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Higher habitual intake of dietary fat and carbohydrates are associated with lower leptin and higher ghrelin concentrations in overweight and obese postmenopausal women with elevated insulin levels

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Abstract

A highly regulated homeostatic system governs body weight; however, it is possible that this system might be impaired by the sustained intake of highly palatable foods. Short-term feeding studies suggest that the appetite stimulating hormone ghrelin is suppressed less effectively by dietary fat intake, and diets high in sucrose decrease levels of the adipose hormone leptin. We hypothesized that higher habitual intake of dietary fat and carbohydrate (CHO) would be associated with elevated concentrations of circulating plasma ghrelin and lower circulating leptin in humans, a hormonal profile which could promote weight gain. To test our hypothesis, we examined the cross sectional associations of ghrelin and leptin with the habitual macronutrient intake of 165 healthy overweight and obese sedentary women and tested the modifying role of insulin in these associations. We observed a significant inverse association between leptin concentrations and percent calories from CHO independent of body mass index (BMI), percent body fat, age, and intra-abdominal fat (ß= −0.11 p=0.04). No significant associations were observed between ghrelin and macronutrients or their subtypes among the total cohort. Among women with insulin concentrations at or above the median, we found a statistically significant positive association between intake of saturated fat and ghrelin concentrations, as well as additional statistically significant associations between leptin concentrations and macronutrients not observed among the total cohort. Our results provide some evidence that diets higher in fat and CHO are associated with a hormonal profile (i.e. lower leptin and higher ghrelin concentrations), which could enhance weight gain; particularly among individuals with higher circulating insulin concentrations.

Keywords

ghrelin; leptin; insulin; dietary carbohydrates; dietary fats; dietary habits; obesity; women

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1. Introduction

Energy balance is governed by a complex homeostatic system that regulates body weight over the long term by affecting energy intake and energy expenditure [1]. Two hormones that play an important role in this energy homeostatic system are leptin and ghrelin [2]. Leptin is produced in adipose tissue while ghrelin arises from the gastrointestinal tract, but both of these peptide hormones affect body weight regulation by initiating signaling cascades in the brain, particularly the hypothalamus [2]. Leptin activates catabolic pathways leading to weight loss, while ghrelin activates anabolic pathways leading to weight gain [3–7]. Leptin and ghrelin circulate in proportion to body fat stores and adaptively respond to changes in body weight [2,8,9]. Though leptin and ghrelin are affected by longer-term changes in body adiposity, these hormones also respond in the short term to other regulatory stimuli such as acute fasting and feeding [2,5].

Despite a highly regulated homeostatic system governing body weight, the prevalence of obesity has been rising rapidly for the last few decades in most parts of the world [10]. While there are many potentially contributing factors, one theory to explain this apparent contradiction is that consumption of highly palatable food containing excess fat and sugar disrupts this system [11]. Palatable foods affect reward pathways in the brain, which also play a role in addictive behaviors, and these reward pathways appear to modulate hypothalamic centers governing energy homeostasis [1]. Indeed, animal and human studies have demonstrated that ingesting highly palatable foods promotes caloric intake in excess of physiological needs [1]. Sustained excess intake of palatable food could potentially lead to weight gain if the long-term response of energy homeostatic hormones promoted an anabolic milieu. In fact, short-term feeding studies suggest that appetite-stimulating ghrelin is suppressed less effectively by dietary fat [12–14], and ghrelin administration increases perceived palatability of food among obese subjects [15]. In rats, a high fat, high sucrose diet has been shown to lower leptin levels [16]. The relationship between long-term dietary intake and ghrelin and leptin is not as clear. There are some observational studies that have reported inverse associations between carbohydrate (CHO) intake and circulating leptin concentrations among both lean and obese males [17,18], but to our knowledge, less is known about the relationship of long-term dietary intake and ghrelin.

The purpose of this study was to evaluate the relationship of plasma ghrelin and leptin with the habitual dietary intake of macronutrients and their subtypes. More specifically, we hypothesized that higher dietary intake of fat and CHO would be correlated with higher ghrelin and lower leptin concentrations based on the premise that intake of palatable foods (containing high fat and sugar content) facilitates changes in energy homeostatic pathways promoting weight gain. To test this hypothesis, we evaluated the cross-sectional associations between fasting plasma ghrelin and leptin concentrations and reported dietary intake of macronutrients and their subtypes in a group of overweight to obese, postmenopausal women. Given the potential role of insulin in the regulation of ghrelin and leptin [19–21], we further examined how insulin might modify these associations.

2. Methods and materials

2.1 Study Design

This was a cross sectional study examining the baseline data from a randomized controlled trial testing the effects of a year long exercise intervention on body weight, body fat, and circulating hormone concentrations among 173 postmenopausal women [22]. Since we were interested in assessing the relationship of habitual dietary intake on hormone levels, using baseline measures assured us that neither our dietary measures nor ghrelin or leptin

concentrations would be impacted by the exercise intervention. Full details of the specific aims, design, and protocol for this study have been described previously [22]. The Fred Hutchinson Cancer Research Center Institutional Review Board reviewed and approved all study procedures and all study participants provided written informed consent.

2.2 Participants

Participants for this study were women living in the greater Seattle area. Eligible participants for this physical activity trial were post-menopausal women between 50–75 years at entry, free from cancer or endocrine-related disease (e.g. diabetes), sedentary (less than 60 min/wk of moderate and vigorous intensity recreational activity and a maximal oxygen consumption ($V0_2$ max) <25.0 mL/kg/min), and with a body mass index (BMI) ≥ 25.0 kg/m^2 (or a BMI ≥ 24.0 and < 25.0 if percent body fat > 33.0%) [22].

2.3 Baseline Measurements

Anthropometric measurements, medical histories, food frequency questionnaires (FFQ), and blood samples were all collected prior to randomization. Trained technicians obtained height and weight using a balance beam scale and stadiometer while participants wore light clothing without shoes [22]. A fiberglass tape measure was used to measure waist and hip circumferences at the greatest circumference [22]. The measurements were completed in duplicate and averaged. Dual energy x-ray absorptiometry (DEXA) (QDR 1500, Hologic, Inc., Waltham, MA) was used to measure body fat and lean body mass. Computed tomography (CT) (General Electric model CT 9800 scanner, Waukesha, WI) measured intra-abdominal fat [23]. All baseline blood samples were obtained from participants in the morning after a 12-hour overnight fast [24]. Within 1 hour of collection, samples were processed and stored at −70°C [24]. Total plasma ghrelin concentrations were measured using a commercial radioimmunoassay (RIA) kit (Phoenix Pharmaceuticals Inc, Belmont, CA) that employs ^{125}I-labelled bioactive ghrelin as a tracer and a polyclonal antibody raised against full-length acylated human ghrelin, as previously described [24]. A single fasting measurement of plasma ghrelin has been validated against repeated samples obtained over the course of 24 hours to determine area under the curve and is well correlated [10]. The intra- and inter-assay coefficients of variation (CV) were 3.5% and 4.9%, respectively with a lower and upper limit of detection of 80 and 2500 pg/mL, respectively [24]. A commercial RIA was used to measure plasma leptin (Linco Research, Inc., St. Charles, MO). The intra- and inter-assay CVs were 8.7% and 11.2%, respectively with a lower and upper limit of detection of 0.5 and 100 ng/mL, respectively [24]. A 48-h, polyethylene glycol-accelerated, double-antibody RIA was used to obtain plasma insulin concentrations. The intra- and inter-assay CVs were 6.5% and 9.3%, respectively with a lower and upper limit of detection of 2.2 and 280 μU/mL, respectively [24].

2.4 Dietary Measurements

Usual intake of fat and other macronutrients were measured using the Women's Health Initiative (WHI) FFQ [22]. Dietary estimates from the WHI FFQ are, on average, within 10% of other dietary methods (food records and 24-hr recalls) [25]. Specifically, the correlation coefficient for percent energy from fat from the WHI FFQ compared to other dietary methods (8 days of food records and 24-hr dietary recalls combined) was 0.62 [25]. The WHI FFQ has a test-retest reliability of 0.74 for percent energy from fat [25]. Furthermore, the adjustment questions in the first section of the WHI FFQ and the summary questions on usual intake of fruits, vegetables, and fat added to foods and used in cooking further refine estimates for fat and fiber [25]. Study participants were asked to complete the FFQ using the prior three months as a reference time point. In the analysis, we excluded participants reporting outside plausible valuesof energy intake(<600 kcals and >4000 kcals) [25].

2.5 Statistical Analyses

In the descriptive analysis, means and standard deviations were calculated for continuous variables and frequencies for categorical variables. The distributions of ghrelin and leptin were normal and did not need further transformation. Univariate associations between hormonal data and demographic, anthropometric, and dietary intake were determined using Pearson correlation coefficients. There were eight participants with values outside the plausible range of energy intake (<600 kcals or >4000 kcals) and three individuals with missing data for intra-abdominal fat and thus were excluded from analyses including these variables.

In our primary analysis, associations between hormones and macronutrient variables and their subtypes were tested using multiple linear regression models. We determined that a sample size of 162 would have sufficient power ($\beta = 0.80$, $\alpha = 0.05$) to detect a partial correlation coefficient of greater or equal to 0.22, including allowing for adjustment of 4 covariates within the model. In order to assess the impact of specific macronutrients on ghrelin and leptin independent of total energy intake, we included total calories per day (kcals/d) in our models whenever appropriate [26]. Potential confounding variables included BMI, % body fat, age, $V0_2$ max, and intra-abdominal fat. To determine the final model, we retained all covariates that significantly altered the β-coefficient by more than 10%. The final model adjusted for energy intake, BMI, % body fat, age, and intra-abdominal fat. Simplified (only energy-adjusted) and full models (energy intake plus all other confounders) are presented in our results. We verified that our predictors (i.e. macronutrients and macronutrient subtypes) met the assumption for linearity by re-fitting our models using dummy variables. To determine the modifying effects of insulin, we also examined our hormone-macronutrient relationships by dividing the study sample by median insulin concentrations. All statistical tests were two-sided with an alpha of <0.05 and all analyses were performed using SAS software, version 9.1 (SAS Institute Inc., Carey, NC).

3. Results

Baseline characteristics of study participants are presented in Table 1. Study participants were on average 61 years old, 87% were Non-Hispanic White, and 47% had at least a college degree. Mean BMI was 30.5 kg/m^2, mean percent body fat was 47.4%, intra-abdominal fat was 146.7 g/cm^2 and VO_2 max was 20.3 mL/kg/min. The average total energy intake was 1663 calories per day, with 36.6% of calories coming from fat, 46.5% from CHO, and 17.1% from protein (Table 1).

Mean ghrelin concentrations were 614 pg/mL, with a median of 560 pg/mL and a 25^{th} to 75^{th} percentile range of 333 – 806 pg/mL. Only one participant had values outside the plausible range for ghrelin and no difference was observed when this participant was excluded from the analysis; therefore, we did not exclude her data. The mean leptin concentrations were 28.0 ng/mL with a median of 28.0 ng/mL and a 25^{th} to 75^{th} percentile range of 22.1 – 33.8 ng/mL and mean insulin concentrations were 20.1μU/mL with a median of 17.5 μU/mL and a 25^{th} to 75^{th} percentile range of 13.3 – 25.3 μU/mL.

Ghrelin was statistically significantly negatively correlated with BMI, % body fat, body weight, insulin, intra-abdominal fat and was positively correlated with $V0_2$ max. Leptin was statistically significantly positively associated with BMI, % body fat, intra-abdominal fat, weight, insulin, and negatively associated with $V0_2$ max. Both hormones were negatively associated with age (Table 2).

Ghrelin concentrations were not associated with reported intake of any macronutrient types in univariate analyses. Leptin concentrations were statistically significantly positively correlated

with % calories from fat, total saturated fat (SFA) (g/d) (r=0.19 p=0.01; r=0.16 p=0.04, respectively) and negatively correlated with % calories from CHO (r=−0.19, p=0.02; Table 2).

We next created regression models to further examine the relationship between ghrelin and macronutrients and their subtypes (Table 3). Models 1 and 2 present the unadjusted and adjusted models (i.e. controlling for BMI, % body fat, age, and intra-abdominal fat) for main macronutrients (expressed as % calories from fat, CHO, or protein) and models 3 and 4 present the energy-adjusted and fully-adjusted (i.e. controlling for energy intake, BMI, % body fat, age, and intra-abdominal fat) models for macronutrient subtypes and ghrelin. We did add total kcals/d to models 1 and 2, but this did not significantly impact estimates (data not shown). Overall, there were no statistically significant associations between ghrelin and macronutrients among the total cohort. Statistically significant positive associations were observed for reported total fiber, water-soluble fiber, and insoluble fiber intake among women with insulin concentrations below the median value (17.5 μU/mL), but none of the associations remained significant after adjusting for energy intake, BMI, % body fat, age, and intra-abdominal fat **(**Table 3**)**. Among women with insulin concentrations at or above the median, we found a statistically positive association between saturated fat and ghrelin in the energy-adjusted model and a positive association nearing significance in the model adjusting for energy intake, BMI, intra-abdominal fat, % body fat, and age (ß= 7.32, p=0.048 and ß=7.36, p=0.051, respectively) **(**Table 3**)** . This indicates that with every one-gram increase per day in saturated fat, there was a 7-pg/mL increase in ghrelin among those in the high insulin group after adjustment for energy intake, BMI, body fat, age, and intra-abdominal fat **(**Table 3**)**.

The unadjusted and adjusted models for the associations between main macronutrients (expressed as % calories from fat, CHO, or protein) and leptin are presented in models 1 and 2, and the energy-adjusted and fully-adjusted models (i.e. energy intake, BMI, % body fat, age, and intra-abdominal fat,) for macronutrient subtypes and leptin are presented in models 3 and 4, respectively (Table 4). A statistically significant inverse association between leptin and % calories from CHO was observed after adjustment for BMI, % body fat, age, and intra-abdominal fat (ß=−0.11 p=0.04) **(**Table 4**)**. This suggests that for every one percent decrease in CHO, there was a 0.1 ng/mL increase per day in leptin among women who were similar in energy intake, BMI, % body fat, age, and intra-abdominal fat. Leptin was significantly positively associated with % calories from fat (ß=−0.19 p=0.01), but the association did not remain statistically significant in our final model (ß=−0.09 p=0.14) adjusted for energy intake, BMI, intra-abdominal fat, % body fat, and age **(**Table 4**)**.

The energy-adjusted models of leptin and macronutrient subtypes showed statistically significant negative associations for all types of fiber, sucrose, and fructose (Table 4). Statistically significant positive associations were observed with monounsaturated fats (MUFA), saturated fat (SFA), and nearly significant associations with alpha-linolenic acid (ALA) and trans fatty acids **(**Table 4**)**. These associations were no longer significant after controlling for BMI, % body fat, age, and intra-abdominal fat in the cohort as a whole; however, significant associations remained among women with insulin concentrations at or above the median value. In the model adjusting for energy intake, BMI, body fat, age, and intra-abdominal fat, leptin was inversely associated with all fiber types (total fiber: ß =−0.34 p=0.02, water soluble: ß =−0.96 p=0.03, insoluble: ß =−0.48 p=0.02) and fructose (ß =−0.13 p=0.048). These results indicate that for every one-gram decrease in total fiber, there was a 0.3 ng/mL per day decrease in leptin among women with insulin concentrations at or above the median value after adjustment for potential confounders. Similar associations were found with the other CHO subtypes as well **(**Table 4**).** Leptin was also positively associated with SFA (ß =0.24 p=0.02) and ALA (ß =4.39 p=0.02). Therefore, for every one-gram increase in SFA and one-gram increase in ALA acid, there was a 0.2 ng/mL and 4.39 ng/mL increase per day in leptin,

respectively, among women with insulin concentrations at or above the median after adjustment for energy intake, BMI, % body fat, age, and intra-abdominal fat **(**Table 4**)**.

4. Discussion

We hypothesized that reported habitual intake of high fat and high sugar foods, suggestive of consuming a highly palatable diet, would be associated with a metabolic profile that could promote weight gain. Such a metabolic profile would consist of relatively higher concentrations of fasting ghrelin and lower concentrations of fasting leptin. We found that habitual CHO intake was inversely associated with fasting leptin concentrations among postmenopausal overweight and obese women even after adjusting for energy intake, % body fat, BMI, age, and intra-abdominal fat, which is consistent with our hypothesis. Findings from other studies assessing the associations between habitual dietary intake and leptin concentrations have generally not been significant after adjustment for confounding [17,18]. However, these studies were conducted in men and might not be generalizable to postmenopausal women since there are sex-specific differences in leptin [27,28]. Further studies among both men and women would help to elucidate this relationship. We did not observe any associations with ghrelin concentrations and macronutrient intake in the cohort as a whole.

We also hypothesized that insulin might modify the hormone-nutrient associations given the potential role of insulin in the regulation of both leptin and ghrelin [19–21]. Insulin has been shown to influence circulating leptin concentrations. Specifically, insulin increases leptin concentrations in rodents, both in cultured adipocytes as well as *in vivo* [29,30]. These associations have also been found in humans, at the level of the adipocyte and in the peripheral circulation wherein experimental hyperinsulinemia using clamp techniques results in increased leptin concentrations [31–33]. Critically, these two hormones act together within the brain to impact homeostatic systems [34]. Evidence also points to a role for insulin in the regulation of circulating ghrelin. The pre and postprandial ghrelin fluctuations inversely correlate with those of insulin [7,35,36], i.e. when insulin concentrations rise after meal intake; ghrelin concentrations fall [37–39]. In rodents, high insulin concentrations decrease ghrelin production by the stomach *in vitro*, and *in vivo* high insulin induced via clamp decreases ghrelin [40,41]. In humans, hyperinsulinemia is inversely correlated with plasma ghrelin [42,43]. The relationship between leptin or ghrelin and insulin is potentially more complex because of the possibility of insulin resistance, wherein comparable concentrations in obese individuals would not have the same effect as in leaner persons [27,44].

In this study, fasting insulin served as a surrogate estimate for insulin sensitivity, a marker that is reasonably correlated with the euglycemic-hyperinsulinemic clamp technique when applied among postmenopausal women [45]. To test the hypothesis that our results might differ by insulin status, we performed analyses based on a median split of insulin concentrations among our study population. While we did not observe statistically significant results with ghrelin and macronutrient intake in the total cohort, we did detect a positive significant association between ghrelin and SFA intake among those with higher insulin concentrations even after adjusting for body fat, BMI, age, and intra-abdominal fat. One reason for this finding may be that SFA intake has a negligible impact on insulin secretion, and therefore, less inhibition of ghrelin. While mechanisms are not fully understood, cross-over design studies have shown diets higher in SFA reduce insulin sensitivity more so than do diets higher in MUFA [46,47]. Insulin resistant individuals consuming higher SFA diets may have higher circulating plasma ghrelin concentrations because their hyperinsulinemia is less effective at suppressing ghrelin.

We also found that associations between leptin and certain macronutrients were strongest among individuals with higher insulin concentrations. We observed significant inverse associations between leptin and CHO, particularly fructose, as well as inverse associations with

all types of fiber. Two observational studies have shown similar associations between fiber intake and leptin. First, in a study of young Japanese women (n=424), higher intake of dietary fiber from vegetables and legumes was associated with lower serum leptin concentration independent of BMI [48]. The second study examined whole grain intake in healthy US adults (n=938) from the Health Professionals study and Nurses Health study and also found lower leptin concentrations among those in the highest quintile of whole grain intake [49]. Conclusions from these studies discuss the potential benefits of fiber and lower leptin concentrations. These studies suggest that higher fiber intake might lead to increased leptin sensitivity resulting in a subsequent decline in leptin production [48,49]. Our hypothesis suggests that higher habitual intake of CHO from “sugars” are likely to be associated with lower leptin concentrations, but perhaps leptin's response to CHO does not differ by type of CHO. Alternatively, lower leptin concentrations may be beneficial in normal weight individuals [48], but the same might not be true in overweight and obese individuals. Future prospective studies where relative changes in these diets can be assessed are needed to better understand this relationship.

When we examined leptin and SFA or ALA intake by median split for insulin concentrations, we found a positive association only among individuals at or above the median insulin concentration. Unlike our study, inverse associations of omega 3 fatty acids (e.g. fish intake) with leptin have been reported [50,51]. However, one of these studies was conducted among indigenous Africans, a very different population than ours, while the other took place within the context of weight loss and hence interpretation of the results is complicated by the effect of weight loss on leptin. In general, no studies that we know of have examined the relationship between dietary fat intake and leptin concentrations accounting for insulin. Based on our findings, the relationship between habitual dietary fat intake and leptin could feasibly depend on the degree of endogenous insulin and leptin resistance.

There are several limitations to this study. The main limitation from using a cross-sectional study design is our inability to draw causal conclusions about habitual dietary intake and hormone concentrations. Another limitation is that the WHI FFQ was not specifically designed for a hypothesis to study some of the macronutrient subtypes we investigated in this study such as ALA. While we did detect an inverse association between ALA and leptin, our estimates might have been slightly attenuated since all of the foods containing this nutrient consumed by our study population might not have been fully captured by the FFQ. Further, while we adjusted for potential confounders, the existence of residual confounding is always a limitation in observational studies. Finally, the present findings may only be generalizable to non-Hispanic white postmenopausal overweight/obese women.

In conclusion, the present study found that habitual carbohydrate intake was negatively associated with leptin among postmenopausal overweight and obese women, independent of body fat, BMI, intra-abdominal fat, and age; a finding consistent with our hypothesis. While no associations were observed with ghrelin and macronutrient intake in the cohort as a whole, we did detect a positive significant association between ghrelin and SFA intake among those with higher insulin concentrations, which also supports our hypothesis. The relationship between habitual macronutrient intake in both leptin and ghrelin concentrations appeared to be more pronounced among women with higher insulin levels. Future studies examining associations of dietary composition with plasma ghrelin and leptin should take into account the potential role of insulin. Our findings provide an initial step towards determining if there is a relationship between sustained palatable food intake among humans and a hormonal profile that could foster weight gain. Such a relationship may be a barrier to long-term weight loss success, and understanding nutrient effects on energy homeostatic pathways should facilitate improving future clinical approaches to dietary weight loss interventions.

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Abbreviations

CHO, carbohydrate
FFQ, Food Frequency Questionnaire
$V0_2$ max, Maximal Oxygen Consumption
BMI, Body Mass Index
DEXA, Dual Energy X-ray Absorptiometry
CT, Computed Tomography
RIA, Radioimmunoassay
CV, Coefficients of Variation
WHI, Women's Health Initiative
SFA, Saturated Fat
MUFA, Monounsaturated Fat
ALA, Alpha-Linolenic Acid

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Table 1

Baseline Demographic, Anthropometric, and Hormonal Characteristics of Women Enrolled in an Exercise Trial (n=165)

Variable	%
Ethnicity (% Non Hispanic White)	87.0%
Education	
High School/ Vocational School	17.5%
Some college/College Graduate	41.0%
Some post college education/Master's or Doctoral Degree	39.3%

	Mean	SD	Minimum	Maximum
Age (y)	60.7	6.7	50.0	75.0
Weight (kg)	81.6	13.0	59.1	132.2
BMI (kg/m2)	30.5	3.9	24.1	42.0
Total Percent Body Fat (kg)	47.4	4.7	34.1	60.1
Intra-abdominal fat, g/cm^2 (CT)	147	57.5	23	341
Maximum VO_2 (mL/kg/min)	20.3	3.2	11.2	32.5
Ghrelin (pg/mL)	613	341	53	1632
Leptin (ng/mL)	28.0	9.8	0.44	50.52
Insulin (μU/mL)	20.1	9.8	3.70	55.20
Total energy intake (kcal/d)	1663	613	674	3830
% calories from fat	36.6	8.4	16.1	68.8
% calories from carbohydrates	46.4	9.4	12.6	72.3
% calories from protein	17.1	3.2	10.2	27.2

Mean ± standard deviation and maximum and minimum range reported for continuous variables

Frequency (expressed as a proportion) reported for categorical variables

Percent energy (% calories) from fat, carbohydrates, and protein are based on self reported dietary data from food frequency questionnaire

Table 2

Correlations between fasting plasma ghrelin and leptin concentrations with dietary intake of macronutrient and macronutrient sub-types

Variables (N=165)	Ghrelin ρ	Leptin ρ
Total caloric intake (kcal/d)	0.04	0.05
% calories from fat	0.01	**0.19***
% calories from CHO	−0.02	**−0.19***
% calories from protein	0.06	0.01
Total fiber (g)	0.11	−0.10
Water soluble fiber (g)	0.09	−0.10
Insoluble fiber (g)	0.12	−0.09
Total PUFA (g)	0.06	0.08
Total MUFA (g)	0.01	0.12
Total SFA (g/d)	0.02	**0.16***
Total ALA (g)	0.07	0.12
Total trans fats (g)	−0.05	0.13
Sucrose (g)	−0.02	−0.09
Fructose (g)	−0.02	−0.13
Alcohol (g)	0.03	0.04
BMI (kg/m2)	**−0.29****	**0.50****
Total Percent Body Fat	**−0.16***	**0.60****
Intra-abdominal fat, g/cm^2 (CT)	**−0.32****	**0.33****
VO2 Max (mL/kg/min)	**0.18***	**−0.29***
Age (y)	−0.10	**−0.24***
Weight (kg)	**−0.30****	**0.53****
Insulin (μU/mL)	**−0.40****	**0.47***

Correlations reported are based on Pearson Correlation Coefficient (ρ).

Percent energy (% calories) from fat, carbohydrates, and protein are based on self reported dietary data from food frequency questionnaire

CHO: carbohydrates

PUFA: polyunsaturated fatty acids

MUFA: monounsaturated fatty acids

SFA: saturated fatty acids

ALA: Alpha-linolenic acids

* indicates p value <.05

** indicates p value ≤.0001

Table 3

Relationship between fasting plasma ghrelin concentrations and macronutrients and macronutrient subtypes by subgroups of insulin

	GHRELIN											
	Insulin*						Insulin*					
	(< 17.5 μU/mL)* n=83		(≥ 17.5 μU/mL)* n=82		total n=165		(< 17.5 μU/mL)* n=81		(≥ 17.5 μU/mL)* n=81		total n=162	
Macronutrients	**Model1[1]**	**P value**	**Model 1[1]**	**P value**	**Model 1[1]**	**P value**	**Model 2[2]**	**P value**	**Model 2[2]**	**P value**	**Model 2[2]**	**P value**
% calories from fat	−1.80	.70	1.33	.69	−.52	.87	2.09	.67	.96	.79	.73	.81
% calories from CHO	3.88	.34	−2.04	.50	.61	.83	.98	.82	−2.55	.41	−.19	.95
% calories from protein	−.34	.98	12.84	.14	6.69	.43	−.64	.96	14.66	.10	5.45	.50
Macronutrient Subtypes	**Model 3[3]**		**Model 3[3]**		**Model 3[3]**		**Model 4[4]**		**Model 4[4]**		**Model 4[4]**	
Total fiber (g)	13.42	.05	−3.03	.56	7.30	.13	11.81	.11	−5.78	.29	2.87	.54
Water Soluble fiber (g)	37.80	.07	−12.14	.44	16.81	.25	33.60	.13	−18.00	.27	7.25	.61
Insoluble fiber (g)	19.90	.05	−3.29	.67	11.63	.10	17.36	.10	−7.64	.33	4.50	.51
Total PUFA (g)	6.21	.48	−1.73	.83	4.42	.49	6.99	.43	−2.55	.75	3.10	.61
Total MUFA (g)	−.83	.90	1.16	.81	−2.00	.65	1.13	.86	−.04	.99	−.87	.83
Total SFA (g)	−5.74	.23	**7.32**	**.049**	−.73	.83	−2.84	.59	7.36	.051	1.71	.60
Total ALA (g)	18.32	.84	42.55	.55	47.92	.44	30.61	.73	42.87	.55	49.35	.40
Trans fats (g)	4.18	.90	−20.38	.22	−25.41	.16	17.10	.64	−18.89	.26	15.88	.36
Sucrose (g)	−2.97	.24	.86	.73	−1.37	.47	−2.90	.25	.20	.94	−1.61	.37
Fructose (g)	3.96	.32	−3.82	.12	−1.41	.57	1.41	.75	−2.56	.11	−2.42	.32

ß coefficients reported for all models

CHO: carbohydrates

PUFA: polyunsaturated fatty acids

MUFA: monounsaturated fatty acids

SFA: saturated fatty acids

ALA: alpha-linolenic acids

[1] Model 1: unadjusted

[2] Model 2: adjusted for BMI, % body fat, age, and intra-abdominal fat

[3] Model 3: adjusted for energy intake

[4] Model 4: adjusted for energy intake, BMI, % body fat, age, and intra-abdominal fat (missing values n=3, excluded from data analysis)

* Insulin subgroups are based on a median split of insulin. The median value of insulin (17.5 μU/mL) was the cut point used to establish the two insulin groups represented in the table

Table 4

Relationship between fasting plasma leptin concentrations and macronutrients and macronutrient subtypes by subgroups of insulin

	LEPTIN											
	Insulin*						Insulin*					
	(< 17.5 μU/mL) n=83		(≥ 17.5 μU/mL) n=82		total n=165		(< 17.5 μU/mL) n=81		(≥ 17.5 μU/mL) n=81		total n=162	
Macronutrients	**Model1[1]**	**p value**	**Model 1[1]**	**p value**	**Model 1[1]**	**p value**	**Model 2[2]**	**p value**	**Model 2[2]**	**p value**	**Model 2[2]**	**p value**
% calories from fat	.06	.55	**.31**	**.002**	**.19**	**.01**	.001	.99	**.22**	**.02**	.09	.14
% calories from CHO	−.06	.48	−.32	**.0003**	**−.17**	**.02**	−.04	.51	−.21	.01	**−.11**	**.04**
% calories from protein	.12	.64	.04	.87	.03	.88	.20	.31	−.06	.80	.06	.49
Macronutrient Subtypes	**Model 3[3]**	**p value**	**Model 3[3]**	**p value**	**Model 3[3]**	**p value**	**Model 4[4]**	**P value**	**Model 4[4]**	**p value**	**Model 4[4]**	**p value**
Total fiber (g/d)	.02	.77	**−.39**	**.01**	−.28	.02	.08	.50	**−.34**	**.02**	−.11	.25
Water Soluble fiber (g)	−.41	.35	**−1.21**	**.01**	−.28	.02	.15	.67	**−.96**	**.03**	−.37	.20
Insoluble fiber (g)	−.14	.50	**−.54**	**.02**	−.38	.03	.13	.44	**−.48**	**.02**	−.14	.31
Total PUFA (g)	.02	.93	.47	.052	.14	.38	.05	.77	.35	.10	.15	.23
Total MUFA (g)	.01	.93	**.36**	**.02**	.22	.04	−.05	.61	.22	.11	.09	.31
Total SFA (g)	.10	.34	**.32**	**.005**	.22	.001	−.006	.94	**.24**	**.02**	.10	.12
Total ALA (g)	1.51	.41	**5.71**	**.008**	.82	.07	.63	.66	**4.39**	**.02**	1.87	.12
Trans fats (g)	−.20	.78	**.97**	**.06**	2.79	.07	−.38	.51	.45	.32	.30	.39
Sucrose (g)	−.06	.23	**−.20**	**.01**	−.10	.03	−.06	.12	−.11	.10	−.07	.06
Fructose (g)	−.09	.23	**−.21**	**.004**	−.14	.02	−.002	.95	**−.13**	**.048**	−.06	.21

ß coefficients reported for all models

CHO: carbohydrates

PUFA: polyunsaturated fatty acids

MUFA: monounsaturated fatty acids

SFA: saturated fatty acids

ALA: alpha-linolenic acids

[1]Model 1: unadjusted

[2]Model 2: adjusted for BMI, % body fat, age, and intra-abdominal fat

[3]Model 3: adjusted for energy intake

[4]Model 4: adjusted for energy intake, BMI, % body fat, age, and intra-abdominal fat (missing values n=3, excluded from data analysis)

*Insulin subgroups are based on a median split of insulin. The median value of insulin (17.5 μU/mL) was the cut point used to establish the two insulin groups represented in the table