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Increased 24-hour *ad libitum* food intake is associated with lower plasma irisin concentrations the following morning in adult humans

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Abstract

Background—The relationship between food intake and irisin concentrations in humans is unclear.

Objectives—To determine whether the previous day's intake impacts fasting plasma irisin concentrations, or whether fasting irisin concentrations associate with subsequent *ad libitum* food intake.

Methods—Sixty-six nondiabetic adults (42 men) were admitted for a study of the determinants of energy intake. After 6 days of a weight maintaining diet, *ad libitum* energy intake over 3 days was assessed using a vending machine paradigm. Fasting plasma irisin concentrations were measured on the morning of the second day of the vending period.

Results—There were no correlations between irisin and demographic or anthropometric parameters. On day 1, subjects consumed $144 \pm 52\%$ of weight maintaining energy needs. Every additional 500 kcal consumed on day 1 associated with a 3.4% lower irisin concentration the following morning (95% CI $-6.2, -0.4\%$, $p = 0.01$; adjusted for age, sex and race). If energy intake was expressed as a percentage of weight maintaining energy needs, every 10% increase associated with a 1.9% lower irisin concentration (95% CI $-3.7, -0.1\%$; adjusted $p = 0.02$). A 100 kcal increase in carbohydrate or fat consumption associated with a 1.3% (95% CI $-2.5, -0.1\%$, $p = 0.01$) and a 0.6% (95% CI $-1.1, -0.0\%$, $p = 0.02$) lower irisin concentration, respectively. There was no association between fasting irisin concentrations and subsequent energy intake on day 2 ($r = 0.19$, $p = 0.1$).

Conclusions—Higher *ad libitum* 24 h energy intake was associated with lower fasting irisin concentrations the following morning, but fasting irisin concentrations did not predict subsequent energy intake. The decrease in irisin concentrations with increased energy intake is consistent with the detrimental metabolic effects of overeating.

Keywords

Irisin; *Ad libitum* food intake

Introduction

Fat-free mass is a primary determinant of energy intake (Blundell et al., 2012; Weise, Hohenadel, Krakoff, & Votruba, 2014). Skeletal muscle is an important component of fat-free mass. In 2012, the myokine irisin, which conveys metabolic signals between various tissues, was identified (Boström et al., 2012). Irisin is a cleaved and secreted fragment of the membrane protein fibronectin type III domain containing 5 (FNDC5), which is stimulated by the master regulator of muscle metabolism, peroxisome proliferator-activated receptor- γ coactivator-1 alpha (PGC1- α), and has been reported to increase after acute exercise (Boström et al., 2012). Overexpression of full-length FNDC5 increases oxygen consumption, improves glycemic control, and modestly reduces weight gain in mice fed a high-fat diet without influencing food intake (Boström et al., 2012). However, it is not yet known if irisin concentrations influence the amount of subsequent food intake in humans, or if the amount of food intake or the food choices consumed affect irisin concentrations. A recent study did assess whether irisin concentrations were associated with a healthy eating index score, and found no association between irisin and that score (Park et al., 2014).

The effects of exercise and overeating on cellular mechanisms regulating metabolic health often vary in opposing directions (Flachs, Rossmeisl, Kuda, & Kopecky, 2013; Ogasawara, Sato, Matsutani, Nakazato, & Fujita, 2014; Wilson, Hargreaves, & Howlett, 2006). For example, increased PGC-1 α transcription mediates many of the beneficial effects of exercise, including decreased inflammation and increased number and efficiency of mitochondria (Handschin & Spiegelman, 2008). Conversely, diet induced obesity has been associated with reduced expression of PGC-1 α , as well as mitochondrial dysfunction in muscle (Bonnard et al., 2008). Given the beneficial effects of the PGC-1 α pathway, we hypothesized that fasting plasma irisin concentration, as a downstream effector of PGC-1 α , might be related to *ad libitum* food intake. The objective of the present study was to investigate whether irisin is associated with *ad libitum* food intake on the day prior to measurement, or if irisin, as a potential mediator of the relationship between fat-free mass and energy intake, associates with subsequent daily food intake in humans.

Subjects and methods

Subjects

This study was part of a larger, ongoing protocol designed to investigate predictors of *ad libitum* food intake and food preferences (registered with ClinicalTrials.gov, number NCT00342732). A total of 210 subjects were enrolled in this study as of December 2013. All subjects, aged 18–65 years, were recruited from the greater Phoenix, AZ area by advertisement, and were non-diabetic, not pregnant, not on medication, and healthy by history, physical, and fasting blood tests. Plasma was drawn daily during the assessment of *ad libitum* food intake from a subset of subjects. Subjects who had accessed the vending

machine during nighttime hours (11:00pm to 05:00am) were excluded ($n = 48$) from this analysis to ensure uniformity of irisin measurements. As a result, irisin measures were available in only 66 individuals (24 women, 42 men).

On admission to the clinical research unit of the National Institute of Diabetes, Digestive and Kidney Disease (NIDDK) in Phoenix, the subjects were placed on a standard weight maintaining diet (50% carbohydrates, 30% fats, and 20% proteins), using unit specific equations based on weight and gender. Body weight was measured each morning using a calibrated scale, and the weight maintaining diet was adjusted to maintain a body weight within $\pm 1\%$ (Ferraro, Boyce, Swinburn, De Gregorio, & Ravussin, 1991). Body composition was measured using dual-energy X-ray absorptiometry (DPX-1, Lunar Corp, Madison, WI, USA) on the second day after admission. Waist-to-thigh ratio was calculated as a measure of body fat distribution, with circumferences measured at the level of the umbilicus (waist) and the gluteal fold (thigh) in the supine and standing position, respectively (Tataranni, Larson, & Ravussin, 1994). After 3 days on the weight maintaining diet, glucose tolerance status was assessed by a 75 g oral glucose tolerance test (American Diabetes Association, 2013). Study participants were asked to restrict their activities to walking to and from their room to the community room, watching TV or playing pool while on the clinical research unit. All participants provided written informed consent prior to beginning the study. The study was approved by the Institutional Review Board of the NIDDK.

***Ad libitum* energy intake assessment using a vending machine paradigm**

Using the food preferences questionnaire (Geiselman et al., 1998), study participants were asked to rate 80 typical breakfast, lunch, dinner, and snack items listed in random order using a 9-point Likert scale on the first day of admission. Vending machines were stocked with 40 food items that the study participants had marked with an intermediate hedonic rating. Measurement of *ad libitum* food intake by an automated food-selection system, which is a validated and reproducible method for objectively assessing energy intake, was performed as previously described (Rising et al., 1992; Venti, Votruba, Franks, Krakoff, & Salbe, 2010). In brief, during the final 3 days on the clinical research unit, study participants had *ad libitum* access to the computer-operated vending machines for 23.5 h/day in a separate eating area. The dietary staff weighed all unconsumed food portions to determine the exact amount of kilocalories and macronutrients consumed. The daily energy intakes, as well as the specific protein, fat, and carbohydrate intakes from foods, and condiments consumed were calculated with the use of the CBORD Professional Diet Analyzer Program (The CBORD Inc, Ithaca, NY, USA). Daily energy, protein, fat and carbohydrate intakes are expressed as both mean kilocalories per day (kcal/day) and mean percentage of weight maintaining energy needs.

Analytical measurements

After 6 days of the weight maintaining diet, subjects chose food *ad libitum* from the vending machines as described above. On the morning of the second day of the vending machine assessment, venous blood samples were drawn from an antecubital vein of each volunteer at ~0530 after an overnight fast. Samples were treated with protease inhibitor cocktail (Sigma-

Aldrich, St. Louis, MO, USA) and dipeptidyl peptidase IV (DPPIV) inhibitor (EMD Millipore Corporation, Billerica, MA, USA), and stored at -70°C . Fasting plasma irisin concentrations were measured in the Clinical Research Core Laboratory of the National Institutes of Health (Bethesda, MD, USA) with a highly sensitive commercial enzyme-linked immunosorbent assays (ELISA) that uses the recombinant irisin protein (Catalog Number EK-067-29) (Phoenix Pharmaceuticals, Belmont, CA, USA). The minimum detectable concentration of the assay is 1.29 ng/ mL. Plasma samples were analyzed in duplicate, and the intraassay CV for the irisin ELISA was 5.61%, and the interassay CV was 14.56%. The plasma glucose concentrations were measured using either an enzymatic oxygen-rate method (Beckman Glucose Analyzer 2; Beckman Instruments, Brea, CA, USA) (before September 2010), or the comparable Analox GM9 glucose oxidase method (Analox Inst. USA Inc., Lunenburg, MA, USA) (after September 2010). Plasma insulin concentrations were measured with the automated Access radioimmunoassay (Beckman Coulter Inc.) or the AT AIA Pack IRI on a TOSOH analyzer (Tosoh Bioscience, King of Prussia, PA, USA).

Statistical analysis

A target sample size of 61 was estimated to provide 80% power with an alpha of 0.05 to detect a modest correlation of 0.35 between variables. Sixty-six subjects were included in the final analysis. All statistical analyses were performed using software from the SAS Institute (SAS version 9.2; Cary, NC). Normally distributed data are expressed as mean $\pm$ SD, and skewed data are presented as medians with interquartile range. Glucose area under the curve (AUC) and insulin AUC were calculated using the trapezoidal method. Correlations between normally distributed and skewed variables were assessed with Pearson and Spearman correlation coefficients, respectively. Differences between groups were assessed with Student's *t*-test or 1-way ANOVA. Multiple linear regression analysis using stepwise selection was used to identify determinants of fasting plasma irisin concentrations. Fasting plasma irisin concentration data used in multivariate analyses were log transformed to meet the assumptions of linear regression. In order to determine anthropometric variables to include as covariates in the model to test our hypothesis, the following were considered as possible determinants of irisin after forcing age, sex, and race into the model: height, weight, fat mass, fat-free mass, waist circumference, thigh circumference, and waist-to-thigh ratio. A linear regression model including age, sex and race was used to determine if energy intake predicts log fasting plasma irisin concentrations the next morning.

Results

Baseline characteristics

General, anthropometric, and energy intake characteristics of the subjects are shown in Table 1. Total energy intake as assessed using the vending machine paradigm did not differ by day ($p = 0.22$), and was 4032 ± 1612 , 3774 ± 1645 , and 3820 ± 1721 kcal for days 1, 2, and 3, respectively. Mean energy intake was 3875 ± 1503 kcal/day or $138 \pm 47\%$ weight maintaining energy needs. Mean *ad libitum* intake did not differ by race ($p = 0.26$), but was higher in men compared to women (4294 ± 1409 vs. 3143 ± 1402 kcal/day, $p = 0.002$; 149 ± 42 vs. $118 \pm 49\%$ weight maintaining energy needs, $p = 0.009$).

We did not find any correlations between fasting irisin concentrations and demographic or anthropometric parameters (Supplementary Table S1). There was no correlation between fasting irisin concentrations and the AUCs of glucose or insulin during the initial oral glucose tolerance test. There was also no correlation between fasting insulin and fasting irisin concentrations drawn on the same day ($\rho = 0.28$; $p = 0.07$). Fasting irisin concentrations did not differ between participants classified as obese and lean ($p = 0.7$), or between those classified as having impaired glucose tolerance versus normal glucose regulation ($p = 0.4$). In a regression model using stepwise selection to identify determinants of log fasting plasma irisin concentrations (and including age, sex and race), no anthropometric parameters were associated with irisin in this small sample.

Association of *ad libitum* food and macronutrient intake with fasting plasma irisin concentrations

On day 1, the subjects consumed $144 \pm 52\%$ of weight maintaining energy needs. There was a negative association between log fasting plasma irisin concentrations on the morning of day 2 and energy intake on day 1 when expressed as either total caloric intake or as percentage of weight maintaining energy needs ($r = -0.26$, $p = 0.04$). After adjusting for age, sex and race, consuming an additional 500 kcal/day or 10 more percent weight maintaining energy needs on day 1 was associated with a 3.4% (95% CI -6.2 , -0.4% , $p = 0.01$) (Fig. 1A) or 1.9% (95% CI -3.7% , -0.1% ; $p = 0.02$) (Fig. 1B) lower fasting plasma irisin concentration the next morning, respectively. A 100 kcal increase in carbohydrate consumption was associated with a 1.3% lower irisin concentration (95% CI -2.5 , -0.1% , $p = 0.01$) (Fig. 2A), and a 100 kcal increase in fat consumption was associated with a 0.6% lower irisin concentration (95% CI -1.1 , -0.0% , $p = 0.02$) (Fig. 2B). We did not observe an association between protein consumption and log fasting plasma irisin concentrations ($p = 0.09$, adjusted for age, sex and race) (Fig. 2C). No correlations between log fasting plasma irisin concentrations (adjusted for energy intake on day 1) and energy intake parameters on day 2 were found (Supplementary Table S2). After multivariate adjustment for age, sex, race, fat mass, fat-free mass, and energy intake on day 1, log fasting plasma irisin concentrations on the morning of day 2 were associated with protein consumption throughout that same day (95% CI -1.8 , -0.1% , $p = 0.02$). However, no relationship was observed with overall subsequent daily energy intake (Fig. 3), carbohydrate or fat consumption on the same day (Supplementary Table S3) after multivariate adjustment.

Discussion

The aim of the present study was to investigate whether irisin is associated with *ad libitum* food intake in humans, and to determine the directionality of any association between irisin and energy intake. We found that greater amounts of 24 hour *ad libitum* food intake were associated with lower irisin concentrations the following morning. This relationship was due to the amount of carbohydrate and fat consumed. In general, fasting irisin concentrations were not associated with subsequent food intake.

Other studies have also assessed for relationships of irisin concentrations with anthropometric characteristics. Similar to one previous report, we did not find a correlation

between irisin levels and body composition measures (Pekkala et al., 2013), although several other studies have found a positive association of circulating irisin with BMI (Choi et al., 2013; Crujeiras et al., 2014; Liu et al., 2013; Park et al., 2013). We also did not detect any correlations between irisin concentrations and measures of glucose homeostasis (Al-Daghri et al., 2013; Lopez-Legarrea et al., 2014), or fasting insulin and irisin, consistent with previous findings (Choi et al., 2013; Liu et al., 2013; Moreno-Navarrete et al., 2013; Schumacher, Chinnam, Ohashi, Shah, & Erickson, 2013). The novel finding from our data is that the greater the 24 hour *ad libitum* energy intake, particularly carbohydrate and fat intake, the lower the fasting irisin concentrations on the following morning.

Lower irisin concentrations the morning after overeating is consistent with the hypothesis that irisin is metabolically beneficial. Irisin has been reported to increase after exercise (Boström et al., 2012), and the differences in the irisin response to overeating and exercise (Flachs et al., 2013; Ogasawara et al., 2014; Wilson et al., 2006) is consistent with differences in other metabolic risk factors between people who chronically overeat (Bonnard et al., 2008; Muoio & Koves, 2007; Sparks et al., 2005) versus those who chronically exercise (Handschin & Spiegelman, 2008). For example, transcription of the master regulator of muscle metabolism PGC1- α , a molecule that plays a role in thermogenesis in both skeletal muscle and brown adipose tissue, is increased in individuals who participate in endurance exercise. This is also the protein which stimulates expression of FNDC5/irisin (Boström et al., 2012). PGC-1 α expression is also known to be (Fernandez-Marcos & Auwerx, 2011) downregulated by overfeeding. The activation of other proteins by irisin, including p38 mitogen-activated protein kinase and extracellular signal-related kinase signaling (Zhang et al., 2014), have been reported to be attenuated during refeeding after a period of fasting (Ueyama, Morikawa, Yasuda, & Senba, 2004). Of note, there is evidence that irisin may not just be released from myocytes but may also be an adipokine with important autocrine and endocrine function (Roca-Rivada et al., 2013).

Because irisin is released from muscle, and fat-free mass is a primary determinant of daily energy intake (Blundell et al., 2012; Weise et al., 2014), we hypothesized that fasting irisin concentrations might be the link by which fat-free mass influences daily energy intake. However, we did not find that irisin concentrations associate with subsequent food intake. This is consistent with the observation by Boström and colleagues that transgenic mice overexpressing FNDC5/irisin and fed a high-fat diet had no change in food intake (Boström et al., 2012). In another human study, irisin was not associated with a healthy eating scale based on average yearly intake self-reported on the Food Frequency Questionnaire (Park et al., 2014). In addition, irisin is not affected by intake of a standardized mixed meal (Anastasilakis et al., 2014). Our results suggest that overeating may be required to impact irisin concentrations. We used an objective method of energy intake assessment that is not reliant on self-reports. Energy intake as assessed by a computerized vending machine paradigm is a consistent and reproducible assessment of *ad libitum* food intake (Venti et al., 2010), although, in general, individuals consume more than their weight maintaining energy needs. A potential irisin resistance has been reported in diet-induced obese rats where over-secretion of FNDC5/irisin in the white adipose tissue was detected (Roca-Rivada et al., 2013). Therefore, it is possible that our findings, including the association of lower irisin

concentrations with increased prior food intake and the lack of a relationship between irisin and subsequent food intake may be due to the high proportion of overweight and obese subjects in our study population.

The results of the current study must be considered in the context of several potential limitations. There were no objective measures of physical activity available, which would have been an interesting addition to the present analysis. It should be noted that the initial finding that irisin is regulated by exercise in humans has only been reproduced in selective cohorts (Aydin et al., 2013; Boström et al., 2012; Huh et al., 2012; Kraemer, Shockett, Webb, Shah, & Castracane, 2014; Lecker et al., 2012; Norheim et al., 2014; Timmons, Baar, Davidsen, & Atherton, 2012), but not in all (Aydin et al., 2013; Besse-Patin et al., 2014; Hecksteden et al., 2013; Hofmann et al., 2014; Huh et al., 2012; Kraemer et al., 2014; Moraes et al., 2013; Norheim et al., 2014; Pekkala et al., 2013; Phillips et al., 2012; Raschke et al., 2013; Timmons et al., 2005). We did not include measures of other known appetitive hormones such as ghrelin, leptin or adiponectin as our primary focus was the association between *ad libitum* food intake and irisin. Additionally, we had no ratings of hunger and satiety. Although visual analog scales to assess hunger have some ability to predict feeding behavior, they are not considered as reliable as direct measures of nutrient intake (Stubbs et al., 2000). Assessment of food intake using subjective means, such as food diaries, can be prone to errors, but we used an objective vending machine paradigm that has a high interindividual consistency (intraclass correlation coefficient = 0.9, $p < 0.0001$). Although most individuals overeat using this methodology (Gluck, Venti, Salbe, & Krakoff, 2008; Pannacciulli et al., 2007; Votruba, Kirchner, Tschöp, Salbe, & Krakoff, 2009; Weise et al., 2014), it can be a reproducible tool for the determination of *ad libitum* energy intake on a clinical research unit (Venti et al., 2010).

As irisin is a relatively recently identified hormone, its function in humans is an area of active investigation. We found that higher *ad libitum* food intake is associated with lower irisin concentrations the following morning consistent with a potential role for irisin in metabolic health. Our findings are in line with the opposing metabolic effects of exercise and overeating, and also the current data proposing a regulatory feedback mechanism between skeletal muscle, adipose tissue and the central nervous system (Dun et al., 2013; Hashemi et al., 2013; Moon, Dincer, & Mantzoros, 2013; Roca-Rivada et al., 2013). Further observational and interventional food intake studies to better understand the relationship between irisin and food intake are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ANOVA	analysis of variance
AUC	area under the curve
DPPIV	dipeptidyl peptidase IV
ELISA	enzyme-linked immunosorbent assay
FNDC5	fibronectin type III domain containing 5
PGC1-α	peroxisome proliferator-activated receptor- γ coactivator-1 α

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Appendix: Supplementary material

Supplementary data to this article can be found online at doi:10.1016/j.appet.2015.03.003.

Fig. 1.

A, Association between fasting plasma irisin concentrations (ng/mL) and 24h energy intake on the preceding day (kcal). B, Association between fasting plasma irisin concentrations (ng/mL) and 24h energy intake on the preceding day (% WMEN).

Fig. 2.

A, Association between fasting plasma irisin concentrations (ng/mL) and 24h carbohydrate intake on the preceding day (kcal). B, Association between fasting plasma irisin concentrations (ng/mL) and 24h fat intake on the preceding day (kcal). C, Association between fasting plasma irisin concentrations (ng/mL) and 24h protein intake on the preceding day (kcal).

Fig. 3.
Association between 24h energy intake on the subsequent day (kcal) and fasting plasma irisin concentrations (ng/mL).

Table 1General, anthropometric, and energy intake characteristics of the study population.^b

	Whole population	Men	Women	p ^a
Race^c				0.33
African Americans	6	3	3	
Whites	21	13	8	
Hispanics	5	5	0	
Native Americans	34	21	13	
Age (year)	33.7 ± 9.7 ^d	32.9 ± 9.6	35.0 ± 10.1	0.42
Body weight (kg)	90.6 ± 22.5	91.0 ± 18.9	90.1 ± 28.1	0.88
Height (cm)	170.4 ± 8.8	175.1 ± 6.0	162.3 ± 6.9	<0.001
Waist (cm)	104.1 ± 18.0	102.7 ± 15.6	106.5 ± 21.7	0.42
Thigh (cm)	25.3 ± 3.2	24.5 ± 2.4	26.8 ± 3.8	0.004
Waist-to-thigh ratio	1.6 ± 0.2	1.6 ± 0.1	1.5 ± 0.2	0.13
BMI (kg/m²)	31.2 ± 7.5	29.6 ± 5.7	34.0 ± 9.5	0.02
Body fat (%)	31.0 ± 9.1	26.4 ± 6.6	38.9 ± 7.3	<0.001
FFM (kg)^d	61.5 ± 12.8	65.9 ± 10.1	53.7 ± 13.6	<0.001
FM (kg)^d	29.1 ± 13.5	25.0 ± 10.3	36.3 ± 15.5	0.001
FPIC (ng/mL)^e	296.9 (219.5–434.8)	314.4 (221.6–433.3)	267.8 (215.2–446.0)	0.67
African Americans	409.7 ± 95.4	413.7 ± 43.0	405.8 ± 144.4	0.83
Whites	367.7 ± 199.3 [*]	378.5 ± 222.7	350.3 ± 167.2	0.72
Hispanics	585.8 ± 140.9	585.8 ± 140.9	–	–
Native Americans	292.9 ± 119.1 [*]	280.1 ± 102.7	313.7 ± 143.8	0.60
WMD (kcal)	2776 ± 244	2857 ± 199	2633 ± 255	<0.001
DEI (kcal/d)	4032 ± 1612	4390 ± 1488	3405 ± 1658	0.02
%WMEN (%)	144 ± 52	153 ± 47	127 ± 58	0.06
24-h CHO (kcal)	2028 ± 796	2196 ± 716	1732 ± 860	0.02
24-h FAT (kcal)	1593 ± 819	1737 ± 819	1341 ± 756	0.06
24-h PRO (kcal)	1035 ± 405	1143 ± 369	864 ± 423	0.01

^a Unless otherwise stated, sex differences were evaluated by unpaired t-test.^b Data are presented as mean ± SD or median (IQR). DEI, *ad libitum* daily energy intake; WMEN, weight maintaining energy needs; %WMEN, an expression of DEI as a percentage of WMEN consumed.^c Race differences between genders were assessed by the Chi Squared test.^d FFM, fat-free mass; FM, fat mass; FPIC, fasting plasma irisin concentrations; 24-h CHO, 24-h carbohydrate intake on day 1 of the vending machine paradigm; 24-FAT, 24-hour fat intake on day 1 of the vending machine paradigm; 24-PRO, 24-h protein intake on day 1 of the vending machine paradigm.^e Data are presented as median with IQR due to skewed distribution. P-values for gender difference were calculated by Wilcoxon rank sum test.^{*} p < 0.05 vs. Hispanics by Bonferroni *post-hoc* test.