females, but hepatosteatosis developed only in males [43], which was consistent with the observation in LiGHR-KO mice [42]. In intestine epithelial cell-specific GHR knockout (IntGHR-KO) mice, occludin level was increased in males, and fecal albumin was decreased in females [44]. It seems that the effects of GHR knockout, either in various tissues or globally, showed sex-dependent responses of various physiological and biochemical parameters. Our current study added more information on the sexually dimorphic response of global GHR-KO mice to mild cold stress, which provides an important reference on the relationship of sex to mild cold resistance.

Acknowledgements We would like to thank Dr. John J. Kopchick for providing animals to start our colonies and Lisa Hensley for editorial assistance.

Funding This work was supported by the National Institutes of Health NIA R01-AG057767 (YF, SM, KNH, ERH) and NIA R01-AG061937 (YF, SM, ERH, KNH, AB), Dale and Deborah Smith Center for Alzheimer's Research and Treatment, Kenneth Stark Endowment (YF, SM, KNH, ERH), NIA R21-AG062985, American Diabetes Association 1–19-IBS-126 (DM, RS, AB), AG031736 and AG051869 (AB).

Declarations

Conflict of interest The authors declare no competing interests.

References

1. Brown-Borg HM. Longevity in mice: is stress resistance a common factor? Age (Dordr). 2006;28(2):145–62.
2. Coschigano KT, Clemmons D, Bellush LL, Kopchick JJ. Assessment of growth parameters and life span of GHR/BP gene-disrupted mice. Endocrinology. 2000;141(7):2608–13.
3. Coschigano KT, et al. Deletion, but not antagonism, of the mouse growth hormone receptor results in severely decreased body weights, insulin, and insulin-like growth factor I levels and increased life span. Endocrinology. 2003;144(9):3799–810.
4. Salmon AB, et al. Fibroblast cell lines from young adult mice of long-lived mutant strains are resistant to multiple forms of stress. Am J Physiol Endocrinol Metab. 2005;289(1):E23-29.
5. Hauck SJ, Aaron JM, Wright C, Kopchick JJ, Bartke A. Antioxidant enzymes, free-radical damage, and response to paraquat in liver and kidney of long-living growth hormone receptor/binding protein gene-disrupted mice. Horm Metab Res. 2002;34(9):481–6.
6. Fang Y, et al. Lifespan of long-lived growth hormone receptor knockout mice was not normalized by housing at 30 degrees C since weaning. Aging Cell. 2020;19(5): e13123.
7. Klein Hazebroek M, Keipert S. Adapting to the cold: a role for endogenous fibroblast growth factor 21 in thermoregulation? Front Endocrinol (Lausanne). 2020;11:389.
8. Meyer CW, Ootsuka Y, Romanovsky AA. Body temperature measurements for metabolic phenotyping in mice. Front Physiol. 2017;8:520.
9. Fang Y, et al. Effects of rapamycin on growth hormone receptor knockout mice. Proc Natl Acad Sci USA. 2018;115(7):E1495–503.
10. Masternak MM, et al. Metabolic effects of intra-abdominal fat in GHRKO mice. Aging Cell. 2012;11(1):73–81.
11. Lee P, et al. Mild cold exposure modulates fibroblast growth factor 21 (FGF21) diurnal rhythm in humans: relationship

between FGF21 levels, lipolysis, and cold-induced thermogenesis. J Clin Endocrinol Metab. 2013;98(1):E98-102.
12. Lee P, et al. Irisin and FGF21 are cold-induced endocrine activators of brown fat function in humans. Cell Metab. 2014;19(2):302–9.
13. Hanssen MJ, et al. Serum FGF21 levels are associated with brown adipose tissue activity in humans. Sci Rep. 2015;5:10275.
14. Salminen A, Kaarniranta K, Kauppinen A. Regulation of longevity by FGF21: interaction between energy metabolism and stress responses. Ageing Res Rev. 2017;37:79–93.
15. Potthoff MJ, et al. FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. Proc Natl Acad Sci USA. 2009;106(26):10853–8.
16. Makela J, et al. Fibroblast growth factor-21 enhances mitochondrial functions and increases the activity of PGC-1alpha in human dopaminergic neurons via Sirtuin-1. Springerplus. 2014;3:2.
17. Ji LL, Kang C. Role of PGC-1alpha in sarcopenia: etiology and potential intervention - a mini-review. Gerontology. 2015;61(2):139–48.
18. Liang H, Ward WF. PGC-1alpha: a key regulator of energy metabolism. Adv Physiol Educ. 2006;30(4):145–51.
19. Cannon B, Nedergaard J. Brown adipose tissue: function and physiological significance. Physiol Rev. 2004;84(1):277–359.
20. Kalinovich AV, de Jong JM, Cannon B, Nedergaard J. UCP1 in adipose tissues: two steps to full browning. Biochimie. 2017;134:127–37.
21. Zhang Y, et al. The starvation hormone, fibroblast growth factor-21, extends lifespan in mice. Elife. 2012;1: e00065.
22. Schlein C, et al. FGF21 lowers plasma triglycerides by accelerating lipoprotein catabolism in white and brown adipose tissues. Cell Metab. 2016;23(3):441–53.
23. Steinbaugh MJ, Sun LY, Bartke A, Miller RA. Activation of genes involved in xenobiotic metabolism is a shared signature of mouse models with extended lifespan. Am J Physiol Endocrinol Metab. 2012;303(4):E488-495.
24. Jarrar YB, Lee SJ. Molecular functionality of cytochrome P450 4 (CYP4) genetic polymorphisms and their clinical implications. Int J Mol Sci. 2019;20(17):4274. https://doi.org/10.3390/ijms20174274.
25. Hsu MH, Savas U, Griffin KJ, Johnson EF. Human cytochrome p450 family 4 enzymes: function, genetic variation and regulation. Drug Metab Rev. 2007;39(2–3):515–38.
26. Hardwick JP. Cytochrome P450 omega hydroxylase (CYP4) function in fatty acid metabolism and metabolic diseases. Biochem Pharmacol. 2008;75(12):2263–75.
27. Huang S, Howington MB, Dobry CJ, Evans CR, Leiser SF. Flavin-containing monooxygenases are conserved regulators of stress resistance and metabolism. Front Cell Dev Biol. 2021;9: 630188.
28. Conti B, et al. Transgenic mice with a reduced core body temperature have an increased life span. Science. 2006;314(5800):825–8.
29. Sanchez-Alavez M, Alboni S, Conti B. Sex- and age-specific differences in core body temperature of C57Bl/6 mice. Age (Dordr). 2011;33(1):89–99.
30. Gagliano-Juca T, et al. Effects of testosterone administration (and its 5-alpha-reduction) on parenchymal organ volumes in healthy young men: findings from a dose-response trial. Andrology. 2017;5(5):889–97.
31. Nucci RAB, et al. Effects of testosterone administration on liver structure and function in aging rats. Aging Male. 2017;20(2):134–7.
32. Bartke A, Steele RE, Musto N, Caldwell BV. Fluctuations in plasma testosterone levels in adult male rats and mice. Endocrinology. 1973;92(4):1223–8.
33. Al-Regaiey KA, Masternak MM, Bonkowski M, Sun L, Bartke A. Long-lived growth hormone receptor knockout mice: interaction of reduced insulin-like growth factor i/insulin signaling and caloric restriction. Endocrinology. 2005;146(2):851–60.
34. Zhou Y, et al. A mammalian model for Laron syndrome produced by targeted disruption of the mouse growth hormone receptor/binding protein gene (the Laron mouse). Proc Natl Acad Sci USA. 1997;94(24):13215–20.
35. Basu R, Qian Y, Kopchick JJ. Mechanisms in endocrinology: lessons from growth hormone receptor gene-disrupted mice: are there benefits of endocrine defects? Eur J Endocrinol. 2018;178(5):R155–81.
36. Allard C, et al. Activation of hepatic estrogen receptor-alpha increases energy expenditure by stimulating the production of fibroblast growth factor 21 in female mice. Mol Metab. 2019;22:62–70.
37. Hannibal KE, Bishop MD. Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation. Phys Ther. 2014;94(12):1816–25.
38. Schooling CM, Leung GM. Testosterone and cardiovascular risk. Lancet Diabetes Endocrinol. 2015;3(9):682.
39 Mariotti A. The effects of chronic stress on health: new insights into the molecular mechanisms of brain-body communication. Future Sci OA. 2015;1(3):FSO23.
40. Junnila RK, et al. Disruption of the GH receptor gene in adult mice increases maximal lifespan in females. Endocrinology. 2016;157(12):4502–13.
41. List EO, et al. Removal of growth hormone receptor (GHR) in muscle of male mice replicates some of the health benefits seen in global GHR-/- mice. Aging (Albany NY). 2015;7(7):500–12.
42. List EO, et al. Liver-specific GH receptor gene-disrupted (LiGHRKO) mice have decreased endocrine IGF-I, increased local IGF-I, and altered body size, body composition, and adipokine profiles. Endocrinology. 2014;155(5):1793–805.
43. Nagarajan A, Srivastava H, Jablonsky J, Sun LY. Tissue-specific GHR knockout mice: an updated review. Front Endocrinol (Lausanne). 2020;11: 579909.
44. Young JA, et al. Characterization of an intestine-specific GH receptor knockout (IntGHRKO) mouse. Growth Horm IGF Res. 2019;46–47:5–15.