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Glucose-dependent leukocyte activation in patients with type 2 diabetes mellitus, familial combined hyperlipidemia and healthy controls

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ABSTRACT

Background. Leukocyte activation has been associated with vascular complications in type 2 diabetes mellitus (T2DM). Hyperglycemia may be involved in this leukocyte activation. Our aim was to investigate the role of elevated glucose concentrations on leukocyte activation in patients with a wide range of insulin sensitivity.

Methods. Leukocyte activation was determined after ingestion of 75 gram glucose in subjects with T2DM, familial combined hyperlipidemia (FCH) and healthy controls. Leukocyte activation markers were measured by flow cytometry. Postprandial changes were calculated as the area under the curve (AUC), and the incremental area under the curve corrected for baseline values (dAUC).

Results. 51 subjects (20 T2DM, 17 FCH and 14 controls) were included. Fasting neutrophil CD66b expression and CD66b-AUC were respectively 36% and 39% higher in T2DM patients than in controls ($p = 0.004$ and $p = 0.003$). Fasting neutrophil CD66b expression correlated positively with glucose-AUC (Spearman's rho 0.481, $p < 0.001$) and HbA1c (rho 0.433, $p = 0.002$). Although fasting monocyte CD11b expression was not significantly different between subjects, monocyte CD11b-AUC was 26% higher in T2DM than in controls ($p = 0.006$). Similar trends were observed for FCH patients. Monocyte CD11b-dAUC correlated positively with glucose-AUC (rho 0.322, $p = 0.022$) and HbA1c (rho 0.319, $p = 0.023$).

Conclusions. These data suggest that both acute and chronic hyperglycemia, associated with insulin resistance as seen in T2DM and FCH, are involved in the increased fasting and postprandial leukocyte activation observed in these conditions.

Abbreviations: AUC, area under the curve; dAUC, incremental integrated area under the curve; FCH, familial combined hyperlipidemia; OGTT, oral glucose tolerance test; T2DM, type 2 diabetes mellitus.

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1. Introduction

Inflammation is important in the development of diabetic complications. Increased leukocyte activation has been established in patients with insulin resistance and type 2 diabetes mellitus (T2DM) [1,2]. This leukocyte activation has been linked to microvascular diabetic complications and atherosclerosis [3–5]. Activated leukocytes are able to adhere to the intact endothelium, and can migrate to the subendothelial space, where the development of atherosclerosis starts [6].

Leukocyte activation can be determined by quantitation of cell surface integrins like CD11b and CD66b. CD11b, or Mac-1, is involved in early adhesion of monocytes and neutrophils to the endothelium. CD66b, or CEACAM8, is a marker of neutrophil degranulation [7].

Postprandial studies have shown that leukocytes are activated by lipids [8–10]. In addition, several studies have demonstrated the effect of glucose on leukocyte activation. An *in vivo* study showed a rapid increase in monocyte CD11b expression following the ingestion of glucose in T2DM patients and in healthy controls [11]. In a different study, administration of glucose during 15 days induced leukocyte activation in non-diabetic volunteers [12].

However, little is known about leukocyte activation in non-diabetic insulin resistant conditions. The aim of this study was to investigate the role of acute and chronic glycemia in leukocyte activation in patients with a wide range of insulin sensitivity, such as T2DM and familial combined hyperlipidemia (FCH) [13] and in healthy controls.

2. Materials and Methods

Subjects visiting our Department of Vascular Medicine, who met the diagnostic criteria for T2DM or FCH, were asked to undergo an oral glucose tolerance test (OGTT). Healthy volunteers were recruited by advertisement. T2DM was defined using the diagnostic criteria of the World Health Organization [14]. FCH was defined as familial hyperlipidemia with a dominant inheritance pattern, elevated plasma apolipoprotein B concentrations (>1.2 g/L) and elevated fasting triglyceride levels (>1.7 mmol/L) [13]. Exclusion criteria were the presence of inflammatory disorders, a plasma C-reactive protein level above 10 mg/L and disorders of kidney, liver and thyroid function.

In order to investigate the effect of statins on leukocyte activation, a second group of subjects was selected. The study design of this statin withdrawal substudy has been published elsewhere [15].

The studies were approved by The Institutional Review Board of the Sint Franciscus Gasthuis in Rotterdam and the Regional Independent Medical Ethical Committee at the Maasstad Hospital in Rotterdam (registered at clinicaltrials.gov under clinical trial numbers NCT02130505 and NCT01634906). All participants gave written informed consent.

Blood samples were obtained fasting, and 1 and 2 hours after ingestion of 75 grams of oral anhydrous glucose. Blood was drawn from a peripheral vein of the forearm. For leukocyte activation markers, blood samples were obtained in sodium EDTA (2 mg/mL).

All clinical chemistry measurements were performed as described previously, according to standard procedures in our laboratory [10]. The cell surface expression of CD11b and CD66b on monocytes and neutrophils was determined by flow cytometry, as described previously [10]. The fluorescence intensity of each cell was expressed as the mean fluorescence intensity (MFI), given in arbitrary units (au).

Data are given as mean ± SEM in the text, table and figure. The total area under the curve (AUC) was calculated by the trapezoidal rule using Graphpad Prism version 5.0 (LA, USA). The incremental integrated AUC (dAUC) was calculated after correction for baseline values. Differences were tested by analysis of variance (ANOVA), with Fisher's least significant difference (LSD) test as post-hoc analysis, or chi-square test for dichotomous variables. Correlation analysis was carried out using Spearman correlation statistics. P-values <0.05 (2-tailed) were considered statistically significant.

3. Results

A total of 51 subjects (20 T2DM patients, 17 FCH patients and 14 healthy volunteers) underwent an OGTT. Their baseline characteristics are listed in Table 1.

T2DM patients had a higher fasting plasma glucose level than FCH patients and healthy controls. Glucose levels increased postprandially in T2DM patients. In FCH patients, glucose increased only slightly. In healthy controls, glucose levels were not different from fasting measurements at 1 and 2 hours postprandially (Fig. 1A and Table 1).

Fasting neutrophil CD66b expression in T2DM was 36% higher than in controls and 27% higher than in FCH ($p = 0.006$, Fig. 1B). Fasting CD11b expression on monocytes or neutrophils was not different between the 3 groups (Fig. 1C and D).

Postprandial leukocyte activation was highest in T2DM patients. Monocyte CD11b expression decreased postprandially in all groups, but the decrease was smallest in T2DM patients. In this group, monocyte CD11b-AUC was 26% higher than in controls ($p = 0.022$, Table 1). Neutrophil CD66b-AUC in T2DM was 39% higher than in controls and 24% higher than in FCH ($p = 0.008$).

For the total group, fasting neutrophil CD66b expression correlated positively with glucose-AUC (Spearman's rho: 0.481, $p < 0.001$) and HbA1c (rho: 0.433, $p = 0.002$). Postprandial changes in monocyte CD11b, reflected by the dAUC, correlated positively with glucose-AUC (rho 0.322, $p = 0.022$) and HbA1c (rho 0.319, $p = 0.023$). No correlations were found between fasting monocyte CD11b expression, or neutrophil CD66b-dAUC, with measurements of acute or chronic glycemia.

In a separate statin withdrawal cohort, 54 subjects were included. Their baseline characteristics have been published elsewhere [15]. The statins used were simvastatin (n = 21), atorvastatin (n = 6), rosuvastatin (n = 21) and pravastatin (n = 6). Discontinuation of statins for 6 weeks did not result in significant changes in fasting leukocyte activation. Monocyte CD11b expression was 30.53 ± 1.03 au before and 28.59 ± 0.81 au after statin withdrawal ($p = 0.108$). Neutrophil CD11b expression was 43.81 ± 1.64 au before and 43.83 ± 1.69 au after statin withdrawal ($p = 0.910$). Neutrophil CD66b expression was 6.14 ± 0.19 au before and 6.06 ± 0.21 au after statin withdrawal ($p = 0.977$).