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No relation between sCD163 levels and insulin resistance in caloric restricted overweight women

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CD163 is a monocyte/macrophage membrane receptor involved in scavenging of haptoglobin-haemoglobin complexes. Its soluble form, sCD163, has been proposed as a biomarker of inflamed adipose tissue (AT) and development of type 2 diabetes mellitus (T2DM). We investigated changes in sCD163 levels before and following caloric restriction in overweight females. We hypothesized that changes in sCD163 would be associated with changes in BMI, AT mass, and insulin resistance (HOMA-IR) following caloric restriction.
Anthropometric (BMI, AT mass) and metabolic parameters (fasting glucose, insulin, HOMA-IR, sCD163) were measured in 40 overweight women before and after three months of caloric restriction to 33% of baseline intake. BMI, AT mass and sCD163 decreased following caloric restriction, whereas HOMA-IR improved. sCD163 levels nor changes in sCD163 levels were associated with (changes in) BMI, AT mass or HOMA-IR. We found no support for the concept that sCD163 is a direct marker of early T2DM in this study.

Keywords: obesity, inflammation, macrophages, diabetes mellitus

Introduction

Obesity is a highly prevalent disorder that predisposes to the development of insulin resistance (IR), type 2 diabetes mellitus (T2DM) and cardiovascular problems. Obesity is associated with chronic low grade inflammation of adipose tissue (AT) and other organs. With increasing adiposity, macrophages infiltrate the AT, leading to secretion of pro-inflammatory cytokines which in turn promote IR and development of T2DM, whereas weight loss improves insulin sensitivity and T2DM (1). CD163 is a monocyte/macrophage membrane receptor involved in scavenging of haptoglobin-haemoglobin complexes. Continuous shedding of the extracellular part of the receptor results in substantial amounts of a soluble form of the receptor, sCD163, in the plasma (2). Besides its role in removal of haemoglobin, CD163 participates in immune modulation by activating intracellular pathways leading to secretion of anti-inflammatory cytokines (3). In several case-control studies it has been shown that sCD163 levels are increased in obese subjects vs. healthy weight controls (4-7). sCD163 has been proposed as a strong predictor of developing T2DM and its metabolic complications (4), and sCD163 was found to be positively associated with insulin resistance, estimated by homeostasis model assessment (IR) (6,8). Until recently no data was available about within-subject changes in sCD163. We investigated changes in sCD163 levels before and following three months caloric restriction in a population of overweight females participating in a dietary intervention, and investigated whether changes in sCD163 were associated with changes in BMI, AT mass, and HOMA-IR. We hypothesized that caloric restriction would decrease circulating levels of sCD163 and that changes in serum sCD163 levels would be associated with changes in BMI, AT mass, and HOMA-IR adding evidence to the concept of sCD163 acting as a biomarker of inflamed AT and T2DM.

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Methods and Procedures

Subjects

The dietary intervention focussed on possible differences in effects of four isocaloric diets with differing caloric intake from macronutrients on body weight (BW) loss and BW maintenance (9). In short, 132 overweight, mostly obese, subjects between 18-88 years of age were included in the intervention. Subjects with a diagnosis of cancer, HIV, or psychiatric disease, subjects who were breastfeeding or pregnant, or subjects who lost more than 10% BW in the preceding six months were excluded. Subjects were allocated to one of four diets and following two weeks of run in, caloric intake was restricted to 33% for three months, followed by a nine-month maintenance period at 67% caloric intake. Subjects were seen every two weeks for dietary advice, coaching and anthropometric measurements, including estimated AT mass by impedance. Blood was drawn each month after an overnight fast and serum was stored in aliquots at -80°C. The Medical Ethics committee of the University Medical Centre Groningen approved the study. Written informed consent was obtained from all subjects. The present study was limited to 40 Caucasian female subjects who participated in the dietary intervention, all without a diagnosis

of T1DM or T2DM (4,8), and equally spread over the four dietary groups.

Laboratory measurements
sCD163 was determined in serum using a sandwich ELISA method (IQ products, Groningen, The Netherlands). Serum was diluted 500x and samples were run in duplicate (CV 6.7%). Washing and reading of the plate were performed using the BEP-2000 Advance Elisa analyser (Siemens Healthcare Diagnostics, The Hague, The Netherlands). C reactive Protein (CRP) was measured in serum by an immunoturbidometric method (Roche Diagnostics, Almere, The Netherlands). Glucose and insulin were assayed by a hexokinase photometric assay (Roche Diagnostics) and a solid phase sandwich chemiluminescence assay (Siemens Healthcare Diagnostics) as described before (9). Insulin resistance was estimated by the HOMA index (fasting insulin (mU/l) x fasting glucose (mmol/L)/22.5).

Statistics
Changes in sCD163, glucose, insulin, HOMA-IR, CRP, and anthropometric measures between start (T0) and following three months of caloric restriction (T3) were calculated and analysed using paired T-tests. T3 was chosen because the dietary intervention showed most weight loss at this time point (9). ANOVA analyses with Bonferroni post-hoc testing were performed to investigate differences between the four dietary groups. Data are reported as mean ± SD. Pearson correlation coefficients were calculated to investigate the association between (changes in) sCD163 and the above mentioned parameters. Normality of the data was assessed by Shapiro Wilcoxon tests and outliers were identified using the median absolute deviate method. The minimum level of significance used was $P < 0.05$ throughout.

Results

Subjects
Before starting caloric restriction two female subjects were heavily overweight (BMI 29) and 38 were obese (BMI≥30). None of the subjects were diagnosed with T1DM or T2DM and HOMA-IR varied between 1.3 and 3.9 (Table 1). Average age of the subjects was 51.7 ± 11.9 y. Metabolic parameters, anthropometric parameters and age were not significantly different between dietary groups (data not shown).

Changes in sCD163 and metabolic or anthropometric parameters following caloric restriction
Serum levels of sCD163 as well as BMI, AT mass, and hip- and waist circumference significantly decreased following three months of caloric restriction. Fasting glucose levels remained unchanged, but fasting insulin levels and HOMA-IR improved following caloric restriction. CRP levels tended to decrease over time (Table 1).

Associations between sCD163 and metabolic or anthropometric parameters
Serum sCD163 levels at T0 and T3 were not associated with HOMA-IR, fasting insulin levels, BMI, AT mass or CRP at T0 respectively T3. We only found an association between sCD163 and hip circumferance or waist circumferance at T0 respectively T3. Changes in sCD163 following three months of caloric restriction were also not related to changes in HOMA-IR and the above mentioned parameters (Table 2). Changes in

Table 1. Metabolic and anthropometric parameters before and after three months of caloric restriction.

Parameter	T0	T3	p value
Fasting glucose (mmol/l)	5.1 ± 0.4	5.0 ± 0.4	0.100
Insulin (mU/l)	11.5 ± 3.5	6.2 ± 2.0	p<0.001
HOMA-IR	2.6 ± 0.8	1.4 ± 0.5	p<0.001
sCD163 (mg/l)	3.82 ± 1.04	2.64 ± 0.80	p<0.001
CRP (mg/l)	4.2 ± 2.8	3.5 ± 2.6	0.061
BW (kg)	96.2 ± 11.1	84.7 ± 10.9	p<0.001
BMI (kg/m^2)	34.5 ± 3.0	30.3 ± 3.1	p<0.001
AT mass (kg)	46.7 ± 2.3	42.7 ± 3.0	p<0.001
Hip circumference (cm)	112.5 ± 7.5	104.0 ± 8.2	p<0.001
Waist circumference (cm)	107.1 ± 7.4	97.3 ± 6.3	p<0.001

Table 2. Association between sCD163 and metabolic and anthropometric parameters before and after three months of caloric restriction.

	T0 sCD163		T3 sCD163		Δ sCD163	
	r	p	r	p	r	p
Fasting glucose (mmol/l)	0.039	0.820	-0.004	0.981	-0.038	0.861
Insulin (mU/l)	-0.055	0.751	-0.125	0.509	-0.057	0.781
HOMA-IR	0.075	0.673	-0.266	0.155	0.082	0.704
CRP (mg/l)	-0.042	0.812	-0.162	0.401	0.026	0.904
BW (kg)	0.190	0.259	0.286	0.107	-0.186	0.342
BMI (kg/m^2)	0.221	0.210	0.134	0.472	0.021	0.922
AT mass (kg)	0.275	0.105	0.146	0.441	-0.207	0.320
Hip circumference (cm)	0.411	0.013	0.275	0.134	0.365	0.080
Waist circumference (cm)	0.260	0.126	0.380	0.035	-0.141	0.541

HOMA-IR correlated with changes in insulin (r=0.873, p<0.001) and BMI (r=0.459, p<0.016), but not with changes in sCD163 as noted above.

Discussion

This study shows that serum sCD163 decreases following caloric restriction in overweight women. Serum sCD163 levels were, however, not related to BMI, AT mass, fasting insulin, HOMA-IR, or changes in these parameters following caloric restriction. sCD163 levels significantly decreased following caloric restriction, whereas CRP levels did not. This shows that sCD163 is a specific macrophage marker, rather than a component of the general acute phase response. This is in agreement with data from Al-Daghri et al. (7), but others showed strong correlations between sCD163 and CRP in obese and normal weight subjects (6,8).

Møller et al. showed in a prospective cohort study that high levels of sCD163 were associated with a high risk of developing T2DM, independent of age and BMI (4). Then Parkner et al. showed that sCD163 levels were related to HOMA-IR, independent of glycaemic status, in subjects with T2DM, subjects with impaired glucose tolerance, and healthy controls (8). While we were writing our manuscript, Fjeldborg et al. (10) published a study on sCD163 levels in obese subjects following weight loss. In their study female and male obese subjects were included who were exposed to strong caloric restriction (600-800 kcal/d) for eight weeks followed by three-four weeks of weight stabilization. They found that serum sCD163 levels were increased in obese subjects (vs lean controls) and decreased with weight loss, similar to our results.

Reference values for serum sCD163 are not known. Møller et al. reported a median of 1.71 mg/l and a 34-66 percentile of 1.40-1.97 mg/l for Danish females aged 50-60 y using an inhouse sandwich ELISA method (4). Our serum sCD163 levels are defined "high-normal" in literature, are comparable to those reported by others in subjects with obesity, diabetes, or atherosclerosis (4,5,8,10), and lower than reported in subjects with acute liver failure (10 fold increase vs control, median 21 mg/l (range 3.6-74.9)) (11). In contrast to others we found no association between sCD163 and metabolic or anthropometric parameters (except for hip and waist circumferance) at baseline or following caloric restriction. Of course one may question the study population and the design of the study. Our subject group was homogenic; only Caucasian females were included with BMI ranging from 29.4-40.4 kg/m^2 and AT mass ranging from 42.1-52.4 kg. This might explain absence of associations between sCD163 and metabolic and anthropometric parameters. Because we aimed to investigate changes in early T2DM, we excluded patients with diagnosed DM. One may, however, argue whether glucose homeostasis was sufficiently disturbed in our population. At T0, 56% of our subjects had a HOMA-IR>2.5, indicative of IR. A subanalysis of these subjects did not reveal different results. Following caloric restriction HOMA-IR improved in 91% of all subjects, and no subject had HOMA-IR>2.5. We interpret this as a significant improvement of glucose homeostasis following 3 months of caloric restriction. The existence of different dietary groups could be pointed out as a flaw of the design, however, we found no evidence for any dietary group effect and therefore exclude this factor as a possible limitation of the study. The lack of association between sCD163 and amongst others HOMA-IR may also be explained by different kinetics of changes in these parameters. The kinetics of changes in CD163 and sCD163 in situations of weight gain or weight loss are largely unknown. Furthermore it is also not known whether a reduction in sCD163 is the result of a decrease in number of macrophages in AT or a phenotypic switch of these macrophages. Kováčiková et al. showed that changes in BMI, AT mass and HOMA-IR were already observed following one month of caloric restriction in obese women, whereas changes in macrophage content and macrophage gene expression occurred much later, following months of weight maintenance (12). It is also possible that the relation between sCD163 and HOMA-IR depends on the state of BW, e.g. in a situation of stable weight sCD163 and HOMA-IR may be associated, but during or following situations of dynamic weight change this relation may disappear. Indeed Fjeldborg et al. recently reported an association between sCD163 and HOMA-IR at baseline, but not following 8 weeks of weight loss nor following 3-4 weeks of additional weight stabilization (10). Their data suggest that sCD163 is not the key marker of changes in HOMA-IR during weight loss as hypothesized before. Our data confirm these findings. In conclusion, we found no evidence for a direct association between changes in sCD163 and changes in BMI, AT mass and HOMA-IR in a population of overweight women following a dietary program to loose weight. Thus we found no support for the concept that sCD163 is a direct marker of HOMA-IR or early T2DM in our population. sCD163 is hereby not yet excluded as a marker of glucose homeostasis, e.g. sCD163 could be an indirect mediator in glucose homeostasis rather than a direct marker of HOMA-IR.

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Samenvatting

Hillebrand JJG, van Berkum FNR, Mulder AHL. Geen relatie tussen sCD163 concentraties en insuline resistance in calorisch beperkte vrouwen met overgewicht. Ned Tijdschr Klin Chem Geneesk. 2014;39:182-185

sCD163 wordt sinds een aantal jaren genoemd als mogelijke marker van ontstoken vetweefsel en de ontwikkeling van type 2 diabetes mellitus (T2DM). Wij bestudeerden concentraties van sCD163 in vrouwen met overgewicht voorafgaand en na afloop van een periode van calorische beperking. Onze hypothese was dat calorische beperking zou leiden tot veranderingen in concentraties van sCD163 welke geassocieerd zouden zijn met veranderingen in BMI, vetmassa en insuline resistentie (HOMA-IR). antropometrisch (BMI, vetmassa) en metabole parameters (gevast glucose, insuline, HOMA-IR, sCD163) werden gemeten in 40 vrouwen met overgewicht voorafgaand aan en drie maanden na het volgen van het dieet (33EN%). BMI, vetmassa en sCD163 concentratie daalden als gevolg van calorische beperking en de HOMA-IR verbeterde. sCD163 en veranderingen in sCD163 concentraties waren echter niet geassocieerd met (veranderingen in) BMI, vetmassa en HOMA-IR. Wij concluderen op basis van deze data dat er geen bewijs is voor het concept dat sCD163 een directe marker is voor vroege T2DM.

Trefwoorden: obesitas, ontsteking, macrofagen, diabetes mellitus

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Biomarkers of excessive alcohol intake in alcohol addicts with a normal nutritional status

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Introduction: **Biomarkers of excessive alcohol intake are generally measured for diagnosis and follow-up of alcohol dependency. However the micronutrient status might influence the biomarker results, especially for MCV. We studied the relationship of commonly used biomarkers with the alcohol intake in the absence of a (micro)nutrient deficiency.**
Methods: **Blood samples were taken from 70 patients (39 males, 31 females) at the start of the clinical period of an alcohol abstinence program. Vitamin B12, folic acid and albumin were used as markers for the nutritional state; γ-GT, ASAT, ALAT, MCV as well as CDT were measured as biomarkers for alcohol use. According to their self reported alcohol intake of the last month (MATE interview) patients were divided into three groups with normal, high (men 4-6 units/d, women 3-4 units/d) and excessive intake.**
Results: **No significant increase in MCV was found in the three groups. Only in excessive users of alcohol, a significant increase in the liver parameters was observed both in men and women. CDT showed a significant increase with alcohol intake especially in men. Deficiencies in albumin, vitamin B12 and folic acid were hardly present and levels of CDT were not related to the levels of the nutritional parameters.**
Conclusions: **In individuals with a normal nutritional status, CDT is the biochemical parameter most related to the reported intensity of drinking for men. ASAT, ALAT and γGT reflect liver toxicity rather than alcohol abuse and MCV increase is probably more related to vitamin and nutritional status than to excessive drinking.**

Keywords: alcohol biomarkers, CDT, MCV, nutrition, vitamin B12, folic acid

Chronic excessive alcohol intake has been associated with a number of hematological and biochemical changes in the blood (1). These changes include an increase in the mean cellular volume (MCV) of erythrocytes and a raise of the enzymes aspartate aminotransferase (ASAT), alanin aminotransferase (ALAT) and γ-glutamyl transpeptidase (γGT). Two decades ago carbohydrate deficient transferrin (CDT)

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