The effect of exercise training on hormone-sensitive lipase in rat intra-abdominal adipose tissue and muscle

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1. Adrenaline-stimulated lipolysis in adipose tissue may increase with training. The rate-limiting step in adipose tissue lipolysis is catalysed by the enzyme hormone-sensitive lipase (HSL). We studied the effect of exercise training on the activity of the total and the activated form of HSL, referred to as HSL (DG) and HSL (TG), respectively, and on the concentration of HSL protein in retroperitoneal (RE) and mesenteric (ME) adipose tissue, and in the extensor digitorum longus (EDL) and soleus muscles in rats.

2. Rats (weighing 96 ± 1 g, mean ± s.e.m.) were either swim trained (T, 18 weeks, n = 12) or sedentary (S, n = 12). Then RE and ME adipose tissue and the EDL and soleus muscles were incubated for 20 min with 4.4 µM adrenaline.

3. HSL enzyme activities in adipose tissue were higher in T compared with S rats. Furthermore, in RE adipose tissue, training also doubled HSL protein concentration (P < 0.05). In ME adipose tissue, the HSL protein levels did not differ significantly between T and S rats. In muscle, HSL (TG) activity as well as HSL (TG)/HSL (DG) were lower in T rats, whereas HSL (DG) activity did not differ between groups. Furthermore, HSL protein concentration in muscle did not differ between T and S rats (P > 0.05).

4. In conclusion, training increased the amount of HSL and the sensitivity of HSL to stimulation by adrenaline in intra-abdominal adipose tissue, the extent of the change differing between anatomical locations. In contrast, in skeletal muscle the amount of HSL was unchanged and its sensitivity to stimulation by adrenaline reduced after training.

Adipose tissue metabolism is under hormonal and sympathetic nervous regulation, and the rate-limiting step in adipose tissue lipolysis is controlled by the enzyme hormone-sensitive lipase (HSL), which catalyses the hydrolysis of triglyceride to monoglyceride and two fatty acid molecules. Another enzyme, a monoglyceride lipase, is responsible for the release of the third fatty acid molecule. It is generally accepted that HSL is regulated acutely by phosphorylation–dephosphorylation, primarily via the β-adrenergic activation of cAMP-dependent protein kinase (Anthonsen et al. 1998). During the last decade, however, it has been shown that long-term adaptations of HSL may also take place during conditions such as fasting (Sztalryd & Kraemer, 1994), obesity (Hellstrom et al. 1996; Large et al. 1995 and 1999) and pregnancy (Martin et al. 1994). We have recently demonstrated that intra-abdominal adipose tissue exhibits a higher adrenaline-stimulated lipolysis in vivo in trained compared with untrained rats (Enevoldsen et al. 2000). In accordance with this finding it has been demonstrated that exercise training increases the in vitro responsiveness of parametrial, epididymal and periumbilical adipocytes to adrenaline in vitro. These findings were ascribed to adaptations in post-receptor mechanisms (Bukowiecki et al. 1980; Crampes et al. 1986; Galbo, 1995). Enhanced cAMP production may be involved in this adaptation (Izawa et al. 1988), but an enhanced HSL activation has not been demonstrated. Neither is it known whether exercise training can modulate the protein concentration and total activity of HSL in intra-abdominal adipose tissue. It is important to fill these gaps in the existing knowledge in order to strengthen the recommendation for physical training in the treatment of diseases associated with visceral obesity (e.g. cardiovascular disease and non-insulin-dependent diabetes; Büemann & Tremblay, 1999).
Recently, it has been shown that HSL is also present in skeletal muscle (Holm et al. 1987; Langfort et al. 1999). Furthermore, as in adipose tissue, HSL activity in skeletal muscle is stimulated by adrenaline via the \( \beta \)-adrenergic activation of cAMP-dependent protein kinase (Langfort et al. 1999). During exercise, there is a greater depletion of intramuscular triglyceride concentration in trained compared with untrained muscle (Hurley et al. 1986), despite the existence of lower plasma adrenaline concentrations in the former. This might reflect an increase in the amount of, or sensitivity to stimulation by adrenaline of HSL in trained compared with untrained muscle. However, until now the effect of training on HSL in muscle was unknown.

In the present study we examined the effect of exercise training on HSL in retroperitoneal (RE) and mesenteric (ME) adipose tissue, and in the extensor digitorum longus (EDL) and soleus muscles in rats. HSL protein concentration was determined by Western blotting. Furthermore, the neutral lipase activity, which reflects HSL activity (Holm & Österlund, 1998; Langfort et al. 1999), was determined. The phosphorylated, activated form of HSL has a much higher activity towards triglyceride substrate than does the non-phosphorylated form, whereas the two enzyme forms are equally active against diglyceride substrate. Accordingly, the active form of HSL can be measured with the triglyceride substrate, tri\(^3\)Hplein, and is referred to as HSL (TG), while the total enzyme activity can be determined with a diglyceride analogue, 1(3)-mono\(^3\)Holeoyl-2-oleylglycerol, and is referred to as HSL (DG). Since we were interested in explaining previous findings obtained during adrenaline stimulation, which might be due to an increase in the amount or sensitivity to stimulation by adrenaline of HSL, and because the total HSL activity is not changed by phosphorylation/stimulation (Holm & Österlund, 1998), we measured HSL activity only during adrenaline stimulation. The HSL (TG)/HSL (DG) ratio was taken as an index of the fraction of overall enzyme being in the active form (Langfort et al. 2000).

**METHODS**

All animal experiments were carried out according to guidelines approved by the Danish Animal Experiments Inspectorate and the Council of the American Physiological Society.

**Experimental groups**

Twenty-four female Wistar rats weighing 96 ± 1 g (mean ± s.e.m.) were divided randomly into two groups, one of which participated in an 18 week swimming program (trained, \( T; n = 12 \)); the other served as sedentary controls (\( S; n = 12 \)).

**Swim training**

The rats swam in tepid water maintained at 36 °C (35.5–36.5 °C). The duration of the daily training (5 days week\(^{-1}\)) was gradually increased up to 6 h day\(^{-1}\) during the first 10 weeks. All rats swam in a tank in water that was 58 cm deep with an average surface area of 200 cm\(^2\) per rat. This ensured that the rats were continuously active during the training sessions. After each training session rats were dried with a towel and placed under a lamp at 31 °C for 1 h with access to food ad libitum.

**Experimental protocol**

The rats were brought to the laboratory at 09.00 h, 40 h after the last training session. After being weighed, the rats were anaesthetized by an I.P. injection of sodium pentobarbital (5 mg (100 g body weight))\(^{-1}\)). A catheter was placed in the left cardiac ventricle and the inferior vena cava was cut open. Rats were perfused with Krebs-Ringer bicarbonate buffer through the cardiac left ventricle for 3 min (25 ml min\(^{-1}\)) to wash out blood. This buffer (pH 7.4) contains 8 mM glucose, 1 mM pyruvate and 0.2 % (w/v) bovine serum albumin (BSA). Subsequently, the left and right RE adipose tissues, the ME adipose tissue, and the left and right EDL and soleus muscles were excised from each rat. Then, for 12 rats (6 T and 6 S) the excised adipose tissues were placed into perforated baskets and incubated in separate test tubes in more Krebs-Ringer bicarbonate buffer at room temperature. The incubation medium was continuously gassed with 95 % O\(_2\)–5 % CO\(_2\). After 1 h of preincubation, the adipose tissues were transferred to 10 ml of fresh incubation medium containing 4.4 \( \mu \)M adrenaline. The left RE adipose tissue was stimulated by adrenaline for 5 min, whereas the right RE and ME adipose tissues were stimulated for 20 min. At the end of the incubation with adrenaline, all of the adipose tissue was freeze-clamped with aluminium tongs that had been precooled in liquid N\(_2\).

The adipose tissues were homogenized (Polytron PT 3100, Kinematica, Switzerland) at maximum speed on ice in 10 volumes of 0.25 M sucrose, 1 mM dithioerythritol, 40 mM \( \beta \)-glycerophosphate, 10 mM sodium pyrophosphate, 310 mM okadaic acid (Boehringer Mannheim, Denmark), 20 \( \mu \)g ml\(^{-1}\) leupeptin (Sigma, Denmark), 20 \( \mu \)g ml\(^{-1}\) antipain (Sigma) and 6.5 \( \mu \)g ml\(^{-1}\) pepstatin (Sigma), pH 7.0. Okadaic acid has previously been shown to double the contraction-induced enhancement of HSL (TG) activity, indicating that it impairs the dephosphorylation of HSL (Langfort et al. 2000). The crude homogenate was centrifuged at 15,800 g in an Eppendorf tube at 4 °C for 45 s. The infranatant was recovered and stored at −80 °C until analysis of HSL activities. The excised muscles from the same rats were freeze-clamped with aluminium tongs that had been precooled in liquid N\(_2\), and stored at −80 °C until determination of HSL protein concentration by Western blotting.

In separate experiments the excised muscles from 12 rats (6 T and 6 S) were incubated as described for the adipose tissue, and muscles from the left and right sides were stimulated with 4.4 \( \mu \)M adrenaline for 5 and 20 min, respectively. At the end of this incubation muscles were freeze-clamped with aluminium tongs that had been precooled in liquid N\(_2\), and then trimmed of connective tissue and visible fat while kept in liquid N\(_2\). The muscles were homogenized and centrifuged as for the adipose tissue, after which the supernatants were recovered and stored at −80 °C until analysis of HSL activities. The excised adipose tissues from the same rats were also rapidly freeze-clamped, weighed and then stored at −80 °C until determination of HSL protein concentration by Western blotting.

**Analysis of HSL activities**

The activities of the total and the activated form of HSL (HSL (DG) activity and HSL (TG) activity, respectively) were determined in the infranatant obtained from adipose tissue and in the supernatant obtained from muscle, as described by Langfort et al. (1999). In brief, the HSL (TG) and HSL (DG) substrates were emulsified with phospholipids by sonication, and BSA was used as a fatty acid acceptor. Adipose tissue infranatant or muscle supernatant was incubated for 30 min with either triglyceride or diglyceride substrate and enzyme dilution buffer. Then hydrolysis was stopped and the mixture was vortexed and centrifuged. A 1 ml portion of the upper phase containing the released fatty acids was mixed with 10 ml of
were significantly lower in T compared with S rats. The weights of the RE and ME adipose tissues
Table 1). Heart weight was significantly higher in T than in S rats or between tissues. The Student-Newman-Keuls test was used as a post hoc test. Student’s  t test for unpaired data (when data were normally distributed) or the Mann-Whitney rank sum test (when data were not normally distributed) was used to test whether the body, heart and adipose tissue weights, and ME HSL (DG) and HSL (TG) activities differed between T and S rats. A significance level of 0.05 in two-tailed testing was chosen a priori.

RESULTS

Characteristics of experimental animals

Body weights did not differ between T and S rats either before or after the 18 week swim training (P>0.05; Table 1). Heart weight was significantly higher in T than in S rats. The weights of the RE and ME adipose tissues were significantly lower in T compared with S rats.

HSL (TG) and HSL (DG) activities

During incubation with adrenaline, HSL (TG) and HSL (DG) activities (expressed per mg protein) increased by about 120% in RE and by about 60% in ME adipose tissue in T compared with S rats (P<0.05, Figs 1 and 2). Correspondingly, HSL (TG) and HSL (DG) activities (expressed per mg wet weight of adipose tissue) were increased significantly in T compared with S rats (RE: HSL (TG) 0.14 ± 0.02 (T) vs. 0.04 ± 0.01 mU mg⁻¹ (S), and HSL (DG) 0.59 ± 0.07 (T) vs. 0.22 ± 0.04 mU mg⁻¹ (S); ME: HSL (TG) 0.16 ± 0.02 (T) vs. 0.07 ± 0.01 mU mg⁻¹ (S), and HSL (DG) 0.80 ± 0.07 (T) vs. 0.34 ± 0.08 mU mg⁻¹ (S)). When measured after 20 min of incubation with adrenaline, the HSL (TG)/HSL (DG) ratio was lower in T compared with S muscles (EDL: 0.06 ± 0.00 vs. 0.07 ± 0.00 for T and S, respectively, P<0.05; soleus: 0.11 ± 0.01 vs. 0.13 ± 0.01 for T and S, respectively, P<0.05). The duration of incubation with adrenaline had no significant effect on the HSL (TG) and HSL (DG) activities in either adipose tissue or muscle (Figs 1 and 2).

HSL (TG) activity (expressed per mg protein) was significantly higher (P<0.05) and HSL (DG) activity tended to be higher (P=0.09) in RE adipose tissue than after 20 min of incubation with adrenaline. The HSL (TG)/HSL (DG) ratio was lower in T compared with S muscles (EDL: 0.06 ± 0.00 vs. 0.07 ± 0.00 for T and S, respectively, P<0.05; soleus: 0.11 ± 0.01 vs. 0.13 ± 0.01 for T and S, respectively, P<0.05). The duration of incubation with adrenaline had no significant effect on the HSL (TG) and HSL (DG) activities in either adipose tissue or muscle (Figs 1 and 2).

The neutral lipase activity against triglyceride substrate, which reflects mainly the activity of the phosphorylated, activated form of HSL, referred to as HSL (TG), was measured in retroperitoneal and mesenteric adipose tissue, and in the EDL and soleus muscles of 6 trained and 6 sedentary rats. Tissues were incubated with 4.4 µM adrenaline. Data are presented as means ± s.e.m. *P<0.05 compared with sedentary rats. #P<0.05 vs. mesenteric adipose tissue. †P<0.05 vs. EDL and soleus muscles.

Figure 1. HSL (TG) activity

The neutral lipase activity against triglyceride substrate, which reflects mainly the activity of the phosphorylated, activated form of HSL, referred to as HSL (TG), was measured in retroperitoneal and mesenteric adipose tissue, and in the EDL and soleus muscles of 6 trained and 6 sedentary rats. Tissues were incubated with 4.4 µM adrenaline. Data are presented as means ± s.e.m. *P<0.05 compared with sedentary rats. #P<0.05 vs. mesenteric adipose tissue. †P<0.05 vs. EDL and soleus muscles.
in ME adipose tissue in T but not in S rats ($P > 0.05$; Figs 1 and 2). Furthermore, HSL (TG) and HSL (DG) activities were significantly higher in the soleus muscle compared with the EDL ($P < 0.05$; Figs 1 and 2). In S rats, HSL (TG) and HSL (DG) activities were 4- to 40-fold higher in adipose tissue than in muscle ($P < 0.05$), and the difference between these tissues was even higher in T rats ($P < 0.05$).

**HSL protein**

HSL protein concentration (expressed per µg protein) was more than doubled in T compared with S rats in RE adipose tissue ($P < 0.05$; Fig. 3). HSL protein levels did not differ significantly between T and S rats in either ME adipose tissue or the two muscles tested. HSL protein concentration was significantly higher in RE than in ME adipose tissue in T rats but not in S rats. HSL protein concentration was significantly higher in the soleus than in the EDL muscle. In S rats, the HSL protein level was 10- to 50-fold higher in adipose tissue than in muscle ($P < 0.05$), and the difference between these tissues was even higher in T rats ($P < 0.05$).

**DISCUSSION**

The major findings of the present study are that exercise training enhances total and adrenaline-stimulated HSL activity in intra-abdominal adipose tissues, whereas in muscle, total HSL activity is unchanged and adrenaline-stimulated activity decreased after training (Figs 1 and 2). Furthermore, training increased the amount of HSL protein in RE adipose tissue, while the amount of HSL protein was unchanged in muscle (Fig. 3).

We have previously demonstrated increases in both mitochondrial enzyme activity (Stallknecht *et al.* 1991) and the concentration of the glucose transporter GLUT4 (Stallknecht *et al.* 1993) in rat adipocytes after exercise training. These adaptations are identical to those known to occur in rat skeletal muscle in response to training (Henriksson *et al.* 1985; Ploug *et al.* 1990). However, the present study demonstrates for the first time that a selective adipocyte adaptation in the total activity and amount of HSL protein, the key enzyme in adipocyte function, can be provoked by prolonged endurance training. These findings add to earlier studies in which it was shown that adipocyte HSL levels may also change during other conditions that are accompanied by altered lipid metabolism (Martin *et al.* 1994; Large *et al.* 1999). In those studies it was found that HSL activity as well as protein concentration were decreased in obese compared with non-obese subjects (Large *et al.* 1999), that HSL activity was increased during early pregnancy but decreased during late pregnancy (Martin *et al.* 1994) and that HSL activity as well as protein concentration were increased after 3–5 days of fasting (Sztalryd & Kraemer, 1994 b).

The increases in total HSL activity per unit adipocyte volume in both RE and ME adipose tissues, as well as increases in the concentration of HSL protein in RE adipose tissue (findings of the present study), indicate an
enhanced lipolytic capacity of the examined adipose tissues. This is in accordance with previous in vitro studies in which it was shown that training increases the adrenaline-stimulated lipolysis of periumbilical adipocytes in humans (Crampes et al. 1986; Riviere et al. 1989) and of parametrial and epidydimal adipocytes in rats (Bukowiecki et al. 1980; Crampes et al. 1986). Furthermore, in a previous in vivo study in rats we found that adrenaline-stimulated lipolysis was increased by exercise training in a number of adipose tissues, including intra-abdominal adipose tissues (Enevoldsen et al. 2000).

In the present study, in addition to the overall increase in HSL activity (as determined by HSL (DG) measurements), the HSL (TG)/HSL (DG) ratio was also higher in adipose tissue from T compared with S rats. In other words, during adrenaline stimulation a greater percentage of HSL was in the activated/phosphorylated form after exercise training. This indicates that the mechanisms leading to stimulation of HSL adapt to exercise training. The number of β-adrenergic receptors on adipocytes does not change with training (Bukowiecki et al. 1980). However, it has been demonstrated that adenylate cyclase activity in rat adipocyte membranes is significantly increased by training (Izawa et al. 1988). Accordingly, training-induced increases in adrenaline-stimulated cellular levels of cAMP probably account for the higher HSL (TG)/HSL (DG) ratio found in T compared with S rats.

In the present study we used female rats to ensure that the body weights of the T and the S groups only differed marginally. If a group of S rats was food-restricted so that the adipose tissue mass was equal to that of the T rats, the body weights and, accordingly, the muscle mass would be much lower than those of the T animals. The animals would be starved and would not fulfil the requirements for a physiological control group. In a previous study of male rats we used an adipocyte cell size-matched sedentary control group, but these rats were much younger than the T rats (6 weeks old compared to 14 weeks old; Stallknecht et al. 1993). In our opinion, to compare rats of very different body weight or age is more problematic than comparing rats of different levels of adiposity. The fact is that in rats physical training results in a lower fat mass, and it is not possible to design control experiments from which it can be determined whether observed differences in adipose tissue composition between T and S groups are due to physical training per se or are secondary to, for example, the reduction in fat mass or increased food intake. Accordingly, it could be speculated that in the present study the HSL activity per milligram of adipose tissue in the T rats was simply upregulated proportionately to the reduction in their fat mass. This possibility concurs with findings in the ME adipose tissue. However, in the RE adipose tissue the training-induced increase in HSL activity per milligram of tissue was higher than the corresponding decrease in fat mass. These findings imply that the ‘total’ lipolytic capacity of visceral adipose tissue is increased by exercise training, a hypothesis that is in accordance with a previous study in which we measured in vivo lipolysis in intra-abdominal adipose tissue in trained rats (Enevoldsen et al. 2000).

In S rats, there was no difference in HSL (TG) and HSL (DG) activities or HSL protein concentration between the adipose tissues studied. However, in the T rats, HSL (TG) and HSL (DG) activities and HSL protein concentration were higher in the RE compared with the ME adipose tissue. This finding suggests regional differences in adaptations and, in turn, regional differences in lipolysis between different intra-abdominal adipose tissue depots after exercise training. Previous studies of adipose tissue metabolism have demonstrated regional differences in basal and adrenaline-stimulated lipolysis between intra-abdominal and subcutaneous adipose tissues both in vivo (Iwao et al. 1997; Enevoldsen et al. 2000) and in vitro (Sztalryd & Kraemer, 1994a) and in untrained rat and man (Richelsen et al. 1991; Hellmer et al. 1992). These regional differences have been explained by different levels of expression of the lipolytic β1- and β2-adrenergic receptors (Hellmer et al. 1992) and the antilipolytic α2-adrenergic receptor (Richelsen et al. 1991) as well as variations in the expression of HSL between intra-abdominal and subcutaneous adipose tissue (Sztalryd & Kraemer, 1994a). Our finding that the activity and protein concentration of HSL adapt differently in different intra-abdominal adipose tissues in response to exercise training corresponds with the observation that after training, lipolytic capacity varies between adipose tissues (Enevoldsen et al. 2000).

While total HSL activity was increased in both RE and ME adipose tissue after exercise training (Fig. 2), the amount of HSL protein determined by Western blotting was significantly increased only in RE adipose tissue (Fig. 3). This finding may reflect analytical difficulties or the fact that training may alter the relationship between HSL protein and activity by recruiting HSL from an inactive pool or inducing post-translational modifications that are tissue specific and influence enzyme activity. Alternatively, in ME adipose tissue, training may increase the amount of a different protein, one that mimics HSL activity. In line with this possibility is the finding that in the HSL-knockout mouse, adipose tissue lipolysis is not completely abolished (Osuga et al. 2000). Others have also observed the lack of covariation between enzyme activity and protein concentration. It has been found that after endurance training the amount of glycogen synthase protein in muscle was unchanged, while the total glycogen synthase activity had increased (Vestergaard et al. 1994).

Interestingly, in contrast to findings in adipose tissue, training resulted in a reduction in adrenaline-stimulated HSL (TG) activity in both the soleus and EDL muscles (Fig. 1), while the total HSL activity was unchanged (Fig. 2). The decrease in the HSL (TG)/HSL (DG) ratio
after training corresponds with the findings from a microdialysis study of rat muscle, which indicated that adrenaline-stimulated lipolysis in muscle is reduced by training (Enevoldsen et al. 2000). This would appear to be at variance with the finding that during exercise the breakdown of muscle triglyceride is higher in trained compared with untrained subjects (Hurley et al. 1986). However, adrenaline is not necessary for the stimulation of triglyceride breakdown in exercising muscle because contractions per se can activate HSL in muscle (Langfort et al. 2000). It may be hypothesized that this mechanism is enhanced by training. In fact, our finding that the adrenaline-induced activation of HSL activity in muscle is reduced after training is meaningful in the sense that the mobilization of muscle triglyceride by adrenaline during a stress other than exercise will be lessened, thus saving muscle triglyceride for use during exercise. On the other hand, during such stress (e.g. fasting, hypoglycaemia, infections and trauma) the augmented capacity for adrenaline-stimulated lipolysis in extramuscular adipose tissues in trained individuals may secure sufficient free fatty acids for oxidation in the face of the complementary downregulation of adrenaline-stimulated lipolysis that occurs in muscle. Training resulted in an increase in the maximal adrenaline-stimulated lipolysis in extramuscular adipose tissue; this may also contribute to the fact that during exercise, peak fat oxidation is higher in trained compared with untrained individuals (Galbo, 1995). In conclusion, training increases the amount of HSL and the sensitivity of HSL to stimulation by adrenaline in intra-abdominal adipose tissues, the extent differing between tissues. In contrast, in skeletal muscle the amount of HSL is unchanged and its sensitivity to stimulation by adrenaline decreased after training.


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