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Temporal changes in plasma markers of oxidative stress following laparoscopic sleeve gastrectomy in subjects with impaired glucose regulation

Running title: Change in oxidative stress post sleeve gastrectomy

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Keywords: obesity; type 2 diabetes; sleeve gastrectomy; oxidative stress

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Abstract

Background: Laparoscopic sleeve gastrectomy (LSG) is an effective treatment for obesity and associated metabolic complications. Obesity and type 2 diabetes are associated with increased oxidative stress. Previous studies have examined changes in plasma oxidative stress following Roux-en-Y Gastric Bypass, but there is limited evidence of the effects of LSG.

Objectives: To examine the effects of LSG on plasma thiobarbituric acid reactive substances (TBARS) and total antioxidant status (TAOS) at 1 and 6 months following LSG in subjects with obesity and impaired glucose regulation.

Setting: University Hospital, United Kingdom.

Methods: Twenty-two participants with impaired glucose homeostasis undergoing LSG (body mass index [BMI] 50.1kg/m^2 , glycated haemoglobin [HbA_{1c}] 53mmol/mol) were studied. Measurements of fasting and 120 minute TBARS and TAOS were performed during an oral glucose tolerance test pre-operatively and post-operatively.

Results: Compared to pre-operative levels, significant decreases were seen 6 months post-operatively in fasting TBARS (61.0 ± 17.9 v $39.4\pm 13.8\text{ng/mL}$, $p=0.04$) and 120 minute TBARS (76.0 ± 29.5 v $46.5\pm 16.3\text{ng/mL}$, $p=0.02$). No significant changes were observed in plasma TAOS. No significant association was observed between changes in TBARS and other clinical or biochemical measures.

Conclusion: We observed a significant reduction in TBARS, a global measure of lipid peroxidation 6 months following LSG in participants with obesity and impaired glucose regulation.

Introduction

Obesity and related impaired glucose homeostasis are associated with increased circulating and tissue levels of reactive oxygen species (ROS) ^(1, 2) and oxidative damage. ROS are associated with systemic inflammation, vascular and endothelial dysfunction ⁽³⁾, β -cell dysfunction ⁽⁴⁻⁶⁾, insulin resistance, type 2 diabetes and associated complications ^(7, 8). Bariatric surgery is an effective treatment for obesity and obesity-associated impaired glucose homeostasis (impaired glucose tolerance and type 2 diabetes) ⁽⁹⁾. This is likely to occur through a reduction in adipose tissue mass and subsequent improvements in systemic inflammation ^(10, 11) and oxidative stress ^(12, 13). Previous publications have demonstrated reductions in systemic measures of inflammation following laparoscopic Roux-en-Y Gastric Bypass (RYGB) ⁽¹⁴⁻¹⁷⁾ and laparoscopic sleeve gastrectomy (LSG) ⁽¹⁷⁻²⁰⁾. LSG is an established and recognized stand-alone bariatric procedure and has an established safety profile comparable to other bariatric procedures ^(21, 22). There are few published studies examining changes in plasma markers of oxidative stress following bariatric surgery, especially LSG. In relation to RYGB, Ueda et al ⁽²³⁾, observed reductions in plasma levels of F₂-isoprostane (8-*iso*-PGF₂ α) and an increase in glutathione peroxidase (GPX) in 14 subjects within the first week of surgery. These changes were also associated with changes in adipocyte levels of 8-*iso*-PGF₂ α and an increase in the adipocyte expression of GPX-3. Carbrear et al ⁽²⁴⁾, reported significant reductions in plasma superoxide dismutase (SOD) and malondialdehyde (MDA) and increases in glutathione and total reactive antioxidant potential (TRAP) in 20 subjects twelve months after surgery. Two other studies have shown that weight reduction per se was associated with improvements in plasma measures of oxidative stress following RYGB ^(12, 13). Kelly and colleagues ⁽¹⁷⁾ reported significant improvements in markers of both inflammation and oxidative stress, but this was in a combined cohort of both RYGB and

vertical sleeve gastrectomy. Our aim was to specifically examine the temporal changes in fasting and 120 minute plasma TBARS and TAOS following a glucose load (an oral glucose tolerance test) pre-operatively and at 1 and 6 months following LSG in a sample of subjects with impaired glucose tolerance or type 2 diabetes. These measures provide a global plasma measure of oxidative stress (TAOS) and a specific measure of lipid peroxidation (TBARS).

Methods

Study participants

Approval for the study was obtained from the Local Research Ethics Committee and the Joint Scientific Research Committee. Participants were identified and recruited from patients undergoing a planned bariatric surgical procedure at our centre. Entry criteria at the outset of the study included:- both sexes, age 20-60 years, BMI $>40\text{kg/m}^2$ and physically fit for surgery. Participants with any acute concurrent illness were excluded. Participants with pre-existing type 2 diabetes treated with diet, oral agents 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)⁽²⁵⁾. Participants with normal fasting glucose values or a normal glucose tolerance test prior to recruitment were excluded. At the time of recruitment no participant was known to be taking any form of vitamin supplementation, and no vitamin supplementations are given routinely following LSG. No participant had any diagnosed or clinically manifest systemic immune or inflammatory disorder. Informed consent was obtained from all individual participants included in the study.

Study design

Participants with a planned LSG were recruited prospectively and consecutively from the bariatric surgical clinic. The LSG was a standard sleeve (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 (within 1 month of surgery) and followed up post-operatively at 1 and 6 months. Baseline questionnaire and all clinical measurements were documented during 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 of Polycal 61.9g/100ml of glucose, Nutricia Clinical Care, Trowbridge, UK). Previous studies have demonstrated that the level of glycaemia reached 2 hours after 75g of glucose is closely related to the level of glycaemia after a standardised meal indicating an OGTT is a valid tool for revealing altered carbohydrate metabolism during a meal ⁽²⁶⁾. There was no standardised meal prescribed for the night before and subjects were asked to fast from midnight before the test with diabetes related medication omitted.

Baseline clinical and biochemical information

At the time of screening the following clinical information was ascertained: age, gender, past medical history, treatment and duration of diabetes. Baseline clinical measurements consisted of weight, height, body mass index (BMI), waist circumference, systolic and diastolic blood pressure. Baseline biochemical measurements (total cholesterol, low density lipoprotein-cholesterol [LDL-C], high density lipoprotein-cholesterol [HDL-C] and triglycerides) were analysed within the local hospital accredited laboratory. Glucose and lipids (Roche Modular P800 Analyzer), and insulin and C-peptide (Roche E170 Modular Analyzer) were also measured locally. Total glucagon-like peptide-1 (GLP-1) was

quantitatively measured using the Total GLP-1 ELISA Kit (Epitope Diagnostics Inc,) which utilizes the two-site sandwich technique with two selected GLP-1 antibodies in order to assess both the intact GLP-1 amide (7-36) and the primary (NH₂-terminally truncated) metabolite GLP-1 amide (9-36). The intra- and inter-assay coefficients of variation were 4.7% and 9.5% respectively.

All blood samples were 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 TAOS and TBARS immediately prior to the glucose load and 120 minutes afterwards. These were collected pre-operatively, and 1 and 6 months post-operatively. Glucose, C-peptide and GLP-1 were measured during each OGTT at time 0, 15, 30, 45, 60 and 120 minutes. This allowed area under the curve analysis at 120 minutes (AUC₁₂₀) to be calculated and examined in relation to changes in TBARS and TAOS.

Measurement of lipid peroxidation (TBARS)

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 a 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- and inter-assay variability coefficients were 5.2% and 16.2% respectively. All samples were assayed in duplicate^(27, 28).

Measurement of plasma total anti-oxidant status (TAOS)

Plasma total anti-oxidant status (TAOS), which is inversely related to oxidative stress, was measured by Sampson's modification of Laight's photometric microassay⁽²⁹⁾. Previously, we have shown that plasma TAOS has a good correlation with plasma F₂-isoprostanes^(30, 31). The TAOS of plasma was determined by its capacity to inhibit the peroxidase-mediated formation of the 2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS⁺) radical. The difference in absorbance (control [saline] minus test [plasma]) divided by the control absorbance (expressed as a percentage) was used to represent the percentage inhibition of the reaction. Intra- 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 20, SPSS Inc., Chicago). Results for continuous variables are presented as mean and standard deviation and in graphical representation as mean (TAOS) or geometric mean (TBARS) and standard error. Continuous variables that did not have a normal distribution (triglyceride and TBARS) underwent log transformation to normalize the data for analysis and are described with the geometric mean and approximate standard deviation. For continuous variables, temporal changes were compared between baseline and 1 or 6 months using a paired t-test. Categorical data were analysed using a Chi-squared test. Changes in the AUC₁₂₀ were analysed during the OGTT pre-operatively and 1 and 6 months for glucose, C-peptide and GLP-1 using the trapezoidal rule. Correlations between temporal variables and changes in variables (delta values) were examined by Pearson's correlation of continuous variables following log transformation if appropriate. In all cases a p<0.05 was considered statistically significant.

The study was explorative in nature and the sample size was dependent on the prospective recruitment of subjects with samples available for the plasma measure of oxidative stress. As described in the introduction, Carbrear et al ⁽²⁴⁾, reported significant increases in total reactive antioxidant potential (TRAP) in 20 subjects twelve months after RYGB surgery, but there are no studies examining this in relation to LSG. Therefore, there are no previous studies to base a power calculation on the primary outcome of this study. With respect to changes in plasma markers of oxidative stress, we have previously observed a significant reduction in plasma TAOS (37%) within 8 hours following vaccination with typhoid vaccine ⁽³²⁾ in a sample of 21 healthy volunteers. All measurements were significantly increased at this time point compared to baseline.

Results

Subject characteristics

A total of 22 participants (7 males/15 females) who underwent a LSG (mean age 48 ± 7 years) with impaired glucose homeostasis (50%) or type 2 diabetes (50%) (median duration of 42 month [interquartile range 21-66 months]) were recruited. Of those with type 2 diabetes, 27% were diet controlled, 46% taking oral hypoglycaemic agents and 27% were using insulin. Table 1 show the temporal changes between pre-operative, 1 and 6 months within the group. As seen in table 1, significant reductions were observed in weight, BMI and waist circumference at both 1 and 6 months post-operatively. We also observed a significant reduction in systolic blood pressure at 1 month but no significant changes in total cholesterol, LDL-C or triglyceride concentrations. Significant changes were observed in fasting glucose, 120 minute glucose, HbA_{1c}, and AUC₁₂₀ for glucose, C-peptide and GLP-1.

Additionally, 32% and 64% of patients had remission of their diabetes at 1 month and 6 months post-LSG, respectively.

Temporal changes in plasma markers of oxidative stress following LSG

The mean temporal changes in fasting and 120 minute TBARS and TAOS between pre-operative, 1 and 6 months are shown in Table 2. With respect to fasting plasma TBARS, there was a linear decrease in TBARS from the pre-operative period to 6 months post-operatively. At 6 months the fasting TBARS had decreased by approximately 35% ($p=0.04$). Similarly, with respect to the 120 minute TBARS, there was a linear decrease with the levels at 6 months being approximately 40% lower than pre-operative levels ($p=0.02$). For TAOS, no significant increases were observed in fasting or the 120 minute levels at 6 months.

Correlations of changes in TBARS with other metabolic parameters

Correlations between TBARS, TAOS and the clinical and biochemical variables listed in table 1 at the temporal time points were examined to determine whether changes in TBARS were related to changes in the other clinical and biochemical parameters following surgery. In addition the correlations between the changes (delta values) in TBARS and TAOS with changes (delta values) in the variables listed in table 1 were also examined. There was a positive correlation between fasting TBARS and 120 minute TBARS ($r=0.58$, $p=0.01$) pre-operatively and 6 months post-operatively ($r=0.58$, $p=0.02$). No other temporal (pre-operative, 1 or 6 months) correlations were observed between TBARS and any other clinical or biochemical measurement. Furthermore, no correlations between the changes (delta values) in TBARS or TAOS and changes (delta values) in the variables listed in table 1 were observed.

Discussion

We observed that LSG was associated with a reduction in fasting and 120 minute glucose-stimulated TBARS. TBARS is an easily measured global plasma marker of lipid peroxidation, a process dependent on oxidative stress and damage. Bariatric surgery in addition to reducing weight effectively reduces morbidity and mortality in obese individuals with favourable effects on vascular and endothelial dysfunction⁽³⁾, β -cell dysfunction⁽⁴⁻⁶⁾, insulin resistance, type 2 diabetes and associated complications^(7, 8). These effects are clearly related to significant reductions in fat mass following surgery, but improvements in circulating levels of oxidative stress and inflammation are also likely to play an important role. We observed a linear decrease in TBARS at 1 and 6 months following LSG. To our knowledge this is the first manuscript that reports changes in a plasma marker of oxidative stress following this procedure that has gained recent popularity as an established operation^(21, 22). Previously, with respect to RYGB, Ueda et al, observed reductions in plasma levels of 8-*iso*-PGF2 α and an increase in GPX in 14 subjects within the first week of surgery⁽²³⁾. In line with this observation, Carbrear et al, reported significant reductions in plasma SOD and MDA levels and increases in glutathione and TRAP in 20 subjects twelve months after surgery⁽²⁴⁾. Furthermore, 2 other studies have shown that weight reduction per se is associated with improvements in plasma measures of oxidative stress following RYGB^(12, 13). Therefore, our study is in keeping with those relating to RYGB surgery.

Within this study we observed no changes in plasma fasting or postprandial TAOS, a measure of global plasma antioxidant status. Of note, within our study sample of morbidly obese participants with impaired glucose regulation, the mean and standard deviation of

plasma TAOS at each temporal point (Table 1) were relatively low compared to studies which have examined this marker previously⁽³⁰⁾. Part of this may be related to the fact that the study group still had significant risk factors associated with increased oxidative burden at 6 months and the antioxidant status may have been influenced by a dietary change post-operatively. Two other published studies have observed no changes in plasma TAC in morbidly obese participants. Tinahones et al, in a sample of 60 subjects with a BMI of $53.5 \pm 7.3 \text{ kg/m}^2$ observed no changes in TAC following a lipid rich meal but did observe changes in TBARS⁽³³⁾. Furthermore, Catoi et al, in a sample of 23 subjects with a BMI of $48.4 \pm 8.4 \text{ kg/m}^2$ undergoing silastic ring vertical gastroplasty, observed no changes in total oxidant status and total antioxidant response 12 months following weight loss surgery⁽³⁴⁾. However, there were differences between the pre-operative measurements in morbidly obese patients compared to a control group (BMI $22.3 \pm 2.1 \text{ kg/m}^2$) at baseline. 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. There has been considerable debate within the literature in relation to the biochemical measurement of plasma oxidative stress and the challenges these offer⁽³⁾. Measuring plasma markers of oxidative stress is a challenge and options available include measuring global markers of oxidative damage such as total antioxidant status/capacity (TAOS/TAS/TAC), measuring products of lipid peroxidation such as thiobarbituric acid reactive substances (TBARS) or 8-*iso*-PGF 2α , or measuring specific antioxidant molecules such as SOD or GPX. Further controversy exists on whether such measurements should be performed on a static fasting sample or following dynamic testing such as with a glucose load^(29, 35, 36). By definition, ROS are highly reactive and are thus difficult to measure in any biological sample, especially in easily accessible specimens such

as serum or plasma, and therefore measuring plasma levels in the face of a pro-oxidant stimulus such as glucose may provide a better measure of the dynamic response within an ex-vivo sample ⁽³⁾.

No correlations were observed between fasting and 120 minute TBARS and other measured clinical and biochemical parameters and furthermore no correlations were observed between changes (delta) in these variables. Therefore, the improvements seen in TBARS would appear to be independent of these factors. Of specific note, no association or correlation was observed with changes in glucose homeostasis, BMI or in AUC₁₂₀ for glucose, C-peptide and GLP-1 in relation to the temporal OGTTs.

It is well established that a close relationship exists between oxidative burden and low-grade systemic inflammation and associated obesity, glucose dysregulation and endothelial dysfunction. We have previously described a reduction in systemic inflammation following LSG within this study sample in a previously published manuscript ⁽²⁰⁾. We therefore examined to see if there were correlations between the previously measured inflammatory markers with TBARS in the current study. Pre-operatively no correlation was observed between fasting and 120 minute TBARS with interleukin-6, interleukin-10, C-reactive protein, adiponectin or leptin. Post-operatively, fasting TBARS had a significant correlation with adiponectin ($r=-0.55$, $p=0.03$) at 1 month and at 6 months this was borderline ($r=-0.48$, $p=0.05$).

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 ^(3, 29). Furthermore, the median duration of diabetes was 42 months [interquartile range 21-66 months] and this might have an effect on levels of inflammatory markers pre-operatively. Other limitations of this observational study include the unbalanced gender distribution and small sample size. However the sample size is in line with previous studies relating to plasma markers of oxidative stress and RYGB surgery. Additionally, we do not have data regarding diet and/or exercise activity following surgery in these participants. Even though plasma F₂-isoprostanes are seen as the gold standard measure of global oxidative stress we have previously demonstrated that plasma TAOS is a comparable methodology ⁽³⁰⁾.

Conclusion

To our knowledge this is the first study to examine temporally at 1 and 6 months the effects of LSG on plasma markers of oxidative stress. 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. Further longer-term prospective studies are required to examine the effects of LSG (alone and versus other bariatric procedures such as RYGB) in relation to biomarkers of inflammation and oxidative stress in relation to the inflammatory mediated complications of obesity in participants with and without impaired glucose regulation.

Conflict of interest: The authors declare that there is no conflict of interest associated with this manuscript.

Keywords: obesity; type 2 diabetes; sleeve gastrectomy; oxidative stress.

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Table 1: Temporal changes in clinical and biochemical measures

Measurement	Baseline	1 month	^a p	6 months	^b p
Weight (kg)	146.6 (29.6)	129.4 (26.9)	<0.001	115.5 (24.4)	<0.001
BMI (kg/m ²)	50.1 (6.6)	44.0 (6.6)	<0.001	39.6 (6.2)	<0.001
Waist (cm)	138 (18)	128 (17)	<0.001	118 (18)	<0.001
Systolic BP (mmHg)	131 (18)	123 (14)	0.04	128 (20)	0.12
Diastolic BP (mmHg)	76 (11)	74 (9)	0.63	73 (14)	0.13
Cholesterol (mmol/L)	4.3 (1.0)	4.4 (1.1)	0.58	4.7 (1.2)	0.09
LDL-C (mmol/L)	2.4 (0.8)	2.7 (1.0)	0.16	2.8 (0.9)	0.05
HDL-C (mmol/L)	1.2 (0.3)	1.1 (0.3)	0.02	1.3 (0.3)	0.10
Triglyceride (mmol/L)*	1.4 (0.4)	1.4 (0.2)	0.92	1.1 (0.2)	0.15
HbA _{1c} (%)	7.0 (1.7)	6.1 (0.8)	0.005	5.7 (0.8)	0.002
HbA _{1c} (mmol/mol)	53 (18.6)	43 (8.7)	0.005	39 (8.7)	0.002
Fasting glucose (mmol/L)	7.6 (3.6)	5.4 (0.9)	0.02	5.0 (1.0)	0.08
120 minute glucose (mmol/L)	11.6 (5.9)	7.8 (3.4)	0.002	5.4 (2.2)	<0.001
AUC ₁₂₀					
Glucose (mmol h L ⁻¹)	23.4 (7.3)	19.1 (3.5)	0.01	16.5 (4.7)	0.003
C-peptide (pmol h L ⁻¹)*	16.9 (3.1)	21.5 (3.3)	<0.001	17.7 (3.7)	0.04
GLP-1 (pmol h L ⁻¹)	5.6 (5.1)	23.3 (10.9)	<0.001	22.1 (12.6)	<0.001

BMI: body mass index; LDL-C: low density lipoprotein cholesterol; HDL-C: high density lipoprotein cholesterol; HbA_{1c}: glycated hemoglobin; AUC₁₂₀: area under the curve at 120 minutes; GLP-1: glucagon like peptide-1; BP: Blood pressure. Mean and standard deviation shown for continuous variables. *log transformed for analysis with the geometric mean and approximate standard deviation shown. ^ap-value comparing baseline with 1 month. ^bp-value comparing baseline with 6 months

Table 2: Temporal changes in fasting and 120 minute TBARS and TAOS

Measurement	Baseline	1 month	^a p	6 months	^b p
TBARS* (ng/mL)					
Fasting	70.0 (17.3)	49.7 (13.0)	0.14	39.4 (13.8)	0.04
120 minutes	76.0 (29.5)	55.6 (14.3)	0.25	46.5 (16.3)	0.02
TAOS (%)					
Fasting	43.1 (8.9)	37.3 (9.1)	0.03	40.9 (7.3)	0.25
120 minutes	42.1 (8.2)	41.4 (7.3)	0.66	40.0 (11.2)	0.29

TBARS: thiobarbituric acid reactive substances; TAOS: total antioxidant status. Mean and standard deviation shown for TAOS. *log transformed for analysis. Geometric mean and approximate standard deviation shown for TBARS. ^ap-value comparing baseline with 1 month. ^bp-value comparing baseline with 6 months.

Figure 1: Temporal changes in inflammatory biomarkers following sleeve gastrectomy

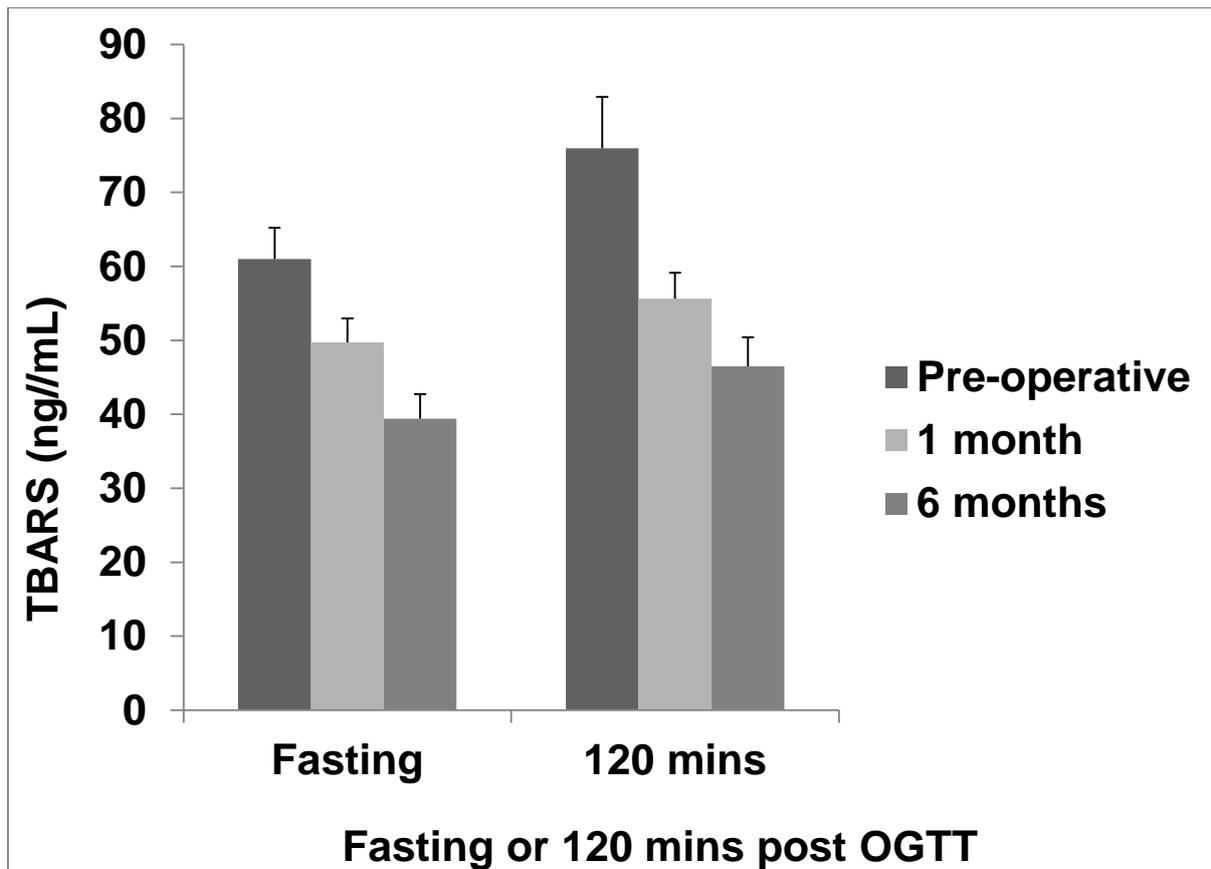


Figure 1a: TBARS

Geometric mean and standard error shown for TBARS. Significant changes relative to baseline: *P=0.04, #P=0.02. See also Table 2.

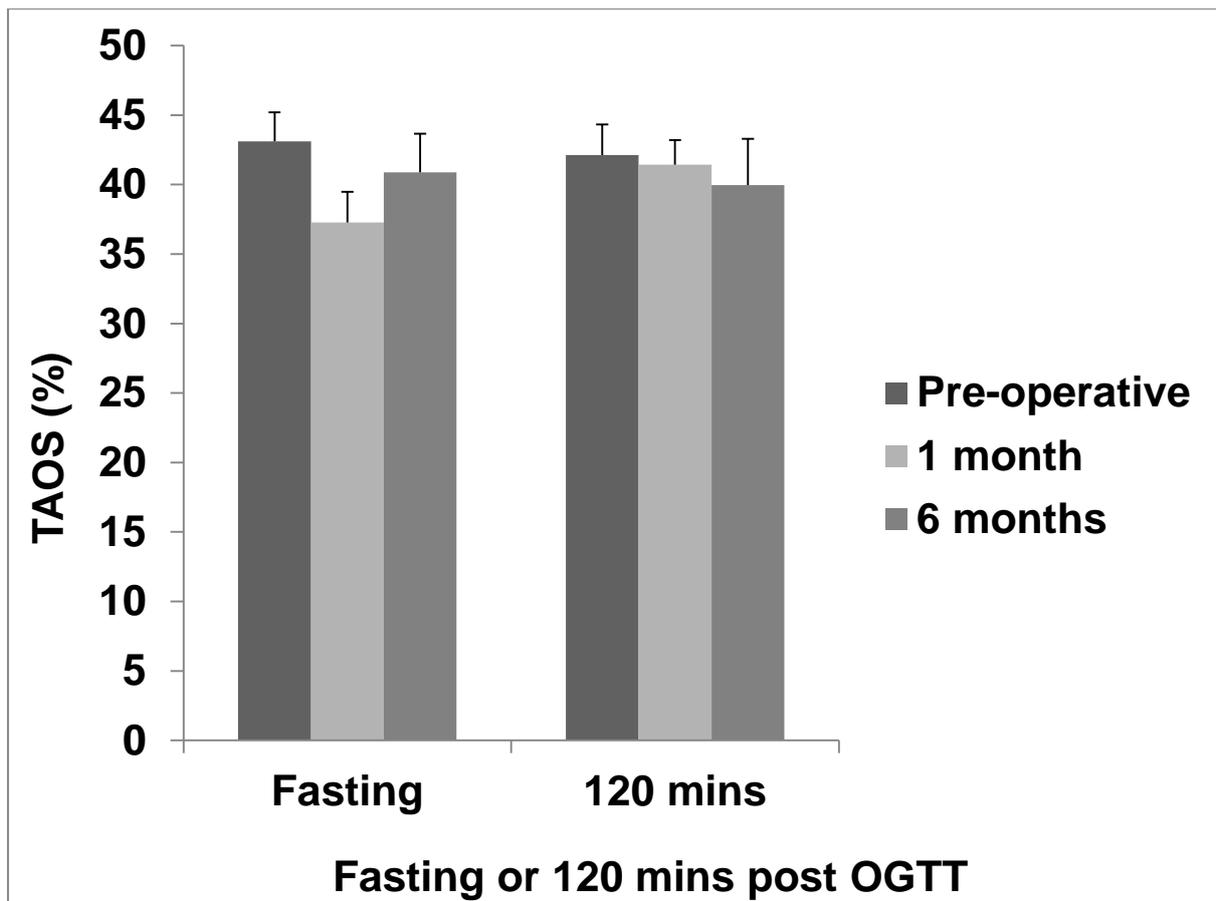


Figure 1b: TAOS

Mean and standard error shown for TAOS.