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Obesity

Intrahepatic Fat Content Correlates with Soluble CD163 in Relation to Weight Loss Induced by Roux-en-Y Gastric Bypass

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Objective: Soluble CD163 (sCD163) is a new marker of obesity-related metabolic complications. sCD163 and CD163 mRNA were investigated in relation to the fat distribution at baseline and 12 months after Roux-en-Y gastric bypass (RYGB).

Methods: Thirty-one obese subjects (BMI: 42.3 ± 4.7 kg/m^2) were enrolled. Subcutaneous (SAT) and visceral adipose tissue (VAT) volume were determined by MRI, intrahepatic lipid content (IHL) by MR-spectroscopy, and body composition by DXA. Fasting blood samples and adipose tissue samples were obtained, and ELISA and RT-PCR were performed.

Results: RYGB-induced weight loss (36 ± 11 kg) was accompanied by a significant reduction in sCD163 (2.1 ± 0.8 mg/l vs. 1.7 ± 0.7 mg/l), SAT, VAT, and IHL (all, $P < 0.001$). At baseline, sCD163 was associated with VAT ($r = 0.40$, $P < 0.05$) but not with SAT or IHL. Moreover, CD163 mRNA was significantly upregulated in VAT compared with SAT at baseline ($P < 0.05$) and significantly downregulated in SAT after RYGB ($P < 0.001$). ΔsCD163 was significantly associated with ΔIHL after RYGB compared with baseline ($r = 0.40$, $P < 0.05$).

Conclusions: RYGB-induced weight loss results in a reduction of sCD163 and CD163 mRNA. The association between ΔsCD163 and ΔIHL may reflect a reduction in sCD163-producing Kupffer cells in the liver. Moreover, sCD163 may be a marker of "unhealthy" fat distribution in obese subjects.

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Introduction

We and others have recently found that the macrophage-specific serum marker soluble CD163 (sCD163) is a good marker for obesity-related metabolic complications since sCD163 is significantly associated with BMI and with insulin resistance (1-6). Furthermore, sCD163 has been found to be a strong predictor for the development of type 2-diabtes (7). CD163 is a hemoglobin scavenger receptor primary expressed on monocytes and macrophages (8,9). sCD163 is found to be elevated in pathological conditions with activation of the monocyte-macrophage system (10-12). However, small amounts of sCD163 are constitutively shed to the circulation and the normal range is between 0.7 and 3.9 mg/l in healthy individuals (13). The metalloproteinase tumor necrosis factor α-converting enzyme (ADAM17) mediates the shedding of sCD163 (and TNF-α) from the plasma membrane (14). CD163 is expressed in macrophages resident in several tissues such as the adipose tissue (AT), liver, spleen, and in blood monocytes (15); however, it is unknown which tissues that primarily contribute to the circulating level of sCD163. CD163 is highly expressed in the macrophages in the AT and the expression of CD163 in the subcutaneous AT (SAT) has been found to be closely associated with circulating sCD163 (16,17).

In obesity, there is an increased amount of activated macrophages in the AT due to adipocyte expansion and possibly local hypoxia (18). The infiltrating macrophages produce inflammatory cytokines that cause systemic inflammation and insulin resistance (19,20).

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Furthermore, accumulation of excessive fat in the visceral adipose depot (VAT) and ectopic fat in the liver (IHL), as compared to the subcutaneous adipose depot (SAT), is strongly associated with systemic inflammation and increased risk of developing metabolic complications (21,22). The obesity-induced systemic inflammation is reversible since weight loss is associated with decreased levels of inflammatory cytokines, reduced SAT and VAT, and improved insulin sensitivity (23,24). Bariatric surgery is the most effective way to achieve a sustained weight loss and to improve the long-term metabolic profile (25,26).

The aim of this study was to evaluate the level of sCD163 and the expression of CD163 in relation to the fat distribution (normal and ectopic) and in relation to Roux-en-Y gastric bypass (RYGB)-induced weight loss in obese subjects.

Methods

Forty-six obese subjects were enrolled, who prospectively had been included in a RYGB program at the Department of Endocrinology at Aarhus University Hospital. Thus, meeting the national criteria of RYGB in Denmark (BMI >50 kg/m^2 or BMI >35 kg/m^2 plus obesity-related comorbidities). Exclusions criteria were glucocorticoid treatment or chronic systemic disease with the exception of type 2 diabetes. 13 subjects failed to lose the required weight (8%) before RYGB and two subjects dropped out of the clinical project. The 31 subjects who completed the study were evaluated at baseline and again at 6 and 12 months after the laparoscopic gastric bypass surgery, which proceeded without any postoperative complications for all subjects. Among the 31 subjects, 11 had been diagnosed with type 2 diabetes. Four of these patients were treated with metformin, one with liraglutide, four with a combination of different treatments, and two did not receive any medical treatment. Two subjects were treated with metformin due to PCOS. Of the 31 subjects, 10 were taking statins at baseline and five of these continued to take the same dose throughout the study. The study took place at the research laboratory at Aarhus University Hospital. All subjects were recruited via our out-patient clinic. The study was approved by the local ethics committee in the county of Aarhus and followed the principles of the Declaration of Helsinki (ClinicalTrialsgov.number:NCT01463449).

AT biopsy

In the beginning of the RYGB surgery, abdominal SAT and VAT biopsies were removed ($n = 25$). Immediately after the removal, the AT was transported to the laboratory where the samples were rinsed in isotonic NaCl and cut into smaller fragments (between 10 and 20 mg). Visible vessels and connective tissue were carefully removed and the AT samples were snap-frozen in liquid nitrogen and kept at −80°C until RNA extraction. About 12 months after RYGB, a new fat sample was taken (needle aspiration) from the abdominal SAT depot as previously described (27) from the same subjects ($n = 25$). No biopsy was obtained from VAT after 12 months.

Quantification of mRNA

RNA was isolated as previously described (17). All analyses ($n = 25$) were performed simultaneously and the mRNA levels of the target genes were expressed relative to the house-keeping gene glyceraldehyde-3-phosphate dehydrogenase (GADPH). The PCR reactions were performed in duplicate using the KAPA SYBR FAST qPCR kit (Kapa Biosystems, Woburn, MA) in a LightCycler 480 (Roche Applied Science) as previously described (17). The relative gene expression was estimated using the default "advanced relative quantification" mode of the software version LCS 480 1.5.0.39 (Roche Applied Science). Following primer pairs were used for amplification of CD163 (5′-3′): CGGCTGCCTCCACCTCTAAGT and ATGAAGATGCTG GCGTGACA, CD68 (5′-3′): GCTACATG GCGG TGGAGTACAA and ATGATGAGAGGCAGCAAGATGG, ADAM17 (5′-3′): ATCTGAACAACGACACCTGCTG and AAGGA CTGTTCCTGTCACTGCAC, and those for GADPH (5′-3′) AATGA AGGGGTCATTGATGG and AAGGTGAAGGTCGGAGTCAA. Before analysis of target genes the house-keeping gene was tested for stability and found stable displaying comparable number of C_T cycles.

Immunohistochemistry (IHC)

SAT ($n = 6$) and VAT ($n = 5$) biopsies were obtained in relation to the RYGB surgery and paired SAT biopsies were obtained 12 months after RYGB surgery ($n = 6$). Immediately after the removal the samples were fixed in 4% formaldehyde (24-48 h at room temperature) and then embedded in paraffin. Sections of 3 μm were deparaffinized, rehydrated and stained with hematoxylin and eosin using routine methods (28). Moreover, 3 μm sections were deparaffinized with EZ prep., and stained for CD163 using anti-CD163 monoclonal mouse antihuman antibody (AbD Serotec, clon edhu-1, MCA1853, dilution 1:450), using standard IHC methods. A single stained whole slide from each biopsy was captured and digitalised at a magnification of 20× using the Hamamatsu Nanozoomer 2.0HT (Hamamamatsu Photonics, Hamamamatsu City, Japan). Virtual slides were loaded into the digital image analysis software Visiopharm Integrator System ver. 4.4.4.0 (Visiopharm, Hørsholm, Denmark). Adipocyte morphology was assessed in HE-slides using a specially designed analysis protocol package (APP). Total tissue area in each slide together with the area and perimeter of all intact adipocytes within the slide were automatically computed and means with standard deviations were calculated for all biopsies, respectively. The numbers of adipocytes were automatically computed and given as number of adipocytes pr. total area adjusted for differences in the total area between biopsies. All digitalized slides were visually screened to ensure correct and satisfactory classification and sufficient intact tissue morphology within each virtual slide after completed automated quantification.

Magnetic resonance (MR) measurements

MR imaging and MR liver spectroscopy were performed using a Signa Excite 1.5 Tesla twin speed scanner (GE Medical Systems, Milwaukee, USA) for all patients and visits ($n = 26$). Five subjects were not MR-scanned due to claustrophobia. Abdominal SAT and VAT data were determined from axial MRI recordings using a fast spin echo sequence (body coil; echo time: 8.5 ms; repetition time: 600 ms; slice thickness: 8 mm; field of view: 48 cm). A multi-slice technique was used, in which slices were obtained from the upper pole of the femoral heads to the upper pole of the kidney. The protocol of the MR imaging and liver spectroscopy has previously been described (29).

Dual-energy X-ray absorptiometry (DXA)

Body composition was measured by using the same DXA scanner (Hologic Discovery, Hologic, Waltham, MA, USA) for all patients

and visits ($n = 30$). One subject was excluded because the preoperative weight exceeded the limit of the DXA scanner (150 kg). Body composition is presented as total fat mass and total lean body mass measured in kilograms and as percent body fat.

Anthropometrics and blood samples

Anthropometrics and fasting blood samples were collected at baseline, 6 months, and 12 months after RYGB. Venous blood samples were collected after an overnight fast and serum were frozen at −80°C. The homeostasis model assessment insulin resistance index (HOMA-IR) was calculated using the formula: serum fasting insulin (μU/ml)* fasting glucose (mmol/l)/22.5 (30,31). Soluble CD163 was quantified in serum samples using an in-house enzyme-linked immunosorbent assay, as previously described (32). Adiponectin was measured using a human-specific high sensitive ELISA method (b-Bridge International, USA), high sensitive-CRP by an hs-CRP enzyme immunoassay (DRG, Germany), and TNF-α and IL-6 were measured using a high sensitivity ELISA (R&D Systems, USA).

Statistical analysis

Descriptive statistics for anthropometric, metabolic markers and fat depots are presented as means ± SD.

Baseline associations were determined by a Spearman's rank correlation. Differences in high- and low-VAT groups were analyzed with an unpaired *t*-test or a Wilcoxon Mann-Whitney rank sum test for those variables, which were not normally distributed. The paired data, VAT versus SAT, was computed with a Wilcoxon signed rank test or a paired *t*-test where appropriated. Changes in 6 and 12 months after RYGB were determined by one-way repeated measures ANOVA (Holm-sidak corrected). Finally, the relationship between the relative changes before and 1 year after RYGB were analyzed by a Spearman's Rank Correlation. The chosen significance level was a two-tailed *P*-value of <0.05. The statistical software packet SPSS (SPSS, Chicago, IL) was used for all calculations. Graphs were made in Sigma-Plot.

Results

Baseline characteristics of the subjects are given in Table 1. Mean BMI was 42.3 ± 4.7 kg/m^2 (range: 35-51 kg/m^2). Age was between 25 and 57 years with a mean of 43.9 ± 7.7 years, and 61% of the subjects were females. There was a relative large individual variation in the fat depots at baseline with a range of SAT at 7941-19422 cm^3 and a range of VAT at 1936-7621 cm^3.

Effect of RYGB-induced weight loss on sCD163

The mean weight loss was 36 ± 11 kg (29%), and the fat mass was reduced from 51.0 ± 9.3 kg to 25.7 ± 7.5 kg after RYGB (Table 1). The weight loss was primary achieved within the first 6 months after RYGB (31 ± 8 kg). The fat depots were significantly decreased (all, $P < 0.001$)—the SAT by 45 ± 16%, the VAT by 57 ± 28%, and the IHL by 86 ± 13%. The relative change in IHL was significantly higher than the relative change in VAT and SAT (both, $P < 0.05$). Furthermore, the change in VAT was significantly higher compared with SAT ($P < 0.05$).

The level of sCD163 was significantly reduced at 12 months compared with baseline (1.7 ± 0.7 mg/l vs. 2.1 ± 0.8 mg/l) ($P < 0.001$). A significant reduction was also seen from baseline to 6 months ($P < 0.001$); however, no significant change was found in sCD163 from 6 to 12 months after RYGB ($P > 0.05$) (Figure 1). The insulin resistance measured by HOMA-IR decreased significantly with a reduction at 69% 12 months after RYGB ($P < 0.001$). Same result was found when excluding those with type 2 diabetes at baseline ($n = 20$) ($P < 0.001$). Diabetes remission was seen in eight out of the 11 subjects with type 2 diabetes (data not shown). The level of IL-6 and hs-CRP were significantly reduced ($P < 0.05$; $P < 0.001$) and adiponectin was significantly increased by 93% after 12 months ($P < 0.05$). The level of TNF-α was not affected by the RYGB-induced weight loss.

TABLE 1 Clinical and metabolic characteristics of the study subjects at baseline, 6, and 12 months after RYGB

	Baseline	6 Months	12 Months
Number	31	31	31
Age	43.9 ± 7.7	A	A
Sex (female/male), *n*	19/12	A	A
BMI (kg/m^2)	42.3 ± 4.7	31.8 ± 3.8^b	29.9 ± 3.7^b
Waist (cm)	118.9 ± 11.5	94.5 ± 9.7^b	91.8 ± 12.3^b
ALAT (U/l)	36.3 ± 13.6	30.1 ± 18.3^a	27.5 ± 12.4^a
Glucose (mmol/l)	7.2 ± 1.7	6.1 ± 1.2^a	5.9 ± 0.9^a
Insulin (pmol/l)	102.0 ± 49.4	45.7 ± 34.2^b	37.7 ± 27.3^b
HOMA-IR	5.5 ± 3.0	2.2 ± 2.1^b	1.7 ± 1.3^b
sCD163 (mg/l)	2.1 ± 0.8	1.8 ± 0.6^b	1.7 ± 0.7^b
Adiponectin (μg/ml)	5.8 ± 3.2	8.5 ± 3.7^b	11.2 ± 5.5^{bc}
IL-6 (pg/ml)	2.7 ± 1.4	1.9 ± 0.8^a	1.8 ± 0.8^a
TNF-α (pg/ml)	1.8 ± 0.4	2.1 ± 1.4	1.7 ± 0.6
hs-CRP (mg/l)	5.3 ± 3.4	2.0 ± 1.9^b	1.2 ± 1.1^b
Lean mass (kg)	67.1 ± 12.8	A	57.2 ± 11.6^b
Fat mass (kg)	51.0 ± 9.3	A	25.7 ± 7.5^b
Fat percent (%)	42.4 ± 6.7	A	30.1 ± 8.4^b
SAT (cm^3)	13944.6 ± 3573.7	A	7654.4 ± 2833.2^b
VAT (cm^3)	4820.9 ± 1550.7	A	1933.8 ± 1112.9^b
VAT/SAT-ratio (%)	38.4 ± 20.1	A	33.7 ± 25.8^b
IHL, arbitrary fraction	0.23 ± 0.23	A	0.021 ± 0.018^b

Data are given for the obese subjects at baseline, 6, and 12 months after RYGB surgery. Data are means ± SD. The difference between baseline, 6, and 12 months after RYGB is measured by one-way repeated measures ANOVA.
Baseline vs. 6 months and baseline vs. 12 months, ^{a}*P*-value < 0.05, ^{b}*P*-value < 0.001.
6 months vs. 12 months after RYGB, ^{c}*P* < 0.001.

Gene expression of CD163 in VAT versus SAT

The gene expression of CD163, ADAM17, and CD68 were examined in VAT and SAT at baseline. The expression of CD163 and the general macrophage marker CD68 was significantly higher in VAT compared with SAT (both, $P < 0.05$) (Figure 2). The metalloproteinase ADAM17, which is known to regulate the shedding of sCD163, was equally expressed in VAT and SAT ($P > 0.05$) (Figure 2).

The morphology of the adipocytes in VAT and SAT were determined in a small subgroup ($n = 5$). The number of adipocytes pr. total area was significantly higher in VAT compared with SAT ($P < 0.05$). The mean area and the mean perimeter of the adipocytes were similar in VAT compared with SAT (both, $P > 0.05$).

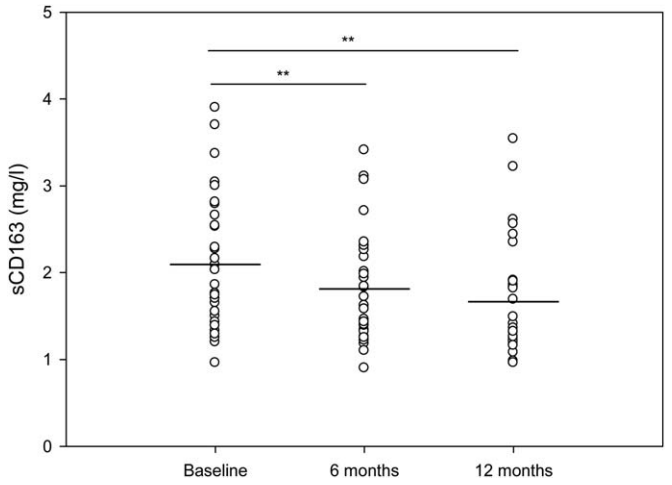

Figure 1 Changes in the level of sCD163 at 6 and 12 months post after RYGB. The Level of sCD163 in obese subjects ($n = 31$) at baseline (mean 2.1 ± 0.8 mg/l), at 6 months after RYGB (mean 1.8 ± 0.6 mg/l), and at 12 months after RYGB (1.7 ± 0.7 mg/l). One-way repeated measures ANOVA, ***P*-value < 0.001. The lines represent mean values.

Changes in CD163 mRNA in SAT after RYGB

Abdominal SAT biopsies were taken in association with the RYGB surgery and 12 months after the surgery. The expression of CD163 was significantly reduced after RYGB by 46% ($P < 0.001$) (Figure 3) and also the expression of CD68 was significantly decreased ($P < 0.05$) (Figure 3). No change was found in the expression of ADAM17 ($P > 0.05$) (Figure 3). In a subgroup ($n = 6$), the adipocyte morphology was determined by IHC. The mean perimeter, diameter and area of the adipocytes were significantly decreased after RYGB compared with baseline (both, $P < 0.001$) (Figure 4). Moreover, the number of adipocytes pr. total area was significantly higher after RYGB compared with baseline ($P < 0.05$) (Figure 4). Crown-like-structures (CLS) with CD163-positive macrophages were observed in five out of six samples at baseline; however, only one sample with CLS was observed after RYGB (Figure 5).

Differences in sCD163 in relation to the amount of VAT

To determine the possible effects of excessive fat in VAT on sCD163 the obese subjects were divided into two groups, one with high amount of VAT and one with low VAT with a cutoff at 4820 cm^3 corresponding to the mean VAT in the obese subjects at baseline (Table 2). Age, BMI, and waist circumference were similar in both groups ($P > 0.05$). In the high-VAT group, there were more men and 64% of the subjects had type 2 diabetes compared with only 13% in the low VAT group. sCD163 was significantly higher in the high-VAT group (2.4 ± 0.9 mg/l vs. 1.8 ± 0.6 mg/l) ($P < 0.05$). Furthermore, IHL was significantly higher ($P < 0.001$), HOMA-IR tended to be higher ($P = 0.05$), SAT was significantly lower, and the level of adiponectin was significantly lower (both, $P < 0.05$) in the high-VAT group compared with the low VAT group. There was no significant difference in the level of TNF-α, hs-CRP, or IL-6 between the high- and low-VAT groups (Table 2).

Baseline correlations

In a Spearman correlation, no association was found between sCD163 and respective sex or age at baseline ($r = 0.08$; $r = 0.21$, $P > 0.05$). A positive association was found between sCD163 and type 2 diabetes ($r = 0.35$, $P = 0.06$). Furthermore, sCD163 was significantly associated with VAT ($r = 0.40$, $P < 0.05$); however, when adjusting for sex and type 2 diabetes, the association between sCD163 and VAT was no longer significant ($P = 0.12$) (data not shown). No association was found between sCD163 and BMI, total fat mass, SAT, or IHL (all, $P > 0.05$). A strong association was found between sCD163 and IL-6 ($r = 0.54$, $P < 0.05$), whereas, no association was found between sCD163 and hs-CRP, adiponectin, and TNF-α ($P > 0.05$).

Relationship between sCD163, CD163 mRNA, and fat depots after RYGB

The relative change in the level of sCD163 was significantly associated with change in IHL ($r = 0.40$, $P < 0.05$) but not with changes in BMI, VAT or SAT ($P > 0.05$) (Table 3). Furthermore, the decrease in CD163 mRNA in SAT was significantly associated with a reduction in the fat percent ($r = 0.41$, $P < 0.05$) (Table 3). No

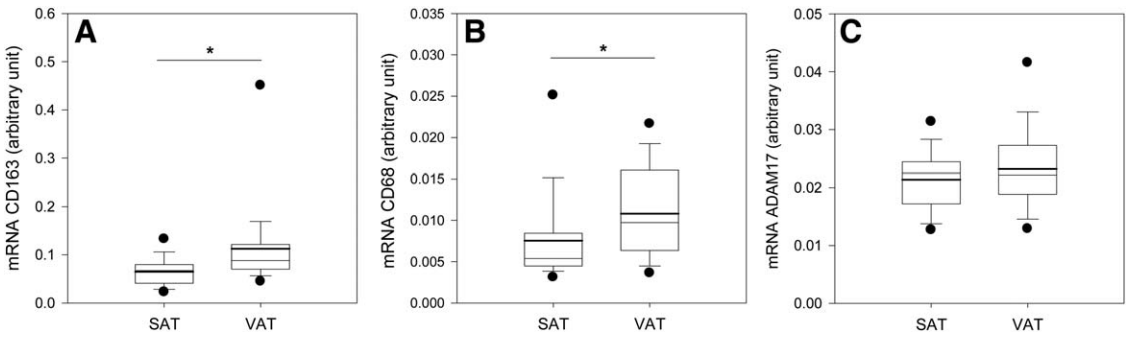

Figure 2 Gene expression levels of CD163 and other macrophage markers in VAT and SAT at baseline. SAT and VAT samples from obese subjects at baseline ($n = 25$). Gene expression levels of macrophage markers measured by RT-PCR relative to the housekeeping gene GADPH. (**A**) Expression of CD163 in SAT vs. VAT, (**B**) expression of the general macrophage marker CD68 in SAT vs. VAT, and (**C**) expression of ADAM17 in SAT vs. SAT. **P*-value <0.05. Mean value with a solid line and outliners represent the 5th/95th percentile.

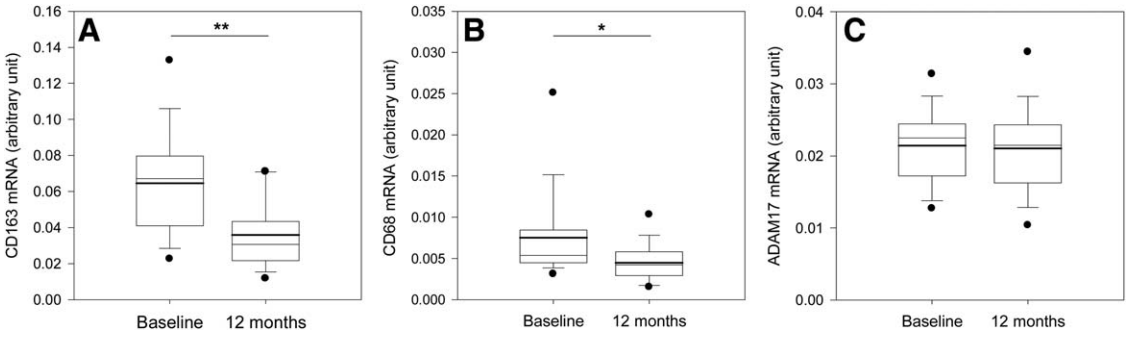

Figure 3 Gene expression levels in SAT 12 months post after RYGB compared with baseline. Gene expression levels relative to the house-keeping gene GADPH were determined by RT-PCR in SAT samples from 25 obese subjects at baseline and 12 months after RYGB. (**A**) Expression of CD163, (**B**) expression of CD68, and (**C**) expression of ADAM17. Differences measured by paired *t*-test or Wilcoxon Mann-Whitney rank sum test where appropriate. **P*-value <0.05, ***P*-value <0.001. Mean value with a solid line and outliners represent the 5th/95th percentile.

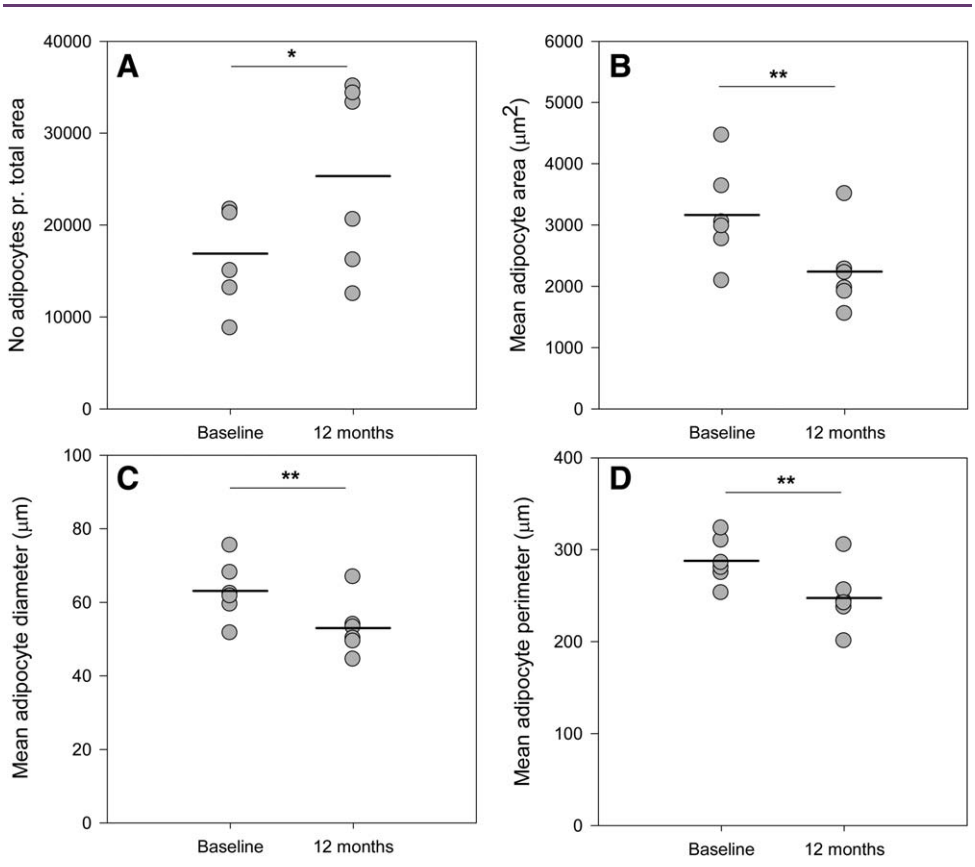

Figure 4 In a subgroup ($n = 6$), the adipocyte morphology was determined at baseline and 12 months after RYGB. SAT samples were formalin-fixed and paraffin-embedded, sectioned (3 μm sections), and stained with hematoxylin and eosin using routine methods. Histopathological evaluation of the adipocytes was performed using Visiopharm integrator system ver. 4.4.4.0. (**A**) Adipocyte number, (**B**) mean adipocyte area, (**C**) mean adipocyte diameter, and (**D**) mean adipocyte perimeter. Paired *t*-test or Wilcoxon Mann-Whitney rank sum test where appropriate. **P*-value<0.05, ***P*-value <0.001. The lines represent mean values.

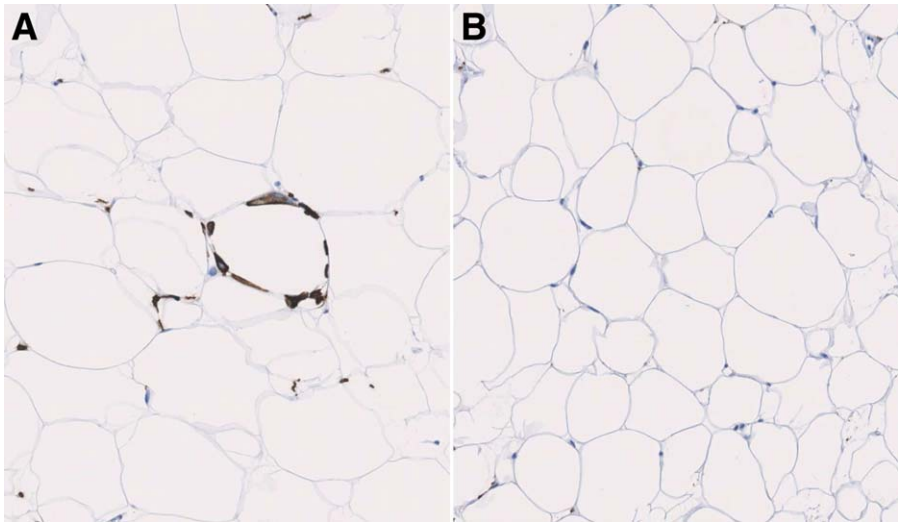

Figure 5 Characterization of CD163-positive macrophages in SAT before and 12 months after RYGB. Human AT was stained with antibodies to CD163 (brown). (**A**) Baseline SAT sample. Adipocytes surrounded by CD163-stained macrophages placed in a crown-like-structure (20×). (**B**) SAT sample 12 months after RYGB. No crown-like-structure observed (20×). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 2 Study subjects divided into high- and low-VAT groups

	Low VAT (<4820 cm^3)	High VAT (>4820 cm^3)
Number, n	15	11
Sex (female/male), *n*	12/3	5/6
DM2, *n*	2	7
Age (years)	41.7 ± 7.3	46.5 ± 8.2
BMI (kg/m^2)	43.1 ± 4.6	41.0 ± 4.5
Waist (cm)	116.2 ± 9.9	121.3 ± 11.9
Fat mass (g)	54726.2 ± 8993.0	46913.6 ± 9467.9[a]
Fat percent (%)	45.3 ± 5.9	39.1 ± 7.0[a]
SAT (cm^3)	15370.9 ± 3118.2	11999.5 ± 3332.2[a]
VAT (cm^3)	3775.4 ± 981.6	6246.5 ± 887.6[b]
VAT/SAT ratio (%)	25.4 ± 9.0	56.1 ± 17.2[b]
IHL, arbitrary fraction	0.12 ± 0.08	0.39 ± 0.26[b]
HOMA-IR	4.1 ± 2.7	6.2 ± 2.2
sCD163 (mg/l)	1.8 ± 0.6	2.4 ± 0.9[a]
Adiponectin (μg/ml)	7.0 ± 3.9	4.8 ± 1.4[a]
IL-6 (pg/ml)	2.3 ± 0.8	3.2 ± 1.9
TNF-α (pg/ml)	1.8 ± 0.4	1.8 ± 0.5
Hs-CRP (mg/l)	5.7 ± 3.6	5.0 ± 3.6

Data are means ± SD. Data are given for the obese subjects, who were MR-scanned at baseline ($n = 26$). The subjects are divided into two groups, one with high VAT and one with low VAT. The cutoff level is 4820 cm^3 VAT corresponding to the mean VAT in the obese subjects at baseline. Comparison of the high- and low-VAT group by unpaired t-test or Wilcoxon Mann-Whitney rank sum test where appropriate.
[a]*P*-value < 0.05.
[b]*P*-value < 0.001.

significant association was found between the change in CD163 mRNA in SAT and the change in circulating sCD163 ($r = 0.31$, $P > 0.05$) (data not shown).

Discussion

Circulating sCD163 is significantly associated with BMI and waist circumference (1-6); however, the level of sCD163 has until now not been examined in relation to the fat distribution in obese subjects. Thus, in this study, we examined the level of sCD163 in relation to the fat distribution at baseline and 12 months after RYGB surgery-induced weight loss.

TABLE 3 Univariate association between relative changes in sCD163, CD163 mRNA, and fat depots

	ΔsCD163	ΔCD163 mRNA
ΔBMI	0.01	0.11
ΔWaist	0.11	0.01
ΔFat mass	−0.03	0.35
ΔFat percent	−0.10	0.41[a]
ΔSAT	−0.10	0.40
ΔVAT	0.27	0.12
ΔIHL	0.40[a]	−0.04

Relative changes in circulating sCD163 and CD163 mRNA expressed in SAT and the association with relative changes in the fat depots in obese subjects ($n = 31$) at baseline compared with 12 months after RYGB. Statistical tests: Spearman's correlation, r = correlations coefficient.
[a]*P*-value < 0.05.

RYGB induced an impressive weight loss at 29% (mean 36 ± 11 kg) and the weight loss was accompanied by a significant reduction in the level of sCD163 (2.1 ± 0.8 mg/l vs. 1.7 ± 0.7 mg/l, $P < 0.001$) which is in line with previous studies showing a reduction in sCD163 in relation to diet-induced weight loss (1,16). Furthermore, the weight loss was accompanied by a significant reduction in the total fat mass, SAT, VAT, and IHL (all, $P < 0.001$) with a preferential reduction of IHL and VAT compared with SAT (both, $P < 0.05$) which has been described previously (33,34). Massive weight loss in relation with RYGB is associated with improvement of the metabolic profile (24,26) and likewise, we found a reduction in the circulating inflammatory cytokines IL-6 and hs-CRP, decreased level of HOMA-IR, diabetes remission, and increased level of adiponectin after RYGB.

Recently, we have demonstrated that the gene expression of CD163 and the general macrophage marker CD68 are elevated in SAT in obese subjects compared with lean subjects (17). In this study, we found that CD163 mRNA was significantly upregulated in VAT compared with SAT at baseline. Furthermore, the expression of CD68 mRNA, which is a close correlate to the total number of macrophages in the AT (35), was also significantly upregulated in VAT compared with SAT at baseline. The expression of CD163 mRNA has previously been found equally upregulated in the two depots; however, in a study by Bauer *et al.,* there was a tendency towards a higher level of CD163 mRNA in VAT compared with SAT (36). Upregulated expression of CD68 mRNA in VAT has also been described previously (35), but the results are conflicting since no depot difference has been described in other studies (6,37). However, other general macrophage markers have been found upregulated in VAT compared with SAT in obese subjects (38) which is in accordance with the general assumption that the VAT is more infiltrated by macrophages than SAT in obese subjects.

RYGB-induced weight loss resulted in a significant reduction of the expression of both CD163 and CD68 mRNA the latter indicates that the total number of infiltrating macrophages was reduced in relation to the weight loss. A study by Cancello *et al*. also found at reduced number of macrophages in SAT 3 months after RYGB compared with baseline (39); however, increased level of CD163-positive macrophages has been described 3 months after RYGB (40). Macrophages expressing CD163 are considered anti-inflammatory since they are elevated in the down-regulatory phase of an inflammatory response. Anti-inflammatory macrophages have a function in tissue repair and remodeling (9); thus, it could be speculated that there is a temporary increased level of CD163-positive macrophages due to a need of tissue repair the first 6 months after RYGB along with the main weight loss. We found indeed that the morphology of the adipocytes had changed with a significant reduction in the adipocyte area, diameter and perimeter after RYGB.

Accumulation of fat in VAT is known to represent an unhealthy obese phenotype compared with accumulation of fat in SAT (22). We found that sCD163 was significantly associated with VAT but not with SAT or IHL. We divided the obese subjects into low- and high-VAT groups and found that the high-VAT group was characterized by a high sCD163 level, high IHL, high HOMA-IR, low SAT, and low adiponectin level, despite similar BMI in the two groups. Moreover, we found that there were more male subjects and a preponderance of type 2 diabetics in the high-VAT group. Thus, we confirm that sCD163 is a marker of obese subjects with an "unhealthy" metabolic phenotype; however, this association may in part be explained by the strong association between sCD163 and type 2 diabetes (7).

Interestingly, the relative reduction in sCD163 in relation to RYGB was not associated with the change in VAT ($r = 0.27$, $P > 0.05$) but significantly associated with the change in IHL ($r = 0.40$, $P < 0.05$). We found a dramatic reduction in IHL after RYBG compared with baseline which coherently may have induced a reduction in the amount of sCD163-producing Kupffer cells (macrophages) in the liver. Thus, activated macrophages in the liver and in the VAT may be responsible for the increased level of sCD163 in obese subjects. We found no difference in the expression of ADAM17 in VAT and SAT at baseline which could indicate that sCD163 is released equally from the two depots; however, the gene expression level may not reflect the enzyme activity. A study by Bauer *et al*. showed comparable levels of sCD163 in portal venous, hepatic venous, and systemic venous blood of liver healthy controls indicating that the VAT, liver, and SAT equally contribute to the circulating sCD163. However, only nine subjects were enrolled in the study, all of them were lean, and six of the subjects had malignant abdominal tumors, which could affect the level of sCD163 (36). Further investigations are needed to sort out which tissue that primarily contributes to the increased production of sCD163 in obese subjects.

Our study had some limitations. First, the SAT samples were not obtained by the same procedure before and after RYGB which could influence the results. Furthermore, it would have been interesting with SAT samples after 3-6 months and VAT samples after 12 months. Secondly, we were not able to differentiate between weight loss-induced changes or changes in relation to RYGB which may induce other hormonal changes that may affect the inflammatory status. Finally, HOMA-IR may not be the best measurement of insulin sensitivity in this study.

In conclusion, we found that the BMI, SAT, VAT, and IHL were significantly reduced after RYGB. A concomitant reduction was seen in the level of sCD163 and in the expression of CD163 mRNA in SAT. The change in sCD163 was significantly associated with the change in IHL after RYGB which may reflect a reduction in sCD163-producing Kupffer cells in the liver. Moreover, we found that sCD163 may be a marker of an "unhealthy" fat distribution in obese subjects at baseline. O

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References

1. Fjeldborg K, Christiansen T, Bennetzen M, Moller HJ, Pedersen SB, Richelsen B. The macrophage specific serum marker, soluble CD163, is increased in obesity and reduced after dietary induced weight loss. *Obesity* 2013;21:2437-2443.
2. Parkner T, Sorensen LP, Nielsen AR, et al. Soluble CD163: a biomarker linking macrophages and insulin resistance. *Diabetologia* 2012;55:1856-1862.
3. Axelsson J, Moller HJ, Witasp A, et al. Changes in fat mass correlate with changes in soluble sCD163, a marker of mature macrophages, in patients with CKD. *Am J Kidney Dis* 2006;48:916-925.
4. Sporrer D, Weber M, Wanninger J, et al. Adiponectin downregulates CD163 whose cellular and soluble forms are elevated in obesity. *Eur J Clin Invest* 2009;39:671-679.

5. Zanni MV, Burdo TH, Makimura H, Williams KC, Grinspoon SK. Relationship between monocyte/macrophage activation marker soluble CD163 and insulin resistance in obese and normal-weight subjects. *Clin Endocrinol (Oxford)* 2012;77: 385-390.
6. Shakeri-Manesch S, Zeyda M, Huber J, Ludvik B, Prager G, Stulnig TM. Diminished upregulation of visceral adipose heme oxygenase-1 correlates with waist-to-hip ratio and insulin resistance. *Int J Obes (Lond)* 2009;33:1257-1264.
7. Moller HJ, Frikke-Schmidt R, Moestrup SK, Nordestgaard BG, Tybjaerg-Hansen A. Serum soluble CD163 predicts risk of type 2 diabetes in the general population. *Clin Chem* 2011;57:291-297.
8. Kristiansen M, Graversen JH, Jacobsen C, et al. Identification of the haemoglobin scavenger receptor. *Nature* 2001;409:198-201.
9. Moestrup SK, Moller HJ. CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. *Ann Med* 2004;36:347-354.
10. Moller HJ, Aerts H, Gronbaek H, et al. Soluble CD163: a marker molecule for monocyte/macrophage activity in disease. *Scand J Clin Lab Invest Suppl* 2002;237: 29-33.
11. Moller HJ. Soluble CD163. *Scand J Clin Lab Invest* 2011;72:1-13.
12. Gronbaek H, Sandahl TD, Mortensen C, Vilstrup H, Moller HJ, Moller S. Soluble CD163, a marker of Kupffer cell activation, is related to portal hypertension in patients with liver cirrhosis. *Aliment Pharmacol Ther* 2012;36:173-180.
13. Moller HJ, Peterslund NA, Graversen JH, Moestrup SK. Identification of the hemoglobin scavenger receptor/CD163 as a natural soluble protein in plasma. *Blood* 2002;99:378-380.
14. Etzerodt A, Maniecki MB, Moller K, Moller HJ, Moestrup SK. Tumor necrosis factor alpha-converting enzyme (TACE/ADAM17) mediates ectodomain shedding of the scavenger receptor CD163. *J Leukoc Biol* 2010;88:1201-1205.
15. Van den Heuvel MM, Tensen CP, van As JH, et al. Regulation of CD 163 on human macrophages: cross-linking of CD163 induces signaling and activation. *J Leukoc Biol* 1999;66:858-866.
16. Kracmerova J, Rossmeislova L, Kovacova Z, et al. Soluble CD163 is associated with CD163 mRNA expression in adipose tissue and with insulin sensitivity in steady state condition but not in response to calorie restriction. *J Clin Endocrinol Metab* 2014;99:E528-E535.
17. Fjeldborg K, Pedersen SB, Moller HJ, Christiansen T, Bennetzen M, Richelsen B. Human adipose tissue macrophages are enhanced but changed to an anti-inflammatory profile in obesity. *J Immunol Res* 2014;2014:309548.
18. Trayhurn P. Hypoxia and adipose tissue function and dysfunction in obesity. *Physiol Rev* 2013;93:1-21.
19. Hajer GR, Van Haeften TW, Visseren FLJ. Adipose tissue dysfunction in obesity, diabetes, and vascular diseases. *Eur Heart J* 2008;29:2959-2971.
20. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol* 2004;25:4-7.
21. Makkonen J, Westerbacka J, Kolak M, et al. Increased expression of the macrophage markers and of 11beta-HSD-1 in subcutaneous adipose tissue, but not in cultured monocyte-derived macrophages, is associated with liver fat in human obesity. *Int J Obes (Lond)* 2007;31:1617-1625.
22. Wajchenberg BL, Giannella-Neto D, da Silva ME, Santos RF. Depot-specific hormonal characteristics of subcutaneous and visceral adipose tissue and their relation to the metabolic syndrome. *Horm Metab Res* 2002;34:616-621.
23. Christiansen T, Paulsen SK, Bruun JM, et al. Comparable reduction of the visceral adipose tissue depot after a diet-induced weight loss with or without aerobic exercise in obese subjects: a 12-week randomized intervention study. *Eur J Endocrinol* 2009;160:759-767.
24. Kopp HP, Kopp CW, Festa A, et al. Impact of weight loss on inflammatory proteins and their association with the insulin resistance syndrome in morbidly obese patients. *Arterioscler Thromb Vasc Biol* 2003;23:1042-1047.
25. Sjostrom L, Lindroos AK, Peltonen M, et al. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med* 2004;351:2683-2693.
26. Manco M, Fernandez-Real JM, Equitani F, et al. Effect of massive weight loss on inflammatory adipocytokines and the innate immune system in morbidly obese women. *J Clin Endocrinol Metab* 2007;92:483-490.
27. Christiansen T, Paulsen SK, Bruun JM, Ploug T, Pedersen SB, Richelsen B. Diet-induced weight loss and exercise alone and in combination enhance the expression of adiponectin receptors in adipose tissue and skeletal muscle, but only diet-induced weight loss enhanced circulating adiponectin. *J Clin Endocrinol Metab* 2010;95: 911-919.
28. Cinti S, Zingaretti MC, Cancello R, Ceresi E, Ferrara P. Morphologic techniques for the study of brown adipose tissue and white adipose tissue. *Methods Mol Biol* 2001;155:21-51.
29. Wamberg L, Pedersen SB, Richelsen B, Rejnmark L. The effect of high-dose vitamin D supplementation on calciotropic hormones and bone mineral density in obese subjects with low levels of circulating 25-hydroxyvitamin d: results from a randomized controlled study. *Calcif Tissue Int* 2013;93:69-77.
30. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412-419.
31. Volund A. Conversion of insulin units to SI units. *Am J Clin Nutr* 1993;58: 714-715.
32. Moller HJ, Hald K, Moestrup SK. Characterization of an enzyme-linked immunosorbent assay for soluble CD163. *Scand J Clin Lab Invest* 2002;62:293-299.
33. Heath ML, Kow L, Slavotinek JP, Valentine R, Toouli J, Thompson CH. Abdominal adiposity and liver fat content 3 and 12 months after gastric banding surgery. *Metabolism* 2009;58:753-758.
34. Busetto L, Tregnaghi A, De Marchi F, et al. Liver volume and visceral obesity in women with hepatic steatosis undergoing gastric banding. *Obes Res* 2002;10:408-411.
35. Harman-Boehm I, Bluher M, Redel H, et al. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *J Clin Endocrinol Metab* 2007;92:2240-2247.
36. Bauer S, Weiss TS, Wiest R, et al. Soluble CD163 is not increased in visceral fat and steatotic liver and is even suppressed by free fatty acids in vitro. *Exp Mol Pathol* 2011;91:733-739.
37. Michaud A, Drolet R, Noel S, Paris G, Tchernof A. Visceral fat accumulation is an indicator of adipose tissue macrophage infiltration in women. *Metabolism* 2012;61: 689-698.
38. Cancello R, Tordjman J, Poitou C, et al. Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. *Diabetes* 2006;55:1554-1561.
39. Cancello R, Henegar C, Viguerie N, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* 2005;54:2277-2286.
40. Aron-Wisnewsky J, Tordjman J, Poitou C, et al. Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. *J Clin Endocrinol Metab* 2009;94:4619-4623.