Human skeletal muscle releases leptin in vivo

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A B S T R A C T
Leptin is considered an adipokine, however, cultured myocytes have also been found to release leptin. Therefore, as proof-of-concept we investigated if human skeletal muscle synthesized leptin by measuring leptin in skeletal muscle biopsies. Following this, we quantified human skeletal muscle and adipose tissue leptin release in vivo.

We recruited 16 healthy male human participants. Catheters were inserted into the femoral artery and vein draining skeletal muscle, as well as an epigastric vein draining the abdominal subcutaneous adipose tissue. By combining the veno-arterial differences in plasma leptin with measurements of blood flow, leptin release from both tissues was quantified. To induce changes in leptin, the participants were infused with either saline or adrenaline in normo-physiological concentrations.

The presence of leptin in skeletal muscle was confirmed by western blotting. Leptin was released from leg skeletal muscle (50.6 ± 12 ng min⁻¹) and the pattern of release was different from subcutaneous adipose tissue. Moreover, during adrenaline infusion the leptin release from leg skeletal muscle was strongly suppressed (20.5 ± 7.9 ng min⁻¹, p < 0.017), whereas the release from fat was unaltered. During saline infusion the adipose tissue release averaged 0.8 ± 0.3 ng min⁻¹ 100 g tissue⁻¹ whereas skeletal muscle release was 0.5 ± 0.1 ng min⁻¹ 100 g tissue⁻¹.

In young healthy humans, skeletal muscle contribution to whole body leptin production could be substantial given the greater mass of muscle compared to fat. An understanding of the role that leptin plays in skeletal muscle metabolism may prove important in light of several late-phase trials with recombinant leptin as an anti-obesity drug.

1. Introduction

Obesity is recognized as a strong predictor of mortality and morbidity. This has fuelled interest in leptin and its centrally modulating effects on food intake and satiety [19,29] and has led to experiments with leptin supplementation to induce or maintain achieved weight loss by keeping leptin at pre-weight loss levels [29]. The finding that many obese people are hyperleptinaemic [18,22] does, however, add to the confusion of whether leptin is capable of producing and releasing leptin [20]. A change in substrate availability is believed to modulate the leptin release from adipose tissue, whereas substrate requirements would be the most likely stimulus for leptin secretion from skeletal muscle. Accordingly, a hyperglycaemic or hyperlipidaemic environment induces leptin secretion in myocytes in vitro [36]. The physiological significance of this finding in humans in health or disease has never been experimentally addressed, which in the light of several late-phase clinical studies may prove clinically important [30].
To study the role of leptin in human metabolism we investigated two different states. We used short-term fasting to look at basal rates of leptin release. In addition, one group was infused with adrenaline as it is known to suppress leptin concentrations acutely [12,16]. Both interventions were carried out avoiding any sudden disruptions in metabolism or major metabolic hormones.

As proof-of-concept that skeletal muscle synthesizes leptin, we used western blotting to confirm the presence of leptin in skeletal muscle biopsies. To quantify the release of leptin from human skeletal muscle and subcutaneous adipose tissue, we used a human in vivo model with veno-arterial differences across the leg and subcutaneous adipose tissue. The patterns of leptin release by skeletal muscle and adipose tissue were examined in response to either saline or adrenaline infusion.

We hypothesized that skeletal muscle is involved in leptin production and furthermore, that skeletal muscle leptin release responds to the adrenaline infusion.

2. Methods

2.1. Ethical approval

The Danish National Committee on Biomedical Research Ethics approved the protocol. The experiment was conducted in compliance with the rules of the Helsinki Declaration as revised in 2000. After giving their written and oral consent, subjects underwent a physical examination, parameters of health were examined in blood, and an ECG was recorded.

2.2. Subjects

We recruited eight non-smoking male subjects for each intervention, age: Control group: 26.0 ± 3.5 years of age, weight: 81.2 ± 10.3 kg, BMI: 24.7 ± 2.0 kg/m². Characteristics of the Adrenaline group were: age: 25.4 ± 3.2 years of age, weight: 72.6 ± 5.6 kg, BMI: 22.2 ± 1.4 kg/m². Subjects were enrolled if they were moderately physically active, medication free, had a balanced diet, and did not suffer from acute or chronic illness. Due to the invasive nature of the study and the technical challenges of placing the superficial epigastric venous catheter, a non-crossover design was chosen.

Body composition of fat and fat free mass was measured using a dual-energy X-ray absorptiometry (DXA) scanner (Lunar Prodigy, GE Medical Systems LUNAR, Prodigy Advance, Milwaukee, Wisconsin USA, software vers. 8.8). Subjects were scanned on the examination day by the same researcher, and for calculation of leg composition a line from symphysis pubis to the proximal part of the trochanter major femoris was used to define the lower extremity.

2.3. Experimental design

Having refrained from meal ingestion after 10:00 p.m. and exercised the previous day, subjects reported to the lab at 7:00 a.m. Subjects voided and remained supine, sedentary, and fasting for the remaining experimental day. Water was allowed ad libitum during the entire study.

The epigastrica superficialis vein and the femoral artery and vein were catheterized using the Seldinger technique as described by Frayn et al. [8] and van Hall et al. [34], respectively. Catheters were kept patent by slow infusion or regular flushing with saline (Arrow Intl Belgium, no.: CS-24301-E and Vygon France, pediatric arterial catheter no.: 115.11).

The catheter in the femoral vein was positioned distal to the confluence of the saphenous vein to ensure that the sampled blood represented skeletal muscle with minimal contamination from subcutaneous adipose tissue, as described by van Hall et al. [35]. A large antecubital vein was used for the infusion of adrenaline or saline. Subjects were infused with either adrenaline or saline for 4 h. Following the infusion, the subjects remained supine for another 2 h. During the experimental day, subjects had their blood pressure and heart rate monitored continuously (HP sphygmomanometer with brachial arm-cuff). Auricular temperature was monitored every hour. Blood was drawn every 30 min the first 3 h, after which blood was drawn every hour.

Adrenaline was infused at a rate of 10 ng kg⁻¹ min⁻¹. The infusion rate was chosen based on a pilot study and prior experience [16]. The adrenaline (Adrenalin “SAD”, 1 mg ml⁻¹, SAD, DK) was dissolved in saline.

2.4. Leg blood flow

Leg blood flow in the femoral artery was measured using the Doppler ultrasound method (CFM-800, Wingmed A/S, Norway) as described by Rådegrén [27], and corrected with leg oxygen extraction data. The calculation of blood velocity in the femoral artery was obtained by recording the Doppler signal at an angle of ultrasound insonation as small as possible to the flow vector to limit errors in measurements. Often only an angle of ~55° was achievable due to anatomical constraints. The true angle of insonation was computed for correction of the recorded Doppler signal (angle correction). As the angle of insonation has a much lower intra-individual than inter-individual variability, we chose to use the Doppler signal as a measure of changes from baseline. To get quantitative measures of leg blood flow, flow was determined using a set value of 5% oxygen extraction rate from the blood during a single pass through the leg. This value corresponded very well to observations carried out at the Copenhagen Centre of Muscle Research (data not shown). By using indirect calorimetry (Quark b², Cosmed, Italy) whole body oxygen consumption at rest was recorded. Using 5% of this value and determining the volume of oxygen extraction across the leg with simultaneous blood sampling (ABL 615, Radiometer, Copenhagen, Denmark), a baseline blood flow was determined:

Baseline leg blood flow: \( Q_{\text{fem}}(\text{ABL}) = \frac{\text{VO}_{2} \times 5\%}{\text{leg}[O_{2\text{art}}] - [O_{2\text{vein}}]} \)

By using the baseline Doppler flow measurement as an index 1, the delta changes over time were multiplied with the quantified baseline leg blood flow from the blood gas measurements:

\[
\begin{align*}
\text{Leg blood flow: } Q(t) &= Q_{\text{fem}}(\text{ABL}) \times \frac{Q(t)}{Q(t)} \\
&= Q_{\text{fem}}(\text{ABL}) \times \frac{Q(t)}{Q(t)}
\end{align*}
\]

where \( Q(t) \) is the leg blood flow at time \( t \), \( Q_{\text{fem}}(\text{ABL}) \) is the baseline leg flow obtained by ABL and indirect calorimetry measurements, and \( Q_{\text{fem}}(\text{ABL}) \) are the Doppler recorded flows at time \( t \) and baseline.

2.5. Adipose tissue blood flow

Abdominal adipose tissue blood flow was measured using the 133-Xenon washout method [2,15]. A tissue/blood partition coefficient (lambda) for xenon of 8 was used [2]. Data was analyzed using GammaScan software (vers. 1.20, FB Engineering, Denmark). Plasma flow for both methods was obtained by using haematocrit values:

\[
\text{Plasma flow} = \text{blood flow} \times (1 - \text{Hct})
\]
2.6. Analytical methods

Blood was drawn into pre-chilled tubes containing EDTA and immediately spun at 3500 g for 10 min at 4 °C before they were frozen at −80 °C until analyses. Blood from all three catheters was collected into syringes with heparin (80 IU) (PICC050, Radiometer, Copenhagen, Denmark) and analyzed immediately for glucose (ABL 615, Radiometer, Copenhagen, Denmark).

Skeletal muscle biopsies from the vastus lateralis were obtained by the percutaneous needle biopsy method at baseline, 1 h into the infusion, and 2 h after the cessation of infusion. Blood and visible connective tissue were quickly removed from the biopsies before being frozen in liquid nitrogen and stored at −80 °C until analyses.

Adrenaline and glucagon were measured in duplicate using commercially available ELISA kits (DakoCytomation, Cambridgeshire, UK (interassay CV of 5.6%, and an intraassay CV of 14.0%), and Linco Research, MI, USA (interassay CV of 13.5% and an intraassay CV of 6.8%), respectively.

Insulin and leptin were measured in duplicate using commercially available ELISA kits (DakoCytomation, Cambridgeshire, UK (interassay CV of 8.9% and an intraassay CV of 7.5%) and R&D systems, MD, USA (interassay CV of 3.5% and an intraassay CV of 3.2%), respectively).

2.7. Western Blotting

Human muscle samples were homogenized in lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 50 mM NaF, 5 mM NaP, pH 7.4.) using a TissueLyser (Qiagen, CA, USA). Phosphatase inhibitor cocktail 1 and 2 (Sigma Aldrich, MO, USA), 3 mM NaV, 1 mM DTT, 0.2% Ipegal-CA-630 (Sigma Aldrich, MO, USA) and protease inhibitor complete mini (Roche, Mannheim, Germany) was added to the buffer immediately before homogenization. Following homogenization, protein lysates were centrifuged at 13,000 g for 15 min at 4 °C and the pellet was discarded.

Protein concentration was measured using a colorimetric protein assay (Bio-Rad, Copenhagen, Denmark). Samples were mixed with 5 × Laemmli buffer and boiled for 5 min before subsequent loading of 15 μg onto a 4–12% gradient bis-Tris NuPage gel (Invitrogen, CA, USA). The gel was run for 1 h and 30 min at 125 V and protein was transferred onto a PVDF membrane using a semi-dry blotting system for 1 h at 20 V (Invitrogen, CA, USA). The membrane was blocked for 1 h at room temperature in 5% skimmed milk. Incubation with primary antibody (anti-leptin ab-2 PA1–052, Thermo Fischer Scientific, IL, USA) took place overnight at 4 °C. Blots were washed in TBS-T and incubated with an anti-rabbit IgG HRP conjugated antibody (1:5000, Dako, Copenhagen, Denmark) for 1 h at room temperature. Specific signal was detected using Supersignal West Femto Luminal/Enhancer Solution (Thermo Fischer Scientific, IL, USA) and subsequent exposure in a charge-coupled device camera (Bio-Rad, Copenhagen, Denmark). Following exposure, blots were briefly rinsed in TBS-T and then incubated in 0.5% Reactive Brown (Sigma Aldrich, MO, USA) for 15 min. Blots were analyzed and quantified using ImageQuant software, with the reactive brown image as a control for equal loading and transfer.

2.8. Calculations

Leptin release from adipose tissue and skeletal muscle was calculated as:

\[
\text{Net leptin release} = (\text{leptin}_{\text{venous}} - \text{leptin}_{\text{arterial}}) \times \text{plasma flow}
\]

Blood was sampled from the femoral vein to measure leptin release from skeletal muscle, while blood was sampled from the superior epigastric vein to measure leptin release from adipose tissue.

Leptin release per mass unit of muscle in leg was calculated as:

\[
\text{Net leptin release per mass unit of muscle} = \frac{\text{net leptin leg release}}{\text{Leg Fat Free Mass (FFM)}}
\]

2.9. Statistical analyses

All values are given as mean ± SEM, except subject characteristics, which are presented as mean ± SD. The following data were transformed to achieve normality: adipose blood flow, leptin concentrations, adipose leptin flux, V-A differences, and glucagon. Transformed data are presented as geometric mean ± SEM. Changes between treatments (time × treatment) and over time (time) were tested with a RM-ANOVA. Differences in leptin concentrations within subjects over time were tested using a paired Student’s t-test, whereas differences between groups were tested using an unpaired design. A probability level of 5% was chosen as being significant.

3. Results

3.1. Body composition and regional blood flow

The two intervention groups were comparable with regard to their whole body fat mass, Control group: 16.7 ± 8.0% and Adrenaline group: 12.8 ± 5.1% (p = 0.27), and leg fat mass, Control group: 19.7 ± 7.1% and Adrenaline group: 15.7 ± 6.1% (p = 0.25).

Over the course of the day, the average blood flow to the leg remained stable in the Control group and was 385 ± 51 ml min⁻¹. There was an effect of the adrenaline infusion on the blood flow to the leg (p < 0.05) averaging 349 ± 42 ml min⁻¹ (Table 1).

Adipose blood flow increased during the course of the day (p < 0.002) and in response to the adrenaline infusion (p < 0.0001). The average blood flow during the entire day was 2.65 ± 0.43 ml min⁻¹ 100 g tissue⁻¹ in the Control group and 4.22 ± 0.81 ml min⁻¹ 100 g tissue⁻¹ in the Adrenaline group (p < 0.001)(Table 1).

3.2. Skeletal muscle leptin protein

Leptin was present in skeletal muscle from both the Control and the Adrenaline group in all biopsy samples (Fig. 1). Leptin protein concentrations, as visualized by band densities, did not differ significantly with regard to intervention or over time.

3.3. Leptin concentrations and tissue release during saline infusion

The leg vein displayed a positive veno-arterial output (V-A), indicating a net release from skeletal muscle to the systemic circulation (p < 0.0001). The average difference in V-A concentration was 248.0 ± 64.1 pg ml⁻¹ between leg venous and arterial blood (p < 0.0001).

Likewise, the abdominal adipose tissue vein displayed a positive V-A output, indicating a net release from the adipose tissue to the systemic circulation (p < 0.0001). The average difference in the leptin concentration between adipose tissue venous and systemic (arterial) blood was 548.0 ± 132 pg ml⁻¹ (p < 0.0001).

Leptin was released from abdominal subcutaneous adipose tissue and the release did not change during the experimental day (p = 0.21). In contrast, the release from skeletal muscle increased during the day (p < 0.001) (Figs. 3 and 4). Combining the leg composition data obtained from the DXA scan, with the release rate of leptin from the leg in the Control group, the release rate of leptin
3.4. Effect of adrenaline on leptin concentrations and tissue release

Adrenaline was infused for 4 h, increasing systemic adrenaline concentrations 4–5-fold. There was an expected difference in adrenaline concentrations \( p < 0.005 \) between the Control and Adrenaline groups. The average adrenaline concentration throughout the trial was \( 0.29 \pm 0.03 \) ng ml\(^{-1}\) in the Control group and \( 0.77 \pm 0.11 \) ng ml\(^{-1}\) in the Adrenaline group \( p < 0.0001 \) (Table 1).

Adrenaline infusion suppressed systemic leptin concentrations \( p < 0.02 \) (Fig. 2). Veno-arterial adipose tissue leptin differed between interventions \( p = 0.0013 \), whereas the leptin release from adipose tissue was not affected by the adrenaline infusion \( p = 0.49 \) (Fig. 3). The average leptin release rate from adipose tissue during the day was \( 752 \pm 276 \) pg min 100 g tissue\(^{-1}\) in the Control group and \( 807 \pm 174 \) pg min 100 g tissue\(^{-1}\) in the Adrenaline group \( p = 0.50 \) (Fig. 3). Conversely, the leg leptin vено-arterial difference \( p < 0.005 \) and release \( p = 0.017 \) from skeletal muscle was significantly suppressed by the adrenaline infusion. The average leptin release rate from skeletal muscle during the day was \( 50.6 \pm 12.0 \) ng min\(^{-1}\) in the Control group and \( 20.5 \pm 7.9 \) ng min\(^{-1}\) in the Adrenaline group (Fig. 4).

3.5. Hormonal and metabolic changes

Glucose decreased during the trial \( p < 0.0001 \) in the Control group. There was a significant increase in blood glucose following

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### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>End-of-infusion</th>
<th>2-h post inf.</th>
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</thead>
<tbody>
<tr>
<td><strong>Blood flow</strong></td>
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<tr>
<td>Leg blood flow (ml min(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (saline)</td>
<td>351 ± 43</td>
<td>386 ± 55</td>
<td>250 ± 50</td>
</tr>
<tr>
<td>A</td>
<td>352 ± 24</td>
<td>398 ± 50</td>
<td>384 ± 58</td>
</tr>
<tr>
<td>Adipose tissue blood flow (ml min(^{-1})100 g tissue(^{-1}))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (saline)</td>
<td>2.71 ± 0.63</td>
<td>4.34 ± 0.77&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.21 ± 0.55</td>
</tr>
<tr>
<td>A</td>
<td>2.53 ± 0.29</td>
<td>2.46 ± 0.50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.10 ± 0.58</td>
</tr>
<tr>
<td>Glucose (mmol l(^{-1}))</td>
<td></td>
<td></td>
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<tr>
<td>Control (saline)</td>
<td>5.0 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>4.8 ± 0.1</td>
</tr>
<tr>
<td>A</td>
<td>5.2 ± 0.2</td>
<td>5.4 ± 0.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.8 ± 0.2</td>
</tr>
<tr>
<td>Insulin (pmol l(^{-1}))</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control (saline)</td>
<td>19.9 ± 2.4</td>
<td>22.3 ± 2.1</td>
<td>25.7 ± 3.3</td>
</tr>
<tr>
<td>A</td>
<td>23.8 ± 2.7</td>
<td>26.9 ± 2.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>19.7 ± 2.2</td>
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<tr>
<td>Glucagon (pg ml(^{-1}))</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control (saline)</td>
<td>46.9 ± 8.3</td>
<td>44.8 ± 11.0</td>
<td>46.6 ± 11.0</td>
</tr>
<tr>
<td>A</td>
<td>60.0 ± 8.1</td>
<td>67.4 ± 5.0</td>
<td>67.4 ± 9.4</td>
</tr>
</tbody>
</table>

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Blood flow, glucose, and hormone data at baseline, end-of-infusion (4-h), and 2-h after cessation of infusion. Values are mean ± SEM, or geometric mean ± SEM. A – Adrenaline infused group \( n = 8 \). C – Control group, saline infused \( n = 8 \). Letters in superscript denote a significant treatment effect of the adrenaline infusion.

- \( a \) \( p < 0.005 \).
- \( b \) \( p < 0.05 \).
- \( c \) \( p < 0.0002 \).
- \( d \) \( p < 0.0001 \).
- \( e \) \( p < 0.002 \).
Differences were affected by the adrenaline infusion (p<0.0001), most likely due to stimulation of glycogenolysis. The average blood glucose concentration was 4.9 ± 0.0 mmol l⁻¹ in the Control group and 5.4 ± 0.1 mmol l⁻¹ in the Adrenaline group (p=0.0001) (Table 1).

Insulin also increased during the course of the day (p<0.003) and during adrenaline infusion (p<0.002), most likely in response to the adrenergically-induced increase in blood glucose. The average insulin concentration throughout the day was 22.6 ± 1.1 pmol l⁻¹ in the Control group and 24.3 ± 1.4 pmol l⁻¹ in the Adrenaline group (p=0.33), indicating that insulin was only slightly affected by the adrenaline infusion (Table 1). Glucagon was not different between groups (p=0.49) and did not change over time (p=0.07). The concentration averaged 49.0 ± 4.0 pg ml⁻¹ in the Control group and 67.0 ± 2.8 pg ml⁻¹ in the Adrenaline group (Table 1).

4. Discussion

The key findings of this study are that (1) leptin protein is present in human skeletal muscle in vivo; (2) healthy individuals release leptin in appreciable amounts from skeletal muscle. The adipose tissue release averaged 0.8 ± 0.3 ng min⁻¹100 g tissue⁻¹ in the Control group, whereas the measured leptin release from skeletal muscle was 0.5 ± 0.1 ng min⁻¹100 g tissue⁻¹. This suggests that skeletal muscle contribution to whole body leptin production could be substantial given the greater mass of muscle compared to fat in the population studied. To our knowledge this is the first quantitative measure of leptin release from skeletal muscle in the literature. The notion that leptin may be produced by skeletal muscle fibers is supported by Wang et al. [36] who showed that leptin was expressed and secreted by myocytes in vitro, and that it was stimulated through a hexosamine energy-sensing pathway. In accordance, Lappas et al. [20] noticed a release of leptin from incubated muscle extracted from women undergoing caesarean section. Clearly, these results were obtained in a milieu physiologically distinct from the human body.

Similar to our study, Jensen and co-workers [14] detected leptin release from the leg, but they ascribed it to subcutaneous and intramuscular fat in the leg and not to skeletal muscle.

In contrast to the design of Jensen et al. [14], we sampled leg blood originating primarily from skeletal muscle, an assumption based on our catheterization method. In comparison, the normally used proximal positioning of the femoral venous catheter will in addition to blood from skeletal muscle, sample blood originating from small veins draining lower abdominal adipose tissue. This has been demonstrated by van Hall et al. [35]. However, when placing our femoral venous catheter in a distal direction, the tip of the catheter bypasses the confluence of blood from the saphenous vein, making the sampling representative of skeletal muscle. Two subjects not included in the study had their femoral venous catheter placed near or in the saphenous vein. This was very
apparent when viewing the uptake of fatty acids. In all other subjects there was a constant uptake of fatty acids into the muscle (average \( -5.9 \pm 2.0 \, \text{nmol min}^{-1} \)), whereas the two excluded subjects displayed a constant release of fatty acids (average \( 12.7 \pm 5.4 \, \text{nmol min}^{-1} \)).

As we cannot rule out *per se* that adipose tissue in the leg contributed to the leptin release, we performed a “worst-case” approximation. By using the release rate of leptin from the abdominal adipose tissue and applying it to the leg fat mass measured by DXA scan, the share of leptin released by the leg fat mass only averaged 42% of the total leg leptin release. As mentioned earlier, we assume that our catheter placement bypasses most of the adipose contribution. Our sampling does include blood from fat interspersed in the muscle, but studies have shown that it constitutes a minor fraction in the skeletal muscle compartment of young healthy men [1,11]. Furthermore, the DXA scan does not discriminate between inert fat droplets in skeletal muscle that do not release leptin and fat stored in leptin-secreting adipocytes. If these biases are considered it would further increase the percentage of skeletal muscle contribution to the leptin release from leg. Conclusively, we believe that our sampling from the femoral vein is representative of skeletal muscle and our main findings are supported by the “worst-case” assumptions. Another finding that supports the above assumptions is that leptin protein was found in all skeletal muscle biopsies sampled.

Previously, leptin has been correlated to fat mass [23], which seems reasonable in light of the classification as an adipokine. However, systemic leptin concentration drops during fasting [38] and leptin is released in a pulsatile fashion [6]. In the present study we saw a 38% decrease in systemic leptin concentrations during the experimental day, which obviously does not equal the fasting-induced utilization of fat stores. This suggests that leptin is not directly related to fat mass, as also whole body nutrient status influences the relationship between fat mass and leptin concentration and could explain why insulin resistant individuals have higher leptin concentrations than healthy weight- and body composition-matched individuals [31]. Another finding that would contradict a simple relationship between fat mass and leptin is that leptin concentrations do not always mirror a weight loss in adults or children [3,7,13]. Importantly, our results on leptin release from muscle were obtained from young healthy males. As the exact stimulus, and hence function of leptin in human muscle is still unknown, our results may not be applicable to patients suffering from metabolic disturbances such as anorexia nervosa or lipatropathy who display low levels of circulating leptin.

Our rate of release from adipose tissue at baseline was 0.9 ± 0.4 \( \text{ng} \, \text{min}^{-1} \, \text{100 g tissue}^{-1} \). In comparison Goossens et al. [12] reported a release rate of 1.7 (0.6–2.1) \( \text{ng} \, \text{min}^{-1} \, \text{100 g tissue}^{-1} \). As such the release rates correspond, and the minor differences could be ascribed to different methods of measuring leptin (RIA vs. ELISA), calculation of adipose blood flow, and differences in the populations studied (body composition and age).

By infusing adrenaline we acutely depressed leptin concentrations. Adrenaline has been shown to suppress systemic concentrations of leptin, most likely through a direct mechanism [12,16,28]. We aimed at a adrenaline concentration mimicking the peak concentrations observed during exercise [10,32,37]. This was done to minimize secondary counter-regulatory responses (e.g. insulin and glucagon) that could potentially cloud the effect on leptin. Unaffected heart rate, blood pressure, and body temperature (data not shown) during the intervention further underlined our attempt to elicit minor secondary effects, a point of concern in previous studies [12].

Leg and adipose tissue clearly responded differently to the adrenaline infusion. Whereas the leg release was suppressed, the abdominal adipose tissue release was unaffected. Thus, our data support the notion of a differential tissue release of leptin. It seems unlikely that adipose tissue located in the leg and the anterior abdominal surface would react differently to adrenaline, reiterating that the observed leptin release from the leg originates from skeletal muscle. Another reason for the apparent suppressed release of leptin from skeletal muscle during the adrenaline infusion could be an increased extraction of leptin by skeletal muscle. The current study design was not able to discriminate between an increase in extraction and/or a decrease in leptin release as a result of the adrenaline infusion.

Carulli et al. [5] infused adrenaline into humans and found suppressed expression of leptin in adipose tissue. In contrast, when Keller and co-workers [16] infused adrenaline or made subjects perform exercise, they noted a decrease in the systemic leptin levels in line with our findings, but they could not detect any concomitant changes in leptin mRNA levels obtained from adipose tissue biopsies [16]. In accordance, Goossens et al. [12], found a similar decrease in systemic leptin following adrenaline infusion, but did not find any change in the leptin release from the abdominal adipose tissue. Our observation of a suppressed skeletal muscle release during an adrenaline infusion would provide an explanation for the apparent discrepancy between suppressed systemic leptin concentrations and unchanged release rates and dissociated leptin expression from abdominal adipose tissue and would certainly support the importance of skeletal muscle as a source of leptin.

The release of leptin from skeletal muscle into the circulation can be part of a muscle-brain cross talk, similar to the believed function of leptin released from adipose tissue. Alternatively, leptin could have autocrine properties in the muscle and the release into the systemic circulation could be a spillover from the skeletal muscle compartment. Leptin receptors are found in skeletal muscle [9] and leptin has been shown to modulate peripheral metabolism directly in humans, rodent and in *in vitro* studies [33,39]. Leptin elicits an increase in \( \beta \)-oxidation and reduces esterification of free fatty acids [25,39]. In short, leptin partitions the fatty acids away from storage in skeletal muscle and into utilization [39].

Whereas the adipose tissue leptin release did not differ over time, the release from skeletal muscle increased over time in our study. This could be a counter-regulatory mechanism preventing lipid accumulation inside the myocellular compartment, as the free fatty acid concentrations in plasma steadily increase during fasting (data not shown). This notion would attribute the role of skeletal muscle-released leptin as an autocrine hormone, working to counter-balance the impact of fatty-acid fluctuations on skeletal muscle. In accordance, Wang et al. [36] showed how leptin expression was increased in rodent skeletal muscle following induced hyperlipidaemia. This would be in line with clinical observations that leptin supplementation tends to normalize the insulin resistance and dyslipidemia seen in HIV lipodystrophic patients [21,24] and reduces hepatic steatosis in Congenital Leptin Deficiency patients [4]. Both diseases involve ectopic fat deposition that can be linked to insulin resistance [17].

The idea of skeletal muscle as an endocrine organ has surfaced in recent years with the advent of Interleukin-6 (IL-6) being characterised as a “myokine” [26]. Leptin is classified as an adipokine in the scientific literature. However, from this study it appears that leptin may also be a myokine.

### 5. Conclusion

The current study was able to confirm the presence of leptin protein in muscle biopsies and quantify the release and compare it with adipose tissue release. We found a clear and constant release from skeletal muscle and observed a response to adrenaline
that differed between the two organs. The leptin release from muscle may cumulatively be substantial and contribute to whole body leptin production.

Authors contribution
EW designed and conducted the study, analyzed data, and prepared the manuscript. HM designed and conducted the study. TSG conducted the study and analyzed data. GvH designed the study, analyzed data and prepared the manuscript. BKP designed the study and prepared the manuscript.

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References