

Short Communication

Monocytic expression of CD14 and CD18, circulating adhesion molecules and inflammatory markers in women with diabetes mellitus and impaired glucose tolerance

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Abstract

Aims/hypothesis. Type 2 diabetes is a major risk factor for cardiovascular disease. Monocyte recruitment and inflammatory activation are crucial steps in the development of atherosclerosis and several receptors are involved in these processes. The aim of this study was to investigate levels of CD14 and the β_2 -integrin subunits CD11b and CD18 on monocytes from women with diabetes or impaired glucose tolerance.

Methods. A population-based sample of 112 Swedish women, who were aged 64 years and had diabetes mellitus or impaired or normal glucose tolerance, was investigated. Cell surface receptors were analysed with flow cytometry and serum inflammation markers and soluble adhesion molecules with enzyme-linked methods.

Results. The monocytic CD14 expression and serum levels of C-reactive protein, IL-6 and soluble adhesion molecules were higher in the diabetes group than in the group with normal glucose tolerance. Monocytic CD18 was elevated both in the diabetes and in the impaired glucose tolerance groups. The levels of monocytic surface markers correlated with BMI and to a lesser extent with glycaemic control.

Conclusions/interpretation. The increased monocytic expression of important surface receptors together with elevated serum inflammation markers supports the concept of increased inflammation in type 2 diabetes and may be an important factor for the risk of atherosclerosis.

Keywords Adhesion molecule · Atherosclerosis · CD14 · CD18 · Diabetes · Integrin · Monocyte

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Abbreviations: CRP, C-reactive protein · CVD, cardiovascular disease · HRT, hormone replacement therapy · ICAM, intercellular adhesion molecule · LPS, lipopolysaccharide · VCAM, vascular cell adhesion molecule

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Introduction

Type 2 diabetes is a major risk factor for cardiovascular disease (CVD). Monocyte recruitment is a crucial step in the development of atherosclerosis [1]. The entering of circulating monocytes into the subendothelial space involves initial rolling followed by firm adhesion and subsequent transmigration through the endothelium and is largely dependent on the interaction of monocytic β_2 -integrins and intercellular adhesion molecule (ICAM) [2]. Another important monocytic surface antigen possibly implicated in the recruitment of monocytes is CD14, a key molecule involved in innate immunity. Lipopolysaccharide (LPS) ligation of CD14 initiates an intracellular signal ultimately leading to the secretion of pro-inflammatory cytokines. Ligation of CD14 with LPS or ceramide leads to clustering of CD14 with other receptors involved in ath-

Table 1. Clinical and laboratory data of the study groups

Characteristic ^a	Diabetes (n=30)	IGT (n=28)	NGT (n=54)
BMI (kg/m ²)	30.4 (28.3–32.5)*	26.2 (24.9–27.4)	24.7 (23.9–25.6)
Weight (kg)	78.6 (74.0–83.2)*	70.0 (66.3–73.7)	66.7 (64.2–69.2)
Waist circumference (cm)	99.8 (95.8–103.8)*	91.6 (87.9–95.4)*	84.4 (82.0–86.8)
WHR	0.91 (0.89–0.93)*	0.88 (0.86–0.90)*	0.84 (0.83–0.85)
SBP (mm Hg)	151 (143–159)*	141 (133–149)	133 (129–138)
DBP (mm Hg)	81 (78–84)	79 (75–83)	77 (74–79)
S-cholesterol (mmol/l)	5.74 (5.28–6.20)	6.58 (6.06–7.09)	5.96 (5.65–6.28)
S-triglycerides (mmol/l)	1.67 (1.30–2.03)*	1.38 (1.10–1.65)	1.14 (1.01–1.26)
LDL-cholesterol (mmol/l)	3.42 (3.03–3.81)	4.25 (3.78–4.72)	3.64 (3.37–3.90)
HDL-cholesterol (mmol/l)	1.50 (1.34–1.66)*	1.70 (1.56–1.85)	1.88 (1.76–1.99)
HbA _{1c} (%)	5.60 (4.30–11.8)*	4.50 (3.40–5.10)	4.45 (3.60–5.00)
FB-glucose (mmol/l)	7.47 (6.36–8.58)*	4.88 (4.64–5.11)*	4.48 (4.33–4.62)
P-insulin (pmol/l)	53.0 (20.6–155.0)*	32.5 (11.6–98.6)	25.9 (11.5–79.2)
Leucocyte count (×10 ⁹ /l)	5.80 (5.27–6.32)	5.76 (5.08–6.43)	5.63 (5.28–5.99)
Smokers (n/%)	14/46.7	4/14.3	10/18.5 ^b
Diabetes duration ^c (years)	2 (0–34)	0 (0–0)	0 (0–0)
Medication (n/%)			
Diet	15/50	0/0	0/0
Metformin	4/13.3	0/0	0/0
SU ^d	1/3.3	0/0	0/0
Insulin	4/13.3	0/0	0/0
Metformin + SU ^d	3/10	0/0	0/0
Metformin + other ^e	2/6.7	0/0	0/0
Metformin + Insulin	1/3.3	0/0	0/0
Statin	7/23.3 ^b	1/3.6	0/0
HRT ^f	5/16.7	5/17.9	15/27.8

^a Data are shown as means (95% CI) except for HbA_{1c}, P-insulin and diabetes duration, for which medians (ranges) are shown. ^b $p < 0.01$ in chi square test; ^c duration of diabetes from diagnosis; ^d sulphonylurea; ^e pioglitazone or repaglinide; ^f sys-

temic hormone replacement therapy; * $p < 0.05$ vs NGT. SBP, systolic blood pressure; DBP, diastolic blood pressure; S, serum; FB, fasting blood; P, plasma

erogenesis, such as CD11b/CD18 and scavenger receptor CD36 [3].

A changed phenotype of monocytes may be one component of a state with an increased tendency to develop atherosclerosis and it may be mediated through vascular monocyte recruitment and increased cellular inflammatory activation. The aim of the present study was to investigate if women with type 2 diabetes or IGT have abnormal levels of CD14 and the β_2 -integrin subunits CD11b and CD18 on their monocytes.

Subjects and methods

Subjects. We selected 64-year-old women with NGT (blood glucose at 2 h after 75 g of oral glucose < 7.8 mmol/l, $n=54$), IGT (blood glucose 7.8–11.1 mmol/l, $n=28$) and diabetes (blood glucose > 11.1 mmol/l, $n=30$) from a newly completed population study in Göteborg, Sweden (DIWA, Diabetes and Impaired glucose tolerance in Women and Atherosclerosis, $n=650$). Exclusion criteria were clinical CVD, chronic inflammatory disease, anti-inflammatory treatment, ongoing infection, or C-reactive protein (CRP) greater than 10 mg/l. The study was approved by the Research Ethics Committee at Sahlgrenska University Hospital and informed consent was obtained from all study subjects. All analyses were obtained after

an overnight fast. The clinical and laboratory data of the study groups are shown in Table 1.

Laboratory analyses. Capillary blood glucose was measured using HemoCue Glucose 201 (HemoCue, Angelholm, Sweden), plasma insulin using the AutoDelfia Immunoassay system (Perkin Elmer Life Sciences, Boston, Mass., USA) and leucocyte count using flow cytometric particle counting (Cell-Dyn 4000; Abbott Laboratories, Abbott Park, Ill., USA). We determined HbA_{1c} with HPLC on a Mono S HR 5/5 column (Amersham Biosciences, Piscataway, N.J., USA), and assessed high sensitive CRP, triglycerides and cholesterol (total and HDL) enzymatically in serum in a Konelab 20 Autoanalyzer (Thermo Clinical Labsystems, Vantaa, Finland). Enzyme-linked immunosorbent assays were used for the analysis of IL-6, ICAM-1, vascular cell adhesion molecule (VCAM)-1 and E-selectin (R&D Systems, Minneapolis, Minn., USA).

Flow cytometry. Mononuclear cells were isolated using density gradient centrifugation and stained with either phycoerythrin-conjugated anti-CD14 antibody, clone MΦP9, and fluorescein-isothiocyanate-conjugated anti-CD16 antibody, clone NKP15, or phycoerythrin-conjugated anti-CD11b antibody, clone D12, and fluorescein-isothiocyanate-conjugated anti-CD18 antibody, clone L130, together with peridinin-chlorophyll-protein-conjugated anti-CD45 antibody, clone 2D1 (Becton Dickinson Biosciences, San Jose, Calif., USA). Monocytes ($n=5000$) were analysed using a FACScan flow cytometer (Becton Dickinson) with CellQuest Software (Becton Dickinson) and

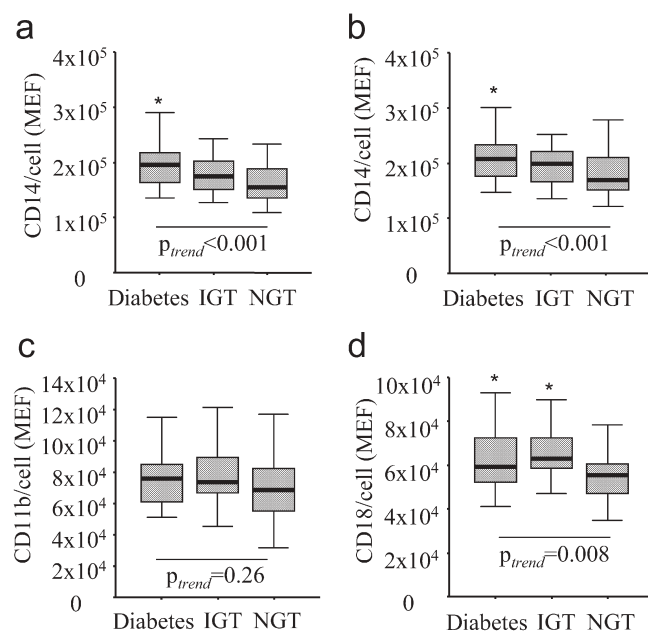


Fig. 1. Box plots of expression levels of (a) CD14 in the total monocyte population, (b) CD14 in the CD14⁺⁺CD16⁻ monocyte population, (c) β_2 -integrin subunits CD11b and (d) CD18 on monocytes from women with diabetes, IGT and NGT. MEF, molecules of equivalent fluorochrome. * $p < 0.05$ vs NGT; $p_{\text{trend}} = p$ value for linear association as tested with chi square test

Table 2. Correlations between CD14, CD11b, CD18 and anthropometric data, glucose metabolism measures and serum lipids

Variable	Spearman's correlation		
	CD14	CD11b	CD18
BMI	0.35***	0.1	0.32***
Waist circ.	0.33***	0.11	0.36***
WHR	0.23*	0.041	0.25**
FB-glucose	0.21*	0.048	0.038
HbA _{1c}	0.25*	0.082	0.05
P-insulin	0.26**	0.076	0.18
S-cholesterol	0.02	0.08	0.18
S-triglycerides	0.18	0.03	0.20*
HDL-cholesterol	-0.13	-0.08	-0.20*
LDL-cholesterol	0.00	0.09	0.21*

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. circ, circumference; FB, fasting blood; P, plasma; S, serum

gated on the basis of forward and side-angle scatter. The populations of CD14⁺⁺CD16⁻ and CD14⁺CD16⁺ cells were defined as described [4]. Median fluorescence of CD11b, CD14 and CD18 was measured in gated cells positive for CD45 and arbitrary units of fluorescence were converted to molecules of equivalent fluorochrome values by construction of a calibration curve with DAKO Fluorospheres (DAKO, Glostrup, Denmark). The day-to-day variance in the instrument was avoided by constructing calibration curves on every analysis occasion.

Statistical analyses. Using the SPSS 11.0 software package for Windows data were analysed with ANOVA using the Bonferroni or Dunnett T3 post hoc tests as appropriate, as well as Spearman's rank correlation and chi square tests. The effects of smoking and medication were analysed with multiple regression analysis.

Results

Monocytic expression of CD14, CD11b and CD18 is shown in Figure 1. CD14 expression was higher in the diabetes group than in the NGT group, as was CD18. The latter was also elevated in the IGT group. The sizes of the CD14⁺CD16⁺ and the CD14⁺⁺CD16⁻ populations did not differ between the groups (data not shown). According to multiple regression analyses, smoking or medication with statins or hormone replacement therapy (HRT) did not influence the results for CD14 nor CD18 (data not shown).

Correlations between anthropometric data, measures of glucose metabolism, serum lipids and monocytic receptors are shown in Table 2. Due to a relatively strong co-variability the present study lacked the power to separate the effect of glucose tolerance states (diabetes, IGT, NGT) from BMI on CD14 ($r_s = 0.44$, $p < 0.001$ for BMI-glucose tolerance state). Different receptors were associated as follows: CD11b-CD18: $r_s = 0.72$, $p < 0.001$; CD14-CD11b: $r_s = 0.63$, $p < 0.001$; and CD14-CD18, $r_s = 0.66$, $p < 0.001$.

The levels of CRP, IL-6 and soluble adhesion molecules ICAM-1, VCAM-1 and E-selectin were elevated in the diabetes group (Table 3). According to multiple regression analyses (data not shown), smoking or medication with statins or HRT did not affect the results for any of these molecules. C-reactive pro-

Table 3. Serum levels of C-reactive protein, IL-6, intercellular and vascular cell adhesion molecule and E-selectin in the study groups

Variable ^a	Diabetes (n=30)	IGT (n=28)	NGT (n=54)	p_{trend}^b
CRP (mg/l)	2.1 (0.3–7.3)*	1.3 (0.2–6.7)	0.7 (0.2–9.9)	0.013
IL-6 (ng/l)	2.2 (0.8–8.2)*	2.3 (0.7–5.9)	1.4 (0.6–6.0)	<0.001
ICAM-1 (μ g/l)	241 (152–693)*	220 (135–413)	209 (133–441)	0.011
VCAM-1 (μ g/l)	643 (454–1610)*	610 (439–877)*	527 (308–1126)	<0.001
E-selectin (μ g/l)	57 (28–151)*	38 (22–70)	39 (16–81)	<0.001

^a Data are shown as medians (ranges). ^b $p_{\text{trend}} = p$ value for linear association as tested with chi square test. * $p < 0.05$ vs NGT. CRP, C-reactive protein; ICAM, intercellular adhesion molecule; VCAM, vascular cell adhesion molecule

tein and IL-6 correlated with CD14 (CRP-CD14: $r_s=0.27$, $p=0.004$; IL-6-CD14: $r_s=0.31$, $p=0.001$). A significant correlation with CD18 was found for CRP but not for IL-6 (CRP-CD18: $r_s=0.28$, $p=0.003$; IL-6-CD18: $r_s=0.18$, $p=0.061$). The only significant association between integrin subunits and soluble adhesion molecules was a weak correlation between CD18 and E-selectin ($r_s=0.21$, $p=0.027$).

Discussion

The results showed that, in comparison to healthy control subjects, women with diabetes had higher monocytic expression of CD14 and CD18, and higher circulating concentrations of both inflammatory markers and adhesion molecules. Subjects with IGT generally had values between those of subjects with diabetes and NGT. After the exclusion of two individuals with elevated GAD-antibodies, all results on monocytic receptors, including associations with other variables, remained valid. We therefore think that the results reported here are valid for type 2 diabetes.

The results for serum inflammation markers confirm previous studies (reviewed in [5]). The new aspect of our observations is that we included women with IGT, a stage in the development of diabetes. In addition, the serum analyses were combined with analyses of circulating monocytes. Our results on these cells strengthen existing ideas that inflammation is increased in patients with type 2 diabetes. A combination of up-regulated monocytic receptors involved in adhesion and an activation of the endothelium strongly suggests the presence of an increased potential for cellular recruitment to the arterial wall. Accordingly, monocytes from type 2 diabetic patients have increased adhesion *in vitro* [6] and the level of macrophages in atherosclerotic lesions from diabetic individuals is increased compared with non-diabetic lesions [7]. The elevation of CD14 in diabetic patients confirms a previous report [8]. CD18 was elevated both in subjects with IGT and in subjects with diabetes. This early elevation of CD18 and graded increase in other factors already present in subjects with IGT may be of importance for the development of atherosclerosis, since the increased risk of CVD is seen very early in type 2 diabetes.

The strongest covariate to CD14 as well as CD18 expression was BMI. It is possible that monocytic receptor expression is influenced by substances secreted from adipose tissue, such as leptin, adiponectin, fatty acids or IL-6. The strong relationship between obesity and expression of proteins involved in adhesion may also be one mechanism involved in the recently discovered high abundance of inflammatory active macrophages in adipose tissue from obese individuals [9]. Body composition is closely associated with the glucose tolerance state and a much larger study is needed

to specify the separate effect of BMI on CD14 and CD18 expression. Importantly, our population of diabetic subjects was in general well controlled and had had diabetes for only a short time. The relation with diabetic state may be different in a population with more severe diabetes.

Our study population comprised only post-menopausal women, and results may be different in younger women. On the other hand, both type 2 diabetes and CVD are diseases of the elderly and are rarely evident in women before the menopause. A substantial proportion of the women in this study were on HRT, but according to our analyses, this did not influence the results. Also, when women with HRT were excluded from the analyses, the results were unchanged except for a minor increase in the correlation between CD18 and triglycerides (data not shown). Treatment with metformin/sulphonylurea also had no influence on the results (data not shown). Data in the literature on the effect of these substances on monocytic receptor expression are scarce. Statin treatment was most common in the diabetes group and these substances have been shown to decrease the expression of CD14 on monocytes [10]. In the event that this influenced the results in this study, it could have been by reducing the differences.

In conclusion, the present study showed that expression of important surface receptors on circulating monocytes in women with diabetes or IGT was increased and that this expression was largely dependent on body composition. These abnormalities support the idea that increased inflammation is common in type 2 diabetes. They may therefore be important factors for the risk of atherosclerosis.

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