Serum free testosterone, leptin and soluble leptin receptor changes in a 6-week strength-training programme

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Strength training is usually associated with a reduction in fat mass and with muscle hypertrophy. The aim of the present study was to examine whether the serum free leptin index (FLI), measured by the molar excess of soluble leptin receptor (sOB-R) over leptin, is increased by 6 weeks of strength training. Eighteen male, physical education students were randomly assigned to two groups: a strength-training (*n* 12) and a control group (*n* 6). Body composition (lean body mass and body fat) determined by dual-energy X-ray absorptiometry (DXA), muscle performance and leptin, sOB-R, total testosterone and free testosterone concentrations were determined before and after training. Fat mass was reduced by 1 kg with strength training ($P < 0.05$). Lean body mass of trained extremities was increased by 3 % ($P < 0.05$), while the concentration of free testosterone in serum was reduced by 17 % ($P < 0.05$) after training. However, despite the reduction in fat mass and free testosterone, serum leptin concentration was not significantly affected by strength training, even after accounting for the differences in body fat. By contrast, for a given fat mass, the sOB-R was increased by 13 % ($P < 0.05$) at the end of the strength-training programme, although the molar excess of sOB-R over leptin remained unchanged. Therefore, the quantity of free leptin available to bind to the target tissues was not significantly affected by the short strength-training programme, which elicited a 7 % reduction in fat mass.

Free leptin index: Obesity: Weight loss: Exercise

Leptin is a hormone released by the adipose tissue that assists in the regulation of body weight and energy homeostasis (Pelleymounter *et al.* 1995). Plasma leptin concentration is directly proportional to adipose tissue mass and acts on the hypothalamus as a signal of the amount of energy stored as fat (Considine *et al.* 1996; Friedman & Halaas, 1998). Increasing fat mass results in higher leptin levels, which induce satiety (Mars *et al.* 2006) and a mild enhancement of basal energy expenditure (Considine *et al.* 1996). Conversely, reducing the body fat stores through regular exercise and/or dieting results in a proportional reduction of circulating leptin (Perusse *et al.* 1997; Houmard *et al.* 2000; Thong *et al.* 2000), although exercise can also elicit changes in leptin concentration without an accompanying change in body composition (van Aggel-Leijssen *et al.* 1999).

A circulating soluble form of the leptin receptor, soluble leptin receptor (sOB-R), is the main leptin-binding protein that determines the free fraction of the circulating hormone, presumably the biologically active form of leptin (Lammert *et al.* 2001; Chan *et al.* 2002; Stein *et al.* 2006). sOB-R facilitates the transport of leptin from its peripheral site of production to the target tissues (Tartaglia, 1997; Maamra *et al.* 2001). The plasma sOB-R concentration increases with weight loss (van Dielen *et al.* 2002; Stein *et al.* 2006). Thus, the concentration of free leptin may be reduced even more than the concentration of total leptin with weight loss.

In children with protein energy malnutrition (PEM), the molar excess of sOB-R over leptin was about seventy times higher than in normal children (Stein *et al.* 2006). Two weeks after refeeding, the plasma leptin concentration was found to have increased by 31 % over the control group while the sOB-R was reduced by about 45 % in the PEM children, even though both weight and BMI mean values were still below those in the control group (Stein *et al.* 2006). An excess of sOB-R inhibits the bioactivity of leptin through its competition with the membrane receptor (Zastrow *et al.* 2003) and may reduce the availability of free leptin for transport to the cerebrospinal fluid (Landt *et al.* 2000).

Regular physical activity (i.e. exercise training) is associated with increased energy demand and changes in body composition that in turn could influence plasma leptin concentration (Landt *et al.* 1997; Leal-Cerro *et al.* 1998; van Aggel-Leijssen *et al.* 1999; Reseland *et al.* 2001; Zaccaria *et al.* 2002). It remains unknown, however, to what extent exercise training affects the plasma concentration of sOB-R and the balance between leptin and its soluble receptor.

Strength training increases muscle mass, strength and power while fat mass is reduced (Hakkinen, 1989; McCall *et al.* 1996). In the short term, a strength-training session elicits a transient increase in basal plasma testosterone concentration in young men (Hakkinen *et al.* 1998; Kraemer *et al.* 1998). In the long term, strength training has also been associated

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with a small increase (8 %) in basal serum total testosterone concentration (Gorostiaga *et al.* 2004), although the free fraction seems not to be affected (Ahtiainen *et al.* 2003; Gorostiaga *et al.* 2004). However, intensive strength training may be associated with a reduction in serum testosterone (Raastad *et al.* 2001), and this effect may be accentuated by energy deprivation (Bergendahl *et al.* 1998). Free plasma testosterone has been reported to be inversely related to sOB-R (Chan *et al.* 2002) and leptin (Luukkaa *et al.* 1998). Moreover, the weekly administration of 200 mg testosterone enanthate over 12 months has been associated with a 44 % decrease in mean serum leptin concentration (Luukkaa *et al.* 1998), probably due to the suppressive effect of testosterone on leptin production (Wabitsch *et al.* 1997). Thus, strength training may cause changes in the relationship between fat mass and the plasma levels of leptin, sOB-R and the molar excess of sOB-R over leptin, an estimation of the free leptin index (FLI; Misra *et al.* 2004; Stein *et al.* 2006), which may be modulated in part by alterations in the androgenic status.

The aims of the present study were therefore: (1) to determine whether a 6-week strength-training programme has an effect on circulating levels of leptin, sOB-R, the FLI and testosterone; (2) to determine to what extent strength training-induced changes in body composition are associated with changes in serum plasma concentrations of leptin, its soluble receptor, and free leptin; (3) to determine whether changes in total or free testosterone influence the response of leptin, sOB-R, and the FLI to strength training.

Methods

Subjects

Eighteen healthy men (age 23 (SD 2) years, height 173 (SD 5) cm, body mass 73 (SD 6) kg, body fat 17 (SD 6) %) participated in this investigation. Written informed consent was obtained from all subjects after they had received a full explanation of the study procedures. The subjects were randomly assigned to two groups: one performed a strength-training (ST, *n* 12) programme and the other was a control group (CON, *n* 6). Their physical characteristics are presented in Table 1. All of the subjects were healthy and physically active and were familiar with strength training. In addition, they all answered a questionnaire providing information about personal data, sports participation (including the number of training hours per week) and medical history (including past injuries and medication). The study was performed in accordance with the Helsinki Declaration of 1975, and was approved by the Ethical Committee of the University of Las Palmas de Gran Canaria.

Table 1. Characteristics of subjects at the beginning of the study

(Mean values with their standard errors)

	Strength group		Control group	
	Mean	SEM	Mean	SEM
Age (years)	22·7	0·6	23·7	0·7
Body mass (kg)	74·7	2·2	70·3	1·8
Height (cm)	173·9	2·2	171·9	1·9

Study design

Each subject performed two test sessions, one before and one after strength training. In both test sessions body composition was determined by dual-energy X-ray absorptiometry (DXA) and by the maximum weight that could be lifted at least once for each exercise (one-repetition maximum, 1RM) used during the training period. Before testing and training, subjects were familiarized with the experimental procedures. The total duration of the study was 8 weeks. Baseline testing was completed during the first week. This was followed by a 6-week period of supervised experimental strength training (Table 2). Resting blood samples were drawn at baseline and at the end of the study, 48–72 h after the last training session. Venous blood samples were taken between 07.00 and 08.00 hours, after 12 h overnight fasting. The blood samples were allowed to clot at 4°C and then centrifuged at the same temperature (Allegra 21R, Beckman Instruments Inc, Fullerton, CA, USA). The serum obtained was separated and frozen at −80°C for later analysis.

Anthropometry and body composition

Anthropometric measurements were obtained on each subject. Height was measured in the upright position to the nearest millimetre (Atlántida, Añó Sayol, Barcelona, Spain). Body mass was determined using a balance with a 50 g imprecision (Atlántida), calibrated with M1 calibration masses (tolerance <0·005 % in mass).

Total and regional body composition was assessed by DXA (Hologic QDR-1500, Hologic Corp., software version 7.10, Waltham, MA, USA) as described elsewhere (Calbet *et al.* 1998; Ara *et al.* 2004, 2006). Lean body mass (g) and body fat were calculated from whole-body scans. Whole-body scans were submitted to a regional analysis to determine the composition of the arm, leg and trunk regions. The arm region included

Table 2. Strength-training programme (sets × repetitions). Percentage of 1RM 000 (the maximum weight that could be lifted at least once for each excercise) lifted during training is given in parentheses. A 90-s rest period was given between exercise sets

Week	Session	Weight lifted per session
1	1	10 (50 %) + 6 (70 %) + 3 (90 %)
	2	10 (50 %) + 6 (70 %) + 3 (90 %)
	3	10 (50 %) + 6 (70 %) + 3 (90 %)
2	4	2 × 10 (50 %) + 2 × 6 (70 %) + 2 × 3 (90 %)
	5	10 (50 %) + 6 (70 %) + 3 (90 %)
	6	10 (50 %) + 6 (70 %) + 3 (90 %)
3	7	2 × 10 (50 %) + 2 × 6 (70 %) + 2 × 3 (90 %)
	8	2 × 10 (50 %) + 2 × 6 (70 %) + 2 × 3 (90 %)
	9	10 (50 %) + 6 (70 %) + 3 (90 %)
4	10	2 × 10 (50 %) + 2 × 6 (70 %) + 2 × 3 (90 %)
	11	2 × 10 (50 %) + 2 × 6 (70 %) + 2 × 3 (90 %)
	12	2 × 10 (50 %) + 2 × 6 (70 %) + 2 × 3 (90 %)
5	13	3 × 10 (50 %) + 3 × 6 (70 %) + 3 × 3 (90 %)
	14	2 × 10 (50 %) + 2 × 6 (70 %) + 2 × 3 (90 %)
	15	2 × 10 (50 %) + 2 × 6 (70 %) + 2 × 3 (90 %)
6	16	3 × 10 (50 %) + 3 × 6 (70 %) + 3 × 3 (90 %)
	17	3 × 10 (50 %) + 3 × 6 (70 %) + 3 × 3 (90 %)
	18	3 × 10 (50 %) + 3 × 6 (70 %) + 3 × 3 (90 %)

the hand, forearm and arm, and was separated from the trunk by an inclined line crossing the scapulo-humeral joint, such that the humeral head was located in the arm region. The leg region included the foot, the lower leg and the upper leg, and was separated from the trunk by an inclined line passing just below the pelvis, which bisected the femoral neck crossing the neck of the femur. The trunk region included the rest of the body minus the arm, leg and head regions. The head region comprised all skeletal parts of the skull and cervical vertebra above a horizontal line passing just below the jawbone. With this analysis, regional body fat and lean mass can be assessed with a coefficient of variation below 5 % (Calbet *et al.* 1998). Lean mass of the upper and lower extremities was assumed to be equivalent to the muscle mass of the upper and lower extremities (Shih *et al.* 2000; Kim *et al.* 2002).

Strength assessment and strength-training programmes

Maximum strength (1RM) for all exercises used during training (parallel squat, leg extension, inclined leg press, leg curl and hip flexors) was assessed immediately before and at the end of the strength-training period. These exercises were performed on weight-lifting equipment (Technogym Ltd, Barcelona, Spain). The parallel squat and the inclined leg press were performed with a range of motion between full extension and a knee angle of 90°. All the available range of motion was used during the leg extension and leg curl exercises. The hip flexors exercise was carried out from the standing position by flexing the knee of one of the extremities, trying to bring it into contact with the chest while a pulley was attached at the ankle. Both limbs were trained alternatively. The 1RM values obtained were subsequently used to calculate the relative loads for the training protocols. The relative loads for each exercise ranged between 50 % and 90 % of the 1RM load (Table 2). Subjects completed a total of 414 sets (parallel squats: 102 sets, leg extension: 102 sets, inclined leg press: 102 sets, leg flexors: 54 sets, hip flexors: 54 sets). A 90-s rest period was taken between exercise sets. All subjects completed the training programme. The energy expenditure during the strength-training sessions was estimated according to the formula of McArdle *et al.* (1991), energy expenditure (kJ) = (0·36) × (body mass in kg) × (duration of exercise in minutes), and ranged between 1047 and 1424 kJ per session.

Leptin, soluble leptin receptor and testosterone assays

Serum leptin, sOB-R and total and free testosterone were determined by ELISA (ELx800 Universal Microplate Reader, Bioteck Instruments Inc., VT, USA). Serum leptin, sOB-R and free testosterone were measured by using reagent kits from Diagnostic Systems Laboratories (Webster, TX, USA), and total testosterone with reagent kits from Diagnostics Biochemical Canada (London, ON, Canada). The sensitivity of the total leptin and sOB-R assays was 0·05 and 0·2 ng/ml, respectively. The intra-assay CVs were 3·8 % and 4·1 % and the interassay CVs were 4·4 % and 4·9 % for leptin and sOB-R, respectively. For the total and free testosterone assays, the sensitivity was 1·74 nmol/l and 0·66 pmol/l, respectively, and the intra- and inter-assay CVs were 10·8 % and 11·4 % for total testosterone and 6·5 % and 3·1 % for free testosterone, respectively. The FLI, the molar excess of

sOB-R over leptin, was calculated as sOB-R divided by leptin multiplied by 0·13 (Stein *et al.* 2006).

Statistical analyses

The statistical analyses was performed using the Statistical Package for the Social Sciences version 11.0 (SPSS Inc., Chicago, IL, USA). All data are reported as means with their standard errors. The effect of strength training on the dependent variables was assessed using ANOVA for repeated measures. The Mauchly's test of sphericity was run before the ANOVA and in case of violation of the sphericity assumption, the degrees of freedom were adjusted according to the Huynh–Feldt test. Pairwise comparisons were carried out with Tukey's test. To determine whether changes in fat mass accounts for the effects observed in leptin, sOB-R and the FRI, ANCOVA was run using the changes in total fat mass as a covariant. Pearson's correlation coefficients were used to assess associations between leptin, sOB-R, the FLI, testosterone and body composition changes. A statistical test was considered significant at the $P \leq 0.05$ level (two-tailed).

Results

Body composition

Total body mass was not significantly modified either in the control or in the strength-training group (Table 3). The strength training resulted in a 3·1 % increase in the muscle mass of trained muscles ($P < 0.05$). Total fat mass decreased by 7 % ($P < 0.05$) and the fat mass in the trunk, lower and upper extremities was reduced by 8, 7 and 13 %, respectively (all $P < 0.05$), following strength training. Consequently, at the end of the study, the percentage of body fat was 1·3 units lower in the strength-training group ($P < 0.05$). Likewise, the percentage of body fat in the trunk, lower and upper extremities was significantly reduced following strength training.

Maximal strength

The strength-training group significantly improved 1RM values in all exercises [inclined leg press (13 %), squats (27 %) leg extension (16 %), leg flexion (12 %) and hip flexors (29 %)] compared with initial 1RM values (all $P < 0.05$; Fig. 1).

Leptin, sOB-R, the FLI and testosterone changes

Strength training did not significantly alter the basal concentrations of serum leptin, sOB-R, and the FLI or mean total testosterone levels (Table 4). Free testosterone concentration was reduced by 17 % in the strength-training group ($P < 0.05$). However, for a given fat mass, sOB-R was increased by 13 % ($P < 0.05$).

There was no relationship between the changes in leptin, sOB-R, the FLI, total and free testosterone, on the one hand, and the changes in body composition on the other. However, the change in leptin correlated with the change in sOB-R concentration ($r -0.50$, $P < 0.05$). The change in sOB-R concentration correlated inversely with the changes in total testosterone ($r -0.56$, $P < 0.05$).

Table 3. Total and regional soft-tissue composition before and after the resistance-training period in all groups

(Mean values with their standard errors)

|	Strength group				Control group			
	Before		After		Before		After	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Lean mass (kg)								
Whole body	55·9	1·2	56·5	1·2	56·8	2·3	55·9	2·9
Trunk	26·8	0·7	27·0	0·7	27·6	0·8	27·0	1·0
Lower extremity	9·5	0·2	9·8	0·2*	9·4	0·6	9·2	0·5
Upper extremity	3·1	0·1	3·1	0·1	3·3	0·3	3·2	0·4
Fat mass (kg)								
Whole body	14·2	1·6	13·2	1·6*	9·51	2·88	10·12	2·73
Trunk	6·0	0·9	5·5	0·9*	3·57	1·14	3·93	0·94
Lower extremity	2·9	0·3	2·7	0·3*	2·07	0·84	2·13	0·84
Upper extremity	0·8	0·0	0·7	0·0*	0·50	0·14	0·54	0·12
Percentage of fat (%)								
Whole body	19·1	1·8	17·8	1·8*	13·6	3·6	14·5	3·1
Trunk	17·3	2·2	16·0	2·2*	11·1	1·4	12·3	1·0
Lower extremity	22·0	1·8	20·4	1·8*	16·8	2·4	17·4	2·3
Upper extremity	18·5	1·5	17·6	1·6*	12·4	0·9	13·7	0·5

* $P < 0.05$ compared to pre-training values.
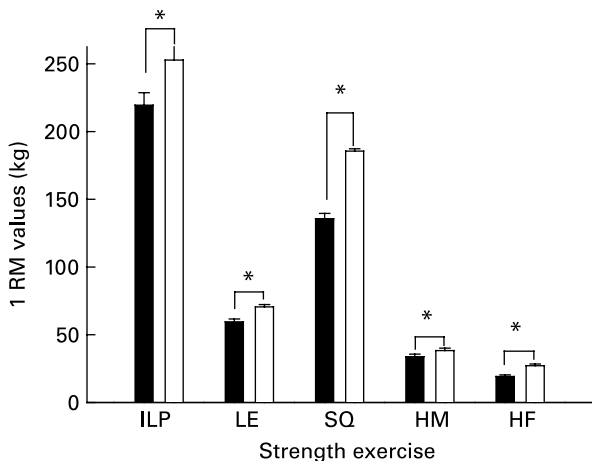
Fig. 1. Effects of strength training. ILP, inclined leg press; LE, leg extension; SQ, parallel squat; HM, leg curl (hamstrings) HF, hip flexors; 1 RM, one-repetition maximum. ■, Before training; □, after training. *$P < 0.05$ compared to pre-training values

When all subjects were pooled together, a close relationship was observed between leptin concentrations (Table 5) and the molar excess of sOB-R over leptin (Fig. 2) with fat mass and the waist circumference. However, no relationship was observed between the sOB-R and fat mass before training. At the end of the follow-up, sOB-R was inversely related to the hip circumference ($r -0.59$, $P < 0.05$) and a trend for a negative correlation was also observed with the waist circumference ($r -0.48$, $P = 0.07$).

Discussion

Although 6 weeks of strength training resulted in a significant reduction in fat mass, an improvement in dynamic muscle strength and an increase in lean body mass of the trained extremities, the present investigation shows that strength training is not associated with significant changes in basal concentration in serum of leptin, sOB-R or the molar excess of sOB-R over leptin (as an estimation of the FLI). Even after adjusting the hormonal response for changes in whole-body fat mass, no effect of strength training was observed.

Table 4. Leptin, soluble leptin receptor (sOB-R), molar excess of sOB-R over leptin and total and free testosterone values in both groups before and after training

(Mean values with their standard errors)

	Strength-training group				Control group			
	Before		After		Before		After	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Leptin (ng/ml)	5·9	1·3	5·4	1·1	3·5	0·4	4·3	0·6
sOB-R (ng/ml)	9·1	0·3	9·4	0·4	10·4	1·2	9·8	0·5
Molar excess of sOB-R over leptin	0·30	0·06	0·30	0·05	0·41	0·04	0·32	0·04
Total testosterone (nmol/l)	29·8	3·8	16·3	1·0*	35·4	7·3	27·8	9·7
Free testosterone (pmol/l)	56·2	5·9	44·4	4·5*	67·0	6·6	62·5	6·9

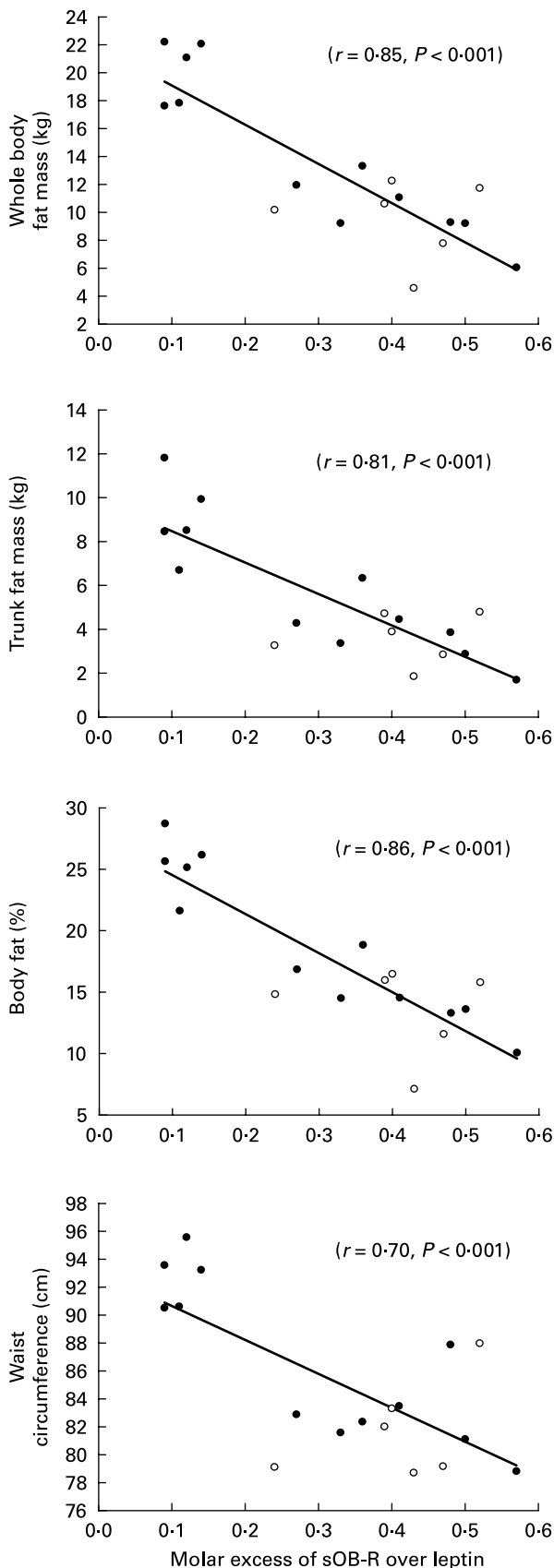* $P < 0.05$ compared to pre-training values.

Fig. 2. Relationship between the molar excess of soluble leptin receptor (sOB-R) over leptin and adiposity.

Lean mass increases of around 3 % have been reported following a 6-month whole-body strength-training programme in young people (Lemmer *et al.* 2001; Ryan *et al.* 2004). However, in the present study the effect at the whole-body level was smaller, probably because only the muscles of the lower extremities were trained. However, the lean mass of the lower extremities was increased by 3 %, showing that despite being of shorter duration than other studies (Lemmer *et al.* 2001; Ryan *et al.* 2004), our strength-training programme was efficient, in agreement with previous studies, as reviewed by Nissen & Sharp (2003).

In accordance with the present results, Kanaley *et al.* (2001) did not observe any change in basal serum leptin concentration following 6 weeks of strength training in middle-aged men. However, in contrast to the present investigation, no reduction in body fat mass or increase in muscle mass was observed with strength training in the study by Kanaley *et al.* (2001). Sixteen weeks of strength training resulted in lower basal serum leptin levels in obese postmenopausal women who lost body fat, while leptin was not altered in the group of women without changes in their body fat mass during the training period (Ryan *et al.* 2000). The authors suggested that the change in basal serum leptin concentration was mediated by the loss of body fat.

The changes observed in whole-body composition with strength training in the present investigation clearly show that our subjects were in negative energy balance. The energy deficit (at least 37 680 kJ, or about 834 kJ/day, assuming no changes in the overall efficiency of the energy metabolism during this period) was covered by the oxidation of part of the fat deposits, as reflected by a 7 % reduction in fat mass. Despite this decrease in fat mass, basal serum leptin concentration was not altered. This could be interpreted as meaning that our subjects developed a state of relatively elevated basal serum leptin concentration by comparison to the fat mass that they had before the start of the training programme. However, we can rule out this possibility because after accounting for the changes in fat mass, no significant effects were observed in leptin concentration. We also explored the possibility of an increase in the concentration of the bioavailable form of leptin, which may change independently of the total serum leptin concentration (Misra *et al.* 2004; Stein *et al.* 2006). An inverse relationship was in fact observed in the present investigation between serum sOB-R and some indexes of adiposity, as reported previously (Sandhofer *et al.* 2003). A high serum concentration of sOB-R, combined with reduced leptin and leptin/sOB-R index, has been reported in anorexic girls (Misra *et al.* 2004). Body weight gain resulted in a reduction in sOB-R and an increase in leptin and leptin/sOB-R index in the anorexic girls (Misra *et al.* 2004). In agreement, sOB-R increases with weight loss, probably due to the reduction in fat mass (Laimer *et al.* 2002; van Dielen *et al.* 2002). An inverse relationship between leptin and sOB-R changes has been reported (Chan *et al.* 2002; Gavrila *et al.* 2003) and confirmed in the present investigation. Thus, sOB-R is somewhat sensitive to changes in body composition (Chan *et al.* 2002; Reinehr *et al.* 2005; Stein *et al.* 2006). However, despite a 7 % reduction in whole-body fat mass with strength training, no significant effects were observed in sOB-R in the present investigation. However, a 13 % increase in sOB-R concentration adjusted for the whole-body fat mass was observed with strength training, meaning that for a given amount of fat mass, sOB-R is increased after strength training. An increase in sOB-R could decrease

Table 5. Relationship between leptin, sOB-R, testosterone and fat mass

	WB fat mass (kg)	Trunk fat mass (kg)	% body fat	Waist circumference (cm)	WHR
Leptin (ng/ml)	0·89*	0·89*	0·91*	0·81*	0·54*
sOB-R (ng/ml)	−0·11	−0·12	−0·12	0·02	0·22
Total testosterone (nmol/l)	−0·13	−0·08	−0·10	−0·07	0·01
Free testosterone (pmol/l)	−0·04	−0·08	−0·05	−0·06	0·00

WB, whole body; sOB-R, leptin soluble receptor; WHR, waist-to-hip ratio.

* $P < 0.05$.

the quantity of free leptin available to interact with the target tissues, and with adipose tissue in particular. However, as the molar excess of sOB-R over leptin remained unchanged with strength training, such a possibility seems unlikely (Yang *et al.* 2004).

In vitro studies indicate that gonadal hormones, such as testosterone, may be important regulators of leptin secretion (Luukkaa *et al.* 1998). Androgens are reported to block adipogenesis (Lea-Currie *et al.* 1998). This endocrine regulation is supported by the presence of specific sex steroid receptors in fat tissue (Wade & Gray, 1978; Dieudonne *et al.* 1998), indicating that adipocytes are targets for testosterone (Rebuffe-Scrive *et al.* 1985). Although not all published studies have found a significant relationship between serum testosterone and leptin, a strong inverse association has been reported (Behre *et al.* 1997; Bhasin *et al.* 1997; Luukkaa *et al.* 1998). It has also been shown that 6 months of treatment with testosterone injections reduces fat mass by 16 % (Young *et al.* 1993), probably because of a direct effect of testosterone at the level of adipocytes (Wabitsch *et al.* 1997). However, the mechanism underlying testosterone influences in leptin secretion remains to be elucidated. In the whole group, the decrease in testosterone concentration was inversely correlated with the changes in sOB-R. In agreement, free plasma testosterone has been reported to be inversely associated with sOB-R (Chan *et al.* 2002).

In summary, an intervention consisting of 6 weeks of strength training associated with a 1 kg reduction in fat mass and increases in lean body mass of the trained extremities did not result in significant changes in serum leptin concentration, even after accounting for the differences in body fat. By contrast, for a given fat mass, the sOB-R was increased at the end of the strength-training programme, although the molar excess of sOB-R over leptin (a measure of the FLI) remained unchanged. Thus, the present study indicates that the quantity of free leptin available to bind to the target tissues was not significantly affected by a short strength-training programme, which elicited a 7 % reduction in fat mass.

Acknowledgements

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