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Review article

Physiological changes due to mild cooling in healthy lean males of white Caucasian and South Asian descent: A metabolomics study

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ABSTRACT

During mild cold exposure, non-shivering thermogenesis increases to maintain core body temperature by increasing utilization of substrates, especially fatty acids (FA), ultimately affecting lipid-associated metabolites. We aimed to investigate whether mild cooling induces changes in other metabolites and whether this response differs between white Caucasians and South Asians, who have a disadvantageous metabolic phenotype. 12 lean male Dutch white Caucasians and 12 matched Dutch South Asians were exposed to mild cold. Before and after 100 min exposure, serum samples were collected for analysis of 163 metabolites and 27 derived parameters using high throughput metabolomics. The overall response to mild cooling between both ethnicities was not different, therefore the data were pooled. After Bonferroni correction, mild cooling significantly changed 44 of 190 (23%) metabolic parameters. Specifically, cooling increased 19 phosphatidylcholine (PC) species, only those containing very long chain FAs, and increased the total class of PC containing mono-unsaturated FAs (+12.5%). Furthermore, cooling increased 10 sphingomyelin species as well as the amino acids glutamine (+18.7%), glycine (+11.6%) and histidine (+10.6%), and decreased short-chain (C3 and C4) acylcarnitines (−17.1% and −19.4%, respectively). In conclusion, mild cooling elicits substantial effects on serum metabolites in healthy males, irrespective of white Caucasian or South Asian ethnicity.

Contents

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1. Introduction

Mammals are programmed to tightly maintain their core body temperature, during high as well as low environmental temperatures. Cold exposure results in a fast physiological response that includes minimizing heat loss by peripheral vasoconstriction and enhancing heat production by thermogenesis (*e.g.* 'cold-induced thermogenesis'). Cold-induced thermogenesis is a tightly regulated process in which a coordinated action of many organs is involved [1]. It can be divided in non-shivering thermogenesis (NST) and shivering thermogenesis. NST is quickly activated upon cold exposure and is largely mediated by activation of UCP1 in brown adipose tissue (BAT), which uncouples the electron transport chain from ATP synthesis resulting in production of heat [2]. Shivering thermogenesis is induced when NST is insufficient to maintain core body temperature and occurs as a consequence of muscle contractions. Shivering thermogenesis can increase human basal metabolic rate (BMR) by as much as 3–5 fold, however, it is generally experienced as uncomfortable. NST can contribute to BMR up to a more modest +30% but can be sustained without appreciable discomfort. Therefore, enhancing NST is generally considered an attractive target to fight obesity [2].

Exposure to mild cold exposure, generally resulting in enhanced NST, has large metabolic implications, for instance it results in high demand of extra fuel, of which fatty acids (FAs) are likely the most important. FAs are released from white adipose tissue (WAT) as a consequence of lipolysis and are subsequently redirected to several metabolic organs, including BAT, for oxidation and subsequent production of ATP or heat [3,4]. Interestingly, we recently showed that the increase in FA upon mild cold-induced thermogenesis shows an ethnic variation in healthy lean men. When exposed to mild cold, serum FA levels significantly increased in white Caucasians but not in South Asians [5].

Metabolomics is an efficient approach to study many separate metabolic pathways in a high-throughput fashion [6]. Therefore, the objective of the current study was to study changes in metabolites upon mild cooling and to assess ethnic differences in this response in white Caucasians versus South Asians.

2. Materials and methods

2.1. Ethics

Blood samples were collected as part of a clinical study aimed at investigating the activity and volume of BAT in Dutch white Caucasian and Dutch South Asian individuals [7]. The study was approved by the Medical Ethical Committee of the Leiden University Medical Center (LUMC) and undertaken in accordance with the principles of the revised Declaration of Helsinki. All volunteers provided written informed consent.

2.2. Participants and study design

Twenty four Dutch healthy, lean (BMI < 25 kg/m^2) males of white Caucasian (n = 12) and South Asian (n = 12) origin between 18 and 28 years of age were included. The study was conducted in The Rijnland Hospital, Leiderdorp (The Netherlands).

Subjects were studied in the morning after a 10-h overnight fast and subjects were not allowed to exercise 24 h prior to the study. Subjects wore standardized clothing, consisting of a T-shirt and boxer shorts. Upon arrival, subjects ingested a telemetric capsule (Jonah™, BMedical, Australia) to measure core body temperature at 1-min intervals. An intravenous cannula was inserted for blood collection.

To establish maximum NST, an individualized cooling protocol was used in which a subject was placed between two water perfused cooling mattresses (Blanketrol® III, Cincinatti Sub-Zero (CSZ) Products, Inc). During the procedure subjects stayed in a clinical examination room (temperature approx. 24 °C) in a semi-supine position. The protocol started with a baseline period of one hour in thermoneutral condition (water temperature cooling mattresses 32 °C), after which subjects were exposed to mild cold. Since the onset temperature of shivering shows a high interindividual variation (*e.g.* due to differences in body composition), an individualized cooling protocol was used to ensure maximum NST for each subject. For the cooling protocol, we gradually decreased the water temperature of the cooling mattresses until shivering occurred. In short, we first decreased the temperature with steps of 5 °C every 5 min. After we reached a water temperature of 17 °C, we decreased the temperature with 2 °C every 10 min. When a water temperature of 11 °C was reached (but not all subjects reached this temperature), we decreased temperature with 1 °C every 10 min. This was continued until shivering occurred. Shivering was detected visually and by asking the subject if he experienced shivering. When the shivering temperature had been reached, the subject was warmed for 3 min with a bathrobe so that shivering stopped after which he was cooled with a water temperature 3 °C higher than the temperature at which shivering occurred. From that moment, a cooling period of two hours was started (defined as t = 0 min). In case of shivering, temperature was raised by steps of 1 °C until shivering just stopped. In this manner NST was maximized for each individual without shivering. At the end of the thermoneutral period and during the stable cooling period (t = 110 min) venous blood was drawn. For metabolomics, we used serum samples from the 22 participants that were BAT positive on the basis of the ^{18}F-FDG PET-CT scan and therefore excluded the 2 (white Caucasian) subjects that exhibited virtually no BAT. These two excluded subjects were both from white Caucasian descent. We measured 190 metabolic parameters (of which 163 small molecule metabolites) in these samples.