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# Acute improvements in glycemic control after gastric bypass occur despite persistent adipose tissue inflammation

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## Abstract

**Objective—**Type 2 diabetes (T2D) commonly goes into remission following Roux-en-Y gastric bypass (RYGB). As the mechanisms remain incompletely understood, we hypothesized that a reduction in adipose tissue inflammation may contribute to these metabolic improvements. Therefore, we investigated whether RYGB reduces adipose tissue inflammation compared to equivalent weight loss from an intensive lifestyle intervention.

**Methods—**Sixteen people with obesity and T2D were randomized to RYGB or lifestyle intervention. Fasting blood and subcutaneous abdominal adipose tissue were obtained before and after the loss of ~7% of baseline weight. Adipose tissue inflammation was assessed by whole-tissue gene expression and flow cytometry-based quantification of tissue leukocytes.

**Results—**At 7% weight loss, insulin and metformin use were reduced among the RYGB but not the Lifestyle cohort, while fasting glucose and insulin declined in both. Adipose tissue

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The authors' responsibilities were as follows— DEC, DEA and DRF designed the CROSSROADS clinical trial; CPC, SS, and EOW: identified and recruited subjects for the main trial; JNK, DEC, KEF-S, and MK: administered the lifestyle intervention; DEC, KEF-S and CPC oversaw all clinical visits; MK and DEC designed and oversaw the ancillary study to the main CROSSROADS trial; MK, DKH and JNK: completed statistical analysis of data and the first draft of the manuscript; DKH and JNK: collected/processed biological specimens and carried out the flow cytometry and ELISA analyses; BvY: measured expression of genes in adipose tissue; KEF-S and DEC performed tissue biopsies. All authors contributed to the preparation of the final manuscript.

inflammation increased modestly after RYGB, and to a similar extent following non-surgical weight loss. In both groups, the number of neutrophils increased several-fold (P<0.001), mRNA levels of the pro-inflammatory cytokine interleukin-1β increased (P=0.037), and mRNA expression of the anti-inflammatory and insulin-sensitizing adipokine, adiponectin, decreased (P=0.010).

**Conclusions—**A reduction in adipose tissue inflammation is not one of the acute weight loss-independent mechanisms through which RYGB exerts its anti-diabetes effects.

### Keywords

Adipose Tissue Inflammation; Roux-en-Y Gastric Bypass; Type 2 Diabetes Mellitus

## Introduction

Adipose tissue inflammation is associated with obesity, and appears to contribute to the development of type 2 diabetes (T2D). A prevailing view is that activated immune cells infiltrate adipose tissue in greater numbers with increasing adiposity leading to a pro-inflammatory milieu that disrupts insulin signaling in adipocytes (1). This inflammation may also affect systemic insulin sensitivity as it reduces adipocyte secretion of the anti-inflammatory, insulin-sensitizing hormone adiponectin, and increases the release of pro-inflammatory cytokines, free fatty acids (FFA) and resistin (2, 3).

Supporting this model, targeted deletion of molecules involved in the inflammatory process often results in improved insulin sensitivity and glucose tolerance in obese mice (4-7). In humans, cross-sectional studies as well as intervention trials with high-dose non-steroidal anti-inflammatory agents also support a role for adipose tissue inflammation in the development of insulin resistance (8-10).

Roux-en-Y gastric bypass (RYGB) surgery offers a unique opportunity to study the links among obesity, insulin sensitivity, and inflammation in humans. Within days of surgery, normal glucose homeostasis is commonly restored in patients with T2D (11). Although the mechanisms behind this remain unclear, the effects of RYGB on caloric restriction, nutrient delivery, hormone changes, bile-acid signaling, and microbiome composition have all been proposed as potential contributors (12). Intriguingly, several studies found that weight loss following RYGB was associated with reduced measures of systemic (3, 13-20) and adipose tissue inflammation (13, 21, 22), suggesting a link between metabolic improvements and reduced inflammation.

However, whether the immediate anti-diabetes effects of RYGB, which appears partly independent of weight loss (23), may result from an attenuation of inflammation remains unclear. This is due to two major limitations. First, most studies assessed changes in inflammatory status before surgery and between 1-24 months afterward (3, 13-22). Because weight loss in the first month following RYGB can be substantial, earlier follow-up assessment is necessary to determine whether this procedure may exert weight loss-independent anti-inflammatory properties. Second, no previous study has compared changes

in adipose tissue inflammation following RYGB versus a non-surgical intervention leading to similar weight loss.

We conducted an ancillary study to the "Calorie Reduction Or Surgery: Seeking Remission for Obesity and Diabetes" (CROSSROADS) randomized controlled trial (RCT) (Cummings et. al, *Diabetologia*, in press), examining adipose-tissue and systemic inflammation in patients with T2D at baseline and again at 7% weight loss from either RYGB or an intensive lifestyle intervention. This design allowed us to address whether RYGB exerts anti-inflammatory effects beyond those that may result from similar weight loss following a non-surgical intervention.

## Methods

### Study design and subjects

Sixteen of 43 participants in the CROSSROADS RCT agreed to participate in this ancillary study. All were randomized to undergo either RYGB or an intensive lifestyle modification. The 1-year lifestyle intervention consisted of regular physical activity (5 times/week for ≥45 min/day) and an intense dietary intervention based on the Diabetes Prevention Program, modified for a population with manifest diabetes (Cummings et. al, *Diabetologia*, in press). No power calculation was conducted for this ancillary study.

Study participants included individuals with established T2D who were 21-65 years old, and with BMI between 30-45 kg/m$^2$. Pregnant women or women planning to become pregnant within the subsequent 2-year period of the trial were excluded. Individuals with any prior bariatric surgery, gastrectomy or bowel resection, as well as other contraindications for RYGB such as dementia, psychosis, HIV, cancer diagnosis within previous 2 years, inflammatory bowel disease, cirrhosis, or dialysis were excluded. All study procedures were approved by the institutional review boards of GHC, the University of Washington, and the Fred Hutchinson Cancer Research Center (FHCRC), and were in compliance with the Helsinki Declaration. All study participants provided written informed consent.

### Clinic procedures and laboratory analyses

Participants attended clinic visits at baseline (pre-intervention) and following the loss of 7% of total body weight. For RYGB patients, 7% weight loss was achieved within 13±2 days after surgery. Because patients scheduled to undergo RYGB are routinely placed in a weight-loss program before surgery, they already had lost 1.2±2.3% pre-operatively, with weight loss after surgery averaging 5.8±1.9%. In contrast, Lifestyle participants lost 4.8±3.0% of their baseline weight over 277±106 days. Three participants never reached 7% weight loss, and were therefore assessed 1 year after baseline. Two of the original 16 participants were excluded from final analysis: one failed to return for follow-up, and the other yielded an insufficient amount of biopsied adipose tissue for analyses. Thus, data from 14 participants (n=7/group) were available for the analyses.

### Blood and Tissue Collection

Blood was collected into EDTA-coated tubes, following an overnight fast of 10h. Periumbilical subcutaneous adipose tissue biopsies were performed as described previously (24). Specimens were stored at −70°C until analysis.

### Plasma Analysis

Total and high molecular weight (HMW) adiponectin and interleukin (IL)-6 were measured in triplicate by ELISA (ALPCO Diagnostics, Salem, NH; and R&D Systems, Minneapolis, MN). Intra- and inter-assay coefficients of variation were 10.8% and 12.6% for IL-6; 4.0% and 8.6% for total adiponectin; and 7.3% and 12.6% for HMW adiponectin. High-sensitivity CRP (immunonephelometry), glucose (Hitachi 917 autoanalyzer; Boehringer Mannheim, Germany) and insulin (Tosoh 1800 autoanalyzer; Tosoh Bioscience, San Francisco, CA) were assayed at Northwest Lipid Laboratories, Seattle, WA.

### Flow Cytometry

Stromal vascular cells were isolated from fresh adipose tissue following a 60min digestion at 37°C with collagenase I (Worthington, Lakewood, NJ), as described previously (25). Labeling for flow cytometry included a combination of nine fluorescently conjugated antibodies (BD Pharmingen, San Jose or Biolegend, San Diego, CA) to identify and characterize different leukocyte populations including CD1c, CD3, CD4, CD8, CD11c, CD14, CD15, CD16, CD45, and CD206. Positive staining was determined based on staining with appropriate isotype controls. Labeled cells were analyzed on a LSRII (Beckton Dickinson, San Jose, CA), collecting up to 30,000 events in a broad gate, defined by forward- and side-scatter attributes. Analysis was conducted with FlowJo (version 9.3.3, TreeStar, Ashland, OR) using histograms and dot plots on live cells defined by fluorescence levels associated with the lower uptake of 4',6-diamidino-2-phenylindole, 2HCl (Calbiochem, EMD Chemicals, Gibbstown, NJ). Leukocytes were identified based on positive expression of CD45, and further segregated into lymphoid ($CD3^{pos}CD4^{pos}$ or $CD3^{pos}CD8^{pos}$ T-cells), or myeloid lineages (**Supplemental Figure 1**). Myeloid cells included neutrophils ($CD15^{pos}CD16^{pos}$), dendritic cells ($CD1c^{pos}CD11c^{pos}$), and adipose tissue macrophages (ATM; $CD14^{pos}CD206^{pos}$). Data were expressed as a percentage of the total leukocyte ($CD45^{pos}$) fraction, and as total cell number per gram of tissue (25).

### RNA Isolation and Gene Expression Analysis

Total RNA was extracted from adipose tissue using RNeasy® mini kits (Qiagen, Hilden, Germany) and quantified using the RiboGreen® RNA Quantitation Kit (Invitrogen, Carlsbad, CA). cDNA synthesis employed 0.5-1.5 μg of total RNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) followed by PCR on an Mx3005P® Multiplex QPCR System (Stratagene, Cedar Creek, TX) using pre-designed ABI TaqMan® Gene Expression Assays. By including a standard curve on each plate, Ct values were converted to copy numbers of all target genes. Data were normalized using two housekeeping genes (β-glucuronidase and 18s rRNA) and expressed as target gene copy number per ng RNA. Target genes included tumor necrosis factor (*TNF)α, IL1β, IL6*,

monocyte chemoattractant protein (*MCP)-1*, intracellular adhesion molecule (*ICAM)-1*, and adiponectin.

### Statistical analyses

Statistical analyses were performed using SPSS software (version 20, SPSS Inc., Chicago, IL). Normality of distributions for all variables was assessed by Shapiro-Wilk tests and by checking histograms and normal plots. Results are expressed as mean±sd for normally distributed variables or as median ($25^{th}$ percentile, $75^{th}$ percentile) for non-normally distributed variables. Baseline characteristics were compared using independent-samples t-tests, or independent-samples Mann-Whitney U-Tests for variables that were not normally distributed. The latter included gender, duration of diabetes, and fasting plasma concentrations of glucose, insulin, and CRP. Changes over time were compared by repeated-measures analysis of variance (RM-ANOVA), with 'time' (baseline vs. 7% weight loss) as the within-subjects factor, and intervention group (RYGB vs. Lifestyle) as the between-subjects factor. Secondary analyses adjusted for weight change were conducted to assess whether the small experimental difference in weight change between the two intervention groups may have affected the outcomes. Variables were log 10-transformed if the residuals were not normally distributed, which was the case for insulin and metformin use; fasting plasma IL-6, CRP and total adiponectin; the adipose tissue gene expression of *TNFα, IL1β, and IL6*; and all leukocyte populations in adipose tissue. For metformin or insulin use, where participants were often not taking these drugs either at baseline or follow up, a value of 1 was added to each reported daily dosage before log-transformation. We did not adjust for multiple testing because all measures of systemic and adipose tissue inflammation were interpreted together, i.e. we did not interpret a change in single biomarker as indicative of a change in 'inflammation'. An alpha-error of $P<0.05$ was considered significant.

## Results

Baseline characteristics of the study population are presented in **Table 1**. Duration of diabetes was significantly longer among the RYGB group ($P=0.009$), consistent with slightly higher baseline glycosylated hemoglobin and CRP concentrations in this group. There were no other significant differences between the groups.

Weight loss from baseline was significant in both groups ($P<0.001$), as reflected by reductions in both lean mass ($P<0.001$) and body fat ($P<0.001$) (**Table 2**). While the loss of lean mass was less pronounced in the Lifestyle versus RYGB group, changes in weight and fat mass were not significantly different between the two. Fasting glucose concentrations were reduced slightly, and fasting insulin more substantially in both groups, with no difference between the groups. The main effects of time were strongly attenuated and no longer significant when adjusted for weight change (data not shown).

Fasting glucose and insulin concentrations changed to a similar degree in both groups. However, whereas metformin use remained largely unchanged among participants in the Lifestyle group, it declined sharply in the RYGB group ($P<0.001$ for the time × intervention interaction; **Table 2**). This reduction in metformin use between the intervention groups remained significant when adjusted for weight change ($P=0.002$ for the time × intervention

interaction, adjusted for weight change; data not shown). Similarly, insulin usage was largely unchanged among Lifestyle participants but tended to be reduced among RYGB participants (P=0.061 for the time × intervention interaction, **Table 2**).

Systemic inflammation was assessed through measurement of circulating levels of IL-6, CRP, and total and HMW adiponectin. We observed no change over time nor any effect of intervention on systemic inflammation markers (**Table 2**), with or without adjustment for weight change (data not shown). Although no consistent trend was apparent within or between groups, it is worth pointing out that some individuals experienced substantial changes from baseline to follow-up (**Supplemental Figure 2**).

The expression of pro-inflammatory genes in whole-adipose tissue changed in some individuals, often substantially (**Supplemental Figure 3**). Among the mediators of inflammation studied, only *IL1β* changed significantly (P=0.037 for main effect of time; **Table 3**). Consistent with this increase in a major pro-inflammatory cytokine, the expression of the key anti-inflammatory and insulin-sensitizing adipokine, adiponectin, decreased over time in both groups (p=0.010 for the main effect of time; **Table 3**). For none of the genes examined did we observe a differential change between the two intervention groups. The change in *IL1β* and adiponectin gene expression was strongly attenuated and became non-significant after adjustment for weight change (data not shown).

Consistent with the increase in gene expression of *IL1β* and decrease in gene expression of adiponectin, the absolute number of neutrophil granulocytes increased dramatically in both groups (P<0.001 for the main effect of time), slightly more so in the Lifestyle than RYGB group (P=0.049 for the time × intervention interaction, **Table 4**). The observed increase in neutrophil number was apparent, independent of whether the number of neutrophils was normalized as a number of cells per g of adipose tissue or as a percentage of $CD45^{pos}$ cells (**Supplemental Figures 4 & 5**). We also observed an increase in the total number of $CD4^{pos}$ T-cells in both groups (P=0.041 for the main effect of time), an increase that was not statistically different between interventions (P=0.408 for the time × intervention interaction). The absolute number of $CD8^{pos}$ T-cells remained unchanged, resulting in an overall increase in the ratio of $CD4^{pos}$ to $CD8^{pos}$ T-cells in both groups (P=0.005 for the main effect of time). This increase was slightly greater in the Lifestyle as compared to the RYGB group (P=0.040 for the time × intervention interaction). The absolute number of total or $CD11c^{pos}$ ATM or dendritic cells did not change significantly. Due to the substantial increase in the number of neutrophils, the proportion of each of the other leukocyte populations (other than $CD4^{pos}$ T-cells) relative to all leukocytes decreased significantly, again to a similar degree in each group. Adjustment for weight change did not change any of the results, other than slightly attenuating the time × intervention group interaction for the ratio of $CD4^{pos}$ to $CD8^{pos}$ T-cells (P=0.069).

## Discussion

We observed no changes in either systemic or adipose tissue-specific inflammation that could explain the rapid and substantially greater metabolic improvements observed in RYGB patients following surgery. In contrast to our hypotheses, the number of leukocytes within

adipose tissue increased markedly immediately after surgery as well as with similarly modest weight loss from a non-surgical intervention. Changes in the numbers of neutrophils seen in both groups were associated with an increase in the expression of the major pro-inflammatory cytokine IL-1β, and a decrease in the expression of the insulin-sensitizing adipokine, adiponectin. We also detected an increase in the number of $CD4^{pos}$ T-cells and the $CD4^{pos}$ to $CD8^{pos}$ T-cell ratio. It remains unclear, however, whether this increase is anti- or pro-inflammatory given that we did not measure Th1, Th2, or regulatory T-cell markers (all of which are $CD4^{pos}$).

Our rationale for this study was based on the presumed causal link between obesity-induced adipose-tissue inflammation, insulin resistance, and glucose intolerance that has been advanced based on extensive work in rodents. RYGB presents a unique, useful model for the investigation of the relationship between adipose tissue inflammation and glucose homeostasis in humans, as it results in massive weight loss and often, complete remission of diabetes (11). In most studies investigating the mechanisms of RYGB-related anti-diabetes effects, follow-up typically occurs 6-12 months after surgery. At this stage, RYGB patients typically have lost between 20-36% of their pre-operative body weight, exhibit reductions in systemic inflammatory markers, and have markedly improved glucose tolerance and insulin sensitivity (3, 15-20). These data are consistent with the hypothesis that obesity is associated with adipose tissue inflammation, and that adipose tissue inflammation is an important etiological contributor to insulin resistance.

However, to understand whether RYGB has anti-inflammatory effects independent of long-term weight loss, studies with shorter time between surgery and follow-up are needed, as metabolic improvements typically precede significant weight loss (11). Such studies could also clarify whether adipose tissue inflammation is indeed a major contributing factor in the etiology of insulin resistance. In one prior study, adipose tissue adiponectin protein levels roughly doubled in the 2 weeks following RYGB. However, this change was not associated with a corresponding increase in serum adiponectin or a reduction in serum TNFα or MCP-1 (26). Miller and colleagues (14) observed no reduction in systemic markers of inflammation within 3-4 weeks after RYGB and a corresponding total body weight loss of 8-9%. At 7% weight loss, we similarly observed no reduction in systemic inflammation, and importantly, no decline in any measure of adipose tissue inflammation in either group. On the contrary, we detected increases in several measures of inflammation, such as a substantially increased number of neutrophil granulocytes, increased IL-1β gene expression and reduced adiponectin gene expression in adipose tissue, despite clear reductions in fasting glucose and insulin, and specifically among the RYGB cohort, decreased metformin use. Notably, in one participant who was studied 10 days after RYGB, there was evidence of systemic inflammation (CRP: 26.9 mg/L, up from 1.2 mg/L at baseline), likely due to surgery. In this same individual, adipose-tissue gene expression of the major pro-inflammatory cytokines TNFα and IL-1β was increased 4.4-fold and 125-fold, respectively, and adiponectin gene expression was reduced by 90%. Nevertheless, fasting glucose in this subject remained constant and fasting insulin was reduced from 22.1 to 5.6 μU/L, all while metformin use had been discontinued (from 2,550 mg/d) and the insulin dose halved. Thus, our data clearly show that acute improvements in glucose homeostasis after RYGB occur in spite of the persistence of adipose tissue inflammation.

How are our findings reconcilable with the prevailing paradigm that inflammation of adipose tissue is a factor in the development of insulin resistance, and that insulin sensitivity clearly improves in response to RYGB? First, the observed decreases in fasting glucose and insulin within days of surgery suggest improved hepatic insulin sensitivity. Consistent with this consideration, there is evidence demonstrating that basal hepatic glucose production decreases, and that postprandial insulin-related suppression of glucose production improves within 30 days of RYGB (27, 28), although the literature is not entirely consistent regarding this (29). Second, there is also evidence that systemic insulin sensitivity improves within the first few weeks following RYGB (29-32). However, these studies predominantly did not specifically assess hepatic versus peripheral insulin sensitivity, while other studies show no acute weight loss-independent improvement in systemic insulin sensitivity (27, 28, 33, 34). It therefore remains possible that RYGB first improves hepatic and only later muscle and adipose tissue insulin sensitivity. Thus, our finding of modestly increased adipose tissue inflammation concurrent with substantially reduced fasting plasma glucose and insulin concentrations is not necessarily inconsistent with the prevailing paradigm that adipose tissue inflammation is a major factor in the etiology of insulin resistance. As a further consideration, our data are consistent with our recent finding that chronic exposure to high concentrations of FFA may contribute to adipose tissue inflammation (35). By definition, negative energy balance and weight loss are associated with lipolysis and increased release of FFA from adipocytes. Thus, weight loss may temporarily lead to increased exposure of ATM to FFA (28), which could plausibly perpetuate inflammatory activation in adipose tissue. As such, reduced adipose tissue inflammation may only occur once body weight stabilizes at a new, lower level, as reported in Belza et al (36). This premise has been supported by several other studies noting that substantial declines in markers of adipose tissue inflammation were apparent only after weight loss in excess of 10% (13, 37, 38).

Notable strengths of our study included the randomization of intervention assignments, rapid follow-up within 2 weeks of surgery, and the assessment of adipose tissue inflammation based on flow cytometry and qPCR. Limitations included a small sample size, which would have been particularly problematic had we seen consistent trends towards a reduction in adipose tissue inflammation that remained non-significant due to a lack of statistical power. However, statistically significant changes consistent in their directionality were observed in several measures of adipose tissue inflammation (in the opposite direction of what we hypothesized), illustrating our ability to detect such changes. Nevertheless, the small sample size prevented subgroup and sensitivity analyses. Therefore differential effects of the interventions based on factors such as gender, medication intake, or duration of disease could not be determined. Also of note, our assessment of inflammation included cell surface antigen expression but not functional measures. Nevertheless, our overall assessment of inflammation was based not on any single measure, but rather a combination of circulating factors, gene expression and cell populations. Also notable, randomization of participants occurred in the main trial, not in this ancillary study. Additional limitations include the absence of state-of-the-art measures of insulin sensitivity, β-cell function, glucose tolerance, and glucose effectiveness, and that gene expression was assessed only in one adipose tissue depot. Although differences, for example in the absolute expression of pro-inflammatory cytokines, have been reported for visceral and subcutaneous adipose tissue in humans, gene

expression patterns in subcutaneous adipose tissue are as strongly associated with obesity and metabolic disease as those in visceral adipose tissue (39). Also, the speed of weight loss was substantially greater among RYGB versus Lifestyle participants, as we sought to study a realistic weight loss from a comprehensive lifestyle modification, as would be seen in a clinical setting. Particularly considering this discrepancy in the rate of weight loss, it is reassuring that the two interventions led to similar changes in measures of systemic and adipose tissue inflammation. Limitations aside, we are confident that we would have detected at least a trend toward resolution of inflammation, either systemically or in adipose tissue, if this were indeed a contributing factor to the metabolic improvements associated with RYGB in particular, or with modest weight loss in general.

In conclusion, in individuals with T2D, we detected no reduction in measures of systemic or adipose tissue inflammation within two weeks of RYGB versus similar weight loss from a non-surgical intervention. Although changes in chronic inflammatory processes that occur with more substantial weight loss 6-12 months following RYGB may contribute to improved metabolic health, this study suggests that a reduction in adipose tissue inflammation is not one of the acute mechanisms through which RYGB exerts its anti-diabetes effects.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What is already known about this subject?

- Evidence from animal models strongly suggests a causal role for adipose tissue inflammation in the development of insulin resistance
- Factors contributing to the rapid metabolic improvements following gastric bypass surgery remain unclear, but appear to include some weight-loss independent mechanisms
- The objective of our study was to test the hypothesis that the immediate anti-diabetic effect of gastric bypass surgery may be due to a resolution of adipose tissue inflammation

What does your study add?

- In contrast to our hypothesis, rapid metabolic improvements associated with gastric bypass surgery are associated with a modest increase in adipose tissue inflammation
- No previous study has compared changes in adipose tissue inflammation following RYGB compared to a non-surgical intervention leading to similar weight loss in a randomized controlled trial

**Table 1**

Clinical characteristics of the study population at baseline in participants randomized to Roux-en-Y gastric bypass surgery (RYGB) or an intensive lifestyle modification combined with best medical care ('Lifestyle')[1].

| | All (n=14) | Lifestyle (n=7) | RYGB (n=7) | Lifestyle vs. RYGB P-value |
|---|---|---|---|---|
| Gender (female / male) | 8 / 6 | 3 / 4 | 5 / 2 | 0.383 |
| Age (y) | 53.1 ± 6.8 | 53.7 ± 7.7 | 52.6 ± 6.2 | 0.767 |
| Body mass index (kg/m$^2$) | 37.7 ± 3.9 | 36.5 ± 2.2 | 38.9 ± 4.9 | 0.265 |
| Lean body mass (kg) | 54.0 (47.5 – 71.4) | 64.4 (49.1 – 74.1) | 53.6 (48.3 – 60.9) | 0.376 |
| Fat mass (kg) | 48.7 ± 9.2 | 46.1 ± 7.0 | 51.4 ± 10.9 | 0.309 |
| Fasting glucose (mmol/L) | 7.8 (6.9 – 11.6) | 7.9 (7.3 – 10.3) | 7.7 (7.1 – 12.0) | 0.710 |
| Fasting insulin (μU/mL) | 23.6 (14.4 – 28.5) | 23.8 (17.2 – 49.0) | 22.1 (15.1 – 25.0) | 0.456 |
| Hemoglobin A1c (%) | 7.7 ± 0.9 | 7.3 ± 0.6 | 8.1 ± 1.1 | 0.114 |
| Fasting high-sensitivity C-reactive protein (mg/L) | 3.0 (1.3 – 4.1) | 2.3 (1.7 – 3.1) | 4.0 (2.4 – 4.6) | 0.383 |
| Fasting interleukin-6 (pg/mL) | 2.1 ± 0.8 | 2.0 ± 0.5 | 2.3 ± 1.0 | 0.483 |
| Fasting total adiponectin (ng/mL) | 2,502 ± 1,048 | 2,388 ± 1,076 | 2,616 ± 1,091 | 0.701 |
| Duration of known diabetes (years) | 12.0 (7.0 – 18.0) | 7.0 (6.0 – 10.0) | 18.0 (13.0 – 21.5) | 0.009 |

[1]Values are means ± standard deviations or medians (25$^{th}$, 75$^{th}$ percentiles). P-values are based on independent samples t-tests or Mann-Whitney U-tests (the latter for gender, fasting glucose, fasting insulin, and fasting high-sensitivity C-reactive protein).

**Table 2**

Metabolic parameters at baseline (pre-intervention) and after 7% weight loss following either Roux-en-Y gastric bypass surgery (RYGB) or an intensive lifestyle modification combined with best medical care ('Lifestyle')[2].

| | Baseline (pre-intervention) | Follow-up (7% wt loss) | RM-ANOVA | |
|---|---|---|---|---|
| | | | P (time) | P (time × intervention) |
| **Body Weight (kg)** | | | | |
| Lifestyle (n=7) | 113.6 ± 17.5 | 108.2 ± 16.7 | <0.001 | 0.202 |
| RYGB (n=7) | 109.7 ± 19.0 | 102.2 ± 18.7 | | |
| **Lean mass (kg)** | | | | |
| Lifestyle (n=7) | 64.4 (49.1 – 74.1) | 61.9 (47.3 – 75.2) | <0.001 | <0.001 |
| RYGB (n=7) | 53.6 (48.3 – 60.9) | 48.6 (43.3 – 57.0) | | |
| **Fat mass (kg)** | | | | |
| Lifestyle (n=7) | 46.1 ± 7.0 | 41.4 ± 3.7 | <0.001 | 0.355 |
| RYGB (n=7) | 51.4 ± 10.9 | 48.1 ± 10.8 | | |
| **Fasting glucose (mmol/L)** | | | | |
| Lifestyle (n=7) | 7.9 (7.3 – 10.3) | 7.9 (6.5 – 9.1) | 0.028 | 0.489 |
| RYGB (n=7) | 7.7 (7.1 – 12.0) | 7.4 (6.9 – 7.8) | | |
| **Fasting insulin (μU/L)** | | | | |
| Lifestyle (n=7) | 23.8 (17.2 – 49.0) | 16.7 (13.3 – 22.7) | 0.009 | 0.401 |
| RYGB (n=7) | 22.1 (15.1 – 25.0) | 6.2 (5.8 – 10.6) | | |
| **Metformin use (mg/d)** | | | | |
| Lifestyle (n=7) | 2,000 (500 – 2,000) | 2,000 (1,500 – 2,000) | 0.005 | <0.001 |
| RYGB (n=7) | 2,000 (1,625 – 2,250) | 0 (0 – 0) | | |
| **Insulin use (U/d)** | | | | |
| Lifestyle (n=7) | 0 (0 – 52) | 10 (0 – 42) | 0.180 | 0.061 |
| RYGB (n=7) | 20 (5 – 43) | 0 (0 – 13) | | |
| **High-sensitivity interleukin-6 (pg/mL)** | | | | |
| Lifestyle (n=7) | 1.9 (1.7 – 2.4) | 1.4 (1.4 – 2.1) | 0.921 | 0.850 |
| RYGB (n=7) | 2.3 (1.6 – 2.9) | 2.3 (1.6 – 3.2) | | |
| **High-sensitivity C-reactive protein (mg/L)** | | | | |
| Lifestyle (n=7) | 2.3 (1.7 – 3.1) | 1.4 (1.0 – 1.9) | 0.929 | 0.059 |

| | Baseline (pre-intervention) | Follow-up (7% wt loss) | RM-ANOVA | |
|---|---|---|---|---|
| | | | P (time) | P (time × intervention) |
| RYGB (n=7) | 4.0 (2.4 – 4.6) | 3.5 (2.3 – 15.4) | | |
| **Total adiponectin (ng/mL)** | | | | |
| Lifestyle (n=7) | 2,524 (1,557 – 2,842) | 2,048 (1,297 – 6,562) | 0.737 | 0.339 |
| RYGB (n=7) | 2,642 (1,739 – 3,271) | 2,074 (1,736 – 2,968) | | |
| **High molecular weight adiponectin (ng/mL)** | | | | |
| Lifestyle (n=7) | 849 (579 – 1,537) | 820 (638 – 1,121) | 0.655 | 0.730 |
| RYGB (n=7) | 922 (548 – 2,568) | 712 (605 – 2,509) | | |

[2]Values are means ± standard deviations or medians ($25^{th}$, $75^{th}$ percentiles). RM-ANOVA: Repeated measures analysis of variance.

**Table 3**

Adipose tissue expression of genes encoding mediators of inflammation at baseline (pre-intervention) and after 7% weight loss following either Roux-en-Y gastric bypass surgery (RYGB) or an intensive lifestyle modification combined with best medical care ('Lifestyle')[4].

| | Baseline (pre-intervention) | Follow-up (7% wt loss) | RM-ANOVA | |
|---|---|---|---|---|
| | | | P (time) | P (time × intervention) |
| **Tumor necrosis factorα (*TNFα*)** | | | | |
| Lifestyle (n=7) | 9.1 (9.0 – 10.8) | 10.2 (9.9 – 10.7) | 0.351 | 0.636 |
| RYGB (n=6) | 10.3 (9.5 – 14.3) | 11.1 (9.7 – 19.8) | | |
| **Interleukin-1β (*IL1B*)** | | | | |
| Lifestyle (n=7) | 4.1 (3.5 – 4.9) | 9.3 (7.4 – 21.5) | 0.037 | 0.926 |
| RYGB (n=6) | 4.6 (3.3 – 8.6) | 8.8 (4.3 – 12.4) | | |
| **Interleukin-6 (*IL6*)** | | | | |
| Lifestyle (n=7) | 8.5 (7.7 – 16.2) | 10.9 (8.8 – 12.0) | 0.597 | 0.726 |
| RYGB (n=6) | 9.5 (6.8 – 14.1) | 12.6 (9.7 – 16.2) | | |
| **Monocyte Chemoattractant Protein-1 (*MCP1*)** | | | | |
| Lifestyle (n=7) | 820 (748 – 870) | 602 (436 – 657) | 0.229 | 0.734 |
| RYGB (n=6) | 807 (520 – 1,055) | 629 (559 – 920) | | |
| **Intercellular adhesion molecule-1 (*ICAM1*)** | | | | |
| Lifestyle (n=7) | 79 (76 – 88) | 86 (74 – 93) | 0.727 | 0.460 |
| RYGB (n=6) | 96 (72 – 103) | 91 (67 – 101) | | |
| **Adiponectin (*ADIPOQ*)** | | | | |
| Lifestyle (n=7) | 24,473 (20,205 – 25,527) | 19,960 (19,373 – 22,983) | 0.010 | 0.125 |
| RYGB (n=6) | 20,436 (19,559 – 21,232) | 15,680 (12,583 – 18,856) | | |

[3] Values are medians ($25^{th}$, $75^{th}$ percentiles), in mRNA copy number of the respective target gene per ng of total RNA, normalized by a normalization factor based on two housekeeping genes, β-glucuronidase and 18s ribosomal RNA. RM-ANOVA: Repeated measures analysis of variance.

**Table 4**

Adipose tissue leukocyte numbers at baseline (pre-intervention) and after 7% weight loss following either Roux-en-Y gastric bypass surgery (RYGB) or an intensive lifestyle modification combined with best medical care ('Lifestyle')[5].

| | Baseline (pre-intervention) | Follow-up (7% wt loss) | RM-ANOVA | |
|---|---|---|---|---|
| | | | P (time) | P (time × intervention) |
| **Total ATM (per g tissue)** | | | | |
| Lifestyle (n=7) | 54,772 (4,421 – 94,555) | 31,938 (19,849 – 45,750) | 0.850 | 0.320 |
| RYGB (n=6) | 30,042 (22,403 – 38,827) | 26,486 (13,672 – 58,831) | | |
| **Total ATM (as % of CD45$^{pos}$ fraction)** | | | | |
| Lifestyle (n=7) | 12.8 (6.6 – 25.7) | 2.5 (1.9 – 5.6) | 0.004 | 0.511 |
| RYGB (n=6) | 11.4 (11.0 – 14.2) | 4.7 (1.7 – 8.2) | | |
| **CD11c$^{pos}$ ATM (per g tissue)** | | | | |
| Lifestyle (n=7) | 5,502 (1,100 – 26,465) | 8,663 (5,833 – 9,798) | 0.793 | 0.209 |
| RYGB (n=6) | 7,163 (3,311 – 10,872) | 3,393 (1,701 – 4,922) | | |
| **CD11c$^{pos}$ ATM (as % of CD45$^{pos}$ fraction)** | | | | |
| Lifestyle (n=7) | 3.6 (1.7 – 5.5) | 0.7 (0.6 – 0.8) | 0.010 | 0.596 |
| RYGB (n=6) | 2.8 (0.4 – 4.0) | 0.9 (0.6 – 1.3) | | |
| **CD1c$^{pos}$ dendritic cells (per g tissue)** | | | | |
| Lifestyle (n=7) | 10,010 (2,455 – 22,830) | 9,582 (8,525 – 18,555) | 0.761 | 0.138 |
| RYGB (n=7) | 10,814 (9,009 – 21,054) | 9,934 (3,346 – 17,014) | | |
| **CD1c$^{pos}$ dendritic cells (as % of CD45$^{pos}$ fraction)** | | | | |
| Lifestyle (n=5) | 3.1 (3.0 – 4.1) | 1.9 (1.4 – 4.1) | 0.003 | 0.440 |
| RYGB (n=6) | 3.7 (3.1 – 6.0) | 1.2 (1.0 – 1.6) | | |
| **Neutrophil granulocytes (per g tissue)** | | | | |
| Lifestyle (n=7) | 6,192 (2,037 – 13,592) | 510,762 (337,602 – 695,103) | 0.001 | 0.049 |
| RYGB (n=7) | 14,913 (9,783 – 57,238) | 84,926 (16,049 – 849,753) | | |
| **Neutrophil granulocytes (as % of CD45$^{pos}$ fraction)** | | | | |
| Lifestyle (n=7) | 4.0 (3.5 – 4.0) | 52.3 (42.7 – 64.0) | <0.001 | 0.090 |
| RYGB (n=7) | 4.8 (4.2 – 15.0) | 33.0 (17.2 – 64.5) | | |
| **CD8$^{pos}$ T cells (per g tissue)** | | | | |
| Lifestyle (n=5) | 40,044 (24,564 – 56,744) | 56,812 (44,842 – 139,448) | 0.110 | 0.680 |

| | Baseline (pre-intervention) | Follow-up (7% wt loss) | RM-ANOVA | |
|---|---|---|---|---|
| | | | P (time) | P (time × intervention) |
| RYGB (n=5) | 24,686 (22,635 – 25,677) | 35,897 (20,671 – 59,698) | | |
| **CD8$^{pos}$ T cells (as % ofCD45$^{pos}$ fraction)** | | | | |
| Lifestyle (n=5) | 12.4 (11.5 – 23.8) | 9.3 (6.9 – 9.6) | 0.023 | 0.632 |
| RYGB (n=5) | 8.1 (7.2 – 9.5) | 7.1 (4.5 – 7.2) | | |
| **CD4$^{pos}$ T cells (per g tissue)** | | | | |
| Lifestyle (n=5) | 61,091 (40,969 – 62,351) | 136,824 (67,707 – 171,496) | 0.041 | 0.408 |
| RYGB (n=5) | 53,817 (51,626 – 54,597) | 92,247 (61,368 – 121,248) | | |
| **CD4$^{pos}$ T cells (as % of CD45$^{pos}$ fraction)** | | | | |
| Lifestyle (n=5) | 17.2 (17.1 – 19.3) | 14.6 (11.1 – 17.6) | 0.118 | 0.628 |
| RYGB (n=5) | 21.7 (19.9 – 22.7) | 11.5 (9.8 – 21.2) | | |
| **CD4/CD8 T- cell ratio** | | | | |
| Lifestyle (n=5) | 0.97 (0.7 – 1.6) | 1.53 (1.2 – 3.0) | 0.005 | 0.040 |
| RYGB (n=5) | 2.21 (2.1 – 2.8) | 2.57 (2.2 – 3.0) | | |

[4] Values are medians (25$^{th}$, 75$^{th}$ percentiles). ATM: Adipose tissue macrophages; RM-ANOVA: Repeated measures analysis of variance.

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