HubLE I MY MENTOR AND I **Perspective**

Bone homeostasis and energy metabolism after cold stress challenge in mice

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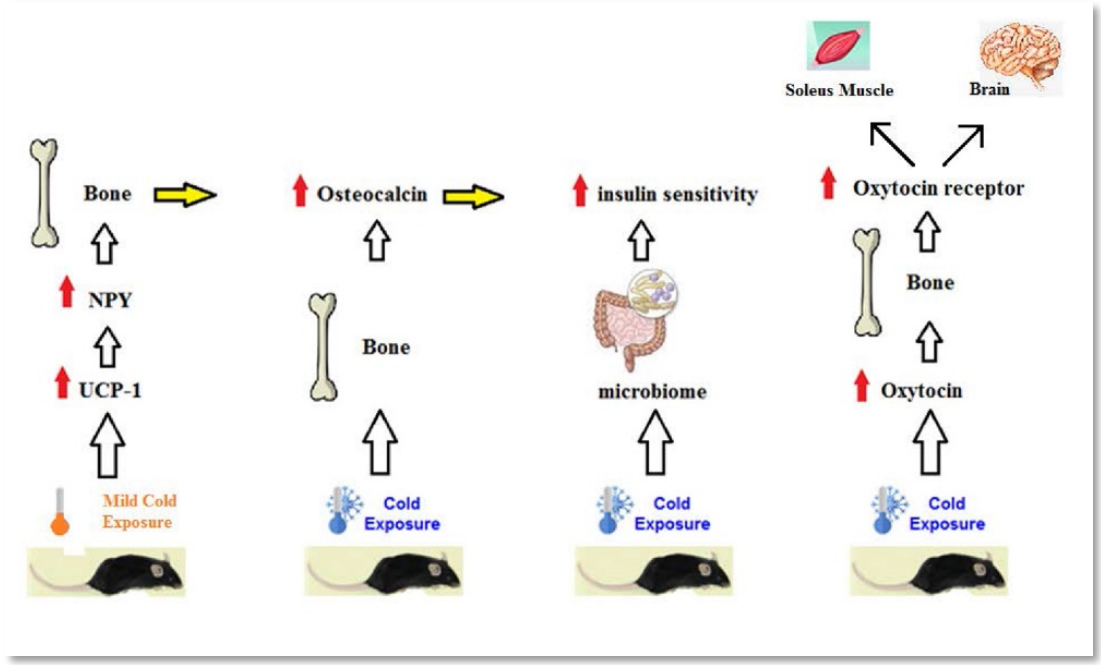
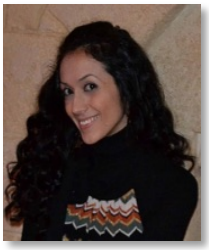
GRAPHICAL ABSTRACT



Claudia Camerino, PhD, is an assistant professor of medicine in the Department of Biomedical Sciences and Human Oncology, School of Medicine, University of Bari and in the Department of Physiology and Pharmacology "V. Erspamer", SAPIENZA University in Rome. Her main research topics are the physiological functions of hormones/neurotransmitters in the regulation of bone and energy metabolism. She developed a special interest for the role of Oxytocin in the development of obesity and muscular diseases.



Elena Conte, PhD, is a post-doctoral fellow in the Department of Pharmacy-Drug Sciences at the University Aldo Moro in Bari. Her research focuses on the characterization of skeletal muscle function and bone metabolism in different pathophysiological conditions through in vivo and ex vivo studies on animal models. Her studies are leading to the identification of molecular targets for new drugs and to the development of individualized treatments in skeletal muscle-related pathologies.

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ABSTRACT

Weight gain occurs when energy intake from food exceeds energy expenditure, while restricting caloric intake to a value below that is needed for daily energy expenditure results in weight loss. Energy expenditure increases during exposure to cold and the regulation of body temperature is one of the most critical function of the nervous system. In this perspective we will describe some of the newly discovered physiological circuits activated by thermogenic challenge and their involvement in bone and energy metabolism.

Introduction

Energy expenditure increases during exposure to cold. Physiologic and metabolic pathways are activated by cold exposure to produce heat and maintain body temperature around 37C.

In human, skeletal muscles can generate heat during cold exposure. This is achieved by voluntary and involuntary muscle contractions[1]. The involuntary activation of skeletal muscle movement is referred to as shivering. Shivering requires a significant amount of energy and therefore generation of ATP to sustain muscle contractions[1]. Characterization of the central neural circuits involved in the involuntary muscle contractions produced from shivering has been the subject of numerous studies[2].

An alternative pathway leading to increased energy expenditure during cold is the induction of non-shivering thermogenesis that relies on the dissipation of heat from brown adipose tissue (BAT). BAT contains significantly more mitochondria than white adipose tissue (WAT) which serves to generate heat through a protein located on the inner mitochondrial membrane called Uncoupling protein-1 (UCP-1)[1]. Recently, it has been reported that the metabolic demands of shivering and non-shivering thermogenesis also relies on an additional type of tissue called “Beige adipose tissue”, which has greater number of mitochondria and is functionally more consistent with BAT than WAT[1].

The thermoregulatory center in the preoptic area of brain[2] receives sensory input from temperature-sensitive receptors in primary nerve endings of the skin and deep organs. These thermal inputs initiate thermoregulatory responses[3].

Thermoregulation of the core of the body combines feed-back and feed-forward mechanisms. Feed-back responses are triggered when the core temperature deviates from the physiological range, for example during exercise, while feed-forward mechanisms are triggered in the absence of any change in core temperature but anticipate thermal challenge, for example following a change in air temperature by thermoreceptors in the skin, which initiate thermoregulatory response that anticipate any change in core-temperature[3]. In this article we will explain some of the most recent and probably unexpected physiological circuit involved in thermoregulation.

Cold stress, bone and the UCP1/NPY axis

BAT dependent and non-shivering thermogenesis is activated during mild cold stress of 22C. This leads to the upregulation of UCP-1 activity, which exerts has a protective effect on bone mass through the modulation of NPY pathway[4]

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Brown adipose tissue (BAT), is essential for the determination of insulin sensitivity and regulation of energy metabolism besides its role in non-shivering thermogenesis and energy dissipation. BAT activity has been demonstrated to positively correlate with bone mass suggesting that energy metabolism regulates bone turnover[5]. In a "Mild cold-stress intervention" protocol, mice were housed at temperature of thermoneutrality or maintained at 22C for 10 weeks[4]. The increase of UCP-1 during mild cold stress is protective of bone mass, thus UCP-1 is a critical mediator of BAT's anabolic action on bone. Indeed, mice lacking UCP-1 show a significant reduction in linear and radial grow of bone under chronic mild-cold stress conditions, a change that is absent when UCP-1-/- mice are kept for under thermo-neutral conditions. The action of UCP-1 in response to cold stress on bone appears to be indirect since no protein expression was found in bone by western blot analysis suggesting an indirect mechanism[4]. Thus, the stimulation of UCP-1 activity triggered by cold exposure appears to result in the activation of a protective pathway capable of stimulating osteoblast activity and preserving bone architecture. UCP-1 is likely to influence bone via an indirect pathways probably triggering the elevation of NPY in the arcuate nucleus of the hypothalamus[4] which may increase food intake that is important when energy demand increases[6,7]. In sum UCP-1 protects bone mass after mild cold stress through increase of NPY and food consumption in mice[4,6].

Cold stress, bone and the Osteocalcin/Oxytocin axis

Osteocalcin and its receptor (Bglap) and oxytocin and its receptor (Oxytocin receptor) share common effects regulating energy, bone mass, reproduction and neuronal functions[8,9,10,11]. The carboxylated osteocalcin has a local effect that leads to bone remodeling and osteoblast activation, while the uncarboxylated form of osteocalcin regulate insulin secretion and sensitivity[8,9,10,11,12]. Oxytocin is involved inthermoregulation[13,14] and the lack of oxytocin receptor may lead to decreased core body temperature after acute exposure to cold[14]. We recently demonstrated that both short-term (6 hours) and long-term (5 days) exposure to 4C cold-stress challenge induces coordinated changes in the mRNA levels of osteocalcin/Bglap and oxytocin/oxytocin receptor in different organs of a mouse model[15]. Osteocalcin and Oxytocin show a beneficial effect on bone and brain after exposure to short term 6 hours or long term 5 days at 4C[15,16]. The up-regulation of the Osteocalcin receptor gene observed after 6 hours of cold stress in brain can be explained by the hormonal proposed action of bone-released osteocalcin as a protector of brain function during stressed-related conditions [17,18,19,20]. Osteocalcin increased consistently in bone in response to cold stress to protect this tissue against the thermogenic insult and potentially improved insulin sensitivity at the face of increased energetic demand[12,15]. On the other end, the up regulation of Oxytocin receptor gene in the brain suggested its role in response to cold stress challenge.

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Oxytocin emerges as an essential factor regulating the coordinated gene response to the paradigm of cold stress. Indeed, Oxytocin receptor regulates gene-response to cold stress through a feed-forward loop in brain mediating the effects of oxytocin in thermoregulation. Oxytocin mRNA is also upregulated in bone after 5 days cold stress[15]. Interestingly, short and long term cold stress induced a potentiation of the slow-twitch phenotype of Soleus muscle by down-regulating Myosin heavy-chain 2b (Myhc2b) and increasing the ratio Myosin heavy-chain 1 (Myhc1) Myhc1/Myhc2b as previously described[21]. This is in line with the metabolic need of the slow-twitch oxidative muscle during thermogenic challenge. Myhc2a was down-regulated in Tibialis Anterioris increasing the ratio Myhc1/Myhc2a. This indicates that cold may trigger the shift of tibialis toward the slow-twitch phenotype. Interestingly, Oxytocin receptor is upregulated in the slow-twitch soleus muscle at 5 days cold stress but not in fast-twitch tibialis anterioris playing a phenotype-dependant protective effect on the slow-twitch muscle (Camerino unpublished data). Myoblasts express oxytocin receptor and oxytocin supports the maintenance of skeletal muscle[22]. In vitro studies on human mesenchymal stem cells show that circulating oxytocin declines with age which contributes to sarcopaenia[23] but significant oxytocin receptor level stays in the old cells allowing myogenesis by ectopic Oxytocin[23]. The increase of Oxytocin in bone[15] after cold stress is in line with increase of Oxytocin receptor in Soleus muscle.

The potentiating effect of Oxytocin on the slow-twitch muscle during cold stress could help explain the normophagic obesity reported for Oxytocin/Oxytocin receptor deficient mice[14,25]. Overall these data show that Oxytocin is adaptive and essential in the physiology of thermogenic stress.

Interestingly, our study also showed that while 6 hours exposure to cold stress induced a significant increase in food intake, there were no significant changes in the abdominal fat pad and body weight. In contrast, exposure to 5 days of cold enhanced food intake and reduced the abdominal fat pad without significantly affecting the mice body weight[15]. These data suggest other mechanisms of cold-tolerance are activated in long-term cold exposed mice.

Cold stress and microbiome

Cold stress influences the composition of the microbiota following the adaptation to the host physiological needs[26]. Indeed the microbiota develops with the host and its composition is influenced by several physiological changes[27]. It has been reported that transplantation of microbiota from mice underwent chronic cold exposure triggers a mechanisms of adaption: increased the intestinal absorptive surface through the elongation of the total intestinal length[26], improved insulin sensitivity in the host and the "browning" of WAT[26,27]. This mechanism regulates energy homeostasis during augmented energy demand. Body fat composition and weight loss in the mice attenuated and normalized over time. Interestingly cold exposure increases the intestinal length and diameter of cold stressed mice[26]. These latter data could

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explain why fat loss is attenuated while body weight normalizes towards control also in our model of cold stressed mice[15].

Conclusion

Overall the use of a cold stress challenge protocol unravel new mechanism of action to counterbalance the increased energy demand including UCP-1 and Oxytocin pathways or the insulin-dependent thermogenesis mediated by microbiome.

This knowledge can be useful in the treatment of obesity or to develop a therapeutic strategy to address nutritional or age-associated detrimental conditions.

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