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Does housing temperature influence glucose regulation and muscle-fat crosstalk in mice?

Geneviève Tavernier

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Biochimie

Does housing temperature influence glucose regulation and muscle-fat crosstalk in mice?

--Manuscript Draft--

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Keywords:	Thermoneutrality, brown adipose tissue, glucose metabolism, myokines, mouse model
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First Author:	Marlène Lac
Order of Authors:	Marlène Lac Geneviève Tavernier, Ph.D. Cedric Moro, Ph.D.
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Abstract:	<p>The robustness of scientific results is partly based on their reproducibility. Working with animal models, particularly in the field of metabolism, requires to avoid any source of stress to rule out a maximum of bias. Housing at room temperature is sufficient to induce thermal stress activating key thermogenic organs such as brown adipose tissue (BAT) and skeletal muscle. BAT covers most of the non-shivering thermogenesis in mice and burns a variety of fuels such as glucose and lipids. A high prevalence of BAT is associated with a strong protection against type 2 diabetes risk in humans, implying that BAT plays a key role in glucose homeostasis. However, thermal stress is poorly and inconsistently considered in experimental research. This thermal stress can significantly impede interpretation of phenotypes by favoring compensatory signaling pathways. Indeed, various studies revealed that thermoneutrality is essential to study metabolism in mice in order to reach a suitable level of "humanization". In this review, we briefly discuss if and how ambient temperature influence blood glucose homeostasis through BAT and muscle-fat crosstalk.</p>
Suggested Reviewers:	
Opposed Reviewers:	

Toulouse, August 18, 2022

RE: Manuscript submission

Dear Editors,

It is our pleasure to submit our manuscript entitled “ Does ambient temperature influence glucose regulation and muscle-fat crosstalk in mice?” as an original mini-review article for publication in Biochimie.

This submission falls into the upcoming special issue entitled " Thermoneutrality: A Confounding Variable in Metabolic Research" with the following handling editors: Katherine J. Motyl, Ormon Mc Dougald, Jeff Gimble, and Claude Forest.

The manuscript has been approved by the co-authors and no conflict of interest have been disclosed.

Please let me know if I can provide any additional information.

Warmest regards,

Cedric Moro, Ph.D.
Research Director Inserm,
Head of Team MetaDiab



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Dear Claude,

Thank you for the review of our manuscript BIOCHI-D-22-00551R1 entitled “Does housing temperature influence glucose regulation and muscle-fat crosstalk in mice?”. We specifically addressed Reviewer 2 remaining comments and comprehensively revised the manuscript. We thank again the reviewers for their insightful and constructive comments and hope that the paper will be now acceptable for publication in the journal.

Sincerely,
Cedric Moro, Ph.D.
On behalf of all co-authors

Reviewer #2: The manuscript has been substantially revised, however, some problems are still present:

We sincerely thank the reviewer for the positive and constructive assessment of the manuscript.

1) On line 57 of the tracked version, you mention that main heat producing organs are "cardiac and skeletal muscles, brain, liver, and adipose tissues". White adipose tissue is definitely not an important producer of heat, modify the text so that it is more accurate.

We agree. Sorry for this omission. The sentence has been changed (line 56).

2) On line 63-64 of the tracked version you use the argument that after the stimulation by norepinephrine BAT is the main source of heat, while the muscle temperature decreases. This is not surprising given the fact that NE is a specific activator of BAT, but you cannot draw from the result of this experiment the conclusion that shivering is not significant contributor to the cold-induced heat production.

This is correct. We modified the sentence as follow:

When mice are exposed to cold (4-6°C), shivering slightly increase (~30%) as recorded by electromyography [5]. However, the contribution of shivering to cold-induced energy expenditure and heat production was minor in comparison to cold-induced non shivering thermogenesis mostly produced by brown fat. Thus, when directly recording tissue temperature with specific probes, norepinephrine increases markedly heat production in BAT (+1°C) while temperature in skeletal muscle did not change significantly [5].

3) On line 204 of the tracked version you use BMR for conditions of measurement of metabolic rate under temperatures below thermoneutrality. By definition, BMR is measured at thermoneutrality.

Yes, we agree and have revised the sentence accordingly for “metabolic rate” (line 190).

4) On line 217 of the tracked version you mention the fact that "... baseline energy expenditure at room temperature was significantly higher in IL-6^{-/-} mice than in wild-type animals...". This fact does not correspond to the sentence on line 221 of the tracked version ("... oxygen consumption at room temperature was identical in wild-type and IL-6^{-/-} mice..." and also does not correspond to the data given in this paper.

Sorry for the confusion. "... baseline energy expenditure" was meant for "RER". The sentence has been changed accordingly (lines 203-205).

BIOCHIMIE conflict of interest declaration and author agreement form

Title of Paper: Does housing temperature influence glucose regulation and muscle-fat crosstalk in mice?

Author (s): Marlène Lac, Geneviève Tavernier, and Cedric Moro

Please delete one of the following two lines:

We have no conflict of interest to declare.


This statement is to certify that all Authors have seen and approved the manuscript being submitted, and agree to the submission to *BIOCHIMIE*. We warrant that the article is the Authors' original work. We warrant that the article has not received prior publication, is not under consideration for publication elsewhere, and will not be submitted for publication elsewhere, in whole or in part, while under consideration for publication in *BIOCHIMIE*. On behalf of all Co-Authors, the corresponding Author shall bear full responsibility for the submission.

We attest to the fact that all Authors listed on the title page have contributed significantly to the work, have read the manuscript, attest to the validity and legitimacy of the data and their interpretation, and agree to its submission to *BIOCHIMIE*. We further attest that no other person has fulfilled the requirements for authorship as stated in the Elsevier Authorship-factsheet (**2019_ETHICS_AUTH02 - attached**), but is not included in the list of authors, and that no other person has contributed substantially to the writing of the manuscript but is not included either among the authors or in the acknowledgements.

All authors agree that no modification to the author list can be made without the written acceptance of all authors and the formal approval of the Editor-in-Chief. All authors accept that the Editor-in-Chief's decisions over acceptance or rejection or in the event of any breach of the Principles of Ethical Publishing in *BIOCHIMIE* being discovered, of retraction are final.

On behalf of all authors (*delete line if not appropriate*)

Corresponding Author Signature:



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Date 11.02.2022

Abstract

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3 The robustness of scientific results is partly based on their reproducibility. Working with animal
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5 models, particularly in the field of metabolism, requires to avoid any source of stress to rule out
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1 **Does housing temperature influence glucose regulation and muscle-fat crosstalk in mice?**

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12 **Abstract**

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38 27 **Keywords:** Thermoneutrality, brown adipose tissue, glucose metabolism, myokines, mouse
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47 **Highlights**

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50 31 • Housing at room temperature induces a significant thermal stress in mice
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52 32 • Brown adipose tissue plays a major role in regulating systemic glucose flux
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55 33 • Housing at thermoneutrality offsets thermal stress and can uncover frank phenotypes
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58 34 • Thermoneutrality is required to “humanize” mice
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1. Introduction

The living conditions on the surface of the earth are different all around the globe. Indeed, animals and especially mammals have to cope with drastic temperature changes during the year or sometimes during a day [1]. The maintenance of their body temperature at a constant value is essential for metabolic and vital functions. This maintenance of body temperature is energy consuming; at “low” ambient temperatures, the body must provide enough substrates, such as glucose and fatty acids to produce heat. In contrast, when the ambient temperature is “high”, the body must increase its energy expenditure in order to dissipate the heat. An ambient “third” temperature exists, the thermoneutrality, over which metabolic rate is minimum and constant, with a balance at equilibrium between heat loss and heat production [2]. However, most experiments in mice are carried out at room temperature (~21-22°C). At room temperature, mice exhibit a ~2-fold increase in metabolic rate which has major influence on systemic metabolism and thermogenic organs such as brown fat and skeletal muscle [3]. This review briefly examines to what extent ambient temperature can influence glucose homeostasis and muscle-fat crosstalk through circulating secreted signals.

2. Notions of thermogenesis and thermoneutrality

The body temperature is maintained at a constant value within the organism by means of a balance that allows the production and dissipation of heat. Heat is virtually produced by every organ having a baseline metabolism. Main heat producing organs involve cardiac and skeletal muscles, brain, liver and **brown and beige fat**. In presence of thermal stress, both shivering and non-shivering thermogenesis is activated. Shivering does not seem to occur significantly at room temperature [4]. When mice are exposed to cold (4-6°C), shivering slightly increase (~30%) as recorded by electromyography [5]. **However, the contribution of shivering to cold-**

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60 induced energy expenditure and heat production was minor in comparison to cold-induced non
61 shivering thermogenesis mostly produced by brown fat. Thus, when directly recording tissue
62 temperature with specific probes, norepinephrine increases markedly heat production in BAT
63 (+1°C) while temperature in skeletal muscle did not change significantly [5]. Brown adipose
64 tissue (BAT) ensures most of the non-shivering thermogenesis. BAT is present in rodents and
65 in infants, but also in adult humans [6]. Indeed, the numerous BAT mitochondria contain
66 uncoupling protein 1 (UCP1) [7]. This protein can be inhibited by purine-based nucleotides, or
67 activated by fatty acids [8]. When UCP1 is activated by fatty acids, the proton gradient
68 produced by the mitochondrial respiratory chain is dissipated, allowing heat production. The
69 muscle is also responsible for thermogenesis through high frequency muscular contraction, also
70 called shivering thermogenesis [9]. Its major role in maintaining body temperature during
71 thermal stress is explained by its large mass in the body reaching up to 35% of total body weight.
72 White adipose tissue (WAT) also plays a role in non-shivering thermogenesis. It has the ability
73 to convert partially into a heat producing tissue, by increasing mitochondria and thermogenic
74 processes. This phenomenon, called browning or beigeing, is characterized by an increase in
75 the expression of UCP1. It occurs under conditions of cold exposure and activation of the
76 sympathetic nervous system [10] or the atrial natriuretic peptide pathway [11]. Others
77 thermogenic mechanisms can occur independently of UCP1 in WAT involving creatine [12]
78 and calcium [5] futile cycles. The energy requirement essential for the survival of living
79 organisms defines the basal metabolic rate (BMR). To measure this parameter, it is essential
80 that people are in fasted conditions, lying down, without clothing protection and awake. Above
81 all, it is necessary to be at an ambient temperature that does not require heat production or
82 dissipation [13]. The ratio of daily energy expenditure to BMR is ~2-fold higher in mice housed
83 at standard animal care facility (between 21°C and 23°C) (Figure 1).

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84 The use of rodent models in research does not allow reproduction of the same metabolic
85 responses as in humans. Minimizing the involvement of thermogenesis in metabolism is
86 therefore essential to "humanize" the animals and thus facilitate the extrapolation of results [3].
87 In mice, thermoneutrality varies from 28°C to 32°C, that is the reason why the term of
88 thermoneutral zone is often used [14]. In fact, in mice, during the light phases or rest phases,
89 the temperature zone where energy expenditure was minimal extends over 4°C. During the dark
90 phases or active phases, it converges towards a point. This point separates temperatures that
91 lead to an increase in body temperature and temperatures that conversely lead to a body
92 temperature loss. The decrease in body temperature is counteracted by an increase in energy
93 expenditure [14]. To rule out the thermogenic role of BAT, experiments must be carried out at
94 the temperature that corresponds to a zero thermoregulatory balance. However, although
95 housing animals at 21°C is recognized as a significant thermal stress, it has also been shown
96 that thermoneutrality must be regulated in a very specific way and depends on many factors,
97 such as the number of animals by cage, the resources available (enrichments and nesting
98 materials). It is also necessary to take into account the active and resting phases, and to adapt
99 the temperature to these phases. According to Speakman and colleagues, the ideal housing
100 temperature would be 25°C in mice arguing that humans usually live 3-5°C below
101 thermoneutrality [2]. This implies that the ratio of daily energy expenditure to BMR is about
102 1.8 in mice at 25°C, mimicking the ratio found in free-living humans.

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103 To define the thermoneutral zone, one must evaluate the temperature at which the blood flow
104 from the center of the body to the skin provides as much heat as the flow from the skin to the
105 core of the body. Thermoneutrality is therefore not strictly identical to thermal comfort
106 temperature which is influenced by behaviors in humans [15]. In mice, thermoneutrality is used
107 and typically ranges between 29 and 32°C in metabolic research. Differences in
108 thermoneutrality temperatures between mice and humans are mainly explained by the large

109 difference in body size [14]. Housing mice at thermoneutrality can help unveil metabolic
110 phenotype since they spend less energy for maintaining their body temperature constant [16].
111 This lower energy expenditure makes them more sensitive to the development of obesity [17]
112 and less sensitive to fasting-induced weight loss [18]. McKie et al. demonstrated that housing
113 temperature influences exercise-induced increase in mitochondrial biogenesis and thermogenic
114 gene expression in inguinal WAT while others tissues such as BAT and skeletal muscle remain
115 unaffected [19]. Remarkably mice housed at thermoneutrality run greater distance on a wheel
116 compared to mice housed at room temperature. Conventional housing temperatures are
117 considered protective against obesity [20]. In a recent study, authors have shown morphological
118 differences both in BAT and muscle according to the housing temperature of mice. At
119 thermoneutrality, mitochondria in both tissues were loosely packed with irregular cristae, while
120 adaptation to cold initiated ultrastructural remodeling of mitochondria including acquisition of
121 more elaborate cristae structure [21]. In summary, housing temperature is critical to unveil
122 metabolic phenotypes in mice since the sustained increase in metabolic rate observed at room
123 temperature favor compensatory mechanisms.

3. Influence of thermoneutrality on blood glucose control

126 Housing temperature is also of major importance in the study of metabolism, because many
127 organs, nervous and hormonal systems are sensitive to temperature variations. It is well known
128 that mice fed a high-fat diet (HFD) develop glucose intolerance within a few days regardless of
129 housing temperature. However, several factors such as blood glucose, food intake and energy
130 expenditure are significantly decreased when housed at thermoneutrality. Indeed, we observed
131 that C57BL/6J mice had reduced fasting blood glucose levels when housed at 30°C compared
132 to 21°C. Overall their glucose excursion during an oral glucose tolerance test was significantly
133 lower, although their responsiveness (AUC OGTT) to the glucose load was similar (Figure 2).

134 This observation is in agreement with previous studies [19, 22]. Some parameters were studied
135 and described in wild-type C57BL/6J male mice, fed either a standard diet or a HFD and during
136 an acute exposure (6h) to 15, 20, 25 or 30°C [22]. As expected, main differences were shown
137 between mice fed a HFD vs standard diet, *i.e.* fasting blood glucose and insulin were increased
138 by the HFD and only fasting insulin was increased in obese mice housed at 30°C compared to
139 other temperatures. Furthermore, glucose excursion during an OGTT was increased in HFD-
140 fed mice housed at 15°C, 20°C or 25°C when compared to mice housed at 30°C. However,
141 housing temperature did not seem to influence insulin tolerance [22]. Another study with acute
142 response to changes in housing conditions combined with HFD was carried out. In addition to
143 classical *in vivo* tests, body composition was analysed. The increase in percent fat mass seen at
144 thermoneutrality was linked to an increase in subcutaneous and perigonadal fat pads while BAT
145 mass was reduced [23]. This effect was accompanied by a reduced BAT expression of
146 thermogenic genes such as *Ucp1*, *Pparγ* (peroxisome proliferator-activated receptor-gamma)
147 and *Pdrm16* (PR domain containing 16). Interestingly, *in vivo* glucose uptake measured using
148 radiolabelled 2-deoxyglucose was mostly reduced in BAT at room temperature (22°C) after 1-
149 day of HFD, while it was mostly reduced in *soleus* muscle at thermoneutrality. Altogether, these
150 studies suggest that thermoneutrality significantly influences blood glucose regulation while
151 increasing the susceptibility of mice to develop obesity and T2D in response to HFD.

152 As previously discussed, UCP1 plays a major role in heat production by uncoupling the
153 respiratory electron chain to ATP production. It was therefore expected that *Ucp1*^{-/-} mice would
154 become obese. First analyses failed to validate this assertion [24]. More than 10 years later,
155 *Ucp1*^{-/-} mice were housed at thermoneutrality (30°C) and the results were remarkable, *Ucp1*^{-/-}
156 mice became obese even when fed a standard diet [16]. This also indicates that robust
157 compensating mechanisms occur when housing mice below thermoneutrality. More recently,
158 Dieckmann et al. produced a new transgenic mouse line of UCP1 knockout mice and

159 demonstrated that loss of UCP1 function does not promote diet-induced obesity in mice [25].

160 In UCP1 deficient mice, administration of CL316,243, a specific beta3 agonist, did not change

161 glucose uptake while O2 consumption was decreased compared to wild-type mice. Glucose

162 uptake in BAT thus appears to be uncorrelated with heat production *via* UCP1 [26].

163 Cold-induced energy expenditure is controlled by several neuro-hormonal mechanisms

164 including the sympathetic nervous system (SNS), the natriuretic peptides and thyroid hormones.

165 The sympathetic nervous system stimulates the cAMP-inducible type 2 deiodinase (D2) that

166 amplifies thyroid hormone signaling in BAT by locally converting the prohormone T4 to the

167 active form of thyroid hormone T3. Mice with targeted inactivation of D2 (D2KO mice) exhibit

168 impaired BAT thermogenesis and hypothermia during cold exposure [27]. However, D2KO

169 mice housed at room temperature do not develop obesity when compared to WT animals both

170 when fed a chow or a HFD. The residual activation of BAT caused by thermal stress at room

171 temperature likely mask the lack of D2. Interestingly, after 60 days of *ad libitum* HFD feeding

172 at thermoneutrality, D2KO mice exhibited developed obesity, glucose intolerance and hepatic

173 steatosis, independently of changes in food intake [28]. In addition, white adipose tissue-

174 specific inactivation of D2 in euthyroid mice lead to excess body weight and fat mass gain on

175 a HFD at room temperature due to impaired BAT thermogenesis and reduced systemic lipid

176 oxidation [29]. This "obese phenotype" is lost when mice are housed at thermoneutrality, thus

177 unraveling the role of thermogenesis in this process. In contrast, skeletal muscle (SKM) D2

178 does not seem to play a significant metabolic role given that SKM-D2KO animals exhibit no

179 particular metabolic phenotype.

180 In summary, several studies highlight that housing temperature is a key factor in the study of

181 "humanized" mice. For a more accurate transposition of findings related to glucose regulation

182 and T2D risk from mice to humans, experiments in mice should be performed in at

183 thermoneutrality. Our data are in agreement with previously published work indicating that

184 glucose tolerance is strongly influenced by housing temperature. Although mice housed at
185 thermoneutrality have reduced fasting blood glucose levels, they are more susceptible to diet-
186 induced obesity and T2D.

4. Influence of thermoneutrality on muscle-fat crosstalk

189 We previously discussed that housing mice at ambient temperature below thermoneutrality lead
190 to a significant increase in **metabolic rate** since mice must defend their core temperature by
191 activating both shivering and non-shivering thermogenesis. Thus, shivering-related muscle
192 contraction may serve to augment BAT activity and WAT browning through circulating
193 secreted signals, *i.e.* referred as myokines. Shivering does not seem to occur at room
194 temperature and can be recorded by electromyography when temperature fall below 7°C [4].
195 Human skeletal muscle is known to secrete various myokines [30]. Among those, several such
196 as Interleukin-6 (IL-6), Fibroblast growth factor-21 (FGF21), Irisin, Meteorin-like, and β -
197 Aminoisobutyric Acid (BAIBA) have been suggested to target WAT and increase non-
198 shivering thermogenesis.

199 IL-6 deficient mice display increased body weight and body fat mass without significant
200 changes in food intake. Thus it is unknown how endogenous IL-6 suppresses body fat, but there
201 is growing evidence that central IL-6 increases sympathetic outflow. To elucidate the possible
202 interactions between endogenous IL-6 and the SNS, the responses of wild type and IL-6^{-/-} mice
203 were studied during stress and cold exposure. **At room temperature, the respiratory exchange
204 ratio (RER) was significantly higher in IL-6^{-/-} compared to wild-type mice, reflecting a fat
205 utilization rate significantly lower in IL-6^{-/-} mice.** At thermoneutrality, the RER was similar in
206 wild-type and IL-6^{-/-} mice. There was a trend for decreased baseline oxygen consumption in
207 IL-6^{-/-} mice at thermoneutrality, whereas oxygen consumption at room temperature was

208 identical in wild-type and IL-6^{-/-} mice [31]. In summary, irrespective of the housing
209 temperature, IL6-deficient mice exhibit reduced cold- and stress-induced EE, indicating that
210 endogenous IL6 is a key regulator of this process. In addition, Knudsen et al. demonstrated
211 using IL-6^{-/-} mice that IL-6 is required for a full induction of UCP1 protein expression in
212 response to cold exposure and influences the UCP1 protein content iWAT of both untrained
213 and exercise trained animals [32].

214 FGF21 has been identified as a myokine correlating with insulin resistance in humans [33].
215 FGF21 deficiency in mice was shown to result in impaired ability to adapt to long-term cold
216 exposure with diminished browning of WAT [34]. Inversely, systemic administration of FGF21
217 in obese mice resulted in reduced adiposity, improved glycemic control, as well as increased
218 energy expenditure. Altogether, these observations suggest that FGF21 may be a key factor
219 linking UCP1 expression to improved glucose metabolism. As discussed earlier, UCP1 KO
220 mice were resistant to diet-induced obesity below thermoneutrality and displayed elevated
221 levels of serum FGF21. Generation of UCP1/FGF21 double knockout (DKO) mice to
222 investigate the role of FGF21 signaling during HFD-induced body weight gain was undertaken
223 [35]. Maintaining these mice during the twelve weeks of HFD intervention at thermoneutrality
224 led to identical body weight and body fat gain in all genotypes (WT, FGF21 KO, UCP1 KO,
225 and DKO) coinciding with the lack of FGF21 effects in BAT and iWAT. Collectively, this
226 study characterizes the effects of endogenous FGF21 that acts as master regulator to protect
227 from diet-induced obesity in the absence of UCP1 [36].

228 Irisin derives from the cleavage of Fibronectin type III domain-containing protein 5 (FNDC5)
229 produced by skeletal muscle during physical exercise [37]. This myokine induces myogenesis
230 and WAT browning together with a concomitant increase in energy expenditure. Although a
231 controversy exists on the physiological role of irisin in humans, administration of irisin in mice
232 can positively affect glucose homeostasis, lipid profile and other metabolic parameters related

233 to obesity. Celi and colleagues [38] suggested that irisin secretion could have evolved from
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2 234 shivering-related muscle contraction during cold exposure, serving to augment brown fat
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8 236 Meteorin-like (Metrn1) is induced after exercise and cold exposure in skeletal muscle and
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10 237 adipose tissue. Elevated circulating Metrn1 causes an increase in whole-body energy
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12 238 expenditure associated with WAT browning and improved glucose tolerance in obese/diabetic
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15 239 mice [39]. Metrn1 expression was measured in BAT, WAT depots of mice chronically housed
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17 240 at 30°C or after an acute (6h, 24h) and chronic challenge (2 wk) at 4°C. Acute cold exposure
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20 241 significantly elevated Metrn1 gene expression in BAT and WAT. Adenovirus-mediated
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22 242 overexpression of Metrn1 significantly improved glucose tolerance when compared to control
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25 243 mice injected with Lac Z. Collectively, these data illustrate that increases in circulating Metrn1
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27 244 causes an increase in energy expenditure and an improvement in glucose homeostasis in
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30 245 obese/diabetic mice [39].
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33 246 β -amino-isobutyric acid (BAIBA) is a non-protein β -amino acid, a metabolite of thymine,
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35 247 released by skeletal myocytes [40]. BAIBA can also be generated by catabolism of the
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38 248 branched-chain amino acid valine. BAIBA enhances WAT browning via PPAR α , and may
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40 249 contribute to exercise-induced protection from metabolic diseases. It has also been found that
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43 250 BAIBA attenuates insulin resistance, inhibits inflammation and induces fatty acid oxidation in
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45 251 skeletal muscle *via* the AMPK (5'-AMP-activated protein kinase)-PPAR β pathway [40].
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48 252 In summary, WAT browning through circulating secreted signals from shivering-related muscle
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51 253 contraction significantly contribute to non-shivering thermogenesis, energy balance and blood
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53 254 glucose homeostasis. This muscle-adipose crosstalk may represent a thermogenic, cold-
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55 255 activated endocrine axis, that is exploitable for obesity and T2D therapeutics development.
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5. Conclusion

Rodents are essential models in preclinical research. They have provided important knowledge on disease mechanisms. However, they have been most often used at room temperature, a temperature known to cause thermal stress. Housing temperature is a critical factor to take into account when studying metabolism as it can significantly mask or unmask phenotypes. For instance, thermoneutrality revealed a frank metabolic phenotype of *Ucp1*^{-/-} mice. The compensatory mechanisms occurring at room temperature at least in part involve a muscle-fat crosstalk orchestrated by secreted signals through shivering-related muscle contraction. Housing at thermoneutrality, in addition to revealing phenotypes, could also help potentiating certain therapeutic approaches. For instance, a recent study compared numerous PPAR activators for their ability to induce WAT browning *in vivo* in lean and obese mice at room temperature and at thermoneutrality. Among them, tesaglitazar, a dual PPAR α/γ activator, induced the highest increase of *Ucp1* mRNA levels in WAT at both housing conditions [41]. In addition, tesaglitazar appeared more efficient to increase of *Ucp1* mRNA levels in BAT, energy expenditure and insulin tolerance in mice housed at thermoneutrality when compared to rosiglitazone. Overall, housing mice at thermoneutrality could help to maximize the scientific outcomes of experimental studies and the transposition to human physiology.

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405 **Figures Legend**

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3 406 **Figure 1.** Components of energy expenditure in % of total (A) and in absolute values (B) in
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5 407 mice as a function of housing temperature adapted from Marc L. Reitman [1].
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11 409 **Figure 2.** (A) Oral glucose tolerance test (2 g/kg) in C57BL/6J mice performed at a housing
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13 temperature of 21°C or 30°C after a 2h fast. Data are expressed as means \pm SEM (n=12-13 per
14 410 group). Two-way ANOVA with housing temperature and time as factors were applied. (B) Area
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16 411 Under the Curve (AUC) of the OGTT.
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1 **Does housing temperature influence glucose regulation and muscle-fat crosstalk in mice?**

2

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12 **Abstract**

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3 13 The robustness of scientific results is partly based on their reproducibility. Working with animal
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5 14 models, particularly in the field of metabolism, requires to avoid any source of stress to rule out
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8 15 a maximum of bias. Housing at room temperature is sufficient to induce thermal stress
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10 16 activating key thermogenic organs such as brown adipose tissue (BAT) and skeletal muscle.
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12 17 BAT covers most of the non-shivering thermogenesis in mice and burns a variety of fuels such
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15 18 as glucose and lipids. A high prevalence of BAT is associated with a strong protection against
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17 19 type 2 diabetes risk in humans, implying that BAT plays a key role in glucose homeostasis.
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20 20 However, thermal stress is poorly and inconsistently considered in experimental research. This
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22 21 thermal stress can significantly impede interpretation of phenotypes by favoring compensatory
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25 22 signaling pathways. Indeed, various studies revealed that thermoneutrality is essential to study
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27 23 metabolism in mice in order to reach a suitable level of “humanization”. In this review, we
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30 24 briefly discuss if and how ambient temperature influence blood glucose homeostasis through
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32 25 BAT and muscle-fat crosstalk.
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38 27 **Keywords:** Thermoneutrality, brown adipose tissue, glucose metabolism, myokines, mouse
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47 **Highlights**

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50 31 • Housing at room temperature induces a significant thermal stress in mice
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52 32 • Brown adipose tissue plays a major role in regulating systemic glucose flux
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55 33 • Housing at thermoneutrality offsets thermal stress and can uncover frank phenotypes
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58 34 • Thermoneutrality is required to “humanize” mice
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1. Introduction

The living conditions on the surface of the earth are different all around the globe. Indeed, animals and especially mammals have to cope with drastic temperature changes during the year or sometimes during a day [1]. The maintenance of their body temperature at a constant value is essential for metabolic and vital functions. This maintenance of body temperature is energy consuming; at “low” ambient temperatures, the body must provide enough substrates, such as glucose and fatty acids to produce heat. In contrast, when the ambient temperature is “high”, the body must increase its energy expenditure in order to dissipate the heat. An ambient “third” temperature exists, the thermoneutrality, over which metabolic rate is minimum and constant, with a balance at equilibrium between heat loss and heat production [2]. However, most experiments in mice are carried out at room temperature (~21-22°C). At room temperature, mice exhibit a ~2-fold increase in metabolic rate which has major influence on systemic metabolism and thermogenic organs such as brown fat and skeletal muscle [3]. This review briefly examines to what extent ambient temperature can influence glucose homeostasis and muscle-fat crosstalk through circulating secreted signals.

2. Notions of thermogenesis and thermoneutrality

The body temperature is maintained at a constant value within the organism by means of a balance that allows the production and dissipation of heat. Heat is virtually produced by every organ having a baseline metabolism. Main heat producing organs involve cardiac and skeletal muscles, brain, liver and brown and beige fat. In presence of thermal stress, both shivering and non-shivering thermogenesis is activated. Shivering does not seem to occur significantly at room temperature [4]. When mice are exposed to cold (4-6°C), shivering slightly increase (~30%) as recorded by electromyography [5]. However, the contribution of shivering to cold-

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60 induced energy expenditure and heat production was minor in comparison to cold-induced non
61 shivering thermogenesis mostly produced by brown fat. Thus, when directly recording tissue
62 temperature with specific probes, norepinephrine increases markedly heat production in BAT
63 (+1°C) while temperature in skeletal muscle did not change significantly [5]. Brown adipose
64 tissue (BAT) ensures most of the non-shivering thermogenesis. BAT is present in rodents and
65 in infants, but also in adult humans [6]. Indeed, the numerous BAT mitochondria contain
66 uncoupling protein 1 (UCP1) [7]. This protein can be inhibited by purine-based nucleotides, or
67 activated by fatty acids [8]. When UCP1 is activated by fatty acids, the proton gradient
68 produced by the mitochondrial respiratory chain is dissipated, allowing heat production. The
69 muscle is also responsible for thermogenesis through high frequency muscular contraction, also
70 called shivering thermogenesis [9]. Its major role in maintaining body temperature during
71 thermal stress is explained by its large mass in the body reaching up to 35% of total body weight.
72 White adipose tissue (WAT) also plays a role in non-shivering thermogenesis. It has the ability
73 to convert partially into a heat producing tissue, by increasing mitochondria and thermogenic
74 processes. This phenomenon, called browning or beigeing, is characterized by an increase in
75 the expression of UCP1. It occurs under conditions of cold exposure and activation of the
76 sympathetic nervous system [10] or the atrial natriuretic peptide pathway [11]. Others
77 thermogenic mechanisms can occur independently of UCP1 in WAT involving creatine [12]
78 and calcium [5] futile cycles. The energy requirement essential for the survival of living
79 organisms defines the basal metabolic rate (BMR). To measure this parameter, it is essential
80 that people are in fasted conditions, lying down, without clothing protection and awake. Above
81 all, it is necessary to be at an ambient temperature that does not require heat production or
82 dissipation [13]. The ratio of daily energy expenditure to BMR is ~2-fold higher in mice housed
83 at standard animal care facility (between 21°C and 23°C) (Figure 1).

1 84 The use of rodent models in research does not allow reproduction of the same metabolic
2 85 responses as in humans. Minimizing the involvement of thermogenesis in metabolism is
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4 86 therefore essential to "humanize" the animals and thus facilitate the extrapolation of results [3].
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7 87 In mice, thermoneutrality varies from 28°C to 32°C, that is the reason why the term of
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9 88 thermoneutral zone is often used [14]. In fact, in mice, during the light phases or rest phases,
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11 89 the temperature zone where energy expenditure was minimal extends over 4°C. During the dark
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13 90 phases or active phases, it converges towards a point. This point separates temperatures that
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15 91 lead to an increase in body temperature and temperatures that conversely lead to a body
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17 92 temperature loss. The decrease in body temperature is counteracted by an increase in energy
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19 93 expenditure [14]. To rule out the thermogenic role of BAT, experiments must be carried out at
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21 94 the temperature that corresponds to a zero thermoregulatory balance. However, although
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23 95 housing animals at 21°C is recognized as a significant thermal stress, it has also been shown
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25 96 that thermoneutrality must be regulated in a very specific way and depends on many factors,
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27 97 such as the number of animals by cage, the resources available (enrichments and nesting
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29 98 materials). It is also necessary to take into account the active and resting phases, and to adapt
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31 99 the temperature to these phases. According to Speakman and colleagues, the ideal housing
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33 100 temperature would be 25°C in mice arguing that humans usually live 3-5°C below
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35 101 thermoneutrality [2]. This implies that the ratio of daily energy expenditure to BMR is about
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37 102 1.8 in mice at 25°C, mimicking the ratio found in free-living humans.
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46 103 To define the thermoneutral zone, one must evaluate the temperature at which the blood flow
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48 104 from the center of the body to the skin provides as much heat as the flow from the skin to the
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50 105 core of the body. Thermoneutrality is therefore not strictly identical to thermal comfort
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52 106 temperature which is influenced by behaviors in humans [15]. In mice, thermoneutrality is used
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54 107 and typically ranges between 29 and 32°C in metabolic research. Differences in
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56 108 thermoneutrality temperatures between mice and humans are mainly explained by the large
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109 difference in body size [14]. Housing mice at thermoneutrality can help unveil metabolic
110 phenotype since they spend less energy for maintaining their body temperature constant [16].
111 This lower energy expenditure makes them more sensitive to the development of obesity [17]
112 and less sensitive to fasting-induced weight loss [18]. McKie et al. demonstrated that housing
113 temperature influences exercise-induced increase in mitochondrial biogenesis and thermogenic
114 gene expression in inguinal WAT while others tissues such as BAT and skeletal muscle remain
115 unaffected [19]. Remarkably mice housed at thermoneutrality run greater distance on a wheel
116 compared to mice housed at room temperature. Conventional housing temperatures are
117 considered protective against obesity [20]. In a recent study, authors have shown morphological
118 differences both in BAT and muscle according to the housing temperature of mice. At
119 thermoneutrality, mitochondria in both tissues were loosely packed with irregular cristae, while
120 adaptation to cold initiated ultrastructural remodeling of mitochondria including acquisition of
121 more elaborate cristae structure [21]. In summary, housing temperature is critical to unveil
122 metabolic phenotypes in mice since the sustained increase in metabolic rate observed at room
123 temperature favor compensatory mechanisms.

3. Influence of thermoneutrality on blood glucose control

126 Housing temperature is also of major importance in the study of metabolism, because many
127 organs, nervous and hormonal systems are sensitive to temperature variations. It is well known
128 that mice fed a high-fat diet (HFD) develop glucose intolerance within a few days regardless of
129 housing temperature. However, several factors such as blood glucose, food intake and energy
130 expenditure are significantly decreased when housed at thermoneutrality. Indeed, we observed
131 that C57BL/6J mice had reduced fasting blood glucose levels when housed at 30°C compared
132 to 21°C. Overall their glucose excursion during an oral glucose tolerance test was significantly
133 lower, although their responsiveness (AUC OGTT) to the glucose load was similar (Figure 2).

134 This observation is in agreement with previous studies [19, 22]. Some parameters were studied
135 and described in wild-type C57BL/6J male mice, fed either a standard diet or a HFD and during
136 an acute exposure (6h) to 15, 20, 25 or 30°C [22]. As expected, main differences were shown
137 between mice fed a HFD vs standard diet, *i.e.* fasting blood glucose and insulin were increased
138 by the HFD and only fasting insulin was increased in obese mice housed at 30°C compared to
139 other temperatures. Furthermore, glucose excursion during an OGTT was increased in HFD-
140 fed mice housed at 15°C, 20°C or 25°C when compared to mice housed at 30°C. However,
141 housing temperature did not seem to influence insulin tolerance [22]. Another study with acute
142 response to changes in housing conditions combined with HFD was carried out. In addition to
143 classical *in vivo* tests, body composition was analysed. The increase in percent fat mass seen at
144 thermoneutrality was linked to an increase in subcutaneous and perigonadal fat pads while BAT
145 mass was reduced [23]. This effect was accompanied by a reduced BAT expression of
146 thermogenic genes such as *Ucp1*, *Pparγ* (peroxisome proliferator-activated receptor-gamma)
147 and *Pdrm16* (PR domain containing 16). Interestingly, *in vivo* glucose uptake measured using
148 radiolabelled 2-deoxyglucose was mostly reduced in BAT at room temperature (22°C) after 1-
149 day of HFD, while it was mostly reduced in *soleus* muscle at thermoneutrality. Altogether, these
150 studies suggest that thermoneutrality significantly influences blood glucose regulation while
151 increasing the susceptibility of mice to develop obesity and T2D in response to HFD.

152 As previously discussed, UCP1 plays a major role in heat production by uncoupling the
153 respiratory electron chain to ATP production. It was therefore expected that *Ucp1*^{-/-} mice would
154 become obese. First analyses failed to validate this assertion [24]. More than 10 years later,
155 *Ucp1*^{-/-} mice were housed at thermoneutrality (30°C) and the results were remarkable, *Ucp1*^{-/-}
156 mice became obese even when fed a standard diet [16]. This also indicates that robust
157 compensating mechanisms occur when housing mice below thermoneutrality. More recently,
158 Dieckmann et al. produced a new transgenic mouse line of UCP1 knockout mice and

159 demonstrated that loss of UCP1 function does not promote diet-induced obesity in mice [25].

160 In UCP1 deficient mice, administration of CL316,243, a specific beta3 agonist, did not change

161 glucose uptake while O2 consumption was decreased compared to wild-type mice. Glucose

162 uptake in BAT thus appears to be uncorrelated with heat production *via* UCP1 [26].

163 Cold-induced energy expenditure is controlled by several neuro-hormonal mechanisms

164 including the sympathetic nervous system (SNS), the natriuretic peptides and thyroid hormones.

165 The sympathetic nervous system stimulates the cAMP-inducible type 2 deiodinase (D2) that

166 amplifies thyroid hormone signaling in BAT by locally converting the prohormone T4 to the

167 active form of thyroid hormone T3. Mice with targeted inactivation of D2 (D2KO mice) exhibit

168 impaired BAT thermogenesis and hypothermia during cold exposure [27]. However, D2KO

169 mice housed at room temperature do not develop obesity when compared to WT animals both

170 when fed a chow or a HFD. The residual activation of BAT caused by thermal stress at room

171 temperature likely mask the lack of D2. Interestingly, after 60 days of *ad libitum* HFD feeding

172 at thermoneutrality, D2KO mice exhibited developed obesity, glucose intolerance and hepatic

173 steatosis, independently of changes in food intake [28]. In addition, white adipose tissue-

174 specific inactivation of D2 in euthyroid mice lead to excess body weight and fat mass gain on

175 a HFD at room temperature due to impaired BAT thermogenesis and reduced systemic lipid

176 oxidation [29]. This "obese phenotype" is lost when mice are housed at thermoneutrality, thus

177 unraveling the role of thermogenesis in this process. In contrast, skeletal muscle (SKM) D2

178 does not seem to play a significant metabolic role given that SKM-D2KO animals exhibit no

179 particular metabolic phenotype.

180 In summary, several studies highlight that housing temperature is a key factor in the study of

181 "humanized" mice. For a more accurate transposition of findings related to glucose regulation

182 and T2D risk from mice to humans, experiments in mice should be performed in at

183 thermoneutrality. Our data are in agreement with previously published work indicating that

184 glucose tolerance is strongly influenced by housing temperature. Although mice housed at
185 thermoneutrality have reduced fasting blood glucose levels, they are more susceptible to diet-
186 induced obesity and T2D.

4. Influence of thermoneutrality on muscle-fat crosstalk

189 We previously discussed that housing mice at ambient temperature below thermoneutrality lead
190 to a significant increase in metabolic rate since mice must defend their core temperature by
191 activating both shivering and non-shivering thermogenesis. Thus, shivering-related muscle
192 contraction may serve to augment BAT activity and WAT browning through circulating
193 secreted signals, *i.e.* referred as myokines. Shivering does not seem to occur at room
194 temperature and can be recorded by electromyography when temperature fall below 7°C [4].
195 Human skeletal muscle is known to secrete various myokines [30]. Among those, several such
196 as Interleukin-6 (IL-6), Fibroblast growth factor-21 (FGF21), Irisin, Meteorin-like, and β -
197 Aminoisobutyric Acid (BAIBA) have been suggested to target WAT and increase non-
198 shivering thermogenesis.

199 IL-6 deficient mice display increased body weight and body fat mass without significant
200 changes in food intake. Thus it is unknown how endogenous IL-6 suppresses body fat, but there
201 is growing evidence that central IL-6 increases sympathetic outflow. To elucidate the possible
202 interactions between endogenous IL-6 and the SNS, the responses of wild type and IL-6^{-/-} mice
203 were studied during stress and cold exposure. At room temperature, the respiratory exchange
204 ratio (RER) was significantly higher in IL-6^{-/-} compared to wild-type mice, reflecting a fat
205 utilization rate significantly lower in IL-6^{-/-} mice. At thermoneutrality, the RER was similar in
206 wild-type and IL-6^{-/-} mice. There was a trend for decreased baseline oxygen consumption in
207 IL-6^{-/-} mice at thermoneutrality, whereas oxygen consumption at room temperature was

208 identical in wild-type and IL-6^{-/-} mice [31]. In summary, irrespective of the housing
209 temperature, IL6-deficient mice exhibit reduced cold- and stress-induced EE, indicating that
210 endogenous IL6 is a key regulator of this process. In addition, Knudsen et al. demonstrated
211 using IL-6^{-/-} mice that IL-6 is required for a full induction of UCP1 protein expression in
212 response to cold exposure and influences the UCP1 protein content iWAT of both untrained
213 and exercise trained animals [32].

214 FGF21 has been identified as a myokine correlating with insulin resistance in humans [33].
215 FGF21 deficiency in mice was shown to result in impaired ability to adapt to long-term cold
216 exposure with diminished browning of WAT [34]. Inversely, systemic administration of FGF21
217 in obese mice resulted in reduced adiposity, improved glycemic control, as well as increased
218 energy expenditure. Altogether, these observations suggest that FGF21 may be a key factor
219 linking UCP1 expression to improved glucose metabolism. As discussed earlier, UCP1 KO
220 mice were resistant to diet-induced obesity below thermoneutrality and displayed elevated
221 levels of serum FGF21. Generation of UCP1/FGF21 double knockout (DKO) mice to
222 investigate the role of FGF21 signaling during HFD-induced body weight gain was undertaken
223 [35]. Maintaining these mice during the twelve weeks of HFD intervention at thermoneutrality
224 led to identical body weight and body fat gain in all genotypes (WT, FGF21 KO, UCP1 KO,
225 and DKO) coinciding with the lack of FGF21 effects in BAT and iWAT. Collectively, this
226 study characterizes the effects of endogenous FGF21 that acts as master regulator to protect
227 from diet-induced obesity in the absence of UCP1 [36].

228 Irisin derives from the cleavage of Fibronectin type III domain-containing protein 5 (FNDC5)
229 produced by skeletal muscle during physical exercise [37]. This myokine induces myogenesis
230 and WAT browning together with a concomitant increase in energy expenditure. Although a
231 controversy exists on the physiological role of irisin in humans, administration of irisin in mice
232 can positively affect glucose homeostasis, lipid profile and other metabolic parameters related

233 to obesity. Celi and colleagues [38] suggested that irisin secretion could have evolved from
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2 234 shivering-related muscle contraction during cold exposure, serving to augment brown fat
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5 235 thermogenesis in concert with FGF21 also in humans.
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8 236 Meteorin-like (Metrn1) is induced after exercise and cold exposure in skeletal muscle and
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10 237 adipose tissue. Elevated circulating Metrn1 causes an increase in whole-body energy
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12 238 expenditure associated with WAT browning and improved glucose tolerance in obese/diabetic
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15 239 mice [39]. Metrn1 expression was measured in BAT, WAT depots of mice chronically housed
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17 240 at 30°C or after an acute (6h, 24h) and chronic challenge (2 wk) at 4°C. Acute cold exposure
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20 241 significantly elevated Metrn1 gene expression in BAT and WAT. Adenovirus-mediated
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22 242 overexpression of Metrn1 significantly improved glucose tolerance when compared to control
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25 243 mice injected with Lac Z. Collectively, these data illustrate that increases in circulating Metrn1
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27 244 causes an increase in energy expenditure and an improvement in glucose homeostasis in
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30 245 obese/diabetic mice [39].
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33 246 β -amino-isobutyric acid (BAIBA) is a non-protein β -amino acid, a metabolite of thymine,
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35 247 released by skeletal myocytes [40]. BAIBA can also be generated by catabolism of the
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38 248 branched-chain amino acid valine. BAIBA enhances WAT browning via PPAR α , and may
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40 249 contribute to exercise-induced protection from metabolic diseases. It has also been found that
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43 250 BAIBA attenuates insulin resistance, inhibits inflammation and induces fatty acid oxidation in
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45 251 skeletal muscle *via* the AMPK (5'-AMP-activated protein kinase)-PPAR β pathway [40].
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48 252 In summary, WAT browning through circulating secreted signals from shivering-related muscle
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51 253 contraction significantly contribute to non-shivering thermogenesis, energy balance and blood
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53 254 glucose homeostasis. This muscle-adipose crosstalk may represent a thermogenic, cold-
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55 255 activated endocrine axis, that is exploitable for obesity and T2D therapeutics development.
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5. Conclusion

Rodents are essential models in preclinical research. They have provided important knowledge on disease mechanisms. However, they have been most often used at room temperature, a temperature known to cause thermal stress. Housing temperature is a critical factor to take into account when studying metabolism as it can significantly mask or unmask phenotypes. For instance, thermoneutrality revealed a frank metabolic phenotype of *Ucp1*^{-/-} mice. The compensatory mechanisms occurring at room temperature at least in part involve a muscle-fat crosstalk orchestrated by secreted signals through shivering-related muscle contraction. Housing at thermoneutrality, in addition to revealing phenotypes, could also help potentiating certain therapeutic approaches. For instance, a recent study compared numerous PPAR activators for their ability to induce WAT browning *in vivo* in lean and obese mice at room temperature and at thermoneutrality. Among them, tesaglitazar, a dual PPAR α/γ activator, induced the highest increase of *Ucp1* mRNA levels in WAT at both housing conditions [41]. In addition, tesaglitazar appeared more efficient to increase of *Ucp1* mRNA levels in BAT, energy expenditure and insulin tolerance in mice housed at thermoneutrality when compared to rosiglitazone. Overall, housing mice at thermoneutrality could help to maximize the scientific outcomes of experimental studies and the transposition to human physiology.

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405 **Figures Legend**

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2
3 406 **Figure 1.** Components of energy expenditure in % of total (A) and in absolute values (B) in
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5 407 mice as a function of housing temperature adapted from Marc L. Reitman [1].
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11 409 **Figure 2.** (A) Oral glucose tolerance test (2 g/kg) in C57BL/6J mice performed at a housing
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14 410 temperature of 21°C or 30°C after a 2h fast. Data are expressed as means \pm SEM (n=12-13 per
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16 411 group). Two-way ANOVA with housing temperature and time as factors were applied. (B) Area
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19 412 Under the Curve (AUC) of the OGTT.
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Figure 1

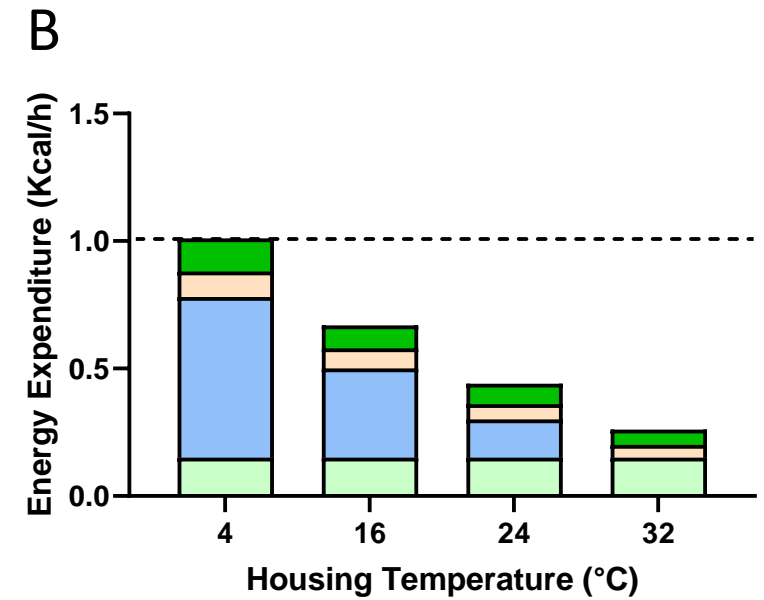
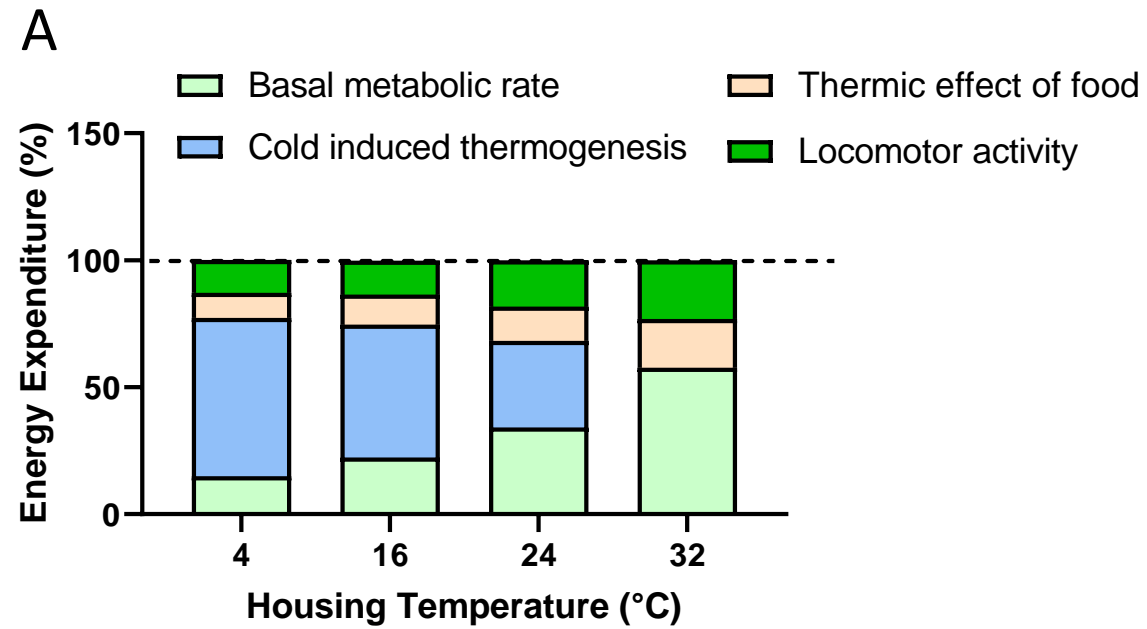


Figure 2

