Long-term regulation of AMP-activated protein kinase and acetyl-CoA carboxylase in skeletal muscle

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Abstract
Evidence is accumulating for roles of AMP-activated protein kinase (AMPK) in controlling glucose uptake, fatty acid oxidation and gene expression in skeletal muscle. Relatively little is known, however, about the control of expression of the AMPK subunit isoforms. Marked differences are noted in subunit expression as a function of muscle fibre type. Expression of the γ3 subunit isoform increases in fast-twitch red fibres of the rat in response to training. All subunit isoforms are expressed to a lesser extent in rats treated with propylthiouracil (PTU; an inhibitor of thyroid hormone synthesis) for 3 weeks compared with rats given excess thyroid hormones for 3 weeks. An approx. 2-fold increase in acetyl-CoA carboxylase was observed in gastrocnemius of hyperthyroid rats compared with experimentally hypothyroid rats. Thyroid state therefore appears to be one important factor controlling expression of these proteins in skeletal muscle.

Introduction
Since 1996, AMP-activated protein kinase (AMPK) has emerged as a key player in both short- and long-term regulation of muscle metabolism [1–3]. The increase in ATP utilization during muscle contraction must be countered by increases in ATP production. AMPK, a heterotrimeric protein, has been shown to be involved in sensing the energy state ([phosphocreatine]/[creatine] and [AMP]/[ATP]) of the muscle fibre [1,2,4,5]. With increases in [AMP] (an activator of AMPK and AMPK kinase) and decreases in [phosphocreatine] (an inhibitor of AMPK), the activated AMPK triggers glucose uptake by causing GLUT4 translocation in the contracting muscle [6–8] and enhances the rate of fatty acid oxidation by phosphorylating acetyl-CoA carboxylase (ACC), decreasing [malonyl-CoA] and relieving inhibition of carnitine palmitoyltransferase 1 [1–3,7,9–12]. Evidence has been presented for a role of chronically activated AMPK in enhancing capacity to synthesize ATP. Key proteins involved in glucose uptake and metabolism (GLUT4, hexokinase) and proteins of the electron transport chain and citric acid cycle increase in response to chronic chemical activation of AMPK [1,13–17]. Thus AMPK appears to play a role in enhancing glucose and fatty acid metabolism acutely in response to exercise, but also may be involved in inducing some of the long-term adaptations to exercise training. Recently, evidence has been obtained for a role of AMPK in enhancing insulin sensitivity in the post-exercise period [18]. In previous reviews on AMPK, we predicted that chemical activation of AMPK, normally activated by exercise or hypoxia, might prove useful in preventing and treating Type 2 diabetes [1,2,19]. Evidence has been presented for AMPK mediation of the effects of metformin, a major oral hypoglycaemic drug, in skeletal muscle and other cell types [20–22]. Chronic chemical activation of AMPK has been reported to dramatically improve the glucose/insulin profile and to normalize plasma triglycerides (triaclylglycerols) and fatty acids in fatty Zucker rats (animal model of Type 2 diabetes) [14]. Leptin has been reported to utilize this signalling system in its stimulation of fatty acid oxidation in skeletal muscle [23], but not in heart [24].

AMPK expression in different fibre types
Despite the identification of key roles of this kinase in regulation of metabolism, little is known regarding factors controlling expression of subunit isoforms in skeletal muscle. Certainly the expression of subunit isoforms is tissue specific and this holds true for skeletal muscle. Three major fibre types are defined in rat muscles [25]. Type I fibres are slow twitch with a high capacity for synthesizing ATP with many mitochondria. These fibres are recruited for postural and endurance activities. Type IIa fibres are fast twitch and also have many mitochondria. These fibres are recruited at low work rates and continue to be utilized at high work rates, and due to the high levels of mitochondrial oxidative enzymes, they do not fatigue quickly. Type IIb fibres are fast twitch but have few mitochondria. They are utilized only during intense physical activity such as sprinting or jumping or near the end of prolonged endurance exercise bouts when the high oxidative fibres begin to fatigue. The IIb fibres fatigue quickly due to low oxidative capacity. A continuum exists between the IIA and IIb fibres, depending on their chronic use. The IIb

Key words: malonyl-CoA, propylthiouracil, thyroxine, tri-iodothyronine.
Abbreviations used: AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; PTU, propylthiouracil.

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fibres may be transformed into IIA fibres with appropriate chronic recruitment. Transformation of type II into type I fibres may be induced by reproducing the normal stimulation pattern of type I fibres using chronic electrical stimulation at a frequency of 10–20 Hz.

The muscle fibre types have markedly different expression patterns with respect to AMPK subunit isoforms (see Figure 1). For example, the soleus, which is composed predominantly of type I fibres, expresses more α1 and less α2 than the type II fibres. As demonstrated first by Chen et al. [26], the β1 peptide is distributed predominantly in type I fibres and less in the type II fibres. β2 shows a greater expression in IIA and IIB fibres than in slow twitch fibres. The γ1 isoform is more prominently expressed in IIA fibres compared with I and IIB fibres. γ3 is found in very low concentrations in IIB fibres and is expressed at approximately equivalent levels in highly oxidative fibres (type I and IIA). Previous studies demonstrated that the γ2 isoform is found in greater abundance in IIB fibres with less in I and IIA fibres [27]. Thus there appears to be a unique pattern of expression of AMPK subunit isoforms in each major fibre type in the rat. Although factors determining this fibre-type specific expression are not well-defined, it is likely that the unique chronic recruitment pattern for each fibre type plays a role.

**Endurance training**

In rats that are endurance trained by running on a treadmill for 2 h/day, 5 days/week for 7–8 weeks, the activation of AMPK in red quadriceps muscle is less during an acute bout of exercise than in non-trained control rats. This decrease in responsiveness is apparently not due to a change in expression of α or β subunits of AMPK, but rather to a better capacity of the muscle to prevent changes in the energy charge of the muscle fibres working at the same absolute work rate. Interestingly, the γ3 isoform was found to increase approx. 3-fold in response to endurance exercise training [27]. This isoform represents a relatively small proportion of the total immunoprecipitable AMPK activity, however, and the significance of this change is not understood. The expression of muscle ACC does not change in response to endurance training, but the degree of inactivation during exercise bouts is attenuated with respect to that seen in untrained rats [27].

**Diet and diabetes**

Although liver ACC is markedly influenced by fasting and refeeding, these same dietary manipulations have no detectable effect on ACC expression in skeletal muscle [28]. To our knowledge, no data are available on the effect of dietary manipulations on AMPK subunit isoform expression.

One study on lean Type 2 diabetic subjects demonstrated no change in α1 or α2 AMPK subunit isoform expression or in exercise-stimulated AMPK activity compared with control subjects [29]. Other subunit isoforms have not been investigated. It will also be of interest to examine subunit expression in obese Type 2 diabetic patients compared with controls.

**Thyroid state**

Previous studies indicate that expression of several skeletal muscle proteins is influenced by the thyroid state [30–37]. Hypothyroidism results in the decreased expression of mitochondrial oxidative enzymes and experimental hyperthyroidism causes an increase in these same mitochondrial enzymes [34–36]. Citrate synthase, for example, increases about 2-fold in the slow twitch soleus over the course of several weeks of treatment with excess thyroid hormones [36,37]. Although food intake increases markedly in response to thyroid hormone treatment, the body fat content is strikingly reduced [38]. Several of the same proteins that increase in response to excess thyroid hormones also increase in response to endurance exercise training and to chronic chemical activation of AMPK using 5-amino-4-imidazolecarboxamide riboside (AICAR) [13–16,25,39]. We were interested to determine whether the expression of all subunits of AMPK and of ACC in skeletal muscle are influenced by thyroid state [38].

Normal rats were studied as well as rats made hypothyroid by administration of 0.01% propylthiouracil in the drinking water. This drug inhibits synthesis of thyroid hormones by preventing iodination and coupling of tyrosines of thyroglobulin [40]. It has also been shown to inhibit one isoform of 5′-diodinase in peripheral tissues which converts thyroxine into tri-iodothyronine, the form of the hormone that binds with high affinity to the nuclear receptors [41]. Rats were made functionally hyperthyroid by administration of thyroxine and tri-iodothyronine in food at a dose that can be tolerated well for at least 6 weeks. Rats were killed after 1 and 3 weeks of treatment. Muscles were frozen and later used for quantification of subunit expression of AMPK and ACC by Western blotting. Phospho-ACC was also determined by Western blotting, along with activity of the enzymes.
Figure 2 | Comparison of AMPK subunit isoform expression

Western blotting was used to determine AMPK subunit isoform expression in gastrocnemius muscle of rats given 0.01% PTU in drinking water or 1 mg of l-triiodothyronine and 3 mg of l-thyroxine/kg of food (T3T4) for 3 weeks. All values for thyroid hormone-treated rats were significantly different from those of PTU-treated rats (P < 0.05). Data are taken from [38].

Figure 3 | Comparison of ACC expression, phospho-ACC and ACC activity in gastrocnemius muscle of rats

Rats were given PTU or excess thyroid hormones for 3 weeks (see Figure 2 legend for details). All values for thyroid hormone-treated rats were significantly different from those of PTU-treated rats. ACC activity is given in terms of V_max. Data are taken from [38].
of AMPK, which is involved in the enhancement of glucose availability and fatty acyl-CoA into the mitochondrial matrix, increases as a function of thyroid state. It is unclear whether the increased AMPK expression is responsible in part for the hypermetabolism, or whether it increases as a consequence of the hypermetabolism. In either case, the increase in AMPK could be mediating the increases in mitochondrial oxidative enzymes and the increases in GLUT4 that are seen with hyperthyroidism.

Summary

There are a number of additional studies that would be of considerable interest regarding expression of proteins in this signalling system. Studies on the expression of AMPK subunit isoforms in muscle of obese patients with Type 2 diabetes are needed. Studies on the effects of long-term exposure to high-fat and high-carbohydrate diets and other models of insulin insensitivity would expand our understanding of the roles of these proteins in induction of the metabolic perturbations of diabetes. Changes accompanying experimentally induced changes in fibre type using disuse models, chronic electrical of diabetes. Changes accompanying experimentally induced changes in fibre type using disuse models, chronic electrical stimulation and hypertrophy would also be of interest relative to the role of muscle fibre recruitment in determining AMPK phenotype.

In summary, AMPK subunit protein expression and ACC protein expression have been found to be greater in thyroid hormone-treated rats than in rats treated with PTU to inhibit thyroid hormone synthesis. Marked variances in expression pattern are also seen in different muscle fibre types of the rat. These observations may provide the basis for determining more specific mechanisms for control of expression of the AMPK subunit genes.

References