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Regulation and role of hormone-sensitive lipase activity in human skeletal muscle

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Hormone-sensitive lipase (HSL) is believed to play a regulatory role in initiating the degradation of intramuscular triacylglycerol (IMTG) in skeletal muscle. A series of studies designed to characterise the response of HSL to three stimuli: exercise of varying intensities and durations; adrenaline infusions; altered fuel supply have recently been conducted in human skeletal muscle. In an attempt to understand the regulation of HSL activity the changes in the putative intramuscular and hormonal regulators of the enzyme have also been measured. In human skeletal muscle at rest there is a high constitutive level of HSL activity, which is not a function of biopsy freezing. The combination of low adrenaline and Ca^{2+} levels and resting levels of insulin appear to dictate the level of HSL activity at rest. During the initial minute of low and moderate aerobic exercise HSL is activated by contractions in the apparent absence of increases in circulating adrenaline. During intense aerobic exercise, adrenaline may contribute to the early activation of HSL. The contraction-induced activation may be related to increased Ca^{2+} and/or other unknown intramuscular activators. As low- and moderate-intensity exercise continues beyond a few minutes, activation by adrenaline through the cAMP cascade may also occur. With prolonged moderate-intensity exercise beyond 1–2 h and sustained high-intensity exercise, HSL activity decreases despite continuing increases in adrenaline, possibly as a result of increasing accumulations of free AMP, activation of AMP kinase and phosphorylation of inhibitory sites on HSL. The existing work in human skeletal muscle also suggests that there are numerous levels of control involved in the regulation of IMTG degradation, with control points downstream from HSL also being important. For example, it must be remembered that the actual flux (IMTG degradation) through HSL may be allosterically inhibited during prolonged exercise as a result of the accumulation of long-chain fatty acyl-CoA.

Intramuscular triacylglycerol: Fat oxidation: Lipolysis

Fat is stored within skeletal muscle myocytes in the form of intramuscular triacylglycerol (IMTG). The energy equivalent of the NEFA stored as IMTG is approximately 67–100% of the energy stored as muscle glycogen in a well-fed individual. The majority of the available evidence from isotope-tracer studies, proton magnetic resonance spectroscopy and direct measures from muscle biopsy samples suggests that IMTG is degraded during low- and moderate-intensity exercise (approximately 30–65% V_{O_2} peak) to provide NEFA for oxidation (Romijn *et al*. 1993; Decombaz *et al*. 2001; Watt *et al*. 2002*a*). The oxidation of IMTG-derived NEFA accounts for approximately 20% of the energy expended during prolonged (2 h) moderate-intensity exercise (Romijn *et al*. 1993; Watt *et al*. 2002*a*). In addition to its role as a metabolic substrate, an association between increased IMTG storage and reduced insulin sensitivity has been demonstrated (for review, see Kelley *et al*. 2002). This finding has generated much interest in the notion that the accretion of IMTG and associated metabolites may contribute to the pathogenesis of insulin resistance in obesity and type 2 diabetes.

Despite the importance of IMTG as a metabolic substrate and its putative role in the pathogenesis of insulin resistance, there is limited information relating to the mechanisms by which IMTG is stored and degraded in skeletal muscle. Hormone-sensitive lipase (HSL) is considered to be the rate-limiting enzyme for IMTG hydrolysis because the enzyme exhibits an approximately 10-fold higher specific activity for diacylglycerol compared with triacylglycerol (Langfort *et al*. 1999) and appears to

Abbreviations: ERK, extracellular regulated kinase; HSL, hormone-sensitive lipase; IMTG, intramuscular triacylglycerol.
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be the only degradation enzyme subject to external regulation. Until recently, very little has been known about the regulation of HSL in skeletal muscle. HSL has a neutral pH optimum and is covalently activated by numerous kinases that add a phosphate and deactivated by a phosphatase that removes a phosphate, as described for adipose tissue. It has also been suggested that HSL can be allosterically regulated by long-chain fatty acyl-CoA and possibly other metabolites (Jepson & Yeaman, 1992). Recently, Langfort *et al.* (1999, 2000) measured the activities of the active fraction of HSL in rodent skeletal muscle in a variety of conditions and suggested that HSL activity is subject to dual control by contractions and adrenergic mechanisms. Their work was the first to examine the regulation of HSL in skeletal muscle and produced several important advances. The purpose of the present review is to examine the body of knowledge relating to HSL activity and regulation in human skeletal muscle and its role in IMTG hydrolysis.

Evidence demonstrating that intramuscular triacylglycerol is a labile pool

Apart from morphological evidence that shows that IMTG resides in close proximity to the mitochondria (Howald *et al.* 2002), there is considerable evidence to suggest that IMTG exists as a labile pool. Resting IMTG stores are heavily influenced by dietary composition, in so much that acute (Starling *et al.* 1997) and long-term (Kiens *et al.* 1987) high-fat diets result in greater IMTG storage, which enhances muscle triacylglycerol-derived fatty acid oxidation both at rest and during moderate exercise (Schrauwen *et al.* 2000). Conversely, when resting IMTG content is reduced by a short-term low-fat (2% total energy intake) diet, non-plasma fatty acid (IMTG) oxidation is reduced (Coyle *et al.* 2001); whereas reduced plasma NEFA mobilisation and oxidation enhance estimated IMTG utilisation (Coyle *et al.* 1998). Finally, isotope-tracer methodology has demonstrated rapid IMTG turnover at rest and during low-intensity exercise (Guo *et al.* 2000; Sacchetti *et al.* 2002).

Although there is some controversy, most studies using various techniques suggest that IMTG is an important metabolic substrate during low- to moderate (50–65% V_{O_2} peak)-intensity exercise (for review, see Watt *et al.* 2002*b*). The controversy relating to the use of IMTG as a metabolic substrate has mainly been a function of biopsy studies that have not found net decreases in IMTG use during prolonged moderate exercise lasting 90–120 min. It is possible that much of this controversy has been a result of the marked variability (approximately 25%) observed in muscle biopsy samples obtained from untrained and recreationally-trained individuals, which precludes the ability to detect marked reductions during prolonged exercise (for review, see Watt *et al.* 2002*b*). A recent study with well-trained subjects has shown that between-biopsy variability is reduced to approximately 12% and there is a net decrease in IMTG following 120 min of cycling at 55% V_{O_2max} (Watt *et al.* 2002*a*). Notably, recent studies utilising proton magnetic resonance spectroscopy technology, which can differentiate between extra- and intracellular triacylglycerol stores, have consistently demonstrated IMTG degradation after prolonged exercise and IMTG repletion during recovery (Décombaz *et al.* 2001; Décombaz, 2003).

Putative control of hormone-sensitive lipase

Until recently, very little was known about the mechanisms controlling the degradation of IMTG in skeletal muscle. One aspect of this control involves the regulation of HSL activity. Recent studies examining HSL regulation in skeletal muscle have followed the lead provided by previous studies conducted in adipose tissue. These studies have confirmed that reversible phosphorylation is an important feature of the short-term regulation of adipose HSL activity (for review, see Holm *et al.* 2000). While this covalent regulation is a common feature of many rate-limiting enzymes associated with carbohydrate metabolism in skeletal muscle, HSL is the only lipase that is controlled through reversible phosphorylation (Langin *et al.* 1996). It is known that adipose HSL activity is subject to complex regulation by covalent modification at multiple phosphorylation sites and by allosteric factors. An outline of the putative control of skeletal muscle HSL activity has been constructed from the existing adipose tissue data and the recent findings in skeletal muscle (Langfort *et al.* 1999, 2000; Fig. 1). β-Adrenergic activation increases cAMP and phosphorylation of HSL by protein kinase A at one of three serine residues (563, 659 and 660) resulting in increased HSL activity. HSL is also a substrate of the extracellular regulated kinase (ERK), which is capable of phosphorylating HSL at Ser^{600} and increasing lipolysis (Greenberg *et al.* 2001). Insulin deactivates HSL via increased cAMP phosphodiesterase activity, decreased cAMP and decreased protein kinase A activity. Another serine residue (Ser^{565}) can be phosphorylated by

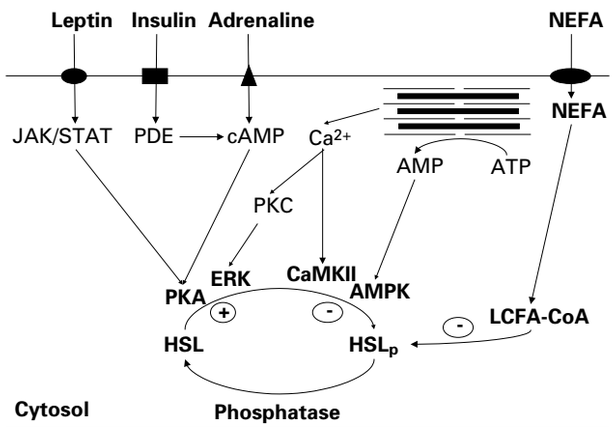

Fig. 1. Putative control of skeletal muscle hormone-sensitive lipase (HSL). HSL_p, phosphorylated HSL; JAK/STAT, janus kinase/signal transducers and activators of transcription; PDE, phosphodiesterase; PKA, PKC, protein kinase A and C respectively; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; AMPK, 5′AMP-activated protein kinase; ERK, extracellular regulated kinase; LCFA-CoA, long-chain fatty acyl-CoA; +, stimulatory effect; −, inhibitory effect.

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Ca^{2+}/calmodulin-dependent protein kinase II, glycogen synthase kinase IV and 5′AMP-activated kinase, which is believed to prevent activation of HSL (Langin *et al*. 1996). Finally, it has been proposed that long-chain fatty acyl-CoA allosterically inhibits HSL activity (Jepson & Yeaman, 1992).

Hormone-sensitive lipase in skeletal muscle

Galbo and colleagues (Langfort *et al*. 1999, 2000), who initiated the early work relating to skeletal muscle HSL activity, have demonstrated that HSL activity in isolated rat skeletal muscle is increased during tetanic contractions (Langfort *et al*. 2000) or with the addition of pharmacological adrenaline to the incubating media (Langfort *et al*. 1999). It has been reported that HSL is activated during moderate to heavy (73–90% V_{O_2} peak) exercise in untrained human subjects, although absolute HSL activity is not different between the exercise power outputs (Kjær *et al*. 2000). The same study has shown that HSL activity does not increase during exercise in adrenaline-deficient adrenalectomised patients; however, when adrenaline is infused to mimic normal exercising levels, HSL activity increases (Kjær *et al*. 2000). Taken together, these data suggest that adrenaline and not contraction-related mechanisms are most important for HSL activation during exercise.

Hormone-sensitive lipase activation quality control

The *in vitro* assay system employed by Langfort *et al*. (1999) has been adapted to measure HSL activity in human skeletal muscle homogenates. Several preliminary experiments have confirmed the robustness and repeatability of the adapted assay. Essentially, a muscle homogenate containing HSL 'trapped' in its *in vivo* phosphorylation state is incubated at 37°C with an emulsified radiolabelled triolein substrate and free albumin. The radiolabelled fatty acids cleaved from triolein bind to albumin and the solution is separated into organic and aqueous phases. An aliquot of the aqueous phase containing the released fatty acids is counted in a β spectrometer and HSL activity is normalised to total cellular protein or creatine (expressed per kg dry muscle). Before undertaking studies in human subjects the measurement of HSL activity was optimised. Time-course experiments have confirmed that the HSL activity assay is linear until 20 min, indicating that measures obtained within this time represent true activity. Substrate availability (100 μl) is not limiting (nmol/min per mg protein; 50 μl, 1·32 (SE 0·21); 100 μl, 1·16 (SE 0·13); 200 μl, 1·21 (SE 0·16), whilst homogenisation of the muscle using a polytron homogeniser for 10 s (0·94 (SE 0·17) nmol/min per mg protein) or a rotating Teflon pestle on glass for 20 s (0·97 (SE 0·20) nmol/min per mg protein) yield similar results. The intra-assay CV for six aliquots, each made in triplicate, determined from one aliquot of freeze-dried muscle is 3·8 (SE 1·5)% at 0·95 (SE 0·15) nmol/min per mg protein. The inter-assay CV (n 9) measured on different days from one subject is 10·3 (SE) 2·6%.

A number of lines of evidence strongly suggest that muscle HSL is being measured and not other neutral lipases. The early studies of Langfort *et al*. (1999, 2000) in rat skeletal muscle demonstrated that contraction- and adrenaline-induced HSL activation is abolished when an anti-HSL antibody is administered. Watt *et al*. (2003*d*) have shown with human skeletal muscle that adrenaline infusion at rest and acute exercise results in marked increases in HSL activity that are similar in magnitude to those observed in rat skeletal muscle by Langfort *et al*. (1999, 2000). Also, the activity values obtained by Watt *et al*. (2003*d*) are consistent with those previously published in human skeletal muscle (Kjær *et al*. 2000). Thus, it can be concluded with confidence that these measurements in human skeletal muscle represent only HSL activity, free from contamination of adipose tissue HSL.

Hormone-sensitive lipase activity at rest

One of the interesting findings from the early studies (Watt *et al*. (2003*b*,*d*) is the high constituent HSL activity in resting skeletal muscle. HSL activity at rest is approximately 40–50% of the rates observed during exercise and/or exogenous adrenaline infusion, which is high compared with other rate-limiting metabolic enzymes. It appears that the increased plasma insulin associated with a small carbohydrate meal (fasted 40 pM *v*. fed 75 pM; Watt *et al*. 2003*b*,*d*) and reduced plasma NEFA availability induced by nicotinic acid ingestion (M O'Neill, MJ Watt, GJF Heigenhauser and LL Spriet, unpublished results) are without effect on resting HSL activity. Similar to the control of adipose tissue HSL, exogenous adrenaline infusion that results in high but physiological plasma levels (3·2 (SE 1·2) nM) increases resting HSL activity to rates observed during contraction (Watt *et al*. 2003*d*). Analogous to the resting situation with glycogen phosphorylase, HSL may be artificially activated by intramuscular Ca^{2+} released during the biopsy procedure. However, when muscle samples are either frozen after sampling, or incubated at room temperature for 1 and 2 min before freezing, HSL activity is not different. Thus, resting HSL activity in skeletal muscle is high, but the factors mediating this effect and the physiological importance of this observation remain unresolved. It appears that the combination of low adrenaline and Ca^{2+} levels and resting insulin concentrations dictate the levels of HSL activity measured at rest.

Hormone-sensitive lipase activity during exercise

Effects of exercise power output

Most of the literature relating to fuel metabolism suggests that fat is not an important fuel source during the initial minutes of exercise or during sustained heavy (>80% V_{O_2} peak) exercise in untrained and recreationally-trained individuals. Initially, HSL activity was assessed during 10 min of exercise at power outputs corresponding to 30, 60 and 90% V_{O_2} peak (Watt *et al*. 2003*b*). Although there are numerous perturbations occurring during exercise and marked disparities in the metabolic signals associated with varied exercise power outputs, these power outputs were

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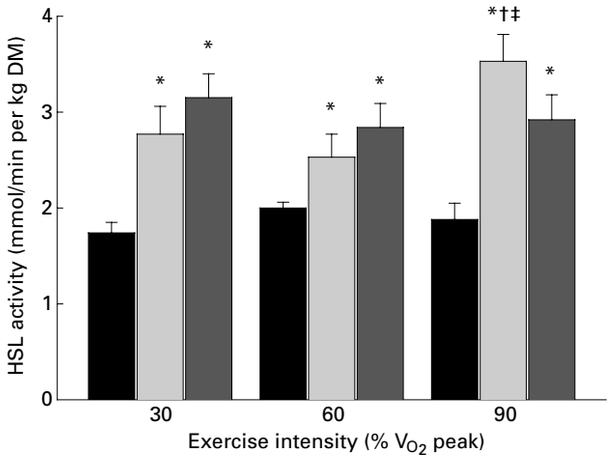

Fig. 2. Hormone-sensitive lipase (HSL) activity at rest (0 min; ■) and during 10 min (1 min, ▒; 10 min, ■) of exercise at various power outputs (30, 60 and 90% V_{O_2} peak). Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those at rest (0 min) at the same energy intensity: $^*P < 0{\cdot}05$. Mean value was significantly different from those at 30 and 60% V_{O_2} peak at the corresponding time point: $\dagger P < 0{\cdot}05$. Mean value was significantly different from that at 10 min for same energy-intensity trial: $\ddagger P < 0{\cdot}05$. (From Watt *et al.* 2003*b*.)

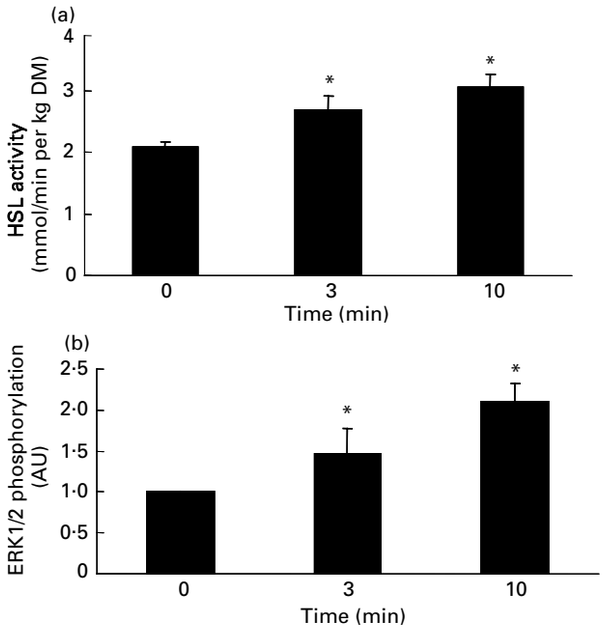

Fig. 3. (a) Hormone-sensitive lipase (HSL) activity and (b) extracellular regulated kinase 1 and 2 (ERK1/2) phosphorylation at rest and immediately after 3 min of exercise at 60% V_{O_2} peak. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from that at 0 min. $^*P < 0{\cdot}05$. (From Watt *et al.* 2003*d*.)

selected to: (1) assess the potential effects of Ca^{2+} and adenylate charge metabolites on HSL activity as these signals are proportional to the contraction intensity in skeletal muscle; (2) to vary the expectations relating to the use of endogenous fat as a fuel. HSL activity increases at 1 min of exercise at 30 and 60% V_{O_2} peak, and to a greater extent at 90% V_{O_2} peak (Fig. 2). HSL activity remains elevated after 10 min at 30 and 60% V_{O_2} peak, and decreases at 90% V_{O_2} peak from the rates observed at 1 min. Venous plasma adrenaline does not increase during the 10 min of exercise at 30 and 60% V_{O_2} peak, but is elevated at 5 min and again at 10 min at 90% V_{O_2} peak. Muscle free AMP, a measure of the adenylate charge, is not increased at 30% V_{O_2} peak and is slightly increased at 60% V_{O_2} peak. At 90% V_{O_2} peak free AMP is higher at 1 min and markedly increased at 10 min.

The finding of an immediate increase in HSL activity at all exercise power outputs, and greater elevation of HSL activity during exercise at 90% V_{O_2} peak is consistent with the premise that Ca^{2+} may be an important early regulator of HSL activity in skeletal muscle. Indeed, Ca^{2+} is the only putative regulator that changes at all power outputs and may activate HSL activity via increasing CaMKII activity or activating the protein kinase C–ERK pathway (Fig. 1). The former mechanism seems unlikely, as work in adipose tissue and resting rat skeletal muscle suggests an inhibitory role for the Ca^{2+}/calmodulin-dependent protein kinase II pathway (Xue *et al.* 2001; Watt *et al.* 2003*c*). In a subsequent study the possibility that ERK mediates the increased HSL activity early in exercise was examined. ERK1/2 phosphorylation is increased after 3 min of exercise at 60% V_{O_2} peak and is associated with a rapid increase in HSL activity (Fig. 3). Moreover, the extent of the increase in ERK1/2 is consistent with HSL activation, and although causality has not been proven, these data indicate that ERK may be partly responsible for the increased HSL activity at exercise onset (Watt *et al.* 2003*d*).

During exercise at 90% V_{O_2} peak HSL activity is reduced from the 1 min value by 10 min, despite marked increases in plasma adrenaline. The mechanism(s) regulating this process is difficult to elucidate. However, the marked increases in the estimated free AMP and decreases in the measured phosphocreatine contents may have stimulated AMP-activated kinase, and it has been shown that AMP decreases HSL activity in adipose tissue (see Holm *et al.* 2000). Such a relationship in skeletal muscle remains to be determined.

Effects of exercise duration

In view of the well-documented net IMTG degradation after prolonged exercise (Décombaz *et al.* 2001; Watt *et al.* 2002*a*), HSL activity has been measured in muscle samples obtained before and during 120 min of cycling at 60% V_{O_2} peak (Watt *et al.* 2003*a*). HSL activity may be reduced late in prolonged exercise because net IMTG utilisation occurs during the first 2 h of moderate-intensity exercise whereas no net IMTG use occurs from 2 h to 4 h (Watt *et al.* 2002*b*). Consistent with this hypothesis and earlier studies, HSL activity increases approximately 25% from resting levels at 10 min of exercise and increases further to rates that are approximately 50% greater than

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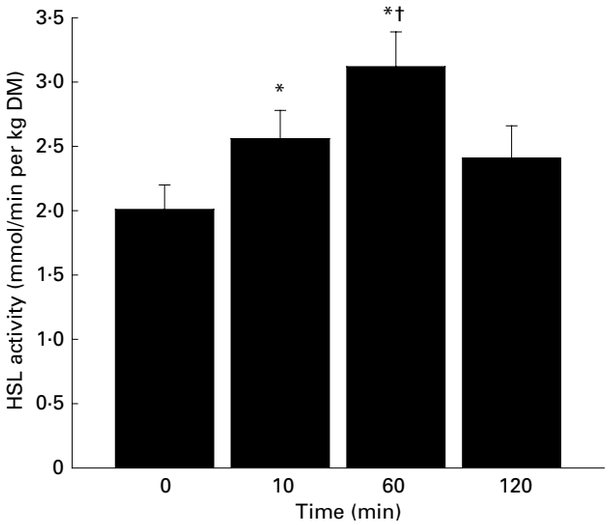

Fig. 4. Hormone-sensitive lipase (HSL) activity during 120 min of cycle exercise at 60% V_{O_2} peak. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from that at 0 min $^*P < 0{\cdot}05$. Mean value was significantly different from those at 10 and 120 min: $\dagger P < 0{\cdot}05$. (From Watt *et al.* 2003*a*.)

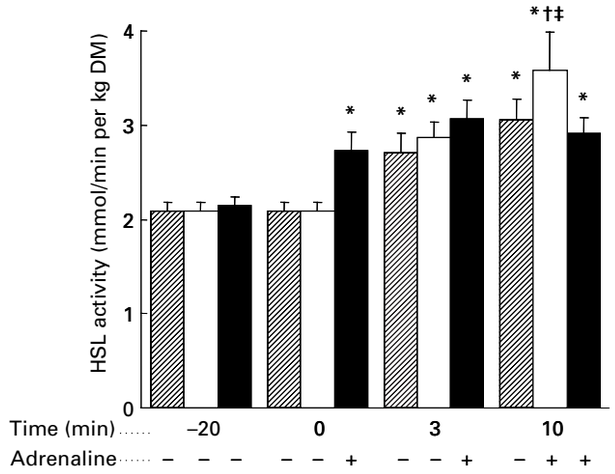

Fig. 5. Hormone-sensitive lipase (HSL) activity before and during exercise at 60% V_{O_2} peak with adrenaline infusion commencing after −20 min (■) or 3 min (□). (▨), Control. Values are means with their standard errors represented by vertical bars for seven determinations. Mean values were significantly different from those at −20 min: $^*P < 0{\cdot}05$. Mean value was significantly different from the corresponding value at 3 min: $\dagger P < 0{\cdot}05$. Mean value was significantly different from the control at the same time point: $\ddagger P < 0{\cdot}05$. (From Watt *et al.* 2003*d*.)

rest by 1 h (Kjær *et al.* 2000; Watt *et al.* 2003*a*). After 120 min of exercise HSL activity decreases from values observed earlier in exercise and is not different from resting rates (Fig. 4). This response has recently been confirmed in endurance-trained men (mU/mg protein; 0 min, 0·72 (SE 0·08); 180 min, 0·71 (SE 0·10)) and untrained men (mU/mg protein; 0 min, 0·74 (SE 0·03); 180 min, 0·68 (SE 0·03)) performing 3 h of cycle exercise at approximately 60% V_{O_2} peak (JW Helge, M Donsmark, H Galbo, T Bipa, M Gaster and B Saltin, unpublished results).

The increased HSL activity from 10 min to 60 min may be mediated by marked increases in plasma adrenaline and decreases in plasma insulin, because there are no changes in the measured putative intramuscular regulators (Watt *et al.* 2003*a*). These hormonal changes are likely to give rise to increased intracellular protein kinase A activity and mediate the increased HSL activity, although it is possible that another intramuscular factor contributes to the further increase in HSL activity from 10 min. Despite further elevations in plasma adrenaline from 60 min to 120 min (approximately 1·5–5 nM) and almost complete suppression of insulin, HSL activity returns to near-resting values. These data further support previous findings that adrenaline is capable of, but does not always, increase HSL activity. The factors mediating the decrease in HSL activity late in prolonged exercise are difficult to determine, although AMP-activated kinase α2 activity is progressively increased during prolonged (approximately 3·5 h) low-intensity exercise (Wojtaszewski *et al.* 2002). Given the marked increase in free AMP (approximately 20-fold) and concomitant decrease in HSL, an inhibitory role for AMP-activated kinase is suggested.

Hormone-sensitive lipase activity during adrenaline infusion

The studies described earlier are essentially descriptive and provide a basis for further mechanistic studies aimed at investigating the acute regulation of HSL. Whilst the initial studies in isolated rat muscle (Langfort *et al.* 1999) and adrenalectomised human subjects (Kjær *et al.* 2000) have demonstrated a stimulatory effect of adrenaline on HSL activity, the early studies of the present authors have indicated that adrenaline is not essential for HSL activation. Thus, the effect of exogenous adrenaline infusion on HSL activity has been investigated in two situations: (1) during rest and subsequent exercise; (2) once exercise has already commenced (Watt *et al.* 2003*d*). The increased HSL activity with elevated adrenaline (3·3 (SE 1·2) nM) in resting human skeletal muscle is of a similar magnitude to the increases previously observed during exercise (Fig. 5). The increased HSL activity with exogenous adrenaline infusion is not further increased by the commencement of exercise at 60% V_{O_2} peak. In contrast, when adrenaline infusion is commenced after the onset of exercise, HSL activity is augmented (Fig. 5). Taken together, these data suggest that skeletal muscle HSL is under dual control by contraction-related mechanisms and adrenaline, and that the order and the magnitude of the stimuli reaching HSL is important in determining enzymic activity. However, it should be stated that the bulk of the evidence from human muscle suggests that intramuscular factors dominate the control, with humoral factors playing a smaller role. Further studies using antibodies specific to the serine phosphorylation sites are required to adequately address the potential effects of multiple phosphorylations on HSL and the existence and/or importance of any 'order' effects.

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Effects of altered substrate availability on hormone-sensitive lipase activity

The effects of decreased plasma NEFA availability following nicotinic acid ingestion on HSL activity during 40 min of cycling at 55% V_{O_2} peak have been examined. When plasma NEFA availability is reduced IMTG use would be expected to increase to compensate for the missing substrate. Thus, it is hypothesised that there would be an increase in HSL activity to facilitate the greater IMTG use. Resting HSL activity is unaffected by nicotinic acid ingestion (averaging 2·22 (SE 0·15) mmol/kg DM per min in the nicotinic acid and control trials). HSL activity increases during exercise in both the nicotinic acid and control conditions, but contrary to the hypothesis, is not elevated in the nicotinic acid condition following 5, 20 or 40 min of exercise, despite a 45% higher plasma adrenaline concentration in the nicotinic acid trial. RER values during exercise reveal that with nicotinic acid ingestion some subjects increase carbohydrate oxidation during exercise, whereas others do not. This finding suggests that with nicotinic acid some individuals have the ability to increase IMTG degradation and oxidation without increasing HSL activity. If this reasoning is correct, it suggests that HSL activation is only one step in the regulation of IMTG degradation and that, as in adipose tissue, factors distal to this step determine the actual rate of IMTG breakdown. This hypothesis will need to be tested with measurements of IMTG degradation over longer time periods and/or direct measures of IMTG oxidation.

In contrast to the large body of work investigating adipose tissue lipolysis and NEFA oxidation during exercise with glucose feeding, no study has examined the mechanisms by which glucose ingestion, and the consequent hormonal and metabolic responses during exercise, affect HSL activity. Thus, HSL activity has been examined before and after 2 h of exercise at 60% V_{O_2} peak when subjects ingest either a placebo or glucose solution. As expected, the exercise-induced decrease in plasma insulin is ameliorated (2 h; control 11·4 (SE 2·4), glucose 35·3 (SE 7·0) pM) and the progressive rise in plasma adrenaline is suppressed (2 h; control 6·1 (SE 2·5), glucose 2·1 (SE 0·9) nM) with glucose ingestion. The changes induced by glucose feeding result in a complete suppression of the exercise-induced rise in HSL activity (Fig. 6). This finding suggests that even with muscle contraction, when local mediators of HSL are activated (Watt *et al*. 2003*a*), the hormonal milieu can play an important role in mediating HSL activity (Watt *et al*. 2004).

Hormone-sensitive lipase activity and intramuscular triacylglycerol hydrolysis

The apparent mismatch between HSL activation and expected IMTG hydrolysis and fat oxidation during varying exercise situations suggests that factors other than HSL activity are also important for IMTG degradation and eventual oxidation (Watt *et al*. 2003*a*,*b*,*d*; JW Helge, M Donsmark, H Galbo, T Bipa, M Gaster and B Saltin, unpublished results). Consistent with the regulation of adipose tissue lipolysis, factors other than HSL activation

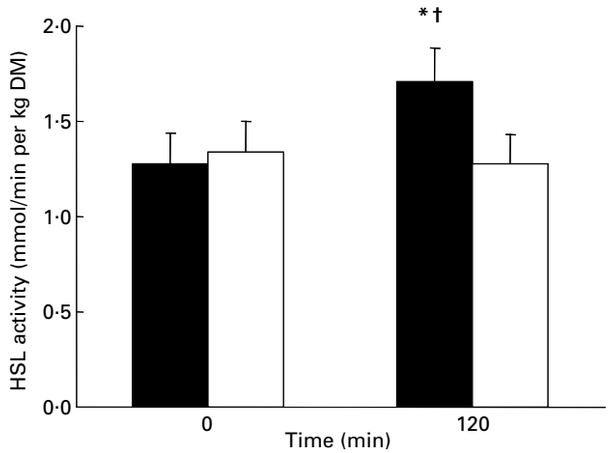

Fig. 6. Hormone-sensitive lipase (HSL) activity at rest and immediately following 120 min of exercise at 65% V_{O_2} peak with (glucose solution; □) or without (control; ■) carbohydrate ingestion. Values are means with their standard errors represented by vertical bars for seven determinations. Mean value was significantly different from the corresponding value for glucose ingestion: $^*P < 0{\cdot}05$. Mean value was significantly different from the corresponding value at 0 min: $\dagger P < 0{\cdot}05$. (From Watt *et al.* 2004.)

are likely to regulate IMTG degradation. Evidence from studies with rodent adipocytes suggests that translocation of HSL from a cytosolic site to the lipid droplet (containing triacylglycerol) and phosphorylation of perilipins may be critical for the access of HSL to triacylglycerol and lipolysis (for review, see Holm *et al*. 2000). The presence of perilipins has recently been identified in human adipose tissue but has not been identified in skeletal muscle (Mottagui-Tabar *et al*. 2003). Thus, HSL activation is unlikely to be the only rate-limiting factor in the regulation of IMTG degradation, and various levels of post-activational or downstream factors are likely to be important in mediating IMTG degradation.

Thus, it is proposed that phosphorylation and activation of HSL is the first step and is essential for IMTG degradation. HSL activation occurs rapidly at the onset of exercise and can be viewed as a gross control level of regulation. This step is accomplished by intramuscular factors that are intricately linked to the cellular fuel and energy status, and to a lesser extent by hormonal factors. Post-activational factors distal to this step (e.g. allosteric control, translocation, phosphorylation of perilipins) 'fine tune' the actual rate of IMTG degradation. This putative control system is akin to the regulation of glycogen phosphorylase activity and the degradation of glycogen, where there is gross covalent regulation followed by post-activational 'fine-tuning' by allosteric and substrate factors.

Conclusions

In human skeletal muscle at rest there is a high constitutive level of HSL activity, which is not a function of biopsy freezing. The combination of low adrenaline and Ca levels and resting levels of insulin appear to dictate the levels of HSL activity measured at rest. During the onset of low and

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moderate aerobic exercise (initial minute) HSL is activated by contractions, in the apparent absence of increases in circulating adrenaline. However, adrenaline may contribute to the early activation of HSL during intense aerobic exercise. The contraction-induced activation appears to be related to the increase in protein kinase C and ERK activity associated with Ca^{2+} and/or other unknown activators. As low- and moderate-intensity exercise continues beyond a few minutes, activation by adrenaline through the cAMP cascade and protein kinase A also appears to occur. With prolonged moderate-intensity exercise beyond 1–2 h and sustained high-intensity exercise, HSL activity decreases despite continuing increases in adrenaline, possibly as a result of increasing accumulation of free AMP, activation of AMP-activated kinase and phosphorylation of inhibitory sites on HSL. Taken together, the human muscle data suggest that intramuscular factors dominate the control of HSL activity, with hormonal factors playing a smaller role.

The existing work in human skeletal muscle also supports the theory that numerous levels of control are involved in the regulation of IMTG degradation, with control points downstream from HSL activation also playing important roles. Phosphorylation of HSL (activation) is the first step and is essential for IMTG degradation. However, factors distal to this step are also important in 'fine tuning' the actual rate of IMTG degradation. HSL activation can be thought of as a gross level of regulation, setting the stage for downstream control by other factors. For example, it must be remembered that actual flux (IMTG hydrolysis) through HSL may be allosterically inhibited during prolonged exercise as a result of the accumulation of long-chain fatty acyl-CoA. In addition, it has been proposed that the actual movement of HSL in the cytosol to the lipid droplet and phosphorylation of a phosphoprotein coat encapsulating the lipid droplet (perilipin, adipose differentiation-related protein) are also necessary steps that permit the physical 'docking' of HSL with the lipid droplet. However, these steps have not been studied in human skeletal muscle.

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