Eur J Appl Physiol (2014) 114:345–357
DOI 10.1007/s00421-013-2779-4

ORIGINAL ARTICLE

Leptin signaling in skeletal muscle after bed rest in healthy humans

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Received: 18 June 2013 / Accepted: 18 November 2013 / Published online: 29 November 2013

Abstract
Purpose This study aimed at determining the effects of bed rest on the skeletal muscle leptin signaling system.
Methods Deltoid and vastus lateralis muscle biopsies and blood samples were obtained from 12 healthy young men (mean ± SD, BMI 22.8 ± 2.7 kg/m^2) before and after 7 days of bed rest. Leptin receptor isoforms (OB-Rs), suppressor of cytokine signaling 3 (SOCS3) and protein tyrosine phosphatase 1B (PTP1B) protein expression and signal transducer and activator of transcription 3 (STAT3) phosphorylation were analyzed by Western blot.
Results After bed rest basal insulin concentration was increased by 53 % ($P < 0.05$), the homeostasis model assessment (HOMA) by 40 % ($P < 0.05$), and serum leptin concentration by 35 % ($P < 0.05$) with no changes in body fat mass. Although the soluble isoform of the leptin receptor (s-OBR) remained unchanged, the molar excess of leptin over sOB-R was increased by 1.4-fold after bed rest ($P < 0.05$). OB-Rs and SOCS3 protein expression, and STAT3 phosphorylation level remained unaffected in deltoid and vastus lateralis by bed rest, as PTP1B in the deltoid. PTP1B was increased by 90 % with bed rest in the vastus lateralis ($P < 0.05$). There was a linear relationship between the increase in vastus lateralis PTP1B and the increase in both basal insulin concentrations ($r = 0.66$, $P < 0.05$) and HOMA ($r = 0.68$, $P < 0.05$) with bed rest.
Conclusions One week of bed rest is associated with increased leptin levels without augmenting STAT3 phosphorylation indicating some degree of leptin resistance in skeletal muscle, which can be explained, at least in part, by an elevation of PTP1B protein content in the vastus lateralis muscle.

Communicated by Jean-René Lacour.

The human experiments were conducted at Copenhagen Muscle Research Centre, Rigshospitalet, Copenhagen, Denmark.

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Keywords Leptin receptors · Leptin signaling · Skeletal muscle · Bed rest · SOCS3 · PTP1B

Abbreviations

AMPK 5′	Adenosine monophosphate-activated protein kinase
AUC	Area under curve
BMI	Body mass index
DEXA	Dual-energy X-ray absorbtiometry
ELISA	Enzyme-linked immunosorbent assay
FLI	Molar excess of leptin over s-OBR
HOMA	Homeostatic model assessment of insulin resistance
JAK2	Janus kinase 2
OB-R128	Leptin receptor isoform of 128 kDa
OB-R170	Leptin receptor isoform of 170 kDa
OB-R98	Leptin receptor isoform of 98 kDa
OB-Rb	Long isoform of the leptin receptor
OB-Rs	Leptin receptor isoforms
OGTT	Oral glucose tolerance test
PTP1B	Protein tyrosine phosphatase 1B
SIRT1	Sirtuin 1
s-OBR	Soluble isoform of the leptin receptor
SOCS3	Suppressor of cytokine signaling 3
STAT3	Signal transducer and activator of transcription 3
TBS-T	Tris-buffered saline with 0.1 % Tween 20
VO_{2max}	Maximal oxygen consumption or maximal oxygen uptake

Introduction

Sedentary lifestyle has been associated with obesity, glucose intolerance, insulin resistance and leptin resistance (Krawczewski Carhuatanta et al. 2011; Krogh-Madsen et al. 2010; Must et al. 1999; Myers et al. 2008; Pedersen 2007; Steinberg et al. 2002). However, the molecular mechanisms by which physical inactivity induces leptin resistance remain unknown. Recently, we have shown that just 7 days of bed rest induce inflammation and muscle insulin resistance (Bienso et al. 2012; Bosutti et al. 2008; Ringholm et al. 2011). However, it remains unknown whether physical inactivity induces muscle leptin resistance in humans.

Human skeletal muscle expresses both long and short isoforms of the leptin receptor (OB-Rs) (Guerra et al. 2007, 2008). Upon binding to the long isoform of the leptin receptor (OB-Rb), leptin stimulates janus kinase 2 (JAK2), which autophosphorylates and phosphorylates several tyrosine residues of OB-Rb (Bjørbæk and Kahn 2004) (Fig. 1). The signal transducer and activator of transcription 3 (STAT3) binds to the phosphorylated Tyr1138 in OB-Rb,

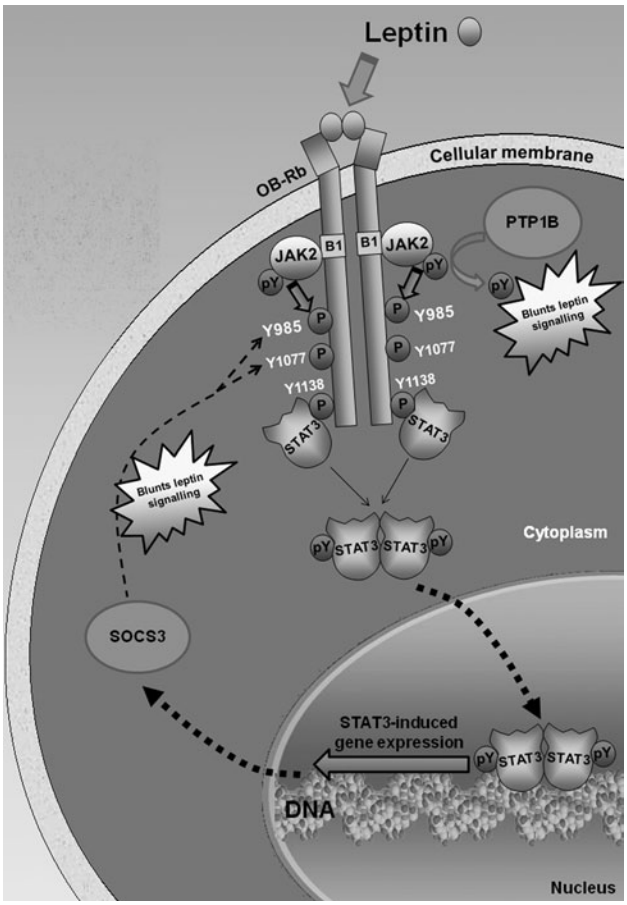

Fig. 1 Signaling pathway activated by leptin through the long isoform of the leptin receptor (OB-Rb). Upon binding to OB-Rb, leptin stimulates Janus kinase 2 (JAK2), which autophosphorylates in tyrosine residues ($Y^{1007/1008}$) and phosphorylates several tyrosine residues in the cytoplasmic domain of OB-Rb (Y^{985}, Y^{1077} and Y^{1138}). The signal transducer and activator of transcription 3 (STAT3) binds to the phosphorylated Y^{1138} and then is phosphorytaled (Y^{705}) and activated by JAK2. Activated STAT3 dimerizes and translocates to the nucleus where it modulates gene expression. Chronically augmented leptin concentrations can stimulate the expression of protein suppressor of cytokine signaling 3 (SOCS3), which binds to Y^{985} and Y^{1077} and blunts JAK/STAT3-dependent leptin signaling. The protein tyrosine phosphatase 1B (PTP1B) is also able to blunt leptin signaling through dephosphorylation of the leptin receptor-associated JAK2

and this interaction is required for tyrosine phosphorylation and activation of STAT3 by JAK2 (Bates et al. 2003) (Fig. 1). Tyr1138-mediated phosphorylation of STAT3 on Tyr705 is required for leptin regulation of energy balance and body weight (Bates et al. 2003) (Fig. 1).

Leptin sensitivity in skeletal muscle can be enhanced by exercise (Olmedillas et al. 2011; Steinberg et al. 2002) and may, therefore, be decreased by physical inactivity. In accordance, bed rest has been associated with increased leptin concentrations, suggesting leptin resistance (Blanc et al. 2000). A mechanism that may explain the potential muscle leptin resistance induced by physical inactivity

is a down-regulation of OB-Rs. In fact, we have recently shown that OB-Rb protein expression is reduced in deltoid and vastus lateralis muscles of obese human (Fuentes et al. 2010). This OB-Rb down-regulation may be responsible, at least in part, for the peripheral leptin resistance typically observed in sedentarism and obesity (Fuentes et al. 2010). This hypothetically negative influence of physical inactivity on skeletal muscle leptin sensitivity may be accentuated in muscles submitted to a high daily use, like the leg muscles, compared with the muscles used more intermittently, like the arm muscles.

Leptin resistance may be also caused by desensitization of OB-Rs, among other mechanisms. In skeletal muscle, chronic augmented leptin concentrations may induce muscle leptin resistance by increasing the expression of protein suppressor of cytokine signaling 3 (SOCS3), which blunts JAK2/STAT3-dependent leptin signaling (Jorgensen et al. 2013; Steinberg et al. 2006) (Fig. 1). Up-regulation of SOCS3 has been observed in vastus lateralis but not in deltoid of humans with obesity (Fuentes et al. 2010). The protein tyrosine phosphatase 1B (PTP1B) may also cause leptin resistance in skeletal muscle (Cheng et al. 2002) by blunting leptin signaling through dephosphorylation of the leptin receptor-associated JAK2 (Dube and Tremblay 2005; Hosoi et al. 2008; St-Pierre and Tremblay 2012) (Fig. 1). PTP1B is also able to down-regulate insulin signaling through dephosphorylation of insulin receptor and insulin receptor substrate 1, which is also activated by leptin through OB-Rb (Dube and Tremblay 2005). Moreover, reduced STAT3 phosphorylation in the presence of chronically increased leptin concentrations (obesity) indicates leptin resistance (Hosoi et al. 2008). In contrast, leptin administration causes a marked muscle STAT3 phosphorylation in healthy humans (Wolsk et al. 2011).

To investigate the effect of physical inactivity on muscle leptin sensitivity, 12 young healthy volunteers were studied before and after 1 week of bed rest. Key signaling proteins involved in the leptin signaling pathway were determined in deltoid and vastus lateralis muscle biopsies obtained before and after bed rest. We aimed at testing the hypothesis that bed rest causes leptin resistance by inducing a down-regulation of OB-R, up-regulation of SOCS3/PTP1B protein expression and reduced STAT3 phosphorylation in the resting human skeletal muscle and that these effects are accentuated in leg compared with arm muscles.

Materials

The complete protease inhibitor cocktail and the PhosphoStop phosphatase inhibitor were obtained from Roche Diagnostics (Mannheim, Germany; #04693116001 and #04906845001, respectively). The polyclonal rabbit anti-human leptin receptor antibody that is raised against the extracellular domain of the human leptin receptor was obtained from Linco Research (St. Charles, Missouri, USA; #4781-L). The polyclonal rabbit anti-human SOCS3 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA; #sc-9023). The monoclonal mouse anti-alpha-tubulin antibody was obtained from Biosigma (Madrid, Spain; #T5168). The monoclonal mouse anti-PTP1B antibody was obtained from Calbiochem (Darmstadt, Germany; #FG6-1G). The polyclonal rabbit anti-Tyr^{705}-STAT3 and the monoclonal mouse anti-STAT3 antibodies were from Cell Signaling Technology (Danvers, MA, USA; #9145 and #9242, respectively). The secondary HRP-conjugated goat anti-rabbit and donkey anti-mouse antibodies were from Jackson ImmunoResearch (West Grove, PA, USA; #111-035-144 and #715-035-150, respectively). The Hybond-P transfer membranes, Hyperfilm ECL and the ECL plus Western Blotting Detection System were from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK). The ChemiDoc XRS System and the image analysis software Quantity One© were obtained from Bio-Rad Laboratories (Hemel Hempstead Hertfordshire, UK).

Methods

Subjects

Twelve young, healthy male subjects with an average (mean ± SD) age 26.2 ± 5.3 year, weight 75.2 ± 11.04 kg, height 181.7 ± 6.1 cm, body mass index 22.7 ± 2.6 and VO_{2max} 52 ± 8 ml kg^{-1} min^{-1} (measured during an incremental exercise test to exhaustion on a cycle ergometer) participated in this study. The subjects were physically active, as they all walked or bicycled for >1 h/day. The subjects were given both written and oral information about the experimental protocol and procedures and were informed about potential risk before they gave their written consent. The study was performed according to the Declaration of Helsinki (2008) and approved by the Copenhagen and Frederiksberg Ethics Committee, Denmark (H-A-2008-0024). This study has previously formed the basis for three publications (Bienso et al. 2012; Kiilerich et al. 2011; Ringholm et al. 2011).

Bed rest

Seven days of bed rest were used as a physical inactivity intervention. The subjects were placed in hospital beds with manual head and leg elevation adjustments. During the bed rest period, the subjects were allowed to sit up for 5 h per day and they were at all times transported in a wheelchair. During the bed rest period, subjects were

served regular healthy food (10–20 % energy from protein, 50–60 % energy from carbohydrates, 25–35 % energy from fat) ad libitum from Righospitalet kitchen, Denmark (Kiilerich et al. 2011).

Body composition

Six to ten days before and immediately after the bed rest period, fat and fat-free tissue mass of the whole body, trunk and extremities were measured using a dual-energy X-ray absorbtiometry (DEXA) scanner (Lunar Prodigy Advance, GE Healthcare, Madison, WI, USA). The total energy content of the body was calculated from body composition assigning energy values of 39.4 and 3.7 MJ/kg to fat and lean mass, respectively (Elia et al. 2003).

Experimental protocol

The day prior to the experimental day before bed rest, the subjects abstained from intense and prolonged exercise. The day before the experimental day after bed rest, the subjects were physically inactive because this was within the bed rest period. The day before the experimental day (both before and after the bed rest period), the subjects consumed a prepackaged, standardized meal and evening snack regulated for body weight (14.3 and 2.9 kcal/kg, respectively). On the experimental day before bed rest, the subjects arrived to the laboratory in the morning by minimum of physical activity, and on the experimental day after bed rest they were transported in wheelchairs. Under local anesthesia (2 % lidocaine) a catheter was placed in one femoral vein as described elsewhere (Bienso et al. 2012; Kiilerich et al. 2011; Ringholm et al. 2011). Three and a half hours after consuming a standardized breakfast (2.9 kcal/kg), muscle biopsies were obtained from the vastus lateralis and deltoid as described elsewhere (Bienso et al. 2012; Kiilerich et al. 2011; Ringholm et al. 2011). Blood samples were taken from the femoral vein just prior to the biopsies. Serum was collected from blood samples and stored at −80 °C.

This protocol was completed between 6 and 10 days before and immediately after the 7 days of bed rest.

Oral glucose tolerance test and HOMA

An oral glucose tolerance test (OGTT) was performed between 6 and 10 days before the onset of bed rest and 6 days into the bed rest. After an overnight fast each subject consumed 1 g of glucose per kg body mass, each gram of glucose was dissolved in 6.67 ml water. After consumption, blood was sampled from an arm vein after 30, 60 and 120 min, and the samples were subsequently analyzed for plasma insulin and glucose (Department of Clinical Biochemistry, Rigshospitalet, Denmark). Homeostatic model assessment of insulin resistance (HOMA) was calculated as HOMA = [glucose (mmol/l)·insulin (mU/l)]/22.5.

Total protein extraction, electrophoresis and Western blot analysis

Muscle protein extracts were prepared as described previously (Guerra et al. 2007) and total protein content was quantified using the bicinchoninic acid assay (Smith et al. 1985). Equal amounts (50 μg) of each sample were subjected to immunoblotting protocol as described previously (Guerra et al. 2007). To determine leptin receptor isoforms (OB-Rs), SOCS3 and PTP1B protein expression, specific anti-human antibodies were used all diluted in 5 % blotting grade blocker non-fat dry milk (Bio-Rad Laboratories, Hercules, CA, USA) in Tris-buffered saline (TBS) with 0.1 % Tween 20 (TBS-T) (blotto blocking buffer) as described previously (Fuentes et al. 2010). As previously reported (Guerra et al. 2008; Guerra et al. 2007), the antibody used in the present study to determine muscle OB-Rs detected three specific bands with a molecular mass of ~170 [OB-R170; which seems to represent a posttranslational modification of the long isoform of the leptin receptor (OB-Rb)], 128 (OB-R128; with a similar molecular mass than the OB-Rb) and 98 kDa [OB-R98; which is likely to correspond to one of the short leptin receptor isoforms (OB-Ra)]. To determine Tyr^{705}-STAT3 phosphorylation level, specific antibodies directed against the phosphorylated and total form of this kinase were used both diluted in 5 % bovine serum albumin in TBS-T (BSA-blocking buffer) as reported elsewhere (Fuentes et al. 2010). To control for differences in loading and transfer efficiency across membranes, membranes were incubated with a monoclonal mouse anti-alpha-tubulin antibody diluted in blotto blocking buffer. Antibody-specific labeling was revealed by incubation with a HRP-conjugated goat anti-rabbit antibody (1:20,000) or a HRP-conjugated donkey anti-mouse (1:10,000) antibody both diluted in blotto blocking buffer and visualized with the ECL chemiluminescence kit (Amersham Bisociences). Specific bands were visualized with the ChemiDoc XRS system (Bio-Rad Laboratories) and analyzed with the image analysis program Quantity one© (Bio-Rad laboratories). The densitometry analysis was carried out immediately before saturation of the immunosignal. Data are reported as the band intensity of immunostaining values (arbitrary units) obtained for OB-R, PTP1B or SOCS3 relative to those obtained for alpha-tubulin or as arbitrary units of band density obtained for the phosphorylated form of STAT3 relative to those obtained for the total STAT3. Alpha tubulin protein expression, used as a loading control in our

immunoblotting assays, was not affected by bed rest and was similar in both muscles. Western blot analysis of all proteins studied was performed in triplicate for each muscle biopsy with a variation coefficient less than 10 %.

Leptin assays

Serum leptin concentration was determined by Enzyme-Linked Immunosorbent Assay (ELISA) (ELx800 Universal Microplate Reader, Bioteck Instruments Inc, Vermont, USA), using reagent kits from Linco Research (#EZHL-80SK, Linco Research St. Charles, Missouri, USA) and following the manufacturer's instructions. The sensitivity of the total leptin assay was 0.05 ng/ml. The intra-assay coefficient variation was 3.8 % and the inter-assay coefficient of variation was 4.4 %.

Soluble leptin receptor (*s*OB-R) assays

Serum OB-Rs were determined by ELISA (ELx800 Universal Microplate Reader, Bioteck Instruments Inc, Vermont, USA), using reagent kits from R&D Systems (#DOBR00, R&D, Minneapolis, MN, USA) and following the manufacturer's instructions. The sensitivity of the soluble leptin receptor (sOB-R) assays was 0.057 ng/ml. The intra-assay coefficient variation was 4.4 % and the inter-assay coefficient of variation was 6.8 %.

Statistical analysis

Variables were checked for normal distribution using the Shapiro-Wilks test. Areas under the curve were determined using the trapezoidal rule. Values in the text are given as mean ± SD. For comparisons between before and after bed rest conditions Students' *t* test for paired observations was employed. Correlations were evaluated using Pearson's linear correlation coefficient. Significance was accepted at $P < 0.05$. The statistical analysis was performed using SPSS (version 15.0) for Windows (SPSS, Chicago, IL, USA).

Results

Physical characteristics and performance

Body mass and body mass index (BMI) were unaffected by 7 days of bed rest (Table 1). Consequently, the changes in energy content (+1.1 % after bed rest) were not significant ($P = 0.4$). Leg lean mass was reduced by 3 % ($P < 0.05$), whereas whole body fat was not significantly increased (+300 g, $P = 0.34$) (Table 1). However, leg fat mass was augmented by 4 % after bed rest ($P < 0.05$) (Table 1).

Table 1 Anthropometric and performance data before and after the bed rest

	Before bed rest	After bed rest
Age (years)[a]	26.2 ± 5.3	–
Height (cm)[a]	181.7 ± 6.1	–
Body mass (kg)[a]	75.2 ± 11.3	75.1 ± 11.4
Body mass index (kg m^{-2})[a]	22.8 ± 2.7	22.7 ± 2.8
Arm lean mass (kg)	7.13 ± 1.34	7.10 ± 1.34
Leg lean mass (kg)[a]	20.6 ± 3.4	20.0 ± 3.2*
Total lean mass (kg)[a]	58.6 ± 8.4	58.3 ± 8.2
Arm fat mass (kg)	1.04 ± 0.5	1.05 ± 0.5
Leg fat mass (kg)	4.7 ± 1.5	4.9 ± 1.6*
Body fat mass (kg)[a]	13.3 ± 6	13.6 ± 6.1
Body fat (%)[a]	18.2 ± 7.3	18.5 ± 7.3
Total energy content of the body (MJ)	741.7 ± 241.5	750.1 ± 248.2
VO_{2max} (ml kg^{-1} min^{-1})	52.0 ± 2.0	49.8 ± 1.7*

Data are mean ± SD. VO_{2max} maximal oxygen consumption

* Significantly different from before bed rest, $P < 0.05$

[a] Previously reported in Ringholm et al. (2011)

Bed rest reduced VO_{2max} per kg of fat free mass by 5 % ($P < 0.05$) (Table 1).

HOMA and oral glucose tolerance test

After bed rest, the basal insulin concentration was increased by 53 % (from 29.8 ± 14.9 to 45.5 ± 11.3 pmol l^{-1}; before and after bed rest, respectively, $P < 0.05$) and the HOMA by 40 % (from 0.9 ± 0.5 to 1.3 ± 0.4; before and after bed rest, respectively, $P < 0.05$) relative to before bed rest (Kiilerich et al. 2011). The area under the curve (AUC) was calculated for plasma glucose and insulin in response to the OGTT, before and after bed rest. The AUC for the glucose response was unchanged; while AUC for insulin was 1.5-fold larger ($P < 0.05$) after bed rest than before (Kiilerich et al. 2011).

Effects of bed rest on body composition, serum leptin, soluble isoform of the leptin receptor (s-OBR) concentrations and free leptin index (FLI)

Neither body fat mass (13.3 ± 6.0 and 13.6 ± 6.1 kg; before and after bed rest, respectively) nor the percentage of body fat (18.2 ± 7.3 and 18.5 ± 7.3 %; before and after bed rest, respectively) was significantly altered by bed rest as previously reported (Bienso et al. 2012; Ringholm et al. 2011). Lean mass in the lower extremities was reduced by 3.1 %, while it remained unchanged in the upper extremities (7.13 ± 1.34 and 7.10 ± 1.34 kg; before and after bed rest, respectively) as previously reported (Kiilerich et al.

2011). Both before and after bed rest, serum leptin concentration was related to the percentage of body fat ($r = 0.87$ and $r = 0.92$, before and after bed rest, respectively, $P < 0.05$). Serum leptin concentration was 35 % higher after bed rest than before bed rest ($P < 0.05$) (Fig. 2a), and this difference remained significant after accounting for the differences in percentage of body fat ($P = 0.01$). There was no relationship between the increase in serum leptin concentration and the non-significant change in fat mass with bed rest ($r = 0.36$, $P = 0.26$). The delta increase in leptin concentration showed a trend to be negatively related to the reduction of lower extremities lean mass with bed rest ($r = -0.52$, $P = 0.08$).

Serum soluble isoform of the leptin receptor (sOB-R) concentration was unaffected by bed rest ($P = 0.57$) (Fig. 2b). The molar excess of leptin over sOB-R (FLI) was increased by bed rest (from 1.06 ± 0.23 to 1.45 ± 0.27; before and after bed rest, respectively, $P < 0.05$). There was no significant relationship between serum s-OBR and serum leptin concentrations at any time point.

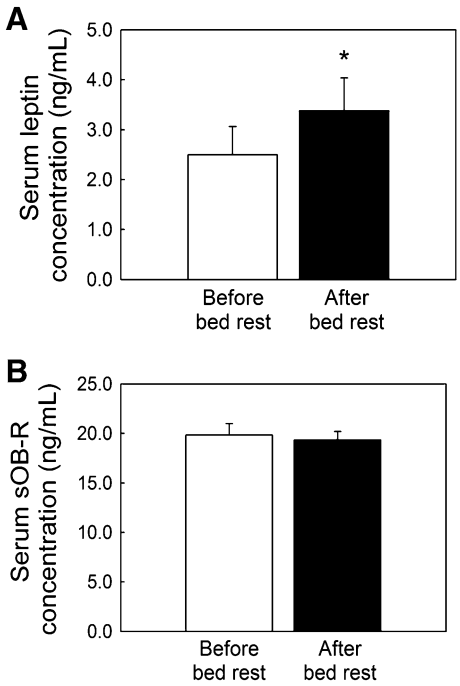

Fig. 2 Serum leptin (**a**) and secreted isoform of the leptin receptor (sOB-R) concentrations (**b**) in healthy subjects before (*white bar*) and after (*dark bar*) 1 week of bed rest ($n = 12$). Values are mean ± SEM. *$P < 0.05$ versus before bed rest

Effects of bed rest on leptin receptor protein content in skeletal muscle

Deltoid and vastus lateralis protein content of the three leptin receptor isoforms (OB-R170, OB-128 and OBR-98) (Fig. 3a) was unaffected by bed rest (Fig. 3b, c and d, respectively). Protein content of OB-R170, OB-R128 and OB-R98 was similar in deltoid and vastus lateralis muscles both before and after bed rest (Fig. 2b, c and d, respectively).

Effects of bed rest on SOCS3 protein content in skeletal muscle

Protein content of SOCS3 tended to be higher in deltoid compared with vastus lateralis before bed rest ($P = 0.091$) (Fig. 4a). However, SOCS3 protein expression was similar in both muscles studied after bed rest ($P = 0.6$) (Fig. 4a). SOCS3 protein content normalized by alpha-tubulin was not affected by bed rest in either the deltoid or the vastus lateralis (Fig. 4a).

Effects of bed rest on PTP1B protein content in skeletal muscle

Deltoid and vastus lateralis muscles had similar protein content levels of PTP1B (Fig. 4b). PTP1B protein expression was unaffected by bed rest in deltoid (Fig. 4b). However, PTP1B protein content was increased by 90 % with bed rest in the vastus lateralis ($P < 0.05$) (Fig. 4b). There was a linear relationship between the increase in vastus lateralis PTP1B protein content and both the increase in basal insulin concentration ($r = 0.66$, $P < 0.05$) and HOMA ($r = 0.68$, $P < 0.05$) with bed rest.

Effects of bed rest on STAT3 phosphorylation (Tyr^{705}) in skeletal muscle

Total STAT3 protein expression normalized by alpha-tubulin was similar in deltoid and vastus lateralis and was not affected by bed rest in either the deltoid (1.96 ± 0.45 and 1.94 ± 0.94 A.U.; before and after bed rest, respectively, $P = 0.95$) or vastus lateralis (1.89 ± 0.44 and 1.77 ± 0.66 A.U., before and after bed rest, respectively, $P = 0.63$). STAT3 phosphorylation level was similar in deltoid and vastus lateralis muscles both before ($P = 0.70$) and after ($P = 0.60$) bed rest (Fig. 5). STAT3 phosphorylation level normalized by total STAT3 protein expression was not affected by bed rest (Fig. 5).

Before bed rest vastus lateralis STAT3 phosphorylation level was linearly related to the OB-R128 protein content ($r = 0.78$, $P < 0.05$), to the OB-R98 protein content ($r = 0.65$, $P < 0.05$) and to before bed rest serum leptin concentration ($r = 0.61$, $P < 0.05$). Moreover, there was a trend for a relationship between before bed rest STAT3 phosphorylation in the vastus lateralis and before bed rest FLI ($r = 0.55$, $P = 0.06$). These correlations were lost after bed rest.

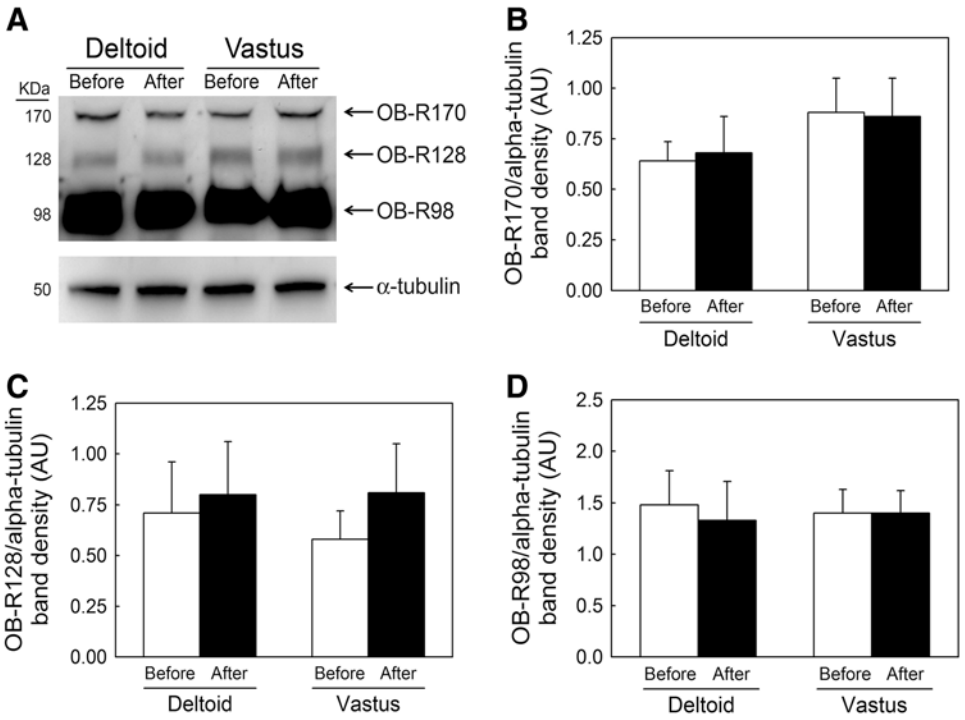

Fig. 3 Leptin receptor isoform (OB-Rs) protein expression in deltoid ($n = 10$) and vastus lateralis muscle biopsies ($n = 12$) obtained from healthy subjects before (*white bars*) and after (*dark bars*) 1 week of bed rest. Representative Western blot assays (**a**) and densitometric immunosignal values (arbitrary units (AU) of band densities) of OB-R170 (**b**), OB-R128 (**c**) and OB-R98 bands (**d**). Protein content is given relative to alpha-tubulin protein content. Values are mean ± SEM

Discussion

In contrast to our hypothesis, this study shows that 1 week of physical inactivity is not associated with down-regulation of the long and short leptin receptor isoforms neither in the deltoid nor in the vastus lateralis muscles, despite the fact that both muscles were differently affected by inactivity, as reflected by the greater reduction of lean mass in the legs. Moreover, Tyr^{705}-STAT3 phosphorylation and SOCS3 protein expression were unaffected by bed rest in the two muscles studied. However, in agreement with our hypothesis, PTP1B protein expression was markedly increased after bed rest in the vastus lateralis muscle, although this response was not observed in the deltoid muscle. This finding may indicate that induction of PTP1B protein expression by physical inactivity affects mainly the muscles for which bed rest imposes a greater change in the daily pattern of activation. Because PTP1B dephosphorylates the insulin receptor and also the janus kinase 2 (JAK2) (first step in leptin signaling) (St-Pierre and Tremblay 2012), the bed rest-induced elevation of PTP1B content in the vastus lateralis may have contributed to causing both insulin and leptin resistance in the vastus lateralis muscle.

Plasma leptin concentrations, sOB-R levels and bed rest

Previous studies have reported conflicting findings regarding the plasma leptin response to bed rest in humans. Bosutti et al. (2008) observed that serum leptin and insulin concentrations did not change in response to bed rest (2 weeks) under either eucaloric or hypocaloric diet. However, it has been shown that plasma leptin levels were augmented in response to 5 weeks of bed rest in subjects who increased their fat mass during the bed rest period (Biolo et al. 2008). In agreement with our results, Blanc et al. (2000) showed that 7 days of physical inactivity induced by head-down bed rest (HDBR) increased leptin and insulin levels in both men and women despite no significant changes in fat mass.

The soluble isoform of the leptin receptor (sOB-R) is the main leptin-binding activity in human blood and regulates the bioavailability of free leptin (Lammert et al. 2001; Sun et al. 2010; Yang et al. 2004; Zastrow et al. 2003; Zhang and Scarpace 2009). Recent studies have reported a predominant inhibitory effect of sOB-R on leptin signaling in cell (Schaab et al. 2012) and animal models (Zhang and Scarpace 2009). The present investigation shows that one-week bed rest does not induce a significant change in sOB-R. Since the latter was accompanied by increased serum leptin concentrations, the molar excess of leptin over sOB-R (free leptin index) was increased after bed rest, implying that more leptin was available to bind its receptor after bed rest. Little information is available on the effects of physical activity on sOB-R. A human strength training study has reported increased sOB-R with no change in leptin concentrations (Ara et al. 2006). In contrast, sOB-R increases with weight loss (Laimer et al. 2002; van Dielen et al. 2002). Thus, changes in leptin sensitivity with bed rest cannot be attributed to an increase in sOB-R, at least for a bed rest period up to 1 week.

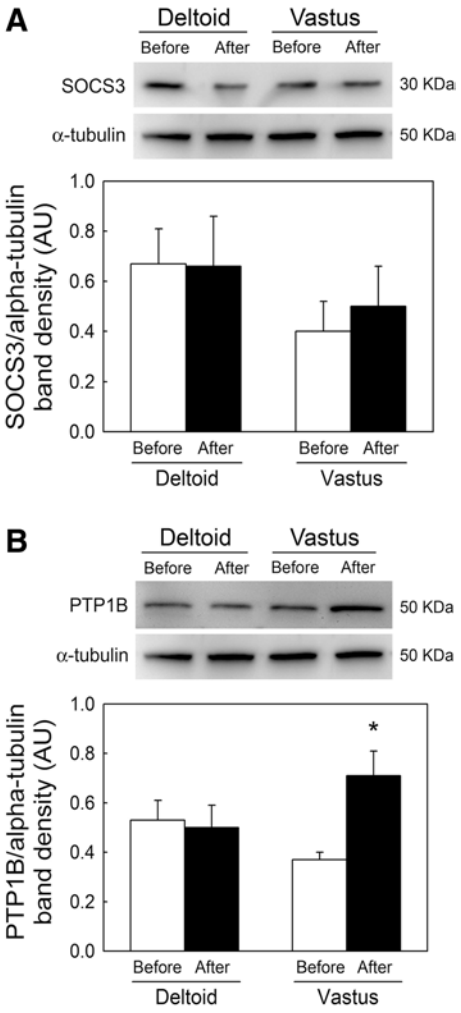

Fig. 4 SOCS3 (**a**) and PTP1B (**b**) protein expression in deltoid ($n = 10$ in A and $n = 9$ in B) and vastus lateralis muscle biopsies ($n = 12$ in A and $n = 11$ in B) obtained from healthy subjects before (*white bars*) and after (*dark bars*) 1 week of bed rest. *Top panel* representative Western blot assay. *Lower panel* densitometric immunosignal values [arbitrary units (AU)]. Protein content is given relative to alpha-tubulin protein content. Values are mean ± SEM. *$P < 0.05$ versus before bed rest

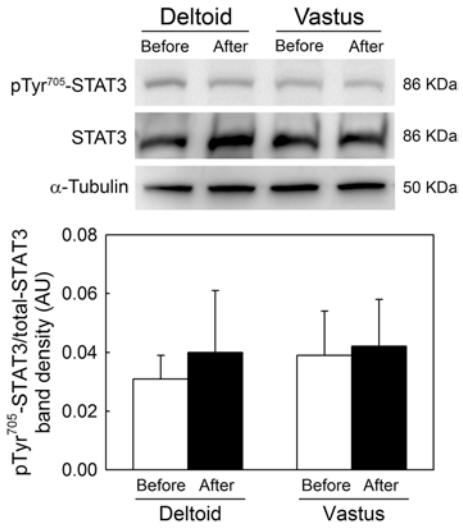

Fig. 5 Determination of pTyr^{705}-STAT3 phosphorylation level in deltoid ($n = 10$) and vastus lateralis ($n = 12$) muscle biopsies obtained from healthy subjects before (*white bars*) and after (*dark bars*) 1 week of bed rest. *Top panel* representative Western blot assay. *Lower panel* densitometric analysis of the immunoblots [arbitrary units (AU)]. Values are relative to total STAT3. Values are mean ± SEM

It has been suggested that leptin levels may not only reflect the quantity of adipose tissue but also disturbances in energy balance (Fuentes et al. 2010; Hilton and Loucks 2000). In accordance with this proposal, the increase in plasma leptin concentration with bed rest in the present study may indicate that free leptin acts as a homeostatic mechanism to reduce appetite and maintain body mass (Myers et al. 2008; Zhang et al. 2005) in response to the reduced energy expenditure during the bed rest period. In addition, it was evident that the subjects experiencing the greatest elevation in serum leptin concentrations tended to have lower reductions in lean mass in the lower extremities, suggesting that the increase in leptin concentration with bed rest may help to preserve the lean mass, although this observation requires further studies to be confirmed. In line with this possibility, some animal (Allen et al. 2008; Arounleut et al. 2013; Hamrick et al. 2010; Sainz et al. 2009) and human studies (Olmedillas et al. 2010) indicate that leptin may have anabolic or anticatabolic effects on skeletal muscle. A greater level of leptin resistance in leg compared with the arm muscles could explain at least in part why the lean mass was only reduced in the legs and not in the arms.

In the present investigation, leg fat mass was increased by bed rest whilst arm fat mass remained unchanged, despite the fact that both territories were exposed to similarly increased circulating leptin concentrations. Leptin stimulates muscle fatty acid oxidation through AMPK activation in human (Wolsk et al. 2011). Therefore, the increased leg fat mass induced by 7 days of bed rest may reflect some degree of leptin resistance in leg muscles, probably due to the increase in PTP1B protein expression induced by bed rest. Several studies have suggested that both the accumulation of intramyocellular lipids (IMCL) and intermuscular adipose tissue (Murphy et al. 2012) are associated with insulin resistance at least in untrained subjects (Dela and Helge 2013; Eckardt et al. 2011). Accumulated IMCL may generate bioactive lipid metabolites, such as diacylglycerol (DAG) and ceramide, which may interfere with insulin signaling causing muscular insulin resistance (Eckardt et al. 2011). However, recent human studies have also suggested that these bioactive lipid metabolites

generated from IMCL are not responsible for muscle insulin resistance (Amati 2012; Helge et al. 2011).

The increase in free leptin may have been facilitated by the increase in insulin levels after bed rest (Bienso et al. 2012). Insulin stimulates leptin production independent of the glucose tolerance status (Aas et al. 2009; Malmstrom et al. 1996; Saad et al. 1998) by both increasing leptin mRNA content and protein release in adipose tissue or by a trophic effect on the adipose tissue (Remesar et al. 1997).

Cell-culture studies have shown that acute leptin administration causes an acute reduction in the expression of leptin receptors indicating that the leptin receptor content is controlled by leptin (Hikita et al. 2000; Liu et al. 2004). Moreover, a reduction in circulating leptin levels by prolonged fasting in humans increases OB-R mRNA in peripheral mononuclear cells, while administration of human recombinant leptin in fasting humans blunts the increase in OB-R in mononuclear cells (Chan et al. 2002). However, we did not find any significant change in the OB-Rs protein expression in skeletal muscle after bed rest despite the observed increase in serum leptin concentration with bed rest. Moreover, no relationship was observed in the present study between leptin levels and the expression of leptin receptors in either muscle as previously reported (Fuentes et al. 2010; Guerra et al. 2007, 2008; Olmedillas et al. 2010). Thus, our findings indicate that the muscle OB-Rs expression appears to be regulated by other mechanisms in addition to circulating leptin levels.

Although leptin signaling elicits an increase in SOCS3 expression (Bjørbæk et al. 2000), no relationship was observed in this study between the increase in serum leptin concentration and the changes in muscle SOCS3 protein expression, neither in the deltoid nor the vastus lateralis muscles. Moreover, the lack of a relationship between leptin concentration and SOCS3 protein expression in the vastus lateralis (Guerra et al. 2008; Olmedillas et al. 2010) both before and after bed rest is in agreement with our previous studies. However, before bed rest there was a positive association between serum leptin concentrations and SOCS3 protein content in the deltoid muscle which was not found after bed rest, indicating that physical inactivity may modulate the normal relationship between serum leptin levels and SOCS3 protein expression in skeletal muscle, as previously suggested (Olmedillas et al. 2010).

OB-R expression, muscle leptin resistance and bed rest

We have recently shown that OB-Rb protein expression is reduced in deltoid and vastus lateralis muscles of humans with obesity (Fuentes et al. 2010), but it is increased in the dominant triceps *brachii* of professional tennis players compared with the non-uploaded triceps (Olmedillas et al. 2010). A down-regulation of OB-Rb protein expression in skeletal muscle could account for some of the peripheral leptin resistance typical of sedentarism and obesity (Fuentes et al. 2010). Conversely, an increase in muscle OB-R expression could be interpreted as an up-regulation of leptin signaling and muscle leptin sensitivity (Olmedillas et al. 2010). However, using immunohistochemistry, an increase in muscle leptin receptor protein expression has also been reported in the human medial gastrocnemius muscles after immobilization for 4–11 days (Chen et al. 2007). Chen et al. (2007) speculated that an up-regulation of the leptin receptor expression may help to increase leptin sensitivity and reduce fatty acid deposition in atrophic muscles, although these results need to be confirmed by western blot. Despite that in the present investigation bed rest was accompanied by muscle atrophy in the vastus lateralis, but not in the deltoid muscle, no change was observed in either muscle in leptin receptor protein expression, indicating that the leptin resistance induced by 7 days of bed rest is not due to downregulation of the amount of leptin receptors in skeletal muscle.

SOCS3 and PTP1B protein expression, muscle leptin resistance and bed rest

Up-regulation of SOCS3 has been implicated as a potential mechanism of leptin resistance and of leptin-induced insulin resistance (Bjørbæk et al. 2000; Jorgensen et al. 2013; Yang et al. 2012). Up-regulation of SOCS3 expression seems to be implicated in central and peripheral leptin resistance because elevated levels are present in hypothalamus, white adipocytes and skeletal muscle of leptin-resistant rodents (Eguchi et al. 2007; Steinberg et al. 2004; Wang et al. 2000; Yang et al. 2012). In fact, muscle-specific overexpression of SOCS3 in mice (MCK/SOCS3 mice) causes both leptin and insulin resistance (Yang et al. 2012). Furthermore, Steinberg et al. (2006) reported that SOCS3 mRNA is up-regulated in human myotubes cultured from skeletal muscle of obese humans compared with myotubes obtained from skeletal muscle of lean subjects. In addition, these authors showed that overexpression of SOCS3 via adenovirus-mediated infection in lean myotubes to a similar degree as observed in obese myotubes prevented AMP-kinase (AMPK) activation by leptin (Steinberg et al. 2006). In contrast to our hypothesis, bed rest for 7 days did not induce a significant change in SOCS3 protein level in either muscle examined, implying that SOCS3 is unlikely to play a significant role in the increase of insulin and leptin resistance with bed rest.

We have observed an increase in PTP1B protein content in the vastus lateralis after bed rest, which may have been facilitated by the concomitant reduction in SIRT1 (Sun et al. 2007) in the m. vastus lateralis, as previously reported (Ringholm et al. 2011). Protein tyrosine phosphatase

1B (PTP1B) is a negative regulator of leptin and insulin signaling (Dube and Tremblay 2005; St-Pierre and Tremblay 2012) and is increased in multiple insulin-and leptin-responsive tissues in mice with diet induced obesity, including the arcuate nucleus and medial hypothalamus, important sites of PTP1B action on body weight regulation, and in peripheral tissues, such as skeletal muscle, adipose tissue and liver (Dube and Tremblay 2005; Zabolotny et al. 2008). Moreover, skeletal muscle PTP1B overexpression in transgenic mice causes impaired insulin signaling and whole body insulin resistance (Zabolotny et al. 2004). In contrast, mice with the whole body deletion of PTP1B were protected against the development of obesity and diabetes (Cheng et al. 2002).

Several studies have reported that PTP1B levels are increased in skeletal muscle and adipose tissue of obese humans (Ahmad et al. 1997a, b; Cheung et al. 1999). In agreement, the present results showing a close relationship between the increase in serum basal insulin concentration and the increase in vastus lateralis muscle PTP1B protein expression indicate that at least part of the bed rest-induced insulin resistance in the present study (Bienso et al. 2012) may be caused by the increased protein amount of PTP1B in some, but not all skeletal muscles.

Because the vastus lateralis muscles are submitted to greater use in normal life it may be speculated that PTP1B is more likely to increase with physical inactivity in the most active muscles. This muscle-specific response of PTP1B to inactivity may also explain why other studies have reported unchanged or decreased PTP1B expression in obese and/or diabetic humans compared with controls (Ahmad et al. 1997a; Fuentes et al. 2010; Kusari et al. 1994; Worm et al. 1999). These differences between arm and leg muscles in inactivity-induced PTP1B protein up-regulation may in part explain why there is better preserved insulin sensitivity in arm than leg muscle in humans with type 2 diabetes (Olsen et al. 2005).

STAT3 phosphorylation and bed rest

The signal transducer and activator of transcription 3 (STAT3) signaling pathway in human skeletal muscle is the signal transducer of numerous stimuli in addition to leptin signaling (Stepkowski et al. 2008) and is involved in the regulation, among other processes, of cellular proliferation, differentiation, programmed cell death, inflammation, muscle hypertrophy and the immune response (Akira 2000; Judd et al. 2006). It has been reported that an acute infusion of leptin in healthy humans causes a 17-fold increase in muscle STAT3 phosphorylation (Wolsk et al. 2011). In the present investigation, despite the increase in free leptin, bed rest had no influence on the level of the STAT3 phosphorylation in leg and arm muscles, indicating some degree of skeletal muscle leptin resistance. In fact, before bed rest there was a positive association between vastus lateralis STAT3 phosphorylation and serum leptin concentrations, which was not found after bed rest. Therefore, our results could indicate that the increased PTP1B expression observed in vastus lateralis after bed rest may have blunted leptin signaling in this muscle, i.e., no increase in STAT3 phosphorylation despite an increase in circulating leptin levels. The lack of correlation between leptin concentrations and STAT3 phosphorylation levels, and the fact that STAT3 phosphorylation is not related to the amount of OB-R in deltoid muscle before and after, could just reflect the influence of other signals overruling the effects of leptin in the deltoid muscle.

Limitations

The main limitation of this study is that neither energy expenditure nor energy intake was controlled during the bed rest period. To maintain the energy balance during bed rest a reduction in energy intake is required to compensate for the lack of physical activity. Our energy balance analysis shows a modest 1.2 MJ/day (non-statistically significant) positive energy balance. This represents ~25 % of the daily energy expenditure due to physical activity in men (Speakman and Westerterp 2010) implying a relatively good adjustment in energy intake to almost match the new energy expenditure. Thus, our energy balance results can only be explained if the energy intake was reduced, indicating good central responsiveness to the increase in leptin concentration. However, for a similar energy content and fat mass of the body a greater level of circulating leptin was required to maintain the energy balance during bed rest indicating some degree of central leptin resistance.

Conclusions

This study shows that 1 week of bed rest is accompanied by increased basal insulin and leptin serum concentrations, despite lack of significant changes in whole body fat mass. The increase in leptin serum concentration without a concomitant increase in STAT3 phosphorylation could indicate some degree of leptin resistance in skeletal muscles, which may be explained, at least in part, by the up-regulation of PTP1B protein expression in vastus lateralis with physical inactivity. In addition, the present investigation suggests that the up-regulation of PTP1B protein expression with inactivity is more likely in previously loaded muscles, like the leg muscles while the arm muscles seem more resistant to this effect.

Acknowledgments The authors would like to thank the subjects for the extraordinary effort and the whole COPENHAGEN BED REST 2008 TEAM for excellent collaboration. Special thanks are given to José Navarro de Tuero for his excellent technical assistance. This study was supported by grants from the Lundbeck Foundation, Denmark, and The Danish Medical Research Council, Denmark, and the Ministerio de Ciencia e Innovación (BFU2006-13784 and FEDER) and FUNCIS (PI/10/07). Borja Guerra was a fellow of the "Recursos Humanos y Difusión de la Investigación" Program (Instituto de Salud Carlos III, Ministerio de Sanidad y Consumo, Spain). The Centre of Inflammation and Metabolism (CIM) is supported by a grant from the Danish National Research Foundation (#02-512-55). The Copenhagen Muscle Research Centre is supported by a grant from the Capital Region of Denmark. CIM is part of the UNIK Project: Food, Fitness & Pharma for Health and Disease, supported by the Danish Ministry of Science, Technology and Innovation.

Conflict of interest The authors report no conflict of interest. The authors alone are responsible for the content and writing the paper.

References

Aas AM, Hanssen KF, Berg JP, Thorsby PM, Birkeland KI (2009) Insulin-stimulated increase in serum leptin levels precedes and correlates with weight gain during insulin therapy in type 2 diabetes. J Clin Endocrinol Metab 94:2900–2906

Ahmad F, Azevedo JL, Cortright R, Dohm GL, Goldstein BJ (1997a) Alterations in skeletal muscle protein-tyrosine phosphatase activity and expression in insulin-resistant human obesity and diabetes. J Clin Invest 100:449–458

Ahmad F, Considine RV, Bauer TL, Ohannesian JP, Marco CC, Goldstein BJ (1997b) Improved sensitivity to insulin in obese subjects following weight loss is accompanied by reduced protein-tyrosine phosphatases in adipose tissue. Metabolism 46:1140–1145

Akira S (2000) Roles of STAT3 defined by tissue-specific gene targeting. Oncogene 19:2607–2611

Allen DL, Cleary AS, Speaker KJ, Lindsay SF, Uyenishi J, Reed JM, Madden MC, Mehan RS (2008) Myostatin, activin receptor IIb, and follistatin-like-3 gene expression are altered in adipose tissue and skeletal muscle of obese mice. Am J Physiol Endocrinol Metab 294:E918–E927

Amati F (2012) Revisiting the diacylglycerol-induced insulin resistance hypothesis. Obes Rev 13(Suppl 2):40–50

Ara I, Perez-Gomez J, Vicente-Rodriguez G, Chavarren J, Dorado C, Calbet JA (2006) Serum free testosterone, leptin and soluble leptin receptor changes in a 6-week strength-training programme. Br J Nutr 96:1053–1059

Arounleut P, Bowser M, Upadhyay S, Shi XM, Fulzele S, Johnson MH, Stranahan AM, Hill WD, Isales CM, Hamrick MW (2013) Absence of functional leptin receptor isoforms in the POUND (Lepr(db/lb)) mouse is associated with muscle atrophy and altered myoblast proliferation and differentiation. PLoS ONE 8:e72330

Bates SH, Stearns WH, Dundon TA, Schubert M, Tso AW, Wang Y, Banks AS, Lavery HJ, Haq AK, Maratos-Flier E, Neel BG, Schwartz MW, Myers MG Jr (2003) STAT3 signalling is required for leptin regulation of energy balance but not reproduction. Nature 421:856–859

Bienso RS, Ringholm S, Kiilerich K, Aachmann-Andersen NJ, Krogh-Madsen R, Guerra B, Plomgaard P, van Hall G, Treebak JT, Saltin B, Lundby C, Calbet JA, Pilegaard H, Wojtaszewski JF (2012) GLUT4 and glycogen synthase are key players in bed rest-induced insulin resistance. Diabetes 61:1090–1099

Biolo G, Agostini F, Simunic B, Sturma M, Torelli L, Preiser JC, Deby-Dupont G, Magni P, Strollo F, di Prampero P, Guarnieri G, Mekjavic IB, Pisot R, Narici MV (2008) Positive energy balance is associated with accelerated muscle atrophy and increased erythrocyte glutathione turnover during 5 wk of bed rest. Am J Clin Nutr 88:950–958

Bjørbæk C, Kahn BB (2004) Leptin signaling in the central nervous system and the periphery. Recent Prog Horm Res 59:305–331

Bjørbæk C, Lavery HJ, Bates SH, Olson RK, Davis SM, Flier JS, Myers MG Jr (2000) SOCS3 mediates feedback inhibition of the leptin receptor via Tyr985. J Biol Chem 275:40649–40657

Blanc S, Normand S, Pachiaudi C, Duvareille M, Gharib C (2000) Leptin responses to physical inactivity induced by simulated weightlessness. Am J Physiol Regul Integr Comp Physiol 279:R891–R898

Bosutti A, Malaponte G, Zanetti M, Castellino P, Heer M, Guarnieri G, Biolo G (2008) Calorie restriction modulates inactivity-induced changes in the inflammatory markers C-reactive protein and pentraxin-3. J Clin Endocrinol Metab 93:3226–3229

Chan JL, Bluher S, Yiannakouris N, Suchard MA, Kratzsch J, Mantzoros CS (2002) Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. Diabetes 51:2105–2112

Chen YW, Gregory CM, Scarborough MT, Shi R, Walter GA, Vandenborne K (2007) Transcriptional pathways associated with skeletal muscle disuse atrophy in humans. Physiol Genomics 31:510–520

Cheng A, Uetani N, Simoncic PD, Chaubey VP, Lee-Loy A, McGlade CJ, Kennedy BP, Tremblay ML (2002) Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. Dev Cell 2:497–503

Cheung A, Kusari J, Jansen D, Bandyopadhyay D, Kusari A, Bryer-Ash M (1999) Marked impairment of protein tyrosine phosphatase 1B activity in adipose tissue of obese subjects with and without type 2 diabetes mellitus. J Lab Clin Med 134:115–123

Dela F, Helge JW (2013) Insulin resistance and mitochondrial function in skeletal muscle. Int J Biochem Cell Biol 45:11–15

Dube N, Tremblay ML (2005) Involvement of the small protein tyrosine phosphatases TC-PTP and PTP1B in signal transduction and diseases: from diabetes, obesity to cell cycle, and cancer. Biochim Biophys Acta 1754:108–117

Eckardt K, Taube A, Eckel J (2011) Obesity-associated insulin resistance in skeletal muscle: role of lipid accumulation and physical inactivity. Rev Endocr Metab Disord 12:163–172

Eguchi M, Gillis LC, Liu Y, Lyakhovsky N, Du M, McDermott JC, Sweeney G (2007) Regulation of SOCS-3 expression by leptin and its co-localization with insulin receptor in rat skeletal muscle cells. Mol Cell Endocrinol 267:38–45

Elia M, Stratton R, Stubbs J (2003) Techniques for the study of energy balance in man. Proc Nutr Soc 62:529–537

Fuentes T, Ara I, Guadalupe-Grau A, Larsen S, Stallknecht B, Olmedillas H, Santana A, Helge JW, Calbet JA, Guerra B (2010) Leptin receptor 170 kDa (OB-R170) protein expression is reduced in obese human skeletal muscle: a potential mechanism of leptin resistance. Exp Physiol 95:160–171

Guerra B, Santana A, Fuentes T, Delgado-Guerra S, Cabrera-Socorro A, Dorado C, Calbet JA (2007) Leptin receptors in human skeletal muscle. J Appl Physiol 102:1786–1792

Guerra B, Fuentes T, Delgado-Guerra S, Guadalupe-Grau A, Olmedillas H, Santana A, Ponce-Gonzalez JG, Dorado C, Calbet JA (2008) Gender dimorphism in skeletal muscle leptin receptors, serum leptin and insulin sensitivity. PLoS ONE 3:e3466

Hamrick MW, Herberg S, Arounleut P, He HZ, Shiver A, Qi RQ, Zhou L, Isales CM, Mi QS (2010) The adipokine leptin increases skeletal muscle mass and significantly alters skeletal muscle miRNA expression profile in aged mice. Biochem Biophys Res Commun 400:379–383

Helge JW, Stallknecht B, Drachmann T, Hellgren LI, Jimenez-Jimenez R, Andersen JL, Richelsen B, Bruun JM (2011) Improved

glucose tolerance after intensive life style intervention occurs without changes in muscle ceramide or triacylglycerol in morbidly obese subjects. Acta Physiol (Oxf) 201:357–364

Hikita M, Bujo H, Hirayama S, Takahashi K, Morisaki N, Saito Y (2000) Differential regulation of leptin receptor expression by insulin and leptin in neuroblastoma cells. Biochem Biophys Res Commun 271:703–709

Hilton LK, Loucks AB (2000) Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. Am J Physiol Endocrinol Metab 278:E43–E49

Hosoi T, Sasaki M, Miyahara T, Hashimoto C, Matsuo S, Yoshii M, Ozawa K (2008) Endoplasmic reticulum stress induces leptin resistance. Mol Pharmacol 74:1610–1619

Jorgensen SB, O'Neill HM, Sylow L, Honeyman J, Hewitt KA, Palanivel R, Fullerton MD, Oberg L, Balendran A, Galic S, van der Poel C, Trounce IA, Lynch GS, Schertzer JD, Steinberg GR (2013) Deletion of skeletal muscle SOCS3 prevents insulin resistance in obesity. Diabetes 62:56–64

Judd LM, Bredin K, Kalantzis A, Jenkins BJ, Ernst M, Giraud AS (2006) STAT3 activation regulates growth, inflammation, and vascularization in a mouse model of gastric tumorigenesis. Gastroenterology 131:1073–1085

Kiilerich K, Ringholm S, Bienso RS, Fisher JP, Iversen N, van Hall G, Wojtaszewski JF, Saltin B, Lundby C, Calbet JA, Pilegaard H (2011) Exercise-induced pyruvate dehydrogenase activation is not affected by 7 days of bed rest. J Appl Physiol 111:751–757

Krawczewski Carhuatanta KA, Demuro G, Tschop MH, Pfluger PT, Benoit SC, Obici S (2011) Voluntary exercise improves high-fat diet-induced leptin resistance independent of adiposity. Endocrinology 152:2655–2664

Krogh-Madsen R, Thyfault JP, Broholm C, Mortensen OH, Olsen RH, Mounier R, Plomgaard P, van Hall G, Booth FW, Pedersen BK (2010) A 2-wk reduction of ambulatory activity attenuates peripheral insulin sensitivity. J Appl Physiol 108:1034–1040

Kusari J, Kenner KA, Suh KI, Hill DE, Henry RR (1994) Skeletal muscle protein tyrosine phosphatase activity and tyrosine phosphatase 1B protein content are associated with insulin action and resistance. J Clin Invest 93:1156–1162

Laimer M, Ebenbichler CF, Kaser S, Sandhofer A, Weiss H, Nehoda H, Aigner F, Patsch JR (2002) Weight loss increases soluble leptin receptor levels and the soluble receptor bound fraction of leptin. Obes Res 10:597–601

Lammert A, Kiess W, Bottner A, Glasow A, Kratzsch J (2001) Soluble leptin receptor represents the main leptin binding activity in human blood. Biochem Biophys Res Commun 283:982–988

Liu ZJ, Endoh A, Li R, Ohzeki T (2004) Effects of leptin and dexamethasone on long and short leptin receptor mRNA. Pediatr Int 46:561–564

Malmstrom R, Taskinen MR, Karonen SL, Yki-Jarvinen H (1996) Insulin increases plasma leptin concentrations in normal subjects and patients with NIDDM. Diabetologia 39:993–996

Murphy JC, McDaniel JL, Mora K, Villareal DT, Fontana L, Weiss EP (2012) Preferential reductions in intermuscular and visceral adipose tissue with exercise-induced weight loss compared with calorie restriction. J Appl Physiol (1985) 112:79–85

Must A, Spadano J, Coakley EH, Field AE, Colditz G, Dietz WH (1999) The disease burden associated with overweight and obesity. JAMA 282:1523–1529

Myers MG, Cowley MA, Munzberg H (2008) Mechanisms of leptin action and leptin resistance. Annu Rev Physiol 70:537–556

Olmedillas H, Sanchis-Moysi J, Fuentes T, Guadalupe-Grau A, Ponce-Gonzalez JG, Morales-Alamo D, Santana A, Dorado C, Calbet JA, Guerra B (2010) Muscle hypertrophy and increased expression of leptin receptors in the musculus triceps brachii of the dominant arm in professional tennis players. Eur J Appl Physiol 108:749–758

Olmedillas H, Guerra B, Guadalupe-Grau A, Santana A, Fuentes T, Dorado C, Serrano-Sanchez JA, Calbet JA (2011) Training, leptin receptors and SOCS3 in human muscle. Int J Sports Med 32:319–326

Olsen DB, Sacchetti M, Dela F, Ploug T, Saltin B (2005) Glucose clearance is higher in arm than leg muscle in type 2 diabetes. J Physiol 565:555–562

Pedersen BK (2007) Body mass index-independent effect of fitness and physical activity for all-cause mortality. Scand J Med Sci Sports 17:196–204

Remesar X, Rafecas I, Fernandez-Lopez JA, Alemany M (1997) Is leptin an insulin counter-regulatory hormone? FEBS Lett 402:9–11

Ringholm S, Bienso RS, Kiilerich K, Guadalupe-Grau A, Aachmann-Andersen NJ, Saltin B, Plomgaard P, Lundby C, Wojtaszewski JF, Calbet JA, Pilegaard H (2011) Bed rest reduces metabolic protein content and abolishes exercise-induced mRNA responses in human skeletal muscle. Am J Physiol Endocrinol Metab 301:E649–E658

Saad MF, Khan A, Sharma A, Michael R, Riad-Gabriel MG, Boyadjian R, Jinagouda SD, Steil GM, Kamdar V (1998) Physiological insulinemia acutely modulates plasma leptin. Diabetes 47:544–549

Sainz N, Rodriguez A, Catalan V, Becerril S, Ramirez B, Gomez-Ambrosi J, Fruhbeck G (2009) Leptin administration favors muscle mass accretion by decreasing FoxO3a and increasing PGC-1alpha in ob/ob mice. PLoS ONE 4:e6808

Schaab M, Kausch H, Klammt J, Nowicki M, Anderegg U, Gebhardt R, Rose-John S, Scheller J, Thiery J, Kratzsch J (2012) Novel regulatory mechanisms for generation of the soluble leptin receptor: implications for leptin action. PLoS ONE 7:e34787

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Anal Biochem 150:76–85

Speakman JR, Westerterp KR (2010) Associations between energy demands, physical activity, and body composition in adult humans between 18 and 96 y of age. Am J Clin Nutr 92:826–834

Steinberg GR, Parolin ML, Heigenhauser GJ, Dyck DJ (2002) Leptin increases FA oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. Am J Physiol Endocrinol Metab 283:E187–E192

Steinberg GR, Smith AC, Wormald S, Malenfant P, Collier C, Dyck DJ (2004) Endurance training partially reverses dietary-induced leptin resistance in rodent skeletal muscle. Am J Physiol Endocrinol Metab 286:E57–E63

Steinberg GR, McAinch AJ, Chen MB, O'Brien PE, Dixon JB, Cameron-Smith D, Kemp BE (2006) The suppressor of cytokine signaling 3 inhibits leptin activation of AMP-kinase in cultured skeletal muscle of obese humans. J Clin Endocrinol Metab 91:3592–3597

Stepkowski SM, Chen W, Ross JA, Nagy ZS, Kirken RA (2008) STAT3: an important regulator of multiple cytokine functions. Transplantation 85:1372–1377

St-Pierre J, Tremblay ML (2012) Modulation of leptin resistance by protein tyrosine phosphatases. Cell Metab 15:292–297

Sun C, Zhang F, Ge X, Yan T, Chen X, Shi X, Zhai Q (2007) SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metab 6:307–319

Sun Q, van Dam RM, Meigs JB, Franco OH, Mantzoros CS, Hu FB (2010) Leptin and soluble leptin receptor levels in plasma and risk of type 2 diabetes in U.S. women: a prospective study. Diabetes 59:611–618

van Dielen FM, van 't Veer C, Buurman WA, Greve JW (2002) Leptin and soluble leptin receptor levels in obese and weight-losing individuals. J Clin Endocrinol Metab 87:1708–1716

Wang Z, Zhou YT, Kakuma T, Lee Y, Kalra SP, Kalra PS, Pan W, Unger RH (2000) Leptin resistance of adipocytes in obesity: role of suppressors of cytokine signaling. Biochem Biophys Res Commun 277:20–26

Wolsk E, Mygind H, Grondahl TS, Pedersen BK, van Hall G (2011) The role of leptin in human lipid and glucose metabolism: the effects of acute recombinant human leptin infusion in young healthy males. Am J Clin Nutr 94:1533–1544

Worm D, Vinten J, Beck-Nielsen H (1999) The significance of phosphotyrosine phosphatase (PTPase) 1B in insulin signalling. Diabetologia 42:1146–1149

Yang G, Ge H, Boucher A, Yu X, Li C (2004) Modulation of direct leptin signaling by soluble leptin receptor. Mol Endocrinol 18:1354–1362

Yang Z, Hulver M, McMillan RP, Cai L, Kershaw EE, Yu L, Xue B, Shi H (2012) Regulation of insulin and leptin signaling by muscle suppressor of cytokine signaling 3 (SOCS3). PLoS ONE 7:e47493

Zabolotny JM, Haj FG, Kim YB, Kim HJ, Shulman GI, Kim JK, Neel BG, Kahn BB (2004) Transgenic overexpression of protein-tyrosine phosphatase 1B in muscle causes insulin resistance, but overexpression with leukocyte antigen-related phosphatase does not additively impair insulin action. J Biol Chem 279:24844–24851

Zabolotny JM, Kim YB, Welsh LA, Kershaw EE, Neel BG, Kahn BB (2008) Protein-tyrosine phosphatase 1B expression is induced by inflammation in vivo. J Biol Chem 283:14230–14241

Zastrow O, Seidel B, Kiess W, Thiery J, Keller E, Bottner A, Kratzsch J (2003) The soluble leptin receptor is crucial for leptin action: evidence from clinical and experimental data. Int J Obes Relat Metab Disord 27:1472–1478

Zhang J, Scarpace PJ (2009) The soluble leptin receptor neutralizes leptin-mediated STAT3 signalling and anorexic responses in vivo. Br J Pharmacol 158:475–482

Zhang F, Chen Y, Heiman M, Dimarchi R (2005) Leptin: structure, function and biology. Vitam Horm 71:345–372