

## Metabolic and hormonal interactions between muscle and adipose tissue

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From the perspective of a muscle physiologist, adipose tissue has long been perceived predominantly as a fuel reservoir that provides muscle and other tissues with NEFA when exogenous nutrients are insufficient for their energy needs. Recently, studies have established that adipose tissue is also an endocrine organ. Among the hormones it releases are adiponectin and leptin, both of which can activate AMP-activated protein kinase and increase fatty acid oxidation in skeletal muscle and probably other tissues. Deficiencies of leptin or leptin receptor, adiponectin and IL-6 are associated with obesity, insulin resistance and a propensity to type 2 diabetes. In addition, a lack of adiponectin has been linked to atherosclerosis. Whether this pathology reflects a deficient activation of AMP-activated protein kinase in peripheral tissues remains to be determined. Finally, recent studies have suggested that skeletal muscle may also function as an endocrine organ when it releases the cytokine IL-6 into the circulation during sustained exercise. Interestingly, one of the apparent effects of IL-6 is to stimulate lipolysis, causing the release of NEFA from the adipocyte. Thus, hormonal communications exist between the adipocyte and muscle that could enable them to talk to each other. The physiological relevance of this cross talk clearly warrants further study.

### Adiponectin: IL-6: AMP-activated protein kinase: Malonyl-CoA: Energy homeostasis: Insulin resistance

For much of the past 50 years adipose tissue has been principally viewed as a fuel reservoir that supplies NEFA for the energy requirements of other tissues and skeletal muscle as a more or less passive recipient of this largesse. From the perspective of a muscle physiologist, the adipocyte has been considered to play a particularly important role during starvation and exercise (Fig. 1(a)) when it increases the release of NEFA in keeping with the fuel needs of the muscle cell. These perceptions have slowly begun to change following the discovery that adipose tissue also functions as an endocrine organ (Spiegelman & Flier, 2001) and, more recently, the realization that skeletal muscle may assume a similar role during exercise (Keller *et al.* 2001).

Adipose tissue releases over twenty substances into the circulation (Gong *et al.* 2003), which perhaps makes it the champion endocrine organ, at least for the present. Of

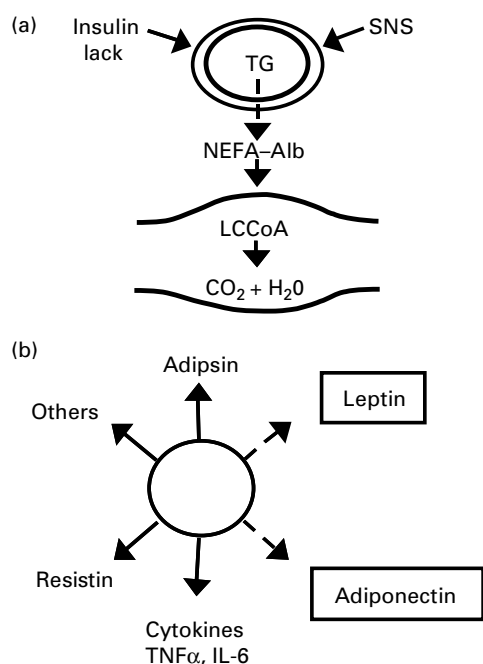
these hormones, leptin and adiponectin have been the most intensively studied (Fig. 1(b)). In the present review, the following questions will be addressed: (1) do leptin and adiponectin act on skeletal muscle and if so, how; (2) what happens to muscle and other tissues when these hormones are deficient; (3) does muscle also function as an endocrine organ. The emphasis will be on known factors released by adipose tissue and skeletal muscle and their effect on the fuel-sensing enzyme AMP-activated protein kinase (AMPK).

### Leptin

It has been appreciated since its discovery (Halaas *et al.* 1995) that leptin diminishes adiposity both by depressing food intake and increasing peripheral energy expenditure. Recently, leptin has been shown to increase fatty acid

**Abbreviations:** AICAR, 5-amino-4-imidazolecarboxamide riboside; AMPK, AMP-activated protein kinase; gAcrp30, the globular subunit of the adiponectin molecule.

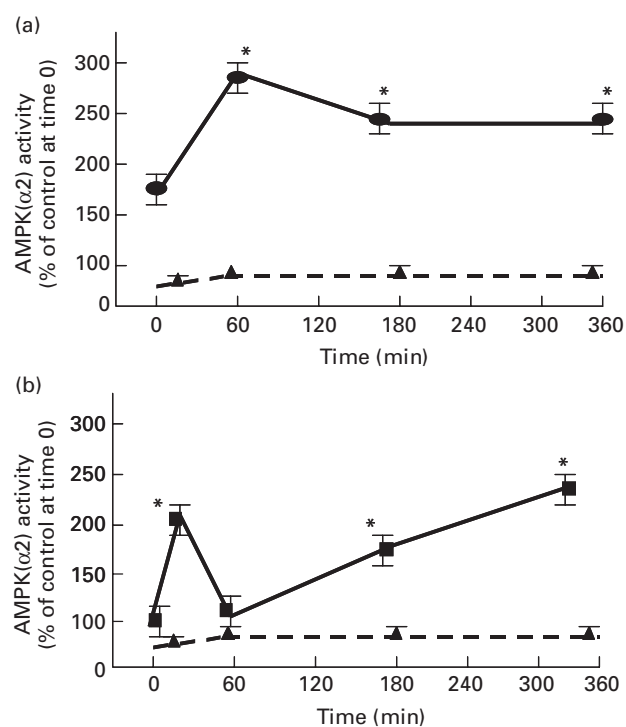
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**Fig. 1.** (a) Adipocyte as a fuel reservoir for muscle during starvation (insulin lack) and exercise. SNS, sympathetic nervous system; TG, triacylglycerols; NEFA-Alb, NEFA-albumin; LCCoA, long-chain fatty acyl-CoA; CO<sub>2</sub> + H<sub>2</sub>O, carbon dioxide and water. (b) Adipocyte as an endocrine organ.

oxidation in skeletal muscle (Muio *et al.* 1999) and to prevent lipotoxicity (the adverse effects caused by the ectopic deposition of lipid in liver, skeletal and cardiac muscle and the pancreatic islets; Lee *et al.* 1994). In addition, it has been shown that many obese individuals are resistant to leptin (Gong *et al.* 2003). In the past year studies by Kahn and her co-workers (Minokoshi *et al.* 2002) have established that leptin may cause these effects by activating the fuel-sensing enzyme AMPK (Fig. 2). Thus, they have shown that infusion of leptin intravenously leads to a transient increase in AMPK activity in skeletal muscle that peaks at 15 min, and a later more sustained increase that persists for upwards of 6 h. They have postulated that the early effect is the result of a direct action of leptin on muscle and the later effect is a result of a central action on the hypothalamus that increases sympathetic nervous system activity. In keeping with this contention, the sustained increase in AMPK activity has been observed when leptin is administered centrally as well as peripherally, and it is markedly diminished both by denervating the muscle and by treating the rat with the  $\alpha$  adrenergic antagonist phentolamine (Minokoshi *et al.* 2002).

Preliminary data (X Yu, R Unger, AK Saha and NB Ruderman, unpublished results) suggest that the effects of leptin on AMPK may extend to tissues other than muscle. In the Zucker diabetic rat, a rodent with obesity and ectopic lipid deposition as a result of a genetic deficiency of leptin receptors (Lee *et al.* 1994), it has been found that treatment for 13 weeks with the AMPK activator 5-amino-4-imidazolecarboxamide riboside (AICAR) markedly diminishes (80%) the accumulation of fat in liver, muscle



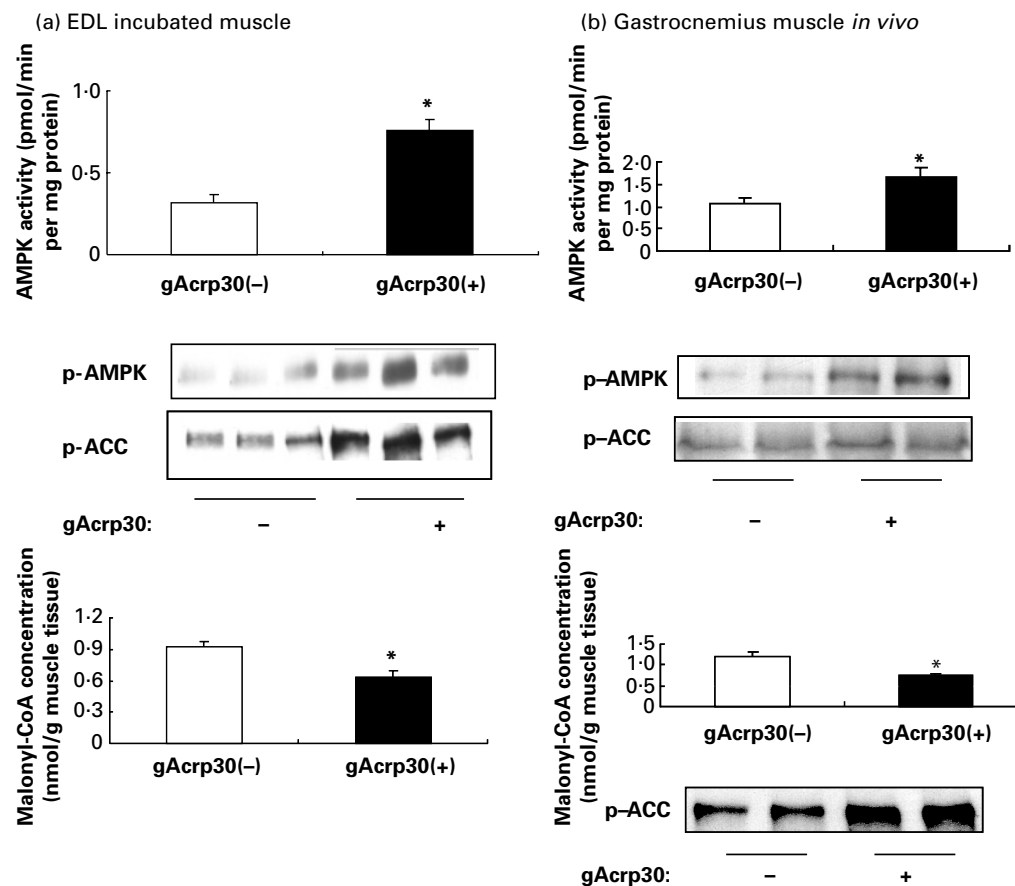
**Fig. 2.** AMP-activated protein kinase (AMPK) activation in rat soleus muscle after injection of leptin intrahypothalamically (a; ●) or intravenously (b; ■). (▲---▲), Saline (9 g NaCl/l). Values are means with their standard errors represented by vertical bars. Mean value was significantly different compared with the saline injection: \* $P < 0.05$ . (Adapted from Minokoshi *et al.* 2002.)

and the pancreatic  $\beta$ -cell and prevents the development of diabetes. Presumably, AMPK activation in each of these tissues accounts for the effects of AICAR; however, direct evidence for this role is still lacking.

## Adiponectin

### Basic description

Another hormone synthesized and released by differentiated adipocytes is the cytokine adiponectin, also termed Acrp30 (Scherer *et al.* 1995). Adiponectin circulates in plasma predominantly as trimeric, hexameric and high-molecular-weight species (Tsao *et al.* 2002). In addition, the globular subunit of the adiponectin molecule (gAcrp30), which is not detectable in plasma, has been shown to have biological activity (Fruebis *et al.* 2001). Early studies have linked adiponectin to energy homeostasis, since the expression of its mRNA is decreased in adipose tissue of obese mice (Hu *et al.* 1996; Arita *et al.* 1999) and human subjects (Hotta *et al.* 2000). In addition, a negative correlation between plasma adiponectin levels, insulin resistance, obesity and a predisposition to diabetes has been repeatedly observed (Hotta *et al.* 2000; Arner, 2003). More recently, it has been shown that adiponectin knock-out mice show delayed clearance of NEFA in plasma, low levels of fatty acid transport protein 1 mRNA in muscle, high levels of TNF $\alpha$  mRNA in adipose tissue and elevated plasma TNF $\alpha$  concentrations (Maeda *et al.* 2002).



**Fig. 3.** Effects of globular adiponectin (gAcrp30) on AMP-activated protein kinase (AMPK) and related variables. (a) AMPK activity and phosphorylation (p-AMPK), acetyl-CoA carboxylase phosphorylation (p-ACC) and malonyl-CoA concentration in rat extensor digitorum longus (EDL) muscles incubated for 30 min in the presence (+) or absence (-) of gAcrp30 (2.5 µg/ml). (b) Effects of gAcrp30 (75 µg per animal) injected retro-orbitally on the same variables in gastrocnemius muscle of C57BL/6J mice. In both studies p-ACC and malonyl-CoA concentration were altered at 30 min but not at 15 min. Values are means with their standard errors represented by vertical bars. Mean values were significantly different from those incubated in the absence of gAcrp30: \*  $P < 0.05$ . (Adapted from Tomas *et al.* 2002.)

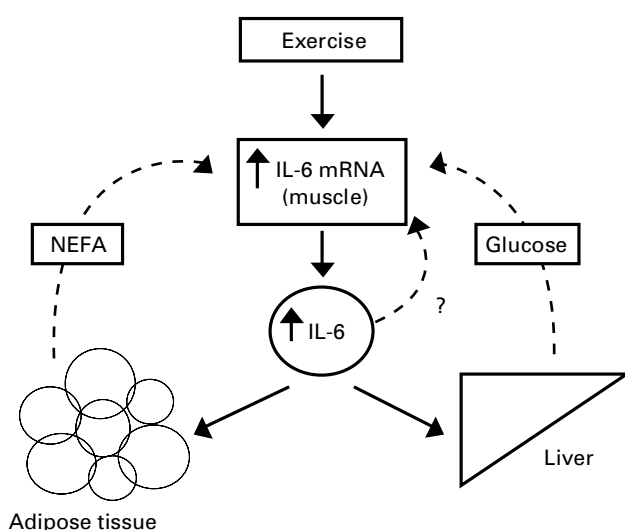
Furthermore, when placed on a high-fat high-sucrose diet these mice develop severe insulin resistance, with increased plasma NEFA levels and decreased insulin-stimulated insulin receptor substrate 1-associated phosphatidylinositol 3-kinase activity in muscle and in cultured myocytes (Maeda *et al.* 2002). Adiponectin has also been reported to have anti-atherogenic properties. Thus, when either the full-length or gAcrp30 is overexpressed, the atherosclerosis observed in apoE-deficient mice is markedly attenuated (Yamauchi *et al.* 2001; Okamoto *et al.* 2002).

Which of the various forms of adiponectin mediate these changes remains to be determined. Fruebis *et al.* (2001) have demonstrated that purified gAcrp30 (bacterially expressed) increases fatty acid oxidation in incubated mouse muscle and cultured cells. They have also found that when administered chronically to mice gAcrp30 causes weight loss without diminishing food intake. In contrast, they have found no effect of full-length adiponectin on muscle fatty acid oxidation or plasma NEFA levels (Fruebis *et al.* 2001). In contrast, in isolated hepatocytes, Berg *et al.* (2001, 2002) have reported that only

the full-length adiponectin enhances suppression of glucose production by insulin. It is likely that the different oligomeric isoforms of adiponectin have different signalling activities. Whereas gAcrp30 and the full-length trimer can activate AMPK in muscle, the hexamer and high-molecular-weight adiponectin can only activate NF-κB. A similar situation may also exist in other tissue types. Alternatively, these different actions of the two oligomers of adiponectin could be related to the presence of different adiponectin receptors in muscle and liver (Yamauchi *et al.* 2003).

#### *Molecular signalling induced by adiponectin*

As gAcrp30 has been reported to have the same effects on glucose and lipid metabolism in muscle as exercise and the administration of AICAR, it has been investigated whether it also activates AMPK. Incubation of the extensor digitorum longus muscle with gAcrp30 (2.5 µg/ml) has been shown to lead to a 2-fold increase in the activity of α2 isoform of AMPK, with comparable increases in the



**Fig. 4.** Proposed actions of IL-6 released from muscle during and after exercise. Not shown is that IL-6 may diminish TNF $\alpha$  expression in muscle.  $\leftarrow$ , Known actions;  $\leftarrow$ -----, less clear, but likely, actions. (Adapted from Steensberg *et al.* 2002.)

phosphorylation of AMPK and acetyl-CoA carboxylase (Fig. 3) and a 30% decrease in the concentration of malonyl-CoA (Tomas *et al.* 2002). Similar findings have been observed after the *in vivo* administration of gAcrp30 to C57 BL/6J mice (Fig. 3). In both situations the activation of AMPK occurs first and the phosphorylation and inhibition of acetyl-CoA carboxylase occurs later. It has also been shown, in the same studies, that gAcrp30 increases glucose uptake in the absence of added insulin (Tomas *et al.* 2002), an effect similar to that of AICAR. Similar effects of gAcrp30 have been described by Yamauchi *et al.* (2002) in C2C12 myocytes and incubated soleus muscle, and by Wu *et al.* (2003) in primary rat adipocytes. gAcrp30 has also been shown to reverse the inhibitory effect of TNF $\alpha$  on insulin-stimulated glucose uptake (Wu *et al.* 2003). The effects of full-length hexameric adiponectin on AMPK activity and acetyl-CoA carboxylase phosphorylation in muscle are controversial (Tomas *et al.* 2002; Yamauchi *et al.* 2002). Only full-length adiponectin appears to activate AMPK in liver (Tsao *et al.* 2002), consistent with the fact that full-length adiponectin receptor is mainly expressed in liver (Yamauchi *et al.* 2003).

#### Muscle as an endocrine organ: IL-6

There is evidence from Pedersen and her coworkers that muscle can function as an endocrine organ (Fig. 4; see Steensberg *et al.* 2002). As shown recently, the transcription rate, mRNA level and the release of IL-6 from skeletal muscle are dramatically increased during and after exercise, especially when the exercise is intense and sustained and muscle glycogen content is low (Steensberg *et al.* 2000; Keller *et al.* 2001). In addition, further work by Pedersen's group has shown that IL-6, at the concentrations found during exercise, stimulates lipolysis in adipose tissue (Van Hall *et al.* 2003), and that it can diminish plasma levels of

TNF $\alpha$  (Starkie *et al.* 2003). In addition, IL-6 deficient mice have been demonstrated to develop late-onset obesity and glucose intolerance (Wallenius *et al.* 2002). Re-administration of IL-6 results in increased energy expenditure, suggesting an important role of IL-6 in maintaining energy homeostasis. In light of these findings and the earlier observation that 30 min after a treadmill run AMPK activity is increased in rat liver and adipose tissue as well as in muscle (Park *et al.* 2002), the effect of IL-6 on AMPK activity in rat tissues has been examined (M Kelly, AK Saha, X Xiang, Z Luo, N Ruderman *et al.*, unpublished results). To date, it has been found that IL-6, at concentrations similar to that observed in plasma during intense exercise, causes 2- to 4-fold increases in AMPK phosphorylation at Thr<sup>172</sup> (an index of activation) in both incubated rat extensor digitorum longus muscle and cultured 443A adipocytes. Whether IL-6 exerts these effects *in vivo* remains to be determined. How these findings relate to the putative linkage between IL-6 and inflammatory events in living organisms will also require further study.

#### Concluding remarks

The evidence that the adipocyte hormones leptin and adiponectin can alter fatty acid oxidation and activate AMPK in skeletal muscle has been reviewed. Conversely, IL-6, which is released from muscle during exercise, has been shown to stimulate lipolysis in fat cells and it can activate AMPK in incubated muscle and cultured adipocytes. It must be emphasized, however, that the activation of AMPK by IL-6 has only been observed *in vitro*. Studies in intact animals are needed to establish whether this effect is physiologically relevant.

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