Secretion of adipokines by human adipose tissue in vivo: partitioning between capillary and lymphatic transport

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Miller NE, Michel CC, Nanjee MN, Olszewski WL, Miller IP, Hazell M, Olivecrona G, Sutton P, Humphreys SM, Frayn KN. Secretion of adipokines by human adipose tissue in vivo: partitioning between capillary and lymphatic transport. Am J Physiol Endocrinol Metab 301: E659–E667, 2011. First published July 12, 2011; doi:10.1152/ajpendo.00058.2011.—Peptides secreted by adipose tissue (adipokines) may enter blood via capillaries or lymph. The relative importance of these pathways for a given adipokine might influence its biological effects. Because this has not been studied in any species, we measured the concentrations of seven adipokines and eight nonsecreted proteins in afferent peripheral lymph and venous plasma from 12 healthy men. Data for nonsecreted proteins were used to derive indices of microvascular permeability, which in conjunction with the molecular radii of the adipokines were used to estimate the amounts leaving the tissue via capillaries. Transport rates via lymph were estimated from the lymph adipokine concentrations and lymph flow rates and total transport (secretion) as the sum of this and capillary transport. Concentrations of nonsecreted proteins were always lower in lymph than in plasma. With the exception of adiponectin, adipokine concentrations were always higher in lymph (P < 0.01). Leptin and MCP-1 were secreted at the highest rates (means: 43 μg/h or 2.7 mmol/h and 32 μg/h or 2.4 mmol/h, respectively). IL-6 and MCP-1 secretion rates varied greatly between subjects. The proportion of an adipokine transported via lymph was directly related to its molecular radius (r, +0.94, P = 0.025, n = 6), increasing from 14 to 100% as the radius increased from 1.18 (IL-8) to 3.24 nm (TNFα). We conclude that the lymph/capillary partitioning of adipokines is a function of molecular size, which may affect both their regional and systemic effects in vivo. This finding may have implications for the physiology of peptides secreted by other tissues.

adipokine secretion; capillary permeability; leptin secretion; lymph flow; molecular radius

There is an emerging view that the role of adipose tissue as an endocrine organ may be of comparable importance to its metabolic role. The secretory functions of adipose tissue were first recognized with respect to steroid hormones (42), but the discovery of the peptide hormone leptin (51) initiated an era of discovery of many potentially biologically active peptides secreted by the tissues now generally known as adipokines (47). The secretion of peptides by adipose tissue has been studied in vitro (13, 19), but it has been more difficult to do so in vivo. For some, it has been possible to measure secretion rates by an arteriovenous sampling technique. For example, secretion of interleukin-6 (IL-6) from subcutaneous abdominal adipose tissue was measured and used to estimate that around one-quarter to one-third of whole body secretion came from adipose tissue (29). Leptin secretion has also been measured in this way (11, 22), and net release of other peptides, for example, soluble tumor necrosis factor-α (TNFα) receptor type I, has been demonstrated (28). On the other hand, it was not possible to demonstrate secretion of TNFα by this technique (29), although TNFα mRNA is present in adipose tissue, and at least in rodents there is good evidence that adipose tissue–derived TNFα has systemic metabolic effects (20). Similarly, it has not been possible to demonstrate a significant arteriovenous difference indicating net adiponectin release, although this is understood to be a peptide that is produced only by adipocytes and circulates at high concentrations (45). It has been argued that the kinetics of adiponectin turnover make detection of its secretion difficult because it has both a low production rate and a low clearance rate (45).

Adipokines can be detected in adipose tissue interstitial fluid by the technique of microdialysis. Use of a large-pore microdialysis probe has demonstrated a local inflammatory response to the trauma of probe insertion, reflected in increased concentrations of several proinflammatory cytokines [IL-1β, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), and TNFα] in the interstitial fluid (6). It has also allowed the study of differences in adipokine concentrations between different fat depots (31).

Molecules secreted into the interstitial fluid of a tissue can leave by one or both of two routes, the lymphatic system and capillaries. Capillary walls are much more selective as molecular sieves than are the walls of terminal lymphatics. Molecules of large radius are more likely to enter the lymph from interstitial fluid than are those of small radius, which can more readily enter capillary blood by diffusion. This is likely to be more relevant to adipose tissue than to other endocrine tissues. In most endocrine glands, hormones of small molecular size are secreted into a tissue perfused by fenestrated capillaries. Adipokines, on the other hand, are relatively large molecules, and their exit from adipose tissue would be expected to occur by both the capillary and lymphatic routes. This in turn might
influence their biological effects. To date, little attention has been given to this issue.

Measurement of the steady-state plasma and lymph concentrations of a range of proteins not secreted or broken down in a tissue can be used to determine indices of the permeability characteristics of its capillaries. In the case of adipose tissue, it should be possible, by combining those indices with information on the concentrations of adipokines in lymph and plasma, their known molecular radii, and lymph flow rate, to calculate the total rates of secretion and lymph/capillary partitioning of the molecules. In the present study, we have used this approach to study the secretion of seven adipokines in a peripheral adipose tissue site in healthy humans. We have also taken the opportunity to compare the results with those of lipoprotein lipase (LPL), an enzyme that is also synthesized in adipocytes but exported by a structured pathway to the luminal surface of capillary endothelium (9).

**EXPERIMENTAL PROCEDURES**

*Subjects.* All studies were carried out in healthy male volunteers not taking any medication or alcohol. Clinical details are presented in Table 1. A medical history was taken from all subjects, and each was given a full clinical examination, including ECG. Blood samples were screened for evidence of renal, hepatic, and endocrine dysfunction and for the presence of recreational drugs. The study was approved by the local ethics committee, and all subjects gave informed consent.

*Clinical procedures.* Subjects were admitted to a metabolic ward at the London Bridge Hospital and placed on a normal solid food diet designed to maintain constant body weight. A prenodal (afferent) lymph vessel in the lower leg was cannulated in a surgical theater either 24 or 48 h after admission, using local anesthesia and sterile conditions, according to previously described procedures (30, 32). A sterile siliconized polyethylene cannula was left in place, draining into a 2-ml polypropylene vial strapped to the leg, and the volunteer returned to the ward. Thereafter, the vial was changed every 2–4 h, except in five subjects who had unusually high or low flow rates (Table 1). (The subjects with unusually high flow rates had no local or systemic evidence of inflammation.) On each occasion the volume of lymph was measured, the sample was centrifuged for 30 min at 1,500 g at 4°C to remove cells, and multiple aliquots were dispensed into cryovials for storage at −80°C until analysis. Lymph collection vials contained 2 mg of lyophilized disodium EDTA. Deep antecubital venous blood samples were collected at the midpoint of each lymph collection period into tubes containing disodium EDTA to give a final concentration of 1 mg/ml. They were then promptly centrifuged under the same conditions as the lymph samples. The samples of lymph and plasma were collected between 6 and 10 AM before breakfast, after the subjects had been supine and fasting since 10 PM the previous night. The air temperature of the ward was at all times controlled by air conditioning. We have shown previously that, although this protocol does not achieve an absolute steady state of lymph flow, the rate is at its lowest and least variable during this period of the 24-h cycle (7).

*Analytical methods.* Pairs of plasma and lymph samples collected concurrently were analyzed together. Metabolite concentrations [triacylglyceride (TG), nonesterified fatty acids (NEFA), glycerol, glucose, and lactate] were measured enzymatically in an ILab 600 or ILab 650 clinical chemistry analyzer (Instrumentation Laboratory UK, Warrington, UK). Albumin was measured by Laurell rocket immunoelectrophoresis, using polyclonal antiserum from International Immunology (Murrieta, CA). Adiponectin (total and high molecular mass) was assayed by an “in house” ELISA, using monoclonal antibodies as described previously (2). Low-molecular mass adiponectin concentrations were calculated by subtraction of high molecular mass from total concentrations. The following proteins were assayed by multiplex immunoassay by Millipore Profiling Services CytokineProfiler (Millipore, Dundee, UK): IL-1β, IL-6, IL-8 (also known as CXCL8), leptin, MCP-1 (also known as CCL2), and TNFα. Because there was a matrix difference between plasma and lymph, the plasma was diluted one-third before the multiplex assay to ensure comparable measurements between the two samples. LPL mass (but not activity) was measured by solid-phase sandwich ELISA (48).

In addition to albumin, six other proteins known to not be secreted by adipose tissue and covering a wide range of molecular masses were quantified to provide estimates of the permeability characteristics of the local adipose tissue capillaries in each subject. Concentrations of C-reactive protein, haptoglobin, α1-acid glycoprotein, α1-antitrypsin, ceruloplasmin, and transferrin were quantified by liquid-phase, double-antibody radioimmunoassays. All of these assays included Nonidet NP-40 (final concentration 2.5% vol/vol; Sigma-Aldrich, St. Louis, MO) to expose cryptic epitopes and reduce nonspecific binding and polyethylene glycol 6000 (final concentration 3% wt/vol; Sigma-Aldrich) to enhance binding kinetics. The primary antisera were polyclonal goat IgGs raised against purified proteins. A common secondary antisemper, donkey anti-goat IgG (Biomedica, Foster City, CA), was used as precipitating antibody. 125I-labeled tracers were prepared by iodinating purified proteins (Lee Biosolutions, St. Louis, MO) with iodine monochloride. Dose-response curves were constructed using the same proteins as for iodination, and the assays were calibrated using multilevel calibrators from International Immunology. Samples and standards were assayed in duplicate with intra-assay CVs of <9%. The proteins whose concentrations were used in the calculations are listed in Table 2.

*Calculations.* All calculations assumed that the concentrations of macromolecules in interstitial fluid are equal to those in lymph (see APPENDIX: THE CALCULATION OF JI AND THE RELATION BETWEEN CI/Cp AND

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**Table 1. Clinical characteristics of the subjects and lymph flow rates**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>Plasma Cholesterol, mmol/l</th>
<th>Duration of Lymph Collection, h:min</th>
<th>Lymph Volume, ml</th>
<th>Lymph Flow Rate, ml/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>67</td>
<td>23.6</td>
<td>4.0</td>
<td>2:00</td>
<td>0.97</td>
<td>0.47</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>84</td>
<td>25.5</td>
<td>3.7</td>
<td>2:00</td>
<td>0.78</td>
<td>0.39</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>68</td>
<td>21.0</td>
<td>4.4</td>
<td>3:00</td>
<td>0.47</td>
<td>0.16</td>
</tr>
<tr>
<td>4</td>
<td>20</td>
<td>70</td>
<td>21.7</td>
<td>3.7</td>
<td>2:15</td>
<td>1.57</td>
<td>0.70</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>62</td>
<td>20.0</td>
<td>4.6</td>
<td>9:06</td>
<td>1.20</td>
<td>0.13</td>
</tr>
<tr>
<td>6</td>
<td>41</td>
<td>67</td>
<td>27.2</td>
<td>3.9</td>
<td>8:42</td>
<td>0.82</td>
<td>0.09</td>
</tr>
<tr>
<td>7</td>
<td>37</td>
<td>67</td>
<td>23.1</td>
<td>4.2</td>
<td>3:00</td>
<td>2.76</td>
<td>0.92</td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td>67</td>
<td>20.7</td>
<td>3.8</td>
<td>3:00</td>
<td>1.92</td>
<td>0.64</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>62</td>
<td>18.0</td>
<td>4.8</td>
<td>3:00</td>
<td>0.48</td>
<td>0.16</td>
</tr>
<tr>
<td>10</td>
<td>37</td>
<td>85</td>
<td>25.4</td>
<td>4.9</td>
<td>4:00</td>
<td>1.55</td>
<td>0.39</td>
</tr>
<tr>
<td>11</td>
<td>26</td>
<td>81</td>
<td>24.2</td>
<td>5.1</td>
<td>0:30</td>
<td>1.70</td>
<td>3.40</td>
</tr>
<tr>
<td>12</td>
<td>34</td>
<td>60</td>
<td>20.2</td>
<td>5.1</td>
<td>0:45</td>
<td>1.25</td>
<td>1.67</td>
</tr>
</tbody>
</table>

BMI, body mass index. All subjects were healthy males.
Table 2. Proteins studied and data used to calculate secretion rates

<table>
<thead>
<tr>
<th>Protein</th>
<th>Molecular Mass, kDa</th>
<th>Molecular Radius (Stokes-Einstein), nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₁-Acid glycoprotein</td>
<td>40</td>
<td>3.01</td>
</tr>
<tr>
<td>α₁-Antitrypsin</td>
<td>55</td>
<td>3.35</td>
</tr>
<tr>
<td>Albumin</td>
<td>66</td>
<td>3.56</td>
</tr>
<tr>
<td>Transferrin</td>
<td>77</td>
<td>3.74</td>
</tr>
<tr>
<td>CRP</td>
<td>120</td>
<td>4.34</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>160</td>
<td>4.78</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td>300*</td>
<td>5.90</td>
</tr>
<tr>
<td>Proteins potentially secreted by adipose tissue (adipokines)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1/CCL2</td>
<td>13.0</td>
<td>1.61</td>
</tr>
<tr>
<td>IL-8/CXCL8</td>
<td>8.0</td>
<td>1.18</td>
</tr>
<tr>
<td>Leptin</td>
<td>16</td>
<td>1.81</td>
</tr>
<tr>
<td>IL-1β</td>
<td>17</td>
<td>1.88</td>
</tr>
<tr>
<td>TNFα</td>
<td>51†</td>
<td>3.24</td>
</tr>
<tr>
<td>IL-6</td>
<td>26</td>
<td>2.34</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>26.8‡</td>
<td>2.38§</td>
</tr>
</tbody>
</table>

CRP, C-reactive protein; MCP-1, monocyte chemoattractant protein-1; CCL2, chemokine (C–C motif) ligand 2. *Molecular weight (MW) of Hp 1–1 = 86; MW of Hp 1–2 = 86–300; MW of Hp 2–2 = 170–900, MW of 300 and molecular radius of 5.9 used; †assumed trimeric; ‡as monomer; §mean of values for dimer (2.26) and trimer (2.62).

RESULTS

Concentrations in plasma and lymph. Glucose and lactate concentrations were similar in plasma and lymph (Table 3). As reported previously (30), glycerol concentrations were significantly higher in lymph, presumably reflecting lipolysis in adipocytes, whereas TG concentrations were markedly lower, reflecting the virtual absence of TG-rich very low-density lipoproteins and chylomicrons, the two largest classes of lipoprotein particles (Table 3). Although concentrations of NEFA were somewhat lower in lymph, the molar ratio of NEFA to albumin was similar in the two matrices (0.32 ± 0.05 and 0.40 ± 0.08, P = 0.35).

As expected, the concentrations of all proteins not secreted by adipose tissue were lower in lymph than in plasma (Table 4). In accord with previous reports (30), their lymph/plasma concentration ratios were inversely related to log molecular mass.

Concentrations of adipokines were significantly higher in lymph than in plasma (Table 4). The only exceptions were total and high-molecular-mass adiponectin, which were consistently lower in lymph (~20 and 15%, respectively; Table 4).

Concentrations of some adipokines in lymph varied greatly between participants. This was particularly so for IL-6 (plasma 2–98 ng/l, lymph 14 ng/l to 20.7 μg/l) and for MCP-1 (plasma 99–415 ng/l, lymph 287 ng/l to 102 μg/l). LPL concentrations were very low in lymph relative to plasma (lymph median 7% of plasma; Table 4).

Secretion rates. Calculated rates of secretion are shown in Table 5. On average, leptin and MCP-1 were secreted at markedly higher rates than were other adipokines, although MCP-1 secretion varied greatly between subjects (0.033–325 μg/h). Average IL-6 secretion was also high compared with others, but again there was great variation between subjects (1.3 ng/h to 13 μg/h). For adiponectin (large molecular mass), there was apparent net uptake by the tissue (values for individual subjects are given in Supplemental Tables S1 and S2; Supplemental Material for this article is available online at the AJP-Endocrinology and Metabolism website). There were no statistically significant correlations between body weight or body mass index and individual adipokine secretion rates.

Striking differences were observed in the relative proportions of different adipokines estimated to be leaving the tissue via lymph and capillaries (Table 5; see Supplemental Tables S1 and S2 for values in individual subjects). For IL-6 and IL-1β, lymphatic transport predominated. For leptin and MCP-1, the two routes were of equal importance. For IL-8, capillary transport predominated. Both TNFα and adiponectin (low molecular mass) showed negative net rates of transport via capillaries (i.e., a net movement from plasma to interstitial fluid).
Table 4. Concentrations of proteins in plasma and lymph

<table>
<thead>
<tr>
<th>Protein</th>
<th>Plasma</th>
<th>Lymph</th>
<th>Ratio (median)</th>
<th>n</th>
<th>P (Wilcoxon)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nonsecreted proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-1-Acid glycoprotein, g/l</td>
<td>0.94 ± 0.08</td>
<td>0.40 ± 0.03</td>
<td>0.40</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin, g/l</td>
<td>1.46 ± 0.08</td>
<td>0.57 ± 0.05</td>
<td>0.38</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>Transferrin, g/l</td>
<td>43.9 ± 0.84</td>
<td>17.1 ± 1.3</td>
<td>0.38</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP, mg/l</td>
<td>2.67 ± 0.07</td>
<td>0.98 ± 0.07</td>
<td>0.36</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ceruloplasmin, g/l</td>
<td>1.32 ± 0.74</td>
<td>0.19 ± 0.08</td>
<td>0.26</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Haptoglobin, g/l</td>
<td>0.262 ± 0.010</td>
<td>0.072 ± 0.004</td>
<td>0.26</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Potentially secreted proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1-CCL2, ng/l</td>
<td>190 ± 23</td>
<td>1760 ± 585</td>
<td>3.89</td>
<td>12</td>
<td>0.003</td>
</tr>
<tr>
<td>IL-8-CXCL8, ng/l</td>
<td>2.6 ± 0.3</td>
<td>75.1 ± 24.8</td>
<td>21.0</td>
<td>12</td>
<td>0.002</td>
</tr>
<tr>
<td>Leptin, μg/l</td>
<td>3.80 ± 0.62</td>
<td>22.9 ± 4.74</td>
<td>5.35</td>
<td>12</td>
<td>0.002</td>
</tr>
<tr>
<td>TNFα, ng/l</td>
<td>0.41 ± 0.04</td>
<td>1.17 ± 0.18</td>
<td>3.02</td>
<td>12</td>
<td>0.002</td>
</tr>
<tr>
<td>IL-6, ng/l</td>
<td>2.8 ± 0.3</td>
<td>13.4 ± 1.8</td>
<td>5.32</td>
<td>12</td>
<td>0.002</td>
</tr>
<tr>
<td>Adiponectin (total), mg/l</td>
<td>22.3 ± 3.0</td>
<td>4.8 ± 0.6</td>
<td>0.22</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>Adiponectin (high molecular mass), mg/l</td>
<td>8.3 ± 1.5</td>
<td>1.0 ± 0.2</td>
<td>0.14</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>LPL, μg/l</td>
<td>29.7 ± 2.8</td>
<td>2.5 ± 0.4</td>
<td>0.08</td>
<td>11</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Results are shown as means ± SE except for the lymph/plasma concentration ratio, for which the median value is shown. LPL, lipoprotein lipase.

Discussion

The lymphatic vessel that we cannulated collects lymph from the superficial tissues of the foot and ankle, which include adipose tissue (30, 33). Lymph vessels have been difficult to demonstrate histologically within adipose tissue (14, 43). The vessels appear to be predominantly in a narrow interstitial space that lies outside the lobules of adipocytes in the enveloping connective tissue (39, 40). This is similar to the arrangement in skeletal muscle, where a narrow interstitial space with the lymphatics lies outside the bundles of muscle fasciuli in the perimsysym. Whatever the precise microscopic anatomy, it is clear from our findings that interstitial fluid finds its way from adipose tissue into the vessel we cannulated. First, as in a previous study (30), we found significantly higher concentrations of free glycerol in lymph than in plasma. The only source of the additional glycerol could have been adipocytes, because peripheral lymph contains almost no triglyceride-rich lipoproteins. Second, in the present study we also observed net secretion of leptin, a specific marker of adipose tissue. The similarity of the observed NEFA/albumin ratios in lymph and plasma despite other evidence for active lipolysis can be explained by rapid movement across the capillary endothelium of NEFA from albumin in interstitial fluid to albumin in plasma.

We have shown previously that in patients with rheumatoid arthritis lymph collected from the same vessel drains inflammatory tissues of the foot joints, with greatly increased concentrations of some of the inflammatory cytokines measured here, including TNFα, IL-1β, and IL-6 (33). Skin may not make a major contribution since lactate concentrations, which are high in superficial blood vessels draining skin (15), were similar in lymph and plasma (from a deep antecubital vein). It is important to note that even within adipose tissue some of the molecules we studied may arise from cells other than adipocytes. For instance, TNFα is known to arise from inflammatory and other cells in the stromal-vascular fraction of adipose tissue rather than from adipocytes (12, 13).

We have used our data to estimate the total secretion rates of several adipokines and their partitioning between the capillary and lymphatic routes of exit from the tissue. To our knowledge, this is the first time that either has been attempted in any fluid in association with net transport from the tissue via lymph. In the case of TNFα there was nevertheless net overall secretion, since transport via lymph exceeded that from plasma into interstitial fluid. For adiponectin, however, transport via lymph was less than that from plasma to interstitial fluid, implying net consumption by the tissue.

The six adipokines for which there was net total secretion, the proportion exiting via the lymphatic route increased from 14 to 100% as molecular radius increased from 1.18 to 3.4 nm (rₑ = 0.943, P = 0.025; Fig. 1).

Table 5. Transport rates of proteins from adipose tissue via capillaries and lymph

<table>
<thead>
<tr>
<th>Protein</th>
<th>Capillary Transport</th>
<th>Lymphatic Transport</th>
<th>Total Transport*</th>
<th>Lymph/Capillary Ratio (Median)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCP-1, μg/h</td>
<td>15.3 ± 12.8</td>
<td>16.2 ± 14.1</td>
<td>31.6 ± 26.9</td>
<td>0.95</td>
</tr>
<tr>
<td>Leptin, μg/h</td>
<td>22.2 ± 7.4</td>
<td>20.5 ± 8.8</td>
<td>42.7 ± 14.3</td>
<td>1.07</td>
</tr>
<tr>
<td>IL-6, ng/h</td>
<td>902 ± 489</td>
<td>2110 ± 1013</td>
<td>3012 ± 1476</td>
<td>2.56</td>
</tr>
<tr>
<td>IL-8-CXCL8, ng/h</td>
<td>660 ± 315</td>
<td>109 ± 61</td>
<td>769 ± 374</td>
<td>0.17</td>
</tr>
<tr>
<td>TNFα, ng/h</td>
<td>−0.98 ± 0.35</td>
<td>9.7 ± 3.6</td>
<td>8.8 ± 3.40</td>
<td>1.17</td>
</tr>
<tr>
<td>IL-1β, ng/h</td>
<td>0.55 ± 0.19</td>
<td>0.82 ± 0.26</td>
<td>1.36 ± 0.36</td>
<td>2.36</td>
</tr>
<tr>
<td>Adiponectin, ng/h</td>
<td>−9.5 ± 2.5</td>
<td>2.6 ± 1.1</td>
<td>−6.90 ± 1.60</td>
<td>n.a.</td>
</tr>
</tbody>
</table>

Results are shown as means ± SE (except in the case of lymph/plasma concentration ratio); n = 10, 11, or 12. In each case, the total secretion rate was significantly different from zero (P < 0.001 by sign test). *Mean total transport rates in molar units were as follows: MCP-1, 2.43 nmol/h; leptin, 2.67 nmol/h; IL-6, 115.8 pmol/h; IL-8-CXCL8, 96.1 pmol/h; TNFα, 172.5 pmol/h; IL-1β, 0.08 pmol/h; adiponectin, −257.5 pmol/h. †Low molecular mass. ‡Lymph/capillary ratios are presented only for those adipokines for which there was net secretion via both routes.
species in vivo. Of the adipokines studied, leptin, MCP-1, and IL-6 were secreted at by far the highest rates whether expressed in mass or molar units. For leptin and MCP-1 the capillary and lymphatic routes were equally important, whereas IL-6 was transported mostly via the lymphatic route. Transport of IL-1β from the tissue was also predominantly via lymph, but total secretion rate was low. IL-8 was transported mostly via capillaries. TNFα was transported entirely via lymph, whereas transport via capillaries was negative; i.e., there was a net movement from plasma to interstitial fluid. This finding could explain why it has not been possible to show net release of TNFα by arteriovenous measurements across human adipose tissue (29), whereas leptin (11, 22) and IL-6 (27, 29) secretion are readily demonstrable by this means. The same approach was not able to demonstrate secretion of MCP-1 into blood by adipose tissue (8). Because the transport of TNFα from the tissue via lymph greatly exceeded transport into it from plasma via capillaries, there was net transport from the tissue overall.

In the pooled data on the six adipokines for which there was net transport from the tissue (i.e., all except adiponectin), the proportion transported by lymph was strongly positively correlated with molecular radius (Fig. 1). A positive trend would be expected, owing to the nature of the formulae used for the calculation. However, this is unlikely to provide the full explanation for such a strong correlation considering that all other variables in the calculations were independent of molecular radius, among which the lymph flow rates varied over an almost 40-fold range of 0.09–3.4 ml/h. Although the relation appears to be linear, it is presumably a section of a curve that would be expected to plateau for molecules with radii greater than TNFα. Similar trends were seen in the data for all but two of the subjects (not shown).

It is important to recognize that these results for the partitioning of adipokines between the two routes (although not those for total secretion rate) are essentially predictions based on current pore theory. Although there is a large body of evidence to support this theory, our conclusions nevertheless require confirmation by other studies using appropriate experimental systems.

Surprisingly, adiponectin (low molecular mass) differed from all the other adipokines in having a negative value for total transport, implying there was net consumption in the tissue, owing to the fact that capillary transport from plasma to interstitial fluid exceeded transport from the tissue via lymph. We have no explanation for this unexpected finding. It has been reported previously that net secretion of adiponectin could not be observed by arteriovenous difference measurements across human adipose tissue (45), and it was postulated that a long plasma half-life combined with a low rate of secretion explained this finding. The half-life of adiponectin has been estimated at about 2.5 h, and its production rate has only a minor influence on its plasma concentration (19). In contrast, leptin has a plasma half-life of 25 min, and its plasma concentration is determined directly by its secretion rate (22). The mechanisms of adiponectin clearance from plasma have not been well defined. Renal clearance appears to be relatively low (38), but there is excretion into bile also (44). Removal by other tissues has not been studied as far as we are aware, but our findings raise the possibility that adiponectin can be removed in the periphery, perhaps after binding to its cell surface receptors. Some technical issues need also to be considered. We modeled the secretion of the low-molecular-mass form only, since it was difficult to estimate a molecular mass for the remaining multimeric forms. There is evidence that the association state of adiponectin is affected by Ca²⁺ concentration (1), which may have varied among lymph samples, because a fixed weight of lyophilized EDTA had been added to each collection tube. In addition, as discussed below, there are issues regarding effects that pulsatile secretion of adiponectin might have had on our estimates. Nevertheless, the fact remains that in all subjects in whom they were measured, concentrations of both total and high molecular mass forms of adiponectin were considerably lower in lymph than in plasma (maximum value was <32% of that in plasma). Whatever the effects of isomeric state and pulsatile secretion, it is difficult to reconcile that observation with secretion by any pathway as currently understood.

We did not attempt to model the data for LPL because it is not secreted in the same way as adipokines. Its very low concentration in lymph is in accordance with a structured pathway for transport to the endothelium rather than diffusion through the interstitial space. Details of this pathway are beginning to emerge with the identification of the glycosylphosphatidylinositol-anchored glycoprotein GPIHBP1, expressed by endothelial cells, as the factor responsible for LPL transport to the capillary lumen (3, 9). In addition, other tissues contribute to plasma LPL mass, and LPL production rate in adipose tissue would be expected to have been low in these fasted subjects. The recent demonstration that inhibition of LPL by angiopoietin-like protein 4 is necessary to protect lymph nodes from massive inflammatory responses to ingested fat (26) may be why lymph LPL concentrations are maintained so low.

As far as we are aware, transport of adipokines from adipose tissue via the lymphatic system has not been considered previously. The partitioning of an adipokine between the lymphatic and capillary routes could have functional significance. Close relationships exist between adipose tissue and lymphatic tissues (10), especially the lymph nodes (35). As we studied prenodal (afferent) lymph, the measured concentrations were

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**Fig. 1.** Relation between molecular radius and %transport via lymph for 6 adipokines. Values for %transport via lymph were calculated from the mean values for lymphatic and total transport presented in Table 5. Spearman rank correlation coefficient = 0.94, \( P = 0.025 \). MCP-1, monocyte chemoattractant protein-1.
those to which downstream lymph nodes were exposed. For different adipokines these were three- to 25-fold greater (median values; see Table 4) than the corresponding concentrations in plasma. This might have significant effects on lymphocyte function. IL-1, IL-6, and TNFα are key molecules in the inflammatory state associated with adipose tissue enlargement and its accompanying insulin resistance (17, 46), and each is in turn capable of activating cells of the immune system (46, 50). Because afferent lymph is considered to represent interstitial fluid, the concentrations in it are presumably also those to which fibroblasts, macrophages, and stromal cells in adipose tissue are exposed. In the case of splanchnic adipose tissue, adipokine molecules leaving the tissue via capillaries will directly enter the portal venous system, exposing the liver to higher concentrations than those that exist in arterial blood. An adipokine’s partitioning between lymph and capillaries is also likely to affect its pharmacokinetics. Changes in systemic effects remote from the adipose tissue following changes in secretion rate are likely to occur more rapidly with adipokines that enter blood predominantly via capillaries.

It is pertinent to note the assumptions used in our calculations. The most important was that the concentrations in plasma and lymph reflected a steady state of transport between the secretion of molecules and their removal by the local blood capillaries and lymphatics. Steady-state transport from a tissue can be achieved only if local blood and lymph flow rates and microvascular permeability remain constant. In this study, all samples were taken in the early morning before breakfast, after around 8–12 h of supine rest. Although variations of local blood flow may have occurred during this period, they are unlikely to have been large. Moreover, the transport of intermediate to large plasma molecules (such as those considered here) is less sensitive to changes in blood flow than that of small molecules and ions (e.g., glucose, Na+) whose exchange may be flow limited at basal levels of tissue blood flow. Lymph flow rates vary with posture and physical activity (7), but changes in these will have been small because the samples were collected while the volunteers remained in bed.

Concentrations of lipoproteins, albumin, and other nonsecreted proteins in lymph have been shown to be much more stable during sleep than during the day (7). However, we cannot exclude the possibility that secretion rates in the tissue varied. The overnight-fasted state is not a true metabolic steady state because there is a continuous rise in adipose tissue lipolysis (24, 41). Leptin secretion falls in early starvation (23), and there is evidence for pulsatile secretion of adiponectin and leptin (16). Pulsatile release of other hormones such as insulin and glucagon (18, 25) and of products of lipolysis from adipose tissue in vivo (21), with periods of 10–15 min, have also been reported. The pulsatile release of an adipokine would compromise the steady-state assumption necessary for our calculation of the secretion rate. Little is known about variations in the secretion rates of the other adipose tissue-derived proteins studied here. In most tissues it is reasonable to assume that microvascular permeability is constant, but changes in leptin secretion have been reported to affect permeability (5). Our adoption of the cylindrical pore model for adipose tissue microvascular permeability is discussed further in the APPENDIX.

We conclude that adipokines are transported from adipose tissue into blood via both capillaries and lymph. In the absence of a specific transport mechanism through the capillary walls, the relative partitioning between the two routes is determined largely by molecular radius. This phenomenon is likely to affect the apocrine actions of adipokines in the adipose tissue itself, some local regional effects, for example, in the liver and lymph nodes, and their systemic pharmacokinetics. Although caution must be exercised in extrapolating from one tissue site to others, similar considerations probably apply to the secretion of other peptides in peripheral tissues. Collection of lymph provides a means for quantifying secretion rates in vivo. Our data confirm the high secretion rate of leptin relative to most other adipokines from human subcutaneous adipose tissue and show that secretion of IL-6 and MCP-1 is extremely variable from person to person. They also raise questions about the pathway for secretion of adiponectin, which warrants further study.

APPENDIX: THE CALCULATION OF $J_S$ AND THE RELATION BETWEEN $C_P$/CP AND MOLECULAR RADIUS

Background. We make the assumption that microvascular permeability of adipose tissue can be described by convection and diffusion through pathways modeled as cylindrical pores of a given molecular radius. Although it bears little structural resemblance to pathways through microvessels (see Ref. 27a), the pore model has been shown to describe the permeability properties of several different types of vessels remarkably well (34, 36, 45a, 49). Whereas other models have been based on two or more populations of pores to account for the permeability to macromolecules, the present observations confirm the conclusion of Rosell (38a) that the small pores of the exchange vessels in adipose tissue are larger than those found in skeletal muscle, small intestine, lung, etc., and the presence of a much larger pore population would have little influence on the exchange of the molecules of interest here.

Estimation of secretion rates of adipokines. Molecules secreted by adipose tissue are cleared into the blood and lymph circulations. Provided that they are not broken down until after they have left their tissue of origin and that steady-state conditions are established between their rate of secretion and their rate of clearance, their rate of secretion, $J_A$, is equal to the sum of their rates of clearance by the blood, $J_B$, and by the lymph. Clearance of molecules by the lymph can be estimated from the product of their concentration in the lymph ($C_L$) and the lymph flow ($J_L$). Thus

$$J_A = J_B + J_L C_L$$

To estimate clearance of a solute by the local blood flow and its rate of secretion into the tissues, it is necessary to make several assumptions. These are that 1) the molecules are secreted at a constant rate, 2) a steady state is achieved between their rate of secretion and their rate of clearance, and 3) their concentration in the interstitial fluid is constant and is equal to their concentration in the lymph. Errors resulting from these assumptions are expected to give rise to relatively small underestimates of the secretion rate into plasma. Only if the secretion of some or all of the molecules into the interstitial fluid is pulsatile are the underestimates likely to be large.

In the absence of specialized mechanisms transporting adipokines into the local capillary blood, $J_S$ is determined by diffusion and convection through pathways in the walls of the capillaries supplying the adipose tissue. For net diffusion to occur, the concentration of adipokines in the interstitial fluid, $C_I$, must be greater than its concentration in the capillary plasma, $C_P$. If $J_D > 0$ and interstitial fluid volume remains constant, there must be net filtration of fluid from capillaries into the tissues, and the diffusion of adipokine molecules into the capillaries occurs against this filtration stream. This reduces the rate of diffusion by an amount that can be calculated from the Péclet number, which is the ratio of the rate of convection to that of diffusion of the
molecule through the permeable pathways in the capillary walls. To
describe the interaction between convection and diffusional transport
of a solute in any one pathway through microvascular walls, let \( J_\nu \) be
the fluid filtration rate through an area \( A \) of microvascular wall, let \( P_d \)
be the diffusional permeability coefficient of solute, and let \( \sigma \) be its
reflection coefficient at the microvascular wall, and then \( J_S \) can be
written as

\[
J_S = J_\nu (1 - \sigma) \left( C_P e^{-Pe} - C_P \right) \quad (2)
\]

where the Pécel number

\[
P_e = \frac{J_\nu (1 - \sigma)}{P_d A}
\]

The symbol \( \sigma \) is the reflection coefficient of the pathway
to the solute, and \( P_d \) is its diffusional permeability coefficient. The
reflection coefficient of a membrane to a particular solute can be
defined as that fraction of solute molecules that is reflected at a
membrane when the ultrafiltration of a solution of that solute through
the membrane gives rise to a concentrated solution upstream and a
more dilute solution in the filtrate. It also describes the fraction of
the osmotic pressure of a solution that can be measured across a particular
membrane. The diffusional permeability, \( P_d \), is a measurement of the
conductance of a membrane to a solute that is passing through it by
diffusion alone. It is defined as the flux of solute passing through unit
area of membrane per unit concentration difference across the mem-
brane.

In the steady state \( J_\nu = J_\nu \), interstitial concentration \( C_I = J_S/J_\nu =
C_L \), where \( J_S \) and \( C_L \) refer to the flow of preportal lymph draining
the fat tissue and the molecule’s concentration in this lymph, respectively.
Substituting for \( C_I \) in Eq. 2 and rearranging the terms yields an
expression for the ratio of \( C_I \) to \( C_P \), i.e.,

\[
\frac{C_I}{C_P} = \frac{1 - \sigma}{1 - \sigma e^{-P_e}} \quad (3)
\]

To evaluate Eqs. 2 and 3, values are required for \( \sigma \) and \( P_e \), which
are permeability coefficients of the adipose tissue capillaries and also
for \( J_\nu /A \), which is the net fluid filtration rate per unit area of
microvascular wall. To estimate \( J_S \) for each adipokine, we have made
the assumptions that 1) these will be the same as those for nonsecreted
molecules of the same molecular radius; 2) these molecules cross
microvascular walls largely via a single type of pathway, and 3) the
transport properties of this pathway can be approximated as that of
a population of uniform cylindrical pores of constant numbers per unit
area of vessel wall (34).

The concentrations of seven endogenous nonsecreted proteins were
determined in the same samples of lymph and plasma as those used
for measuring concentrations of the secreted molecules, and the values
of \( C_I/C_P \) for the nonsecreted molecules were plotted against their
molecular radii for each subject. The points could be fitted to a curve
based on Eq. 3, and the shape of this curve can be predicted from the
pore theory if the value of the pore radius, \( r \), is known (see Ref. 36 and
Fig. 2).

A preliminary estimate of pore radius was made from inspection of
the points and knowing that when molecular radius is zero, \( C_I/C_P \)
should have a value of 1.0 and \( C_I/C_P \) should be zero when molecular
radius, \( a \), is equal to the radius of the pores. From this starting point,
values of \( \lambda \), the equilibrium partition coefficient of a molecule
between the solution within the pore and an open solution, and of \( \sigma \)
for all molecules with radii, \( a \), less than \( r \) can be calculated from the relations

\[
\lambda = (1 - a/r)^2 \quad (4)
\]

and

\[
\sigma = (1 - \lambda)^2 \quad (5)
\]

(see Ref. 7a).

For a population of pores of radius, \( r \), pore theory (7a) allows us
to write an expression for the Pécel number, \( P_e \), as

\[
P_e = \frac{r^2 \cdot \Delta P(1 - \sigma)}{8 \eta D \lambda f(a/r)} \quad (6)
\]

where \( \Delta P \) is the difference in hydrostatic and osmotic pressures
driving fluid through the capillary walls, \( \eta \) = the viscosity of the
solution within the pores, \( D \) = the free diffusion coefficient of the
solute in aqueous solution, \( a \) = the molecular radius, and \( f(a/r) \) is a
function of the ratio of molecular radius to pore radius that determines
the degree to which \( D \) is reduced in a pore of molecular dimensions
and is given by

\[
f(a/r) = 1 - 2.1((a/r) + 2.09(a/r)^3 - 0.95(a/r)^5) \quad (7)
\]

An initial estimate is made for \( \Delta P \) (e.g., between 2 and 10 cm
H\(_2\)O), and when this is substituted in Eq. 6, values of \( P_e \) for all
molecules with radii less than \( r \) can be calculated. The values of \( P_e \)
together with corresponding values for \( \sigma \) (from Eq. 5) can now be
used in Eq. 3 to define a theoretical relation between \( C_I/C_P \) and \( a \),
which can be compared with the values observed for the seven
nonsecreted proteins. As values of \( a \) approach \( r \), the
theoretical relation is dominated by \( \sigma \) and insensitive to
changes in \( P_e \), whereas the value of \( P_e \) determines the steepness of the decline
in \( C_I/C_P \) when \( a/r \) lies between 0.2 and 0.7. This makes it relatively easy
to adjust values of \( P_e \) and \( \sigma \) to obtain the best fit of the theoretical
curve to the observed values of \( C_I/C_P \) for the seven nonsecreted
proteins. Once this has been determined, the curve is used to estimate
the values of \( P_e \) corresponding to the secreted molecules. Assuming
that \( J_\nu = J_\nu \) and \( C_3 = C_L \), Eq. 2 can now be used to evaluate \( J_S \)
for each of the secreted proteins. Finally, substitution of \( J_S \) in Eq. 1
and the evaluation of \( J_\nu C_3 \) yields the value of \( J_{III} \).

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LYMPHATIC AND CAPILLARY TRANSPORT OF ADIPOKINES

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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