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Obesity

Inverse Association Between Carbohydrate Consumption and Plasma Adropin Concentrations in Humans

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Objective: The role of metabolic condition and diet in regulating circulating levels of adropin, a peptide hormone linked to cardiometabolic control, is not well understood. In this study, weight loss and diet effects on plasma adropin concentrations were examined.

Methods: This report includes data from (1) a weight loss trial, (2) an evaluation of acute exercise effects on mixed-meal (60% kcal from carbohydrates) tolerance test responses, and (3) a meta-analysis to determine normal fasting adropin concentrations.

Results: Distribution of plasma adropin concentrations exhibited positive skew and kurtosis. The effect of weight loss on plasma adropin concentrations was dependent on baseline plasma adropin concentrations, with an inverse association between baseline and a decline in concentrations after weight loss (Spearman's $\rho = -0.575$; $P < 0.001$). When ranked by baseline plasma adropin concentrations, only values in the upper quartile declined with weight loss. Plasma adropin concentrations under the main area of the bell curve correlated negatively with habitual carbohydrate intake and plasma lipids. There was a negative correlation between baseline values and a transient decline in plasma adropin during the mixed-meal tolerance test.

Conclusions: Plasma adropin concentrations in humans are sensitive to dietary macronutrients, perhaps due to habitual consumption of carbohydrate-rich diets suppressing circulating levels. Very high adropin levels may indicate cardiometabolic conditions sensitive to weight loss.

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Introduction

Studies using mice suggest the peptide hormone adropin has metabolic (1-3) and vascular functions (4). Circulating adropin concentrations have been measured in several mammalian species using commercially produced enzyme immunoassays (5-22). Human studies have screened for associations between plasma adropin concentrations and cardiovascular disease (5,8,12,17), endothelial function (10,11), type 2 diabetes (T2D) (7,9,14,17), obesity and aging (6), and exercise response (23). These studies suggest associations between cardiometabolic disorders of obesity and altered circulating adropin concentrations (24,25).

Adropin expression in a human liver cell line (Hepg2) is suppressed following the activation of liver receptor (LXRα), suggesting sensitivity to carbohydrate and lipid metabolism (26). We reported increased plasma adropin concentrations following Roux-en-Y gastric bypass (6), changes following sugar consumption (20), and

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Author contributions: PJH, KLS, M-PS-O, JPT, JAK, and EPW contributed materials for the collection of data, assisted with data interpretation, and reviewed the manuscript. JRS and MLK collected data, assisted with data analysis, and reviewed the manuscript. AAB contributed materials for collection of data, analyzed and interpreted data, and prepared the first draft of the manuscript. AAB also had full access to the data and takes responsibility for the integrity and accuracy of the analysis.

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associations between plasma adropin concentrations and fat and carbohydrate intake (16). Metabolic condition and feeding behavior thus influence plasma adropin levels in humans. However, the specific metabolic and dietary factors influencing plasma adropin concentrations in humans remain unclear.

Here we report investigating whether weight loss and improved insulin sensitivity would affect plasma adropin concentrations in humans using plasma samples before and after 6% to 8% weight reduction (27). We also further investigated associations between plasma adropin concentrations and macronutrient intake previously observed in women participating in a sleep restriction study (16), using habitual intake data from a larger cohort of men and women. Finally, we report results from a meta-analysis to establish normal values for fasting plasma adropin.

Methods

Data from three studies were included in this report: (1) a randomized intervention trial to determine whether weight loss and habitual diet affect plasma adropin concentrations (Caloric Restriction, Exercise, and Glucoregulation in Humans; CREG study), (2) a meta-analysis to determine normal plasma adropin concentrations, and (3) an acute exercise study in diabetics evaluating the effects of a single bout of exercise on plasma adropin responses to a mixed-meal tolerance test (MMTT study).

The rationale for examining whether weight loss affects plasma adropin concentrations in humans is based on experiments in mice showing regulation in response to fasting (26) and caloric restriction (28). The rationale for the meta-analysis was based on data from the weight-loss study suggesting a clustering of values below 5 ng/mL. The intent of the MMTT was to examine whether a meal with carbohydrate content similar to the habitual intake of participants with low plasma adropin concentrations would have an inhibitory effect

CREG study

Participants and intervention. Selection criteria, interventions, and primary outcomes were reported previously (27) (ClinicalTrials.gov #NCT00777621). In brief, the study involved sedentary, men and women with excess weight aged 45 to 65 years. Subjects were randomized with stratification for sex, and assigned to groups with the established goal of achieving 6 to 8% weight loss using either calorie restriction (CR), endurance exercise training (EX), or a combination (CREX) to maintain a 20% negative energy balance relative to estimated total energy expenditure. The study was reviewed and approved by the Institutional Review Boards (IRB) of Washington University and Saint Louis University.

Plasma samples used in this study were collected at baseline and follow-up; follow-up samples were collected after 2 weeks of weight stability to eliminate confounding effects of negative energy balance. For CREX and EX participants, samples were collected 12 to 24 h after the last exercise bout. Sera were analyzed in a Clinical Laboratory Improvement Amendments (CLIA)-certified clinical laboratory for concentrations of total, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) cholesterol, and glycerol-blanked triglyceride concentrations using automated enzymatic/colorimetric assays (Roche/Hitachi Modular Analytics System, Roche Diagnostics Corporation, Indianapolis, IN). Plasma glucose was measured using the glucose oxidase method (YSI STAT Plus; YSI Life Sciences, Yellow Springs, OH); insulin was measured using IMMULITE Chemiluminescence Kits (Diagnostics Products Corporation, Los Angeles, CA). Fat mass and fat-free mass were measured by DXA (Lunar iDXA, software version 13.31; GE Healthcare, Madison, WI).

TABLE 1 Composition of the breakfast meal (breakfast wrap and orange juice; 60% energy as carbohydrates, 30% as fats, and 10% as protein) used for the MMTT

Food	Carbohydrates (g)	Fats (g)	Protein (g)	Energy (kcal)
Flour tortilla	23.4	3.3	3.7	140.5
Egg Beaters®	0.0	0.0	0.2	1.1
Egg yolk	0.2	1.2	0.7	14.5
Cheese	1.7	4.3	2.6	60.0
Margarine	0.0	4.5	0.0	38.6
Orange juice	34.8	0.0	2.7	147.1
Total	60.1	13.3	9.9	401.8

Food diaries. CREG study participants maintained food diaries for 3 days before starting weight loss intervention; the sample size is higher ($n = 62$) compared with that used for the weight loss study ($n = 54$) owing to noncompliance of eight participants. Nutrient intakes were quantified by analyzing the 3-day food diaries (2 weekdays, 1 weekend day) with Food Processor SQL (ESHA Research, Salem, OR).

MMTT study

Participants. Sedentary (0-1 sessions/week of physical activity lasting >30 min; not employed in physically active jobs or hobbies), weight-stable, male ($n = 2$) and female ($n = 7$) subjects aged 48 to 67 years who had overweight or obesity (body mass index, BMI 25.0-37.0 kg/m^2) and physician-diagnosed T2D and HbA1c <10% were recruited for participation. Subjects were nonsmokers, not on insulin therapy, had no previous cardiac events, and did not skip breakfast or have other irregular dietary patterns. The study was reviewed and approved by the IRB at the University of Missouri in Columbia, MO.

Intervention. Plasma adropin concentrations during the MMTT were compared before (pre) and after (post) a 7-day exercise intervention, allowing within subjects comparisons. Seven days of aerobic exercise training is commonly used to examine the effects of added daily exercise before changes in body composition and training adaptations that occur with chronic training (29). Subjects exercised under the supervision of trained personnel for 1 h/day at 60% of heart rate reserve (monitored by telemetry) over 7 consecutive days between tests. Exercise involved combining brisk treadmill walking and stationary cycling (29). The last exercise session was completed 14 to 16 h before the day of the MMTT.

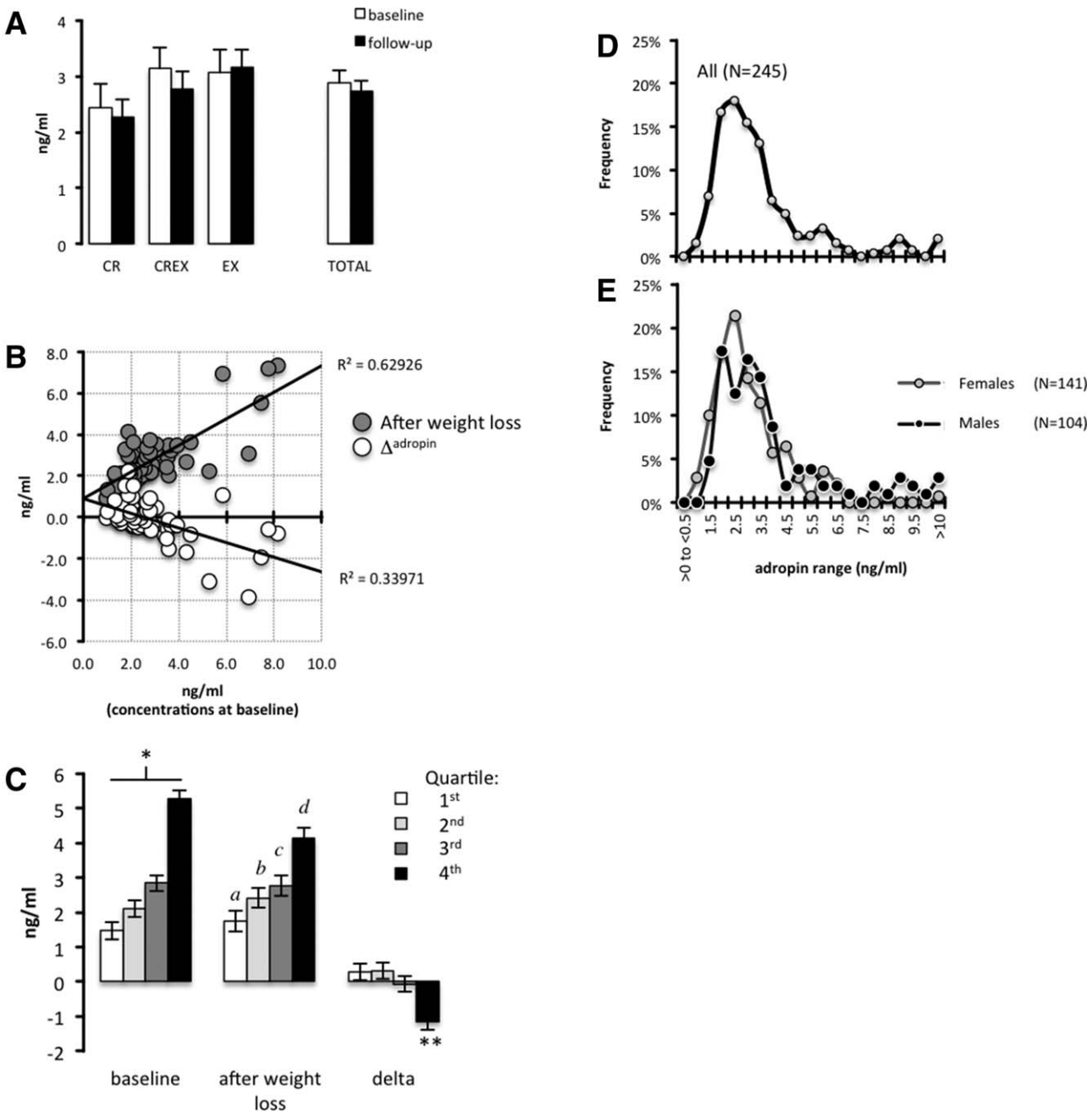

Figure 1 An inverse association between baseline plasma adropin concentrations and the effect of weight loss. (**A**) Plasma adropin concentrations at baseline and after achieving 6% to 8% weight loss ("follow-up") by calorie restriction (CR), calorie restriction plus exercise (CREX), and exercise only (EX). White bars = adropin values at baseline; black bars = adropin values after weight loss. (**B**) Scatterplot showing associations between baseline plasma adropin concentrations and concentrations after weight loss (follow-up) or the difference in concentration at follow-up from baseline ($\Delta^{adropin}$). Gray circles = adropin values after weight loss; white circles = $\Delta^{adropin}$. (**C**) Plasma adropin concentrations quartiled by ranking levels at baseline from low (1st quartile) to high (4th quartile). There was a significant interaction between weight loss and quartile, with the 4th quartile exhibiting a decline in plasma adropin concentrations. *$P < 0.005$ between all quartiles; [a] $P < 0.005$ vs. 3rd, 4th quartile; [b] $P < 0.005$ vs. 4th quartile; [c] $P < 0.005$ vs. 1st quartile; [d] $P < 0.005$ vs. 1st, 2nd quartile; **$P < 0.001$ compared with the 1st and 2nd quartile, $P < 0.05$ to 3rd quartile. (**D**) Frequency distribution of plasma adropin data pooled from the current and previously published experiments ($n = 245$). (**E**) Frequency distribution of plasma adropin data by sex. The values for the *x*-axis are the same in panels D and E.

After an overnight fast, blood samples were taken at time zero and subjects then consumed a ~400 kcal mixed meal containing 60% energy as carbohydrates (2/3 as simple sugars) (Table 1). Blood was sampled from the arterialized venous site, collected in EDTA tubes containing aprotinin and dipeptidyl peptidase IV inhibitors; aliquots of plasma and serum were stored at −80°C. Plasma adropin

TABLE 2 Subject demographics of the CREG participants for whom plasma adropin values were measured for the weight loss study

Demographic; laboratory measurement	All participants	CR	CREX	EX
Gender (F/M, *n*)	42/12	13/3	16/4	13/5
Age (years)	57.0 ± 0.7	57.4 ± 1.4	57.2 ± 1.2	56.9 ± 1.3
Weeks taken to attain weight loss goals[a]	17.0 ± 1.1	18.6 ± 1.8	13.5 ± 1.7*	19.4 ± 1.8
BMI (kg/m^2)[b]				
Pretreatment	27.7 ± 0.2	27.7 ± 0.4	28.3 ± 0.4	27.0 ± 0.4
Post-treatment	25.9 ± 0.2	25.9 ± 0.4	26.3 ± 0.4	25.4 ± 0.4
Change	−1.8 ± 0.1	−1.7 ± 0.2	−2.0 ± 0.2	−1.6 ± 0.2
Body weight (kg)[b]				
Pretreatment	78.7 ± 1.4	77.2 ± 2.0	81.8 ± 1.8	76.5 ± 1.9
Post-treatment	73.5 ± 1.4	72.2 ± 2.0	75.9 ± 1.8	72.0 ± 1.9
Change	−5.2 ± 0.3	−5.0 ± 0.5	−5.9 ± 0.5	−4.5 ± 0.5
Fat mass (kg)[b]				
Pretreatment	31.8 ± 0.7	32.0 ± 1.3	33.1 ± 1.1	30.3 ± 1.2
Post-treatment	27.6 ± 0.7	28.1 ± 1.3	28.3 ± 1.1	26.4 ± 1.2
Change	−4.3 ± 0.3	−3.9 ± 0.5	−4.8 ± 0.4	−3.9 ± 0.4
Fat-free mass (kg)[b]				
Pretreatment	46.5 ± 1.2	44.9 ± 1.1	48.3 ± 1.0	45.9 ± 1.1
Post-treatment	45.8 ± 1.2	44.0 ± 1.1	47.5 ± 1.0	45.7 ± 1.0
Change	−0.6 ± 1.2	−0.8 ± 0.3	−0.8 ± 0.2	−0.2 ± 0.3
Fat%[b]				
Pretreatment	42.2 ± 0.8	43.4 ± 1.0	42.2 ± 0.9	41.2 ± 0.9
Post-treatment	39.1 ± 0.9	40.7 ± 1.1	38.8 ± 1.0	37.9 ± 1.0
Change	−3.2 ± 0.2	−2.7 ± 0.4	−3.4 ± 0.4	−3.3 ± 0.4

Data are mean ± SE.
[a]Among group comparison by ANOVA, $P < 0.05$; CREX vs. CR, EX, $P < 0.05$.
[b]Sex was used as a covariate in the analysis to adjust for differences in proportion of males and females in each group.

concentrations were measured at baseline ($T = 0$) and then at $T = 30$, 60, and 90 min postmeal.

Meta-analysis of plasma adropin concentrations

Distribution of plasma adropin concentrations in a mixed population was estimated by pooling data from this study with published data (6,16,20) (ClinicalTrials.gov #NCT01165853; NCT01103921; NCT00935402, and NCT00936130), and data obtained from measurement of plasma adropin concentrations in samples obtained from studies performed at the University of Missouri-Columbia (30-33). The samples used for these measurements were collected at baseline. The original studies were reviewed by the IRB at UC Davis, Syracuse University, St. Luke's-Roosevelt Hospital Center, Pennington Biomedical Research Center, and the University of Missouri in Columbia.

Measurement of plasma adropin concentrations

Adropin concentrations were determined in the plasma fraction of blood as previously described (6,16,20) using a commercially available enzyme immunoassay (s-1385, Peninsula Laboratories, San Carlos, CA) validated previously using plasma from adropin knockout mice, and tested using a spike and recovery of synthetic adropin[34-76] (1,6). The intra- and interassay coefficient of variation are <5% and 25 to 30%, respectively.

Statistical analysis

Data were analyzed using SPSS Statistics Version 23 (IBM). Effects of weight loss on plasma adropin concentrations were assessed using repeated measures ANOVA, including treatment (CR, EX, CREX) as an independent variable and sex and glucose tolerance state as covariates, and using linear regression modeling including plasma adropin data and other metrics. When grouped into quartiles based on ranking plasma adropin concentrations treatment, sex, and glucose tolerance status were used as covariates. Associations between changes in plasma adropin concentrations ($\Delta^{adropin}$ calculated by subtracting the baseline value from final value) and fasting measurements indicating glucose control and lipid metabolism were evaluated using Spearman correlations. Associations between baseline and $\Delta^{adropin}$ and food intake data were first evaluated by converting all data into *Z*-scores (standard deviations [SD] from the mean), and further evaluated by separation into quartiles or tertiles, and ranked by baseline plasma adropin values from lowest to highest.

For the meta-analysis, distribution, skew, and kurtosis were determined using SPSS. Effects of sex were assessed using univariate analysis with age, BMI, and glucose tolerance status as covariates.

Associations between plasma adropin concentrations with macronutrient intake were initially analyzed using linear and nonlinear associations using Microsoft Excel. We initially converted macronutrient intake data

TABLE 3 Demographics of the subjects used for the meta-analysis of plasma adropin concentrations

Demographic; laboratory measurement	All subjects ($n = 245$)	Males ($n = 104$)	Females ($n = 141$)	P
T2D (n)	46	8	38	
Age (years)				
Mean	34.9	33.3	36.1	n.s
SD	12.8	13.6	12.2	0.001
Range	18-67	18-70	20-67	0.001
BMI (kg/m^2)				
Mean	30.7	28.4	32.4	0.001
SD	9.1	7.4	9.9	
Range	17.6-71.5	17.6-62.6	19.4-71.5	
Adropin (ng/ml)				
Mean	3.3	3.9	2.9	0.001
SD	2.3	3	1.6	
Range	0.6-20.0	1.1-20.0	0.6-10.9	

into Z-scores, allowing for comparisons of protein, carbohydrate, and fat intake as g/d or relative to total calorie intake as a function of plasma adropin values. Nonlinearity appeared to be driven by participants with very high plasma adropin concentrations; these individuals were treated as outliers (values >2 SD from the mean). Associations between plasma adropin concentrations and nutrient intake were further investigated by separating the tertiles ranked by plasma adropin concentrations; the outliers were not included in the tertiled data, but were treated as a separate group. Comparisons of macronutrient intake between groups were then assessed by ANCOVA with total caloric intake, sex, and glucose tolerance status used as covariates.

Between-group differences were tested using *post hoc* comparisons (Bonferroni). All the statistical tests reported were two-tailed, with significance accepted at $P \leq 0.05$.

Results

Circulating adropin concentrations before and after weight loss (CREG study)

Demographics and metrics of the 54 participants in CREG study who completed the weight loss program and for whom plasma adropin concentrations were measured are shown in Table 2; groups were matched for body weight, body composition, and weight loss (27). Weight loss and treatment method (CR, EX, CREX) had no significant effect on plasma adropin concentrations (Figure 1A). However, an analysis of baseline, postintervention, and $\Delta^{adropin}$ (change in plasma adropin concentrations after weight loss) suggested an effect of weight loss dependent on baseline values (Figure 1B). There was a strong negative correlation between $\Delta^{adropin}$ and baseline concentrations ($\rho = 0.575$, $P < 0.001$). There was also a strong linear correlation between baseline plasma adropin concentrations with values after weight loss was also observed ($\rho = 0.680$, $P < 0.001$). Further analysis using linear regression modeling with $\Delta^{adropin}$ as the dependent variable and metrics recorded during the study (baseline adropin values, body weight, fat and fat-free mass, BMI), demographics, and treatment indicated that baseline adropin was the only significant coefficient ($R = 0.722$; $B = -0.391$; standard error [SE], 0.073; $P = 0.001$).

We next separated participants into quartiles ranked by baseline plasma adropin concentrations from low (1st quartile) to high (4th quartile) (Figure 1C). While the effect of weight loss was still not significant, there was a significant interaction between quartile and weight loss ($P < 0.001$). Plasma adropin concentrations in the 4th quartile declined with weight loss, with no change in the 1st to 3rd quartile (Figure 1C). Demographics in the quartiles were similar (males/females and n for quartile 1, 9/4; 2, 11/3; 3, 11/3; 4, 11/2; mean age for quartile 1, 55.9 years; 2, 56.2 years; 3, 59.0 years; 4, 57.0 years; mean BMI adjusted for sex for quartile 1, 27.7 kg/m^2; 2, 27.8 kg/m^2; 3, 27.5 kg/m^2; 4, 27.8 kg/m^2). There was also no correlation between $\Delta^{adropin}$ and measures of glucose control or blood lipids, either at baseline or in response to weight loss (data not shown).

Normal values for fasting plasma adropin concentrations (meta-analysis)

Inspection of plasma adropin concentrations suggested that plasma adropin concentrations in most individuals are <5 ng/mL (Figure 1B). This observation was confirmed in a meta-analysis using data from 245 individuals pooled from current and previously published studies (6,16,20) (Figure 1D, Table 3). Plasma adropin concentrations exhibit a unimodal Gaussian distribution profile with positive skew and high kurtosis (concentration in ng/mL; mean, 3.30; median, 2.73; SD, 2.33; skewness, 3.262; kurtosis, 16.23, range 0.57 to 19.95 ng/mL, $n = 245$) (Figure 1D). As observed previously (6), plasma adropin concentrations are higher in males compared with females (concentrations in ng/mL adjusted for age, BMI, and glucose tolerance status for males, 3.8 ± 0.2; females, 2.9 ± 0.2; $P < 0.01$). A more pronounced positive skew and kurtosis may contribute to sex differences, as both are more pronounced in males (mean, 3.86; median, 2.93; SD, 2.93; skewness, 2.91; kurtosis, 11.18, $n = 104$) compared with females (mean, 2.88; median, 2.50; SD, 1.58; skewness, 1.97; kurtosis, 5.866; $n = 141$) (Figure 1E). While 46 of the 245 participants had been diagnosed with T2D (Table 3), there was no significant difference in plasma adropin concentration in participants with or without diabetes (concentrations in ng/mL for diabetic vs. nondiabetic, 2.9 ± 0.3 vs. 3.4 ± 0.2).

Negative association between habitual carbohydrate intake and plasma adropin levels (CREG study)

We observed a nonlinear association between plasma adropin concentrations and self-reported habitual carbohydrate intake in participants of the CREG study (correlation coefficient between plasma adropin concentrations and carbohydrate intake, 0.5155; for relative carbohydrate intake, 0.4514) (Figure 2A). In contrast, no associations were evident between plasma adropin concentrations and either fat or protein intake (Figure 2B, C). When examined as tertiles ranked by plasma adropin concentration, there were significant differences in absolute and relative carbohydrate intake between the 1st and 3rd tertiles (Figure 2D), with no difference in fat (Figure 2E) or protein intake (Figure 2F).

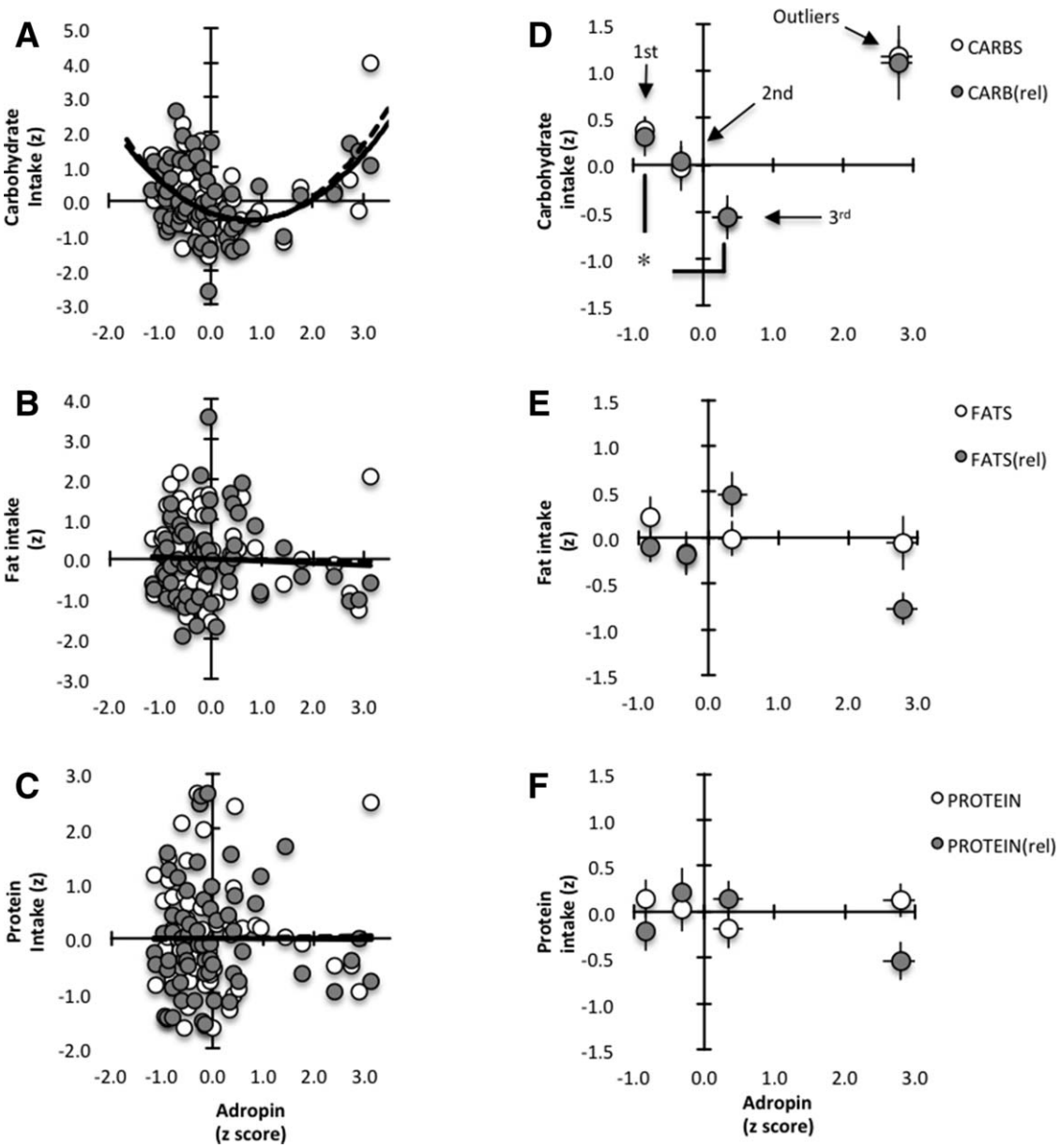

Figure 2 Association between plasma adropin concentration and carbohydrate intake. The macronutrient intake data (in kJ per person, or expressed relative to other macronutrients) and plasma adropin concentration data were converted into *Z*-scores (±SD from the mean) to allow plotting total and relative intake data on the same graph. White circles = total intake; gray circles = intake relative to other macronutrients; $n = 62$. (**A-C**) Scatterplots showing a nonlinear association between plasma adropin concentration (*x*-axis) and carbohydrate intake (*y*-axis); no associations were evident for intake of either fats or protein. (**D**) Carbohydrate intake of the 58 participants whose plasma adropin concentrations were within 2 SD of the mean separated into tertiles with low-normal (1st tertile, $n = 19$), normal (2nd tertile, $n = 20$), or high-normal (3rd tertile, $n = 19$) plasma adropin concentrations. Carbohydrate intake (in total or relative to other macronutrients) in the 1st and 3rd tertiles was significantly different (*$P < 0.01$). The means of the four participants whose plasma adropin concentrations were >2 SD from the mean ("outliers") are also shown. (**E**,**F**) Intake of fats and protein by tertile.

The difference in carbohydrate intake between the 1st and 3rd tertiles was due to simple sugars (mono- and disaccharides; 34% difference) and complex carbohydrates (oligo- and polysaccharides; 30% difference); fiber intake was similar between tertiles (Table 4). Analysis of the different fatty acid species for which reliable data were available from the study (saturated, unsaturated) also indicated no significant differences between tertiles. As diet may affect glucose control and lipid profile, we also compared blood chemistries between tertiles (Table 4). There was no evidence for differences in insulin sensitivity between tertiles; however, blood lipids (triglycerides, cholesterol, LDL) were significantly lower in the 3rd tertile compared with 1st tertile. In general, the tertiles had similar demographics; while people in the 3rd tertile weighed significantly less, their BMI and body composition were normal compared with the other groups (Table 4).

TABLE 4 Demographics, blood chemistries, and self-reported nutrient intake of CREG study participants separated into tertiles ranked by plasma adropin concentrations

Demographic; laboratory measurement	1st tertile	2nd tertile	3rd tertile	Outliers (>2 SD)	*P*
Gender (F/M, *n*)	14/5	13/7	18/1	3/1	
T2D	1	0	1	0	
Prediabetes	10	9	13	3	
Age (years)	55.9 ± 1.2	57.2 ± 1.3	57.6 ± 1.1	54.7 ± 0.8	n.s.
Weight (kg)[a]	81.3 ± 1.8	79.9 ± 1.8	73.8 ± 1.9*	87.4 ± 4.0	<0.01
BMI (kg/m^2)[b]	27.7 ± 0.4	27.8 ± 0.4	27.4 ± 0.4	28.8 ± 0.8	n.s.
Fat%	42.5 ± 1.0	42.2 ± 0.9	41.5 ± 1.0	43.6 ± 2.1	n.s.
SBP	119.2 ± 2.4	115.7 ± 2.4	114.2 ± 2.5	119.4 ± 5.3	n.s.
DBP	75.0 ± 1.9	74.9 ± 1.9	75.8 ± 2.0	75.5 ± 4.1	n.s.
Blood chemistries[b]					
Adropin (ng/mL)[c]	1.5 ± 0.1	2.4 ± 0.1	3.7 ± 0.1	7.6 ± 0.3	<0.001
Insulin (μU/mL)	9.1 ± 1.4	8.4 ± 1.4	6.5 ± 1.5	15.2 ± 2.8	(0.063)
Glucose (mg/d)	96.9 ± 1.5	95.0 ± 1.5	93.8 ± 1.6	101.6 ± 3.3	n.s.
HOMA-IR	2.25 ± 0.35	1.98 ± 0.36	1.51 ± 0.37	4.12 ± 0.7**	<0.05
HbA_{1c}	5.64 ± 0.06	5.65 ± 0.06	5.68 ± 0.06	5.63 ± 0.12	n.s.
Triglycerides (mg/dL)	135.2 ± 12.9	116.2 ± 13.1	83.3 ± 13.5***	92.5 ± 28.2	(0.063)
Total cholesterol (mg/dL)	217.3 ± 9.7	195.7 ± 9.7	174.4 ± 10.1***	205.8 ± 21.0	<0.05
LDL-C (mg/dL)	136.4 ± 6.6	118.4 ± 6.7	104.7 ± 6.9***	135.1 ± 14.4	<0.05
HDL-C (mg/dL)	53.9 ± 3.3	54.7 ± 3.3	61.1 ± 3.4	51.9 ± 7.1	n.s.
Nutrient intake[d]					
Total kcal	2,250 ± 118	1,915 ± 117	2,164 ± 128	2,298 ± 263	n.s.
Carbohydrates (g)	269 ± 10	254 ± 10	218 ± 10*	309 ± 22	<0.001
Fats (g)	80 ± 4	81 ± 4	92 ± 4	67 ± 9	=0.05
Protein (g)	81 ± 4	84 ± 4	85 ± 4	75 ± 8	n.s.
Carbohydrates by class					
Sugars (g)	96 ± 7	87 ± 7	76 ± 7	103 ± 15	n.s.
Other carbs (g)	114 ± 8	116 ± 9	98 ± 9	144 ± 18	n.s.
Fiber (g)	18 ± 1	19 ± 2	18 ± 2	31 ± 3#	<0.01
Fats by class					
Saturated (g)	26 ± 2	27 ± 2	30 ± 2	18 ± 4##	(0.057)
Unsaturated (g)	52 ± 3	52 ± 3	60 ± 3	47± 6	n.s.

[a]Sex included as a covariate in the analysis to adjust for differences in proportion of males and females in each group.
[b]Sex and glucose tolerance status included as covariates in the analysis.
[c]The differences in plasma adropin concentrations between all groups were highly significant ($P < 0.001$).
[d]For total caloric intake, body weight, sex, and glucose tolerance status were used as covariates; for macronutrients, total caloric intake, sex, and glucose tolerance status were included as covariates.
*$P < 0.05$ vs. 1st tertile and outliers; **$P < 0.05$ vs. 3rd tertile; ***$P < 0.05$ vs. 1st tertile; #$P < 0.05$ vs. all other tertiles; ##$P < 0.05$ vs. 3rd tertile.

Reduced plasma adropin concentrations during the MMTT study

Analysis using repeated measures indicated a significant effect of meal ($P < 0.01$) but not of exercise (mean ± SE plasma adropin concentrations at T0, pre-exercise, 2.73 ± 0.30 ng/mL; postexercise, 2.94 ± 0.30 ng/mL) (Figure 3A, B). Plasma adropin levels at $T = 30$ and $T = 60$ were 13% and 14% lower compared with baseline (mean ± SE of the delta in plasma adropin concentrations in ng/mL at $T = 30$, −0.37 ± 0.13 ng/mL; at $T = 60$, −0.41 ± 0.19 ng/mL), returning to normal levels at $T = 120$ (−0.01 ± 0.22 ng/mL) (Figure 3C, D). The meal effect was predominantly observed in individuals with baseline adropin values >2.5 ng/mL (Figure 3E, F).

Discussion

Our initial objective was to determine whether weight loss reduces plasma adropin concentrations in humans. A simple association between weight loss and plasma adropin concentrations was not observed. However, further analysis suggested asymmetry in effects of interventions known to alter systemic metabolism on plasma adropin concentration. In individuals with adropin values at the high end of the range, weight loss reduced values (Figure 1B, C).

A secondary objective of this study was to further investigate the association between diet and plasma adropin concentrations. In lean women (BMI 22-26 kg/m^2) aged between 30 and 45 years participating in a

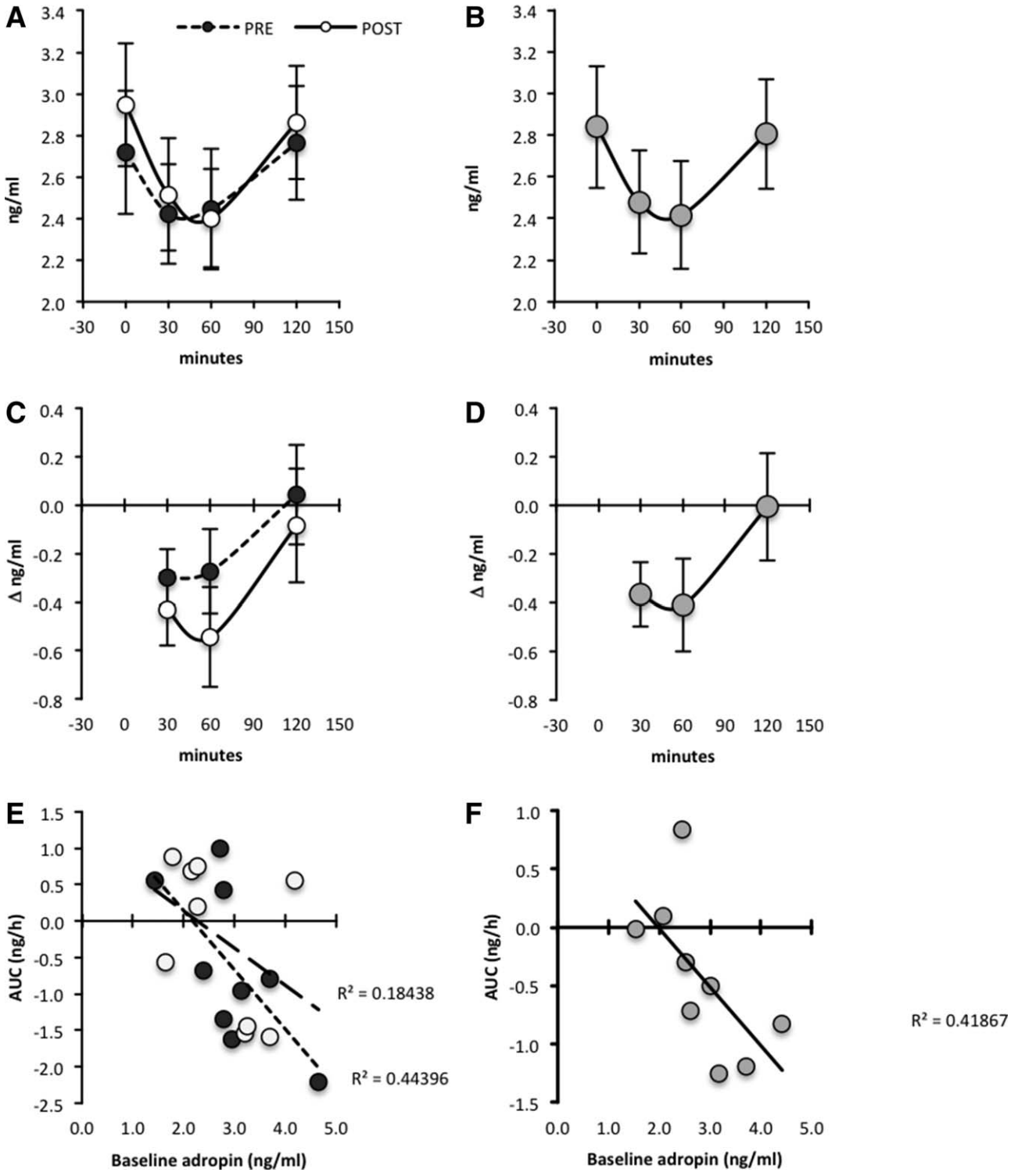

Figure 3 Reduced plasma adropin concentrations following a high-carbohydrate (60% energy), low-fat (30%), and low-protein (10%) meal. (**A, C, E**) Actual values for the two arms of the study and (**B**,**D**,**F**) least square means of the overall meal effect. Panels **A** and **B** show actual plasma adropin values, while panels C and D show the change in plasma adropin concentrations relative to baseline ($\Delta^{adropin}$). A negative association between the area under the curve (AUC) for plasma adropin concentrations following the meal and baseline values is shown in panels E and F. White circles = postexercise; black circles = pre-exercise; gray circles = averaged meal effect.

sleep restriction study, we reported strong associations between plasma adropin concentrations and macronutrient intake (16). When expressed relative to total calories, there was a positive association between plasma adropin values and fat intake and a negative association with carbohydrate intake. Here we screened for associations between diet and plasma adropin concentrations using a larger sample size ($n = 62$). Results from this study suggest habitual dietary preferences are a factor determining plasma adropin concentrations. In participants with adropin values under the main peak of the distribution, there was a negative association with carbohydrate consumption. Individuals with low plasma adropin concentrations exhibited higher intake of carbohydrates as simple sugars and complex carbohydrates, with no difference in fiber intake.

The lipid profile between tertiles was also significantly different, with the 3rd tertile having lower blood lipids compared with the 1st tertile (Table 4). An inverse association between fasting triglycerides and plasma adropin concentrations has been reported (6). Whether differences in dietary preferences explain the association between plasma adropin concentrations and lipid profiles is unclear, with further studies needed.

The impact of the high-carbohydrate breakfast on plasma adropin values was more pronounced in participants with values >2.5 ng/mL (Figure 3E, F). While speculative, if plasma adropin values are an indicator of long-term food selection preferences, then a supervised change in feeding behavior (e.g., a participant who normally consumes diets high in saturated fat consumes a high-carbohydrate breakfast) could have a proportionately greater impact on plasma adropin concentrations compared with someone who regularly consumes carbohydrate-rich diets. Thus, study participants with adropin values between 3 and 4 ng/mL and lower self-reported carbohydrate consumption would exhibit a decline in plasma adropin values following a high-carbohydrate meal. On the other hand, people with low plasma adropin concentrations who self-report habitual consumption of high-carbohydrate diets will be less responsive when consuming a supervised breakfast that matches their regular diet.

Data from studies using mice suggest inhibitory effects of simple carbohydrates on adropin expression. *In silico* analysis using the GEO database (34) suggests an 80% reduction of *Enho* expression ($P < 0.001$) in livers of C57BL/6J mice maintained on a very-low-fat (1% w/w), high-carbohydrate diet (50% sucrose) for 10 days relative to chow-fed controls (35) (GEO accession GDS1517). Dietary sugars may therefore suppress adropin synthesis in mice. However, the regulation of plasma adropin concentrations by sugars in humans is not clear. Consumption of glucose as 25% of daily energy requirements for 2 to 10 weeks reduces plasma adropin concentration, while consumption of fructose as 25% of daily energy requirements has the opposite effect (20). Plasma adropin concentrations in humans may be affected by multiple factors, including diet composition and secondary effects of diet on metabolic condition.

It is important to note some of the weaknesses of these studies. Most of the participants of the CREG study were women (48 out of 62); whether associations between carbohydrate intake and circulating adropin occur in men is not clear. Relying on self-reported feeding data is also a weakness, as underreporting of total energy intake and macronutrient intakes is common (36). Carbohydrate intake when expressed as a percentage of total energy intake does not appear to be susceptible to bias from underreporting (37). Underreporting is thus unlikely to be responsible for the association between carbohydrate intake and plasma adropin concentrations in this study. Furthermore, this weakness is offset by replication of the finding in participants recruited in different clinical settings (New York City) in which food intake was objectively measured (16). Further studies of macronutrient-specific and meal-related effects using healthy individuals who have nondiabetes are needed, as the impact of altered carbohydrate metabolism on the parameters being investigated is not clear. Finally, this study did not determine whether plasma adropin concentrations alter eating behavior. Studies using visual analog scales could be useful in determining whether plasma adropin concentrations correlate with altered appetite and/or food preferences.

The positive skew observed in the distribution of plasma adropin concentrations may suggest a "normal range" of plasma adropin concentrations. Important questions raised by this result concern the metabolic conditions of people with values in the extreme low or high ends of the distribution profile. Determining whether plasma adropin values at the low or high end ("hypo/hyperadropinemia") are associated with increased metabolic risk and clearly defined metabolic phenotypes could establish whether adropin has significant physiological roles in humans. This study suggests that low plasma adropin concentrations may indicate a situation of excess carbohydrate consumption and dyslipidemia. The inverse association between plasma adropin and serum TG was observed previously (6). The current results suggest that the association may not be causative, as carbohydrates appear to have an inhibitory on plasma adropin levels. Further studies examining whether extremely high or low plasma adropin concentrations are associated with dyslipidemias are needed.

We previously reported an asymmetric impact of sugar consumption and high-fat meals on plasma adropin concentrations (20). Combining the results of these studies leads us to propose that obesogenic or leptogenic interventions either increase or reduce the distribution in plasma adropin concentrations at the high end of the range (Figure 2A). Interventions causing weight loss appear to reverse the positive skew observed in the distribution of plasma adropin values (Figure 2B). These results are consistent with hyperadropinemia resulting from a metabolic condition that may be related to changes in systemic lipid metabolism. The exact nature of this metabolic condition requires further analysis. However, we can use published data from animal studies to suggest two hypotheses. First, if adropin regulates fuel selection in skeletal muscle in humans as observed in mice (2,3), then abnormalities in carbohydrate and/or lipid metabolism are possible in situations of hyperadropinemia. Consistent with this theory, the changes in plasma adropin concentrations in response to sugars may be linked to systemic lipid metabolism (20). A second possibility is vascular; adropin enhances vascular function in mouse models (4). Elevated plasma adropin concentrations have been observed with heart failure (5), while myocardial infarction in a rat model increases adropin expression (12).

Conclusion

These results provide further evidence supporting a link between circulating adropin concentrations, dietary macronutrient intake, and systemic lipid metabolism in humans. Further investigation will be required to determine how carbohydrate intake affects plasma adropin concentrations, as the response may be specific for different sugar species (20). Further studies focusing on individuals with

plasma adropin concentrations at either end of the spectrum may also provide important information on the role of this peptide in human physiology. O

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