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Original Article

Short-term cooling increases serum angiopoietin-like 4 levels in healthy lean men

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KEYWORDS:
Angiopoietin-like 4;
Brown adipose tissue;
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Sympathetic nervous system

BACKGROUND: Cold exposure enhances sympathetic outflow to peripheral tissues, thereby stimulating intracellular lipolysis in white adipose tissue and increasing the lipoprotein lipase–dependent uptake and combustion of triglyceride-derived fatty acids (FAs) by brown adipose tissue. Angiopoietin-like 4 (ANGPTL4) inhibits lipoprotein lipase and can be regulated by cold exposure, at least in mice.

OBJECTIVE: In the present study, we examined the effect of short-term mild cooling on serum ANGPTL4 levels in healthy lean men of White Caucasian and South Asian descent.

METHODS: Healthy, lean White Caucasian ($n = 12$) and South Asian ($n = 12$) men were exposed to an individualized cooling protocol for 2 hours. Serum ANGPTL4 levels were measured before and after cooling, and its relation with previously measured parameters (ie, free fatty acid [FFA] levels, body fat percentage, and resting energy expenditure) was determined.

RESULTS: Short-term cooling increased ANGPTL4 levels (+17%, $P < .001$). Thermoneutral ANGPTL4 levels positively correlated with FFA levels ($R^2 = 0.250$, $P < .05$) and body fat percentage ($R^2 = 0.338$, $P < .05$). Furthermore, ANGPTL4 negatively correlated with resting energy expenditure ($R^2 = 0.235$, $P < .05$). The relative increase in ANGPTL4 levels was higher in White Caucasians compared with South Asians (25 ± 4 vs 9 ± 4%, $P < .05$).

CONCLUSION: Short-term cooling increases ANGPTL4 levels in healthy lean men. We anticipate that FFA liberated from white adipose tissue during cooling increases ANGPTL4 to limit uptake of triglyceride-derived FA by this tissue.

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Conflicts of interest: The authors declare no conflict of interests.

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Introduction

Cold exposure enhances the sympathetic outflow toward peripheral metabolic organs, including white adipose tissue (WAT) and brown adipose tissue (BAT).[1,2] In WAT, sympathetic stimulation increases intracellular lipolysis and subsequent release of free fatty acids (FFAs) into the bloodstream.[3] In contrast, sympathetic stimulation of BAT increases the uptake and combustion of triglyceride (TG)-derived FA.[4,5]

Uptake of TG-derived FA from TG-rich lipoproteins by metabolic organs is determined by the activity of lipoprotein lipase (LPL). LPL is tightly regulated by many factors including angiopoietin-like 4 (ANGPTL4). ANGPTL4 is expressed by metabolic tissues (ie, liver, WAT, and BAT[6,7]) and inhibits LPL resulting in limited uptake of TG-derived FA uptake by tissues.[8,9] ANGPTL4 expression is stimulated by elevated intracellular FFA levels,[10] through activation of peroxisome proliferator-activated receptors (PPARs).[9] In mice, we recently showed that cold exposure decreases ANGPTL4 protein content in BAT while increasing ANGPTL4 protein content in WAT.[6,11] This opposite regulation of ANGPTL4, and thus LPL activity, in BAT and WAT, makes physiological sense to secure the shuttling of FA toward BAT to meet the increased energy demand of activated BAT for heat production.[11]

Although the regulation of ANGPTL4 on cooling is well described in mice, the effect of short-term cooling on circulating ANGPTL4 levels in humans remained to be elucidated. Moreover, ANGPTL4 levels may be differentially regulated in subjects of South Asian descent, in which we previously showed lower resting energy expenditure (REE) and BAT volume on cold exposure compared with White Caucasians.[12] The aim of the present study was, therefore, to determine the effect of short-term mild cooling on serum ANGPTL4 levels in healthy lean men of White Caucasian and South Asian descent.

Materials and methods

For the present study, blood samples were used from a previous clinical study[12] that was approved by the Medical Ethical Committee of the Leiden University Medical Center and undertaken in accordance with the principles of the Declaration of Helsinki. All volunteers provided written informed consent (Netherlands Trial Register 2473).

An extensive description of the materials and methods has been described previously.[12] In short, 24 healthy, young (age 18–28 years), lean (body mass index <25 kg/m^2) White Caucasian ($n = 12$) and South Asian ($n = 12$) men, participated in a prospective case–control study. Body composition was assessed by bioelectrical impedance analysis. An individualized water cooling protocol (2 hours mild cooling) followed by [^{18}F]FDG-PET-CT imaging was performed to quantify BAT volume and activity. Before and after cooling, REE was measured (Oxycon) and fasted blood samples were collected. Pre- and post-cooling blood samples were analyzed for ANGPTL4 by enzyme-linked immunosorbent assay [10] and related to previously measured FFA levels, lipoprotein constituent levels (TG, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C]), body fat percentage, and REE. The effects of cooling on ANGPTL4 and FFA, TG, LDL-C, and HDL-C were assessed using paired (effect of cold) and unpaired (effect of ethnicity) Student *t*-tests with the SPSS 20 software. Correlations were analyzed using linear regression analysis, both with and without correction for the effect of ethnicity, by respectively including/excluding ethnicity as a covariate. For lipid and lipoprotein measurements 1 South Asian and 1 white Caucasian individual were excluded because of familial hypercholesterolemia and hyperventilation, respectively. Data on body fat are missing for 4 South Asians and 2 White Caucasians because bioelectrical impedance analysis measurements could not be performed. Values of $P < .05$ were considered statistically significant. Data are presented as mean ± standard error of the mean.

Results

Clinical characteristics

Clinical characteristics of the participants and REE, BAT volume, and lipid and lipoprotein levels were described previously.[12,13] In short, we observed lower thermoneutral REE in South Asians compared with White Caucasians (1297 ± 36 vs 1689 ± 56 kcal/d, $P < .001$). Short-term cooling significantly increased REE in White Caucasians (2027 ± 136 kcal/d, +20%, $P < .01$) but not in South Asians (1462 ± 37 kcal/d, +13%, n.s.). In addition, body fat percentage was significantly higher in South Asians compared with White Caucasians (16 ± 1 vs 12 ± 1%, $P < .05$) and BAT volume was lower in South Asians compared with White Caucasians (188 ± 23 vs 287 ± 49 mL, $P < .05$). Thermoneutral serum FFA levels were not different between White Caucasians and South Asians (0.66 ± 0.09 vs 0.88 ± 0.12 mmol/L, n.s.). However, cooling strongly increased FFA levels in White Caucasians (0.99 ± 0.09 mmol/L, +49%, $P < .001$), but not in South Asians (0.97 ± 0.11 mmol/L, +10%, n.s.). In addition, South Asians had comparable thermoneutral serum TG levels (1.33 ± 0.77 vs 1.27 ± 0.12 mmol/L) as well as HDL-C levels (0.86 ± 0.04 vs 0.92 ± 0.07 mmol/L), whereas South Asians tended to have higher thermoneutral LDL-C levels (2.39 ± 0.14 vs 2.01 ± 0.13 mmol/L, $P = .06$), compared with White Caucasians. Cooling significantly increased serum TG in both ethnicities (+18%, $P < .05$ in White Caucasians and +24%, $P < .05$ in South Asians), whereas HDL-C (+9%, $P < .05$) and LDL-C (+8%, $P < .01$) were only increased in White Caucasians on cooling.