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PRELIMINARY REPORT

Acute Cold Exposure Decreases Plasma Leptin in Women

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We investigated whether cold exposure affects circulating leptin in humans. Five women (age, 32 ± 4 years; body mass index, 23.1 ± 1.7 kg/m^2) participated in two separate trials. Subjects sat at room temperature ([RT] 24.8° ± 0.3°C) or in the cold (6.3° ± 0.5°C) for 90 minutes. During RT exposure, plasma leptin and norepinephrine were unchanged over time. Cold exposure significantly decreased plasma leptin by 14%, 17%, and 22% at 30, 60, and 90 minutes, respectively (temperature × time interaction, $P < .04$). Plasma norepinephrine increased by 400% to 500% ($P < .001$) and plasma glycerol increased by 110% over baseline during cold exposure (temperature effect, $P < .005$). We conclude that circulating leptin decreases during cold exposure, probably as a result of activation of the sympathetic nervous system (SNS).

THE SYMPATHETIC nervous system (SNS) appears to play a role in regulating leptin expression. Administration of β-adrenergic receptor agonists and cold exposure reduced plasma leptin and leptin mRNA in the white adipose tissue of rodents.[1,2] In humans, infusion of isoproterenol (a nonselective β-adrenergic agonist) decreased plasma leptin by 19% to 27% over 120 minutes in lean men and women.[3,4] Furthermore, in vitro data demonstrate that isoproterenol directly decreases the amount of leptin secreted from human adipose tissue[5] and isolated human adipocytes.[6] Since plasma norepinephrine in humans markedly increases during acute cold exposure,[7] we have examined whether physiological stimulation of the SNS via acute cold exposure decreases circulating leptin in humans.

SUBJECTS AND METHODS

Subjects

The study was approved by the Rutgers University Institutional Review Board for Human Subjects Research, and all subjects provided informed consent. Five healthy women (age, 32 ± 4 years; body mass index, 23.1 ± 1.7 kg/m^2) participated in two separate trials, both of which were performed during the luteal phase of the subject's menstrual cycle.

Experimental Protocol

Since circulating leptin exhibits a diurnal rhythm with a decline in the morning hours,[8] our investigations were performed in the early afternoon when plasma leptin is stable. Subjects were fed a standardized breakfast (~600 kcal) at 9 AM (86% carbohydrate, 10% protein, and 4% fat). After consuming a banana at approximately 11 AM, the subjects returned to the laboratory in the early afternoon and changed into a light shirt, shorts, and socks. A venous catheter was inserted into the subject's arm at the antecubital space for subsequent blood sampling. The core temperature (T_{es}) was measured as described previously.[9]

After instrumentation, the subjects sat at room temperature (RT) for 20 minutes, after which baseline measurements (0 minutes) and blood samples were taken. Subjects then either remained seated at RT (24.8° ± 0.3°C) or were seated in an environmentally regulated cold room (6.3° ± 0.5°C) for 90 minutes. The order in which subjects completed the two trials was randomized, with 3 subjects completing the cold trial first and 2 completing the RT trial first. Blood was collected every 30 minutes and T_{es} was measured every 5 minutes.

Blood Collection and Analyses

Prior to centrifugation to obtain plasma, 120 μL reduced glutathione (75 ng/mL) and EGTA (75 mg/mL) were added to approximately 10 mL blood for subsequent norepinephrine analysis. After centrifugation, the plasma was collected and stored at −70°C for radioimmunoassay of leptin (Linco, St Louis, MO), insulin,[10] and cortisol (Diagnostic Products Corp, Los Angeles, CA). Plasma glucose was determined using a Beckman (Fullerton, CA) glucose analyzer, and plasma glycerol was determined via an enzymatic fluorometric assay.[11] After extraction of catecholamines by alumina extraction, plasma norepinephrine was quantified by high-performance liquid chromatography. Leptin and glycerol analysis was performed on plasma from all 5 subjects, but data

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Table 1. Plasma Variables and T_{es} Over Time

Variable	RT				Cold			
	0	30 min	60 min	90 min	0	30 min	60 min	90 min
Leptin (ng/mL)	8.7 ± 2.1	9.1 ± 2.2	9.2 ± 2.4	9.0 ± 2.4	10.1 ± 2.8	9.0 ± 2.8†	8.7 ± 2.8†	7.8 ± 2.2†
Norepinephrine (nmol/L)	2.8 ± 0.5	3.3 ± 0.5	3.1 ± 0.6	3.8 ± 0.4	2.2 ± 0.2	8.0 ± 1.4†	9.3 ± 1.3†	12.2 ± 2.4†
Glycerol (μmol/L)	158.5 ± 32.4	174.2 ± 26.4	172.0 ± 20.9	192.3 ± 34.7	178.3 ± 37.9	245.7 ± 15.9†	315.4 ± 58.3†	375.1 ± 81.1†
Glucose (mg/dL)	89.5 ± 6.8	81.0 ± 9.1	82.3 ± 7.3	81.8 ± 3.1	87.5 ± 3.2	91.8 ± 3.4	89.0 ± 2.6	87.5 ± 1.7
Insulin (μU/mL)	27.5 ± 2.6	20.8 ± 3.8	21.3 ± 4.3	20.5 ± 1.9	22.4 ± 2.2	22.3 ± 3.4	21.3 ± 1.9	20.4 ± 1.7
Cortisol (μg/dL)	16.1 ± 5.5	13.1 ± 4.3	11.8 ± 3.7	10.4 ± 3.6	18.8 ± 9.0	17.6 ± 7.3	20.9 ± 6.2	21.7 ± 4.0*
T_{es} (°C)	37.1 ± 0.1	37.1 ± 0.0	37.1 ± 0.1	37.1 ± 0.1	37.2 ± 0.1	37.3 ± 0.1	37.0 ± 0.1‡	36.7 ± 0.3†‡§

*$P < .05$ *v* corresponding time at RT.
†$P < .05$ *v* baseline within treatment.
‡$P < .05$ *v* 30 min within treatment.
§$P < .05$ *v* 60 min within treatment.

on other plasma metabolites are from 4 subjects, due to storage problems.

Statistical Analyses

Results are presented as the mean ± SEM. The effects of temperature and time were determined by 2-way ANOVA with repeated measures on both factors on logarithmically transformed values. Because baseline values differed and treatment effects were multiplicative, the data were log-transformed prior to statistical analysis. When significant main effects were found, post hoc analyses with the Bonferroni method were performed (Sigma Stat; SPSS, San Rafael, CA). Significance was set at a P level of .05 or less.

RESULTS

Data for all plasma metabolites and T_{es} are presented in Table 1. Leptin levels were stable at RT but declined during cold exposure (temperature × time interaction, $P \leq .04$). Post hoc analysis showed that during cold exposure, plasma leptin significantly decreased to 86% ± 6%, 83% ± 8%, and 78% ± 5% of baseline values at 30, 60, and 90 minutes, respectively ($P < .05$ for all).

Plasma norepinephrine significantly increased by 375% ± 69% over baseline ($P < .001$) after 30 minutes during cold exposure and remained elevated throughout ($P < .001$). In contrast, plasma norepinephrine did not change over time in the RT trial. Consistent with these results, plasma glycerol increased by 70% ± 42%, 104% ± 40%, and 134% ± 42% (temperature effect, $P \leq .005$) at 30, 60, and 90 minutes of cold exposure, respectively, while no change occurred during the RT trial.

At 90 minutes, cortisol was significantly higher during cold exposure compared with RT (temperature × time interaction, $P = .04$). Plasma insulin and glucose were stable over the experimental period in both trials. A significant effect of time and a temperature × time interaction was noted for T_{es}. T_{es} did not change during the RT trial. In cold exposure, T_{es} was significantly decreased after 60 minutes and continued to decline at 90 minutes ($P < .05$).

DISCUSSION

These data suggest that activation of the SNS (as evidenced by the large increase in plasma norepinephrine and increase in plasma glycerol) by acute cold exposure rapidly decreases plasma leptin levels in healthy women. The magnitude and rapidity of the plasma leptin response to cold reported here (−22% in 90 minutes) is similar to that due to isoproterenol infusion, which decreased plasma leptin by 19% to 27% over 120 minutes in lean men and women.[3,4] Also consistent with these in vivo results, we previously reported that isoproterenol significantly decreased leptin release from human adipose tissue in vitro by 22% and 35% after 1.5 and 3 hours of incubation, respectively.[5] Thus, the effect of β-adrenergic receptor stimulation on leptin release likely results from a direct effect on the adipocyte.

The cold-induced decrease in plasma leptin we report was not related to a decrease in plasma insulin or cortisol. Plasma insulin was not affected by temperature or time. Plasma cortisol was significantly higher at 90 minutes during cold exposure versus RT, which would be predicted to increase, rather than decrease, plasma leptin.[12]

Whereas we observed a rapid suppression of plasma leptin in response to activation of the SNS via cold exposure, several investigations found no effect of SNS activation via exercise on plasma leptin. These disparate results are not likely attributable to differences in SNS activity between these two treatments. Plasma levels of norepinephrine can reach approximately 10 to 15 nmol/L during prolonged submaximal exercise[13] and about 20 nmol/L during short-term (15 to 30 minutes) maximal exercise,[14] while we found that the levels reached 12 nmol/L by 90 minutes of cold exposure. Our ability to detect an effect of increased catecholamines on plasma leptin in the present study is likely due to the fact that we examined the plasma leptin response to cold in the afternoon hours, after a standardized breakfast and snack. Thus, we avoided the possible difficulty in detecting a decrease in leptin during the morning hours—when plasma leptin is decreasing.[8] Also, it may be easier to detect decreases in plasma leptin in women, who have higher leptin levels than men.[15] Further studies are needed to address these interactions.

We considered the possibility that the decline in plasma leptin was related to factors other than adipose tissue production. Cold exposure (15°C) is reported to decrease renal plasma flow in humans[16]; thus, it is unlikely that leptin clearance by the kidney was increased during cold.[17] Changes in peripheral blood flow may have contributed to the decline in plasma leptin. Cold exposure, unlike exercise, results in peripheral vasoconstriction, and it is therefore possible that the decrease in plasma leptin

resulted from a decrease in blood delivery to subcutaneous adipose tissue depots.[18] The present data do not allow a separation of the potential blood flow effects from the direct effects of β-adrenergic stimulation at the level of the adipocyte. The 110% increase in plasma glycerol (from adipocyte lipolysis) during cold exposure suggests adequate blood flow to adipose tissue. While central adipose depots may also release glycerol during cold exposure, their contribution is likely minor in lean young women.

In summary, exposure to cold rapidly decreased plasma leptin in lean healthy women. The swift change in leptin with cold exposure occurs in a much shorter time interval (30 minutes) than previously reported for the decreases during fasting (~12 hours)[19] or increases during overfeeding (>5 hours)[20] or with glucocorticoid administration in the fed state (7 hours).[12] These data suggest that the SNS may play a role in modulating acute alterations in plasma leptin in humans.

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