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Original article

The effect of bariatric surgery on serum TRAIL and osteoprotegerin levels in obesity complicated by glucose disorders

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SUMMARY

Background and aims: Bariatric surgery improves health outcomes in the obese and reduces some aspects of obesity-associated systemic inflammation. Little is known however about its effects on circulating TNF-related apoptosis-inducing ligand (TRAIL) and osteoprotegerin level, which regulate apoptosis and are implicated in atherogenesis. Our objective was to identify whether circulating TRAIL and osteoprotegerin levels are influenced by the energy restriction and weight loss that follows bariatric surgery in obese patients with glucose disorders.

Methods: 15 morbidly obese individuals with type 2 diabetes mellitus (T2D) or glucose intolerance were recruited for bariatric surgery. Participants were assessed for weight, waist circumference and BMI at baseline, then 2 and 12 weeks following energy restriction with bariatric surgery. Laparoscopic adjustable gastric band placement was performed. Fasted blood samples were collected and an oral glucose tolerance test was performed at each visit. Metabolic parameters and plasma chemistries were assessed. Circulating TRAIL, osteoprotegerin and leptin levels were measured.

Results: A significant increase in circulating TRAIL levels was observed at 12 weeks relative to baseline in participants who suppressed leptin levels. The percentage change in TRAIL was inversely related to the percentage change in fasting insulin and HOMA-β. In contrast, osteoprotegerin levels and the osteoprotegerin:TRAIL ratio were significantly reduced following bariatric surgery. The change in osteoprotegerin:TRAIL ratio positively related to the percentage change in fasting glucose.

Conclusions: Energy restriction after bariatric surgery is associated with increased circulating TRAIL levels and reduced osteoprotegerin levels and osteoprotegerin:TRAIL ratio in obese humans with dysglycaemia. Changes in the TRAIL and osteoprotegerin:TRAIL ratio related to changes in fasting insulin, suggesting a possible role in glucose improvements after bariatric surgery. Mechanistic studies will clarify the role of TRAIL and osteoprotegerin in health and disease.

1. Introduction

Obesity united with type-2 diabetes mellitus (T2D) is becoming the single biggest health threat to the Western world, with obesity greatly increasing the risk and accelerating the onset of T2D. While bariatric surgery can result in long-term weight loss and improve both cardiovascular risk and T2D [1–3], urgent new measures are still required to address the morbidity and mortality associated with these debilitating conditions.

TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) is a protein discovered and named with regards to its ability to promote cell death by binding its specific death receptors. TRAIL belongs to the tumour necrosis factor (TNF) cytokine superfamily, existing in both membrane-bound and soluble forms. Dependent on TRAIL levels, receptor expression and cell types involved, TRAIL can also promote survival pathways [4,5]. These apparently

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opposing effects are not surprising, since TRAIL is expressed on most tissues and cells and, in humans, it binds five different receptors, making it's signalling the most complex of all the TNF family members. These include death receptor- 4 and -5, decoy receptor -1 and -2 and osteoprotegerin, the only known soluble receptor for TRAIL.

Recent studies implicate TRAIL in regulating beneficial metabolic responses. For example, in response to a Western diet in an atheroma-prone knockout mouse model, we have shown that TRAIL-gene deficiency resulted in accelerated weight gain, greater adiposity, inflammation and diabetes [6]. Notably, circulating TRAIL levels are reduced in human subjects with newly diagnosed T2D [7], and TRAIL administration into obese C57Bl6 mice resulted in reduced adiposity with improvements in glucose and insulin tolerance, indicating a potential anti-obesity and anti-diabetic effect [8]. While circulating osteoprotegerin levels are also altered in metabolic diseases, its relationship with TRAIL is unclear; the osteoprotegerin:TRAIL interaction in obesity and diabetes is currently unknown.

In this study, we aimed to identify whether circulating TRAIL levels are influenced by energy restriction with bariatric surgery in obese T2D patients. We also assessed levels of circulating osteoprotegerin to examine the relationship between osteoprotegerin and osteoprotegerin;TRAIL ratio following weight loss. Given the paucity of information on TRAIL and obesity in humans, we measured a range of metabolic makers to elucidate its relationships and changes following weight loss.

2. Materials and methods

2.1. Subjects

Fifteen morbidly obese participants (8 males, 7 females) were recruited from ambulatory diabetes or surgery clinics in a tertiary referral hospital for a study of weight reduction by bariatric surgery. The inclusion criteria were: age >18 years, body mass index (BMI) exceeding 35 kg/m^2, either T2D or impaired glucose tolerance (IGT) and the wish to undertake bariatric surgery. The study protocol was approved by the institutional Research and Ethics Committee and all participants gave written informed consent. The study was registered at www.clinicaltrials.gov (NCT00592735).

Of the 15 participants, 10 had T2D for at least 5 years, confirmed by medical record examination. In participants with T2D, the following medications were used: metformin ($n = 8$), sulfonylureas ($n = 5$), lipid-lowering medications ($n = 4$) and antihypertensives ($n = 7$). Five participants had IGT by 75 g oral glucose tolerance testing (OGTT) [10]; one IGT participant had prior gestational diabetes. Participants with IGT took no medications.

Weight, waist circumference and height were measured with the participant barefoot and in a hospital gown. BMI was calculated (weight/height^2, kg/m^2). Participants were studied at baseline, then at 2 and 12 weeks following bariatric surgery. All subjects underwent laparoscopic adjustable gastric band placement by a single surgeon (RVL). Blood samples were collected following a 10-hour overnight fast. A 75 g OGTT was performed at each visit (Carbotest 75 g per 300 mL, Lomb Scientific, Sydney, Australia); samples were collected at 0, 30, 60, 90 and 120 min.

2.2. Laboratory measures

Plasma glucose was determined by the glucose oxidase method (YSI glucose analyzer, model 2300 STAT PLUS 230V, YSI Inc., Yellow Springs, OH, USA). Serum was stored (−80 °C). Serum insulin was measured by radioimmunoassay (Linco, St. Charles, MO, USA). Serum TRAIL, osteoprotegerin, and leptin were measured by ELISA (R&D Systems, Minneapolis, MN, USA). All CVs were <7.5%. Insulin sensitivity was estimated using the oral glucose insulin sensitivity index (OGIS, as described by Mari et al. [9]), using the calculator spread sheet downloaded at http://webmet.pd.cnr.it/ogis. Insulin secretion was estimated using the homeostasis model assessment [10].

2.3. Statistical analysis

Data presented are mean ± SEM for comparisons between groups. Analyses were performed using GraphPad PRISM Version 6 (GraphPad Software, San Diego, CA, USA). The effect of bariatric surgery on serum TRAIL, osteoprotegerin and osteoprotegerin:-TRAIL ratio was examined by paired *t*-tests for normally distributed variables and Wilcoxon Signed Ranks tests for non-normally distributed variables. Relationships between serum TRAIL, osteoprotegerin, osteoprotegerin:TRAIL ratio, as well as multiple other parameters were assessed using the Spearman correlation coefficient. Differences were considered significant if $P < 0.05$.

3. Results

We have previously reported the significant reductions in weight, BMI, waist circumference and glucose levels at 2 and 12 weeks, which are shown now for reference (Table 1) [11]. The effect of weight loss by gastric banding surgery on serum TRAIL, osteoprotegerin, and osteoprotegerin-TRAIL ratio levels at 2 and 12 weeks is shown in Fig. 1. There was no effect on circulating TRAIL concentrations after gastric banding at 2 or 12 weeks (Fig. 1A). In contrast, there were significant reductions in osteoprotegerin and the osteoprotegerin:TRAIL ratio at 12 weeks (Fig. 1B and C).

The impact of weight reduction on TRAIL levels was examined further in a subgroup of participants with consistent energy restriction, defined by reduction in serum leptin levels at 12 weeks. Six participants did not suppress leptin concentrations below baseline at 12 weeks and were excluded from further analyses. Data for clinical and metabolic parameters for the 9 participants who suppressed their leptin levels are shown in Table 2. In participants with consistent energy restriction, there was a significant increase in circulating TRAIL at 12 weeks (Fig. 2).

Relationships were examined between changes in TRAIL concentrations and metabolic markers. The % change in TRAIL at 12 weeks normalised to baseline was negatively related to the % change in fasting insulin (Fig. 3A) and HOMA-ß (Fig. 3B). The relationship between % change in TRAIL with fasting insulin

Table 1
Clinical and metabolic parameters in all participants with T2D at baseline, 2 and 12 weeks after gastric banding surgery.

$N = 15$	Baseline	2 weeks after surgery	12 weeks after surgery
Weight (kg)	127.5 ± 5.7	116.0 ± 4.9†	111.5 ± 5.1†
BMI (kg/m^2)	43.4 ± 1.3	39.6 ± 1.2†	38.1 ± 1.3†
Waist (cm)	132.4 ± 3.8	122.1 ± 3.5†	120.4 ± 3.7†
Systolic BP (mmHg)	132 ± 2	120 ± 3	125 ± 3
Diastolic BP (mmHg)	80 ± 3	77 ± 2	77 ± 2
Fasting glucose (mmol/L)	5.9 ± 0.3	5.3 ± 0.2*	5.1 ± 0.3*
Fasting insulin (μU/ml)	30.3 ± 5.4	19.0 ± 2.2*	22.6 ± 2.7
Insulin secretion (HOMA-β)	322.7 ± 71.6	263.4 ± 38.9	383.4 ± 58.2
Insulin sensitivity (OGIS)	268 ± 25	349 ± 20	360 ± 25*

Data are mean ± SEM. T2D: type 2 diabetes mellitus.
HOMA-β: insulin secretion measured by homeostasis model assessment.
OGIS: oral glucose insulin sensitivity index (ml min^{-1} m^{-2}).
Compared to baseline: *$p < 0.05$; **$p < 0.01$, †$p < 0.0001$, using paired t-tests for normally distributed variables and Wilcoxon Signed Ranks tests for non-normally distributed variables.