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SHORT COMMUNICATION

Reduced adipose tissue lymphatic drainage of macromolecules in obese subjects: a possible link between obesity and local tissue inflammation?

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The aim of this study was to investigate subcutaneous adipose tissue lymphatic drainage (ATLD) of macromolecules in lean and obese subjects and, furthermore, to evaluate whether ATLD may change in parallel with adipose tissue blood flow. Lean and obese male subjects were studied before and after an oral glucose load. Adipose-tissue blood flow was measured in the anterior subcutaneous abdominal adipose tissue by the ^{133}Xe-washout technique. ATLD was measured as the disappearance rate of ^{99m}Tc-labelled nanoaggregated human albumin, during fasting and after an oral glucose load. A significant increase in ATLD was seen after the glucose load in the lean subjects. In the obese subjects, ATLD remained constant throughout the study and was significantly lower compared to the lean subjects. These results indicate a reduced ability to remove macromolecules from the interstitial space through the lymphatic system in obese subjects. Furthermore, they suggest that postprandial changes in ATLD taking place in lean subjects are not observed in obese subjects. This may have a role in the development of obesity-related inflammation in hypertrophic adipose tissue.

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INTRODUCTION

Obesity is connected with a mild, chronic state of inflammation both at whole-body level and in adipose tissue. The inflammation in adipose tissue seems to have an important role in the development of cardiovascular and metabolic complications connected with obesity.[1] In the adipose tissue a vicious cycle seems to exist with adipocytes and macrophages producing various pro-inflammatory macromolecules (for example, cytokines and chemokines). These promote attraction and accumulation of further macrophages.[2] Some of these macromolecules are released to the systemic circulation in considerable amounts. This has been shown by the arterio-venous sampling technique. However, in the case of some cytokines like tumour necrosis factor-alpha, no net release can be measured by this technique.[3] On the contrary, a high interstitial concentration in the adipose tissue can be measured by the microdialysis technique.[4]

In a recent study by Miller *et al.*,[5] the role of lymphatic drainage in the removal of macromolecules from adipose tissue in lean subjects was investigated. Interestingly, the larger the molecular radius, the greater the proportion that is transported via the lymphatic system. Tumour necrosis factor-alpha, which has a molecular radius of 3.24 nm, was found to be transported away from the adipose tissue entirely via the lymphatic capillaries.

It has not been studied whether the ability to remove macromolecules from the interstitial space through the lymphatic system in the adipose tissue is changed in obese subjects. Therefore, our aim in this study was to elucidate whether abdominal subcutaneous adipose tissue lymphatic drainage (ATLD) of macromolecules is changed in obese subjects compared with lean subjects.

It is well known that the subcutaneous adipose tissue blood flow (ATBF) increases postprandially in lean subjects. The response is furthermore markedly blunted in obese subjects.[6] As the increase in lean subjects involves capillary recruitment, it is likely that this leads to an increased filtration of fluid into the interstitial space.[7] Most probably this increases interstitial pressure and thus lymphatic flow. Hence, another aim was to investigate whether the postprandial increase in abdominal subcutaneous ATBF is followed by an increase in ATLD.

MATERIALS AND METHODS

Subjects

Six lean, healthy men and six obese, but otherwise healthy men with normal glucose tolerance (determined by an oral glucose tolerance test) were studied (body mass index: 22.3 ± 1.2 and $35.7 \pm 4.5\,kg\,m^{-2}$, age: 22.1 ± 2.5 and 34 ± 8.1 years, respectively). The whole-body fat was determined by dual-energy X-ray absorptiometry ($17.2 \pm 2.4\%$ and $34.2 \pm 3.4\%$, respectively). The thickness of the periumbilical abdominal skinfold was measured with a Harpenden Skinfold Caliper (Baty International, Burgess Hill, UK) (15.4 ± 4.4 and 36.5 ± 12.0 mm, respectively).

The study was performed according to the Declaration of Helsinki II and was approved by the Scientific Ethics Committees of the Capital Region, Denmark (project no. H-3-2011-019).

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Protocol

After an overnight fast, the subjects were studied in supine position with the ambient temperature maintained at 24 °C. Anterior abdominal subcutaneous ATBF and ATLD were measured simultaneously during fasting (time 0) and for approximately 140 min after a 75-g oral glucose load.

Measurements

ATLD of macromolecules. To measure ATLD we used ^{99m}Tc-albumin-nanocolloid with a mean radius of 6–8 nm (Nanocoll, GE Healthcare, Milano, Italy). A volume of 1 MBq Nanocoll in 0.1 ml isotonic sodium chloride (containing 50 μg particles) was injected intradermally into the lower left-abdominal quadrant. The washout rate was measured continuously with a scintillation-counting device (Mediscint, Oakfield Instruments, Oxford, UK).[8]

Adipose tissue blood flow. ATBF was measured by the ^{133}Xe-washout technique. A volume of 1 MBq gaseous ^{133}Xe mixed in 0.1 ml air (The Hevesy Laboratory, Risø National Laboratory, Roskilde, Denmark) was injected subcutaneously into the upper right-abdominal quadrant. The washout rate was measured continuously with a scintillation-counting device (Mediscint, Oakfield Instruments, Oxford, UK).[9]

Calculations

ATLD of macromolecules. When the washout curve of the Nanocoll depot appeared to represent only the subcutaneous biological half-time of Nanocoll (removal through the lymphatic system) and the physical half-time of ^{99m}Tc, the fasting measurement was performed. By correcting for the decay of radioactive ^{99m}Tc, we achieved a mono-exponential curve, from which we calculated ATLD as the washout-rate constant (k) from the depot. The ATLD was calculated in 20-min periods.

Adipose tissue blood flow. ATBF was calculated by multiplication of the tissue-blood partition coefficient of ^{133}Xe (which was assumed to be 10 g ml^{-1}) and the monoexponential rate constant from the ^{133}Xe washout curve.[10] ATBF was calculated in 20-min periods.

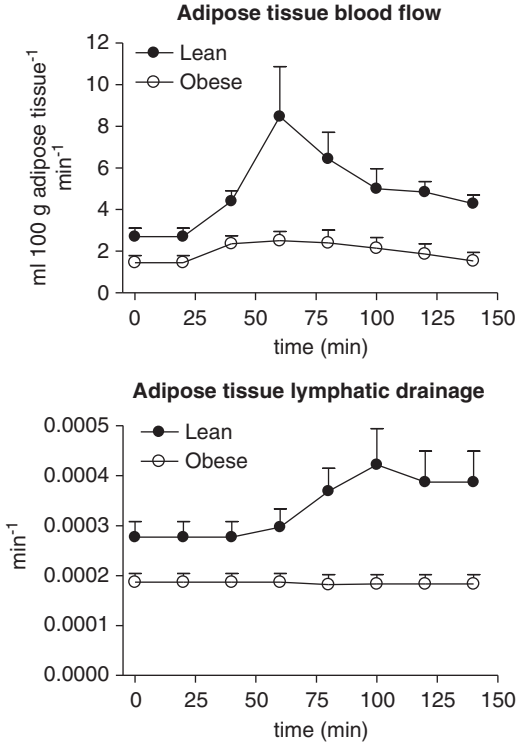

Figure 1. Time course of changes in adipose tissue blood flow (upper panel) and adipose tissue lymphatic drainage (lower panel) in lean and obese subjects after glucose intake. Mean ± s.e.m. are given.

Statistical analysis

t-test for unpaired data was used for comparing the groups. *t*-test for paired data was used for comparing different time points within the groups. Statistical significance was set at $P<0.05$. Data are expressed as mean ± s.d. unless otherwise stated.

RESULTS

Figure 1 shows the average time course of ATBF (upper panel) and ATLD (lower panel) in the two experimental groups. However, owing to a large interindividual variation in the time delay between glucose intake and change in ATBF and later ATLD, the average maximal individual values are given in Figure 2.

In the fasting state, ATBF was significantly higher in lean subjects compared to obese subjects (2.7 ± 1.0 and 1.5 ± 0.8 ml 100 g^{-1} min^{-1}, respectively, $P=0.03$). In the lean subjects the maximal ATBF increase took place about 40 min after the glucose intake (6.5 ± 5.3 ml 100 g^{-1} min^{-1}, $P=0.03$). In the obese subjects the ATBF increase peaked at a similar time, the maximal increase being 1.4 ± 0.6 ml 100 g^{-1} min^{-1} ($P<0.01$). This increase was significantly lower compared to the lean subjects ($P=0.04$) (Figure 2, upper panel).

In the fasting state ATLD was significantly lower in the obese subjects compared to the lean subjects (0.00019 ± 0.00004 and 0.00028 ± 0.00009 min^{-1}, respectively, $P=0.03$). In the lean subjects the increase in ATBF was followed by a significant increase in ATLD to 0.00047 ± 0.00015 min^{-1} ($P=0.01$). Despite the increase in ATBF, the ATLD in the obese subjects remained constant throughout the study (Figure 2, lower panel).

DISCUSSION

Here we report that the lymphatic removal of macromolecules from the anterior subcutaneous abdominal adipose tissue is significantly lower in the fasting state in obese subjects compared to lean subjects. In addition, the postprandial increase in adipose tissue blood flow is followed by an increase in the lymphatic

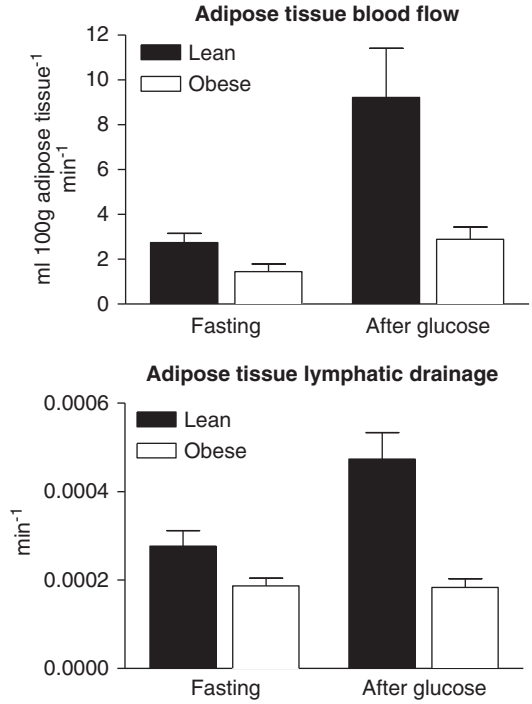

Figure 2. Fasting and glucose induced maximal adipose tissue blood flow (upper panel) and adipose tissue lymphatic drainage (lower panel) in lean and obese subjects. Mean ± s.e.m. are given.

removal of macromolecules from the subcutaneous abdominal adipose tissue in lean subjects, while this could not be observed in the obese subjects.

The nanoaggregated albumin (Nanocoll) is currently used for scintigraphic diagnosis of lymphoedema in lower and upper extremities. It is well validated with respect to the pharmacokinetics, as it is only removed by the lymphatic drainage when injected intradermally or subcutaneously.[8] The pathway for macromolecules away from the interstitial space involves movements between the extracellular matrix structures, movements into the initial lymphatic vessels and further into the proper lymphatic collectors.[8] Several sites in this pathway may give rise to restriction of the lymphatic drainage capacity. One restriction could be the re-modelling of the extracellular matrix. Such re-modelling has been demonstrated in hypertrophic adipose tissue in which especially elastin fibres are often broken down, and the collagen structure is changed in connection with development of fibrosis.[11,12] The reduced lymphatic removal of macromolecules found in the obese subjects compared to the lean subjects in the fasting state and the lack of meal-related changes in this removal could be due to such restrictions. We did not investigate the degree of fibrosis and possible loss of elasticity in the tissue in the present experiment.

Another mechanism that may influence the lymphatic removal of macromolecules from the interstitial space is the interstitial flow rate. This flow rate is dependent on the amount of fluid being extravasated from the adipose tissue capillaries, and thereby the pressure changes generated in the tissue.[13] In a recent study we have shown that the postprandial increase in ATBF involves capillary recruitment in lean subjects,[7] while this recruitment is blunted in obese subjects.[14] Capillary recruitment increases the effective capillary surface area, and thereby the capillary filtration coefficient increases. This mechanism may explain the delayed but significant increase in the lymphatic removal of macromolecules found in the lean subjects. As capillary recruitment is blunted in obese subjects under the applied experimental circumstances,[14] the modest increase in the adipose-tissue blood flow found in the present experiment most likely takes place in larger microvessels (thoroughfares?), which seem to be more abundant in hypertrophic adipose tissue at the expense of capillary area.[11] Therefore, we suggest that, in contrast to what takes place in lean subjects, in obese subjects the capillary filtration rate does not increase to the same extent postprandially, and thereby does not give rise to an increased interstitial flow rate.

The perspectives of the present findings are that both the basically reduced lymphatic drainage of macromolecules and the lack of postprandial changes in this drainage may give rise to a prolonged residence time for inflammatory macromolecules and extravasated macrophages in the adipose tissue. This may cause a high local production and thus increased release of small pro-inflammatory cytokines and chemokines to the systemic circulation via the adipose tissue venous drainage.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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