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ORIGINAL ARTICLE

Muscle hypertrophy and increased expression of leptin receptors in the musculus triceps brachii of the dominant arm in professional tennis players

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Abstract In rodents, endurance training increases leptin sensitivity in skeletal muscle; however, little is known about the effects of exercise on the leptin signalling system in human skeletal muscle. Thus, to determine whether chronic muscle loading increases leptin receptor (OB-R170) protein expression, body composition dual-energy X-ray absorptiometry was assessed in nine professional male tennis players (24 ± 4 years old) and muscle biopsies were obtained from the dominant (DTB) and non-dominant (NDTB) arm *triceps brachii* (TB), and also from the right *vastus lateralis* (VL). In each biopsy, the protein content of OB-R170, perilipin A, suppressor of cytokine signalling 3 (SOCS3), protein tyrosine phosphatase 1B (PTP1B) and signal transducer and activator of transcription 3 (STAT3) phosphorylation were determined by western blot. The DTB had 15% greater lean mass ($P < 0.05$) and 62% greater OB-R170 protein expression ($P < 0.05$) than the NDTB. SOCS3 and PTP1B protein expression was similar in both arms, while STAT3 phosphorylation was reduced in the NDTB. OB-R170 protein content was also higher in DTB than in VL ($P < 0.05$). In summary, this study shows that the functional isoform of the leptin receptor is up-regulated in the hypertrophied TB. The latter combined with the fact that both SOCS3 and PTP1B protein expression were unaltered is compatible with increased leptin sensitivity in this muscle. Our findings are also consistent with a role of leptin signalling in muscle hypertrophy in healthy humans.

Keywords Leptin · SOCS3 · PTP1B · STAT3 · Hypertrophy · Tennis

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Introduction

Leptin is a hormone produced by the adipocytes with a critical role in the regulation of appetite, energy expenditure and fat deposition (Zhang et al. 2005). Functional leptin receptors have been identified in human skeletal muscle (Fuentes et al. 2009; Guerra et al. 2007, 2008) and in vitro assays using human abdominal muscle strips have shown that in lean but not in obese subjects leptin is able to stimulate fatty acid oxidation, indicative of skeletal muscle leptin resistance in obesity (Steinberg et al. 2002). Leptin may also play a role in the regulation of muscle mass (Sainz et al. 2009). The ob/ob mouse, which does not produce leptin, and the db/db mouse, which lacks functional leptin receptors, have lower muscle mass than comparable wild-type lean mice (Madiehe et al. 2002; Trostler et al. 1979). Leptin administration to these mice promotes muscle hypertrophy (Madiehe et al. 2002; Sainz et al. 2009). However, very little is known about the influence that regular

exercise has on leptin signalling in the skeletal muscle of healthy humans.

Leptin may bind to leptin receptors (OB-R), of which six OB-R isoforms have been identified (Tartaglia et al. 1995) and classified into the categories of: secreted, short and long. The secreted isoform or soluble leptin receptor (sOB-R) is mostly secreted into the bloodstream by the liver (Cohen et al. 2005) where it binds circulating leptin and regulates the concentration of free leptin (Ge et al. 2002). The short and long isoforms contain identical extracellular and transmembrane domains and differ in the length of the intracellular amino acid sequence (Tartaglia 1997; Zhang et al. 2005). Only the long isoform of the leptin receptor (OB-Rb) contains intracellular motifs required for activation of the janus kinase, the first step in leptin signalling (Tartaglia 1997).

Upon binding to the long form of its receptor (OB-Rb), leptin stimulates janus kinase 2 (JAK2), which in turn, autophosphorylates and activates the signal transducer and activator of transcription 3 (STAT3). Reduced Tyr^{705}-STAT3 phosphorylation in the presence of increased leptin concentrations is indicative of leptin resistance (Hosoi et al. 2008). Leptin signalling may be attenuated by an increase of the protein suppressor of cytokine signalling 3 (SOCS3), which blunts JAK2/STAT3-dependent leptin signalling (Bjorbaek et al. 2000) and causes leptin resistance in the skeletal muscle (Steinberg et al. 2006). Protein tyrosine phosphatase 1B (PTP1B) is also a negative regulator of leptin and insulin signalling (Dube and Tremblay 2005) which may be increased in skeletal muscle by inflammation (Zabolotny et al. 2008). Similarly, the activation of PTP1B, which causes dephosphorylation of the leptin receptor-associated JAK2 (Dube and Tremblay 2005), could also lead to leptin resistance.

Therefore, the purpose of this study was to determine if chronic muscle loading alters OB-R protein expression, STAT3 phosphorylation and the protein expression of SOCS3 and PTP1B as negative regulators of leptin signalling. To account for the influence of genetic, environmental, nutritional and endocrine factors that cannot be controlled for in longitudinal studies, we decided to use a unilateral loading model in humans. In so doing, we obtained muscle biopsies from the *triceps brachii* (TB) of the dominant (DTB) and non-dominant (NDTB) arm of tennis players. To account for the influence of muscle fibre composition on these signalling pathways, we also obtained muscle biopsies from the m. *vastus lateralis* (VL), which in these tennis players had a higher percentage of type 1 (2/3 of all fibres) than type 2 fibres (Sanchís-Moysi et al. 2009), compared to both TB that had a higher percentage of type 2 (2/3 of all fibres) than type 1 muscle fibres. Our hypothesis was that leptin receptors would be up-regulated in the dominant arm and/or SOCS3 and PTP1B reduced, allowing for increased leptin signalling in this muscle.

Methods

Materials

The Complete protease inhibitor and the Phospho-Stop phosphatase inhibitor cocktails were obtained from Roche Diagnostics (Mannheim, Germany). The polyclonal rabbit anti-human leptin receptor antibody that recognizes three isoforms of the human leptin receptor present in skeletal muscle (Guerra et al. 2007) was obtained from Linco Research (St Charles, MO, USA). The polyclonal rabbit anti-perilipin A antibody was kindly provided by Dr Andrew S. Greenberg. The polyclonal rabbit anti-human SOCS3 antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The polyclonal rabbit anti-Tyr^{705}-STAT3 and the monoclonal mouse anti-STAT3 antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA). The monoclonal mouse anti-PTP1B was from Calbiochem (San Diego, CA, USA). The monoclonal mouse anti-alpha-tubulin antibody was obtained from Sigma-Aldrich (St Louis, MO, USA). The secondary HRP-conjugated goat anti-rabbit and donkey anti-mouse antibodies were from Jackson ImmunoResearch (West Grove, PA, USA). 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).

Subjects

Nine male tennis players aged 24 ± 4 (mean ± SD) agreed to participate in this investigation (Table 1). Written informed consent was obtained from each subject after they received a full explanation of the nature and the possible risks associated with the study procedures. The study was approved by the ethical committee of the University of Las Palmas de Gran Canaria. All subjects began tennis practice before 12 years of age and had been trained and participated in professional tennis competitions of the International Tennis Federation (Futures and Challengers tournaments).

Assessment of body composition

Body composition was determined by dual-energy X-ray absorptiometry (Hologic QDR-1500, Hologic Corp., software version 7.10, Waltham, MA, USA) as described elsewhere (Perez-Gomez et al. 2008a, b). The lean mass of the extremities was assumed to be equivalent to the muscle mass (Calbet et al. 1998; Kim et al. 2006). From the whole

Table 1 Body composition, basal plasma glucose and endocrine variables

	Mean ± SD
Age (years)	24.1 ± 3.6
Height (cm)	181.9 ± 8.5
Body mass (kg)	75.8 ± 9
% Body fat	11.8 ± 6.7
Lean mass DA (g)	3,643 ± 407
Lean mass NDA (g)	3,168 ± 359*
Leptin (ng ml^{-1})	1.9 ± 2.3
Glucose (mmol l^{-1})	5.0 ± 0.1
Insulin (pmol l^{-1})	6.0 ± 1.9
HOMA (mmol l^{-1})	1.3 ± 0.1

DA dominant arm, *NDA* non-dominant arm

* $P < 0.05$ DA versus NDA

body scans, the percentage of body fat (%) was also determined.

Muscle biopsies

On a different day, subjects reported to the laboratory at 8.00 after an overnight fast. After 10 min rest in the supine position, a venous blood sample was obtained and the skin over the lateral aspect of both TB (short head) and the middle portion of the VL was anaesthetized with 2% lidocaine. Thereafter, muscle biopsies were obtained using the Bergstrom technique as described elsewhere (Guadalupe-Grau et al. 2009). The muscle specimens were cleaned to remove any visible blood, fat and connective tissue. The muscle tissue was then immediately frozen in liquid nitrogen and stored at −80°C for later analysis.

Assessment of insulin resistance

In each subject, the degree of insulin resistance was estimated by the homeostasis model assessment (HOMA) according to the method described by Matthews et al. (1985). In brief, fasting plasma insulin and fasting plasma glucose values were used to calculate an index of insulin resistance. The HOMA index was calculated as fasting insulin concentration (μU ml^{-1}) × fasting glucose concentration (mmol l^{-1})/22.5, assuming that normal young subjects have an insulin resistance of 1.

Total protein extraction, electrophoresis and western blot analysis

For total protein extraction from human skeletal muscle, a piece of frozen tissue was homogenized as described elsewhere (Guerra et al. 2007). After centrifugation at 20,000*g* to remove tissue debris, total protein extracts were transferred to clean tubes and an aliquot of each extract was preserved for protein quantification by the bicinchoninic acid assay (Smith et al. 1985). Proteins were solubilized in sample buffer containing 0.0625 mM Tris–HCl, pH 6.8, 2.3% [w/v] sodium dodecyl sulfate (SDS), 10% [v/v] glycerol, 5% [v/v] β-mercaptoethanol and 0.001% [w/v] bromophenol blue. Equal protein amounts (50 μg) of each sample were electrophoresed on 7.5–10% SDS polyacrylamide gel electrophoresis using the system of Laemmli (1970) and transferred to Hybond-P membranes according to the method of Towbin et al. (1979). For immunoblotting, membranes were pre-incubated with 5% blotting grade blocker non-fat dry milk (Bio-Rad Laboratories, Hercules, CA, USA) in Tris-buffered saline (TBS) with 0.1% Tween 20 (blotto blocking buffer) for 1 h at room temperature (20–22°C). To detect the leptin receptor isoforms (OB-Rs), membranes were incubated with a rabbit polyclonal-specific anti-human OB-R (long form) antibody. To detect SOCS3 protein expression, membranes were incubated with a rabbit polyclonal-specific anti-human SOCS3 antibody. To detect PTP1B protein expression, membranes were incubated with a mouse monoclonal-specific anti-human PTP1B antibody. To detect Tyr^{705}-STAT3 phosphorylation, membranes were incubated with a rabbit polyclonal antibody that recognizes this kinase only when the residue Tyr^{705} is phosphorylated. To detect total STAT3, membranes were incubated with a mouse monoclonal antibody that recognizes the total form (phosphorylated and non-phosphorylated) of this kinase. To control for differences in loading and transfer efficiency across membranes, an antibody directed against alpha-tubulin was used to hybridize on the same samples. Membrane incubations with polyclonal rabbit anti-OB-R (diluted 1:1,500 in blotto blocking buffer), polyclonal rabbit anti-Tyr^{705}-STAT3 [diluted 1:500 in 5% bovine serum albumin (BSA) in TBS with 0.1% Tween 20 (BSA blocking buffer)], monoclonal mouse anti-STAT3 (diluted 1:750 in BSA blocking buffer) and the monoclonal mouse anti-PTP1B (diluted 5:1,000 in blotto blocking buffer) were performed overnight at 4°C. Membrane incubations with polyclonal rabbit anti-SOCS3 (diluted 1:500 in blotto blocking buffer) and with monoclonal mouse anti-alpha-tubulin (diluted 1:50,000 in blotto blocking buffer) were performed for 1 h at room temperature. To control for the presence of adipose tissue protein in the muscle extract, a polyclonal rabbit anti-perilipin A antibody was used (Guerra et al. 2007; Wang et al. 2003). To explore the expression of this protein in human skeletal muscle, membranes were blocked with BSA blocking buffer for 1 h at room temperature. Membrane incubations with polyclonal rabbit anti-perilipin A antibody (diluted 1:1,500 in BSA blocking buffer) were performed for 1 h at room temperature. Antibody-specific labelling was revealed by incubation

with an HRP-conjugated goat anti-rabbit antibody (1:20,000) or an HRP-conjugated donkey anti-mouse (1:10,000) antibody both diluted in blotto blocking buffer. Specific bands were visualized with the ECL chemiluminescence kit (Amersham Biosciences), visualized with the ChemiDoc XRS system (Bio-Rad Laboratories) and analysed 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 band intensity of immunostaining values (arbitrary units) obtained for OB-R, Perilipin, SOCS3 or PTP1B relative to those obtained for alpha-tubulin. Alpha-tubulin and total STAT3 protein contents in the DTB, NDTB and VL muscle were similar (data not shown, $P > 0.05$). Western blots were always performed in triplicate with a variation coefficient less than 10%. Samples from the same subject were run in the same gel.

Leptin assays

Serum leptin was determined by enzyme-linked immunosorbent assay (ELx800 Universal Microplate Reader, Bioteck Instruments Inc, Winooski, VT, USA), using reagent kits from Linco Research (#EZHL-80SK, Linco Research, St Charles, MO, USA) following the manufacturer's instructions. The sensitivity of the total leptin assays was 0.05 ng ml^{-1}. The intra-assay coefficient of variation was 3.8% and the inter-assay coefficient of variation was 4.4%.

Statistical analysis

Comparisons between biopsies were performed using ANOVA for repeated measures with post hoc comparisons carried out using the Fisher's LSD test. Since the dominant TB OB-R98 and PTP1B did not follow a Gaussian distribution, they were logarithmically transformed. Side-to-side differences in tissue composition were analysed using paired Student's *t* test. Correlations between variables were determined by calculating the Pearson's correlation coefficient. Results are presented as mean ± SD, except on the bar figures which are presented as mean ± SE of the mean. Data were analysed using the SPSS statistical program (SPSS 14.0 Inc., Chicago, IL, USA). Statistical significance level was set at $P < 0.05$.

Results

Dual-energy X-ray absorptiometry

Body composition and anthropometrics are reported in Table 1. The lean mass of the dominant arm was 15.0% greater than that of the contralateral arm (3,643 ± 407 versus 3,168 ± 359 g, $P < 0.001$). However, the lean mass of both lower extremities was similar (10,351 ± 1,294 versus 10,239 ± 1,123g, right and left legs, respectively, $P = 0.49$).

Serum leptin concentrations, glucose, insulin and HOMA

Serum leptin, insulin and glucose concentrations as well as HOMA are reported in Table 1. Serum leptin correlated with the percentage of fat in arms, limbs, trunk and whole body ($r = 0.88$, 0.99, 0.96 and 0.99, all $P < 0.01$ respectively).

Between-arm differences

The protein expression of the long isoform of the leptin receptor was 62% greater in DTB than in NDTB (Figs. 1a, 2a) ($P < 0.05$). Between-arm differences in OB-R128 ($P = 0.28$) and OB-R98 ($P = 0.36$) did not reach statistical significance (Figs. 1a, 2b, c, respectively). Perilipin A content was similar in both arms, indicating a similar degree of contamination by protein coming from adipocytes in the biopsies from both TB ($P = 0.36$) (data not shown).

Suppressor of cytokine signalling 3 (Figs. 1d, 3b) ($P = 0.34$) and PTP1B (Figs. 1e, 3c) ($P = 0.57$) protein

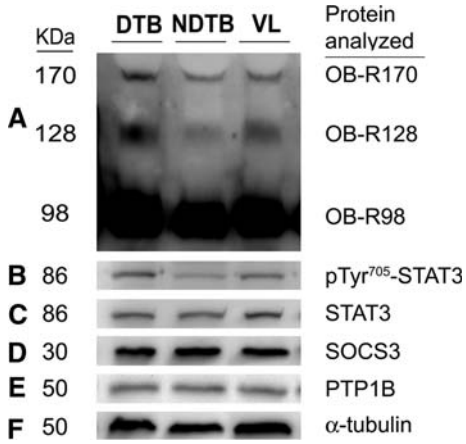

Fig. 1 Representative western blot assays to determine leptin receptor (OB-R), STAT3, SOCS3, PTP1B and alpha-tubulin protein expression and the $pTyr^{705}$-STAT3 phosphorylation level in the *triceps brachii* from the dominant arm (DTB), non-dominant arm (NDTB) and from the *vastus lateralis* (VL). **a** Representative immunoblot assay after incubation with a polyclonal rabbit anti-OB-R antibody specifically raised against the long isoform. **b** Representative western blot after incubation with a polyclonal rabbit anti-$pTyr^{705}$-STAT3 antibody in the same samples used in A. **c** Representative western blot after incubation with a monoclonal mouse anti-STAT3 antibody in the same samples used in A. **d** Representative western blot after incubation with a polyclonal rabbit anti-SOCS3 antibody in the same samples used in A. **e** Representative western blot after incubation with a monoclonal mouse anti-PTP1B antibody in the same samples used in A. **f** Representative western blot after incubation with a monoclonal mouse anti-alpha-tubulin antibody in the same samples used in **a**

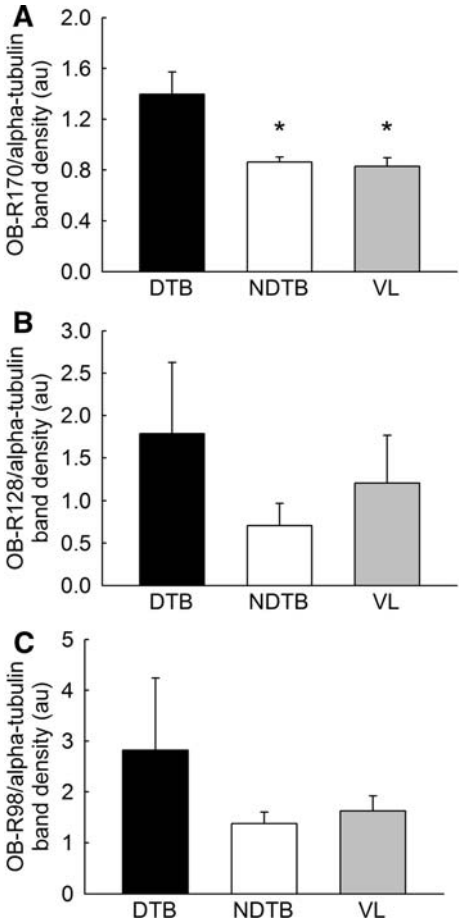

Fig. 2 Determination of the leptin receptor (OB-R) protein expression in the *triceps brachii* from the dominant arm (*DTB*), non-dominant arm (*NDTB*) and from the *vastus lateralis* (*VL*). **a** Densitometric immunosignal values (arbitrary units of band densities) of OB-R170 bands relative to those obtained for alpha-tubulin. **b** Densitometric immunosignal values (arbitrary units of band densities) of OB-R128 bands relative to those obtained for alpha-tubulin. **c** Densitometric immunosignal values (arbitrary units of band densities) of OB-R98 bands relative to those obtained for alpha-tubulin. $^{*}P < 0.05$ versus DTB

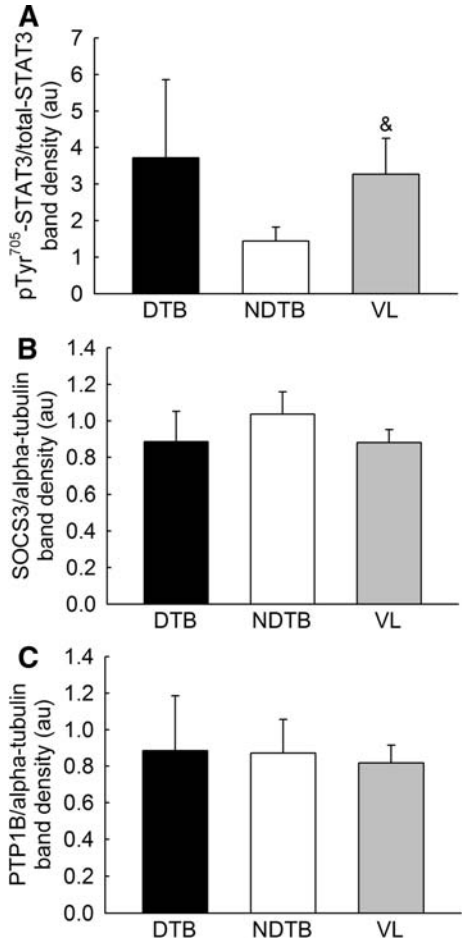

Fig. 3 Determination of pTyr^{705}-STAT3 phosphorylation level and SOCS3 and PTP1B protein expression in the *triceps brachii* from the dominant arm (*DTB*), non-dominant arm (*NDTB*) and from the *vastus lateralis* (*VL*). **a** Densitometric analysis of pTyr^{705}-STAT3 immunoblot (arbitrary units of band densities). Values are relative to total STAT3. **b** Densitometric immunosignal values (arbitrary units of band densities) of SOCS3 bands relative to those obtained for alpha-tubulin. **c** Densitometric immunosignal values (arbitrary units of band densities) of PTP1B bands relative to those obtained for alpha-tubulin. $^{\&}P < 0.05$ versus NDTB

expression as well as Tyr^{705}-STAT3 phosphorylation (Figs. 1b, 3a) ($P = 0.50$) were similar in both arms. A correlation matrix is presented in Table 2. In the DTB, there was a correlation between leptin and leptin receptors. However, in the NDTB and VL, there was no correlation between leptin and leptin receptors. Leptin correlated with SOCS3 in the NDTB ($r = 0.73$, $P < 0.05$). PTP1B was inversely related to leptin ($r = -0.78$, $P < 0.05$) in the DTB.

Comparison between *vastus lateralis* and both triceps brachii

OB-R128 (Figs. 1a, 2b), OB-R98 (Figs. 1a, 2c), Perilipin A (data not shown), SOCS3 (Figs. 1d, 3b) and PTP1B protein expression (Figs. 1e, 3c) were similar in the three muscles. However, OB-R170 protein content was higher in the DTB compared to the VL ($P < 0.05$), and similar in NDTB and VL (Figs. 1a, 2a). Tyr^{705}-STAT3 phosphorylation level was 2.3-fold higher in the VL than in the NDTB ($P < 0.05$) (Figs. 1b, 3a).

Discussion

In agreement with our hypothesis, this study shows that chronic muscle loading causing muscle hypertrophy is associated with up-regulation of the long isoform, i.e. the functionally active form, of the leptin receptor. Moreover, basal Tyr^{705}-STAT3 phosphorylation was reduced in the NDTB compared to the m. *vastus lateralis*, indicating that unloaded (or less loaded) muscles may have reduced STAT3

Table 2 Correlation matrix

		OB-R170	OB-R128	LgOB-R98	SOCS3	pSTAT3	LgPTP1B
Dominant musculus *triceps brachii*							
Leptin	*r*	0.78	0.80	0.81	−0.55	0.00	−0.78
	P	0.01	0.01	0.008	0.12	1.00	0.02
OB-R170	*r*		0.78	0.71	−0.50	−0.002	0.83
	P		0.01	0.03	0.17	1.00	0.01
OB-R128	*r*			0.93	−0.56	−0.14	0.87
	P			0.001	0.11	0.71	0.005
LgOB-R98	*r*				−0.73	−0.16	0.74
	P				0.03	0.78	0.04
SOCS3	*r*					−0.15	−0.61
	P					0.70	0.11
pSTAT3	*r*						0.13
	P						0.76
		OB-R170	OB-R128	OB-R98	SOCS3	pSTAT3	PTP1B
Non-dominant musculus *triceps brachii*							
Leptin	*r*	0.00	0.05	0.17	0.73	−0.22	0.14
	P	0.99	0.90	0.66	0.03	0.58	0.74
OB-R170	*r*		0.15	0.11	0.29	0.47	−0.14
	P		0.70	0.78	0.45	0.20	0.75
OB-R128	*r*			0.38	−0.31	−0.35	−0.26
	P			0.31	0.41	0.35	0.54
OB-R98	*r*				−0.30	−0.34	−0.47
	P				0.43	0.36	0.24
SOCS3	*r*					0.17	0.21
	P					0.66	0.61
pSTAT3	*r*						−0.26
	P						0.53
		OB-R170	OB-R128	OB-R98	SOCS3	pSTAT3	PTP1B
Musculus *vastus lateralis*							
Leptin	*r*	−0.28	−0.10	−0.51	−0.56	−0.38	0.34
	P	0.46	0.80	0.17	0.12	0.31	0.37
OB-R170	*r*		0.60	0.44	0.01	0.10	0.05
	P		0.09	0.24	0.98	0.81	0.91
OB-R128	*r*			0.52	−0.02	0.08	−0.48
	P			0.15	0.97	0.83	0.19
OB-R98	*r*				0.79	0.49	−0.48
	P				0.01	0.18	0.19
SOCS3	*r*					0.51	−0.43
	P					0.16	0.25
pSTAT3	*r*						−0.67
	P						0.05

LgOB-R98 logarithm of OB-R98, *LgPTP1B* logarithm of PTP1B

phosphorylation under basal conditions, which is compatible with reduced leptin signalling. This study also shows that SOCS3 and PTP1B protein expression are similar in the three muscles analysed, indicating that muscle loading has little effect on the basal concentrations of these proteins in skeletal muscle at least in healthy physically active humans.

In agreement with our previous study, there was no relationship between leptin concentration and SOCS3 protein expression in the VL (Guerra et al. 2008). However, there was a positive association between leptin and SOCS3 in the non-dominant triceps ($r = 0.73$). This suggests that other factors dominate over leptin to regulate SOCS3 in loaded

muscles. This may be necessary, since an increase in SOCS3 could limit protein synthesis in the muscle (Leger et al. 2008). What is clear is that correlations appear to be different in the loaded TB compared to the unloaded TB, but also in the loaded TB and the loaded VL implying that this signalling system in skeletal muscles is affected by loading depending on the muscle fibre type composition.

Very little is known about the regulation of the expression of leptin receptors in human skeletal muscles. Leptin, insulin, insulin-like growth factor I (IGF-I), testosterone and estradiol are among the known regulators of OB-R expression, but the effects of circulating hormones on OB-R expression show tissue specificity (Alonso et al. 2007; Garofalo et al. 2006; Hikita et al. 2000; Ishikawa et al. 2007; Liu et al. 2007). For example, leptin administration at high doses to cultures of hepatic stellate cells stimulates OB-R expression (Tang et al. 2009). In human skeletal muscle, OB-Rb protein expression in the musculus VL is almost twice as high in women as in men (Guerra et al. 2008). However, OB-Rb protein content is reduced in the VL and deltoid muscle of obese humans compared to non-obese controls (Fuentes et al. 2009). In this study, we found a positive correlation between OB-Rb and circulating leptin in the loaded TB, which was absent in the other two muscles. Thus, muscle loading facilitates the expression of leptin receptors when accompanied by muscle hypertrophy, at least in muscles with a high proportion of type 2 fibres, as in the TB (Sanchís-Moysi et al. 2009).

It has also been shown that oxidative stress may contribute to stimulate OB-R expression in hepatic stellate cell cultures (Tang et al. 2009). Since both the TB and the VL are subjected to exercise-induced oxidative stress, other factors must also stimulate OB-Rb expression in the hypertrophied human TB. Another possible mechanism that could explain the up-regulation of OB-R is related to the process of muscle hypertrophy. In breast cancer cell lines, immunoprecipitation of OB-R with subsequent immunoblotting for IGF-I receptor (IGF-IR) showed that OB-Rb is pulled down with IGF-IR and that IGF-IR immunoprecipitation pulled down OB-Rb (Ozbay and Nahta 2008). These experiments suggested that at least in cancer cells IGF-IR and OB-Rb interact. Moreover, IGF is able to induce OB-Rb phosphorylation by IGR-IR kinase which is activated upon IGF binding to IGF-IR (Ozbay and Nahta 2008). However, leptin cannot signal through IGF-IR (Ozbay and Nahta 2008). Muscle contraction and stretching stimulate IGF-I and II production in skeletal muscles which by an autocrine mechanism promote muscle hypertrophy (Adams and McCue 1998; Goldspink 1999; Matheny et al. 2009). Since IGF-I is able to stimulate OB-Rb expression, at least in breast cancer cell lines (Garofalo et al. 2006), it is likely that at the same time it elicits an increase in OB-Rb expression also in the skeletal muscles. However, this hypothesis needs to be verified experimentally.

As previously reported (Sanchís-Moysi et al. 2009), type 2 muscle fibres were predominantly hypertrophied in the DTB, and type 1 muscle fibres in the m. *vastus lateralis*. Thus, this could indicate that the increase in OB-Rb protein expression in loaded muscles is mostly occurring in the hypertrophied type 2 muscle fibres. Unfortunately, it was not possible to carry out immunohistochemical experiments to confirm this indirect finding.

It should be taken into consideration that STAT3 is the signal transducer of numerous stimuli in addition to leptin (Stepkowski et al. 2008). Serrano et al. (2008) have shown that interleukin-6 (IL-6) is necessary for satellite cell-mediated muscle hypertrophy by a Tyr^{705}-STAT3-dependent mechanism. IL-6 is produced locally by skeletal muscle fibres in response to muscle contraction (Hiscock et al. 2004; Steensberg et al. 2000) and lengthening (McKay et al. 2009), and also in response to reactive oxygen species (Fischer et al. 2004). The exercise-induced muscle IL-6 production is accentuated after resistance training when the weight-lifting exercise is performed at the same relative intensity (Izquierdo et al. 2009). It has also been reported that in the m. *vastus lateralis* an increase of Tyr^{705}-STAT3 phosphorylation (and SOCS3 mRNA) occurs 2 h after a strength training session that is normalized by 4 h into the recovery period (Trenerry et al. 2007).

In the present investigation, Tyr^{705}-STAT3 phosphorylation was similar in the dominant TB and in m. *vastus lateralis*; however, it was reduced in the NDTB compared to m. *vastus lateralis* (the comparison between the DTB and NDTB did not reach statistical significance due to the high variability observed in the dominant arm). This between-muscle difference in Tyr^{705}-STAT3 phosphorylation could be explained by lower local production of IL-6 in unloaded muscles. Exercise-induced IL-6 production mainly occurs in type 2 fibres (Hiscock et al. 2004). Despite the fact that our subjects had a similar proportion of type 2 fibres in both TB and a lower amount of type 2 fibres in their m. *vastus lateralis* (Sanchís-Moysi et al. 2009), Tyr^{705}-STAT3 phosphorylation was similar in their loaded TB and in their m. *vastus lateralis* (also a loaded muscle). Thus, the present investigation shows that basal Tyr^{705}-STAT3 phosphorylation is increased in the trained muscle regardless of its fibre type composition.

Another interesting aspect of this study is that our Tyr^{705}-STAT3 phosphorylation results are consistent with the known increase in muscle oxidative profile with exercise training (Gollnick et al. 1973): VL (predominantly composed by type 1 fibres) expressing a greater Tyr^{705}-STAT3 phosphorylation to OB-Rb abundance than the DTB (predominantly composed by type 2 fibres) expressing larger receptor abundance but similar albeit variable Tyr^{705}-STAT3 phosphorylation. These data indicate that there is no need for high levels of leptin receptors in the

trained leg muscle that has increased Tyr^{705}-STAT3 phosphorylation (compatible with effective leptin signalling). In the untrained TB, leptin-SOCS3 feedback loop appears to be active, given the correlation observed between these two variables, and leptin sensitivity may be reduced compared to the contralateral trained muscle. In contrast, in the trained TB, leptin signalling is facilitated by the elevated content of leptin receptors combined with lack of elevation of either SOCS3 or PTP1B. This is opposite to what is observed in the VL and *deltoid* muscles of obese humans, who have reduced OB-Rb content and leptin resistance (Fuentes et al. 2009).

It remains to be determined what function the up-regulation of OB-Rb subserves in the hypertrophied human skeletal muscle: is OB-Rb involved in skeletal muscle hypertrophy in response to mechanical loading? Indirect evidence lends support for such a possibility. For example, the ob/ob mouse, which does not produce leptin, and the db/db mouse, which lacks functional leptin receptors, both have lower muscle mass than comparable wild-type lean mice, despite ob/ob and db/db mice weighing twice as much as lean mice (Madiehe et al. 2002; Trostler et al. 1979). Leptin administration, even to db/db mice strains, promotes muscle growth by a mechanism likely mediated by short OB-R isoforms (Madiehe et al. 2002). Likewise, muscle accretion in response to leptin treatment has been recently reported in ob/ob mice (Sainz et al. 2009). Thus, an increased OB-Rb expression in overloaded skeletal muscle may facilitate muscle growth by a mechanism involving leptin signalling through JAK2/PIK3/Akt (Maroni et al. 2005), elicited either by leptin itself or by IGF-I. In contrast, Warmington et al. (2000) reported no effect of leptin treatment on muscle mass and morphology in ob/ob mice, despite reducing body mass. Moreover, treatment with low and high doses of leptin (without changes in mechanical loading) does not increase lean body mass in humans with human immunodeficiency virus-associated lipoatrophy and hypoleptinemia (Mulligan et al. 2009). Perhaps, the theoretical pro-hypertrophic effect of leptin is only observed when the action of leptin is combined with mechanical loading. This notion is supported by human cross-sectional studies showing an association between hypertension-induced cardiac hypertrophy and plasma leptin concentration (Paolisso et al. 1999). However, in cardiac myocytes from ob/ob mice, leptin has been reported to have anti-hypertrophic effects in pressure overloaded hearts (Mascareno et al. 2009).

Another possible role for the increase in OB-Rb in the loaded m. *triceps brachii* is to facilitate leptin signalling in this muscle to increase the capacity to oxidize fat to cope with the increased energy demand caused by tennis participation. However, this hypothetical explanation should be tested in future studies.

In summary, this study shows that TB hypertrophy is accompanied by up-regulation of the functional isoform of the leptin receptor. Given the cross-talk between IGF-I signalling and leptin signalling, this finding is compatible with a role for leptin signalling in muscle hypertrophy in healthy humans. Since hypertrophy occurred predominantly in type 2 fibres in the loaded TB and in type 1 fibres in the VL, our findings are consistent with a greater increase in OB-Rb content in hypertrophied type 2 muscle fibres. We have also shown that SOCS3 and PTP1B protein expression in skeletal muscle is similar in both TB and VL, implying that muscle loading has little influence on the basal expression of these two negative regulators of leptin signalling in the healthy human. In contrast, muscle loading appears to increase Tyr^{705}-STAT3 phosphorylation similarly in slow and fast muscle fibres. These results are compatible with increased leptin sensitivity in the hypertrophied skeletal muscles.

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