In situ profiling of adipokines in subcutaneous microdialysates from lean and obese individuals

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1The Lundberg Laboratory for Diabetes Research, Center of Excellence for Cardiovascular and Metabolic Research, Department of Molecular and Clinical Medicine, The Sahlgrenska Academy at Göteborg University, Göteborg, Sweden; 2Department of Internal Medicine, Section of Internal Medicine, Endocrine and Metabolic Sciences, Perugia University, Perugia, Italy; 3Institute for Clinical Diabetes Research, German Diabetes Center, Leibniz Center at Heinrich Heine University Düsseldorf, Düsseldorf; and 4Department of Anesthesiology and Intensive Care Medicine Mannheim, Heidelberg University, Heidelberg, Germany

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Murdolo G, Herder C, Wang Z, Rose B, Schmelz M, Jansson PA. In situ profiling of adipokines in subcutaneous microdialysates from lean and obese individuals. Am J Physiol Endocrinol Metab 295: E1095–E1105, 2008. First published September 9, 2008; doi:10.1152/ajpendo.90483.2008.—Adipose tissue (AT) had emerged as an endocrine organ and a key regulator of the metabolically triggered inflammation. The aims of this study were 1) to investigate the usefulness of a multiplexed bioassay in characterizing a panel of adipokines in subcutaneous (sc) microdialysate samples and 2) to determine whether lean and obese individuals differ in their interstitial adipokines levels following microdialysis (MD) probe insertion. Ultrafiltrating MD membranes were inserted in opposite sites of the sc abdominal AT of six lean (L) and six obese (OB) males at the beginning (M1) and during the last 120 min (M2) of the study. Interstitial and serum concentrations of adipokines were quantified using the Luminex technique and ELISA at 60-min intervals for 5 h. In comparison with L subjects, OB subjects exhibited elevated interstitial leptin (P < 0.001), IL-8 (P < 0.05), and IL-18 levels (P = 0.05), as well as higher serum concentrations of leptin (P < 0.0001), IL-6 (P < 0.0001), tumor necrosis factor-α (P < 0.001), IL-8 (P = 0.01) and interferon-γ-inducible protein 10 (P < 0.05). In samples from the M1 membranes, leptin decreased and IL-1α, IL-18, and RANTES (regulated on activation, normal T cell expressed and secrete) remained relatively stable, whereas IL-6, IL-8, and monocyte chemoattractant protein-1 significantly increased after the first hour (P < 0.0001 vs. baseline). Notably, either the magnitude of increase from the initial values or the time pattern of all the adipokines in M1 and M2 dialysates were similar between the groups. In conclusion, the current work provides valuable information on the optimal time frame to collect in situ AT microdialysate samples. Further studies are needed, however, to unravel the intricate interplay of cytokines in AT interstitial fluid.

adipose tissue; inflammation; subcutaneous microdialysis

OBESITY IS A CHRONIC INFLAMMATORY ILLNESS, as manifested by accumulated evidence indicating increased levels of acute phase reactants and immune mediators in the circulation, as well as the dysregulated expression and production of inflammation-related genes and molecules in the adipose tissue (AT) (24, 26, 39). The relevance of the low-grade inflammation in linking obesity with its complications is emphasized by the demonstration that chronically elevated circulating levels of cytokines and chemokines are independently associated with increased risk for type 2 diabetes (T2D) and cardiovascular disease (CVD) (22, 23, 27, 51, 53). In this scenario, the unfolding role of AT as a bioactive endocrine organ prompted us to speculate that the systemic low-grade inflammation may be “initiated” by, and reside into, the “inflamed/dysregulated” fat (24, 26, 39). Indeed, many of the so-called “adipokines” are recognized as key modulators of both the metabolic and vascular homeostasis (18).

In human obesity, it appears yet controversial to what extent the “spillover” of the adipose-derived signals contributes to the systemic inflammatory burden (15, 37, 38). However, although many adipokines only act as autocrine/paracrine regulators, the inflammatory molecules locally generated in strategically located fat microdeposits (e.g., the perivascular fat) may well reach surrounding target organs bypassing the systemic circulation (“outside-to-inside” cellular cross talk) (20, 35, 59). Therefore, the concentration in the bloodstream of the inflammatory biomarkers may not mirror the local milieu at the level of an inflamed fat depot.

Subcutaneous microdialysis (MD) is a validated technique for sampling adipose-derived molecules in the intercellular water space, providing unique information at the cellular level (31–33, 38, 49). Although the procedure is minimally invasive, probe implantation into the tissue almost induces a mechanical cell damage that itself may increase levels of inflammatory molecules in dialysate (41, 44). Therefore, the confounding effect of the AT response to probe insertion trauma appears of importance when investigations on temporal changes of proinflammatory mediators are addressed. On the other hand, a number of studies suggest that, independently of any immunological challenge, cytokines production varies spontaneously across the day, substantiating the hypothesis that the rate of adipokine production in situ also may reflect circadian or ultradian variation in immune function (9, 19, 42, 50).

New potential insights for further characterizing the AT-derived mediators was provided by the recent development of bead-based flow cytometric assays, which allow the simultaneous detection of multiple proteins in small sample volume of...
body fluids (10, 30). Remarkably, previous human studies underscored the effectiveness of a bench-top multiplexed bioassay (Luminex 100) at measuring, in vivo, a wide pattern of inflammatory biomarkers in microdialysates obtained from either oral mucosa (44) or cerebral extracellular fluid (36). Moreover, by using the open-flow microperfusion technique, a proinflammatory response of cytokines related to the metabolic syndrome [e.g., IL-6, IL-8, and tumor necrosis factor (TNF)-α] was recently described after a trauma induced by catheter insertion into the subcutaneous abdominal AT (SCAAT) of lean individuals (40). Nevertheless, it remains unclear whether the generation of different adipose-derived molecules in situ reflects diurnal variations of production/release or the kinetics of locally produced adipokines may be driven by the tissue response to probe implantation trauma. Finally, the observation that, after minimally invasive laparoscopic abdominal procedures, obese subjects exhibit a more prominent inflammatory response compared with nonobese individuals implies a close relationship between adiposity excess and changes in circulating concentrations of inflammatory molecules acutely after an operation (16). However, it has not been elucidated whether the “obese” AT, as a result of an increased inflammatory burden, may exhibit an exaggerated response to MD membrane insertion compared with a non-inflamed fat depot from lean individuals.

Based on the foregoing information, the overall aims of the present study were 1) to investigate the usefulness of a bead-based multiplexed bioassay in characterizing a panel of adipokines in subcutaneous MD and serum samples and 2) to determine whether lean and obese subjects exhibit a different local response following the insertion of a MD membrane in the subcutaneous abdominal AT.

MATERIALS AND METHODS

Subjects. Twelve male volunteers were recruited through an advertisement in a local newspaper. The participants were enrolled in the study if they met the following eligibility criteria: 1) a healthy state, as determined by medical history, physical examination, and screening laboratory evaluations; 2) a fasting plasma glucose concentration <5.6 mmol/l; 3) absence of changes (≥10%) in body weight during the last year preceding the screening; 4) normal exercise and drinking habits; and 5) no current regular medication.

The selected participants were then divided into a group of lean (L; n = 6; body mass index (BMI) = 18.5–24.9 kg/m²) and obese (OB; n = 6; BMI = 30–40 kg/m²) individuals. Clinical characteristics of the study subjects are given in Table 1. Informed written consent was obtained from all volunteers before their participation in the study, which was approved by the Ethical Committee of Göteborg University and carried out according to the principles of the Declaration of Helsinki.

Study design. Figure 1 depicts the design of the study. The participants were asked to refrain from any major physical exercise 2 days before the study session. All subjects were admitted to the research center at 0800 after an overnight fast and were investigated in the supine position, reclining in a comfortable bed in a quiet and temperature-controlled room (26 ± 2°C). A peripheral indwelling intravenous catheter was inserted into an antecubital vein of one forearm for intermittent blood sampling.

AT MD for in situ measurement of adipokines was performed as previously described (38, 49). Briefly, two custom-made MD linear probes (Plasmapho OP-02, polyethylene, outer diameter 440 μm, inner diameter 340 μm, pore size 0.3 μm; Asahi Medical, Tokyo, Japan) with a molecular mass cutoff of 3,000 kDa were inserted 40 mm apart into the periumbilical SCAAT; without anesthetics, by using a fine needle (25 gauge). The distance between the entrance and the exit sites in the skin was ~7 cm, and each membrane ran for a length of ~40 mm within the subcutaneous fat at a depth of ~10–20 mm. The inlet of the MD membranes was connected to a microinjection pump (CMA Microdialysis, Stockholm, Sweden) and perfused at a rate of 1.0 µl/min with 1% human albumin (vol/vol) in isotonic saline. After insertion, a period of 30 min was allowed for equilibration, and dialysate fractions were consecutively collected at 60-min intervals for 300 min. During the study, two separate membranes also were inserted in the opposite site of the periumbilical region with the same procedure used for the probes placed at the beginning of the experiment. Accordingly, after the placement of these catheters, a 30-min equilibration period was observed, and dialysate samples were collected at 60-min intervals during the last 120 min of the study (180–300 min; Fig. 1). Hereinafter, the probes inserted at the beginning of the experiment are referred to as “membrane 1” (M1), and the membranes placed later, as “membrane 2” (M2). The main aim of M2 was to find out whether any changes in adipokine concentrations in microdialysates drained from M1 during the 5 h of the experiment could be attributed to diurnal variations (then similar adipokine levels from M1 and M2 at the same time points would be expected) or, rather, to a local response to catheter insertion (then the adipokine levels from the first M2 sample would be similar to those observed in the initial sample from M1 taken 3 h before). Throughout the entire study, the period for the collection of dialysates was paralleled by intermittent blood samplings, and the fractions were stored at −70°C until analyzed.

Finally, to better elucidate the kinetics of adipokines generated in situ after the probe placement, we performed a control study in three obese volunteers who also participated in the previous study session. During this experiment, dialysate sampling started immediately after the placement of the probes, to avoid the equilibration time. Moreover, the MD membranes were perfused at a rate of 2.5 µl/min, and microdialysate samples were collected at 20-min intervals for a period of 180 min. These methodological adaptations were required to obtain sufficient sample volumes for subsequent Luminex measurements.

Analytical procedures. The interstitial concentrations of IL-6, IL-18, IL-1α, IL-8/CXCL8, monocyte chemoattractant protein-1/C-C chemokine ligand 2 (MCP-1/CCL2), and regulated upon activation normal T cell expressed and secreted/C-C chemokine ligand 5 (RANTES/CCL5), as well as the serum levels of IL-8, IL-18, MCP-1, interferon-γ-inducible protein 10/CXC-chemokine ligand 10 (IP-10/CXCL10), and RANTES/CCL5, were simultaneously quantified using a bead-based multiplex assay on a Luminex 100 analyzer (Luminex, Austin, TX) essentially as described previously (22, 23).

The protocol for microdialysates was adapted from previously described protocol for cell culture supernatants and serum (10, 21, 54). Briefly, calibration curves using recombinant proteins were

Table 1. Clinical characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Age, yr</td>
<td>38.2±4.2</td>
<td>41.3±4.9</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>80.2±4.3</td>
<td>117.2±4.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>22.9±1.1</td>
<td>33.4±1.4</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WHR</td>
<td>0.87±0.04</td>
<td>1.03±0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>FPG, mmol/l</td>
<td>4.9±0.1</td>
<td>5.2±0.1</td>
<td>NS</td>
</tr>
<tr>
<td>FSI, mU/l</td>
<td>5.1±0.6</td>
<td>14.3±2.6</td>
<td>0.01</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.1±0.1</td>
<td>3.3±0.5</td>
<td>&lt;0.01</td>
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</table>

Data are means ± SE. WHR, waist-to-hip ratio; FPG, fasting plasma glucose; FSI, fasting serum insulin; HOMA-IR, homeostasis model of assessment-insulin resistance; NS, not significant.
Table 2. Interstitial concentrations of adipokines drained from M1 membranes throughout the whole study period

<table>
<thead>
<tr>
<th>Adipokines</th>
<th>Lean</th>
<th>Obese</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) Leptin, pg/ml</td>
<td>673.2 (346.3; 988.2)</td>
<td>1,368 (749; 1,990.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(i) IL-1α, pg/ml</td>
<td>103.2 (56.6; 177.8)</td>
<td>69.2 (37.3; 203.4)</td>
<td>NS</td>
</tr>
<tr>
<td>(i) IL-18, pg/ml</td>
<td>69.8 (41.3; 117.7)</td>
<td>112.7 (53.7; 184.2)</td>
<td>0.05</td>
</tr>
<tr>
<td>(i) RANTES, pg/ml</td>
<td>318.2 (186.8; 574.5)</td>
<td>4836.8 (189; 760.4)</td>
<td>NS</td>
</tr>
<tr>
<td>(i) IL-6, pg/ml</td>
<td>6,331.2 (2,513.9; 11,717.1)</td>
<td>7,300.1 (2,017.5; 10,846.6)</td>
<td>NS</td>
</tr>
<tr>
<td>(i) IL-8, pg/ml</td>
<td>34.6 (12.6; 83.7)</td>
<td>88.9 (24.9; 148.2)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(i) MCP-1, pg/ml</td>
<td>1,018.1 (219.3; 2,089)</td>
<td>932.7 (296.5; 2,199.1)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are medians, with 25th and 75th percentiles in parentheses, of interstitial [(i)] concentrations of adipokines. RANTES, regulated upon activation normal T cell expressed and secreted; MCP-1, monocyte chemoattractant protein-1. See text for explanation of M1 membrane.
concentrations were substantially stable (Fig. 2, A–D). By contrast, the dialysate concentrations of IL-6, IL-8, and MCP-1 significantly rose from 60–120 min onward \((P < 0.0001\) vs. baseline) and remained stable afterward (Fig. 3, A–C). Notably, the magnitude of interstitial cytokine increase from the starting levels was similar in both groups \((\sim 20\text{-fold for IL-6 and IL-8 and } \sim 15\text{-fold for MCP-1})\). When all study subjects were analyzed together, the AUC\(_{0–300\text{ min}}\) of interleukin (IL)-6, IL-8, and IL-18 were significantly correlated (Table 3). Moreover, interstitial IL-6 and IL-8 AUC values were positively associated, and a strong correlation among interstitial IL-6, MCP-1, and IL-18 AUC values was also found (Table 3).

To address the question whether changes in adipokine concentrations over time would be due to diurnal patterns in AT production or to a local response to catheter insertion, we inserted two additional MD membranes (M2) at a different abdominal location 3 h after the first membrane (M1) implantation. In dialysates from the M2 membranes, interstitial levels and changes of adipokine concentrations from the initial values \((180–240\text{ min})\) were reproducible and almost comparable with the early values observed in M1 (Figs. 2 and 3). More specifically, the concentration of interstitial leptin in M2 fractions was lower \((P < 0.0001)\) than that observed in M1 dialysate at baseline but similar to interstitial leptin levels measured at the same time points in M1 samples. Finally, in M2 dialysates, the levels of IL-6, IL-8, and MCP-1 significantly rose \((P < 0.0001)\) at 240–300 min, and the M2 concentration of these adipokines was lower \((P < 0.0001)\) than that seen in the time-paired samples from M1 (Fig. 3, A–C). Altogether, these data indicate that leptin in situ production may reflect circadian variations, whereas the time profiles of IL-6, IL-8, and MCP-1 likely implicate a local inflammatory response to probe insertion.

Adipokines in dialysates collected at 20-min intervals. To more formally ascertain the temporal resolution of adipokine changes after probe insertion, we consecutively collected dialysate samples at 20-min intervals in an additional control study. In this experiment, interstitial leptin levels remained stable during the first 60 min and then decreased significantly \((P < 0.01)\) from 80–100 min onward, whereas the

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**Fig. 2.** Time profile of interstitial leptin (A), IL-1α (B), IL-18 (C), and regulated upon activation normal T cell expressed and secreted (RANTES; D) in lean and obese individuals. Data are means ± SE. *\(P < 0.0001\) vs. baseline. †\(P < 0.01\); ‡\(P = 0.05\) vs. lean individuals. (i), Interstitial concentrations.
concentrations of interstitial IL-1α and IL-18 did not significantly change (Fig. 4, A–C). Finally, the dialysate concentrations of IL-6, MCP-1, and IL-8 were stable for 90 min, and a significant rise ($P < 0.01$) from the starting level was seen only after 100, 120, and 160 min onward, respectively (Fig. 5, A–C). Therefore, probe insertion may not be reflected in the response of IL-6, MCP-1, and IL-8 as long as dialysate fractions are collected within a reasonable time window (i.e., 100 min) following the MD membrane placement.

Serum adipokines. In comparison with L individuals, OB individuals presented higher circulating leptin, IL-8, IL-6, IP-10, and TNF-α concentrations but similar serum IL-18 and MCP-1 levels (Table 4). In both groups, serum concentrations of TNF-α, IL-8, and IL-18 (Fig. 6A) did not significantly change over time, whereas serum MCP-1 (Fig. 6B) and serum IP-10 ($P < 0.01$ vs. baseline) decreased from 120 and 240 min onward, respectively. Interestingly, in OB subjects, we found a fall of serum leptin at 240 and 300 min ($P = 0.01$ vs. baseline; Fig. 6C). Furthermore, serum IL-6 levels increased significantly at 240 and 300 min ($P < 0.01$ vs. baseline) in L but not in OB individuals (Fig. 6D).

When both groups were combined, the serum and interstitial leptin AUC _0–300 min_ values were strongly correlated (Fig. 7). Finally, serum leptin AUC showed a strong association with HOMA-IR ($r = 0.81; P < 0.01$), fasting serum insulin ($r = 0.81; P < 0.01$), and waist circumference ($r = 0.69; P = 0.01$).

**DISCUSSION**

In the present study, the combination of the MD technique with a bead-based multiplex bioassay (Luminex) provided a powerful tool to assess a complex panel of proinflammatory adipokines in a limited volume ($<25$ μl) of microdialysate samples. Moreover, we have shown that probe implantation microtrauma may induce the local generation of some inflammatory adipokines, namely, IL-6, IL-8, and MCP-1, levels of which increase similarly in lean and obese individuals. However, the use of dialysate samples within a reasonable time after the membrane placement (i.e., ~100 min) appears to more accurately reflect the interstitial concentrations of these inflammatory molecules, enabling the characterization of obesity-linked inflammatory milieu in situ beyond the AT response to probe insertion.

AT increasingly emerges as a key endocrine organ at the interface between metabolism and immune system (26). The demonstration that different effector cells (i.e., macrophages and T cells) and molecules (cytokines and chemokines) of the immune response are constitutively present in the AT and more abundantly represented in the “dysregulated” fat of obese individuals supports the paradigm that AT per se may play a key role in the generation of metabolically triggered inflammation (“metaflammation”) (24, 26, 39, 56, 57). Although the mechanisms whereby AT in obesity becomes inflamed are still not fully understood, fat infiltration by immune cells leads to increased production and secretion of different inflammatory mediators, which, in turn, may detrimentally modulate insulin sensitivity and vascular homeostasis (18, 23, 27, 51, 53). Therefore, knowledge of in situ concentrations of chemokines, proteins known to be strongly implicated in the chemotaxis of immune cells (43), may be very helpful to better understand this intriguing aspect of AT biology.

Our group validated the use of novel MD linear membranes with high cutoff properties for sampling macromolecules such as IL-6 and MCP-1 in the interstitial fluid of the SCAAT in
humans (38, 49). However, compromise between requirements of conventional immunoassays (e.g., volume of dialysate sample and detection limit) and the need to optimize the dialysis efficiency to recover macromolecules (e.g., slow perfusion rate) limited the number of analytes assayable at one time within a single sample. This study emphasizes the capability of the Luminex technology to characterize a panel of proinflammatory adipokines in a limited volume (25 μl) of subcutaneous interstitial fluid samples, in line with the data of recent microdialysis and open-flow microperfusion experiments (1, 36, 40, 44). The combination of these techniques allowed us to iden-

**Table 3. Pearson correlation coefficients between AUC of the interstitial concentrations of cytokines/chemokines drawn on the M1 membrane**

<table>
<thead>
<tr>
<th></th>
<th>AUC (i) Leptin</th>
<th>AUC (i) IL-8</th>
<th>AUC (i) IL-1α</th>
<th>AUC (i) MCP-1</th>
<th>AUC (i) RANTES</th>
<th>AUC (i) IL-18</th>
<th>AUC (i) IL-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (i) leptin</td>
<td>0.599*</td>
<td>-0.190</td>
<td>0.359</td>
<td>-0.303</td>
<td>0.632*</td>
<td>0.466</td>
<td></td>
</tr>
<tr>
<td>AUC (i) IL-8</td>
<td>0.599*</td>
<td>-0.065</td>
<td>-0.046</td>
<td>0.008</td>
<td>0.589*</td>
<td>0.847‡</td>
<td></td>
</tr>
<tr>
<td>AUC (i) IL-1α</td>
<td>-0.190</td>
<td>0.051†</td>
<td>0.217</td>
<td>-0.427</td>
<td>-0.174</td>
<td>-0.271</td>
<td></td>
</tr>
<tr>
<td>AUC (i) MCP-1</td>
<td>0.359</td>
<td>-0.065</td>
<td>-0.046</td>
<td>0.008</td>
<td>0.589*</td>
<td>0.847‡</td>
<td></td>
</tr>
<tr>
<td>AUC (i) RANTES</td>
<td>-0.303</td>
<td>0.0217</td>
<td>-0.427</td>
<td>0.008</td>
<td>-0.174</td>
<td>-0.271</td>
<td></td>
</tr>
<tr>
<td>AUC (i) IL-18</td>
<td>0.632*</td>
<td>0.637*</td>
<td>-0.131</td>
<td>0.589*</td>
<td>-0.174</td>
<td>0.713§</td>
<td></td>
</tr>
<tr>
<td>AUC (i) IL-6</td>
<td>0.466</td>
<td>0.669*</td>
<td>-0.103</td>
<td>0.847‡</td>
<td>-0.271</td>
<td>0.713§</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05. †P = 0.06. ‡P = 0.001. §P < 0.01.

Fig. 4. Time profile of leptin (A), IL-1α (B), and IL-18 (C) in dialysate fractions collected at 20-min intervals. *P < 0.01 vs. baseline. NS, not significant.

Fig. 5. Time profile of IL-6 (A), IL-8 (B), and MCP-1 (C) in dialysate fractions collected at 20-min intervals. *P < 0.0001; §P < 0.05; †P < 0.01 vs. baseline.
tify, for the first time, abundant interstitial concentrations of the cytokine IL-1\(\alpha\), along with detectable levels of the chemokines RANTES, IL-8, and IL-18, in harmony with the results of previous ex vivo studies on human isolated adipocytes and AT explants (5, 6, 26, 48, 57). Notably, the sensitivity of the Luminex technique at measuring these adipokines was almost similar to that reported elsewhere using conventional ELISA assays (7, 10, 13). In harmony with the observation that all the fluorescence signals were in the linear range of the assays, the differences, or lack of, observed presently does not likely reflect the result of inaccurate sample detection. These in vivo findings implicate the subcutaneous abdominal fat depot as a potential source of molecules that, in situ, are importantly engaged in the regulation of adipogenesis and in AT infiltration by immune cells, whereas when appearing in the bloodstream, may contribute to the increased risk of T2D and CVD (5, 23, 26, 51, 57).

Since the concentration of the molecules measured initially in AT interstitial space was above the limit of detection, we were interested to evaluate whether obesity was associated with a dysregulated adipokines milieu and whether, as a result of a different inflammatory burden, the kinetics of proinflammatory mediators in situ might differ between L and OB individuals after the implantation of a MD probe. The presence of an “inflamed” fat depot in OB subjects was evident by the increased dialysate concentrations of leptin, IL-8, and IL-18, molecules that are well known to favor a proinflammatory milieu (11, 14, 43, 56). Moreover, inflammation in the bloodstream was concurrently evidenced by the elevated serum levels of leptin, IL-6, IL-8, TNF-\(\alpha\), and IP10. Of note, in situ profiling of adipokines exhibited important individual differences. In a first study that assessed the 5-h profile of adipokines, we found that interstitial IL-1\(\alpha\), IL-18, and RANTES remained rather constant and leptin tended to decrease,

**Table 4. Characterization of serum adipokines**

<table>
<thead>
<tr>
<th></th>
<th>Lean</th>
<th>Obese</th>
<th>(P) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(s) Leptin, pg/ml</td>
<td>5,743 (4,899; 6,896)</td>
<td>18,194 (13,751; 19,958)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(s) IL-8, pg/ml</td>
<td>9.3 (5.8; 12.3)</td>
<td>11.9 (7.2; 15.1)</td>
<td>0.01</td>
</tr>
<tr>
<td>(s) IL-18, pg/ml</td>
<td>162.5 (144.7; 193.8)</td>
<td>132 (93.6; 212.4)</td>
<td>NS</td>
</tr>
<tr>
<td>(s) IL-6, pg/ml</td>
<td>0.8 (0.6; 1.2)</td>
<td>1.6 (1; 3.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(s) MCP-1, pg/ml</td>
<td>223.1 (193.9; 279.3)</td>
<td>223.7 (200.9; 319.5)</td>
<td>NS</td>
</tr>
<tr>
<td>(s) IP-10, pg/ml</td>
<td>304.8 (251.7; 380.4)</td>
<td>371.1 (303.8; 424.9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(s) TNF-(\alpha), pg/ml</td>
<td>2.3 (1.9; 3.2)</td>
<td>3.8 (2.8; 5.3)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are medians, with 25th and 75th percentiles in parentheses, of serum [s] concentrations of adipokines. IP-10, interferon-\(\gamma\)-inducible protein 10.
whereas IL-6, IL-8, and MCP-1 concentrations increased substantially over time. The placement of a second MD membrane (M2) at a different location within the subcutaneous abdominal fat depot allowed us to investigate whether local generation of cytokines was reflected by “spontaneous” circadian variations or accounted for by the response to tissue injury induced by probe insertion. The results suggest that although the time course of leptin might well reflect diurnal rhythms of production (9, 19, 42, 50), the temporal profile of IL-6, IL-8, and MCP-1 was likely due to a local inflammatory response to insertion, which, in turn, did not induce consistent variations of IL-1α, IL-18, IL-8, and RANTES interstitial concentrations. Finally, despite these differences, the overall AT response to probe insertion was rather similar in L and OB individuals.

In humans, MD- and open-flow microperfusion-based studies indicate that probe implantation into different living tissues (i.e., skin, brain, and AT) might result in “artifactual” interstitial concentrations of some inflammatory mediators (1, 3, 8, 36, 40, 44). From a functional standpoint, catheter insertion in a living tissue induces a microtrauma that may perturb the local microenvironment by inducing the overflow of “constitutively” prestored or cell-associated molecules or by stimulating the production of inflammatory and chemotactic proteins. In this study, the inconsistent time course or even the decrease of interstitial leptin, a prototypical adipokine with established intracellular compartmentalization into the adipocyte (58), implicates subcutaneous MD as a minimally traumatic procedure for the adipose cells. Indeed, previous histological studies on a surgically removed MD probe showed that implantation trauma into the SCAAT did not result in major bleeding or edema, and no signs of foreign body reaction were observed after a period of 6 h following catheter insertion (34). Furthermore, the measurements of subcutaneous interstitial levels of adenosine (32), pyruvate (25), LDH (12), and ATP (2), biomarkers of tissue ischemia and cell destruction, indicated that tissue trauma leads only to minor and transient damage to the adipose cells. Therefore, the observation that interstitial leptin concentrations during the last 120 min of the study were virtually identical in M1 and M2 dialysates, along with the finding that in OB individuals serum and interstitial leptin levels showed a concomitant decrease over time, support the concept that leptin is a periodic hormone (9, 19, 46, 47). Moreover, the reduction in serum and interstitial leptin levels makes sense considering that the subjects were fasting and the study lasted 5 h. Accordingly, the fall of circulating leptin has been indicated as one of the adaptive physiological responses to fasting (29). On the other hand, it is unlikely that the interstitial time profile exhibited by leptin or other adipokines was secondary to a methodological rather than to a “biological” phenomenon. Indeed, although a prolonged time of dialysis or a reduced blood supply may induce a partial depletion of the dialyzable molecule (8, 55), each cytokine exhibited a different and individual trend that appeared in both the M1/M2 membranes and was similarly reproduced in each individual from either study group. Moreover, variations in the degree of protein “recovered” also can be reasonably excluded because, in vivo, dialysis efficiency to recover a given molecule is constant over time and independent of changes in outside concentration as long as membrane perfusion rate remains unchanged (52). Finally, the decrease in leptin was paralleled by an increase of IL-6, IL-8, and MCP-1, yet these proinflammatory mediators were positively correlated to leptin, suggesting that neither variation of recovery nor induction of local inflammation underlies the decrease in interstitial leptin levels. It is thus conceivable that temporal changes of leptin in situ represented the biological consequence of variations in the cellular production/release of the molecule into AT intercellular water space.

As opposed to leptin, the temporal profile of interstitial IL-6, IL-8, and MCP-1, molecules that are mainly produced by the non-adipose cells, reflected a local inflammatory response to membrane insertion rather than a circadian variation. Indeed, IL-6 is a well-known marker of cellular injury (4), whereas IL-8 and MCP-1 are chemokines implicated in acute inflammation (43). However, it also should be noted that the interstitial levels of IL-1α, IL-18, and RANTES, molecules that are known to be present at sites of acute inflammation (11, 14, 43, 56), showed a lack of increase or even an unexpected decrease, indicating that the AT response to probe insertion may be reflected in the levels of only some inflammatory molecules, namely, IL-6, IL-8, and MCP-1. Although these three proinflammatory mediators were clearly correlated, only IL-8 appeared increased in obese subjects and showed a significant correlation with leptin levels. The differential role and the potential contribution of these proinflammatory adipokines to the pathophysiology of obesity should be focused on in further studies.

As a corollary to these findings, we performed an additional study to achieve a higher resolution of the aforementioned temporal changes. Our objective was to find out whether there was a “time window” within which the adipokine concentrations might be more accurately measured, limiting or avoiding insertion artifacts. To this end, we used somewhat different experimental conditions, namely, lack of equilibration, shorter sampling time, and higher flow perfusion rate of the MD membranes. As expected, following catheter insertion, we found an increase of interstitial IL-6, MCP-1, and IL-8 from the starting values, but this was significant only after 100, 120, and 160 min, respectively. These observations suggest that although tissue injury evoked by MD probe placement might stimulate the production of inflammatory agents, a microtrauma-related response appears to contribute minimally to
the generation of the cytokine concentration measured in interstitial fluid within the first 90 min after insertion. Therefore, sampling of dialysate fractions within this time window may more accurately reflect the local concentration of these immune mediators over and beyond the tissue response to microtrauma.

Another relevant observation in this study was that local induction of IL-8 and MCP-1 in AT interstitial fluid did not result in significant changes of the serum levels. By contrast, in L individuals, the rise of interstitial IL-6 was accompanied by a significant increase of the systemic concentrations. This finding may have different explanations. First, the SCAAT has been indicated as an important contributor to the circulating IL-6 levels (37). Thus the overspill of IL-6 from AT interstitial space into the bloodstream might have contributed to the rise of systemic IL-6. Alternatively, the increase of serum IL-6 may reflect local alterations of the endothelium after the placement of an indwelling intravenous cannula (17, 19, 28, 45). However, the increase of serum IL-6 was not accompanied by changes of acute-phase (i.e., TNF-α) or other inflammatory cytokines (i.e., IL-8 and IP-10) and was evident only after a period of 240 min in L but not in OB individuals. Further studies are thus warranted to better clarify the mechanisms by which placement of an indwelling venous cannula may lead to artificial levels of IL-6 or other inflammatory mediators.

Regarding the study limitations, it needs to be mentioned that the concentration of the adipokines measured in dialysates does not mirror the actual interstitial levels, since the values were not adjusted for the in vivo recovery factor. Therefore, we may have missed the true tissue level of cytokines such as IL-6 due to lack of the recovery rate. Indeed, in a previous study performed in nonobese volunteers, we showed that interstitial IL-6 levels correlated with fat cell size (49), and we hence expected higher interstitial IL-6 in OB than in L subjects. On the other hand, relative recovery adjustment results in an equal multiplication of the dialysate levels, and the ability of the technique to detect a correct trend is also important. Second, due to the small sample size of the participants, we may have missed significant differences or associations between the groups. Third, the relatively large interassay CVs for some adipokines may have attenuated the differences over time between the groups.

Despite these constraints, our study has several strengths that should be outlined briefly. The complex experimental design using two membranes (M1/M2) enabled us to distinguish between the effects due to circadian variations (i.e., leptin) from those explained by the generation of adipokines caused by catheter insertion (e.g., IL-6, IL-8, MCP-1). In addition, the current work does provide valuable information for other researchers on the optimal time frame to collect in situ AT microdialysis fractions. Indeed, the control study based on 20-min sampling intervals demonstrated that there may be a time window in which proteins from AT can be sampled using the MD technique with minimal interference of a local immune response. Finally, comparison between L and OB individuals enabled us to better validate the findings that a different local adipokine milieu is accompanied by a similar AT response to probe insertion.

In conclusion, our study demonstrates the usefulness of subcutaneous microdialysis combined with the Luminescotechnique in assessing interstitial concentration and time kinetics of several adipokines in small volumes of dialysate samples. After the insertion of a microdialysis membrane, adipokine levels remain constant within a reasonable time window, whereas in later sampling, increases of some inflammatory markers (namely, IL-6, IL-8, and MCP-1) as well as decreases in leptin concentrations implicate a local inflammatory reaction and the presence of circadian patterns, respectively. With this proviso, the microdialysis technique for sampling adipokines may have important application in clinical trials to monitor the effects of lifestyle-based or pharmacological interventions on the modulation of obesity-linked inflammatory burden in situ, enabling a better understanding of the adipose tissue biology.

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