Differential impact of weight loss and glycemic control on inflammasome signaling

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Author Contributions Statement

Luca Antonioli has made substantial contributions to conception, interpretation of data and he has been involved in drafting the manuscript.
Diego Moriconi, Dario Bottazzo, Stefano Masi, Carolina Pellegrini, Matteo Fornai, Marco. Anselmino carried out the experiments, have made substantial contributions to data collection.
Ele Ferrannini, Corrado Blandizzi, Stefano Taddei have been involved in revising it critically for important intellectual content.
Monica Nannipieri has made substantial contributions to conception and design of the study, data interpretation, and she has been involved in writing of the manuscript.

What is already known about the subject?

An increase in caspase-1 activation and IL-1β levels has been detected in peripheral blood monocytes from patients with obesity and type 2 diabetes (T2D), as compared with healthy subjects, thus suggesting a potential role of the pro-inflammatory cytokine IL-1β in obesity-associated inflammation and T2D

What does the study add?

Caspase-1 levels normalized after surgically-induced weight loss, regardless of the presence of diabetes, while IL-1β normalized in the group without T2D, but not in patients with T2D, suggesting the persistence of a systemic inflammatory condition in the latter group.

Abstract

Objective: IL-1β is involved in obesity-associated inflammation and in the pathogenesis of type 2 diabetes (T2D) mellitus. Our aim was to correlate serum IL-1β and caspase-1 levels with weight-loss, glucose metabolism and insulin resistance (IR) after bariatric surgery.

Methods: Thirty-two patients with obesity and T2D (Ob-T2D) and 29 patients with obesity without T2D (Ob-NGT), treated by Roux-en-Y Gastric Bypass, were studied before and 1-year after surgery. Sixteen healthy individuals served as control group (HC). IR was assessed by the Oral-Glucose-Insulin-Sensitivity-OGIS- method. Plasma IL-1β levels and caspase-1 were measured.

Results: Pre-surgery BMI was similar in Ob-NGT and Ob-T2D. IR was progressively impaired in Ob-NGT and Ob-T2D ($p<0.0001$). Fasting plasma IL-1β and Caspase-1levels were lower in HC than in Ob-NGT and Ob-T2D ($p< 0.02$; $p=0.05$), and both were inversely correlated with IR ($p=0.01$; $p=0.02$). Post-surgery, BMI decreased and IR improved to a similar extent in Ob-NGT and Ob-T2D ($p<0.0001$). Plasma caspase-1 concentrations normalized in both groups ($p<0.0001$), while plasma IL-1β levels normalized only in Ob-NGT.

Conclusions: Plasma IL-1β and caspase-1 levels are inversely correlated with IR. Caspase-1 levels normalized after weight loss, while IL-1β normalized only in people without T2D, suggesting the persistence of a systemic inflammatory condition in people with T2D.

Introduction

Over ~~in~~ the last few decades, the prevalence of obesity has reached epidemic proportions, becoming a serious challenge for global public health (1). Beyond an excessive fat accumulation, obesity is characterized by a chronic systemic inflammation, and there is evidence that such a condition contributes to the development of insulin resistance and the pathogenesis of type 2 diabetes mellitus (T2D) (2, 3, 4). In particular, it has been shown that patients with obesity are characterized by a marked unsettlement of immune cell functions, displaying an uncontrolled activation of innate immune cells (i.e. macrophages), which promote a massive release of pro-inflammatory cytokines (i.e. interleukin [IL]-6, TNF and IL-1β), interfering in an autocrine and paracrine manner with several metabolic processes, with particular regard for insulin synthesis and signaling (5, 6, 7). Among these inflammatory mediators, IL-1β has been found to be pivotally involved in the onset of obesity-associated inflammation as well as in the pathogenesis of T2D (5, 6, 7). Of note, IL-1β production is strictly related with the transcription and subsequent storage of inactive pro-IL-1β into cells, and by caspase-1, a cysteine protease, that converts the inactive proform of IL-1β to the active inflammatory cytokine. In turn, caspase-1 activation is mediated by a repertoire of proteins, through the formation of a multiprotein complex designated as inflammasome (8, 9). It is also noteworthy that a number of recent evidences pointed out a critical role of purines in triggering the inflammasome assembly and activation (8), as well as in orchestrating the activity of several immune cell populations involved in the inflammatory condition associated with obesity and diabetes (10, 11, 12, 13).

Current clinical evidence has documented an increased caspase-1 activity and IL-1β secretion in adipose tissue macrophages from patients with obesity and T2D, and these patterns were tightly correlated with a condition of insulin resistance (14, 15, 16). Interestingly, an increase in caspase-1 activation and IL-1β levels has been detected in peripheral blood monocytes from patients with obesity and T2D, as compared with healthy subjects, thus confirming a critical role of the pro-inflammatory cytokine IL-1β in obesity-associated inflammation and T2D (15, 16).

At present, people with morbid obesity, with or without T2D, undergo often to bariatric surgery, with particular regard for Roux-en-Y gastric bypass (RYGB), which results in a significant body weight loss and glycaemic control improvement, without relevant malabsorption symptoms (17). Observational

studies on bariatric surgery have shown a complete remission rate of T2D of 44% after RYGB at 12 months, about 41% at 2 years (18), and 31% at 5 years (19). The mechanism underlying such an improvement of glycemic control appears to be complex and to involve an improvement in ß-cell function (17) and insulin sensitivity, with a marked decrease in insulin levels, which may be linked to the attenuation of chronic inflammation, as suggested by the significant reduction of high-sensitivity C-reactive protein (CRP) in patients with bariatric surgery (20).

Based on the above background, a crucial role in the improvement of insulin sensitivity might be played by the reduction of chronic inflammation. Indeed, surgically induced weight loss is known to improve the systemic inflammatory status, and inflammatory mediators were found to be normalized in patients with morbid obesity after bariatric surgery (21, 22, 23, 24). However, whether, and to what extent, the improvement of the clinical condition in patients with obesity and T2D following bariatric surgery could, at least in part, depend on an improvement of inflammation remains presently unclear. Therefore, the present study was specifically designed to correlate plasma caspase-1 and IL-1β levels with weight loss, as well as with changes in glucose metabolism and insulin resistance, in people with morbid obesity, with or without T2D, after bariatric surgery.

Materials and methods

Subjects The study group included 32 patients with morbid obesity and normal glucose tolerance (Ob-NGT), 29 patients with morbid obesity and T2D (Ob-T2D), who were wait-listed for laparoscopic RYGB, and 16 healthy people (HC), as a control group. Diabetes was diagnosed according to the ADA criteria (25). Insulin-taking patients, whose age of diabetes onset was ≥40 years, with BMI was >30 $kg \cdot m^{-2}$ and negative for the presence of islet autoantibodies were also considered to have T2D. Diabetes duration ranged from 2 to 14 years. The antidiabetic treatment was based on insulin (basal bolus) in 9 patients, oral antidiabetic agents in 15 (sulphonylurea plus metformin), and diet alone in 5 subjects. The exclusion criteria included: (a) medical conditions requiring acute hospitalization; (b) blindness; (c) severe medical conditions (liver cirrhosis, end-stage renal failure, malignancy, connective tissue diseases, endocrine diseases such as hypo- or hyperthyroidism) or diseases such as chronic congestive heart failure, recent myocardial infarction or stroke, unstable angina pectoris. The study protocol was

approved by the local Ethics Committee (number. 2360) and all patients signed a written consent form prior the study.

Study Design After screening, patients with morbid obesity were requested to attend our Clinical Research Unit for the baseline study two weeks before surgery; healthy subjects were requested to take part to the baseline study as control group. Twelve months later, the metabolic study was repeated at the Clinical Research Unit in all subjects which underwent bariatric surgery. Post-surgery diabetes complete remission was defined as a HbA_{1c} <6.0%, a fasting glucose <5.6 mmol/l without antidiabetic treatment for one year, and partial diabetes remission as a HbA_{1c} <6.0%, a fasting glucose <5.6 mmol/l without antidiabetic treatment for one year, according to 2009 consensus statement criteria (25).

Study Protocol For the metabolic study, all subjects were instructed to not exercise for 48 hours prior to study, and were examined in the morning after an overnight (12-14 hours) fast. Patients with T2D on oral hypoglycemic agents were requested to discontinue these medications for 48-72 hours before the study; in those on insulin, injections were discontinued 16 hours before the metabolic study (patients on bedtime glargine had been switched to NPH two days before the study). The metabolic study consisted of a frequently sampled oral glucose tolerance test (OGTT). After an overnight fast, blood samples were collected through an indwelling cannula. Peripheral blood samples were taken for the assessment of routine blood chemistry and plasma glucose, insulin, C-peptide, HbA_{1c} and cytokines (Il-1β and caspase-1) concentrations. After ingestion of 75 g of glucose in aqueous solution, venous blood was sampled at 10, 20, 30, 45, 60, 90, 120, 150, and 180 min for glucose, insulin, and C-peptide assay. In people with morbid obesity, laparoscopic RYGB was performed as described elsewhere (17).

Methods Plasma glucose concentration was measured on a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA, USA). Fasting concentrations of serum total-cholesterol low-density-lipoprotein (LDL)-cholesterol, and high-density lipoprotein (HDL)-cholesterol were measured by standard techniques (Synchron CX4, Beckman Instruments, Inc., Brea, CA, USA). Plasma insulin and C-peptide were measured by Cobas e411 (Roche Diagnostics S.p.A., Milan, Italy).

Measurements of plasma caspase-1 and IL-*1β levels.* Caspase-1 and IL-1β levels in plasma samples were measured by Quantikine ELISA assay kits (R&D system), as previously described (27, 28). For this purpose, 3 ml of fasting venous blood was collected into tubes containing 30 μ L (0.5 mol/L),

EDTA K_2-coated tubes (0.5 mol / L; 30 µ l) plus 2000 KIU of aprotinin. Samples were mixed, centrifuged at 2000 x g for 20 min. After centrifugation, 2 mL of cold acetone (4°C) was added to 1 mL of plasma, mixed, and centrifuged at 2000 x g per minute for 20 min at 4°C. The supernatant was added to 4 mL of cold petroleum ether, mixed, centrifuged, and dried under a vacuum to remove any residual acetone. The samples were then stored at −20 ° C until use. Plasma caspase-1 and IL-1β levels were expressed as picogram per milliliters.

Modeling Insulin sensitivity and ß-cell function parameters were derived from mathematical modeling of the plasma glucose, insulin, and C-peptide concentrations measured during the frequently sampled OGTT, as previously described (17). In brief, insulin sensitivity was calculated as the oral glucose insulin sensitivity (OGIS) index, which estimates plasma glucose clearance rate (in $ml \cdot min^{-1} \cdot m^{-2}$) at a level of hyperinsulinemia in the range of that achieved during a standard (240 $pmol \cdot min^{-1} \cdot m^{-2}$) euglycemic hyperinsulinemic clamp, against which this index has been validated in subjects with normal glucose tolerance, impaired glucose tolerance or overt diabetes (29). The ß-cell function model was computed as elsewhere described (30).

Statistical analysis Results are expressed as mean ±SD or median [interquartile range], for variables with normal or skewed distribution, respectively. Group differences were compared by the χ^2 test for categorical variables, by the Mann Whitney U test for continuous variables, and by Wilcoxon signed rank test for paired data. Analysis of changes over time (before, early, and late after surgery) in the two subject groups (NGT and T2D) was carried out by ANOVA for repeated measures; for this test, parameters with a skewed distribution were log-transformed. The output of this ANOVA model is a p value for the time factor (i.e., overall changes over time), a p value for the group (i.e., between-group differences), and a p value for the time x group interaction (i.e., differential changes between groups over time). A p value <0.05 was considered to be significant.

Results

Baseline anthropometric and metabolic parameters. The degree of obesity was similar in patients with Ob-T2D and Ob-NGT, and significantly different as compared to the HC group (**Table 1**). Patients with

Ob-T2D were older (p<0.001), and had higher baseline HbA_{1c}, fasting and mean plasma glucose concentrations than patients with Ob-NGT and HC (p<0.001) (**Table 1**). Baseline ß-cell glucose sensitivity was significantly lower in people with Ob-T2D than Ob-NGT and HC (p=0.0001), without differences between Ob-NGT and HC. Fasting insulin concentrations differed significantly among groups, with progressive reduction from patients with Ob-NGT to Ob-T2D to HC (p=0.008), whereas mean plasma insulin concentrations during OGTT were higher in patients with Ob-NGT as compared to Ob-T2D and HC (p<0.0001). Fasting and total insulin secretion rate did not differ substantially among the three groups, although total insulin secretion rate was reduced in Ob-T2D as compared to the HC group (p=0.03) (**Table 1**). Finally, insulin progressively sensitivity impaired progressively from HC to Ob-NGT to Ob-T2D (p<0.0001).

Baseline plasma IL-1β concentrations were significantly and progressively higher in patients with Ob-T2D as compared to Ob-NGT (p=0.03), and to HC (p<0.0001), being significantly higher in Ob-NGT than HC (p=0.017) (median and IQR: 2.52 (1.99-2.85) vs 3.78 (3.53-5.63) vs 4.07 (4.08-7.35) pg/ml, p=0.004, respectively in HC vs Ob-NGT vs Ob-T2D) (**Figure 1 panel a**). Similarly, plasma caspase-1 concentrations were lower in HC, than in Ob-NGT and Ob-T2D (mean±SD: 13.8± 3.2 vs 19.2±7.7 vs 20.5±8.8 pg/ml, p=0.042, respectively), but without any difference in patients with morbid obesity according to the glucose tolerance status (**Figure 1 panel b**).

Regression analysis. In the linear regression analysis of the data from the overall population, mean plasma glucose levels were correlated positively with plasma IL-1 β concentrations (r = 0.35, p<0.0001), while insulin sensitivity was correlated negatively with plasma IL-1 β concentrations (r=0.30, p=0.0009). IL-1 β plasma concentrations were slightly correlated with caspase-1 (r=0.22, p=0.05). In a multivariate model analysis including age, BMI, fasting insulin secretion, insulin sensitivity, mean plasma glucose and HbA1c, only the latter parameter was a significant predictor of plasma IL-1β concentrations (p=0.0063) (**Figure 2, panel a**).

Plasma caspase-1 concentrations were correlated positively with mean plasma glucose levels (r = 0.27, p=0.0045) and BMI (r=0.45, p<0.0001) and negatively with insulin sensitivity (r=0.35, p<0.0001). In a multivariate model analysis including age, BMI, insulin sensitivity, fasting insulin secretion, HbA1c and

mean plasma glucose, only BMI was a significant predictor of plasma caspase-1 concentrations (p=0.0035) (**Figure 2, panel b**).

Effects of RYGB in patients with morbid obesity with T2D and without T2D (**Table 2**). One year after surgery, BMI decreased similarly in all patients with morbid obesity; the respective values were -13.9 kg/m^2 in Ob-NGT and -12.1 kg/m^2 in Ob-T2D (p<0.0001). Fasting and mean plasma insulin concentrations decreased at a similar extent in both groups of patients (p<0.0001 and p<0.0009, respectively). Glycemic control – as indexed by HbA_{1c}, fasting, and mean plasma glucose levels – improved more significantly in patients with Ob-T2D than Ob-NGT (p=0.035, p=0.0009, p=0.004, respectively) (**Table 2**).

Insulin sensitivity improved both in patients with Ob-NGT and Ob-T2D (p=0.0001); in the latter, however, it remained lower than in subjects with Ob-NGT (p<0.0001) (**Table 2**). ß-cell glucose sensitivity showed a marked improvement in both groups (p=0.001); in patients with Ob-T2D it remained significantly lower than in subjects with Ob-NGT (p=0.002). Fasting insulin secretion declined post-surgery in both groups (p=0.0008), whereas total insulin output did not change (**Table 2**).

Fasting plasma IL-1β concentrations decreased after surgery in both groups (median and IQR: 2.97 (1.65-3.22), vs 3.66 (1.67-6.58) pg/ml, p=0.008, respectively in patients with Ob-NGT and Ob-T2D), normalized in patients with Ob-NGT, but remaining still significantly higher in patients with Ob-T2D compared to subjects with Ob-NGT and HC (p=0.03 and p=0.0058, respectively) (**Fig.1 panel a**). After surgery, fasting plasma caspase-1 levels fell similarly down in both groups, reaching similar plasma concentrations to HC subjects (12.08±6.01 vs 11.34±4.13 pg/ml, p<0.0001 effect of surgery, in patients with Ob-NGT and Ob-T2D respectively) (**Fig. 1 panel b**).

Diabetes remission was observed in 15 of the 29 patients 1 year after surgery. With regard to glucose lowering agents, all subjects on diet treatment and 10 of the 15 patients on oral agents had diabetes remission, whereas none of the patients on insulin were in remission, although their metabolic control improved and insulin was stopped. HbA1c was significantly reduced after surgery in all patients with Ob-T2D (people with remission vs no-remission: 5.8±0.4 vs 7.0±0.95%, p=0.0001). However, during the oral glucose tolerance test, within the group of patients with partial diabetes remission, plasma glucose concentrations were above than 11.1 mmol/l 30 min after glucose load in 30% of them, at 60 min in 58%

of them and at 90 min in 34%. Moreover, within patients with complete remission, plasma glucose concentrations were above 11.1 mmol/l at the same times after glucose load in 23%, 24% and 26% of them, respectively. All patients with partial or complete diabetes remission had plasma glucose concentrations lower than 11 mmol/l at 120 min after glucose load.

No differences were observed for plasma IL-1β and caspase-1 concentrations when comparing subjects with remission and non-remission (IL1β median (IQR): 3.52 (1.7-9.5) vs 4.30 (0.8-5.7) pg/ml, p=ns; caspase-1: 112.73±7.48 vs 10.35±2.25 pg/ml, p=ns, respectively). Finally, a positive correlation was found between plasma glucose concentrations at 60 min (after glucose load) and IL-1β levels after surgery (r= 0.33, p=0.026).

Discussion

The major findings of the present study are that (a) subjects with morbid obesity display higher plasma caspase-1 concentrations than healthy controls independently from the glucose tolerance status, while plasma IL-1 β levels were progressively higher when moving from healthy to subjects with morbid obesity with normal glucose tolerance, and from the latter to patients with morbid obesity with T2D (Fig. 1); (b) interestingly, in a multivariate model adjusted for age, BMI, insulin sensitivity and HbA1c, only the BMI ~~explained~~ correlated with the changes in caspase-1 concentrations; whereas at variance, in a similar model analysis, HbA1c was the only variable correlated with IL-1 β (**Fig. 2**) ; (c) after significant weight loss by surgery, plasma caspase-1 levels fell down in all patients with morbid obesity, independently from the glucose tolerance status, reaching plasma concentrations similar to those of healthy individuals; by contrast, IL-1 β plasma concentrations, while decreasing after weight loss in both groups, normalized only in patients with Ob-NGT (**Fig. 1**) and remained significantly higher in patients with Ob-T2D as compared to the healthy group.

It is well known that obesity is linked to a variety of disorders including T2D (31). Recent studies have suggested that the persistent low-grade inflammation found in people with obesity is a major contributor towards the progression to insulin resistance and T2D, and several pro-inflammatory cytokines, including IL-1β, have been strongly associated with this progression (4, 32, 33). In particular, an

enhanced IL-1β production and release in the presence of hyperglycemia has been reported in different cell types (34), including β-cell, suggesting a role of IL-1β in β-cells dysfunction (33, 34, 35, 36). Since there is evidence that IL-1β production is regulated mainly by cytosolic molecular complexes, designed as NLRP3 inflammasome (nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 or Nod-like receptor protein 3), some studies have shown that the activation of NLRP3 inflammasome could play a relevant role in obesity (14, 37, 38), insulin resistance and progression to T2D (39). However, conflicting results have been reported with regard for the circulating concentrations of IL-1β in humans, so that it has been found to be increased or unchanged in people with obesity without T2D versus HC (40, 41, 42). In patients with T2D , elevated plasma IL-1β levels were found in presence of hyperglycemia and insulin resistance (16), but in a large prospective study (EPIC Study) it has been reported that plasma IL-1β per se did not differ in subjects with incident T2D or not; by contrast a combined elevation of IL-6 and IL-1β predicted the risk of T2D (43).

In the present study, circulating IL-1β concentrations were significantly higher in patients with morbid obesity as compared to HC, in agreement with previous data reported by Shen XP (40), and they increased progressively passing through morbid obesity with normal glucose tolerance to morbid obesity with T2D, being inversely correlated with insulin sensitivity and positively associated with mean plasma glucose. In particular, in a multivariate model analysis, only HbA1c was associated with IL-1β concentrations, suggesting a major role of hyperglycemia in determining the circulating IL-1β concentrations, independently from BMI. These data are in line with findings reported by Ruscitti et al., showing that hyperglycemia can lead to an increased secretion of IL-1β in monocytes from patients with T2D (44). Furthermore, other authors have shown an enhanced release of IL-1β from $CD14^{+}$ macrophages freshly isolated from adipose tissue biopsies of patients with obesity and diabetes, as compared with HC, and with a positive correlation between IL-1β and glycated hemoglobin (HbA_{1c}) values (45). Based on these data, it is not surprising that, in the present study, after the weight loss induced by bariatric surgery, circulating IL-1β levels normalized in patients with obesity without T2D, but not in patients with obesity and T2D. Indeed, after RYGB, a high glucose variability (GV), as a consequence of the anatomical changes induced by surgery, has been reported (46), even though some

patients full-filled the agreed criteria of diabetes remission. In our study, patients that fulfilled the criteria for diabetes remission according to the ADA criteria (complete or partial together) [25, 26] 50% had plasma glucose concentrations at 90 min (PG-90') after glucose load ≥200 mg/dl, and 82% had PG-60' ≥200 mg/dl. To the best of our knowledge, the present study is the first one describing that patients, who underwent diabetes remission after surgery but retained a high GV (30-60-90 minutes after oral glucose load), showed persistent evaluations of had plasma IL-1β concentrations versus patients Ob-NGT, and that plasma IL-1β levels did not differ among patients with or without diabetes remission after surgery, suggesting a high risk of vascular damage. On the other hand, as suggested by other authors (46), HbA1c levels in the normal range are not able to predict a wide GV, and silent GV could be responsible for a persistent detrimental inflammatory stimulus. Indeed, pathophysiological and clinical studies have shown that high GV can be involved in the pathogenesis of diabetic vascular complications via activation of inflammatory pathways, increased oxidative stress and endothelial dysfunction (47, 48, 49, 50). Therefore, these data support the findings that several patients, fulfilling diabetes remission criteria 1 year after surgery according to the ADA criteria [25], still retain a substantial risk of diabetes relapse, supporting the need for redefining diabetes remission. As far as we know, circulating caspase-1 has not been investigated in patients after bariatric surgery, and data on tissue NLRP3 expression are quite conflicting. Indeed, while some authors described, a decrease in NLRP3 expression in the abdominal subcutaneous adipose tissue after weight loss in patients with obesity and T2D, in concomitance with an improved insulin sensitivity (51, 52), others (36) did not found any variation of NLRP3 expression in subcutaneous adipose tissue, visceral adipose tissue or liver from patients with severe obesity, 6 months after adjustable gastric banding surgery.

In order to better evaluate the pathophysiological significance of NLRP3 inflammasome, we measured also caspase-1 concentrations, which were higher in plasma from patients with obesity independently of the glucose tolerance status, and resulted to be independently correlated with BMI. After surgery, in concomitance with the weight loss, we observed a significant reduction and normalization of caspase-1concentrations, independently of glucose tolerance status, while BMI remained in the range of first-degree obesity. Therefore, in the present study, different patterns have emerged for circulating caspase-1

and IL-1β, highlighting a major role of body weight on caspase-1 concentrations, and hyperglycemia on circulating IL-1β levels. These different patterns could be explained by the literature evidence that the activation of IL-1β precursor depends not only on inflammasome-mediated caspase-1 activity, but also on extracellular serine proteases (i.e. proteinase-3, elastase and cathepsin G) related to the presence of activated neutrophils (53).

A potential limitation of the present findings is determined by the small number of study patients and by the lack of a continuous glucose monitoring, even if variations in plasma glucose have been detected during OGTT. Furthermore, another limitation of the study is the lack of a control group achieving diet-induced weight loss, in order to exclude the effects of altered gastrointestinal anatomy on cytokine concentrations.

Conclusions

Plasma IL-1β and caspase-1 concentrations are elevated in people with morbid obesity and are inversely related to insulin sensitivity. However, while caspase-1 was found to be linked to body weight, independently of glucose tolerance status, IL-1β was associated with plasma hyperglycemia. In addition, caspase-1 levels normalized after surgically-induced weight loss, regardless of the presence of diabetes, while IL-1β normalized in people without T2D, but not in those with T2D, suggesting the persistence of a systemic inflammatory condition in the latter group. On the basis of the present findings, an accurate evaluation of early post-prandial glucose control should be suggested also in patients with diabetes remission after surgery, in order to be more incisive in modifying the lifestyle and undertake appropriate pharmaceutical therapy by virtue of a high vascular risk.

Overall, our findings support the need for further studies aimed at assessing the impact of glucose variability on inflammatory status and to critically re-define the condition of diabetes remission.

Legend to the figures

Figure 1, Panel a- Plasma IL1β concentrations in healthy control (HC), patients with morbid obesity and type 2 diabetes (Ob-T2D) and patients with morbid obesity without diabetes (Ob-NGT) (median and CI: 2.52 (1.99-2.85), vs 4.07 (4.08-7.35) vs 3.78 (3.53-5.63), p=0.018, respectively). **Panel b**- Plasma caspase-1 concentrations in healthy control (HC), patients with morbid obesity and type 2 diabetes (Ob-T2D) and patients with morbid obesity without diabetes (Ob-NGT) (mean±SD: 13.8± 3.2, vs 20.5±8.8, 19.2±7.7, p=0.042, respectively). Plots are mean±SD and quantiles.

Fig. 2 – Multiple regression analyses. Panel a: (independent variables: age, BMI, insulin sensitivity, HbA1c) HbA1c correlation between and IL1β concentrations in all population (p= 0.0063); panel b: (independent variables: age, BMI, insulin sensitivity, HbA1c) correlation between BMI and caspase-1 concentrations in all population (p=0.0035);

List of abbreviations: Patients with normal glucose tolerance and morbid obesity (Ob-NGT), patients with type 2 diabetes and morbid obesity (Ob-T2D), type 2 diabetes mellitus (T2D), interleukin-1β (ILβ), glycated haemoglobin (HbA1c), body mass index (BMI), nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 or Nod-like receptor protein 3 (NLRP3), healthy control (HC), ß-cell glucose sensitivity (ß-GS), glucose insulin sensitivity (OGIS), oral glucose tolerance test (OGTT).

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Cytokines

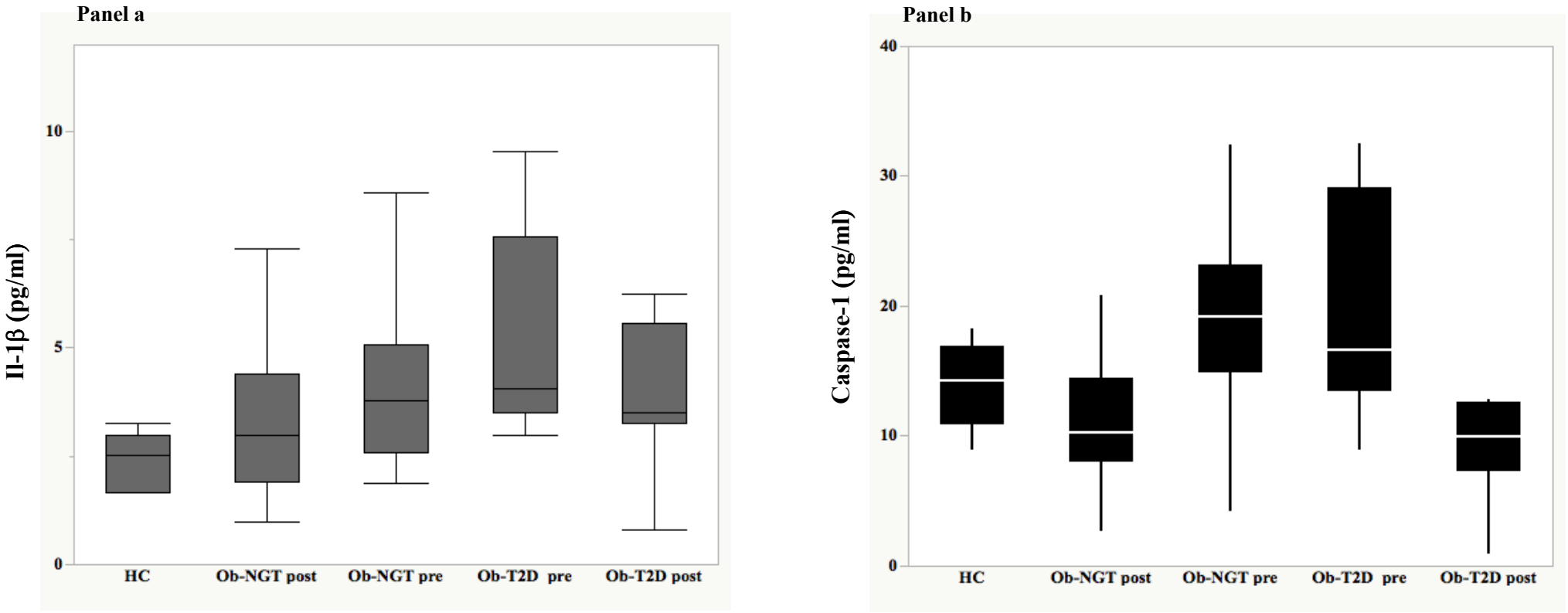

Figure 1

Multiple Regression Analysis