

Immune cell-mediated inflammation and the early improvements in glucose metabolism after gastric banding surgery

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Abstract

Aims/hypothesis The contribution of immune cells to the inflammasome that characterises type 2 diabetes mellitus and obesity is under intense research scrutiny. We hypothesised that early changes in glucose metabolism following gastric banding surgery may relate to systemic inflammation, particularly cell-mediated immunity.

Methods Obese participants (BMI 43.4 ± 4.9 kg/m², $n=15$) with diabetes or impaired glucose tolerance (IGT) underwent laparoscopic adjustable gastric banding surgery. Measurements taken before, and at 2 and 12 weeks after surgery included: fasting glucose, glucose levels 2 h after a 75 g oral load, glucose incremental AUC, oral glucose insulin sensitivity index (OGIS), circulating immune cell numbers and activation, and adipokine levels. Subcutaneous and visceral adipose tissue were collected at surgery, and macrophage number and activation measured.

Results There were significant reductions in fasting and 2 h glucose, as well as improved OGIS at 2 and 12 weeks. At

12 weeks, 80% of the diabetic participants reverted to normal glucose tolerance or IGT, and all IGT participants had normalised glucose tolerance. The 12 week fall in fasting glucose was significantly related to baseline lymphocyte and T lymphocyte numbers, and to granulocyte activation, but also to the magnitude of the 12 week reduction in lymphocyte and T lymphocyte numbers and TNF- α levels. In a model that explained 75% of the variance in the change in fasting glucose, the 12 week change in T lymphocytes was independently associated with the 12 week fall in fasting glucose.

Conclusions/interpretation Rapid improvements in glucose metabolism after gastric banding surgery are related to reductions in circulating pro-inflammatory immune cells, specifically T lymphocytes. The contribution of immune cell-mediated inflammation to glucose homeostasis in type 2 diabetes and its improvement after bariatric surgery require further investigation.

Keywords Adipokine · Bariatric surgery · Diabetes · Glucose · Immune cells · Inflammation · Insulin resistance · Lymphocyte · Obesity · Tumour necrosis factor- α · Weight loss

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Abbreviations

CRP	C-reactive protein
IGT	Impaired glucose tolerance
OGIS	Oral glucose insulin sensitivity index
SAT	Subcutaneous adipose tissue
VAT	Visceral adipose tissue

Introduction

Observational studies have shown that bariatric surgery is associated with long-term improvement in obesity-associated outcomes, including type 2 diabetes mellitus, cardiovascular

disease, cancer and death [1–10]. Data from recent randomised controlled studies demonstrate the superiority of bariatric surgery in inducing diabetes remission or optimising glucose control over standard medical intervention at 1 [11] and 2 [12, 13] years. Several randomised studies have also compared different bariatric approaches, with diabetes remission reported at 1 [11, 14], 2 [13] and 4 [15] years. In these studies, rapid post-operative improvements in glucose metabolism were observed. Moreover, studies in type 2 diabetes have shown that bariatric surgery induces clinically meaningful improvements of cardiac risk factors [11–15] and vascular dysfunction [16–19]. Diabetes-specific outcomes and remission following bariatric surgery have been reviewed elsewhere [20].

Obesity-associated type 2 diabetes is characterised by low-grade circulating and tissue-based inflammation, with evidence of increased levels of adipokines and of pro-inflammatory circulating and adipose tissue-based immune cells [21]. Inflammation interferes with insulin signalling and induces insulin resistance, a fundamental component of glucose regulation [21]. Circulating pro-inflammatory adipokines, which are reduced after bariatric surgery [22–24], contribute to insulin resistance and predict incident diabetes [25, 26].

Cell-mediated inflammation is also implicated in the inflammasome that characterises obesity. Our previous work has shown that the degree of insulin resistance in obesity is related to circulating numbers of a pro-inflammatory T lymphocyte subset, T helper-1 cells [27]. We have also found that energy intake restriction markedly diminished T helper-1 cell numbers and decreased cell surface expression of activation markers on granulocytes, monocytes and T lymphocytes [28]. In keeping with the notion that obesity-associated inflammation contributes to glucose dysregulation, we hypothesised that the degree of circulating and adipose tissue-based inflammation in obesity-associated type 2 diabetes and its dynamic response after bariatric surgery may be related to early improvements in glucose metabolism.

Methods

Participants We recruited 15 morbidly obese participants with type 2 diabetes or IGT from ambulatory diabetes or surgery clinics in a tertiary referral hospital. Inclusion criteria were age >18 years, BMI >35 kg/m², and either type 2 diabetes or IGT. The study protocol was approved by the institutional research and ethics committee. The study was registered at www.clinicaltrials.gov (NCT00592735). Participants gave written informed consent.

According to patient history and an examination of the records, ten participants had had type 2 diabetes for at least 5 years. Five participants had IGT as determined by a 75 g

OGTT; one of these reported a history of gestational diabetes. Medications were documented at each visit. Metformin was continued in diabetes participants, regardless of post-operative glucose levels.

Metabolic studies were performed following a 10 h overnight fast at baseline, and at 2 and 12 weeks after gastric banding surgery. All participants receiving glucose-lowering medications had withdrawn their glucose-lowering medications for 3 days prior to metabolic tests. Weight, waist and height were measured with the participant barefoot and in a hospital gown. BMI was calculated as weight/height² (kg/m²).

Blood samples were taken for fasting glucose, insulin, adipokines, inflammatory markers and HbA_{1c}. A 75 g OGTT was performed at each visit (Carbotest 75 g per 300 ml; Lomb Scientific, Sydney, NSW, Australia), with blood samples collected at 0, 30, 60, 90 and 120 min. Consumption of the glucose load was supervised to ensure it was consumed within 120 s and not regurgitated.

Laboratory measures Plasma glucose was determined by the glucose oxidase method (YSI glucose analyser 2300 STAT PLUS 230V; YSI, Yellow Springs, OH, USA). Serum was stored (−80°C). Serum insulin was measured by radioimmunoassay (Linco Research, St Charles, MO, USA). High-sensitivity C-reactive protein (CRP) was measured by a high-sensitivity assay (Synchron LX System Chemistry Analyser; Beckman Coulter, Sydney, NSW, Australia). HbA_{1c} was measured by high-performance liquid chromatography (Pharmacia LKB Biotechnology, Uppsala, Sweden). Adiponectin was measured by radioimmunoassay (Linco Research), and IL-1β, IL-6 and TNFα by ELISA (R&D Systems, Minneapolis, MN, USA). All CVs were <5%.

Insulin sensitivity was estimated using the oral glucose insulin sensitivity index (OGIS) as described by Mari et al [29] and the calculator spreadsheet downloaded at <http://webmet.pd.cnr.it/ogis> (last accessed 24 December 2012). Insulin secretion was estimated using insulin measures obtained during the OGTT, i.e. the insulinogenic index (Δinsulin 30–0:Δglucose 30–0) [30] and the insulin incremental AUC using the trapezoid rule.

Immune cell phenotyping Immune cells were measured as described previously [27, 28]. Fresh whole blood was collected at baseline and 12 weeks after surgery. T lymphocyte helper-1 and T lymphocyte helper-2 cell numbers were quantified by intracellular cytokine staining for interferon-gamma and IL-4, respectively (BD Biosciences Pharmingen, San Diego, CA, USA), as previously described by us [27, 28]. Immune cells were stained with fluorochrome-conjugated antibodies to the following cell surface markers: granulocyte CD66, monocyte CD11b and T lymphocyte CD25 (interleukin-2 receptor) (BD Biosciences Pharmingen); analyses were

performed with argon and red diode lasers (excitation at 488 and 635 nm, respectively) (FACSCalibur; BD Biosciences), using CellQuest software (version 3.3; BD Biosciences Pharmingen) and FlowJo software (Version 7; Tree Star, Ashland, OR, USA) as previously described by us [27, 28]. Complete immune cell data were available for 13 participants; two participants missed these studies as the required instruments were not available on the study day.

Subcutaneous and visceral fat macrophage activation status Subcutaneous adipose tissue (SAT) and visceral adipose tissue (VAT) samples were collected at surgery from all participants, SAT from peri-umbilical depots and VAT from perigastric depots. Adipose tissue-based macrophages were separated as previously described [27, 28]. Briefly, adipose samples were immediately placed in warm physiological buffer (37°C, modified Krebs–Ringer phosphate buffer: 10 mmol/l CaCl_2 , 6 mmol/l Na_2HPO_4 , 125 mmol/l HEPES, 12 mmol/l MgSO_4 , 4 mmol/l NaH_2PO_4 , 1.2 mol/l NaCl, 60 mmol/l KCl, 3 g BSA and 0.09 g D-glucose in 100 ml H_2O , pH 7.4). Samples were finely cut and digested for 30 min with collagenase IV (0.75 mg/ml) at 37°C. The stromovascular fraction (SVF) was separated from mature adipocytes by slow centrifugation (300 g). Samples were analysed by flow cytometry using a validated staining protocol to quantify distinct cell subsets and activation markers. Macrophages were stained for the monocyte/macrophage antigen CD14. Macrophage activation was determined by surface expression of CD11b [27, 28].

Statistical analysis Data presented are mean \pm SEM for comparisons between groups. Analyses were performed using SPSS (PASW Statistics, Chicago, IL, USA). The effect of bariatric surgery on variables at 2 and 12 weeks was examined by paired *t* tests for normally distributed variables and Wilcoxon's signed ranks tests for non-normally distributed variables. The effect of gastric banding on metabolic measures over time was also examined using repeated measures ANOVA. The associations between immune cell and inflammation measures and glucose metabolism were examined using linear regression analysis after data normalisation. Changes in fasting glucose from baseline were examined as absolute change, but also as the percentage change from baseline. Univariate model analysis was performed to examine the effect of immune cell and inflammation measures on the change in glucose levels, after transformation of non-normally distributed data. A value of $p < 0.05$ was considered significant.

Results

Participant characteristics The mean characteristics were: age 50.3 ± 2.7 years (range 35–65); weight 127.5 kg (range

92.1–172.0); and BMI 43.4 kg/m^2 (range 35.1–50.8). Metabolic and lipid variables, and medication use at baseline and at 2 and 12 weeks after gastric banding are shown in Table 1. There were significant reductions in weight, BMI, waist, systolic blood pressure, total cholesterol, LDL-cholesterol and triacylglycerol, and increases in HDL-cholesterol, accompanied by a reduction or cessation of sulfonylurea and anti-hypertensive and lipid-lowering medications.

Glucose metabolism, insulin secretion, insulin sensitivity and adipokines Significant reductions were observed for fasting glucose, 2 h glucose, HbA_{1c} , glucose incremental AUC and fasting insulin, accompanied by significant increases in insulin sensitivity (Table 1, Fig. 1).

At 2 weeks after gastric banding, seven of ten diabetic participants had shifted to normal glucose tolerance or IGT, and eight of ten had remitted at 12 weeks. The two participants who did not remit at 12 weeks had been receiving maximal sulfonylurea doses and had the highest baseline HbA_{1c} levels (8.3% and 9.2% [67 and 77 mmol/mol]).

Insulin secretion assessed by the insulinogenic index and insulin incremental AUC did not significantly change (Table 1). The effect of higher baseline insulin secretion on glycaemic responses after bariatric surgery was determined using regression analysis. Higher baseline insulin secretion was associated with a greater HbA_{1c} improvement at 12 weeks (insulinogenic index $\beta = 0.67$, $p = 0.035$; insulin incremental AUC $\beta = 0.67$, $p = 0.035$).

Figure 2 shows changes in circulating adipokine levels at 2 and 12 weeks after bariatric surgery. IL-6 levels decreased at 2 weeks only. There was a non-significant trend towards increased adiponectin levels at 12 weeks ($p = 0.053$). Fasting CRP, IL-1 β and TNF α levels did not differ at 2 or 12 weeks after bariatric surgery.

Relationships to immune cell-mediated inflammation The relationships between baseline circulating and adipose tissue immune cell-mediated inflammation and improved fasting glucose at 12 weeks were examined. Relationships were found between higher baseline indices of immune cell-mediated inflammation and greater absolute reduction in fasting glucose for lymphocyte numbers ($\beta = -0.67$, $r^2 = 0.45$, $p = 0.01$), T lymphocyte numbers ($\beta = -0.73$, $r^2 = 0.52$, $p = 0.005$) and granulocyte cell surface expression of the activation marker CD66 ($\beta = -0.69$, $r^2 = 0.47$, $p = 0.009$). Similar results were found when the change in fasting glucose was expressed as percentage change (Fig. 3).

The relationships between changes in circulating immune cell numbers and the fall in fasting glucose at 12 weeks were also examined. The absolute fall in fasting glucose was related to the reduction in numbers of lymphocytes ($\beta = 0.63$, $r^2 = 0.41$, $p = 0.02$) and T lymphocytes ($\beta = 0.73$, $r^2 = 0.52$, $p = 0.005$), and to the rise in anti-inflammatory T helper-2

Table 1 Clinical, metabolic and inflammatory variables in participants with type 2 diabetes and IGT at times indicated after gastric banding surgery

Variable	Baseline	2 Weeks after surgery	12 Weeks after surgery
Clinical			
Weight (kg)	127.5±5.7	116.0±4.9 ^c	111.5±5.1 ^{c f}
BMI (kg/m ²)	43.4±1.3	39.6±1.2 ^c	38.1±1.3 ^{c f}
Waist (cm)	132.4±3.8	122.1±3.5 ^c	120.4±3.7 ^{c f}
Systolic BP (mmHg)	132±2	120±3 ^c	125±3 ^{a e}
Diastolic BP (mmHg)	80±3	77±2	77±2
Metabolic			
Fasting glucose (mmol/l)	5.9±0.3	5.3±0.2 ^a	5.1±0.3 ^{a d}
2 h glucose (mmol/l)	11.9±0.73	8.6±0.7 ^c	8.2±0.7 ^{c f}
HbA _{1c} (%) ^g	6.9±0.3	6.3±0.3 ^b	6.3±0.3 ^{b e}
HbA _{1c} (mmol/mol) ^g	52±2	45±2 ^b	45±2 ^{b e}
OGIS (ml min ⁻¹ m ⁻²)	268±25	349±20 ^b	360±25 ^{c f}
Glucose iAUC (mmol/l×h) ^h	1253±83	1093±74 ^b	1224±210 ^{a e}
Fasting insulin (pmol/l)	208±45	128±19 ^a	155±22 ^d
Insulin iAUC (log pmol/l×h) ^h	4.14±0.12	4.02±0.09	4.04±0.09
Insulinogenic index ⁱ	12.1±3.2	12.6±2.6	16.9±5.4
Total cholesterol (mmol/l)	4.5±0.3	3.7±0.2 ^a	4.4±0.3 ^d
HDL-cholesterol (mmol/l)	1.13±0.08	1.01±0.05 ^a	1.24±0.07 ^c
LDL-cholesterol (mmol/l)	2.7±0.2	2.2±0.2 ^a	2.6±0.2
Triacylglycerol (mmol/l)	1.5±0.2	1.2±0.1 ^a	1.2±0.1 ^{a d}
Immune cell measures			
Granulocytes (%) ^j	39.4±3.9	—	46.7±1.9
Monocytes (%) ^j	3.1±0.6	—	3.2±0.2
Lymphocytes (%) ^j	33.5±2.1	—	35.2±2.2
T lymphocytes (%) ^j	16.2±2.5	—	25.7±1.9
T helper-1 cells (%) ^j	5.2±1.6	—	1.1±0.2
T helper-2 cells (%) ^j	2.6±2.4	—	5.0±2.1
Adipose tissue			
SAT macrophages (%) ^k	6.2±1.7	—	—
SAT macrophage CD11b ^l	64.7±14.4	—	—
VAT macrophages (%) ^k	2.8±1.0	—	—
VAT macrophage CD11b ^l	58.3±11.6	—	—
Medication use (T2D) (n)			
Metformin	8	8	8
Sulfonylurea	5	2	2
Lipid-lowering	4	0	0
Antihypertensive	7	6	5

Unless otherwise stated, data are mean ± SEM (n=15)

Comparisons are by paired *t* tests, compared with baseline (^a*p*<0.05, ^b*p*<0.01, ^c*p*<0.0001) and repeated-measures ANOVA (^d*p*<0.05, ^e*p*<0.005, ^f*p*<0.0001)

^g Measured in ten type 2 diabetic participants

^h Measured during a 75 g OGTT

ⁱ Insulinogenic index: Δ (insulin 30–insulin 0)/Δ (glucose 30–glucose 0), derived during a 75 g OGTT [30]

^j Circulating immune cell counts are expressed as a percentage of leucocyte count

^k Adipose tissue macrophage counts are expressed as a percentage of total viable cells in the stromovascular fraction

^l Adipose tissue macrophage expression of the activation marker CD11b is expressed as relative mean fluorescence intensity

iAUC, incremental AUC

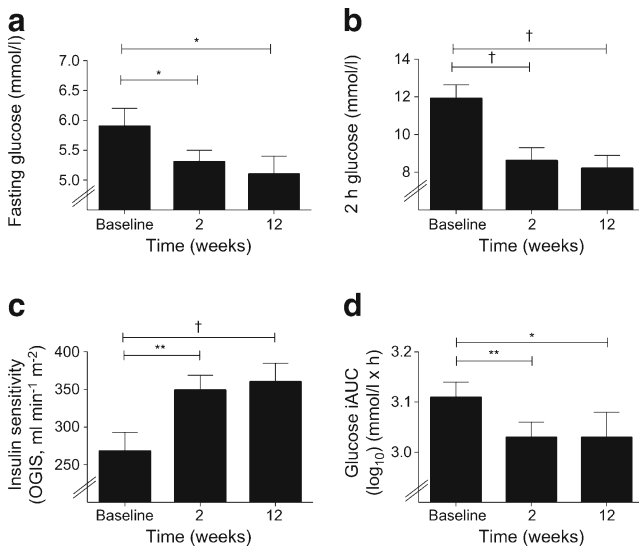


Fig. 1 The effect of gastric banding surgery on variables of glucose metabolism at 2 and 12 weeks. **(a)** Fasting glucose, **(b)** 2 h glucose, **(c)** insulin sensitivity (OGIS) and **(d)** glucose incremental AUC during a 75 g OGTT. * $p<0.05$, ** $p<0.01$, † $p<0.0001$

lymphocytes ($\beta=-0.83$, $r^2=0.69$, $p=0.001$). A weak relationship of borderline significance was found between the fall in fasting glucose and the fall in TNF α levels ($\beta=0.51$, $r^2=0.25$, $p=0.05$). No relationship was found between the change in fasting glucose and adipose tissue macrophage numbers or activation ($p>0.10$; data not shown). Results were similar when the change in fasting glucose was expressed as percentage change (Fig. 3). The relationship between the fall in fasting glucose and the fall in TNF α levels did not remain after Bonferroni's correction.

To examine for independence, the fall in fasting glucose at 12 weeks was examined in a model that included ΔT

lymphocyte numbers and $\Delta TNF\alpha$ levels, with baseline glucose status, age and sex as covariates. The model explained 75% of the variance in the fall in fasting glucose at 12 weeks ($p=0.04$); the fall in T lymphocytes was independently related ($r^2=0.63$, $p=0.01$). To examine whether the observed metabolic improvement associated with T lymphocyte changes was independent of insulin secretion, the model was expanded to include baseline insulin secretion and the change in insulin secretion at 12 weeks. However, the model lost significance and the fall in T lymphocytes was no longer independently related.

The fall in glucose incremental AUC after bariatric surgery was also examined against changes in T lymphocyte numbers. A significant relationship was found between the reduction in glucose incremental AUC and the decline in circulating T lymphocytes ($\beta=0.74$, $r^2=0.55$, $p=0.010$).

The data were examined to determine whether immune cell-mediated inflammation was related to improved insulin secretion following gastric banding (Fig. 4). Significant relationships were found between the improvement in insulin incremental AUC at 12 weeks and the reduction in immune cell-mediated inflammation as measured by the reduction in T lymphocyte numbers ($\beta=0.68$, $p=0.021$), the reduction in T lymphocyte surface expression of the IL-2 receptor ($\beta=0.58$, $p=0.039$), the reduction in granulocyte surface expression of the activation marker CD11b ($\beta=0.60$, $p=0.029$) and lower baseline VAT macrophage activation ($\beta=0.67$, $p=0.033$).

Improvements in OGIS after gastric banding were examined against changes in immune cell-mediated inflammation. The increase in insulin sensitivity at 12 weeks was related to the reduction in T lymphocyte numbers ($\beta=-0.62$, $p=0.043$) and to the reduction in T lymphocyte surface expression of IL-2 receptor ($\beta=-0.60$, $p=0.031$).

Relationships between baseline adipocyte monocyte activation and circulating immune cell profiles were examined. VAT monocyte CD11b expression was related to T helper-1 numbers at baseline ($\beta=0.78$, $p=0.012$) and to the reduction in T helper-1 numbers at 12 weeks ($\beta=0.90$, $p=0.002$).

Discussion

Bariatric surgery is a potent intervention, which improves type 2 diabetes and mitigates many of its adverse cardiometabolic risk factors, with proven efficacy over standard medical therapies in the treatment of obesity-related type 2 diabetes [11–13]. It is a useful in vivo human model for studying 'diabetes in reverse', and, as such, may provide insights into the pathogenesis of diabetes and its associated metabolic and inflammatory perturbations [31].

This study reports the relationships between fasting and post-challenge glucose levels and circulating and adipose tissue-based inflammation in the early phase of weight loss

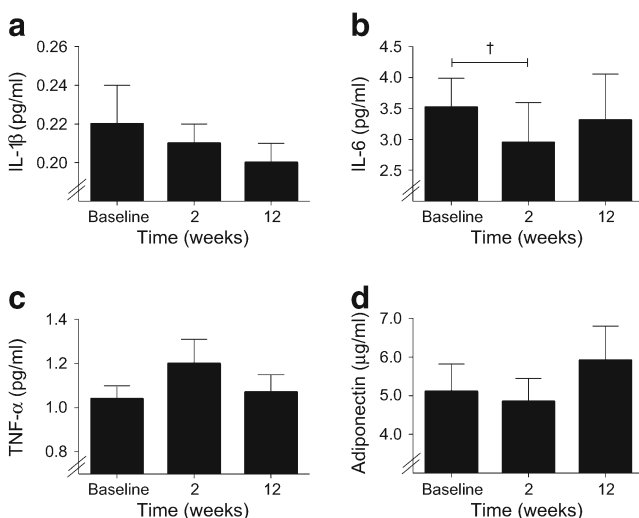


Fig. 2 The effect of gastric banding surgery on fasting adipokine levels at 2 and 12 weeks. **(a)** IL-1 β , **(b)** IL-6, **(c)** TNF- α and **(d)** adiponectin. * $p=0.03$; † $p=0.53$ for baseline vs 12 weeks

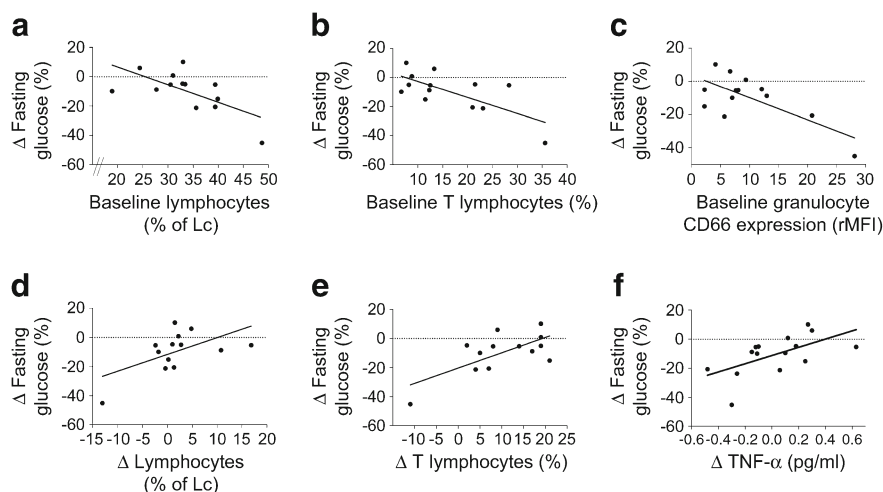


Fig. 3 Relationships between the percentage fall in fasting glucose following gastric banding surgery and baseline (a) lymphocyte count ($r^2=0.42$, $p=0.01$), (b) T lymphocyte count ($r^2=0.49$, $p=0.007$) and (c) granulocyte CD 66 expression ($r^2=0.50$, $p=0.007$). Relationships

between percentage fall as above and the change in (a) circulating lymphocytes ($r^2=0.33$, $p=0.04$), (e) circulating T lymphocytes ($r^2=0.47$, $p=0.009$) and (f) TNF- α levels ($r^2=0.35$, $p=0.02$). Lc, leucocytes; rMFI, relative mean fluorescence intensity

when dynamic changes in glucose levels are described. This study found rapid improvements in fasting and post-challenge glucose levels in obesity-associated type 2 diabetes at 2 and 12 weeks after gastric banding, as has been observed for other bariatric interventions, including gastric bypass [32, 33] or biliopancreatic diversion [34], but also with energy intake restriction by diet [35]. The magnitude of the fall in fasting glucose levels was greater in participants who at baseline had higher indices of circulating inflammation, specifically higher T lymphocyte numbers and higher granulocyte activation. The

fall in fasting glucose at 12 weeks was also related to greater reductions in markers of cell-mediated inflammation, specifically the size of the reduction in numbers of circulating lymphocytes and T lymphocytes. The reduction in fasting glucose at 12 weeks was also related to the increase of an anti-inflammatory T lymphocyte subset, T helper-2 cells. Changes in insulin secretion were also related to reductions in measures of T lymphocyte inflammation. These results support links between changes in glucose metabolism and shifts in the inflammasome towards less cell-mediated inflammation following gastric banding surgery.

Our understanding of the contribution of T lymphocytes in the inflammasome of obesity and type 2 diabetes is growing. In animal models of obesity, adipose tissue macrophage infiltration, inflammation and the development of insulin resistance are preceded by and dependent upon prodromal adipose tissue infiltration by T lymphocytes [36, 37]. In addition to promoting macrophage-mediated inflammation, T lymphocyte sensing of nutrient excess promotes pro-inflammatory T cell phenotypes. Intracellular nutrient pathways have been shown to be master regulators of T lymphocytes, skewing towards pro-inflammatory phenotypes. In humans, T lymphocyte polarisation in obesity and obesity-associated diabetes has been described [27, 28, 38], with higher circulating T helper-1 cells in some [27, 28], but not all [38] studies. Circulating T helper-1 cell numbers have been found to be related to the degree of insulin resistance in humans [27].

To our knowledge, no study has previously reported metabolic outcomes in relation to circulating and tissue-based immune cell phenotypes specifically in patients with glucose disorders. Energy intake restriction has been shown to repress circulating pro-inflammatory immune cell phenotypes in obesity (with reduced T helper-1 [28] lymphocytes or other [39] T

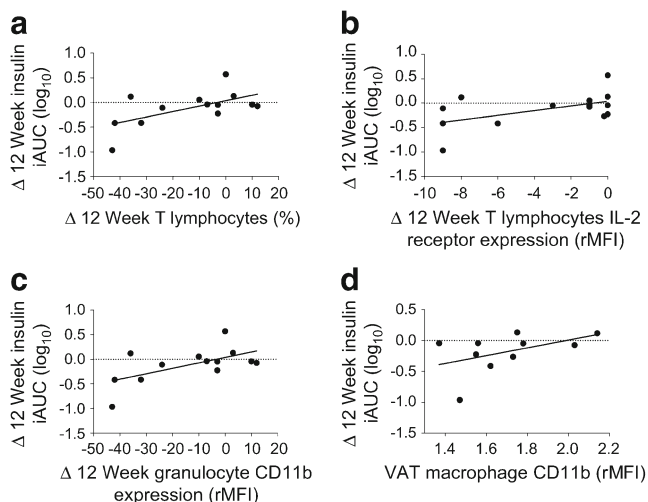


Fig. 4 Relationships between the change in insulin secretion at 12 weeks after gastric banding surgery and indices of immune cell-mediated inflammation. Change in (a) T lymphocyte numbers ($r^2=0.46$, $p=0.021$), (b) T lymphocyte expression of the IL-2 receptor ($r^2=0.34$, $p=0.039$), (c) granulocyte expression of CD11b ($r^2=0.36$, $p=0.029$) and (d) baseline VAT monocyte expression of CD11b ($r^2=0.45$, $p=0.033$). Values (a–e) are in pmol/l \times h, logarithmically transformed. rMFI, relative mean fluorescence intensity

lymphocyte subsets) and rheumatoid arthritis [40]. A longitudinal study of type 2 diabetes patients undergoing bariatric surgery showed that higher baseline leucocyte levels were associated with diabetes non-remission up to 45 months after surgery [41]. Our study builds on the published literature. We found that higher numbers of baseline lymphocytes and T lymphocytes were associated with greater early glucose responses after bariatric surgery. We also found that the size of the fasting glucose reduction was independently related to the concurrent fall in T lymphocytes. These novel findings support a potential role for T lymphocytes in the early recovery of fasting and post-challenge glucose levels after bariatric surgery in type 2 diabetes. Our analyses suggest that the association between improved fasting glucose and reduced T lymphocytes might be contingent on improved insulin secretion, since insulin has anti-inflammatory properties under usual circumstances. A causal relationship between reduced cell-mediated inflammation and improved glucose variables could not be established by this observational study. Mechanistic studies in animal models are needed to clarify the associations between immune cells and glucose metabolism in the setting of restricted energy intake. It is plausible that energy intake restriction, with improved insulin secretion may mediate improved glucose levels and reduced immune cell-mediated inflammation.

Numerous studies have reported adipokine responses after bariatric surgery, but only a few have examined changes within 12 weeks of surgery. We found a transient reduction in IL-6 at 2 weeks and a trend towards increased adiponectin at 12 weeks, but no significant change in IL-1 β or TNF- α , despite significant improvements in glucose levels. Adiponectin levels have been reported to be increased at 12 to 52 weeks after bariatric surgery [24, 42–44], whereas IL-6 levels were decreased at 12 to 60 weeks [22, 24, 43, 45, 46]. Consistent with our findings here, no changes in TNF- α levels have been observed after bariatric surgery [24, 44, 45]. Differences between our findings and previous studies may be explained by the heterogeneity of cohorts, with variable numbers of participants with glucose disorders in other studies. The wide variations in selected cytokines seen in the current study may also have contributed to discrepant findings. Moreover, in the current study, cytokine measures at 2 weeks could have been confounded by post-operative inflammation. Overall, however, serum adipokine changes seem to occur by at least 12 weeks after bariatric surgery, perhaps reflecting changes in adipose mass rather than changes in response to nutrient flux. In contrast, alterations in cell-mediated inflammation occur early, as do changes in glucose metabolism.

Gastric banding is a more conservative form of bariatric surgery. This small study observed that the majority of participants with type 2 diabetes improved their glucose tolerance to normal or IGT by 12 weeks after surgery. There are no randomised studies of gastric banding in comparison with

other bariatric surgery techniques in patients with diabetes, so comparisons of efficacy are lacking. One randomised study reported that gastric banding was superior to medical management in optimising glucose control in early type 2 diabetes at 24 months [12]. Two randomised studies compared other bariatric procedures and medical intervention [11, 13]. These studies showed that surgical intervention induced (strictly defined) target diabetes control or diabetes remission more frequently than did medical management [11, 13]. Gastric bypass, sleeve gastrectomy and biliopancreatic diversion were equally effective in achieving diabetes remission at 12 to 24 months [11, 13], although greater weight loss was observed with biliopancreatic diversion than with the other surgical interventions [11, 13]. A compelling finding in each of these studies was that weight reduction was only relatively modest by the time marked improvements in glucose metabolism were observed. Other studies have also shown rapid improvements of glucose levels in diabetes following bariatric surgery or restriction of energy intake. For example, the Counterpoint study of dietary restriction in obesity-associated diabetes reported a normalisation of fasting glucose levels after 7 days of rigorous energy intake restriction [35]. A study of type 2 diabetes participants compared 4 days of restricted energy intake with the first 4 days after gastric bypass (the period in which a rapid improvement of diabetes is observed); the rapidity and degree of glucose improvement were contingent on the degree of energy intake restriction in both study arms [47]. The current study shows that early and substantive improvements in glucose tolerance are also possible after gastric banding. Together with the published literature, the findings of the current study support the notion that, as with strict restriction of energy intake, rapid early improvements in glucose levels in diabetes are possible across the range of bariatric approaches.

The strengths of this study include: (1) documentation of the early changes in fasting and post-challenge glucose levels; and (2) detailed phenotyping of the inflammasome after bariatric surgery, including detailed measures of circulating immune cells, adipose tissue macrophages and adipokines. Weaknesses include the observational nature of the study, and its use of indirect estimates of insulin sensitivity and insulin secretion. The models of insulin secretion used reflect not only insulin secretion, but also insulin clearance, which may also be increased after bariatric surgery. The small number of participants is also a potential weakness, raising the possibility of type 2 errors for negative findings. Our sample size was restricted due to the technically challenging and labour-intensive studies of circulating and adipose tissue immune cells. Thus, this report could be seen as a pilot study, highlighting the link between lymphocyte-mediated inflammation and the recovery of glucose levels following bariatric surgery.

In summary, glucose tolerance improves by 2 weeks after gastric banding in obese participants with type 2 diabetes

mellitus or IGT. Greater improvements in fasting and post-challenge glucose levels were evident in participants with greater reductions in immune cell-mediated inflammation. Given our current understanding of the role of T lymphocytes in the inflammason of obesity and type 2 diabetes, future studies examining the interface between immunology and glucose metabolism are required.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

Contribution statement All authors contributed to study conception and design, data acquisition or analysis and interpretation, as well as to the drafting or critical revising of the manuscript for important intellectual content. All authors gave final approval of the version to be published.

References

- Adams TD, Davidson LE, Litwin SE et al (2012) Health benefits of gastric bypass surgery after 6 years. *J Am Med Assoc* 308:1122–1131
- Adams TD, Gress RE, Smith SC et al (2007) Long-term mortality after gastric bypass surgery. *N Engl J Med* 357:753–761
- Adams TD, Pendleton RC, Strong MB et al (2010) Health outcomes of gastric bypass patients compared to nonsurgical, nonintervened severely obese. *Obesity (Silver Spring)* 18:121–130
- Iaconelli A, Panunzi S, de Gaetano A et al (2011) Effects of biliopancreatic diversion on diabetic complications: a 10-year follow-up. *Diabetes Care* 34:561–567
- Lee WJ, Hur KY, Lakadawala M, Kasama K, Wong SK, Lee YC (2012) Gastrointestinal metabolic surgery for the treatment of diabetic patients: a multi-institutional international study. *J Gastrointest Surg* 16:45–51, discussion 51–52
- Leslie DB, Dorman RB, Serrot FJ et al (2012) Efficacy of the Roux-en-Y gastric bypass compared to medically managed controls in meeting the American Diabetes Association composite end point goals for management of type 2 diabetes mellitus. *Obes Surg* 22:367–374
- Pontioli AE, Folli F, Paganelli M et al (2005) Laparoscopic gastric banding prevents type 2 diabetes and arterial hypertension and induces their remission in morbid obesity: a 4-year case-controlled study. *Diabetes Care* 28:2703–2709
- Scopinaro N, Adami GF, Papadia FS et al (2011) The effects of biliopancreatic diversion on type 2 diabetes mellitus in patients with mild obesity (BMI 30–35 kg/m²) and simple overweight (BMI 25–30 kg/m²): a prospective controlled study. *Obes Surg* 21:880–888
- Torgerson JS, Hauptman J, Boldrin MN, Sjostrom L (2004) XENical in the prevention of diabetes in obese subjects (XENDOS) study: a randomized study of orlistat as an adjunct to lifestyle changes for the prevention of type 2 diabetes in obese patients. *Diabetes Care* 27:155–161
- Sjostrom L, Narbro K, Sjostrom CD et al (2007) Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med* 357:741–752
- Schauer PR, Kashyap SR, Wolski K et al (2012) Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med* 366:1567–1576
- Dixon JB, O'Brien PE, Playfair J et al (2008) Adjustable gastric banding and conventional therapy for type 2 diabetes: a randomized controlled trial. *J Am Med Assoc* 299:316–323
- Mingrone G, Panunzi S, de Gaetano A et al (2012) Bariatric surgery versus conventional medical therapy for type 2 diabetes. *N Engl J Med* 366:1577–1585
- Lee WJ, Chong K, Ser KH et al (2011) Gastric bypass vs sleeve gastrectomy for type 2 diabetes mellitus: a randomized controlled trial. *Arch Surg* 146:143–148
- Pinheiro JS, Schiavon CA, Pereira PB, Correa JL, Noujaim P, Cohen R (2008) Long-long limb Roux-en-Y gastric bypass is more efficacious in treatment of type 2 diabetes and lipid disorders in super-obese patients. *Surg Obes Relat Dis* 4:521–525
- Ikonomidis I, Mazarakis A, Papadopoulos C et al (2007) Weight loss after bariatric surgery improves aortic elastic properties and left ventricular function in individuals with morbid obesity: a 3-year follow-up study. *J Hypertension* 25:439–447
- Rider OJ, Francis JM, Ali MK et al (2009) Beneficial cardiovascular effects of bariatric surgical and dietary weight loss in obesity. *J Am Coll Cardiol* 54:718–726
- Samaras K, Viardot A, Lee PN et al (2013) Reduced arterial stiffness after weight loss in obese type 2 diabetes and impaired glucose tolerance: the role of immune cell activation and insulin resistance. *Diab Vasc Dis Res* 10:40–48
- Shargorodsky M, Fleed A, Boaz M, Gavish D, Zimlichman R (2006) The effect of a rapid weight loss induced by laparoscopic adjustable gastric banding on arterial stiffness, metabolic and inflammatory parameters in patients with morbid obesity. *Int J Obes (Lond)* 30:1632–1638
- Samaras K (2013) Bariatric surgery for type 2 diabetes: to whom and when? *Minerva Endocrinol* 38:47–58
- Donath MY, Shoelson SE (2011) Type 2 diabetes as an inflammatory disease. *Nat Rev Immunol* 11:98–107
- Swarbrick MM, Stanhope KL, Austrheim-Smith IT et al (2008) Longitudinal changes in pancreatic and adipocyte hormones following Roux-en-Y gastric bypass surgery. *Diabetologia* 51:1901–1911
- Trakhtenbroit MA, Leichman JG, Algahim MF et al (2009) Body weight, insulin resistance, and serum adipokine levels 2 years after 2 types of bariatric surgery. *Am J Med* 122:435–442
- Illan-Gomez F, Gonzalez-Ortega M, Orea-Soler I et al (2012) Obesity and inflammation: change in adiponectin, C-reactive protein, tumour necrosis factor- α and interleukin-6 after bariatric surgery. *Obes Surg* 22:950–955
- Mather KJ, Funahashi T, Matsuzawa Y et al (2008) Adiponectin, change in adiponectin, and progression to diabetes in the Diabetes Prevention Program. *Diabetes* 57:980–986
- Spranger J, Kroke A, Mohlig M et al (2003) Inflammatory cytokines and the risk to develop type 2 diabetes: results of the prospective population-based European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study. *Diabetes* 52:812–817
- Viardot A, Heilbronn LK, Samocha-Bonet D, Mackay F, Campbell LV, Samaras K (2012) Obesity is associated with activated and insulin resistant immune cells. *Diabetes Metab Res Rev* 28:447–454
- Viardot A, Lord RV, Samaras K (2010) The effects of weight loss and gastric banding on the innate and adaptive immune system in type 2 diabetes and prediabetes. *J Clin Endocrinol Metab* 95:2845–2850

29. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ (2001) A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. *Diabetes Care* 24:539–548
30. Seltzer HS, Allen EW, Herron AL Jr, Brennan MT (1967) Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *J Clin Invest* 46:323–335
31. Taylor R (2013) Banting Memorial Lecture 2012. Reversing the twin cycles of type 2 diabetes. *Diabet Med* 30:267–275
32. Matarese G, Procaccini C, de Rosa V (2012) At the crossroad of T cells, adipose tissue, and diabetes. *Immunol Rev* 249:116–134
33. Schauer PR, Burguera B, Ikramuddin S et al (2003) Effect of laparoscopic Roux-en Y gastric bypass on type 2 diabetes mellitus. *Annals Surg* 238:467–484
34. Guidone C, Manco M, Valera-Mora E et al (2006) Mechanisms of recovery from type 2 diabetes after malabsorptive bariatric surgery. *Diabetes* 55:2025–2031
35. Lim EL, Hollingsworth KG, Aribisala BS, Chen MJ, Mathers JC, Taylor R (2011) Reversal of type 2 diabetes: normalisation of beta cell function in association with decreased pancreas and liver triacylglycerol. *Diabetologia* 54:2506–2514
36. Kintscher U, Hartge M, Hess K et al (2008) T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. *Arterioscler Thromb Vasc Biol* 28:1304–1310
37. Nishimura S, Manabe I, Nagasaki M et al (2009) CD8⁺ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity. *Nature Med* 15:914–920
38. Procaccini C, de Rosa V, Galgani M et al (2010) An oscillatory switch in mTOR kinase activity sets regulatory T cell responsiveness. *Immunity* 33:929–941
39. Cottam DR, Schaefer PA, Shaftan GW, Angus LD (2003) Dysfunctional immune-privilege in morbid obesity: implications and effect of gastric bypass surgery. *Obes Surg* 13:49–57
40. Fraser DA, Thoen J, Reseland JE, Forre O, Kjeldsen-Kragh J (1999) Decreased CD4⁺ lymphocyte activation and increased interleukin-4 production in peripheral blood of rheumatoid arthritis patients after acute starvation. *Clin Rheumatol* 18:394–401
41. Hirsch FF, Pareja JC, Geloneze SR, Chaim E, Cazzo E, Geloneze B (2012) Comparison of metabolic effects of surgical-induced massive weight loss in patients with long-term remission versus non-remission of type 2 diabetes. *Obes Surg* 22:910–917
42. Brethauer SA, Heneghan HM, Eldar S et al (2011) Early effects of gastric bypass on endothelial function, inflammation, and cardiovascular risk in obese patients. *Surg Endoscopy* 25:2650–2659
43. Marantos G, Daskalakis M, Karkavitsas N, Matalliotakis I, Papadakis JA, Melissas J (2011) Changes in metabolic profile and adipoinular axis in morbidly obese premenopausal females treated with restrictive bariatric surgery. *World J Surg* 35:2022–2030
44. Miller GD, Nicklas BJ, Fernandez A (2011) Serial changes in inflammatory biomarkers after Roux-en-Y gastric bypass surgery. *Surg Obesity Rel Dis* 7:618–624
45. Kopp HP, Kopp CW, Festa A et al (2003) Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. *Arterioscler Thromb Vasc Biol* 23:1042–1047
46. Moschen AR, Molnar C, Geiger S et al (2010) Anti-inflammatory effects of excessive weight loss: potent suppression of adipose interleukin 6 and tumour necrosis factor alpha expression. *Gut* 59:1259–1264
47. Isbell JM, Tamboli RA, Hansen EN et al (2010) The importance of caloric restriction in the early improvements in insulin sensitivity after Roux-en-Y gastric bypass surgery. *Diabetes Care* 33:1438–1442