Adipose Tissue Metabolism and CD11b Expression on Monocytes in Obese Hypertensives

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Abstract—At a given degree of adiposity, metabolic and cardiovascular risk varies markedly between individuals. Animal studies suggest that differentially expressed systemic activation of monocytes contributes to the obesity-associated risk variability. We tested the hypothesis that systemic monocyte activation is associated with changes in adipose tissue and skeletal muscle metabolism. In 17 obese hypertensive patients, we assessed CD11b expression on circulating monocytes, gene expression in adipose tissue biopsies, and obtained blood samples and adipose tissue and skeletal muscle microdialysis samples in the fasted state and during a glucose load. Patients were stratified into groups with higher and lower CD11b expression on monocytes. Expression of the macrophage marker CD68 was increased markedly in adipose tissue of subjects with higher CD11b expression. Although no differences in systemic insulin sensitivity were found between both groups, patients with higher peripheral CD11b expression showed a markedly augmented increase in dialysate glucose in adipose tissue during oral glucose tolerance testing and increased adipose tissue lipolysis as well. Our data demonstrate that human monocyte activation is associated with tissue-specific changes in glucose and lipid metabolism. These findings may be explained in part by monocyte/macrophage infiltration of adipose tissue, which appears to interfere with insulin responsiveness. (Hypertension. 2005;46:130-136.)

Key Words: adipose tissue • macrophages • gene expression

Obesity is a major risk factor for metabolic and cardiovascular disease; however, the risk varies markedly between individuals. In susceptible subjects, a moderate increase in adiposity may dramatically raise cardiovascular and metabolic risk, whereas others may not experience complications with an even more severe degree of adiposity. A differentially expressed systemic inflammatory response to the development of obesity may contribute to this risk variability. Systemic inflammation may induce insulin resistance and predispose to diabetes mellitus. Systemic C-reactive protein and interleukin-6 (IL-6) concentrations are increased with high body weight. IL-6 stimulates adipocyte expression of chemotactic factors, such as the monocyte chemoattractant protein-1 (MCP-1). Circulating mononuclear cells are also activated in obese compared with lean subjects. In contrast, anti-inflammatory molecules such as adiponectin decrease as adiposity and inflammatory cytokines increase. Animal studies suggest that the imbalance between proinflammatory and anti-inflammatory molecules may be caused or sustained by monocyte/macrophage adipose tissue infiltration (“adipositis”) and that monocyte infiltration precedes systemic hyperinsulinemia. CD11b is an established marker of monocyte activation and plays an important role in monocyte adhesion to the vascular wall. Adhesion is an early step in tissue infiltration. Adipocyte MCP-1 secretion may be one of the signals causing monocyte activation and infiltration. Whether or not similar mechanisms operate in humans is unknown. However, a recent study demonstrated increased expression of MCP-1 and a macrophage marker (CD68) in adipose tissue of obese subjects. We tested the hypothesis that increased CD11b expression on systemic monocytes in obese hypertensive patients is associated with changes in adipose and skeletal muscle tissue metabolism. Given the central role of adipose tissue in obesity-associated inflammation, we expected a predominant change in adipose tissue metabolism.

Methods

The Charité Campus Buch institutional review board approved the study, and previous written informed consent was obtained from all participants. We recruited 17 obese, nondiabetic, hypertensive patients (see Table 1). No patients with secondary causes of hypertension or obesity were included. All antihypertensive and other concomitant medication was withdrawn 2 weeks before testing. No lipid-lowering drugs were allowed for ≥6 months before study entry.

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All specimens were obtained from patients on the same day after an overnight fast.

One catheter was placed in a large antecubital vein for blood sampling. One microdialysis probe was inserted into abdominal subcutaneous adipose tissue. Another microdialysis probe was inserted into skeletal muscle (quadriceps femoris, vastus lateralis) as described previously. Changes in blood flow were determined using the ethanol dilution technique, which is based on Fick’s principle. A decrease in the outflow/infusion ratio (“ethanol ratio”) is equivalent to an increase in blood flow and vice versa. Changes in glucose and lactate were used to assess changes in lipolysis or lipid mobilization, and changes in glucose and lactate were used to assess changes in carbohydrate metabolism. In situ recovery for glycerol, glucose, and lactate in the microdialysate was assessed by near-equilibrium dialysis. For all 4 metabolites, we found recoveries of ∼30% in adipose tissue and 50% in muscle.

A total of 100 μL of whole blood was transferred into polypropylene tubes within 2 hours after collection. Cells were stained with fluorescent isothiocyanate–conjugated monoclonal antibodies against CD11b and isotype controls (ImmunoTech). Blood samples were hemolysed, fixed, and washed twice (fluorescence-activated cell sorter [FACS] lysing solution and Cell Wash; Becton Dickinson). Flow cytometry was performed using a FACScan (Becton Dickinson, Heidelberg, Germany), and 10,000 events per sample were collected. Data were analyzed using CellQuest software. Subsets of white blood cells were gated in the light-scatter diagram and displayed as single-color histograms. Mean fluorescence channels were determined.

After completion of the metabolic evaluation, we obtained abdominal subcutaneous adipose tissue samples (0.5 to 1.0 g) by needle biopsy from the periumbilical region. Specimens were washed twice in 0.9% NaCl and separated from blood cells and blood clots by centrifugation at room temperature for 5 minutes at 200g as described previously. Adipose samples were then snap-frozen in liquid nitrogen. Total RNA was isolated from adipose tissue by the Qiagen RNeasy Mini Kit (including the RNase-free DNase set; Qiagen), followed by determination of quality and quantity with the Agilent 2100 Bioanalyzer and the RNA 6000 Nano Chip (Agilent Technologies). Two μg total RNA were reverse-transcribed in 20 μL final volume for 1 hour at 37°C using 100 U Superscript Reverse Transcriptase, 5.4 μg random primer, 0.5 mmol/L deoxyribonucleotidetriphosphate, 10 mmol/L dithiothreitol, and 1×RT buffer (all reagents by Invitrogen). Relative quantitation of gene expression was performed with the ABI 5700 sequence detection system for reverse-transcriptase polymerase chain reaction (PCR) using the standard curve method. Human GAPDH was chosen as the endogenous control (“housekeeping gene”) to normalize gene expression data (presented in arbitrary units). PCRs were performed with the TaqMan Universal Master Mix and the TaqMan assay reagent for GAPDH in a total volume of 25 μL. Primers and fluorescently labeled probes for CD14, CD68, MCP-1, and MCP-1:stimulating factor-1 (M-CSF1) were “assays-on-demand” (TaqMan technology; Applied Biosystems). The 2-step PCR conditions were 2 minutes at 50°C, 10 minutes at 95°C, 45 cycles with 15 s at 95°C, and 1 minute at 62°C. All primer pairs were spanning exon–intron boundaries. Interassay coefficients of variation were 1.9% for GAPDH, 1.1% for CD14, 2.4% for CD68, 2.0% for MCP-1, and 1.8% for M-CSF1.
All data are expressed as mean±SEM. Group differences were compared by unpaired t test (parametric data) or the Mann–Whitney test (nonparametric data). Two-way ANOVA was used for multiple comparisons followed by Bonferroni’s post hoc test. If necessary, data were logarithmically transformed before analysis. The relationship between variables was assessed with linear regression analysis. A P value <0.05 indicated statistical significance.

**Results**

Sixteen of 17 patients completed the metabolic evaluation and determination of monocyte CD11b expression. Fourteen patients had successful adipose tissue biopsies. Patients were divided by half in groups with lower and higher CD11b expression on circulating monocytes. CD11b expression on circulating monocytes was 420±43 (range 235 to 583) mean log channel in the group with lower CD11b expression and 740±47 (range 595 to 995) mean log channel in the group with higher CD11b expression. As described in Table 1, gender distribution, anthropometric data, blood pressure, and blood levels of fasting insulin, fasting glucose, adiponectin, IL-6, resistin, and hsCRP were similar in both groups. All subjects fulfilled the criteria of visceral obesity (waist circumference >88 cm in women and >102 cm in men). Six patients in each group fulfilled the Adult Treatment Panel III criteria for the metabolic syndrome. The systemic glucose, free fatty acid, and insulin response to oral glucose tolerance testing were also similar between the groups with lower and higher CD11b expression (Figure 1). All subjects had normal fasting glucose levels. Six patients in the group with lower CD11b expression and 7 patients in the group with higher CD11b expression had impaired glucose tolerance. Insulin levels were elevated, suggesting the presence of insulin resistance in both groups.

Ethanol ratio in skeletal muscle and in adipose tissue was similar in patients with lower or higher CD11b expression on circulating monocytes and did not change with oral glucose tolerance testing (Figure 2, top). Dialysate glucose concentrations in skeletal muscle were similar in both groups at baseline and after the glucose load. In contrast, patients with higher CD11b expression showed a markedly augmented increase in dialysate glucose in adipose tissue after the glucose load (Figure 2, bottom). Dialysate lactate concentrations increased after glucose ingestion in skeletal muscle and in adipose tissue. Dialysate lactate concentrations in skeletal muscle were slightly increased in the group with higher CD11b expression on monocytes but more so in adipose tissue (Figure 3, top). Dialysate glycerol concentration in skeletal muscle was moderately increased in the group with higher CD11b expression but raised markedly in adipose tissue. The insulin release during oral glucose tolerance testing was associated with a reduction in glycerol release in both groups and tissues (Figure 3, bottom).

Data on CD11b expression on circulating monocytes and gene expression in adipose tissue were available in 13 patients (Table 2). Adipose tissue CD68 and resistin mRNA expression were significantly increased in patients with higher CD11b expression on circulating monocytes. In contrast, adiponectin mRNA expression was reduced in the group with higher CD11b expression. CD14, MCP-1, IL-6, and M-CSF1 mRNA expression were similar in both groups.

Adipose tissue mRNA expression of MCP-1 was highly correlated with adipose CD14 and CD68 mRNA expression (Figure 4, top). Adipose tissue resistin mRNA expression was also correlated with CD14 and CD68 mRNA expression (Figure 4, bottom).
CD11b expression on circulating monocytes and adipose CD68 mRNA expression were correlated with each other (n=13; \( r^2=0.30; P<0.05 \)). CD11b expression on circulating monocytes tended to correlate with CD14 (\( r^2=0.25; P=0.08 \)), MCP-1 (\( r^2=0.18; P=0.14 \)), and M-CSF1 (\( r^2=0.24; P<0.09 \)) mRNA expression in adipose tissue.

**Discussion**

Our microdialysis data are consistent with the idea that increased CD11b expression on circulating monocytes is associated with tissue-specific changes in glucose and lipid metabolism in humans. The gene expression data suggest that this phenomenon may be explained in part by adipose tissue infiltration by monocytes/macrophages. The infiltration may alter the local production of metabolically active cytokines, such as resistin and adiponectin. Our data also support the idea that signals causing monocyte infiltration (e.g., MCP-1) may be generated in adipose tissue. The findings complement and extend recent animal models of obesity to man.\(^6,10-13\) We stratified our patients into groups with lower and higher monocyte CD11b expression. Both groups were well matched for age, gender, anthropometric variables, blood pressure, and the systemic glucose and insulin responses during an oral glucose tolerance test. Remarkably, systemic hsCRP, IL-6, and adiponectin concentrations were also similar in both groups. However, we observed major differences between patients with higher and lower CD11b expression in adipose tissue metabolism. This finding suggests that a more detailed...
phenotypic characterization may be necessary to interpret experimental data of an at-first-sight homogenous group of patients. In particular, determination of hsCRP and IL-6 may not be sufficient to assess systemic inflammatory responses in obesity.

The interpretation of microdialysis data are not straightforward because metabolite concentration in the dialysate is influenced by tissue perfusion, local metabolite production, and local metabolite utilization. We used the ethanol dilution technique to account for possible differences in tissue perfusion. This method has been validated against direct blood flow measurements. The ethanol ratio before and during the oral glucose challenge was similar in patients with higher and lower CD11b expression in adipose tissue and in skeletal muscle. Thus, differences in microdialysate metabolite concentrations cannot be explained by differences in tissue perfusion. Adipose tissue blood flow depends strongly on the degree of adiposity.

Dialysate glucose concentrations in adipose tissue during the glucose load were increased markedly in patients with higher CD11b expression. Because systemic glucose concentrations and tissue perfusion were similar in both groups, the difference in adipose dialysate glucose cannot be explained by a difference in glucose supply. Instead, our data suggest that glucose uptake by adipocytes may be impaired in patients with higher CD11b expression compared with patients with lower CD11b expression. Although glucose uptake was reduced in patients with higher CD11b expression, lactate production at baseline and during the glucose challenge was increased. A possible explanation for this paradoxical observation is that in the group with higher CD11b expression, less glucose was used for triglyceride synthesis, and a larger proportion of the glucose was fueled into glycolytic degradation.

In the group with higher CD11b expression, dialysate glycerol concentrations were increased at baseline and during the glucose load. This observation is consistent with increased adipose tissue lipolysis. Skeletal muscle metabolism was much better maintained than adipose tissue metabolism in patients with higher monocyte CD11b expression. For example, dialysate glucose concentration in muscle was similar between the groups with higher and lower CD11b expression in adipose tissue and in skeletal muscle. Thus, differences in microdialysate metabolite concentrations cannot be explained by differences in tissue perfusion. Adipose tissue blood flow depends strongly on the degree of adiposity.

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### Table 2. Adipose Tissue mRNA Expression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low CD11b</th>
<th>High CD11b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (n)</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>CD68 (a.u.)</td>
<td>5.4±0.91*</td>
<td>10±2.0</td>
</tr>
<tr>
<td>CD14 (a.u.)</td>
<td>1.4±0.20</td>
<td>1.5±0.19</td>
</tr>
<tr>
<td>MCP-1 (a.u.)</td>
<td>0.018±0.003</td>
<td>0.021±0.004</td>
</tr>
<tr>
<td>Adiponectin (a.u.)</td>
<td>8.3±1.3*</td>
<td>4.7±0.3</td>
</tr>
<tr>
<td>Resistin (a.u.)</td>
<td>5.2±1.2*</td>
<td>8.8±1.3</td>
</tr>
<tr>
<td>IL-6 (a.u.)</td>
<td>0.17±0.03</td>
<td>0.24±0.06</td>
</tr>
</tbody>
</table>

Patients were stratified in 2 groups of equal size according to CD11b expression on circulating monocytes. The cut-off value was median CD11b expression in this group. Data are given as mean±SEM. *P<0.05 by unpaired t test. a.u. indicates arbitrary units.
expression, suggesting that the balance between glucose supply and glucose uptake was similar in both groups.

Insulin stimulates cellular glucose uptake and inhibits adipose tissue lipolysis. Systemic insulin concentrations were similar in patients with higher and lower CD11b expression. Therefore, decreased cellular glucose uptake and increased lipolysis in patients with higher CD11b expression are suggestive of reduced adipose tissue insulin responsiveness. We speculate that the alteration in adipose tissue metabolism in patients with higher CD11b expression may be the consequence of a low-grade inflammatory response. Monocytes, adipose tissue macrophages, and adipocytes release proinflammatory cytokines, such as IL-6 and tumor necrosis factor-α (TNF-α). TNF-α interferes with insulin signaling.\textsuperscript{20} TNF-α infusion into the brachial artery inhibited insulin-mediated glucose uptake in the forearm in humans.\textsuperscript{21} Yet we did not observe a major difference in systemic IL-6 concentrations or IL-6 mRNA expression in adipose tissue between patients with higher and with lower CD11b expression. Given the small sample of patients, we cannot exclude a subtle difference in these measurements.

The changes in adipose tissue metabolism in patients with higher CD11b expression might be related to locally reduced adiponectin or increased resistin production. In humans, resistin is produced mainly in macrophages,\textsuperscript{22} which may explain the correlation between resistin expression and monocyte/macrophage marker expression in our study. Recombinant human resistin has been shown recently to stimulate lipolysis in experimental animals.\textsuperscript{23} There was a discrepancy between systemic adiponectin and resistin concentrations and gene expression data in our study. A possible explanation is that the production was only increased locally, thus eliciting a localized metabolic abnormality.

We studied obese hypertensive patients. The majority fulfilled the diagnostic criteria for the metabolic syndrome. Our data may not be applicable to patients at a lower metabolic and cardiovascular risk.

\section*{Perspectives}

Systemic markers of a low-grade systemic inflammation are associated with an increased risk to develop type 2 diabetes mellitus.\textsuperscript{4} Recent animal studies suggest that the interaction between low-grade inflammation and adipose tissue function may take place at the tissue level and precedes the development of systemic insulin resistance. In these studies, increasing adiposity in various animal models was associated with increased adipose tissue macrophage infiltration.\textsuperscript{10,11} The macrophages appear to interact with adipocytes in a paracrine fashion. Our study suggests that a similar mechanism may be operative in humans. In our study, systemic CD11b expression on circulating monocytes was correlated significantly with adipose tissue expression of the CD68 gene, which is an established macrophage marker. A possible explanation for this correlation is that activated monocytes attach to endothelial cells in adipose tissue, infiltrate into the tissue, and then differentiate to macrophages. Indeed, expression of the macrophage marker CD68 was correlated with expression of the monocyte marker CD14 in adipose tissue in our study. The correlation between adipose CD68 or CD14 and MCP-1 expression suggests that MCP-1 production in adipose tissue enhanced monocyte recruitment.\textsuperscript{12} Visceral adipose tissue appears to release more MCP-1 than subcutaneous adipose tissue.\textsuperscript{24} We speculate that monocyte recruitment may be increased in patients with visceral adiposity. However, further studies with larger numbers of patients are required to confirm this notion.

\section*{Acknowledgments}

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\section*{References}


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