

Temporal effects of laparoscopic sleeve gastrectomy on adipokines, inflammation and oxidative stress in subjects with impaired glucose homeostasis

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Conflicts of interest: None.

Funding: This study was supported by a project Research Grant from The BUPA Foundation (33NOV06).

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Short title: Sleeve gastrectomy, inflammation & oxidative stress

Abstract

Background: Bariatric surgery is an effective treatment for morbid obesity and metabolic dysfunction.

Objectives: The aim of this work was to examine the early temporal effects of laparoscopic sleeve gastrectomy (LSG) on adipokines (adiponectin, leptin), inflammatory cytokines (IL6, CRP, IL10), and global plasma measures of oxidative stress (thiobarbituric acid reactive substances [TBARS] and total antioxidant status [TAOS]) in a sample of 55 participants pre-operatively, and 1 month and 6 months post-operatively. The focus was on a sample of patients with impaired glucose tolerance and type 2 diabetes, which is associated with increased low-grade systemic inflammation and oxidative stress.

Setting: University Hospital, United Kingdom.

Methods: This was a prospective study comprising of 55 participants with impaired glucose homeostasis and type 2 diabetes undergoing LSG (mean body mass index [BMI] 50.4kg/m², mean glycated hemoglobin [A1c] 7.4%). Serial measurements of the above markers were made pre-operatively, 1 and 6 months post-operatively (43 had measureable cytokines and oxidative stress at 1 and 6 months follow-up).

Results: We observed a significant reduction in IL6, CRP, leptin and TBARS; along with an increase in adiponectin 6 months post-operatively.

Conclusions: To our knowledge the effects of LSG on inflammatory cytokines and plasma markers of oxidative stress have not been examined temporally in a sizeable sample of participants who have undergone LSG. This current study supports the

role of LSG for the treatment of the pro-inflammatory and pro-oxidant status associated with obesity-related glucose dysregulation.

Keywords: Type 2 diabetes, Impaired glucose tolerance, Inflammation, Oxidative stress, Laparoscopic sleeve gastrectomy.

Introduction

Obesity and its related metabolic complications are associated with chronic low-grade systemic inflammation, abnormalities in adipokines and increased oxidative stress. Bariatric surgery results in improvements in metabolic dysfunction, including type 2 diabetes mellitus (T2D), and its associated complications and cardiovascular risk factors ^[1]. This beneficial effect occurs through a reduction in adipose tissue mass, improvement in cardiovascular and endothelial function, and is likely to be modulated by improvements in the inflammatory milieu ^[2, 3]. Within the available literature relating to chronic low-grade systemic inflammation, the most studied obesity-related inflammatory markers include: the adipokines, leptin and adiponectin, which are cytokines secreted by adipose tissue that modulate the immune response, insulin sensitivity and energy balance; and the obesity-related inflammatory cytokines interleukin-6 (IL6), interleukin-10 (IL10) and C-reactive protein (CRP) ^[4-6]. The plasma levels of these obesity-related inflammatory biochemicals are associated with adipose tissue mass ^[7, 8]. Whilst the available literature has examined the effects of bariatric surgery on metabolic outcome, there remains a deficit in published studies examining the effects on inflammation, adipokines and plasma markers of oxidative stress. Previous reports have shown that Roux-en-Y gastric bypass (RYGB) ^[9-11] is associated with improvements in metabolic inflammatory markers. With respect to laparoscopic sleeve gastrectomy (LSG) and its effects on obesity-related inflammatory cytokines, the available literature contains limited information with small study numbers ^[12-15]; or with a single measure of inflammation ^[16]; or where LSG has been analyzed in combination with other

bariatric procedures ^[17]. With respect to plasma markers of oxidative stress, controversy exists within the available literature. Catoi *et al*, observed no change in global measures of oxidative stress (nitrite and nitrate [NOx], total oxidant status [TAOS/TOS], total antioxidant response [TAR], and oxidative stress index [OSI]) 6 months after LSG ^[18]. Banazadeh *et al*, observed a significant reduction in pro-oxidant antioxidant balance (PAB), 6 months after RYGB ^[19]. Similarly, Schmatz *et al* reported a reduction in the concentrations of lipid peroxidation, as well as increased superoxide dismutase (SOD) and catalase (CAT) activity following RYGB ^[20].

Our aim was to examine the early temporal effects of LSG on adipokines (adiponectin, leptin), inflammatory cytokines (IL6, CRP, IL10), and global plasma measures of oxidative stress (thiobarbituric acid reactive substances [TBARS] and total antioxidant status [TAOS]) in a sample of 55 participants temporally at 1 month and 6 months post-operatively. The focus was on a sample of patients with impaired glucose tolerance and T2D, which are associated with increased low-grade systemic inflammation and oxidative stress ^[21].

Methods

Study participants

Approval for the study was obtained from the Local Research Ethics Committee. Participants were identified and recruited from patients undergoing a planned bariatric surgical procedure at our locality. Entry criteria at the outset of the study included:- both sexes, age 20-60 years, body mass index (BMI) >40kg/m² and

physically fit for surgery. Participants with any acute concurrent illness were excluded. Participants with pre-existing T2D treated with diet, oral agents, GLP-1 analogues or insulin were included. Participants with impaired glucose regulation were those with either impaired fasting glycaemia (5.6-6.9 mmol/L) or impaired glucose tolerance (2-hour glucose 7.8-11.0 mmol/L) [22]. Participants with normal fasting glucose values or a normal glucose tolerance test prior to recruitment were excluded. This study was an extension of a previous study [13] where 22 participants were recruited. The current study included 55 participants.

Study design

Participants with a planned LSG were recruited prospectively and consecutively from the bariatric surgical clinic. LSG was a standard sleeve i.e. sleeve fashioned around a 32F bougie taken from 5cm proximal to the pylorus and up to the left crus. All participants were recruited pre-operatively and followed up post-operatively at 1 and 6 months. All participants, with the help of the research nurse, completed a baseline questionnaire and all clinical measurements were documented during the visits. All blood samples were collected after stopping any prescribed insulin or oral hypoglycaemic agent for 24 hours prior to an oral glucose tolerance test (OGTT) performed with 75g of glucose (122mL Polycal; 61.9g/100mL glucose, Nutricia Clinical Care, Trowbridge, UK).

Baseline clinical and biochemical information

At the time of first study visit the following clinical information was ascertained: age, gender, past medical history, treatment and duration of diabetes. Baseline clinical measurements consisted of weight, height, BMI, waist circumference, systolic and diastolic blood pressure. Baseline biochemical measurements (glucose, total cholesterol, low density lipoprotein-cholesterol [LDL-C], high density lipoprotein-cholesterol [HDL-C] and triglycerides) were analyzed within the local hospital accredited laboratory (Roche Modular P800 Analyzer). Insulin was measured using an Invitron Insulin luminescence immunometric assay. The analytical sensitivity of the assay was 0.02mU/L with a dynamic range of 0.02-250mU/L. The inter-assay coefficient of variation was $\leq 7.1\%$. Cross reactivities of related proteins were as follows: 1.2% with intact proinsulin and 0% with C-peptide.

The Homeostasis Model Assessment (HOMA) was used to estimate steady state beta cell function (%B) and insulin sensitivity (%S). These were calculated using the Oxford University on-line calculator (<http://www.dtu.ox.ac.uk/homacalculator>, accessed 1st June 2015).^[23] HOMA-insulin resistance (IR) is the reciprocal of HOMA-%S. The HOMA-%B and HOMA-%S represent values of 100% in normal young adults when using currently available assays for insulin, specific insulin or C-peptide. The accuracy of these measures has been validated and they have been shown to correlate with clamp-derived indices of insulin sensitivity and secretion.^[24] They estimate steady state function.

All blood samples were collected on ice, centrifuged and separated within one hour of collection and subsequently stored at -80°C until analysis. Fasting EDTA samples

were collected for the measurement of cytokines and markers of oxidative stress during the OGTT at baseline, 1 and 6 months.

Measurement of total cytokines

Fasting plasma levels of IL6, IL10 and leptin were measured with high sensitivity ELISA kits (R&D Systems). Intra-assay and inter-assay variability coefficients were as follows: IL6 $\leq 4.2\%$ and $\leq 6.4\%$; IL10, $\leq 5.0\%$ and $\leq 7.5\%$; leptin, $\leq 3.3\%$ and $\leq 5.4\%$. Fasting plasma levels of total adiponectin and CRP were measured with high sensitivity ELISA kits (Immundiagnostik AG). Intra-assay and inter-assay variability coefficients were as follows: adiponectin, $\leq 3.4\%$ and $\leq 6.3\%$; CRP, $\leq 6.0\%$ and $\leq 13.8\%$. All samples were assayed in duplicate.

Measurements of plasma markers of oxidative stress

Measurement of lipid peroxidation (TBARS)

Plasma malondialdehyde (MDA) concentration, as a product of lipid peroxidation, was measured using a commercially available TBARS Assay (thiobarbituric acid reactive substances assay) (Caymen Chemical, MI, USA). Using an MDA standard curve, concentrations in plasma samples were calculated. A higher concentration of MDA is indicative of higher levels of lipid peroxidation, and therefore higher oxidative stress within the sample. Intra-assay and inter-assay variability coefficients were 5.2% and 16.2% respectively. All samples were assayed in duplicate [25, 26].

Measurement of plasma total anti-oxidant status (TAOS)

Plasma total anti-oxidant status (TAOS) [27, 28], which is inversely related to oxidative stress, was measured by Sampson's modification of Laight's photometric microassay [29]. Previously, it has been shown that plasma TAOS has a good correlation with plasma F₂-isoprostanes [27]. The TAOS of plasma was determined by its capacity to inhibit the peroxidase-mediated formation of the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS⁺) radical. The difference in absorbance (control [saline] minus test [plasma sample]) divided by the control absorbance (expressed as a percentage) was used to represent the percentage inhibition of the reaction. Intra-assay and inter-assay variability coefficients were 4.3% and 10.1% respectively. All samples were assayed in duplicate.

Statistical methods

Statistical analysis was performed using SPSS (version 25, SPSS Inc., Chicago). Results for continuous variables are presented as mean and standard deviation and in graphical representation as median and interquartile range. Continuous variables that did not have a normal distribution (HDL-C, triglyceride, fasting plasma glucose, 2-hour plasma glucose, A1c, fasting insulin, 2-hour insulin, HOMA measurements, CRP, IL6, IL10, adiponectin, leptin, TBARS, TAOS) are described with the median and interquartile range. For continuous variables, the mean temporal changes were compared between baseline and 1 or 6 months using a paired t-test. The Wilcoxon signed-rank test was used to compare the temporal changes in variables that did not

have a normal distribution. Of the 55 participants, 43 had measureable results of plasma cytokines and oxidative stress at 1 and 6 months follow-up. The losses were due to inadequate sample collection during venesection or failure of analytical analysis of samples from 1 or 6 months. Correlations were performed using Spearman correlation. In all cases a $p < 0.05$ was considered statistically significant.

Results

Participant characteristics

A total of 55 participants (31 females and 24 males) with impaired glucose homeostasis (n=13) or T2D (n=42) and underwent LSG, completed the study with a mean age of 46 ± 8 years. The baseline characteristics, along with the changes in anthropometric and clinical measures, are summarised in Table 1. As can be observed, significant reductions were observed at 1 and 6 months following LSG for measures of obesity, plasma triglyceride and a significant increase in HDL-cholesterol. In addition, significant reductions were observed in fasting plasma glucose, 2-hour plasma glucose, A1c, fasting, 2-hour insulin levels along with HOMA-IR and HOMA-%S.

Temporal changes in adipokines, inflammatory cytokines and plasma markers of oxidative stress following LSG

As shown in Figure 1, we observed a significant reduction in median leptin of approximately 26% at 1 month and 53% at 6 months. There was an approximate 44% increase in adiponectin levels observed at 6 months. We also observed a significant reduction in IL6, 1 month following surgery, with a continued decrease at 6 months. CRP also showed a linear decrease post-operatively with a significant reduction at 6 months. No change was seen in IL10. With respect to plasma markers of oxidative stress, there was a temporal linear decrease in TBARS with a significant reduction observed at 6 months (Table 2).

Discussion

Bariatric surgery reduces morbidity and mortality in severely obese individuals with favorable effects on T2D, hypertriglyceridemia and hypertension. There is growing evidence to support the hypothesis that this is likely to be related to an improvement in the inflammatory profile caused by the rapid and significant reductions in fat mass following surgery [30]. LSG has gained recent popularity as an independent bariatric procedure [30, 31]. We observed significant improvements in CRP, IL6, adiponectin and leptin, 6 months following LSG. Circulating levels of IL6 are raised in insulin resistant states such as obesity [32], impaired glucose tolerance [33] and T2D [34-36]. We observed no difference in IL10, which is in line with our previous study [13] and a study demonstrating that changes in IL10 mRNA expression were not observed until 12 months following LSG [37].

We also examined the correlations between the change (Δ values) in measures of obesity and inflammatory biomarkers. Of interest we observed no significant correlations between the changes in weight and BMI with inflammatory cytokines (BMI with CRP [$r=0.25$, $P=0.13$], IL6 [$r=-0.03$, $P=0.85$], adiponectin [$r=-0.16$, $P=0.33$], leptin [$r=0.13$, $P=0.41$]). This observation is consistent with the findings of Catoi *et al* [18]. We did observe correlations between the Δ values for HOMA-%S and leptin ($r=-0.37$, $P=0.03$) and CRP ($r=-0.48$, $P=0.006$). As expected Δ CRP and Δ IL6 had a significant correlation ($r=0.45$, $P=0.003$).

With respect to plasma markers of oxidative stress, there was a temporal linear decrease in TBARS with a significant reduction observed at 6 months. No significant change was observed in plasma TAOS. This result has been observed previously by us [38] and other authors [18, 39, 40]. Plasma TAOS is a measure of global plasma antioxidant status and reactive oxygen species (ROS). There has been considerable debate within the literature in relation to the biochemical measurement of plasma oxidative stress. By definition, ROS are highly reactive and are thus difficult to measure in any biological sample, especially in easily accessible ex-vivo specimens such as serum or plasma [21]. Published studies utilize different techniques such as measuring a marker of global damage (TAOS, TAS), or a marker of end-damage such as TBARS or specific antioxidant molecules (SOD, glutathione). These observations suggest that measuring markers of global antioxidant status may not be the best measure of plasma oxidative stress in subjects with morbid obesity and where a relatively high BMI remains present. A recent study examining the measurement of urinary F₂-isoprostanes demonstrated a reduction following LSG in 21 participants,

however it should be noted that this measurement requires considerable effort and skill [41].

One limitation of the study was that the participant group comprised of those with impaired glucose tolerance and type 2 diabetes, however our aim was to examine changes in plasma markers of oxidative stress in a sample of subjects with glucose dysregulation, which is itself associated with increased oxidative burden [21, 29]. One further limitation is that we did not have a control non-surgical group to compare the effects of LSG on the variables measured. To our knowledge, the effect of LSG on inflammatory cytokines and plasma markers of oxidative stress has not been examined temporally in 55 participants previously. This current study contributes to the available literature supporting the role of LSG for the treatment of impaired glucose regulation and pro-inflammatory conditions associated with morbid obesity.

Conclusion

To our knowledge the effects of LSG on inflammatory cytokines and plasma markers of oxidative stress have not been examined temporally in a group sizeable sample of participants who have undergone LSG. This current study supports the role of LSG for the treatment of the pro-inflammatory and pro-oxidant status associated with obesity-related glucose dysregulation.

Acknowledgements

We would like to thank to Dr Rachel Still and the staff of the Department of Clinical Chemistry for their assistance and collaboration in measuring glucose, A1c and lipids; and Jane Griffiths, Kathie Wareham, Nia Jenkins, Scott Caplin and James Morgan for subject recruitment and data collection.

Disclosures

None of the authors have any conflict of interest or financial disclosure relating to this study or manuscript.

Author contributions

JS and TM developed the study design, ensured the approval process were in place, and undertook participant recruitment. GD, RC and SP undertook sample analysis for cytokines and markers of oxidative stress. JB supported participant recruitment and performed the routine operations. JS and SP analysed the data. All authors contributed to the creation of the manuscript.

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Figure 1 legend

Figure 1: Temporal changes in inflammatory biomarkers following laparoscopic sleeve gastrectomy

Median and interquartile shown. *P<0.05: Significant changes relative to baseline.

Figure 1a: C-reactive protein (CRP)

Figure 1b: Interleukin-6 (IL6)

Figure 1c: Interleukin-10 (IL10)

Figure 1d: Adiponectin

Figure 1e: Leptin

Table 1: Baseline, 1 month and 6 months clinical and biochemical measurements

Measurement	Baseline	1 month	^a p	6 months	^b p
Weight (kg)	148.3 (27.7)	128.8 (26.6)	<0.001	114.4 (24.1)	<0.001
BMI (kg/m ²)	50.4 (7.2)	43.7 (6.7)	<0.001	38.7 (6.4)	<0.001
Waist (cm)	140 (16)	126 (16)	<0.001	117 (17)	<0.001
Systolic BP (mmHg)	128 (18)	121 (13)	0.02	124 (17)	0.13
Diastolic BP (mmHg)	76 (12)	73 (9)	0.09	72 [12]	0.06
Cholesterol (mmol/L)	4.2 (1.0)	4.2 (1.0)	0.85	4.5 (1.0)	0.10
LDL-C (mmol/L)	2.3 (0.8)	2.5 (0.9)	0.26	2.7 [0.9]	0.02
HDL-C (mmol/L) ^c	1.1 [0.9-1.3]	1.0 [0.9-1.2]	0.002	1.2 [1.0-1.4]	<0.001

Triglyceride (mmol/L) ^c	1.5 [1.1-2.4]	1.4 [1.1-1.9]	0.10	1.2 [0.9-1.6]	0.002
A1c (mmol/mol) ^c	57.0 [46.0-89.3]	46.0 [38.5-56.0]	<0.001	40.0 [36.0-49.0]	<0.001
A1c (%) ^c	7.4 [6.4-10.3]	6.3 [5.6-7.3]	<0.001	5.8 [5.4-6.6]	<0.001
Fasting glucose (mmol/L) ^c	7.1 [5.9-11.7]	5.6 [4.6-6.8]	<0.001	5.2 [4.5-5.8]	<0.001
2-hour glucose (mmol/L) ^c	13.4 [9.2-18.4]	8.8 [5.2-12.7]	<0.001	5.8 [4.2-9.4]	<0.001
Fasting insulin (mU/L) ^c	21.8 [13.7-29.5]	12.0 [8.9-19.3]	<0.001	5.4 [9.0-14.1]	<0.001
2-hour Insulin (mu/L) ^c	52.8 [27.4-102.4]	47.1 [27.7-122.6]	0.21	29.7 [16.2-56.9]	0.01
HOMA-IR ^c	3.1 [1.9-4.1]	1.61 [1.22-2.56]	<0.001	1.33 [0.83-1.91]	<0.001
HOMA-%B ^c	90.1 [36.2-131.6]	110.0 [73.0-147.0]	0.34	99.5 [81.0-150.2]	0.29
HOMA-%S ^c	32.4 [24.5-52.3]	62.3 [39.0-81.7]	<0.001	75.5 [52.3-121.0]	<0.001

Mean and standard deviation shown for continuous variables. ^aP-value comparing baseline with 1 month. ^bP-value comparing baseline with 6
5 months. ^cMedian and interquartile values shown as data did not have a normal distribution. BMI= Body mass index; LDL-C= Low density
lipoprotein-Cholesterol; HDL-C= High density lipoprotein-Cholesterol; A1c= Glycated hemoglobin; BP=blood pressure. HOMA-IR=HOMA insulin
resistance; HOMA-%S: HOMA insulin sensitivity; HOMA-%B: HOMA-beta cell function.

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Table 2: Inflammatory cytokine and oxidative stress measurements

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Measurement	Baseline	1 month	^a P	6 months	^b P
CRP (ng/mL)	6.9 [4.1-17.0]	5.0 [2.3-14.3]	0.183	4.4 [1.9-9.9]	<0.001
IL6 (pg/mL)	4.2 [2.4-8.0]	3.8 [2.3-8.0]	0.032	3.1 [1.7-7.3]	0.002
IL10 (pg/mL)	4.1 [2.3-8.1]	5.1 [2.9-8.7]	0.158	4.9 [2.7-7.7]	0.484
Adiponectin (ng/mL)	7.6 [5.2-10.5]	7.1 [5.0-11.6]	0.07	11.0 [7.0-14.3]	0.003
Leptin (pg/mL)	41.7 [26.7-61.9]	31.0 [15.1-43.4]	<0.001	19.5 [10.9-38.3]	<0.001
TAOS (%)	41.7 [36.1-47.8]	41.4 [33.5-50.2]	0.798	41.0 [35.6-47.3]	0.831
TBARS (ng/mL)	58.6 [33.4-99.2]	46.9 [33.1-72.7]	0.053	46.8 [33.8-66.9]	0.007

Median and interquartile ranges shown. ^aP-value comparing baseline with 1 month.

^bP-value comparing baseline with 6 months. CRP=C-reactive protein; IL6=Interleukin-6; IL10=Interleukin-10; TAOS=Total Antioxidant status; TBARS= Thiobarbituric acid reactive substances.

25

30

Figure 1a: C-reactive protein

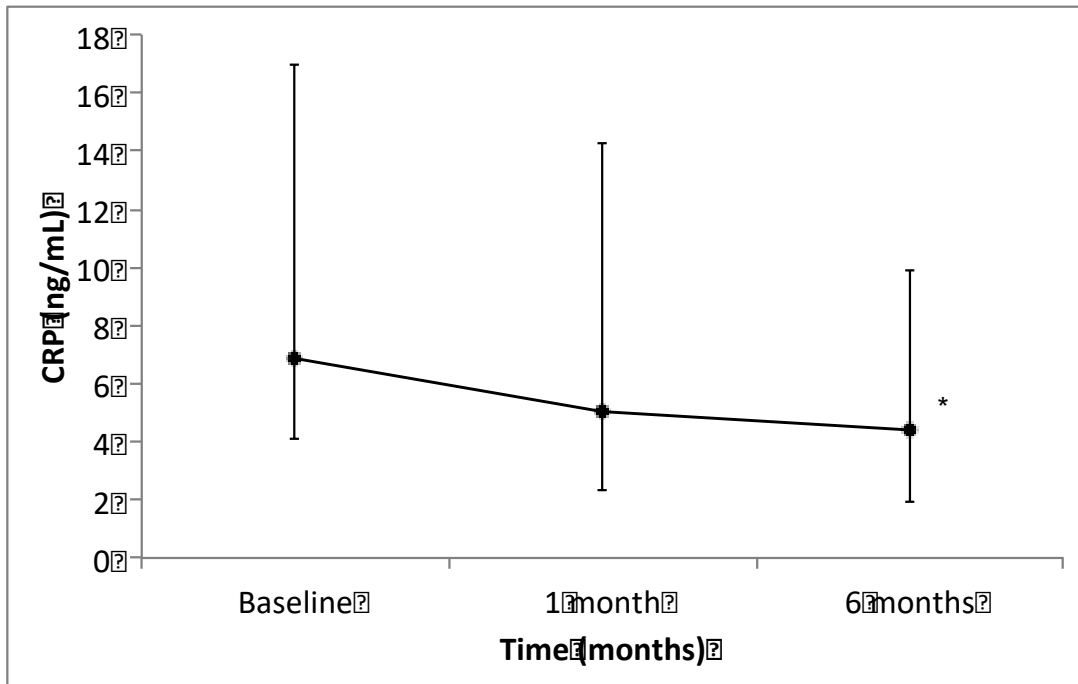


Figure 1b: Interleukin-6

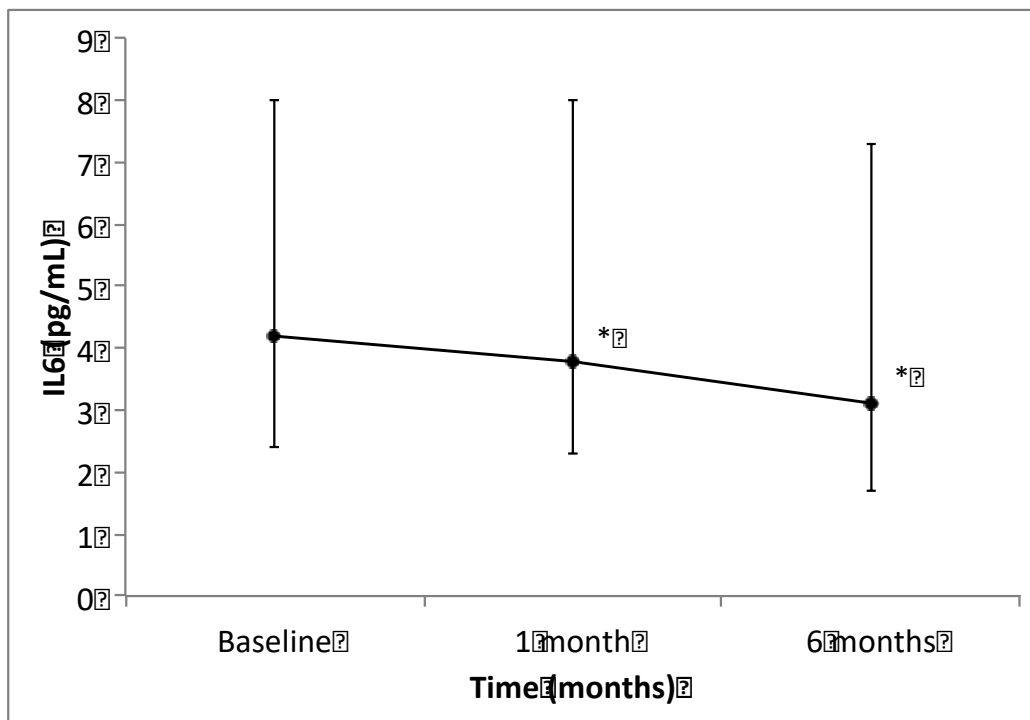


Figure 1c: Interleukin-10

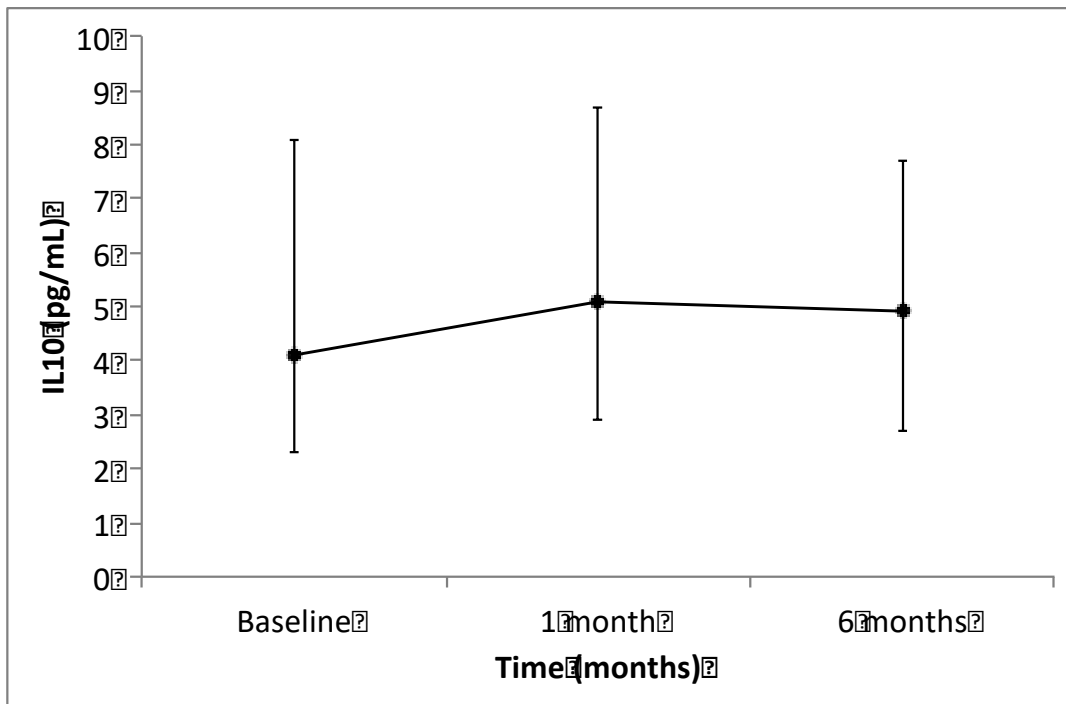


Figure 1d: Adiponectin

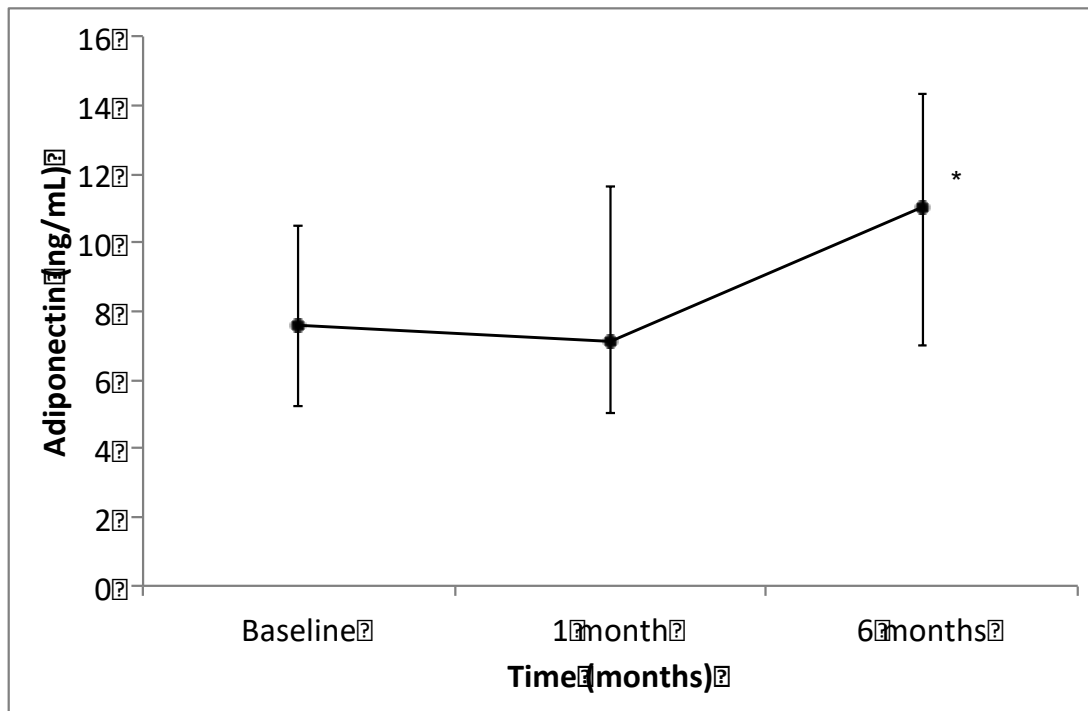


Figure 1e: Leptin

