Sex differences in nonshivering thermogenesis in the wild

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ABSTRACT

Nonshivering thermogenesis (NST) is a key mechanism that allows mammals to control their body temperature. Sex can frequently affect thermoregulatory requirements; therefore, males and females can be expected to differ significantly in their NST capacity. Several sex-related differences in NST have been described in laboratory animals and humans; however, these parameters are relatively rarely studied in animals living under natural conditions. Here, I briefly review factors that may be responsible for this disparity and point out two situations that should be particularly promising in searching for sex differences in NST under natural conditions: the lactation period and potential mitonuclear conflicts over NST control in species with genetic polymorphism.

1. Introduction

Endotherms can maintain a high and constant body temperature using internally generated heat. In mammals, a key role is played by nonshivering thermogenesis (NST), which generates heat through biochemical reactions involving the UCP1 protein. A detailed description of the biochemical processes responsible for the generation of heat through UCP1-based NST is presented elsewhere (e.g., Cannon and Nedergaard, 2004; Klingenspor and Fromme, 2012; Tapia et al., 2018). Briefly, the main thermogenic organ in many mammalian species is brown adipose tissue (BAT), a specialized type of adipose tissue that is found mainly (though not exclusively) in the interscapular region and is characterized by a higher density of mitochondria than that in white adipose tissue. The UCP1 protein is located on the inner membrane of BAT mitochondria and allows protons transferred by the electron transport chain into the intermembrane space to return to the mitochondrial matrix, bypassing ATP synthase. Thus, the UCP1 protein uncouples the mitochondrial electron transport chain from oxidative phosphorylation; in effect, energy processed by mitochondria is not used for ATP synthesis but released as heat. The functional activity of BAT is controlled primarily by norepinephrine, which is bound by β-adrenergic receptors and initializes a series of biochemical reactions, resulting in the conversion of triacylglycerols into free fatty acids and subsequent activation of the UCP1 protein.

Endothermy exerts a profound effect on the physiology of mammals, and their thermal requirements can be affected by environmental conditions, behaviour or reproduction (reviewed in McNab, 2002; Withers et al., 2016; Clarke, 2017). Not surprisingly, NST is a highly flexible trait, and simple acclimation of laboratory mice to different ambient temperatures can lead to individuals differing in UCP1 amounts by several orders of magnitude (Kalinoeitch et al., 2017). NST capacity shows phenotypic plasticity in wild animals in response to changing environmental conditions, primarily being related to ambient temperatures (Koonfeld-Schor et al., 2000; Wang et al., 2006a, 2006b; Zhao et al., 2010; Velotta et al., 2016). Since males and females can differ significantly in their thermal needs, one could expect that sex differences in NST should be a common phenomenon in nature. However, studies on sex differences in NST from animals living under natural conditions (or even caught recently) are relatively rare. Several reasons can be provided to explain this lack of available data. First, field experiments are more exposed to factors that researchers cannot control; thus, they are usually more demanding logistically and more difficult to carry out and finish as they were planned than laboratory experiments. Moreover, researchers usually have little impact on the sex of animals that are caught and become the subject of the study. Therefore, in field experiments focusing on the seasonal, geographic or altitudinal variation in NST, the sex effect is usually seen as a potential confounding factor. In many papers authors simply state that no significant effect of sex was found and thus males and females can be pooled together in subsequent analyses. Indeed, even translational studies that are easier to carry out and finish as they were planned than laboratory experiments. However, studies on sex differences in NST from animals living under natural conditions (or even caught recently) are relatively rare. Several reasons can be provided to explain this lack of available data. First, field experiments are more exposed to factors that researchers cannot control; thus, they are usually more demanding logistically and more difficult to carry out and finish as they were planned than laboratory experiments. Moreover, researchers usually have little impact on the sex of animals that are caught and become the subject of the study. Therefore, in field experiments focusing on the seasonal, geographic or altitudinal variation in NST, the sex effect is usually seen as a potential confounding factor. In many papers authors simply state that no significant effect of sex was found and thus males and females can be pooled together in subsequent analyses. Indeed, even translational studies that are easier logistically tend to focus on NST in only one sex (Horvath and Wolfrum, 2020). The lack of a significant sex difference still has implications for studies that otherwise report significant variation in NST since we can at least conclude that the effect of sex is weaker than the effect of other studied factors (e.g., seasonal variation in Wang et al., 2006b; the effect of altitude in Velotta et al., 2016).

Abbreviations: NST, nonshivering thermogenesis; BAT, brown adipose tissue; RMR, resting metabolic rate.

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Another reason might be that unambiguous quantification of such a complex trait as NST is difficult to achieve experimentally (Cannon and Nedergaard, 2011; Nedergaard and Cannon, 2013; Trayhurn, 2017). For example, some field studies only report changes in BAT mass (Tidemann, 1982). However, the total mass of BAT represents only a rough index of NST capacity, as it is not always proportional to more direct estimates of NST capacity, such as the quantity of UCP1 protein (Kaliovitch et al., 2017). Finally, our knowledge of the mechanisms responsible for NST is far from complete, even in relatively thoroughly studied laboratory rodents and humans (e.g., recent discussion about the importance of beiging or UCP1-independent NST; Lin et al., 2017; Nowack et al., 2017; Campbell and Dicke, 2017; Betz and Enerbäck, 2018; Chouchani et al., 2019; Bal and Periasamy, 2020; Ikeda and Yamada, 2020). The present knowledge on these mechanisms is far from complete even in laboratory rodents, and the focus lies on unravelling the molecular mechanism rather than identifying sex-specific differences. However, the very presence of the significant effect of sex and thyroid hormones on NST suggests that any situation in which the level of these hormones varies between sexes represents a potential opportunity for the finding of sex-specific differences in NST. Therefore, all studies of sex-specific changes in NST in wild-living animals should involve (whenever possible) collecting blood samples from males and females at one time for hormone assays (e.g., Richardson et al., 2018). Such data about simultaneous changes in NST and in hormone levels in both sexes should increase our understanding of the hormonal mechanisms controlling sex-specific differences in NST in different groups of mammals.

2. Effect of sex on NST in translational studies

Most of what is known about the effect of sex on NST comes from translational experiments carried out in laboratory mice and rats, as well as from studies on humans. Although their conclusions can be helpful in predicting how sex differences can affect NST in animals living under natural conditions, some important potential caveats need to be considered. First, controlled and very energetically comfortable conditions usually met by laboratory rodents and humans in Western societies are clearly different from conditions in the wild. Thus, laboratory mice usually have lower NST than wild mice (Richardson et al., 1994). Similarly, both sexes are maintained in the laboratory under the same conditions, which can conceal potential effects of sex-specific differences in habitat or diet preferences on thermal needs and NST. Translational studies usually focus on the potential role of NST and UCP1 in preventing obesity and therefore explore the effect of sex on links between NST, energy metabolism, and obesity; thus, these studies do not see NST as adaptive thermogenesis but rather as a tool for burning spare calories. Finally, changes in NST observed during translational studies may not be representative of all mammals because some transcriptional regulatory elements of the UCP1 gene described in murid rodents (i.e., laboratory mice and rats) are not conserved among other eutheriens (Gaudry and Campbell, 2017).

A detailed description of sex effects on NST observed in laboratory rodents and humans can be found in several review papers (e.g., Quarta et al., 2012; Bloor and Symonds, 2014; Law et al., 2014; Palmer and Clegg, 2017; Valencak et al., 2017; Horvath and Wolfrum, 2020; Moreno-Pais et al., 2020). Some general conclusions can be drawn. Female laboratory rodents seem to have higher NST than males (e.g., Rodriguez-Cuenca et al., 2002), and the same pattern was observed in monkeys (Rothwell and Stock, 1985) and in humans (e.g., Cypress et al., 2009). NST can also be more flexible in females (e.g., Quevedo et al., 1998; Rodriguez et al., 2003; Rodriguez-Cuenca et al., 2002; Valle et al., 2005), which is not unexpected since female mammals must deal with large changes in energy metabolism during pregnancy and lactation. In rats, higher NST in females can also be related to sex-specific differences in the size and structure of the mitochondria in BAT (Rodriguez-Cuenca et al., 2002; Justo et al., 2005). Therefore, studies looking for sex effects in NST in the wild should not overlook potential differences in mitochondrial parameters. Sex can also affect phospholipid fatty acid composition in BAT in laboratory mice (Hoene et al., 2014). Such differences can modulate properties of mitochondrial membranes and thus affect the BAT function. However, no sex differences in the fatty acid composition of BAT were found in the common pipistrelle bat Pipistrellus pipistrellus (Arevolo et al., 1990).

One very important conclusion from translational studies is the fact that although NST is directly stimulated by norepinephrine (as described in the first paragraph), the functional activity of BAT can also be modulated by sex hormones (reviewed in Valencak et al., 2017; Moreno-Pais et al., 2020) and thyroid hormones (reviewed in Yau and Yen, 2020; Sentis et al., 2021). The latter potentially contribute to NST capacity, but the level of these hormones varies between sexes. The present knowledge on these mechanisms is far from complete even in laboratory rodents, and the focus lies on unravelling the molecular mechanism rather than identifying sex-specific differences. However, the very presence of the significant effect of sex and thyroid hormones on NST suggests that any situation in which the level of these hormones varies between sexes represents a potential opportunity for the finding of sex-specific differences in NST. Therefore, all studies of sex-specific changes in NST in wild-living animals should involve (whenever possible) collecting blood samples from males and females at one time for hormone assays (e.g., Richardson et al., 2018). Such data about simultaneous changes in NST and in hormone levels in both sexes should increase our understanding of the hormonal mechanisms controlling sex-specific differences in NST in different groups of mammals.

3. Sex-specific changes in NST during reproduction

The difference in energy metabolism between females and males in mammals is most significant during pregnancy and lactation. Therefore, this is also the time when the largest differences in NST capacity should be expected. Recently, a comprehensive review on the effect of lactation on NST and BAT in mammals was published by Kröl and Speakman (2019). Kröl and Speakman expected that significant and continuous heat production during milk synthesis should reduce the need for dedicated thermogenesis, and thus lactating females should have lower NST. They analysed almost 50 papers and concluded that NST capacity in lactating females is on average reduced by half (though the magnitude of this effect varied widely). Notably, the studies included in the review quantified different parameters relating to NST capacity, from BAT mass to the total UCP1 amount and the activity of BAT mitochondrial enzymes, although only seven studies directly measured NST as the increase in oxygen consumption after injection of norepinephrine. Similarly, maternal effort was quantified differently (from the simple effect of lactation itself to experimental manipulation of litter size). Thus, the general concordance of the results of such diverse experiments suggests that lactation thoroughly modulates the whole metabolic machinery responsible for NST. Moreover, such changes can be rapid, as lactation in small rodents sometimes lasts only 3 weeks. Unexpectedly, the experimental manipulation of sex hormones alone did not affect NST capacity (Gerardo-Gettens et al., 1986). In all reviewed studies, lactating females were compared to nonbreeding females rather than males, but observed changes can be safely interpreted as sex-specific. Most studies reviewed in Kröl and Speakman (2019) were carried out in laboratory rats and mice, and even in experiments on other species, the subjects were maintained in captivity for some generations. Therefore, the only study involving animals that were wild caught but studied in the laboratory is of particular interest (Zhu et al., 2015). Zhu and colleagues followed changes in NST in Chevrier’s field mouse (Apodemus chevrieri) over the entire lactation period and found that during the peak of lactation, it was 33 % lower than in nonlactating controls but returned to the level observed in nonbreeding females one week after weaning. Interestingly, the results presented by Zhu et al. (2015) show that the sum of resting metabolic rate (RMR, i.e. lowest metabolic rate measured within the thermoneutral zone, which in lactating females included also cost of milk production) and NST varied less than either RMR or RMR separately and never differed by more than 15 % from values observed in nonreproductive females. This pattern supports the view that changes in NST are adjusted to changes in the rate of ‘constitutive’ heat production by animals. Overall, this study confirms that females of wild species also considerably downregulate their NST during lactation;
therefore, sex-specific changes in NST during the reproductive period should also be expected in animals living in the wild, and the magnitude of these changes should be large enough to allow their detection during field experiments.

Most likely, the best study of sex-specific changes in NST under natural conditions was carried out by Richardson et al. (2018) in the big brown bat (Eptesicus fuscus). Interestingly, the authors found a significant decline in body mass-controlled NST in females between early May and the second half of July, which presumably overlaps with lactation, whereas NST in males was constant. Moreover, absolute mass-specific values of NST at that time seemed to be lower in females than in males. Furthermore, NST in females, but not in males, showed a clear increase in late summer. All these changes occurred in parallel with changes in the thyroid hormone T3 in blood plasma. This study thus confirms the presence of more pronounced seasonal changes in NST in females than in males and, in particular, a significant reduction in NST during lactation. Unfortunately, the sample size for males was much lower than that for females, and almost no males were caught during the second half of the experimental period. Therefore, this study also reveals common problems with obtaining a balanced experimental design during field studies on wild-caught animals that can potentially impede statistical analyses of the obtained results.

The above pattern can be observed more generally. For example, Tidemann (1982) described seasonal changes in BAT size in the Australian bat Eptesicus vulturinus. This study confirmed that BAT mass in females was lowest during lactation and increased during late summer, although BAT mass in males did not show seasonal changes. Interestingly, Tidemann (1982) related these sex-specific differences not only to energetic costs of pregnancy and lactation but also hypothesized that higher NST capacity during winter can enable males to copulate with hibernating females.

4. NST, genetic polymorphism and the mother’s curse

NST is a complex trait that is affected by both nuclear and mitochondrial genes (UCP1 itself belongs to nuclear-encoded mitochondrial proteins). It was recently shown that the nuclear and mitochondrial lineages in common voles (Microtus arvalis). Bize et al. (2018) compared two evolutionary lineages of common voles occurring in Switzerland and observed that their differences in BAT mass depended on the nuclear genome, but the NST values could be explained by the mitochondrial genome. Nuclear genes responsible for heavier BAT and mitochondrial genes responsible for higher NST were both found in voles living in colder habitats. Bize et al. (2018) specifically stated that they did not find any effect of sex on NST. However, since mitochondria are inherited only maternally, natural selection can favour genotypes that are beneficial for females, even if they are harmful for males (‘mother’s curse’; Havird et al., 2019). A significant sex-specific interaction between mitochondrial DNA and NST was indeed observed in the greater white-toothed shrew Crocidura russula (Fontanillas et al., 2005). Haplotype H1, which is much more common at higher altitudes, correlates with higher thermogenic capacities in females at the beginning of the breeding season and with lower thermogenic capacities in males. Even though females can decrease NST later during lactation, a better thermogenic capacity can be beneficial in early spring, and thus, the H1 haplotype can be potentially beneficial for females and detrimental for males.

Both Bize et al. (2018) and Fontanillas et al. (2005) warn that their results should be treated with some caution. Nevertheless, these studies suggest some intriguing potential for sex-specific conflict over NST. Efficient metabolism requires close interaction between the mitochondrial and nuclear genomes (Hill et al., 2019), and NST capacity represents an important component of fitness in the wild (Haim et al., 1984; Wang et al., 2006; Zhao et al., 2010). There is some evidence that the geographic distribution of nuclear genetic variation responsible for variation in NST can depend on ambient temperature. For example, there is a close correlation between NST capacity and the range of ambient temperatures in four potential cryptic species of blind mole-rat Spalax ehrenbergi, with a different number of chromosomes in Israel (Haim et al., 1984). Climate can also affect polymorphisms of UCP1 genes in humans (Nishimura et al., 2017; Sellayah, 2019). At the same time, the geographic distribution of mitochondrial lineages in many species is determined by climatic variables (McDevitt et al., 2012; Tarnowska et al., 2016; Czarnomska et al., 2019), and I hypothesize that sometimes this effect can be mediated by NST capacity. In conclusion, situations where the geographic distribution of mitochondrial lineages or nuclear genetic variation is limited by ambient temperature offer interesting research opportunities. This may give rise to identifying both geographic variation in NST and sex-specific effects in those genes that are responsible for regulating NST. Consequences of the ‘mother’s curse’ make the presence of such effects particularly likely in the case of the mitochondrial genome.

5. Conclusions and future directions

In summary, our present knowledge of sex-specific differences in NST in the wild is only fragmentary; however, the results of both laboratory experiments and scanty studies carried out on wild-living or wild-caught animals suggest that these discrepancies should be very common. Although studies of NST under natural conditions are more challenging than laboratory experiments, field studies of sex-specific differences in NST offer an interesting scientific opportunity. I like to emphasize that such field projects need not be limited to simple descriptive studies but can easily be related to testing more general biological problems (and thus be well cited). For example, several papers over the last decade tested the validity of the heat dissipation limit theory, predicting that lactation performance in small mammals is limited by their capacity to efficiently dissipate heat generated during milk production (reviewed in Król and Speakman, 2019; Sadowska et al., 2019). However, no single study of changes in NST during lactation under natural conditions was cited by Król and Speakman (2019), and I found only one paper that presumably found the effect of lactation on NST in wild-living mammal (Richardson et al., 2018). However, such studies are necessary for evaluating the ecological and evolutionary importance of the limit set to reproductive performance by the capacity of heat dissipation. Similarly, studies of sex-specific interactions between nuclear and mitochondrial genes controlling NST may have implications for the fundamental problem of fitness consequences of mitonuclear interactions and conflicts (Havird et al., 2019; Hill et al., 2019). Finally, studies of sex-specific differences in NST in different groups of mammals under natural conditions may also benefit translational experiments. As I mentioned earlier, many biomedical studies have investigated problems related to UCP1-mediated NST. However, the thermal biology of laboratory rodents is very different from that of humans (Gordon, 2017; Reitman, 2018). Therefore, a better understanding of how mechanisms controlling NST evolved in different groups of mammals may potentially improve our knowledge of how the results of biomedical experiments can be translated from small rodents to humans.

Declaration of competing interests

The author declare no conflicts of interest.

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