Cold-induced metabolism

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Purpose of review

Cold response can be insulative (drop in peripheral temperature) or metabolic (increase in energy expenditure). Nonshivering thermogenesis by sympathetic, norepinephrine-induced mitochondrial heat production in brown adipose tissue is a well known component of this metabolic response in infants and several animal species. In adult humans, however, its role is less clear. Here we explore recent findings on the role and variability of nonshivering thermogenesis in adults.

Recent findings

Large individual differences exist in mild cold response with respect to the relative contribution of the insulative response and the metabolic (nonshivering) response. In search for the possible explanations of this variation, recent studies on potential mechanisms of nonshivering thermogenesis in humans are presented. Emphasis is given to the role of uncoupling proteins, mitochondrial ATP-synthase, and calcium cycling. The potential contribution of human skeletal muscle to nonshivering thermogenesis is discussed. The differences in nonshivering thermogenesis can partly be attributed to factors such as age, gender, physical fitness, adaptation, and diet. There are indications that genetic variation affect cold response.

Summary

The implications of the observed large individual variation in cold response is that a low metabolic response to cold can partly explain increased risk to develop obesity. Both the effect of environmental factors and genetic factors on nonshivering thermogenesis require more well controlled studies. With extended knowledge on these factors it can be ascertained if a pharmacological regimen is possible which would mimic the effects of chronic cold or elevated catecholamine levels, without attendant side effects.

Keywords

adaptive thermogenesis, nonshivering thermogenesis, cold response, energy expenditure, uncoupling proteins, brown adipose tissue

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Abbreviations

β3-AR	β3 adrenoceptor
BAT	brown adipose tissue
NST	nonshivering thermogenesis
UCP	uncoupling protein
WAT	white adipose tissue

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Introduction

Humans show a combination of behavioural [1] and physiological responses to cold exposure. Two types of physiological responses can be distinguished, which can occur alone or in combination: (1) insulative response, characterized by a decrease in mean skin temperature, in particular of the extremities; (2) metabolic response, or adaptive thermogenesis, characterized by an increase in energy expenditure by so-called shivering thermogenesis or by nonshivering thermogenesis (NST).

It is well established that individuals may differ in the relative contribution of the metabolic and insulative response to cold. Most studies on this topic were performed under extreme conditions (water immersion, arctic climate). Recently, such inter-individual variation was shown in response to mild cold under circumstances met in daily life in a temperate zone. Individuals that responded with increased metabolism in the cold showed less insulative response and vice versa [2•]. The average increase in energy metabolism amounted to 4–6% of 24 h energy expenditure, close to the results of one of the few studies conducted in the past showing an increase of 7% [3]. More interesting, the observed range in changes in metabolism between individuals ranged from 0.15 to 1.45 MJ/day, which can have significant metabolic consequences in the long term.

Insulation

One of the major (nonmetabolic) physiological adjustments in response to cold exposure is peripheral vasoconstriction. This restricts heat transfer from the internal organs to the skin and from the skin to the environment. The vasoconstrictive response is controlled by both core and skin temperatures [4]. When the extremities are exposed to extreme cold for a prolonged period, the vasoconstrictor activity is interrupted periodically, probably to protect against local cold injuries [5]. It is mostly the sympathetic part of the autonomous nervous system that is involved in the cutaneous vasoconstriction. During cold stress norepinephrine is

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released from the sympathetic nerve endings, which induces vasoconstriction via α-receptors [6].

Thermogenesis

Thermogenesis has an obligatory component and a facultative component. Obligatory thermogenesis refers to the heat produced for normal functions of cells and organs, including heat generated due to 'vital' physical activity that is not aimed at extra heat production. The facultative component, or adaptive thermogenesis refers to heat production in response to environmental temperature and is subdivided into NST, shivering thermogenesis and voluntary increased physical activity. In this review the focus will be on the effect of cold on NST in humans. Special attention will be given to individual differences in cold response. Apart from the type of cold exposure (air, water immersion) it is well established that body composition, age, gender, diet, physical fitness and cold adaptation influence the metabolic cold response (see below). A large part of the variation remains unexplained, however, and can potentially be attributed to genetic differences.

Nonshivering thermogenesis

There is still controversy whether humans use NST. It is important to note that some studies refer to NST only when no increase in electromyographical activity is found in response to cold. Under conditions of severe cold, however, both shivering and NST are activated simultaneously and cooperate to ensure thermal homeostasis. In the past it has been shown that daily cold exposure results in an increase in metabolism with a gradual decrease in shivering, which indicates the existence of NST [7]. Several more recent studies indicate the existence of NST in humans [8,9] (A.M.J. Ooijen, W.D.v. Marken Lichtenbelt, A.A. Steenhoven, *et al.*, in preparation). A distinction can be made between 'classical' NST, that is sympathetic, norepinephrine-induced mitochondrial heat production in brown adipose tissue (BAT), and hormonal thermogenesis, associated with epinephrine, glucagons, thyroid, growth hormone and adrenocoricotropic hormone [10••]. This classification is somewhat artificial, since the different hormones cooperate in response to cold within cells, even affecting single genes of different tissues, as excellently reviewed by Lowell *et al.* [11]. BAT thermogenesis dominates in some rodent species and human infants, while in adult humans BAT is probably not the major site of adaptive thermogenesis.

Brown adipose tissue thermogenesis and its role in men

BAT is activated by norepinephrine via β3 adrenoceptors (β3-ARs), resulting in heat production through a mitochondrial uncoupling protein (UCP-1). UCP-1 is a mitochondrial inner-membrane protein that uncouples proton gradient from ATP synthesis. Thereby, 'proton gradient energy' is dissipated as heat and thus increasing energy metabolism [12•].

Direct evidence is provided, using so-called triple knockout mice, that norepinephrine-mediated sympathetic thermogenesis is accomplished by the three known adrenergic receptors (β1-AR, β2-AR, and β3-AR) [13•,14•,15]. In humans both β1-AR and β2-AR are known to contribute to thermogenesis. Although adult humans have β3-ARs, their role in the regulation of thermogenesis is still inconclusive and currently no β3 agonist or antagonist is available for use in humans. In contrast, in adult man, β3 mRNA is present in both BAT and white adipose tissue (WAT) [16].

UCP-1 mRNA has been detected in adipose tissue in adults, indicating that brown adipocytes are interspersed in tissue previously thought to contain white adipocytes only [17]. Another study showed that UCP-1 mRNA was detectable in isolated human adipocytes, and treatment with a peroxisomal proliferator-activated receptor γ(PPARγ), agonist (thiazolidinedione) resulted in a marked increase in UCP-1 expression in these cultured adipocytes [18].

It can be concluded that brown adipocytes are present in adult humans in different densities in all fat depots studied. Although BAT abundance increases with long-term exposure to catecholamines [19] and to cold [20], direct evidence of BAT thermogenesis in humans comes only from infant studies [21]. Could the amount of BAT in adult humans be augmented by pharmacological means? If there were enough brown adipocyte precursor cells in WAT depots, expressing enough β3-ARs, then β3-AR agonists might be able to augment thermogenesis in these cells. Assessment of the number of such cells available in any of the many WAT depots in humans awaits the development of a suitable β3-AR agonist [21].

Alternative mechanisms of nonshivering thermogenesis in humans

Skeletal muscle constitutes up to 40% of the total body mass and has a significant volume density of mitochondria. In adult humans it is known that skeletal muscle plays an important role in the adaptive thermogenesis. It has been demonstrated that in adults the contribution of skeletal muscle accounts for 40% of adrenal infusion induced thermogenesis [22]. The mechanism involved could include effects on uncoupling, Ca^{2+} cycling or both.

Uncoupling protein 1 homologues

Recently, homologues of UCP-1 have been described that are more abundant than UCP-1 in adult humans and are active in other tissues than BAT. New uncoupling proteins are: UCP-2, UCP-3, UCP-4, and UCP-5 [23•–

25•]. Their (regulatory) roles are still not elucidated [26]. While it seems clear that the role of UCP-1 is to produce heat to maintain body temperature, the biological functions of the other UCPs are likely to be different [12•,26]. UCP-3 is mainly active in skeletal muscle and is a serious candidate to play a role in human adaptive thermogenesis. We demonstrated that UCP-3 protein content in skeletal muscle is related to the change in energy metabolism in response to cold [27].

The emergence of UCP knowledge has had a large influence on our outlook on bioenergetics and stresses the importance of uncoupling respiration. The present knowledge suggests that apart from a role in thermoregulation in response to cold or diet, UCPs may participate in the regulation of ATP syntheses, the control of oxidative stress, and fatty acid and glucose metabolism. They could play a role in pathological situations where the availability of circulating fatty acids is very high (starvation, infection, cancer), which may result in very high levels of UCP-3 [26] and UCP-2. High levels of UCPs in hepatocytes could play a role in protection of the liver against excess ATP, decrease the redox potential and reactive oxygen species. Another possibility is that UCP-3 may serve as a mechanism to export fatty acid anions from muscle and BAT mitochondria when fatty acids are the predominantly used substrates [28•]. In these cases UCPs still uncouple and produce heat, but the regulatory function is not thermogenesis.

Mitochondrial ATP synthase

Apart from the energy-dissipating mechanism by mitochondrial uncoupling of ATP synthesis from respiratory chain oxidation through UCPs, it is possible that mitochondrial F_1F_0-ATPase itself can be a target protein for uncoupling mechanisms. Recently it has been shown in rats that enterostatin, an anorectic peptide, can bind to F_1F_0-ATPase. This causes inhibition of ATP production, an increased thermogenesis and an increased oxygen consumption [29•]. Apart from modulation of ATP production resulting in increased thermogenesis, it is also possible that the interaction with F_1F_0-ATPase promotes the expression of UCPs [30].

Ca^{2+}-cycling

In skeletal muscle, the ATP-driven pump Ca^{2+}-ATPase efficiently couples the hydrolysis of ATP to the vectorial transport of Ca^{2+} across the sarcoplasmic membrane. The mechanism of Ca^{2+} cycling is proposed to consist of Ca^{2+} leaks from the sarcoplasmic reticulum and heat would therefore be derived from hydrolysis of the extra ATP needed to maintain a low myoplastic Ca^{2+} concentration [31]. Ca^{2+}-cycling mediated thermogenesis in skeletal muscle cells is a well characterized mechanism for NST in fish and recently in birds [32]. The thermogenic potential for Ca^{2+} cycling in mammals is evident from the pathological syndrome of malignant hyperthermia, a dominant genetic disorder of humans and pigs [33,34]. Recently, this process has been described in healthy rabbits as well [35•]. In mammals lacking large amounts of BAT, as adult humans, the hydrolysis of ATP by Ca^{2+}ATP-ase of skeletal muscle sarcoplasmic reticulum could be an important source of heat.

Factors affecting nonshivering thermogenesis

Cold-induced NST can be affected by experimental factors such as ambient temperature and the cooling medium (water versus air), and by individual factors such as body composition, age, gender, physical fitness, adaptation/acclimatization, and diet.

Body composition

The rate of core cooling is related to physical properties such as surface area/body mass ratio, total mass, and subcutaneous fat thickness. Accumulation of subcutaneous body fat can occur in cold adapted individuals, and there are indications that body fat can affect the degree of cold adaptation [36].

Results from water immersion experiments show that body fat is protective against body core cooling, but the results of an effect of body fat on metabolism (mainly shivering thermogenesis) are less clear. No differences in metabolism during immersion were found between high-fat and low-fat groups of female subjects [37].

Comparable results were obtained when females were exposed to cold air [38]. We recently found a relation between change in core temperature and core peripheral temperature gradients with body fat percentage, under mild cold (air) conditions (A.M.J. Ooijen, W.D.v. Marken Lichtenbelt, A.A. Steenhoven, *et al.*, in preparation). Again, however, there was no relation between percentage of body fat and the change in NST.

In conclusion, body composition can protect the core temperature, but there is no evidence that cold induced metabolism, and more specifically NST, is affected by body fat. More well controlled studies are needed.

Age

Human infants have significant amounts of active BAT. They show an elevated metabolism in a cool environment, as well as a higher skin temperature over the region of interscapular BAT [39]. This results in an increased thermogenic capacity compared with adults.

The elderly have in general an attenuated cold-induced increase in metabolic rate [40•], increased vulnerability to a decreased thyroid function, decreased insulating properties of the skin, and a delayed and relatively slow

vasoconstrictive response to a cool environment [10••,40•]. Some of these factors are related to the aging process itself; others appear to be secondary to other factors for which the risk increases with aging, notably a decreased level of physical activity. In general, older persons may require more intense stimulus to perform protective actions against cold stress.

Gender

Much of the differences between females and males are related to body composition, especially body fat. There are no well controlled studies, correcting for body composition, available showing differences in cold induced metabolism between males and females.

A recent study in rats indicates that gender dimorphism is evident both at BAT adrenergic receptor and at post receptor levels. These changes in the adrenergic control could be responsible for the higher thermogenetic capacity in female rats compared with males [41•].

Physical fitness

Cold thermoregulatory responses can be altered by the extent and nature of physical training as indicated by maximal oxygen consumption (VO2max) [42,43], and endurance level [44]. Discrepancies in the results in studies on physical training and cold response are due to the conditions under which the cold tests are performed and the characteristics of the physical training undertaken. Recent studies show that cold thermoregulatory responses can be altered by training under different climatic conditions [45•], and the type of exercise [46]. There are indications that exertional fatigue has its effect on cold response through a blunted sympathetic nervous responsiveness [46]. None of these studies, however, discriminate between shivering thermogenesis and NST.

Cold adaptation

Phenotypic cold adaptation, or acclimation, are changes that reduce the physiological strain produced by cold [47•]. The number of studies focussing on NST changes due to prolonged cold exposure are scarce. A recent study indicates that winter swimmers during water immersion (1 h, 13°C) show an increase in peripheral vasoconstriction, reduced core temperatures, and increased NST during cold exposure, compared with non-cold adapted humans [9].

Cold air (2 h, 10°C, nude) adaptation has been shown to consist of a reduction of norepinephrine and reduced skin temperatures after a period of 11 days of daily cold exposure [48].

Response to mild cold (3 h, 15°C, clothing) revealed on average no differences in insulation, no hypothermia, but a mean extra increase in NST of 8% in winter compared with summer in a temperate climate (A.M.J. Ooijen, W.D.v. Marken Lichtenbelt, A.A. Steenhoven, *et al.*, in preparation). A large individual difference in the relative contribution of NST was evident, however.

In conclusion, under extreme conditions (such as winter swimmers exposed to 13°C water) individuals exhibit hypermetabolic, hypothermic and insulative types of cold adaptation. Recent work indicates that metabolic adaptation dominates under mild cold conditions.

Diet

It is well known that diet is a potent regulator of adaptive thermogenesis. Both diet and cold activate thermogenesis by the sympathetic nervous system in comparable ways, though different organs may be targeted. Food restriction decreases resting metabolic rate, while overfeeding increases energy expenditure. The amount of increase depends on the quality (especially protein content) of the food [49]. Stock *et al.* [49] provide a very interesting review on how diet composition affects diet-induced thermogenesis. There are indications that overeating at low ambient temperature not only compensates for the increased energy expenditure, but also attenuates a decrease in body temperature [50].

There are, to our knowledge, no human studies investigating diet-induced changes in cold response. A recent study in rats suggests that UCP-1 dependent thermogenic capacity in BAT is influenced by high-fat-diet induced overweight, and that UCP-2 and UCP-3 could also be involved in the thermogenic response [51]. The study does not show that the diet had a direct effect on the cold response, but the cold response could potentially be mediated by large amounts of circulating fatty acids, which can activate UCPs.

Genetics

In the past, several comparative studies on cold response have been performed showing differences between human populations and races. Many physiological adaptations seem to have occurred for survival advantages in some ethnic groups living in harsh environments. Compared with Caucasians, Australian Aborigine men have lower rectal temperatures during sleep [52], Pima Indians have lower body core temperature [53], and black males were found to have lower finger temperatures during a cooling experiment [54]. These physiological adaptations may be contributing to the recent onset of obesity in some of these ethnic groups. In the past, a reduced core temperature or an insulating response, and resulting reduced heat loss, during sleep in a food-restricted environment or during cool nights might have been an adaptive strategy in conserving energy. In modern times, however, those people with

inherited low body temperatures can be more prone to obesity than their warm-blooded conspecifics. This may hold for differences between races but also for individual differences within a (Caucasian) population [2•,55], as mentioned in the Introduction.

A recent study comparing populations examined the evidence for elevations in basal metabolic rate among indigenous northern (Arctic) populations compared with nonindigenous groups [56•]. Their results suggest that genetic factors play a role in the metabolic adaptation to northern climes. The authors relate recent findings on genetics of thyroid function with the potential of genetic adaptations.

Polymorphism linkage or association studies with the UCPs also give indications for an effect of genetic make up on cold-induced metabolism. For instance, genetic studies on human UCP-1 polymorphisms have resulted in at least eight different polymorphisms. The most abundant –3826 A/G UCP-1 polymorphism is associated with fat and weight gain [57–59], and lower expression of UCP-1 mRNA in intraperitoneal depots [17,60]. Moreover, this polymorphism of UCP-1 and one in the β3-AR (Trp64Arg) have been shown to have additive effects on increased risk of weight gain [61], probably as a result of reduced levels of energy expenditure and NST. There is no direct evidence on differences in cold response, however. Also UCP-2, UCP-3, and UCP-5 polymorphisms exist that are linked to body mass indices or energy metabolism [12•,25•].

Conclusion

It is evident that large individual differences in physiological reactions to cold exposure exist. In some individuals heat retention dominates, while cold-induced metabolism is predominant in others. The differences in cold-induced metabolism can partly be attributed to factors such as body composition, age, gender, physical fitness, adaptation/acclimatization, and diet. Studies on the effect of diet and even of gender and body composition are scarce.

There are indications that genetic variation may affect cold response, but no direct evidence is available. To our knowledge there are no polymorphism studies on metabolic cold response carried out in humans.

Many studies are focused on mean values, but the individual differences in response to cold are of great interest. These subject specific characteristics, especially those related to mild cold, can have important consequences for health, pharmacology and comfort. For instance, adaptive thermogenesis is increasingly important for the development of antiobesity therapies. It is possible that individual differences in weight gain are related to multiple defects in UCP-1 gene or other related genes. Such individuals might be at increased risk of developing obesity, but might also respond less well to treatment with β3-adrenergic agonists and other thermogenic agents. With more insight into possible recruitment of BAT in adults, it can be ascertained if a pharmacological regimen is possible which would mimic the effects of chronic cold or elevated catecholamine levels, without attendant side effects.

The role of the novel UCPs and Ca^{2+} cycling still needs to be elucidated. Can they have a protective role against obesity?

Another still open question is to what extent the human skeletal muscle, and possibly other sites (brain, liver), contribute to adaptive thermogenesis.

Finally, it is important to be able to measure cold-induced metabolism without the shivering component. One way is to use mild cold so that shivering does not occur. This of course has to be shown by electromyographic measurements. If uncoupling is the important means of adaptive thermogenesis next to shivering, however, direct noninvasive measurement of uncoupling processes by magneto resonance spectroscopy seems a promising possibility.

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