

# Different Estradiol Level's Impact on Appetite Regulation, Food Intake, and Concentrations of Leptin, Glucagon-Like Peptide-1, and High-Sensitivity C-Reactive Protein During a Long Agonist IVF Protocol

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## Research Article

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# Abstract

**Background:** Do different hormonal phases affect appetite regulation, food intake, and concentrations of leptin, glucagon-like peptide-1 (GLP-1), and high-sensitivity C-reactive protein (hs-CRP) during a long agonist in vitro fertilization (IVF) protocol? Does the IVF stimulation induce metabolic changes, which might impact maternal health?

**Methods:** Fifty-four infertile women were encountered thrice, the first of which was at the beginning of their period (low estradiol). The other two were during a gonadotrophin-releasing hormone (GnRH) analog downregulation (low estradiol) and at the end of a follicle-stimulating hormone (FSH) stimulation (high estradiol). The first visit was the reference; the women served as their controls. The concentrations of leptin, GLP-1, and hs-CRP were assessed from plasma. Dietary intake was assessed using food records (FRs) three days before each visit. In addition, weight, height, body mass index (BMI), and plasma levels of estradiol, glucose, HbA1c, insulin, and lipids were monitored. Twenty-six of the subjects also had a postprandial test, in which the blood samples were taken at five time points at every three visits. They also filled visual analog scales to exhibit satiety and appetite. Twenty-eight of the subjects had only fasting blood samples.

**Results:** During the stimulation protocol, leptin concentrations elevated ( $P<0.001$ ), and energy intake decreased ( $P=0.03$ ) while estradiol levels increased ( $P<0.001$ ). GLP-1 levels unchanged ( $P=0.75$ ) and hs-CRP ( $P=0.03$ ) concentrations diminished while estradiol levels increased.

**Conclusions:** No increased food intake or weight gain occurred during the stimulation protocol; thus, leptin may protect from overeating during high estradiol levels, and leptin resistance may not occur during a short follow-up. Also, a favorable anti-inflammatory effect was detected. During this study, no harmful metabolic effects occurred, which might have a disproportionate impact on maternal health.

**Trial registration:** Not applicable. This study is a clinical study without intervention.

## Background

Sex hormones have essential roles in regulating appetite, food intake, and energy metabolism by interacting with gastrointestinal peptides, neurotransmitters, and adipocytes [1, 2]. Estrogen inhibits food intake, but in the presence of estrogen, progesterone enhances appetite and promotes weight gain [2]. Thus, food intake varies during the menstrual cycle [2]. A mean food intake is reduced during the periovulatory phase of the menstrual cycle when estradiol levels are high [3, 4].

Adipocytes produce leptin. It inhibits food intake and involves energy expenditure, storage of fat, and insulin signaling [5]. When bodyweight is steady, leptin levels are an indicator of body fat mass. In turn, during weight loss or weight gain phases, leptin signals for an energy imbalance. Leptin informs the central nervous system about the abundance and availability of energy deposits [6]. Decreased leptin

concentrations induce hunger, whereas high leptin concentrations in obese individuals due to chronic overfeed lead to leptin resistance [5].

Glucagon-like peptide-1 (GLP-1) is released in response to a meal from the intestinal mucosa enteroendocrine cells. It is one of the gut peptides that increases satiety and suppresses appetite [7]. In addition, it stimulates insulin release in pancreatic  $\beta$ -cells [7].

Hs-CRP is a marker of low-grade inflammation, and it is chronically elevated in patients with atherosclerosis and obesity [8]. It is a robust independent predictor of future atherothrombotic events among people without known cardiovascular disease [8]. Hs-CRP facilitates the adhesion and migration of monocytes into the arterial wall, and it also has an inhibitory effect on nitric oxide synthesis following altered vascular reactivity [6].

Estradiol levels are deficient at the beginning of a menstrual cycle and the beginning of a long agonist IVF protocol while patients use GnRH-agonist medication. After that period, patients start FSH-stimulation, and estradiol levels exaggerate.

Our study aimed to examine appetite regulation, food intake, and release of leptin, GLP-1, and hs-CRP in different hormonal phases during a long agonist IVF protocol. Does the long agonist protocol induce metabolic changes, affecting maternal health?

## Methods

### Participants

We studied 54 infertile women, ranging in age from 24 – 40 years ( $33 \pm 4$  years, mean  $\pm$  SD), who attended a long agonist IVF protocol in the Kuopio University Hospital infertility clinic. Subjects were recruited from the infertility clinic while meeting the doctor for IVF treatment planning. Twenty-six of the subjects participated in a postprandial test, and the rest twenty-eight patients had only fasting blood samples of the different variables.

### Study protocol and meal

The subjects were encountered thrice. The first time (Visit 1) was at the beginning of the menstrual cycle without any hormonal treatment (from the second to the fifth day of the period). Then, agonist medication (GnRH-analogue, nafarelin acetate 800 mcg/day) was initiated approximately one week before the next period. The patient came to the subsequent control (Visit 2) about one month after Visit 1. Then patient's estradiol level was supposed to be very low, like in postmenopausal level. In Visit 2, subjects started the controlled ovarian stimulation with FSH. The FSH dose varied from 125 IU to 300 IU, and it was adapted to the patient's body mass index (BMI), ovarian reserve, and age. In Visit 3, subjects met the doctor for the third time after administering FSH daily for nine days. Then the estradiol level was supposed to be ascended compared to levels in the menstrual cycle. Blood samples and clinical

measurements were obtained at every three visits. For the hormonal intervention, primary outcome variables were the concentrations of leptin, GLP-1, hs-CRP, and the satiety feeling.

For 26 of the 54 patients, a postprandial test was performed. First, fasting samples were taken (0 minutes) and then subjects ate a standard breakfast (185 grams (g) porridge, 150 g yogurt, and a glass of water). Next, they had a cannula in the antecubital vein, and a nurse took blood samples at 30, 60, 120, and 240 minutes (min). At all three visits, plasma levels of leptin, GLP-1, and hs-CRP were determined at those five time points.

Secondary outcomes were serum levels of glucose, HbA1c, insulin, estradiol, and plasma lipid levels. If data from three visits was missing, the patient was excluded from the study, except levels of glucose and HbA1c (n=53), insulin (n=48), and leptin (n=44).

Height and weight were measured at each visit, and BMI was calculated. All laboratory tests and data about appetite profile and dietary intake were blindly analyzed by personnel who had no information of the IVF cycle phase.

#### Appetite profile and dietary intake

Patients (n=26), who participated in postprandial tests, filled visual analog scales to exhibit the appetite profile, satiety, and desire to eat. Scales were filled before the meal (0 min) and at four time points (30, 60, 120, and 240 min). Dietary intake was assessed using food records (FRs), in which food and beverage intakes were reported for three consecutive days before each study visit. Participants who failed to fill FRs beforehand were advised to fill their FR on the days following the study visit. Portion sizes were weighed or estimated using household measures. Thirteen participants who failed to fill all three FRs were excluded from the nutrient intake analyses. Nutrient intake from the diet (i.e., vitamin and mineral supplements excluded) was calculated using AivoDiet software (version 2.2.0.0., AivoFinland Oy, Turku, Finland) based on national and international analyses, and international food composition tables (fineli.fi).

#### Blood samples

Overnight fasting blood samples (12h) were taken and analyzed in the laboratory. Samples were centrifuged at 2000 rpm for 10 minutes, and serum plasma was separated. All lipid, estradiol, and hs-CRP determinations were analyzed using standard methods, as previously described [9, 10]. GLP-1 was analyzed with Cloud-Clone Corp., ELISA (LOT: L190104265, Exp. Sep. 2019), with a working range of 12.35 pg/ml-1000 pg/ml. Leptin was determined with R&D systems (a Bio-technie brand, Quantikine ELISA. Catalog No. DLP00, LOT: P221489, Exp. 03 Nov 2020), with measurement lower boundary under 7.8 pg/ml. Glucose was analyzed by enzymatic method with hexokinase using Cobas 6000 (c 501) analyzer (Hitachi High Technology Co, Tokyo, Japan). Reagent assessed was Glucose HK (GLUC3), (cat. nro 04404483 190, Roche Diagnostics GmbH, Mannheim, Germany. ACN 717). The measuring range was 0.1-41.6 mmol/l. HbA1c was determined by Turbidimetric inhibition immunoassay (TINIA) with Cobas

6000 –analyzer (Hitachi High Technology Co, Tokyo, Japan). Reagents used: 1. Tina-quant Hemoglobin A1c Gen.3 (A1C-3), (Cat. no. 05336163 190, Cobas c systems, Roche Diagnostics GmbH, Mannheim, Germany), 2. Hemolyzing Reagent Gen.2 (A1CD2), (Cat. No. 04528182 190, Cobas c systems, Roche Diagnostics GmbH, Mannheim, Germany), and 3. Special Cell Cleaning Solution (SCCS), (Cat. No. 04880994 190, Cobas c systems, Roche Diagnostics GmbH, Mannheim, Germany). Measuring range was Hb 40-400 g/l, HbA1c 3.0-26.0 g/l, unit mmol/mol. Insulin was assessed by ECLIA with a Cobas e 601 - analyzer (Hitachi High Technology Co, Tokyo, Japan). Reagent assessed was Insulin, (cat nro 12017547 122, Roche Diagnostics GmbH, Mannheim, Germany). The measuring range was 0.2-1000 mU/l.

## Statistical analyses

Statistical analyses and calculations were done with IBM SPSS Statistics (version 27 for Macintosh, Armonk, NY.). Descriptive data are expressed as means $\pm$ SD in tables and as means $\pm$ SEM in figures. Kolmogorov-Smirnov-test was performed to test distribution's normality. This test exhibited skewed distributions in main variables, and statistical analyses were accomplished with non-parametrical tests. Friedman's test was used to detect the difference in the values between separate time points. If the significance was observed between time points, Wilcoxon's test analyzed differences between two time points. Univariate correlations were defined using Spearman correlation.  $P < 0.05$  was considered statistically significant.

Dietary intake data were analyzed using IBM SPSS Statistics (version 27, IBM inc., Armonk, New York) and RStudio Version 1.4.1106 (RStudio, Inc.) software. Shapiro-Wilk test was used to assess the normality of the data. Non-normally distributed variables were log<sub>10</sub>-transformed. Repeated measures ANOVA and Friedman test were used for normally and non-normally distributed variables, respectively. Bonferroni correction was applied to all posthoc tests. A two-tailed P-value of  $< 0.05$  was considered statistically significant.

## Results

The clinical characteristics in visits 1, 2 and 3, and responses to a long agonist IVF protocol are shown in Table 1.

The values in leptin, GLP-1, and hs-CRP at three visits are shown in Figure 1. Leptin levels varied significantly during the IVF stimulation, and those levels were at the lowest in Visit 2. The highest leptin levels were reached at the third Visit. The changes in GLP-1 values were statistically non-significant. The values in hs-CRP declined significantly throughout the protocol from visit 1 to visit 3.

The changes in insulin and HbA1c levels were non-significant (Table 1). Fasting glucose levels diminished between Visits 2 and 3 (Table 1).

Lipid profile changed through Visits 1, 2, and 3 (Table 1). Total and LDL cholesterol concentrations decreased towards the end of stimulation, and also, the total/HDL cholesterol ratio diminished between

Visits 2 and 3. However, HDL cholesterol and triglyceride level were unchanged significantly.

The values of leptin, GLP-1, and hs-CRP during a postprandial test in three visits are presented in Figure 2.

The changes in GLP-1 levels in three visits were non-significant.

The number of patients for leptin values in the postprandial test were 22, 22, and 21 patients in Visits 1, 2, and 3 since some values were missing. Leptin levels were unchanged in Visit 1. In Visits 2 and 3, leptin values changed significantly. The most notable change in leptin values in Visit 2 was between 30 and 60 min ( $P=0.02$ ), and in Visit 3, between 0 and 30 min ( $P=0.04$ ).

The hs-CRP values decreased in Visits 1 and 2, but at the third Visit, the change was non-significant. The most notable change in hs-CRP levels in Visit 1 was between 0 and 30 min ( $P=0.002$ ) and in Visit 2 between 0-240 min ( $P<0.001$ ).

The changes in nutrient intake are presented in Table 2 and Figure 3. The energy intake differed significantly between Visits 1 and 3, and it was at the lowest during the third Visit. The intake of energy nutrients and fiber was unchanged between the three Visits. Totally 21 patients reported using alcohol during stimulation, and intake diminished significantly during three Visits ( $P=0.02$ ), and only seven persons reported using alcohol at Visit 3.

The GLP-1 values correlated only with HbA1c in Visit 3 ( $r=0.279$ ,  $P=0.04$ ). No correlations between GLP-1 and other variables existed.

Statistically significant correlations between leptin and hs-CRP and different variables are presented in Table 3.

## Discussion

We found out that during the stimulation phase of a long agonist IVF protocol, when the estradiol level was low and then increased, leptin concentrations peaked, and energy intake decreased. GLP-1 values unchanged during a long agonist protocol. Toward the end of stimulation, hs-CRP concentrations decreased. During a postprandial test, GLP-1 levels remained. Leptin values varied significantly during low and high estradiol levels. Hs-CRP values descended during the low estradiol levels.

Our findings are novel, with no earlier studies about appetite regulation and food intake during a long agonist IVF protocol. Therefore, we had to compare our results to other hormonal statuses when estradiol levels are high and low. Hormonal fluctuations during the menstrual cycle influence appetite control and food intake [4, 11]. A mean food intake decreases at the periovulatory phase with high estradiol levels [2]. That finding is similar to our results.

During the menopausal transition, weight gain is common, and total and abdominal fat increases [2]. In our study, the weight remained, which may arise due to a short follow-up period. However, Reimer et al. (2005) have published that nutrient and energy intake is similar between postmenopausal women using or not using hormonal treatment [12]. That result differs from our findings of increased energy intake during the low estradiol level compared to the high level.

Food intake and appetite increase during pregnancy, and those increases are due to a rise in progesterone levels and high estradiol levels [13]. Also, leptin levels increase during pregnancy, and resistance to central anorectic actions of leptin occurs [14]. Those findings differ from our results about increasing leptin levels and diminished energy intake during the higher estradiol levels. We did not determine progesterone levels in our study. Still, the rise in those levels during a long agonist treatment is usually moderate at most, and their impact on appetite may appear minor [15, 16].

It has been shown that leptin is necessary for human reproduction since starvation-induced leptin suppression and genetic models of leptin deficiency are associated with hypogonadism due to hypothalamic GnRH deficiency [17]. Leptin also modulates steroidogenesis in human ovaries [17]. In our study, the secretion of GnRH was blocked first, and then exogenous FSH was used, which induced a rise in estradiol levels and also an increase in leptin levels; hence our findings were similar. Results about leptin levels during the menstrual cycle are controversial, but some studies have published a slight increase in leptin during the late follicular phase compared with the early follicular phase. That finding is similar to our conclusions about leptin [18, 19]. It has been demonstrated that leptin levels remained relatively constant for two hours postprandially in lean subjects and decreased gradually over the first 90 min in obese subjects [20]. Our results were different, with the most notable decrease between 30 and 60 minutes in Visit 2 and between 0 and 30 minutes in Visit 3. Anyhow, we did not separate obese and lean subjects, and the median BMI was 25.3.

GLP-1 is an anorexigenic hormone, and it increases insulin secretion from the pancreatic b-cells, satiety and suppresses appetite [7, 21]. GLP-1 and insulin levels remained in our study, and energy intake diminished during the stimulation while rising estradiol levels. Evidence about GLP-1's stimulatory impact on a hypothalamic-pituitary level of the reproductive axis in animals exists [17]. GnRH agonist is used in a long agonist protocol, and the secretion of endogenous FSH and LH is blocked, and then exogenous FSH is used. The reason why GLP-1 levels remained in our study remains unclear. Also, the postprandial GLP-1 levels remained, and that finding differs from the previous results. It has been shown that GLP-1 levels rise pretty fast (10 min) postprandially [7, 20]. On the other hand, leptin has been shown to interact with GLP-1 and its receptor antagonist to induce satiation [22]. Leptin and GLP-1 seem to play an important role together in modulating appetite [22]. However, in our study, leptin levels peaked during the high estradiol level, whereas GLP-1 remained.

Toward the end of stimulation, while estradiol levels rose, proinflammatory factor hs-CRP diminished. This result conflicts with previous findings [23, 24]. They concluded that controlled ovarian stimulation

induces a state of systemic inflammation. An inflammatory response may also be stimulated by IVF-related treatments [25, 26]. Our findings are, however, contrary.

Leptin correlated significantly with insulin, lipids, weight, and hs-CRP in our study. Leptin has a similar anorexigenic effect than insulin has, and it is involved with insulin signaling, which explains the positive correlation [1, 5]. Leptin levels are associated with a body fat mass, and during a weight gain, leptin levels rise [6]. These things explain the positive correlation between leptin and lipids or weight in our results. The positive correlation between leptin and hs-CRP remains unclear in our study. Hs-CRP correlated negatively with HDL cholesterol concentration and positively with triglyceride concentration and weight. As a marker of low-grade inflammation, the hs-CRP level is chronically elevated in people with obesity, which may explain the positive correlation. Hs-CRP also correlated negatively with insulin at Visit 3, and this finding remains unclear in our results. Fasting glucose levels decreased between Visits 2 and 3, and lipid profile improved during the stimulation, and these findings support our earlier results about the safety of IVF stimulation [9, 10].

The validity of this study can be considered good since all these patients underwent the same IVF protocol in one unit, and they served as their controls. All laboratory tests and data about appetite profile and dietary intake were blindly analyzed by personnel who had no information of the IVF cycle phase. The diet was reported with eating diaries three days before each Visit, hence the impact of a long agonist protocol on food intake could be examined. The cause of underlying infertility (for example, obesity, polycystic ovaries syndrome) may play a role in appetite regulation. A limitation of this study is the relatively small study population; limited statistical power in the analyses can affect our results.

## Conclusion

We found that, during a long agonist IVF protocol, the energy intake increased when the estradiol level was first low. During the stimulation, estradiol levels rose, leptin concentrations elevated, energy intake decreased, and weight remained. Thus, leptin may protect from overeating during the high estradiol levels, and leptin resistance may not occur during a short period. Also, the fasting glucose decreased, and lipid profile improved during the high estradiol levels. All these metabolic changes could be considered favorable regarding the possible IVF pregnancy. In addition, hs-CRP concentrations diminished while estradiol levels increased. Thus an anti-inflammatory response was detected in our study. These results support our earlier findings on the safety of IVF stimulation.

## Abbreviations

GLP-1 = Glucagon-like peptide-1

hs-CRP = high-sensitivity C-reactive protein

IVF = *In vitro* fertilization



FSH = Follicle stimulating hormone

GnRH = Gonadotrophin-releasing hormone

BMI = Body mass index

NS = non-significant

LDL = Low-density lipoprotein

HDL = High-density lipoprotein

SD = Standard deviation

SEM = Standard error of mean

## **Declarations**

### **Ethics approval and consent to participate**

The study protocol was approved by the Ethics Committee of the Northern Savo Hospital District.

### **Consent for publication**

Written informed consents were obtained on forms.

### **Availability of data and materials**

Not applicable.

### **Competing interests**

None of the authors have competing interests.

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J.L. has received the Medical Society of Finland's Grant for this study (grant number 3368, 2019). J.L. has worked as a half-time researcher at the University of Eastern Finland 2021.

### **Authors' contributions**

J.L. conceived the study, collected and analyzed the data and wrote the paper. P.N. analyzed nutritional part of the data and wrote that part of the paper. K.R., S.H., T.L., U.S., and J.P. conceived the study and participated on writing. L.K.-N. participated writing the paper. J.R. checked the laboratory analyses and participated on writing.

### **Acknowledgements**

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## Tables

Due to technical limitations, table 1 and 3 is only available as a download in the Supplemental Files section.

Table 2. Changes in nutrient intake based on 3-day dietary records (n = 52).

Visit(s)	Mean $\pm$ SD or median (Q1; Q3)			p-value	p-value of post-hoc tests <sup>a</sup>		
	1	2	3		1 vs. 2	2 vs. 3	1 vs. 3
Energy (kcal)	1940 $\pm$ 422	1914 $\pm$ 426	1795 $\pm$ 337	<b>0.025</b>	1.000	0.172	<b>0.026</b>
Energy (kJ)	8112 $\pm$ 1776	8006 $\pm$ 1781	7506 $\pm$ 1408	<b>0.025</b>	1.000	0.166	<b>0.027</b>
Protein (E%) †	18.1 $\pm$ 4.0	18.1 $\pm$ 3.6	17.8 $\pm$ 3.8	0.690	–	–	–
Carbohydrate (E%)	43.8 $\pm$ 6.7	43.3 $\pm$ 7.1	44.2 $\pm$ 6.9	0.688	–	–	–
Total fat (E%)	35.3 $\pm$ 5.9	35.5 $\pm$ 7.3	35.4 $\pm$ 6.9	0.977	–	–	–
UFA (% of fat) ‡	57.0 $\pm$ 6.2	56.1 $\pm$ 6.7	57.5 $\pm$ 7.4	0.584	–	–	–
Cholesterol (mg) †	232 $\pm$ 88	246 $\pm$ 104	242 $\pm$ 100	0.742	–	–	–
Fiber (g)	21.8 $\pm$ 6.9	20.6 $\pm$ 6.9	19.7 $\pm$ 6.3	0.103	–	–	–

UFA = unsaturated fatty acids; <sup>a</sup> Bonferroni-corrected pairwise comparison between visits; † variable has been log<sub>10</sub>-transformed; ‡ Friedman test was used.

## Figures

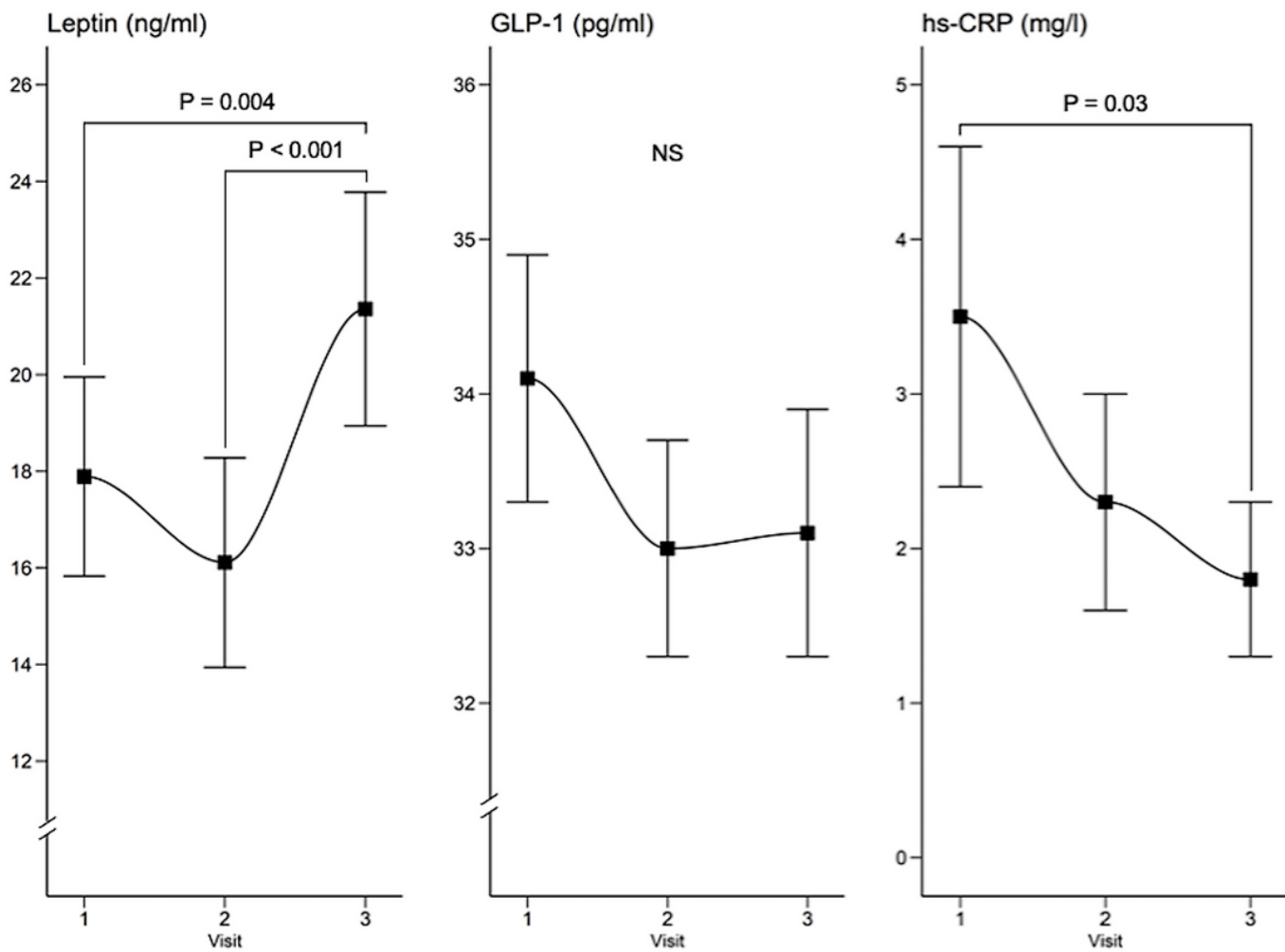


Figure 1. The values in leptin, glucagon-like peptide 1 (GLP-1), and high-sensitivity C-reactive protein (hs-CRP) at three Visits. The values are as means  $\pm$ SEM

## Figure 1

See image above for figure legend

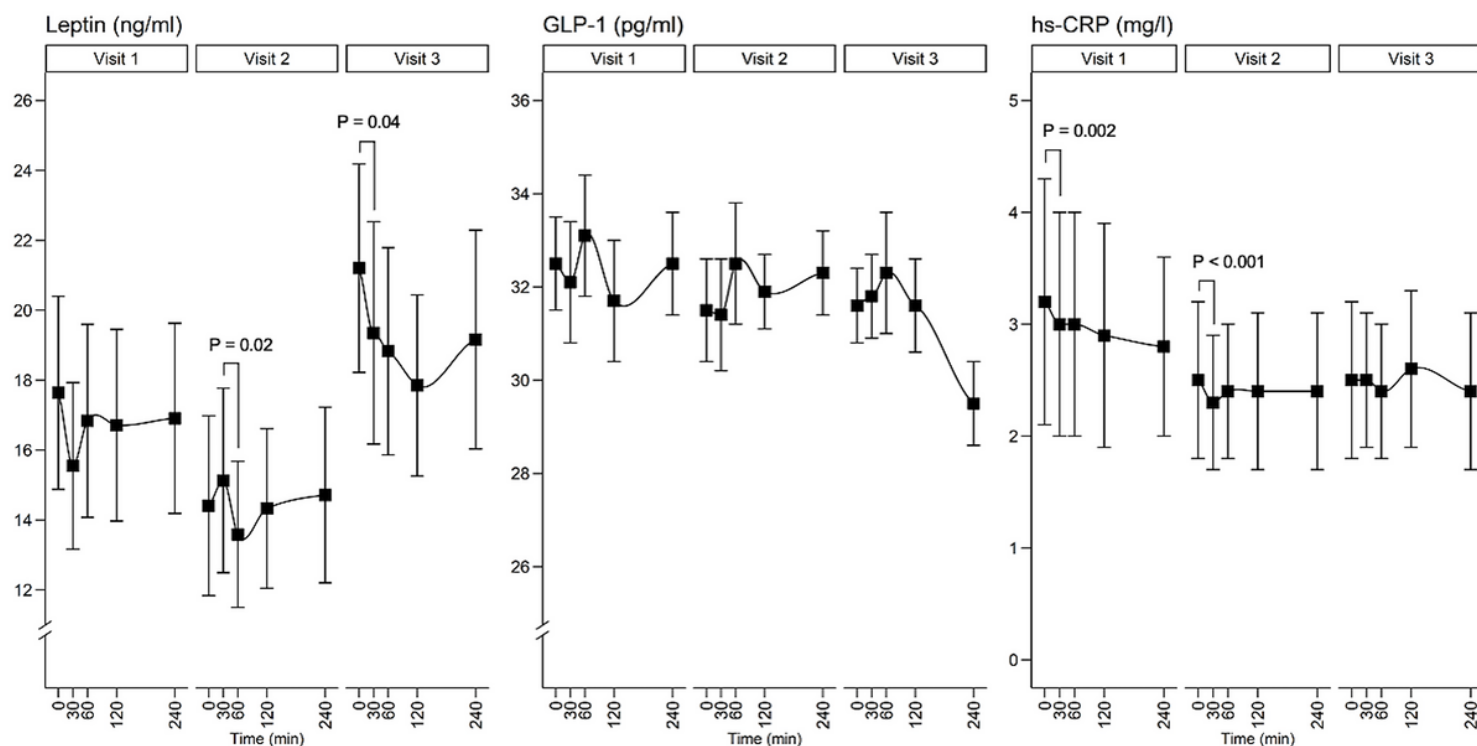
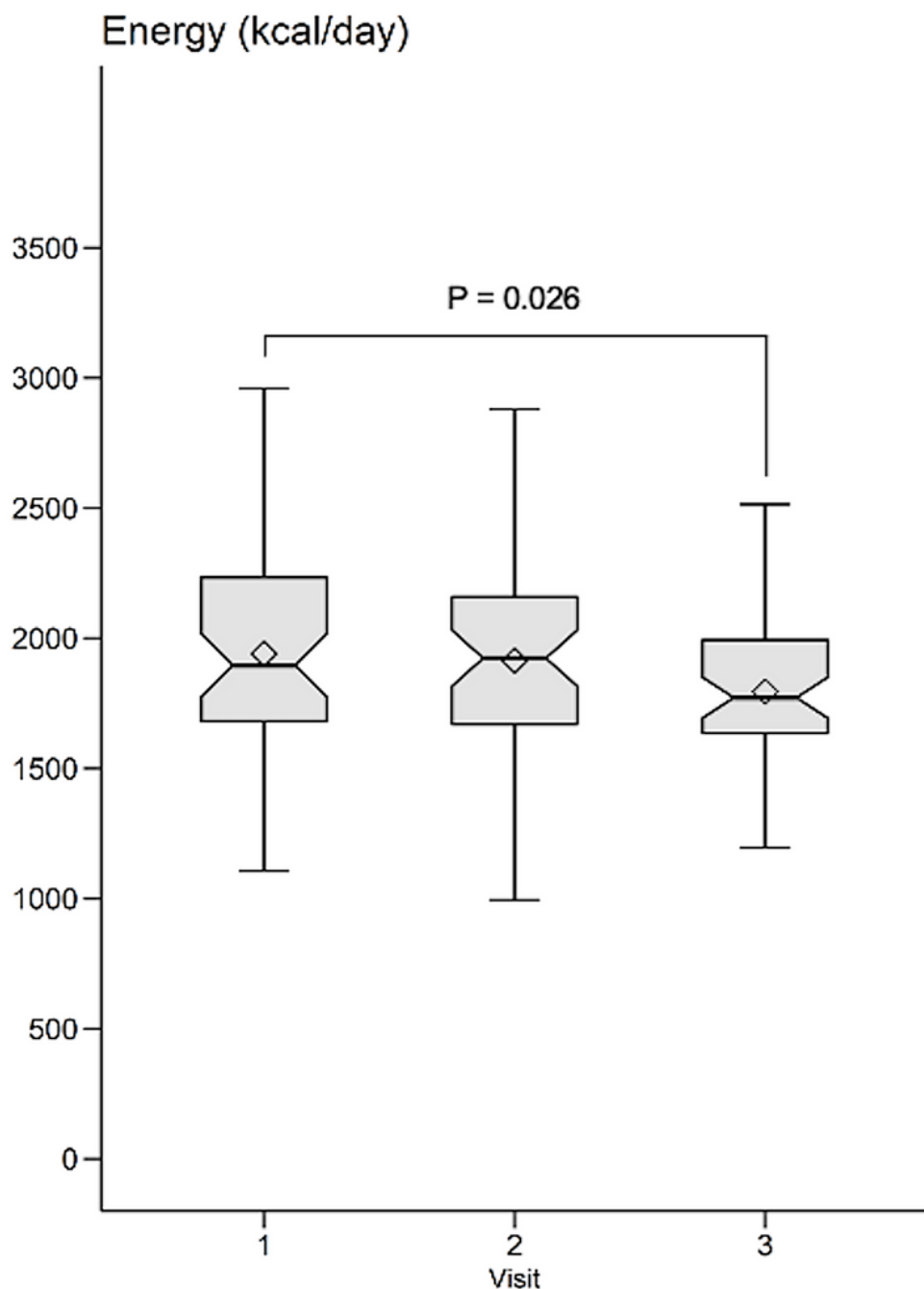


Figure 2. The values in leptin, glucagon-like peptide 1 (GLP-1), and high-sensitivity C-reactive protein (hs-CRP) during the postprandial test in three Visits. The values are as means  $\pm$  SEM.

Figure 2

See image above for figure legend



**Figure 3.** The changes in energy intake in three Visits. The values are as means  $\pm$  SEM.

### Figure 3

See image above for figure legend

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Table13a.docx](#)
- [Table33art.docx](#)