An alternatively activated macrophage marker CD163 in severely obese patients: the influence of very low-calorie diet and bariatric surgery

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Short title: CD163 in obesity and type 2 diabetes mellitus

Summary

CD163 is a marker of macrophages with anti-inflammatory properties and its soluble form (sCD163) is considered a prognostic predictor of several diseases including type 2 diabetes mellitus (T2DM).

We explored sCD163 levels at baseline and after very low-calorie diet (VLCD) or bariatric surgery in 32 patients with obesity (20 undergoing VLCD and 12 bariatric surgery), 32 obese patients with T2DM (22 undergoing VLCD and 10 bariatric surgery), and 19 control subjects. We also assessed the changes of CD163 positive cells of monocyte-macrophage lineage in peripheral blood and subcutaneous adipose tissue (SAT) in subset of patients.

Plasma sCD163 levels were increased in obese and T2DM subjects relative to control subjects (467.2 ± 40.2 and 513.8 ± 37.0 vs. 334.4 ± 24.8 ng/ml, p=0.001) and decreased after both interventions. Obesity decreased percentage of CD163+CD14+ monocytes in peripheral blood compared to controls (78.9 ± 1.48 vs. 86.2 ± 1.31 %, p=0.003) and bariatric surgery decreased CD163+CD14+HLA-DR+ macrophages in SAT (19.4 ± 2.32 vs. 11.3 ± 0.90 %, p=0.004).

Our data suggest that increased basal sCD163 levels are related to obesity and its metabolic complications. On the contrary, sCD163 or CD163 positive cell changes do not precisely reflect metabolic improvements after weight loss.

Key words: Obesity – type 2 diabetes mellitus – macrophages – adipose tissue

Introduction

Obesity induces chronic low-grade inflammation connected with macrophage accumulation in adipose tissue (Weisberg *et al.* 2003). These macrophages may contribute to inflammatory and metabolic complications of obesity (Olefsky and Glass 2010). It has been shown that both the number of macrophages and their pro-inflammatory phenotype can be changed by modifications of energy intake, energy expenditure and adipose tissue mass (Aron-Wisnewsky *et al.* 2009). Macrophages can exist in two main activation states: the classically activated pro-inflammatory (M1) and the alternatively activated anti-inflammatory (M2) macrophages regulated by pro-inflammatory and anti-inflammatory cytokines, respectively (Gustafsson *et al.* 2008). In our study, we focused on alternatively activated macrophages with potentially anti-inflammatory marker CD163.

CD163 receptor is a member of the scavenger receptor cysteine-rich superfamily (SRCR), class B. The SRCR domain forms a common structural fold with six-stranded β sheet cradling an alpha helix. In humans, CD163 contains extracellular region of nine SRCR domains, cytoplasmic tail and one transmembrane element (Fabriek *et al.* 2005). The best characterized function of CD163 is endocytosis of hemoglobin-haptoglobin complexes and its expression is connected with anti-inflammatory processes (Schaer *et al.* 2006). Although its biological action in inflammation is not precisely known, its soluble form sCD163 was suggested as a useful predictor of the development or prognosis of several diseases including type 2 diabetes mellitus (T2DM) (Moller *et al.* 2011), infection by human immunodeficiency virus (Burdo *et al.* 2011) or several types of cancer (Lan *et al.* 2013, Wang *et al.* 2015). Interestingly, despite its suggested anti-inflammatory and scavenging properties CD163 was found to be expressed mainly on the classical pro-inflammatory monocytes (CD14++CD16-) as compared with the non-classical subset

(CD14dimCD16++) despite the latter's role in tissue patrolling and control of vascular integrity (Ziegler-Heitbrock 2007).

Thus, we hypothesized that circulating sCD163 and its mRNA expression in adipose tissue may be connected to inflammatory state and modified by weight-reducing interventions. To this end, we explored the changes of plasma sCD163 levels, its mRNA expression and CD163 positive cells in circulating monocyte subsets and adipose tissue in subject with simple obesity and obesity complicated by T2DM relative to lean healthy control subjects and its modulation by very low-calorie diet (VLCD) and bariatric surgery.

Methods

Study subjects

Thirty two obese patients, 32 obese patients with T2DM and 19 age-matched lean healthy control subjects were included into the study. Control subjects had been free of any medication and had no history of obesity or diabetes mellitus. The inclusion criteria for study subjects were age between 40 and 60 years and BMI (Body mass index) higher than 35 kg/m^2. Patients of both genders were included as previous studies have shown that changes of CD163 are not on gender-dependent (Aristoteli *et al.* 2006, Fjeldborg *et al.* 2013). Written informed consent was signed by each subject and the studies were approved by the Human Ethics Committee, First Faculty of Medicine and General University Hospital, Prague, Czech Republic.

Very low-calorie diet

Forty two patients – 20 with obesity and 22 obese patients with T2DM underwent 3 weeks of VLCD during hospitalization at the Third Department of Medicine, General University Hospital, Prague, Czech Republic. The energy content of VLCD was 2500 kJ per day. Anthropometry, blood and subcutaneous adipose tissue (SAT) samples were taken before and after VLCD.

Bariatric surgery

Twenty two patients – 12 patients with obesity and 10 obese patients with T2DM underwent selected bariatric surgical procedures. The operations included gastric plication (12 patients), sleeve gastrectomy (6 patients), gastric bypass (3 patients) and gastric banding (1 patient). Anthropometry, blood and SAT samples were taken before (visit 1) and 1 month, (visit 2), 6 months (visit 3) and 1 year after bariatric surgery (visit 4). During bariatric surgery, samples of visceral adipose tissue (VAT) and SAT were taken for the evaluation of mRNA expression differences between these depots.

Anthropometric examination, blood and adipose tissue sampling

All subject enrolled in the study underwent anthropometric examination and their BMI was calculated. Blood samples were taken after an overnight fasting and centrifuged for 10 min at 1000 x g within 30 min after withdrawal. Serum or plasma aliquots were subsequently stored at -80 °C.

Samples of SAT were obtained by needle aspiration biopsy from abdominal region after an overnight fasting and subsequently stored at -80 °C, as were samples of SAT and VAT obtained during bariatric surgery.

Hormonal and biochemical assays

Plasma sCD163 levels were measured by sandwich enzyme immunoassay using a commercial ELISA kit (Aviscera bioscience, Inc., Santa Clara, USA). The whole assay including plasma dilution was done according to the manufacturer's protocol. Sensitivity was 50.0 pg/ml. Serum leptin levels were measured by a commercial ELISA kit (Biovendor, Brno, Czech Republic). Sensitivity was 0.2 ng/ml. Serum C-reactive protein (CRP) levels were measured by high sensitive ELISA kit (Bender Medsystems, Vienna, Austria) with a sensitivity of 3 pg/ml. Insulin levels were measured by RIA kit (Cis Bio International, Gif-sur-Yvette, France). Sensitivity was 2.0 μIU/ml. The intra- and interassay variabilities for all assays were between 5.0 and 10.0 %.

Biochemical parameters (blood glucose, glycated hemoglobin – HbA1c, HDL cholesterol, total cholesterol and triglycerides) were measured and LDL cholesterol was calculated at the Department of Biochemistry, General University Hospital, Prague, Czech Republic by standard laboratory methods. The homeostasis model assessment (HOMA) index was calculated according to standard equation: (fasting insulin levels (mIU/l) x fasting glucose levels (mmol/l))/22.5.

Quantitative real time PCR

CD14+ cells were isolated from plasma using human CD14 MicroBeads and MACS columns (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) as described previously (Mraz *et al.* 2011). Samples of adipose tissue were homogenized on MagNA Lyser Instrument (Roche Diagnostics GmbH, Mannheim, Germany). Total RNA was extracted on MagNA Pure instrument using Magna Pure Compact RNA Isolation kit (tissue) (Roche Diagnostics GmbH, Mannheim, Germany). RNA concentration was determined from absorbance at 260 nm on NanoPhotometer (Implen, Munchen, Germany). Reverse transcription was performed using random primers according to the manufacturer's protocol of the High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, CA, USA). Input amount of RNA was 250 μg per reaction in case of adipose tissue and 200 μg per reaction in case of CD14+ cells. Gene expression was performed on a 7500 Real-Time PCR System using TaqMan® gene Expression Assays (Applied Biosystems, Foster City, CA, USA). For reaction, a mix of TaqMan® Universal PCR Master Mix II, NO AmpErase® UNG (Applied Biosystems, Foster City, CA, USA), nuclease-free water (Fermentas Life Science, Vilnius, Lithuania) and specific TaqManGene expression Assays (Applied Biosystems, Foster City, CA, USA) were used. Beta-2 microglobulin (B2M) was applied as endogenous reference. The formula 2^{-ddCt} was used to calculate relative gene expression.

Isolation of stromal vascular fraction from subcutaneous adipose tissue and flow cytometry

Standard 1.5 g amount of adipose tissue was minced with sterile scissors and visible blood vessels were removed. Samples were washed in PBS (0.01 M PBS, pH 7.4) and digested by 0.01

% collagenase (Collagenase from Clostridium histolyticum, St. Louis, MO, USA) for 30 min at 37 °C. After adipocyte removal, SVF fraction was washed in PBS two times.

Flow cytometry was performed from freshly isolated and filtered SVF (Falcon® 40µm Cell Strainer, Becton, Dickinson and Company, Franklin Lakes, USA). A total amount of 100 µl of cell suspension with average 10^6 cell content was labeled by monoclonal antibodies conjugated with FITC (fluorescein isothiocyanate), PE (phycoerythrin), PerCP (peridinin-chlorophyll protein complex), and APC (allophycocyanin). Used primary antibodies (Exbio Praha, a.s., Vestec, Czech republic) included CD16 FITC, CD163 PE, HLA-DR PerCP and CD14 APC. The samples were labeled in the dark for 30 min at 2 – 8 °C and then red cells were lysed using Excellyse I (Exbio Prague, a.s., Vestec, Czech republic) according to manufacturer's instructions. Finally, labelled cells were analyzed on BD Accuri^{TM} C6 (Becton, Dickinson and Company, Franklin Lakes, USA). Data analysis was performed using FlowJo X 10.0.7r2 software (FlowJo, LCC. Ashland, USA). Gating strategy for SAT was as follows: doublets were excluded, monocytes/macrophages were gated according to scatter properties, HLA-DR+ cells were determined on histogram plot and then CD163+CD14+HLA-DR+ and CD163+CD14-HLA-DR+ cells were determined as a percentage of gated monocytes/macrophages. In peripheral blood the percentage of classical, non-classical and intermediate monocyte subsets based on CD14 and CD16 expression and the percentage of CD163+CD14+ cells were determined after doublet exclusion from the monocyte gate set by scatter properties.

Statistical analysis

Statistical analysis was performed on SigmaStat 3.0 and graphs were drawn using SigmaPlot 8.0 software (SPSS Inc., Chicago, IL, USA). Results are expressed as means ± standard error of the mean (SEM) or Median (interquartile range). One way ANOVA/One way RM ANOVA followed by Holm-Sidak test, One way ANOVA on Ranks/One way RM ANOVA on Ranks followed by Dunn's method, unpaired t-test or Mann-Whitney Rank Sum Test, paired-test or Wilcoxon Signed-Rank were used for the assessment of intergroup differences, as appropriate. Spearman or Pearson correlation test was used to assess the association between CD163 and other parameters. The combined group of patients with healthy control subjects was used for correlation analyses. Multiple linear regression analysis using backward stepwise variable selection method was performed in combined group using parameters with significant result from Spearman or Pearson correlation test. Statistical significance was assigned to $p<0.05$.

Results

Baseline characteristics of the study subjects

Obese patients had higher BMI, insulin, leptin and CRP levels and HOMA index and reduced HDL cholesterol levels compared with control subjects (Table 1). Plasma sCD163 levels were increased relative to control subjects (Fig. 1a).

Obese patients with T2DM had higher BMI, blood glucose, glycated hemoglobin, triglycerides, insulin, leptin and CRP levels and HOMA index and decreased HDL cholesterol compared with control subjects (Table 1). Plasma sCD163 levels were increased (Fig. 1a). Obese patients with

T2DM had higher blood glucose, glycated hemoglobin, triglycerides and HOMA index relative to subjects with simple obesity; however, no difference in plasma sCD163 (Fig. 1a) or its mRNA expression in either SAT or CD14+ cells (Table 1) could be seen between the diabetic and obese non-diabetic group. When comparing depot-specific differences in CD163 mRNA expression in subjects enrolled in the bariatric surgery sub-study, SAT showed lower expression levels relative to VAT (2.14 ± 0.17 vs. 3.24 ± 0.41, p=0.019).

The effect of very low-calorie diet

Three weeks of VLCD reduced BMI, blood glucose, CRP and leptin levels and HOMA index in obese subjects both with and without T2DM (Table 2). Also, their plasma sCD163 levels were reduced after VLCD (Fig. 1b). Additionally, in obese diabetics reduced total, HDL and LDL cholesterol were found after VLCD (Table 2).

The effect of bariatric surgery

Bariatric surgery in obese patients without T2DM decreased BMI, glycated hemoglobin, leptin (Table 3) and plasma sCD163 levels (Fig. 1c) relative to baseline and these changes lasted throughout the study.

Bariatric surgery in obese patients with T2DM decreased BMI, glycated hemoglobin, insulin and leptin levels and HOMA index relative to baseline and these changes lasted throughout the study (Table 3). HDL cholesterol decreased transiently 1 month after surgery. Plasma sCD163 levels

tended to decrease after bariatric surgery but the difference did not reach statistical significance (Fig. 1d).

Relationship of CD163 to other studied parameters

In a combined group of obese patients with and without T2DM and control subjects CD163 mRNA expression in SAT positively correlated with serum triglycerides ($R=0.266$, $p=0.017$). Plasma sCD163 levels positively correlated with BMI ($R=0.407$, $p<0.001$), waist circumference ($p=0.007$, $R=0.395$), blood glucose ($R=0.413$, $p<0.001$), glycated hemoglobin ($R=0.567$, $p<0.001$), triglycerides ($R=0.307$, $p=0.005$), insulin ($R=0.592$, $p<0.001$), leptin ($R=0.389$, $p<0.001$), CRP levels ($R=0.525$, $p<0.001$) and HOMA index ($R=0.587$, $p<0.001$) and negatively with HDL cholesterol ($R=-0.478$, $p<0.001$). CD163 mRNA expression in CD14+ cells did not correlate with any of the measured parameters.

Multiple linear regression analysis was performed in the same group. None of the included parameters were independent predictors of CD163 mRNA expression in SAT or CD14+ cells, while sCD163 levels were independently associated with glycated hemoglobin ($p<0.001$, Standard $\beta=3.489$) and CRP ($p<0.001$ Standard $\beta=43.530$). The adjusted Adj R^2 was 0.522 for the whole analysis.

Furthermore, we found a positive correlation of alanine aminotransferase levels and sCD163 ($R=0.440$, $p=0.040$) in subjects who underwent bariatric surgery. In addition, we found a strong positive correlation between CD163 mRNA expression in VAT and basal sCD163 levels ($R=0.803$, $p=0.016$).

Changes of immunocompetent cell composition in peripheral blood and subcutaneous adipose tissue

We studied the changes of immunocompetent cell composition by flow cytometry in 11 obese patients with and without T2DM from the bariatric sub-study, who underwent gastric plication.

In peripheral blood populations of classical, non-classical and intermediate monocytes based on CD14 and CD16 expression and populations of CD163+ monocytes were defined. At baseline, the patients had decreased classical monocytes (CD14++CD16-) and CD163+CD14+ cells and increased non-classical monocytes (CD14dimCD16++) compared with control subjects and these changes lasted also after gastric plication (Table 4).

CD163+CD14+ cells are probably recruited from classical monocyte subset. A strong positive correlation between the classical monocyte subset and CD163+CD14+ monocytes was found ($R=0.939$, $p<0.001$), while only weak inverse correlation between intermediate and CD163+CD14+ monocytes could be seen ($R=-0.302$, $p=0.049$). CD163+CD14+ cells and non-classical monocytes did not correlate.

In the stromal vascular fraction of SAT CD163+CD14+HLA-DR+ and CD163+CD14-HLA-DR+ populations were determined from the gate for monocytes/macrophages. Percentage of CD163+CD14+HLA-DR+ cells decreased and percentage of CD163+CD14-HLA-DR+ cells increased 6 months after gastric plication relative to baseline and 1 month after surgery (Table 4).

Discussion

Subclinical inflammation occurring primarily in the adipose tissue plays an important role in the development of metabolic complications of obesity and ultimately in the increased risk of cardiovascular morbidity and mortality. Here we studied the changes of sCD163 (a marker of alternatively activated macrophages), peripheral blood CD163+ cells and two subpopulations of CD163+ cells in the stromal vascular fraction of subcutaneous adipose tissue in patients with obesity with or without type 2 diabetes mellitus. To explore the role of CD163 in the regulation of systemic and adipose tissue inflammatory response, we prospectively studied the changes of the above mentioned parameters after selected weight-reducing interventions that included very low-calorie diet and bariatric surgery.

Plasma sCD163 is produced by shedding of the membrane form of CD163 receptor (Droste *et al.* 1999). Alternatively activated macrophages expressing CD163 exert anti-inflammatory properties based on the function of CD163 as erythroblast adhesion receptor, receptor for tumor necrosis factor-like weak inducer of apoptosis or receptor for distinct pathogens (Van Gorp *et al.* 2010). Furthermore, soluble sCD163 has potential anti-inflammatory effects as an inhibitor of phorbol ester-induced T lymphocytes activation and proliferation as well as matrix metalloproteinase-9 activity (Frings *et al.* 2002, Hogger and Sorg 2001). It was previously described that sCD163 levels are increased in several diseases including obesity and T2DM (Moller *et al.* 2011, Parkner *et al.* 2012, Zanni *et al.* 2012). In agreement with these data, we found increased baseline sCD163 levels in both obese patients with and without T2DM and positive correlations between sCD163 and assessed biochemical parameters such as serum triglycerides, blood glucose and glycated hemoglobin confirming its association with metabolic dysregulations present in obesity. Moreover, multiple linear regression analysis revealed that glycated hemoglobin and C-reactive

protein levels were independent predictors of sCD163 levels. As expected (Fjeldborg *et al.* 2013, Kazankov *et al.* 2015), both VLCD and bariatric surgery decreased sCD163 which corresponds with global metabolic improvements after both interventions. Taken together, our results suggest that sCD163 could be useful as a marker of global metabolic risk of obese subjects reflecting both the inflammatory response and the metabolic control.

In contrast to changes of circulating sCD163 levels, mRNA expression of CD163 in SAT or in CD14+ cells was not affected by obesity, diabetes or weight reducing interventions. These results are in disagreement with some previously published studies (Fjeldborg *et al.* 2014, Shakeri-Manesch *et al.* 2009), which found increased mRNA expression of CD163 in SAT in obese subjects. In our study, only patients enrolled in the VLCD sub-study tended to have elevated mRNA expression in SAT compared with control subjects, but the difference did not reach statistical significance. We also did not find the previously described association between circulating sCD163 levels and its mRNA expression in SAT (data not shown) (Kracmerova *et al.* 2014). The lack of association between circulating sCD163 and its mRNA expression in SAT in our study may suggest that sCD163 could come from other substantial sources than subcutaneous adipose tissue. For instance, enhanced production of sCD163 was previously described in obese subjects with non-alcoholic fatty liver disease (NAFLD) (Kazankov *et al.* 2014, Kazankov *et al.* 2015). In line with this possibility, we found a positive correlation of alanine aminotransferase levels and sCD163 indirectly supporting a possible interconnection between circulating sCD163 and NAFLD. Furthermore, another potential source of sCD163, which can respond differently to metabolic state than SAT, might be the visceral adipose tissue depot (VAT). Here, we observed a higher mRNA expression of CD163 in VAT relative to SAT as well as a positive correlation between CD163 mRNA expression in VAT and basal sCD163 levels suggesting that VAT could

be in fact a more important source of circulating sCD163 than subcutaneous fat in our patients. These data are in line with the previously described positive correlation between VAT CD163 mRNA expression or VAT to SAT depot ratio and sCD163 and with a multivariate analysis proving VAT CD163 mRNA expression as independent predictor of sCD163 in obese T2DM males (Sorensen *et al.* 2015). The difference between SAT and VAT CD163 mRNA expression might be most probably explained by increased macrophage numbers in VAT versus SAT (Harman-Boehm *et al.* 2007).

We also explored the differences in circulating monocyte subsets between obese and lean subjects based on previously described findings suggesting their modulation by obesity and its complications (Hristov and Weber 2011, Poitou *et al.* 2011). Similarly to previous studies (Hristov and Weber 2011, Rogacev *et al.* 2010), we found lower proportion of classical monocytes (CD14++CD16-) and higher percentage of non-classical monocytes (CD14dimCD16++) in obese patients relative to lean control subjects. In a selected subset of patients, we studied the influence of gastric plication on different subsets of CD163 positive monocytes in peripheral blood. Interestingly, weight loss induced by gastric plication did not have any significant effect on the monocyte subsets. These results are in disagreement with a previously published study by Poitou et al. (Poitou *et al.* 2011), where the percentage of both non-classical and intermediate monocytes decreased after gastric bypass surgery. Moreover, we confirmed that CD163+CD14+ cells are recruited from a subset of classical monocytes by analyzing the CD163 fluorescence signal from individual monocyte subsets (data not shown) (Ziegler-Heitbrock 2007). Lower percentage of CD163+ cells was also found in the subset of intermediate monocytes (CD14++CD16+), but we conclude that these cells might present a reservoir for classical monocytes (Hijdra *et al.* 2013).

In the stromal vascular fraction of SAT, we evaluated the changes of two populations of CD163 positive cells differing by CD14 expression after bariatric surgery. CD163 is generally used as a marker of alternatively activated (M2) macrophages with anti-inflammatory properties and it was suggested that CD163 is expressed exclusively on myeloid lineage (Fabriek *et al.* 2005). Here, M2 macrophage subpopulation (defined as CD163+CD14+HLA-DR+ cells) decreased 6 months after bariatric surgery as compared with baseline and 1 month after surgery. Interestingly, the second population of CD14 negative cells (CD163+CD14-HLA-DR+) significantly increased 6 months after bariatric surgery relative to baseline and 1 month after surgery. The precise origin of the CD14 negative population is unknown and our study design does not allow us to directly answer this question. The most likely explanation is that these CD163 positive cells represent a population of dendritic cells derived from myeloid lineage (Maniecki *et al.* 2006), which share some antigens with cells of monocyte/macrophage system. Other possible origins of CD14 negative cells may include 1) a population of macrophages developed in adipose tissue from preadipocytes (Charriere *et al.* 2003), 2) a population of preadipocytes with macrophage-like phenotype (Cousin *et al.* 1999) or 3) a population of macrophages, which do not express CD14 similarly to resident macrophages in the intestine (Smith *et al.* 1997). In general, these cells have some features of macrophages such as phagocytosis, anti-microbial activity or expression of antigens typical for macrophages (Cousin *et al.* 1999, Charriere *et al.* 2003). Another important question is the possible phenotypic difference in the CD14 positive and the CD14 negative cell populations in the adipose tissue. In our study, bariatric surgery decreased the entire CD163 positive population as well as its CD14 positive subset, while only its CD14 negative fraction was elevated. These data suggest that, that the association of M2 macrophage phenotype with positive metabolic effects in humans may be more complex than anticipated by some previously published studies (Bourlier *et al.* 2008, Zeyda *et al.* 2007). Our results also differ from a recently

published immunochemistry study (Aron-Wisnewsky *et al.* 2009) where M2 macrophages increased after Roux-en-Y gastric bypass. Nevertheless, the disagreement between this and our study could be due to the usage of the CD206 marker for the identification of alternatively activated macrophages and from different methodologies used in these two studies.

In conclusion, we have demonstrated that obesity both with and without T2DM increases sCD163 levels without affecting CD163 mRNA expression in subcutaneous adipose tissue or CD14+ monocytes in blood. Furthermore, obesity increased the non-classical subset and decreased the classical subset of monocytes in blood. The decrease of classical monocytes was in relation with the reduction of CD163+CD14+ monocytes in peripheral blood. In subcutaneous adipose tissue of obese subjects, we identified two CD163 positive cell populations, which responded differently to body weight reduction after gastric plication. Taken together, increase of sCD163 levels could be useful for prediction of obesity and its metabolic complications while sCD163 or CD163 positive cells changes in peripheral blood or subcutaneous adipose tissue do not reflect precisely metabolic improvements after weight loss.

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Table 1. Anthropometric, biochemical, and hormonal characteristics of study subjects at baseline.

	Controls	OB	T2DM
Number	19	32	32
Age (year)	49.95 ± 1.43	49.90 ± 1.65	53.04 ± 1.42
Body mass index (kg/m^2)	24.5 (21.8-25.5)	43.9 (39.4-51.3)*	46.6 (42.7-53.4)*
Waist circumference (cm)	---	128.6 ± 3.11	134.2 ± 3.10
Blood glucose (mmol/l)	4.80 (4.53-5.15)	5.00 (4.70-5.73)	8.75 (6.65-11.65)*^{x}
HbA1c (mmol/mol)	37.0 (37.0-41.0)	41.0 (38.3-41.0)	76.5 (50.0-93.5)*^{x}
Cholesterol (mmol/l)	5.43 ± 0.23	5.02 ± 0.23	4.83 ± 0.22
Triglycerides (mmol/l)	1.01 (0.71-1.89)	1.39 (0.92-1.95)	1.75 (1.36-2.60)*^{x}
HDL cholesterol (mmol/l)	1.56 (1.35-1.65)	1.22 (1.04-1.45)*	1.03 (0.93-1.26)*
LDL cholesterol (mmol/l)	3.35 ± 0.19	3.04 ± 0.16	2.83 ± 0.21
Insulin (mIU/l)	16.2 (14.5-18.3)	35.7 (25.2-58.4)*	41.9 (30.5-67.6)*
Leptin (ng/ml)	13.56 ± 2.12	48.18 ± 4.36*	55.90 ± 3.98*
CRP (mg/l)	0.08 (0.07-0.22)	1.76 (0.90-3.25)*	2.36 (1.75-3.60)*
HOMA index	3.42 (2.93-3.94)	7.01 (5.26-15.77)*	18.74 (13.43-33.46)*^{x}
CD163 mRNA expr. in SAT	1.20 ± 0.17	1.39 ± 0.14	1.58 ± 0.17
CD163 mRNA expr. in CD14+ cells	1.07 ± 0.12	0.88 ± 0.09	1.03 ± 0.09

OB: non-diabetic obese subjects. T2DM: obese subjects with type 2 diabetes mellitus. CRP: C-reactive protein. SAT: subcutaneous adipose tissue. Values are Mean ± SEM or Median (interquartile range). * $p<0.05$ vs. healthy control subjects; One way ANOVA/ANOVA on Ranks, ^{x} $p<0.05$ vs. OB subjects; unpaired t-test or Mann-Whitney Rank Sum Test.

Table 2. The effect of VLCD on anthropometric, biochemical, and hormonal characteristics of obese subjects with and without type 2 diabetes mellitus.

	OB before	OB after	T2DM before	T2DM after
Number	20	20	22	22
Age (year)	51.9 ± 1.87	---	55.1 ± 1.53	---
Body mass index (kg/m^2)	48.4 ± 1.95*	46.2 ± 1.98*°	50.0 ± 1.62*	46.9 ± 1.52*°
Blood glucose (mmol/l)	5.00 (4.70-6.25)	4.90 (4.45-5.20)°	9.05 (7.20-12.0)*^{x}	7.00 (5.60-9.50)*°^{x}
Waist circumference (cm)	135.6 ± 4.63	136.9 ± 3.36	131.8 ± 4.36	126.03 ± 3.45
HbA1c (mmol/mol)	43.0 (40.0-48.5)	---	83.5 (58.0-97.0)*^{x}	---
Cholesterol (mmol/l)	5.13 ± 0.33	4.67 ± 0.32	4.83 ± 0.29	3.76 ± 0.16*°^{x}
Triglycerides (mmol/l)	1.44 (0.93-2.17)	0.96 (0.90-1.66)	1.87 (1.61-2.70)*^{x}	1.77 (1.41-2.36)*^{x}
HDL cholesterol (mmol/l)	1.18 (1.04-1.46)*	1.13 (0.96-1.44)*	1.01 (0.90-1.26)*	0.96 (0.84-1.00)*°^{x}
LDL cholesterol (mmol/l)	3.09 ± 0.23	2.84 ± 0.26	2.71 ± 0.23*	2.05 ± 0.17*°^{x}
Insulin (mIU/l)	32.0 (23.4-62.3)*	34.5 (20.0-45.8)*°	40.7 (32.5-57.8)*	44.0 (31.3-52.1)*
Leptin (ng/ml)	54.2 ± 5.83*	49.8 ± 6.27*	60.1 ± 4.51*	51.9 ± 5.05*
CRP (mg/l)	2.09 ± 0.53*	1.63 ± 0.46*°	2.32 ± 0.29*	1.43 ± 0.23*°
HOMA index	6.6 (5.1-18.3)*	7.9 (3.7-10.8)*°	18.1 (14.7-32.9)*^{x}	11.8 (10.8-17.1)*°^{x}
CD163 mRNA expr. in SAT	1.59 ± 0.20	1.59 ± 0.13	1.93 ± 0.21	1.85 ± 0.19
CD163 mRNA expr. in CD14+ cells	0.89 ± 0.11	0.81 ± 0.07	0.92 ± 0.14	0.77 ± 0.12

OB: non-diabetic obese subjects. T2DM: obese subjects with type 2 diabetes mellitus. CRP: C-reactive protein. SAT: subcutaneous adipose tissue. Values are Mean ± SEM or Median (interquartile range). * $p<0.05$ vs. healthy control subjects; One way ANOVA/ANOVA on Ranks; data are presented in Table 1, column Controls, ° $p<0.05$ vs. before VLCD; paired t-test or Wilcoxon Signed Rank Test, ^{x} $p<0.05$ vs. OB subjects; unpaired t-test or Mann-Whitney Rank Sum Test.

Table 3. The effect of bariatric surgery on anthropometric, biochemical, and hormonal characteristics of obese subjects with and without type 2 diabetes mellitus.

	OB V1	OB V2	OB V3	OB V4	T2DM V1	T2DM V2	T2DM V3	T2DM V4
Number	12	12	12	7	10	10	10	7
Age (year)	46.58 ± 2.97	---	---	---	47.30 ± 2.15	---	---	---
Body mass index (kg/m2)	42.23 ± 2.04*	39.34 ± 2.43*[1]	35.98 ± 2.12*[1 2]	37.04 ± 3.53*[1 2]	43.1 (40.4-45.2)*	40.4 (38.8-42.8)*[1]	34.6 (32.0-39.6)*[1 2]	39.8 (34.4-41.0)*[1 2]
Waist circumference (cm)	121.6 ± 3.18	117.4 ± 3.47	110.8 ± 4.80[1 2]	114.9 ± 5.21[1]	137.8 ± 4.10	131.2 ± 3.65[1]	122.9 ± 3.58[1 2]	121.71 ± 3.78[1 2 3]
Blood glucose (mmol/l)	4.95 (4.65-5.35)	4.70 (4.35-5.05)	4.60 (4.30-4.95)	5.20 (4.10-5.50)	7.35 (5.60-11.00)*	6.35 (5.50-7.40)*	5.55 (4.60-7.40)	4.80 (4.73-7.88)
HbA1c (mmol/mol)	39.0 (34.0-41.0)	35.5 (33.5-37.0)	36.0 (31.0-40.0)[1]	34.0 (30.3-39.8)[1 2 3]	54.5 (44.0-89.0)*	46.0 (40.0-60.0)*[1]	41.5 (40.0-60.0)*[1]	44.0 (38.0-59.0)[1]
Cholesterol (mmol/l)	4.57 (4.22-5.34)	4.36 (3.64-5.215)*	4.60 (4.24-5.22)	4.42 (4.15-4.70)*	4.84 ± 0.36	4.56 ± 0.45	4.37 ± 0.42	4.38 ± 0.16
Triglycerides (mmol/l)	1.39 (0.80-1.73)	1.09 (1.01-1.47)	1.21 (0.76-1.81)	0.97 (0.58-1.44)	1.36 (0.88-2.26)	1.36 (1.05-2.45)	1.11 (0.98-1.71)	1.38 (0.72-2.46)
HDL cholesterol (mmol/l)	1.22 (1.05-1.37)*	1.07 (0.95-1.36)*	1.34 (1.09-1.51)	1.30 (1.25-1.65)	1.21 ± 0.10*	1.04 ± 0.08*[1]	1.31 ± 0.10[2]	1.22 ± 0.09
LDL cholesterol (mmol/l)	2.85 (2.51-3.41)	2.75 (2.15-3.59)	2.91 (2.32-3.52)	2.57 (2.20-2.79)	3.03 (1.81-3.40)	2.31 (1.89-3.36)	2.49 (2.17-3.23)	2.55 (2.07-2.86)
Insulin (mIU/l)	42.47 ± 6.44*	30.87 ± 2.88*	39.75 ± 8.70*	57.88 ± 11.12*	55.40 ± 7.84*	36.10 ± 2.40*[1]	32.48 ± 5.16*[1]	30.45 ± 3.65*[1]
Leptin (ng/ml)	48.4 (20.1-54.2)*	17.2 (12.7-29.9)[1]	17.2 (7.4-41.2)[1]	25.03 (4.24-42.5)[1]	47.50 ± 7.44*	32.93 ± 5.12*[1]	25.48 ± 4.95[1 2]	23.40 ± 5.11[1 2]
C-reactive protein (mg/l)	1.76 (1.36-5.70)*	1.26 (0.54-1.81)*	1.69 (0.59-2.01)*	2.00 (1.26-3.33)*	2.97 (2.00-6.03)*	2.20 (0.92-5.01)*	0.53 (0.25-4.00)*	2.01 (1.18-2.78)*
HOMA index	8.8 (5.8-11.6)*	5.9 (5.1-8.2)*	6.8 (3.5-11.6)*	10.6 (1.5-11.0)	21.2 (12.2-33.9)*	8.9 (8.0-16.4)*[1]	7.2 (4.8-12.1)[1]	7.3 (4.6-9.6)[1]
CD163 mRNA expr. in SCAT (2^{-ddCt})	1.05 ± 0.15	1.11 ± 0.16	1.04 ± 0.15	0.82 ± 0.23	1.02 ± 0.22	0.97 ± 0.19	1.14 ± 0.23	1.21 ± 0.21
CD163 mRNA expr. in CD14+ cells (2^{-ddCt})	0.89 ± 0.13	0.83 ± 0.11	0.89 ± 0.08	0.92 ± 0.09	1.15 ± 0.12	0.88 ± 0.11	1.06 ± 0.18	1.25 ± 0.19

OB: non-diabetic obese subjects. T2DM: obese subjects with T2DM. V1: before bariatric surgery, V2: 1 month after bariatric surgery, V3: 6 months after bariatric surgery, V4: 1 year after bariatric surgery. Values are Mean ± SEM or Median (interquartile range).* $p<0.05$ vs. healthy control subjects; One way ANOVA/ANOVA on Ranks; data are presented in Table 1, column Controls, [1] $p<0.05$ vs. V1; One Way RM ANOVA/One way RM ANOVA on Ranks, [2] $p<0.05$ vs. V2; One Way RM ANOVA/One way RM ANOVA on Ranks.

Table 4. The influence of gastric plication on flow cytometry findings in obese patients with and without type two diabetes mellitus: cell populations in peripheral blood and stromal vascular fraction of SAT.

		Controls	OB + T2DM V1	OB + T2DM V2	OB + T2DM V3
Peripheral blood	Number	12	11	10	10
	Classical monocytes (%)	80.9 ± 1.37	74.7 ± 1.48*	75.7 ± 1.58*	75.8 ± 1.17*
	Intermediate monocytes (%)	6.17 ± 0.58	7.40 ± 0.61	6.95 ± 0.47	7.01 ± 0.38
	Non-classical monocytes (%)	3.30 ± 0.61	7.02 ± 0.96*	7.04 ± 0.92*	8.53 ± 1.04*
	CD163+CD14+ cells (%)	86.2 ± 1.31	78.9 ± 1.48*	81.1 ± 1.65*	80.7 ± 1.07*
Stromal vascular fraction of SAT	Number		11	11	9
	CD163+CD14+HLA-DR+ (%)	---	19.4 ± 2.32	16.7 ± 1.80	11.3 ± 0.90 [1 2]
	CD163+CD14-HLA-DR+ (%)	---	4.17 ± 0.49	3.83 ± 0.52	7.56 ± 0.99 [1 2]

OB: non-diabetic obese subjects. T2DM: obese subjects with type 2 diabetes mellitus. V1: before bariatric surgery, V2: 1 month after bariatric surgery, V3: 6 months after bariatric surgery. SAT: subcutaneous adipose tissue. Values are Mean ± SEM. * $p<0.05$ vs. healthy control subjects; One way ANOVA/ANOVA on Ranks, [1] $p<0.05$ vs. V1; One Way RM ANOVA/One way RM ANOVA on Ranks, [2] $p<0.05$ vs. V2; One Way RM ANOVA/One way RM ANOVA on Ranks.

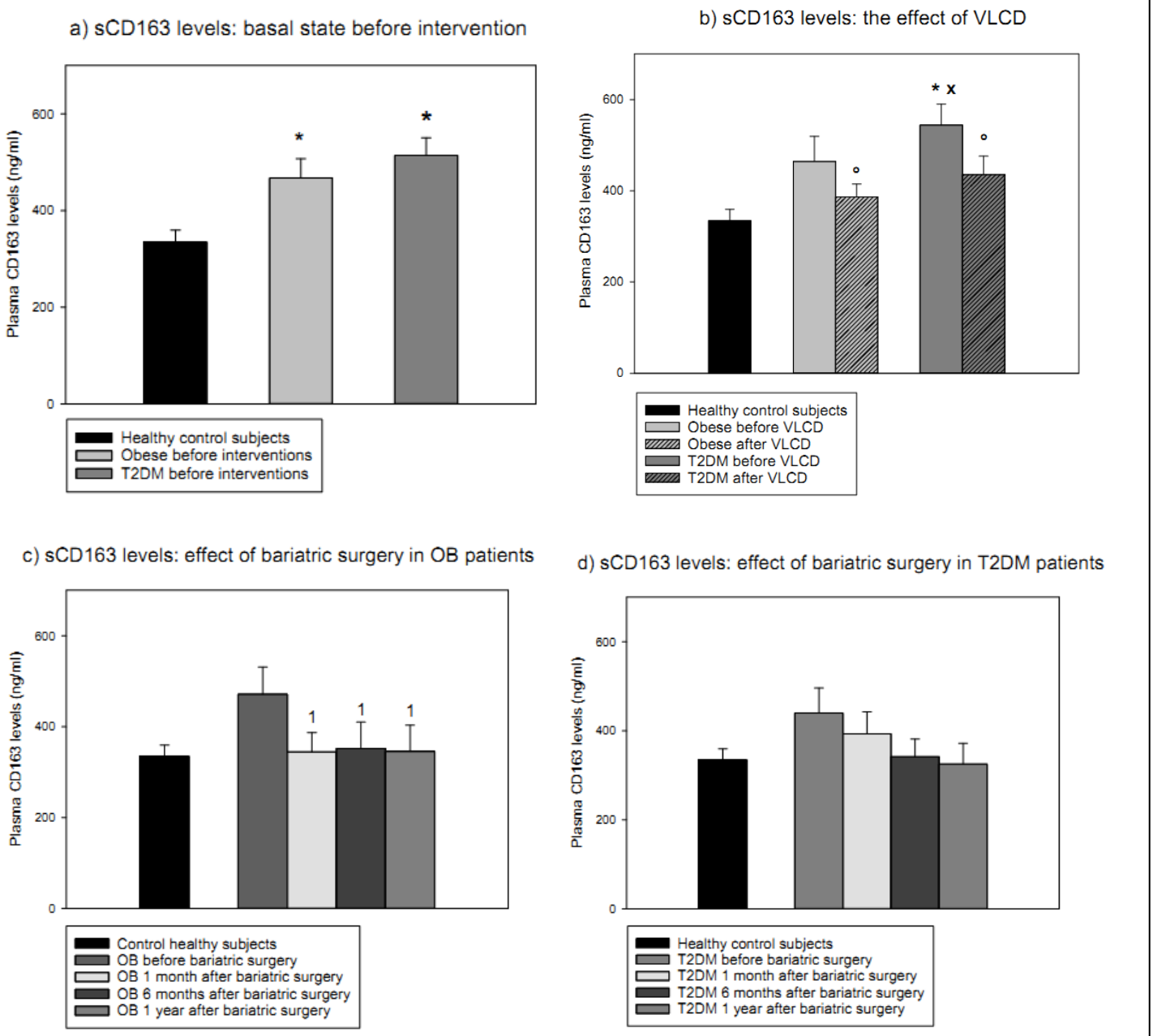

Figure 1. Plasma sCD163 levels: before interventions (a), effect of VLCD (b) and bariatric surgery in obese (c) and T2DM subjects (d).

OB: non-diabetic obese subjects. T2DM: obese subjects with type 2 diabetes mellitus. V1: before bariatric surgery, V2: 1 month after bariatric surgery, V3: 6 months after bariatric surgery, V4: 1 year after bariatric surgery. Values are Mean ± SEM or Median (interquartile range). * $p<0.05$ vs. healthy control subjects; One way ANOVA/ANOVA on Ranks, ° $p<0.05$ vs. before VLCD; paired t-test or Wilcoxon Signed Rank Test, ^{x} $p<0.05$ vs. OB subjects; unpaired t-test or Mann-Whitney Rank Sum Test. ^{1} $p<0.05$ vs. V1; One Way RM ANOVA/One way RM ANOVA on Ranks.