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Fasting glucagon-like peptide 1 concentration is associated with lower carbohydrate intake and increases with overeating

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Abstract

Purpose—Glucagon-like peptide 1 (GLP-1) is an incretin hormone that appears to play a major role in the control of food intake. The aim of this investigation was to evaluate and quantify the association of circulating GLP-1 concentration with ad libitum total calorie and macronutrient intake.

Methods—One-hundred fifteen individuals (72 men) aged 35 ± 10 years were admitted for an inpatient study investigating the determinants of energy intake. *Ad libitum* food intake was assessed during 3 days using a reproducible vending machine paradigm. Fasting plasma GLP-1 concentrations were measured on the morning of the first day and on the morning of the fourth day after *ad libitum* feeding.

Results—Plasma GLP-1 concentrations increased by 14% after 3 days of *ad libitum* food intake. Individuals overate on average 139 ± 45% of weight-maintaining energy needs. Fasting plasma GLP-1 on day 1 was negatively associated with carbohydrate intake ($r=-0.2$, $p=0.03$) and with daily energy intake from low fat-high simple sugar ($r=-0.22$, $p=0.016$).

Conclusion—Higher plasma GLP-1 concentrations prior to *ad libitum* food intake were associated with lower carbohydrate intake and lower simple sugar ingestion, indicating a possible role of the GLP-1 in the reward pathway regulating simple sugar intake.

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Author contributions

A.B. analyzed and interpreted data and wrote the manuscript. P.P. and E.S. assisted with the interpretation of the data and revised the manuscript. M.H. and S.H. supported with the interpretation of the data and reviewed the manuscript. S.V. and J.K. designed, implemented and conducted the study. All authors read and approved the final manuscript. All authors critically revised the draft and approved the final manuscript. A.B. had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis

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Keywords

Glucagon-like peptide 1; ad libitum food intake; carbohydrates intake

Introduction

A chronic positive energy balance is responsible for weight gain leading to obesity and its associated co-morbidities [1]. Physiological, environmental and genetic factors play an important role as determinants of weight gain [2]. Physiological effectors of weight gain include neuronal and humoral signals from peripheral organs, such as the pancreas, gut, and adipose tissue which carry information about satiety and hunger to the CNS and are involved in the regulation of food intake. Glucagon like peptide 1 (GLP-1), an incretin hormone released from the L cells of the intestinal mucosa after meal ingestion, may modulate food intake by conveying meal-related information to the brain, with no substantial effect on energy metabolism [3]. Pharmacologic doses of GLP-1 decreased food intake in rodents [4] and in humans [5] and GLP-1 increases satiety and reduces gastric emptying [6]. Additionally, GLP-1 contributes to postprandial glucose regulation by improving meal related insulin production and secretion from pancreas [7] suppressing glucagon secretion in a glucose dependent manner [8]. Subjects with type 2 diabetes and obesity have been reported to have a lower fasting GLP-1 concentration compared to healthy volunteers [9]. Regardless of diabetes status, infusion of GLP-1 decreased food intake in subjects with and without obesity [10, 11].

In humans, GLP-1 secretion responds to positive energy balance acting as a compensatory or possibly a protective mechanism to reduce appetite and increase satiety. To date, only few studies have investigated the link between circulating GLP-1 concentrations and overfeeding in humans and the results have been conflicting. For instance, previous studies have shown no change in GLP-1 concentration in response to short term overfeeding [12, 13], whereas another study found a significant increase in circulating GLP-1 after 7 days of overfeeding diet with 70% above weight maintaining calories [14].

We sought to investigate the relationship between objectively measured *ad libitum* food intake and circulating plasma GLP-1 concentrations. The aims of the present study were to evaluate whether fasting GLP-1 concentrations predict subsequent food or macronutrient intake over a 3-day period of *ad libitum* food intake and whether the degree of overconsumption is related to changes in fasting GLP-1 concentrations.

Methods

Participants and study design

The volunteers in this analysis were part of a larger inpatient study investigating determinants of food intake (Clinical Trials # NCT00342732) [15] conducted between 2003 and 2015 at the Obesity and Diabetes Clinical Research Center of the NIDDK/NIH in Phoenix, AZ. The protocol was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Out of 250 subjects who

completed the study, 115 (age < 55 years with BMI 30.3 ± 9.5) had available blood samples as well as measurements of *ad libitum* food intake. Due to interruption of the study between 2005 and 2009, we have two subsets of blood samples from individuals (before 2005 and after 2009).

Prior to admission all subjects signed written and informed consent. Based on medical history, physical examination and laboratory testing, all subjects had no evidence of medical diagnoses other than obesity and/or impaired glucose tolerance, were non-smokers, and were not currently taking medications.

On the day of admission to our metabolic unit, subjects were fed a standard weight maintaining diet (50% carbohydrates, 30% fat and 20% protein) for 3 days prior to any metabolic testing. For each individual, weight maintaining energy needs (WMEN) were calculated based on body weight and gender as previously demonstrated [16]. Glucose tolerance was assessed using a 75-g oral glucose tolerance test according to the American Diabetes Association criteria [17] and only data from individuals without diabetes were analyzed. Body composition (percentage of body fat, PFAT) was assessed using dual energy x-ray absorptiometry (DPX-1; Lunar Radiation Corp, Madison, Wisconsin) to calculate body fat mass (FM) and fat free mass (FFM).

Following four days of initial testing, *ad libitum* food intake was assessed for three days (described in detail below) (Supplemental, Figure S1). Blood samples were drawn at 530AM on day 7 of the study before starting the 3-day ad libitum vending machine paradigm (day 1 of *ad libitum* period) as well as on the discharge day (named day 4, after completion of 3-day ad libitum period). True overnight fasting measures can only be considered in the first blood draw due to the unlimited free access to food during the vending machine paradigm for the 3 consecutive days. Total GLP-1 was measured by immunoassay kit (version 2) from MSD (Rockville MD). The intra-assay CV was 3.32% and the inter-assay CV was 5.01%. Fasting insulin concentrations were assessed by using an automated immunoenzymometric assay (Tosoh Bioscience Inc., Tessenderlo, Belgium).

Ad libitum food intake

Measurement of *ad libitum* food intake was assessed over 3 days using a computerized vending machine paradigm which has been shown to be highly reproducible and valid as previously described [18]. On the day of the admission, a food selection questionnaire was provided to each volunteer to assess food preferences. Subjects were asked to rate each item using a 9-point Likert scale (1=dislike extremely, 5=neutral, 9=like extremely). 40 different foods given an intermediate rating were used to stock the vending machines for *ad libitum* food intake. Subjects were given free access to the computer-operated vending machine system for 3 days. Volunteers had 23.5h *ad libitum* access to select food items. All food was weighed prior to placement in the vending machines and returned food leftovers were also weighed to determine actual intake. The CBORD Professional Diet Analyzer Program (CBORD, Inc., Ithaca, NY, USA) and the Food Processor database (ESHA version 10.0.0, ESHA Research, Salem, OR, USA) were used to calculate the daily total and individual macronutrient kilocalories consumed. The recorded measurement of eating time during the 3 days of vending machine paradigm was able to identify night eaters, defined as individuals

who ate after 1100 PM during the day 3 of *ad libitum* food intake. In these volunteers with nighttime eating episodes, GLP-1 measurements collected the morning (530 AM) on day 4 may be altered since they were not in a true fasting state.

The average of total *ad libitum* food intake over 3 days vending period was taken and expressed as total kcals eaten daily. Similar calculations were performed for the ratio to the WMEN prior to the 3-day vending period and for each macronutrient intake.

Macronutrient Categories

Six groups of food were categorized from each food on the vending machine questionnaire, based on the content of the macronutrient as a percentage of the total energy intake. Food categories were identified as low in fat (<20% kcal) or high in fat (≥45% kcal). Furthermore, food groups were categorized as high in protein (≥13% kcal), high in complex carbohydrates (≥30% kcal), high in simple sugars (≥30% kcal). From these classifications, six different groups were formed: low-fat/high-simple sugar (LF/HSS), low-fat/high-complex carbohydrate (LF/HCC), low-fat/high-protein (LF/HP), high-fat/high-complex carbohydrate (HF/HCC), high-fat/high-protein (HF/HP) and high-fat/high-simple sugar (HF/HSS) [19].

Statistical analysis

Analyses were performed using SAS software (SAS 9.3, Enterprise guide version 5.1; SAS Institute, Cary, NC). Data are expressed as means ± standard deviations. Normally distributed data are expressed as mean ± SD, and skewed data are presented as medians with 95% CI. Correlations between normally distributed and skewed variables were assessed with Pearson and Spearman correlation coefficients, respectively.

Fasting plasma GLP-1 and insulin data were log10 transformed to meet the assumptions of linear regression (i.e., homoscedasticity and normal distribution of residuals). Student t test or 1-way analysis of variance were used to assess the differences between groups. Due to the differences in time and conditions of sample storage, fasting GLP-1 at baseline (pre-*ad libitum* food intake period) was adjusted for cohort (before 2005 and after 2009).

To assess determinants of fasting plasma GLP-1 prior to *ad libitum* food intake, we used a multivariate linear regression model including age, sex, FM, FFM, and race.

Glucose area under the curve (AUC) and insulin AUC were calculated using the trapezoidal method. A linear model was used to correlate the log fasting GLP-1 prior to 3-day *ad libitum* energy intake with glucose AUC and insulin AUC (adjusting for fasting glucose during oral glucose tolerance test). Similar analyses were performed to evaluate whether the fasting GLP-1 response on day 1 to glucose and insulin was different between ethnicities.

Change in GLP-1 concentrations was defined as the difference between morning GLP-1 measured on day 4 (after 3-day *ad libitum* food intake period) and GLP-1 measured on day 1 (prior to *ad libitum* food intake period).

Linear regression analysis was used to calculate residuals of total *ad libitum* food intake after adjustments for age, sex, FFM, and FM. Similar analyses were performed to calculate

residuals of individual macronutrient intakes and for the different food groups. Linear regression models were used to evaluate the relationships between fasting GLP-1 prior to *ad libitum* intake and residuals of total *ad libitum* food intake, macronutrients (fat, carbohydrate, or protein) intake and different food groups after adjustment for age, gender, FM, FFM. Similar linear regression models were used to correlate change in plasma GLP-1 concentrations (before and after *ad libitum* period) and residuals of ad libitum food intake and macronutrients.

Results

Baseline characteristic

General and anthropometric characteristics for all subjects are reported in Table 1. On average, volunteers were young (34.9 ± 10.4 yrs), were overweight (30.1±6.9 kg/m^2), and the expected differences in body composition between males and females were observed. Twenty-seven out of 115 individuals had at least one episode of nighttime eating during the last day of the *ad libitum* period, characterized as food intake between 2300 at 0500. There was no significance difference between nighttime eaters and non-nighttime eaters in age, weight or body composition (FM, FFM and percent of body fat). In the analysis of change in GLP-1, there was no difference in the results if nighttime eaters were excluded from the analysis. Night eaters were excluded from the analysis when examining the relationship between the last day (day 3) of the *ad libitum* period and plasma GLP-1 on day 4.

Fasting GLP-1 concentrations and body composition

Mean plasma GLP-1 concentrations on day 1 and day 4 were 12.5 pg/ml (CI 95%: 11.4 to 13.8) and 14.7 pg/ml (CI 95%: 13.3 to 16.2), respectively. The fasting GLP-1 concentration on day 1 was not correlated with body weight ($p = 0.13$), FFM ($p = 0.16$), FM ($p = 0.29$), PFAT ($p = 0.5$) or BMI ($p = 0.23$) after adjustment for differences in individual storage time. Additionally, the pre-*ad libitum* feeding GLP-1 concentrations did not differ significantly by race and sex.

Glucose regulation and association with GLP-1 concentrations

After adjustment for all covariates (age, sex, percentage of body fat and cohorts), fasting GLP-1 concentration before the *ad libitum* period was associated with glucose AUC ($r = 0.2$, $p = 0.01$) and with fasting insulin concentrations ($r = 0.5$ $p = 0.03$). Fasting GLP-1 concentration was also associated with insulin AUC after further adjustment for glucose AUC ($r = 0.54$ $p = 0.002$) during OGTT and with fasting insulin measured on day 1 *ad libitum* period ($r = 0.33$, $p = 0.009$). In those with normal glucose regulation ($n = 91$), we did not find any correlation between fasting insulin and 2 h insulin and morning GLP-1 when we stratified by race (Native Americans, Caucasian and other races).

Association of *ad libitum* food and macronutrient intake with GLP-1 concentrations

Energy intake data are described in Table 2. Average 3-day *ad libitum* energy intake was 3849±1431 Kcal/d (range: 1273 to 9544 Kcal/d) and 138.7 ± 45.4 % expressed as %WMEN. Fasting day 1 GLP-1 concentrations (prior to *ad libitum* period and adjusted for cohort) were not associated with residuals of total food intake ($r = -0.15$, $p = 0.12$, Figure 1A), residuals

of fat intake (r = −0.06, p = 0.5, Figure 1C) or residuals of protein intake (r = −0.05, p = 0.5, Figure 1D), but was negatively associated with residuals of carbohydrate intake (r = −0.2, p = 0.03, Figure 1B). Consistent with this finding, day 1 fasting GLP-1 concentrations were negatively associated with daily energy intake from LF/HSS (r = − 0.22, p = 0.016, Figure 2A) but not with any of the other groups, LF/HCC, LF/HP, HF/HP, HF/HSS and HF/HCC (all p >0.3, Figure 2 B-C-D-E-F, respectively). Results were similar after adjustment for total daily energy intake. Similarly, no changes in GLP-1 results were observed after further adjustment for fasting insulin measured on day 1 (first day of ad libitum period). There was no association between morning plasma GLP1 concentrations (measured on day 1 and defined as baseline) and residuals of food intake (p = 0.56).

GLP-1 concentrations increased by 1.8 pg/ml (CI 95%: 0.4 to 3.3, p = 0.01, Figure 3) after 3 days of *ad libitum* food intake (an increase of nearly 14 %) with no difference according to gender (p=0.7) and ethnicity (p=0.2). We observed a positive correlation between change in GLP-1 concentrations with the residuals of total food intake (r = 0.25, p = 0.002, Figure 4A), carbohydrate intake (r = 0.21, p = 0.0006, Figure 4B), protein intake (r = 0.24, p = 0.012, Figure 4C) and fat intake (r = 0.22, p = 0.017, Figure 4D). Similar results were observed after adjustment for the change in fasting insulin from day 1 to day 3 of ad libitum period. No associations were found between change in GLP-1 concentrations and daily energy intake from the 6 food groups. There was no association between *ad libitum* food intake on day 3 and the morning plasma GLP-1 measured on day 4 (p = 0.09).

Discussion

In the current study, we investigated whether fasting GLP-1 concentrations was associated with subsequent *ad libitum* total energy and macronutrient intake and the response of circulating GLP-1 concentrations during this *ad libitum* period in 115 non-diabetic individuals. We found that fasting GLP-1 concentration prior to *ad libitum* food intake was a negative predictor of carbohydrate intake and was also negatively associated with daily energy intake from foods categorized as high simple sugar-low fat but did not predict either the total daily food intake or the fat and protein intake. Fasting GLP-1 concentrations also increased in association with degree of overfeeding across all macronutrient groups.

Previous studies have demonstrated an effect of GLP-1 receptor agonists on energy intake [10, 20] through both central and peripheral mechanisms in rats and humans [21, 22]. Our findings that GLP-1 was negatively associated with carbohydrate and LF/HSS intake and not total energy intake indicates a possible different mechanism by which GLP-1 regulates intake and may be explained by the potential role of GLP-1 in food reward and satiety. Recently, the gut-brain axis has been identified as a possible mediator of satiety and food reward [23]. Lower responsiveness to palatable food in reward regions might lead to overeating to attempt to compensate for the relative hypostimulation of these regions [24]. GLP-1 receptor agonists potently decreased the intake of palatable carbohydrates in lean and obese mice due to a hypothesized role of GLP-1 in the food reward system [25]. In line with this finding, we observed that higher circulating GLP-1 concentrations predict lower ingestion of carbohydrates, mainly high simple sugars, suggesting GLP-1 as possible mediator in the food reward system.

Hypothalamic and brainstem nuclei have been identified as GLP-1 targets for anorexic signals [26, 27]. However, GLP-1 receptors have also been identified in mesolimbic areas, such as the ventral tegmental area with its dopaminergic projections to the striatum and in the nucleus accumbens, both of which are implicated in reward behavior [28–30]. Thus, GLP-1 may convey signals from the gut about the nutritional status of the body to the brain [30, 31].

Another possible mechanism is GLP1 signaling mediation sweet taste perception [32] and that sweet taste receptors may play an important role in both food intake and glucose regulation. Sweet taste receptors (TRC), found in the oral cavity [33] and in the gastrointestinal tract [34], are activated by sugars, convey signals to the brain via sensory afferent neurons [35] and are involved in the central processing of food reward [36]. Brain centers may receive signals from the gastrointestinal tract via the vagal nerve coordinating with satiety hormones, such as GLP-1, to “alert” the digestive system for incoming carbohydrate intake [37]. Our results might suggest a possible role for GLP-1 in the reward system by increasing “sensitivity” to reward circuitry of carbohydrate intake and, hypothetically, this heightened sensitivity may occur via simple sugars ingestion leading to GLP-1 secretion and activation of neuronal afferent fiber.

In our study GLP-1 concentrations significantly increased by 14% after 3-days of *ad libitum* intake in which the subjects ate almost 40% more than their weight maintaining energy needs. This was not due to an effect of energy intake on the last day (day 3) of the *ad libitum* period on the day 4 GLP-1 concentrations, thus indicating a sustained effect of overeating.

GLP-1 concentrations are known to increase in the context of single mixed meal studies but the relationship between circulating GLP-1 concentrations and long term food intake in humans is controversial. We have found a more pronounced effect of energy intake on GLP-1 changes than was previously reported. Moreover, we have shown that this increase is directly proportional to the degree of overfeeding using a validated reproducible ad libitum paradigm [18].

Consistent with our results, Wadden et al [14] showed an increase in GLP-1 concentrations in a cohort of 72 males after a 7-day overfeeding period, but others have not demonstrated this effect. After five days of high-fat diet overfeeding, fasting GLP-1 concentrations did not change [13] and no significant changes in GLP-1 levels were found after an overfeeding liquid diet in lean males [38]. In a previous study from our group [39], we also did not observe any changes in GLP-1 concentrations after the *ad libitum* period in a smaller sample size (n=30). In the current study, however, we were able to more precisely quantify GLP-1 response in a larger cohort, demonstrating an important effect of *ad libitum* intake on change in GLP-1.

GLP-1 is the most potent incretin hormone involved in the regulation of glucose stimulated-insulin secretion and its differential secretion may play a role in the reported racial differences in glucose regulation after a meal ingestion. Previous groups have reported racial differences, especially in regard to the interaction between GLP-1 and glucose regulation

[40–42]. However, in our study, after stratifying by race, we did not observe any association between incretin secretion and glucose dependent insulin secretion.

One of the strengths of the present study is the large cohort of individuals with a variety of ages and ethnicities, making this investigation one of the larger studies to examine circulating GLP-1 as a predictor of and in response to short term *ad libitum* intake in humans. Furthermore, no prior studies have shown an association between the degree of change in circulating GLP-1 concentrations and *ad libitum* food intake. We were also able to exclude night eaters from the analysis, due to the precise measurement of eating time recorded by vending machine system, yet still measure the effect of GLP-1 post *ad libitum* feeding.

However, several limitations must be acknowledged. Due to the very short half-life of active GLP-1 and due to its rapid degradation, we measured total GLP-1, which gives an indication of the secretion from intestinal cells. However, active GLP-1 has been previously shown to positively correlate with total GLP-1 [43]. Additionally, during the *ad libitum* food intake period on the vending machines, some volunteers had at least one episode of nighttime eating and, thus, the morning GLP-1 measured on day 4 was not have been drawn in "fasting" state for these individuals. Yet, because of the precise timing measurements of the vending machine paradigm, we were able to exclude those volunteers when necessary. Unfortunately, we did not have assessments of levels of hunger or satiety during the ad libitum period, which might be helpful to further understand the relationship between GLP-1 and reward mechanisms of palatable food.

Conclusion

In conclusion, we investigated whether fasting plasma GLP-1 predicted energy intake and its response to short term *ad libitum* food intake in 115 individuals without type 2 diabetes. Fasting GLP-1 concentrations measured prior to ad libitum period was a negatively associated with carbohydrate intake and percentage of LF/HSS foods. We also observed a significant increase in GLP-1 concentrations after 3 days of *ad libitum* overeating. Our results indicate that GLP-1 may have a role in central reward processing limiting the desire to ingest processed high carbohydrate foods while also serving as a protective mechanism against ongoing overeating following energy intake excess.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

GLP-1	Glucagon-like peptide 1
FM	fat mass
FFM	fat free mass
OGTT	oral glucose tolerance test
DXA	dual energy X-ray absorptiometry
CNS	central nervous system
LF	low fat
HSS	high simple sugar
HF	high fat
HCC	high complex carbohydrate
HP	low fat/high protein

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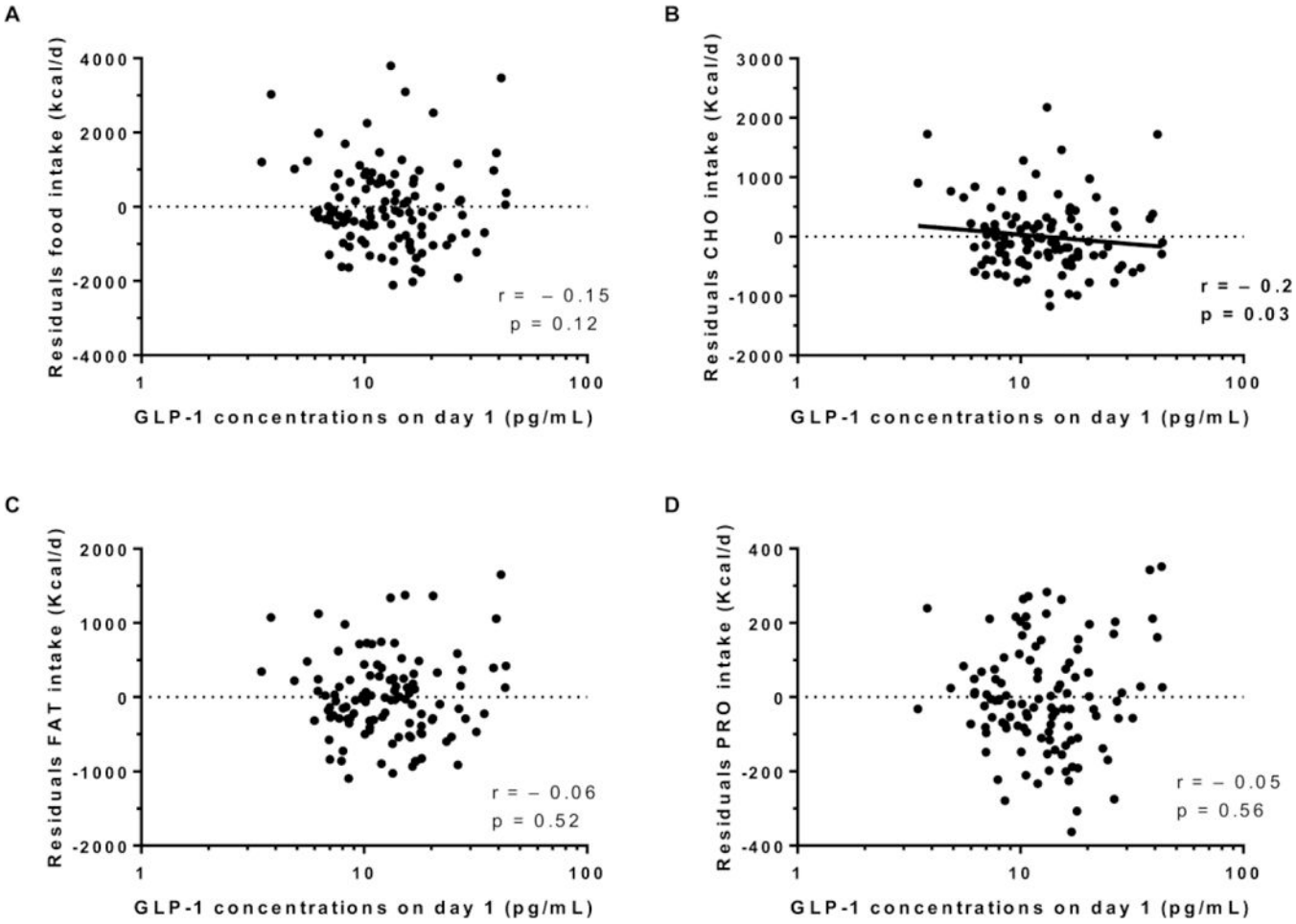

Figure 1.

Relationship between morning GLP-1 (day 1) and residuals of total food intake (A, carbohydrates intake (B), fat intake (C) and protein intake (D). The total *ad libitum* food intake during the 3-day vending period is expressed as the average over 3 days in Kcal per day. In each panel, the Pearson's correlation coefficient (r) is reported along with its significance (p). All correlations were adjusted for storage time. The plasma GLP-1 is expressed in logarithmic scale.

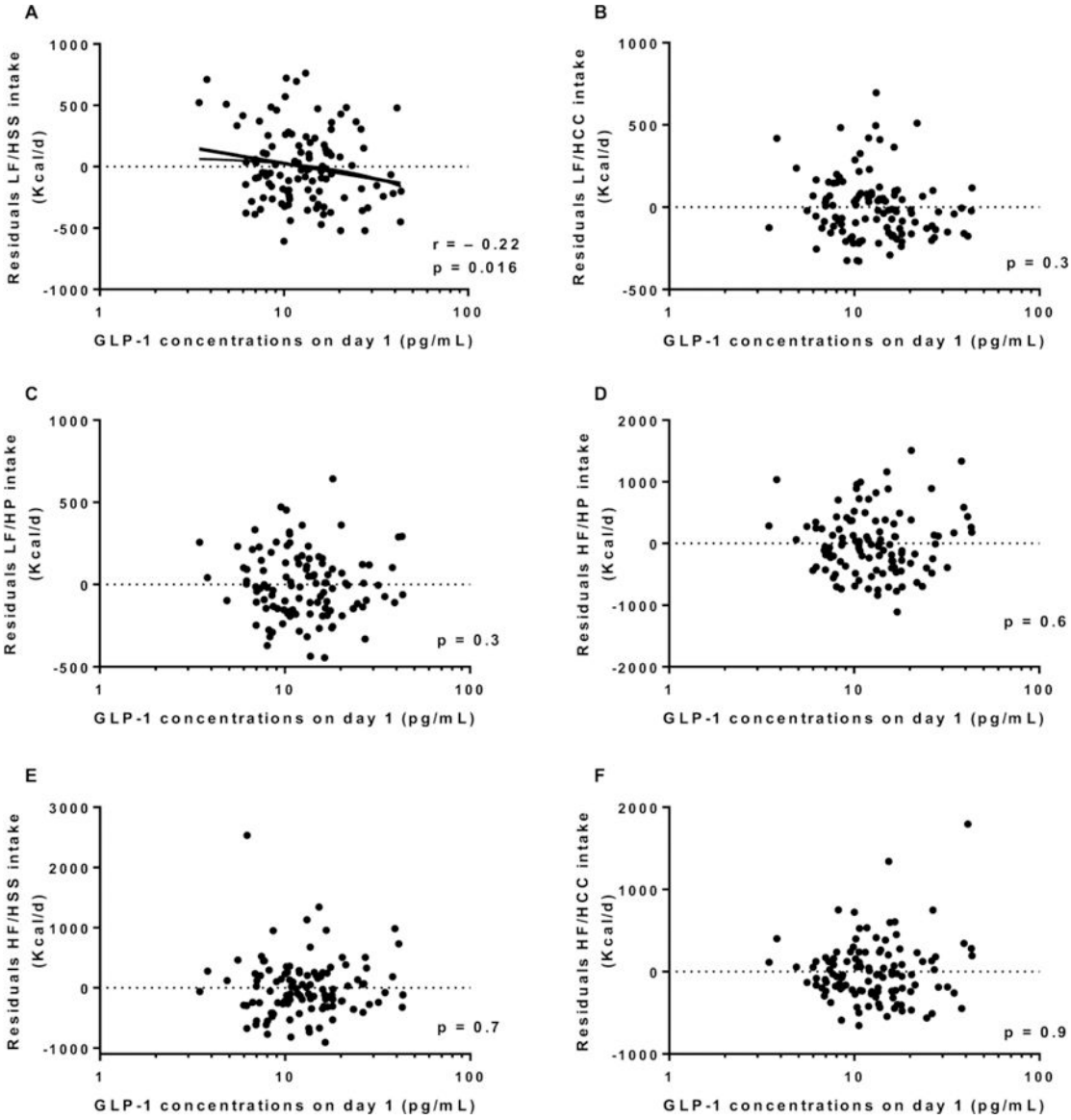

Figure 2.

Relationship between day 1 GLP-1 and 3-day daily average of energy intake from the LF/HSS food group (A), the LF/HCC food group (B), the LF/HP food group (C), the HF/HP food group (D), the HF/HSS food group (E) and the HF/HCC food group (F).

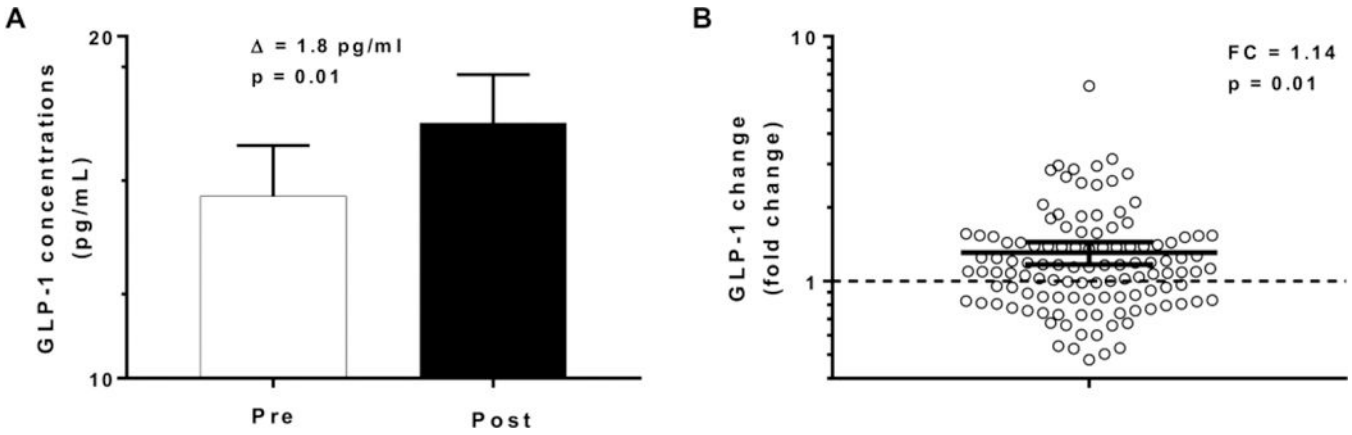

Figure 3. Change in plasma GLP-1 concentrations pre- and post-*ad libitum* food intake.

Panel A, the white bar represents GLP-1 concentrations on day 1 (PRE, prior to 3-day ad libitum period) and the black bar represents GLP-1 concentrations on day 4 (POST, after 3-day ad libitum period). The βs indicate the absolute values of the change in GLP-1 concentrations between pre and post ad libitum food intake.

Panel B, fold change in GLP-1 from day 4 to day 1. The dotted line represents, in a logarithmic scale, the separation between individuals who increase GLP-1 concentration (above the line) and individuals who decrease GLP-1 (below the line).

The night eaters (n=27) were excluded from this analysis

Error bars represent the % confidence interval of the mean.

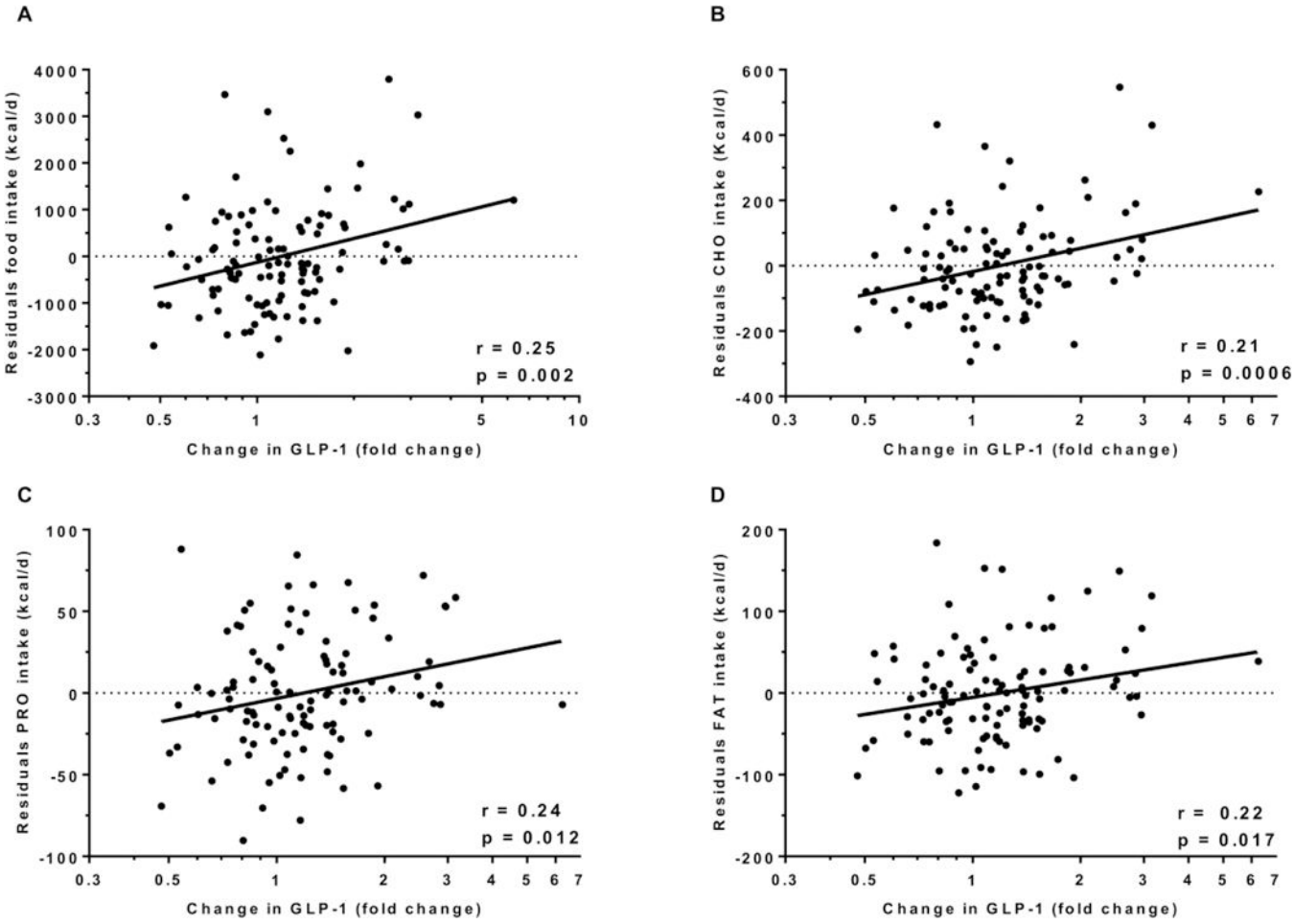

Figure 4.

Change in GLP-1 and total food intake (A), carbohydrates intake (B), protein intake (C) and fat intake (D).

In each panel, the Pearson's correlation coefficient (r) is reported along with its significance (p).

The night eaters on day 3 (n=27) were excluded from this analysis

Table 1.

Demographic and anthropometric measures of the study group.

	Whole study group (n=115)	Men (n=72)	Women (n=43)	P*	Night eaters (n=27)
Ethnicity	10 AA, 42 W, 9 H, 47 NA, O 9	5 AA, 27 W, 6 H, 27 NA, 7 O	5 AA, 13 W, 3 H, 20 NA, 2 O	0.5	4 AA, 12 W, 1 H, 10 NA
Sex (F/M)	43/72	72	43		
Age (years)	34.9 ± 10.4	34.8 ± 10.7	34.5 ± 10.0	0.8	31.0 ± 10.5
Body weight (kg)	86.9 ± 20.6	89.0 ± 19.5	83.6 ± 22.1	0.1	82.3 ± 16.7
BMI (kg/m^2)	30.1 ± 6.9	28.8 ± 6.1	32.2 ± 8.0	0.04	28.1 ± 6.7
FFM (kg)	59.8 ± 12.5	64.8 ± 10.5	50.5 ± 10.3	<.0001	59.2 ± 9.1
FM (kg)	27.1 ± 12.2	23.9 ± 10.7	33.0 ± 12.9	<0.001	23.1 ± 12.0
Body fat (%)	30.2 ± 8.9	25.8 ± 6.9	38.3 ± 6.8	0.0002	26.7 ± 10.6
Fasting glucose (mg/dL)	93.1 ± 6.5	92.5 ± 7.4	95.8 ± 8.4	0.7	89.2 ± 5.9
2-h glucose (mg/dL)	127.6 ± 29.2	121.5 ± 28.8	131.9 ± 27.5	0.07	124.7 ± 27.4
Fasting GLP-1 pre (pg/mL)[1]	14.4 (12.9–16.0)	15.4 (13.4–17.3)	12.9 (10.3–15.5)	0.1	13.27 (10.2–16.4)
Fasting GLP-1 post (pg/mL)[2]	16.8 (15.1–18.5)	18.2 (15.9–20.5)	14.6 (12.2–17.0)	0.03	17.4 (14.7–20.1)
Fasting OGTT Insulin[3]	11.7 (9.8–13.7)	11.6 (8.6–14.4)	12.1 (9.9–14.2)	0.8	9.5 (6.7–12.3)
Fasting insulin pre (mU/L)[1]	11.9 (9.9–13.8)	11.5 (8.7–14.2)	12.7 (10.3–15.0)	0.3	10.0 (4.5–13.5)
Fasting insulin post (mU/L)[2]	15.7 (11.9–19.4)	14.9 (11.2–18.7)	17.1 (8.6–25.5)	0.4	22.0 (6.5–37.7)

Table 1. Data are presented as the mean ± SD, unless otherwise indicated. AA, African American; H, Hispanic; NA, Native American; W, white: O, other

*P values are for differences between male/female groups as determined by Student t test.

[1]Hormones concentration was measured in fasting state (530 AM) on day 7 before starting the day ad libitum vending machine paradigm (day 1 of *ad libitum* period).

[2]Hormones concentration was measured in fasting state (530 AM) on day 10 (discharge day) after completion of 3-day ad libitum period.

[3]Fasting insulin concentration was measured in fasting state during OGTT.

[1,2,3]Data are log expressed with mean ± 95% confidence limits.

Table 2.

Ad Libitum Food Intake Measures of the Study group

	Whole study group (n=115	Men (n=72)	Women (n=43)	p	Night eaters (n=27)	p
Computerized vending machine system						
Total energy intake (Kcal/day)	3849.3 ± 1431	4272.7 ± 1395.6	3130.5 ± 1195.3	**<0.001**	4270.6 ± 1327.9	0.2
Total energy intake (% WMEN)	138.7 ± 45.4	149.8 ± 43.2	119.9 ± 44.0	**<0.001**	155.9 ± 44.4	0.08
CHO intake (kcal/day)	495.9 ± 176.3	551.4 ± 168.3	401.7 ± 148.7	**<0.001**	550.8 ± 159.8	0.1
FAT intake (kcal/day)	162.79± 75.0	180.6 ± 76.4	132.9 ± 62.6	**<0.001**	131.8 ± 38.9	0.3
PRO intake (kcal/day)	117.1 ± 43.7	127.9 ± 42.8	98.8 ± 39.2	**<0.001**	179.6 ± 73.8	0.1
HF/HCC (kcal/day)	473.3 ± 400.3	545.8 ± 461.9	351.9 ± 223.9	**0.003**	499.4 ± 379.8	0.7
HF/HP (kcal/day)	1054.2 ± 589	1201.5 ± 603.6	807.7 ± 475.6	**0.002**	1139.4 ± 665.3	0.2
HF/HSS (kcal/day)	713.6 ± 535.3	774 ± 483.6	612.5 ± 604.7	0.14	898 ± 682.9	0.4
LF/HCC (kcal/day)	307.5 ± 193.9	323.9 ± 201.8	280.1 ± 178.7	0.2	265.6 ± 167	0.2
LF/HP (kcal/day)	437.5 ± 210.9	475.5 ± 197.1	374 ± 220.	**0.01**	523.1 ± 221.1	0.2
LF/HSS (kcal/day)	683.3 ± 327.4	773.6 ± 311.6	532.1 ± 299.2	**<0.001**	761.4 ± 355.1	0.1

Table 2

Data are presented as mean±SD, unless otherwise indicated.

[1] *Ad libitum* food intake measures are reported as the average of three days on the vending machines. HF=High Fat; HCC=High Complex Carbohydrate; HP=High Protein; HSS=High Simple Sugar; LF=Low Fat