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Improved acylated ghrelin suppression at 2 years in obese patients with type 2 diabetes: effects of bariatric surgery vs standard medical therapy

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Abstract

OBJECTIVE—Roux-en-Y gastric bypass (RYGB) produces more durable glycemic control than sleeve gastrectomy (SG) or intensive medical therapy (IMT). However, the contribution of acylated ghrelin (AG), a gluco-regulatory/appetite hormone, to improve glucose metabolism and body composition in patients with type 2 diabetes (T2D) following RYGB is unknown.

DESIGN—STAMPEDE (Surgical Treatment and Medication Potentially Eradicate Diabetes Efficiently) was a prospective, randomized controlled trial.

SUBJECTS—Fifty-three (body mass index: 36 ± 3 kg m^{-2}, age: 49 ± 9 years) poorly controlled patients with T2D (HbA_{1c} (glycated hemoglobin): 9.7 ± 2%) were randomized to IMT, IMT + RYGB or IMT + SG and underwent a mixed-meal tolerance test at baseline, 12, and 24 months for evaluation of AG suppression (postprandial minus fasting) and beta-cell function (oral disposition index; glucose-stimulated insulin secretion × Matsuda index). Total/android body fat (dual-energy X-ray absorptiometry) was also assessed.

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CONFLICT OF INTEREST

All the other authors declare no conflict of interest.

RESULTS—RYGB and SG reduced body fat comparably (15–23 kg) at 12 and 24 months, whereas IMT had no effect. Beta-cell function increased 5.8-fold in RYGB and was greater than IMT at 24 months ($P < 0.001$). However, there was no difference in insulin secretion between SG vs IMT at 24 months ($P = 0.32$). Fasting AG was reduced fourfold following SG ($P < 0.01$) and did not change with RYGB or IMT at 24 months. AG suppression improved more following RYGB than SG or IMT at 24 months ($P = 0.01$ vs SG, $P = 0.07$ vs IMT). At 24 months, AG suppression was associated with increased postprandial glucagon-like peptide-1 ($r = -0.32$, $P < 0.02$) and decreased android fat ($r = 0.38$; $P < 0.006$).

CONCLUSIONS—Enhanced AG suppression persists for up to 2 years after RYGB, and this effect is associated with decreased android obesity and improved insulin secretion. Together, these findings suggest that AG suppression is partly responsible for the improved glucose control after RYGB surgery.

Keywords

bariatric surgery; gastrointestinal hormones; diabetes; insulin resistance; insulin secretion; glycemic control

INTRODUCTION

The accumulation of body fat in ectopic depots is linked with insulin resistance and impaired beta-cell function. Defects in obesity-related insulin action are clinically relevant, as they contribute to the development of cardiometabolic disease and type 2 diabetes (T2D).[1] Diet and exercise are recommended as first-line therapies for treating and managing T2D;[2] however, the long-term compliance to lifestyle modification is poor and often requires additional pharmacological treatment. Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) are widely used surgeries[3] that have proven efficacy in promoting not only long-term weight loss but also remission of T2D.[4–7] The rapid rate of glucose lowering, before significant weight loss, suggests that restoration of glucose metabolism is achieved through an endocrine-related mechanism.

Although the foregut (‘anti-incretin’ and gastric-inhibitory peptide) and hindgut (glucagon-like peptide-1 (GLP-1)) hypotheses are widely invoked to explain improvements in beta-cell function and insulin sensitivity after bariatric surgery,[8,9] they do not fully account for the near complete remission of diabetes after surgery.[10,11] Ghrelin may represent an alternative, or complimentary, endocrine mediator of diabetes remission, as it is related to metabolic syndrome, obesity, insulin resistance and insulin-secretion capacity.[12] Ghrelin is a gastric hormone produced primarily by the A cells in the oxyntic glands of the stomach, with secondary secretion emanating from the proximal small intestine. In obesity, total ghrelin levels are generally low compared with lean healthy controls owing to hyperinsulinemia. Subsequently, the normal rise in total ghrelin levels before food intake and meal-induced suppression (for example, carbohydrate being more effective than protein or fats) is lost. However, the acylated form of ghrelin is elevated in obese individuals during both fasted and fed states. Acylated ghrelin appears to be more important in appetite regulation, as it has potent effects on hypothalamic food initiation centers and adipogenesis.[13,14] In addition, total/acylated ghrelin (AG) reduces glucose-stimulated insulin secretion and attenuates

glucose uptake.[15,16] Thus, given the improvement in body fat and glycemic control following bariatric surgery, it seems reasonable to expect that bariatric surgery could suppress AG in obese individuals with T2D. However, there are no data regarding the effects of RYGB or SG surgery on AG in people with T2D. In the Surgical Treatment and Medication Potentially Eradicate Diabetes Efficiently (STAMPEDE) trial, we recently demonstrated that RYGB surgery produced superior glycemic control compared with intensive medical therapy (IMT) or SG surgery.[5,17] We suggested that the improvement in glycemic control after RYGB surgery was primarily due to improvements in beta-cell function, which were directly related to enhanced meal-stimulated GLP-1 stimulation.[5,17] However, it remains possible that some of the increased pancreatic beta-cell endocrine function following RYGB surgery may be due to changes in stomach-derived factors.[18] To address this knowledge gap, we tested the hypothesis that RYGB surgery would improve AG suppression more than SG surgery or IMT at 24 months. We also hypothesized that improved ghrelin suppression would be associated with enhanced beta-cell function, GLP-1 stimulation and reductions in body fat.

MATERIALS AND METHODS

Subjects and design

This substudy included a randomly selected group of 60 individuals, providing 20 subjects per treatment arm, who were enrolled in the main STAMPEDE trial[5] (see Table 1 for group characteristics). The STAMPEDE study rationale and design has been previously reported.[19] It was a single-center prospective study in which 150 patients were randomized in a 1:1:1 ratio to IMT alone or RYGB surgery + IMT or SG surgery + IMT with stratification by the use of insulin at screening. During this time, all subjects received nutritional counseling by a certified diabetes educator and were encouraged to participate in Weight Watchers for additional nutritional counseling. Patients underwent a psychological evaluation before randomization to assess qualification for bariatric surgery. Subjects randomized to bariatric surgery had periodic evaluation by nutrition, psychology, bariatricians and the surgery team as clinically indicated. The bariatric procedures were performed by a single primary surgeon (PRS) and have been previously described.[5] IMT included use of the latest lifestyle guidelines by the American Diabetes Association, frequent home monitoring and titration strategies, use of the latest Food and Drug Administration-approved drug therapy, including incretin analogs or mimetics and insulin sensitizers for treatment of hyperglycemia. A diabetes specialist at the Cleveland Clinic (SRK) saw patients in the outpatient clinic every 3 months. Subjects were verbally briefed about the study and signed informed consent documents were approved by the Cleveland Clinic Institutional Review Board.

Body composition

Anthropometric measures (weight and height) were obtained in a standard hospital gown on a calibrated scale and wall-mounted stadiometer (Veeder-Root, Elizabethtown, NC, USA) at baseline, 12 and 24 months. Body mass index was calculated as body mass (kg) divided by height $(m)^2$. Total body fat and android body fat (that is, upper portion of the iliac crest and 20% above) was assessed by using dual-energy X-ray absorptiometry (Lunar Prodigy, Madison, WI, USA).

Metabolic studies

Subjects refrained from pharmacological treatment 24 h before metabolic testing to minimize impact on gastrointestinal hormones and insulin action. After a 12–14-h overnight fast, a mixed meal tolerance test (Boost; 8 oz; 350 kcal; 55% CHO, 25% PRO, 20% Fat) was performed to assess glucose tolerance, insulin sensitivity (Matsuda index) and beta-cell function (Insulinogenic index × Matsuda index), as previously described.[17] Blood samples were collected at 0, 30, 60, 90 and 120 min for determination of glucose and insulin from an antecubital vein. Acylated ghrelin concentrations were determined at 0 and 60 min. Ghrelin suppression was defined as: Postprandial ghrelin (60 min) − fasting ghrelin.

Blood analysis

Whole-blood glucose was measured immediately after collection using the glucose oxidase method (YSI 2300 STAT Plus, Yellow Springs, OH, USA). The remaining blood was centrifuged at 4 °C for 10 min and frozen at −70 °C until subsequent analysis. Plasma insulin was assayed by radioimmunoassay (Linco Research, St Charles, MO, USA). Blood collected for AG was treated immediately with a DPP-4 and protease cocktail inhibitor (Sigma, St Louis, MO, USA), and assayed using an enzyme-linked immunosorbent assay (Kamiya Biomedical Company, Seattle, WA, USA). Hydrochorlic acid was not added to collection containers for AG samples, as recent work demonstrates that it is not required.[20] GLP-1 (active) was assayed using an enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH, USA). To minimize inter-assay variability, all pre-measurement and post measurements for each subject were analyzed on the same plate.

Statistical analysis

Ghrelin analysis was performed on subjects who completed both 12- and 24-month follow-up. There was a 10% attrition rate by 24 months leaving 16 subjects in IMT, 18 in RYGB and 19 in SG as previously described.[17] A mixed model for repeated measures analysis of variance was used to analyze the AG data across baseline, 12- and 24-month visits. Normality was tested, and in the event of non-normal distribution (for example, ghrelin), a rank transformation was applied before analysis. Sex and fasting ghrelin was used as a covariate on ghrelin suppression after the intervention. No adjustments were made for *post hoc* pairwise multiple comparisons. Spearman's rank correlations were used to determine associations between outcomes across groups. Significance was accepted as $P \leq 0.05$, and trends are reported as $0.05 \leq x \leq 0.10$.

RESULTS

Metabolic characteristics

The original data for the 53 subjects with complete data at 12 and 24 months for body fat, glucose tolerance, insulin sensitivity and beta-cell function have previously been published.[17] In short, compared with IMT at 12 and 24 months, RYGB and SG surgery decreased body weight by approximately 15–23 kg ($P < 0.0001$), and total body fat was reduced at 12 and 24 months by 10–12% after RYGB and SG but not after IMT ($P < 0.001$). Importantly, android body fat was significantly reduced after RYGB and SG surgery, but not

after IMT ($P < 0.001$). RYGB induced more android fat loss than SG ($P = 0.02$). All interventions reduced fasting glucose and glycated hemoglobin (HbA_{1c}) at 24 months. However, only RYGB surgery led to significantly lower fasting glucose and HbA_{1c} levels, compared with IMT at 24 months ($P < 0.05$). Pancreatic beta-cell function increased 24 months after RYGB surgery compared with IMT ($P < 0.01$) and was not different between SG surgery and IMT. Postprandial GLP-1 stimulation also increased 24 months after RYGB and SG surgery compared with IMT ($P < 0.01$) and tended to be higher in RYGB compared with SG ($P = 0.07$). Insulin sensitivity was improved in both RYGB and SG surgery at 24 months compared with IMT ($P < 0.01$). Patients undergoing RYGB surgery also had greater whole-body insulin sensitivity at 24 months compared with SG ($P < 0.01$).

Acylated ghrelin analysis

Before the intervention, fasting, postprandial and AG suppression outcomes were not different across treatments. Although fasting ghrelin concentrations were lower at 12 and 24 months across treatments, this was only statistically significant after SG ($P < 0.001$). SG had lower ghrelin levels at 12 and 24 months, compared with IMT and RYGB ($P < 0.01$, Figures 1a and b). However, all treatments lowered postprandial AG compared with baseline at 12 and 24 months (all $P < 0.05$). RYGB and SG surgery each lowered postprandial AG compared with IMT ($P < 0.004$) at 12 months, while ghrelin levels showed a non-significant trend toward reduction after SG compared with RYGB surgery ($P < 0.08$, Figures 1c and d). At 24 months, RYGB and SG surgery tended to lower postprandial AG more than IMT ($P < 0.10$ and $P < 0.05$, respectively; Figures 1c and d). After adjusting for fasting ghrelin and sex, meal-induced AG suppression was improved by RYGB surgery at 12 and 24 months compared with baseline ($P < 0.05$). RYGB surgery also enhanced ghrelin suppression at 24 months compared with IMT (absolute $P = 0.07$; Figure 1e and percentage of change $P < 0.01$; Figure 1f) and SG surgery (absolute $P = 0.01$; Figure 1e and percentage of change $P = 0.05$; Figure 1f).

Correlation analysis

At 12 months, reductions in fasting AG did not correlate with changes in android body fat or enhanced GLP-1 stimulation (Figures 2 and 3). However, improved AG suppression correlated with reduced android body fat ($r = 0.31$, $P = 0.03$) and enhanced insulin sensitivity ($r = -0.31$; $P < 0.03$). In addition, reduced postprandial AG concentrations correlated with increased GLP-1 stimulation ($r = -0.34$, $P = 0.01$, Figure 3). At 24 months, decreased fasting AG did not correlate with changes in android body fat or enhanced GLP-1 stimulation (Figures 2 and 3). But improved AG suppression correlated with reduced android body fat ($r = 0.38$, $P < 0.006$; Figure 2), postprandial GLP-1 stimulation ($r = -0.32$, $P < 0.02$; Figure 3) and decreased total cholesterol ($r = 0.34$, $P = 0.01$). Lower fasting glucose and HbA_{1c} levels were associated with improved beta-cell function at 24 months ($r = -0.37$, $P < 0.01$ and $r = -0.54$, $P < 0.001$, respectively).

DISCUSSION

The effect of RYGB and SG surgery on plasma ghrelin concentrations is controversial. Although some studies show no change[21] or slight increases in ghrelin concentrations

following surgery,[22,23] most show that ghrelin decreases in concert with lower glucose levels.[16,24–26] To date, however, there are limited data on the long-term effects of RYGB or SG surgery on AG.[27,28] The major finding in this randomized, controlled trial is that RYGB surgery improves and maintains AG suppression to a greater extent than either SG or IMT in obese adults with T2D, and this effect persists for up to 2 years (Figure 1). We also demonstrate that RYGB surgery had no effect on fasting AG concentrations, which suggests that the RYGB technique preserved pre-surgery fasting ghrelin levels despite significant weight loss. These findings differ from those of Barrazzoni *et al.*,[27] who reported elevated acylated ghrelin at 12 months post-RYGB surgery in obese subjects. The discrepancy between studies is likely related to different populations (T2D vs normoglycemic obese subjects) and/or surgical techniques. In either case, our work is consistent with Lee *et al.*[28] who reported significant improvements in AG suppression up to 24 months after RYGB surgery that paralleled greater reductions in central fat (via waist circumference) and insulin resistance (Homeostasis Model of Assessment—Insulin Resistance). Although these latter findings provide insight into the endocrine-related mechanism responsible for better glycemic control post-RYGB surgery, subjects in the study by Lee *et al.*[28] were not randomized to the surgical procedure, and there was no standard medical therapy control group. As a result, our data extend this previous clinical trial and show that RYGB surgery effectively enhances glycemic control in parallel with improved AG suppression in adults with T2D up to 24 months.

The improvement in ghrelin following surgery is clinically relevant, as high or impaired meal-suppressed AG concentrations compromise weight management.[9] At 24 months, there were no statistical differences in weight loss between RYGB and SG surgery, despite stark differences in the ghrelin response to SG and RYGB. Interestingly, android body fat was significantly lower following RYGB compared with SG surgery, and lower android body fat at 24 months was significantly associated with enhanced AG suppression (Figure 2). This association at 12 and 24 months suggests that AG has a direct role in regulating central adipose tissue mass. Indeed, ghrelin has been reported to enhance adipogenesis,[13] promote fat storage enzymes[14] and reduce fat utilization/lipolysis[29] in rodents and humans. Moreover, ghrelin has been reported to modify adipose tissue volume by affecting lipid efflux.[13] We detected a significant correlation between increased ghrelin suppression and lower plasma cholesterol levels at 24 months, suggesting that RYGB surgery may regulate fat mass via reduced substrate availability.[30] Regardless of the exact mechanism, approximately 30% of ghrelin is secreted from the small intestine, which is in close proximity to visceral adiposity.[31] Thus, it is reasonable that fat loss would be specific to the central region.[32]

Gastrointestinal hormones have been strongly implicated in augmenting beta-cell function and inducing T2D remission after bariatric surgery. We previously reported that RYGB surgery enhanced GLP-1 levels at 24 months to a greater extent than SG surgery or IMT, while SG increased gastric-inhibitory peptide.[17] Enhanced meal-stimulated GLP-1 may be important, because GLP-1 is linked with elevated pancreatic insulin secretion and glycemic control.[10,11] However, GLP-1 is unlikely to be the sole endocrine factor explaining the improved glycemic control after surgery,[33] as the interaction of gastrointestinal hormones

are likely to explain the reversal of T2D. For example, despite intravenous infusion or meal-stimulated GLP-1 being shown to suppress ghrelin concentrations,[24,34] ghrelin has also been shown to attenuate GLP-1 action.[35,36] Although we cannot prove causality or directionality from the correlation between GLP-1 and ghrelin (Figure 3), lower postprandial ghrelin levels would be expected to decrease 'fight or flight' hormones known to suppress meal-stimulated pancreatic insulin secretion and alleviate impaired skeletal muscle insulin action.[15] Thus, the mechanism for diabetes remission following RYGB and SG surgery appears to go beyond simple enforced caloric restriction and likely involves the interaction of GLP-1 and ghrelin to improve glucose homeostasis.[28,34]

Despite RYGB surgery appearing to improve glucose regulation and meal-induced AG suppression to a larger extent than SG, weight loss after SG surgery may still be related to lower ghrelin concentrations during fasting and postprandial states.[37] The current study suggests that SG surgery effectively reduces fasting and postprandial ghrelin concentrations up to 2 years.[28,37] A likely explanation for the relatively flat acylated-ghrelin response after meal intake is the near full removal of the gastric fundus, which is the primary site of ghrelin-producing cells.[31] The impact this ghrelin response has on glycemic control is unclear, as low ghrelin concentrations after SG surgery did not mirror enhanced beta-cell function or insulin sensitivity when compared with RYGB surgery. We do not exclude the possibility that lower ghrelin concentrations exerted some favorable effects on metabolic health and body composition, but our data suggest that enhancing meal-induced ghrelin suppression is more tightly linked to metabolic health (for example, beta-cell function and lower android fat) than reduced hormone levels *per se*.

Intensive lifestyle therapy improves glycemic control in adults with T2D.[38,39] The reason our standard medical treatment induced smaller improvements in glycemic control, compared with SG or RYGB surgery, may be related to combining medication (for example, metformin) with exercise.[40] However, it is unlikely that metformin impacted our study outcomes to a large extent after IMT, given that previous work allowed medication use during intensive lifestyle modification treatment and effectively improved glycemic control.[38,39] Alternatively, we reported that individuals with T2D had blunted exercise-related improvements in beta-cell function and insulin sensitivity.[41] Thus, we caution that our findings do not imply that healthy lifestyle choices (with or without medication) are unsuccessful at improving glycemic control in adults with T2D. Rather, we suggest that surgery be considered as a means to correct underlying metabolic disturbances (for example, glucotoxicity) that blunt diet and exercise responsiveness.

This study has limitations that warrant discussion. We acknowledge that the associations observed do not equate to causality, and further work is necessary to elucidate the mechanistic role that ghrelin may have in improving beta-cell function and reducing body fat. Further, due to limited measurements of postprandial ghrelin, it is possible that our calculation of ghrelin suppression is underestimated despite the observation that ghrelin generally nadirs within the first 60-min post-meal consumption.[21,23,25,26] Moreover, glucose profiles are elevated post surgery and generally reflect differences in glucose absorption.[42] Subsequently, our calculations of insulin sensitivity and beta-cell function may be overestimated despite the observation that glycemic control is generally improved post

surgery. There were more females in the SG vs RYGB group in this randomized clinical trial. Women generally have higher ghrelin levels in response to weight loss, and this is a potential reason women struggle to lose weight compared with men.[43] If true in our study, then the SG group would have been expected to have higher ghrelin and poorer weight loss. This did not occur, and adding sex as a co-variate did not affect our overall findings. Therefore, our data suggest that bariatric surgery (that is, SG and RYGB) have distinct effects on gut physiology independent of sex. Finally, this was a single-center trial, and further studies are required to confirm the effects of RYGB and SG on beta-cell function, gastrointestinal hormones and body fat. However, it is noteworthy that our study is unique in that it assessed AG post-bariatric surgery up to 2 years in obese T2D subjects. All subjects were randomly assigned to medical therapy or bariatric surgery, thereby strengthening the conclusion that RYGB uniquely alters gastrointestinal hormones compared with SG or IMT.

In conclusion, RYGB surgery improves AG suppression more than SG surgery or IMT, while SG results in low ghrelin concentrations in the fasting and postprandial states. The increase in AG suppression was paralleled by greater improvements in pancreatic beta-cell function and reductions in android body fat in our obese patients with T2D. Thus, our data highlight plasma AG as a potential modifying factor in RYGB-induced diabetes remission. SG surgery also produced better glycemic control compared with the standard medical therapy, and this surgical technique may be an effective alternative to RYGB surgery in obese men and women with T2D. Future work is necessary to address the exact mechanism by which AG alters insulin secretion capacity and android body fat, as this may lead to personalized therapeutic interventions that optimize metabolic health and prevent/reverse cardiometabolic disease and T2D.

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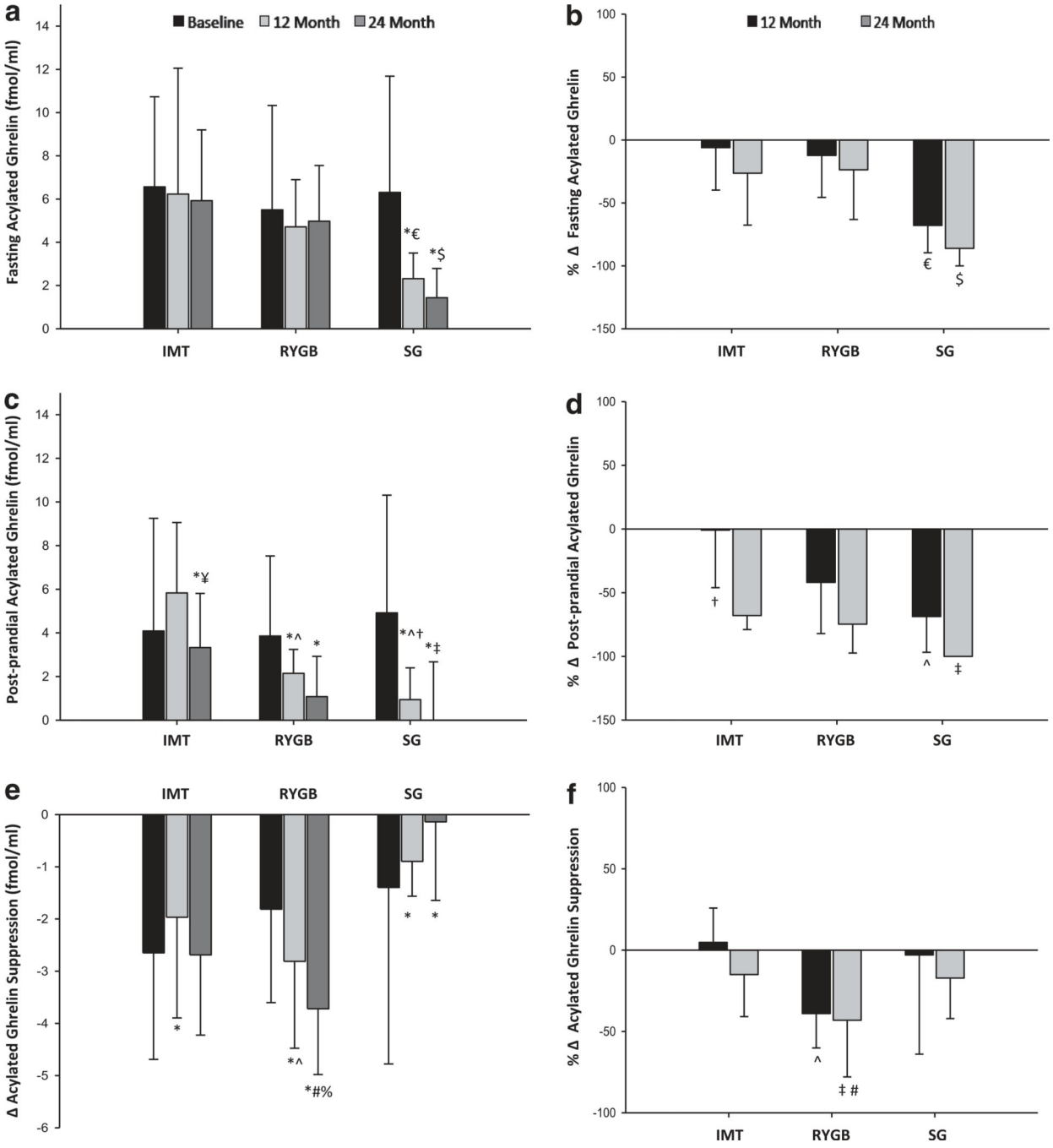

Figure 1.

The effects of medical therapy vs surgery on absolute and percentage change of AG: fasting (**a** and **b**), postprandial (**c** and **d**) and suppression (**e** and **f**) at 0, 12 and 24 months. To convert ghrelin from fmol ml^{-1} to pg ml^{-1}, multiply data by 3.37. Data are median ± interquartile range (IQR). Note: at 24 months, SG IQR was equivalent to lower median. *Compared with baseline ($P < 0.05$). €Compared with IMT and GB at 12 months ($P < 0.001$). ¥Compared with 0 and 12 months IMT ($P < 0.001$). †Compared with GB at 12 months ($P = 0.07$). ^Compared with IMT at 12 months ($P < 0.004$). $Compared with IMT

and GB at 24 months ($P < 0.001$). ‡Compared with IMT at 24 months ($P < 0.05$). %Compared with IMT 24 months ($P = 0.07$). #Compared with SG 24 months ($P < 0.003$).

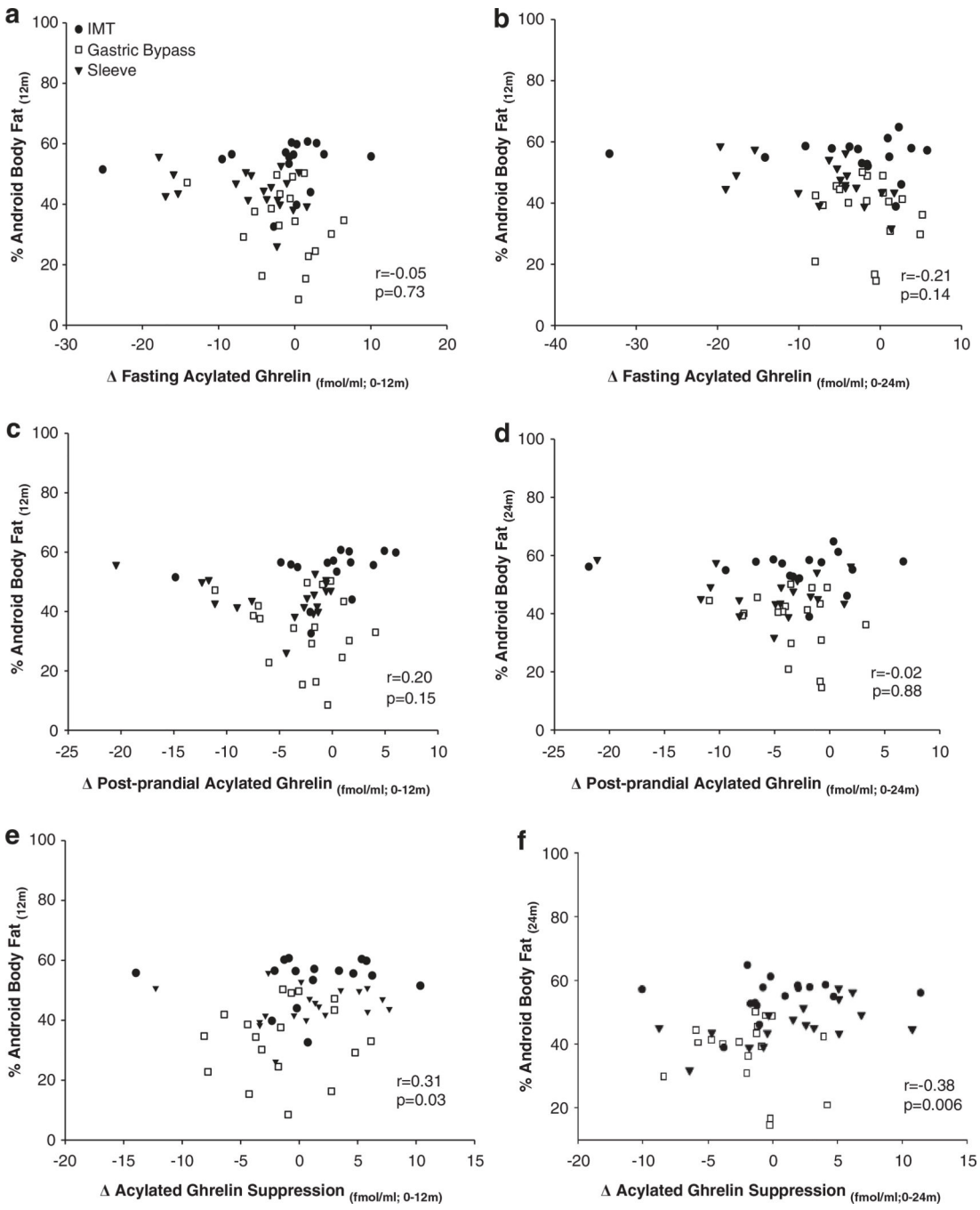

Figure 2.

Correlation between the change in fasting (**a** and **b**), postprandial (**c** and **d**) and AG ghrelin suppression (**e** and **f**) vs android body fat at 12 and 24 months, respectively. Δ = change between 24 month – baseline. Ghrelin suppression defined as postprandial – fasting.

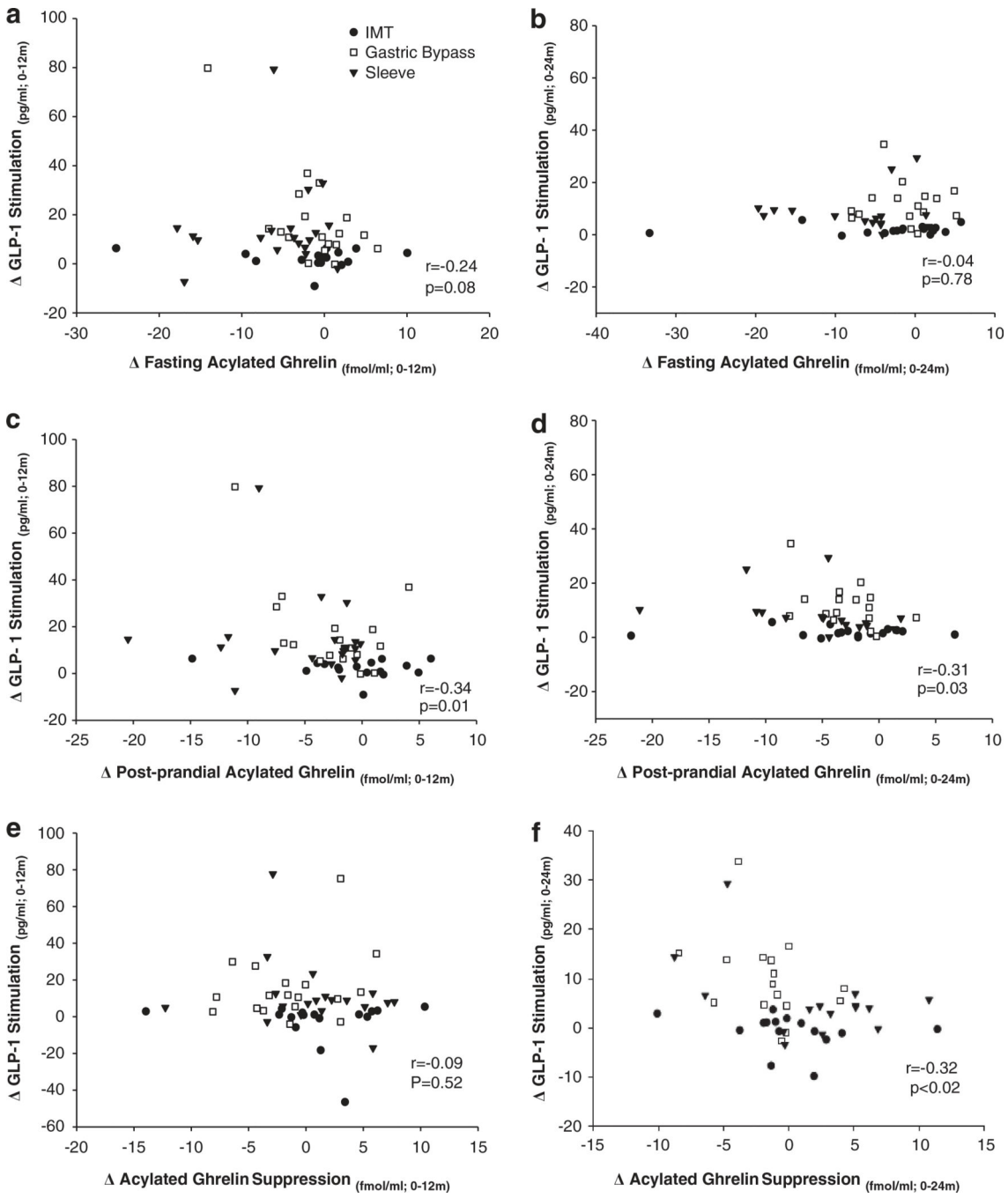

Figure 3.

Correlation between the change in fasting (**a** and **b**), postprandial (**c** and **d**) and AG ghrelin suppression (**e** and **f**) vs the change in GLP-1 at 12 and 24 months, respectively. Δ = change between 24 month – baseline. Ghrelin suppression defined as postprandial – fasting. GLP-1 stimulation defined as postprandial – fasting.

Table 1

Subject characteristics

	IMT	RYGB	SG
Population (*n*)	16	18	19
Age (years)	51.2h ± 7.1	47.9 ± 9.7	47.5 ± 10.0
Female sex (%)	50.0	44.4[a]	84.2[b]
Duration of T2DM (years)	10.6 ± 5.2	7.4 ± 5.0	7.6 ± 4.5
Body weight (kg)	107.9 ± 14.5	105.3 ± 13.6	100.0 ± .5
BMI (kg m^{-2})	36.1 ± 2.6	36.1 ± 2.6	36.4 ± 3.2
Total body fat (%)	42.2 ± 4.6	41.1 ± 4.7	46.1 ± 4.9
Android body fat (%)	54.6 ± 5.3	55.0 ± 4.3	57.8 ± 4.9
Fasting glucose (mg dl^{-1})	173.2 ± 62.63	198.3 ± 48.7	148.5 ± 66.9

Abbreviations: BMI, body mass index; IMT, intensive medical therapy; RYGB, Roux-en-Y gastric bypass; SG, sleeve gastrectomy; T2DM, type 2 diabetes mellitus. Data are mean ± s.d. From reference Kashyap et al.[17]

[a]Significant compared to SG ($P < 0.05$).

[b]Significant compared to IMT ($P < 0.05$).