

Adrenaline is a critical mediator of acute exercise-induced AMP-activated protein kinase activation in adipocytes

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Exercise increases AMPK (AMP-activated protein kinase) activity in human and rat adipocytes, but the underlying molecular mechanisms and functional consequences of this activation are not known. Since adrenaline (epinephrine) concentrations increase with exercise, in the present study we hypothesized that adrenaline activates AMPK in adipocytes. We show that a single bout of exercise increases AMPK α 1 and α 2 activities and ACC (acetyl-CoA carboxylase) Ser⁷⁹ phosphorylation in rat adipocytes. Similarly to exercise, adrenaline treatment *in vivo* increased AMPK activities and ACC phosphorylation. Pre-treatment of rats with the β -blocker propranolol fully blocked exercise-induced AMPK activation. Increased AMPK activity with exercise and adrenaline treatment *in vivo* was accompanied by an increased AMP/ATP ratio. Adrenaline incubation of isolated adipocytes also increased the AMP/ATP ratio and AMPK activities, an effect blocked by

propranolol. Adrenaline incubation increased lipolysis in isolated adipocytes, and Compound C, an AMPK inhibitor, attenuated this effect. Finally, a potential role for AMPK in the decreased adiposity associated with chronic exercise was suggested by marked increases in AMPK α 1 and α 2 activities in adipocytes from rats trained for 6 weeks. In conclusion, both acute and chronic exercise are significant regulators of AMPK activity in rat adipocytes. Our findings suggest that adrenaline plays a critical role in exercise-stimulated AMPK α 1 and α 2 activities in adipocytes, and that AMPK can function in the regulation of lipolysis.

Key words: adrenaline, AMP-activated protein kinase (AMPK), cAMP-dependent protein kinase (PKA), exercise, fatty acid synthesis, lipolysis.

INTRODUCTION

It is widely accepted that physical exercise is an efficient way to reduce visceral fat, the fat depot that is thought to contribute to obesity-associated complications such as Type 2 diabetes and cardiovascular disease. A single bout of exercise decreases fatty acid synthesis and increases lipolysis in adipocytes [1,2], suggesting that regular exercise training reduces adiposity by repeated activation of the lipolytic process and the reduction of fatty acid synthesis [3]. The underlying molecular mechanisms by which an acute bout of exercise decreases fatty acid synthesis and increases lipolysis in adipocytes are not fully understood.

Physical exercise increases the concentration of several blood hormones, neurotransmitters and other circulating factors, including adrenaline (epinephrine). Adrenaline binds to and acts through the adrenergic receptor pathway, increasing lipolysis via a mechanism that involves activation of PKA (cAMP-dependent protein kinase). PKA phosphorylates HSL (hormone-sensitive lipase) at Ser⁵⁶³, Ser⁶⁵⁹ and Ser⁶⁶⁰ [4], two of which are critical sites for HSL activity [4]. Adrenaline also inhibits fatty acid synthesis via inhibition of ACC (acetyl-CoA carboxylase), the rate-limiting enzyme in fatty acid synthesis, although the mechanism by which adrenaline regulates ACC activity has not been elucidated.

AMPK (AMP-activated protein kinase) is a member of an energy-sensing serine/threonine protein kinase family. AMPK is activated by an increase in the AMP/ATP ratio, via a complex mechanism that involves allosteric modification, phosphorylation by one or more AMPK kinases and a decrease in phosphatase

activities [5]. There have been relatively few reports of AMPK function in adipocyte metabolism, and the reports that do exist have been somewhat inconsistent. Activation of AMPK by AICAR (5-amino-4-imidazolecarboxamide riboside) in isolated fat cells has been shown to inhibit isoprenaline (isoproterenol)-induced lipolysis [6,7], suggesting that AMPK may be a negative regulator of lipolytic activity. On the other hand, AMPK activation in 3T3-L1 cells did not inhibit adrenaline-stimulated HSL activity or glycerol release [8]. Overexpression of a dominant-inhibitory mutant of AMPK in 3T3-L1 cells impaired isoprenaline-induced lipolysis, suggesting that AMPK activity is a positive regulator of lipolysis in adipocytes [9]. Furthermore, compared with wild-type mice, AMPK α 2 whole-body knockout mice have greater increases in adiposity and adipocyte hypertrophy in response to high-fat feeding [10].

Previous studies have demonstrated that exercise increases AMPK in rat [11] and human [8] adipocytes. In addition, two studies have indicated that adrenergic receptor activation can stimulate AMPK in L6 cells [12] and brown adipocytes [13]. Since adrenaline is a hormone induced by exercise and acts through β -adrenergic receptors, we hypothesized that adrenaline is a mediator of exercise-induced AMPK activation in adipocytes.

In the present study, we investigated the effects of both acute exercise and chronic exercise training on AMPK activities in rat adipocytes isolated from epididymal fat pads. We also tested the hypothesis that exercise-induced increases in circulating adrenaline are responsible for increased AMPK activity in adipocytes. To study further the effects of adrenaline on adipocyte metabolism, we incubated isolated rat adipocytes *in vitro* and assessed

Abbreviations used: ACC, acetyl-CoA carboxylase; AICAR, 5-amino-4-imidazolecarboxamide riboside; AMPK, AMP-activated protein kinase; CaMKK, Ca²⁺/calmodulin-dependent protein kinase kinase; CREB, cAMP-response-element-binding protein; HRP, horseradish peroxidase; HSL, hormone-sensitive lipase; KRB, Krebs–Ringer bicarbonate; PKA, cAMP-dependent protein kinase.

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the regulation of AMPK, ACC, the AMP/ATP ratio and lipolysis. Our results demonstrate that both a single bout of exercise and chronic exercise training are major regulators of AMPK activity in adipocytes, and that adrenaline plays an important role in the effects of acute exercise to activate AMPK in rat adipocytes *in vivo*. Furthermore, AMPK activity is necessary for maximal activation of adrenaline-induced lipolysis in rat adipocytes.

MATERIALS AND METHODS

Animals

Sprague–Dawley rats were obtained from Taconic. Rats were provided with standard rodent chow and water *ad libitum* and were housed under normal laboratory conditions (12 h light/12 h dark cycle). All experimental procedures were conducted in accordance with NIH (National Institutes of Health) guidelines and approved by the Institutional Animal Care and Use Committee of the Joslin Diabetes Center.

Acute exercise experiments

Male and female rats weighing ~150 g were used for the acute exercise studies. Rats were familiarized with the treadmill (Quinton model 42) by running at a slow pace for 5–10 min on the 2 days before the experiment. The exercised groups performed steady-state treadmill running at 20 m/min up a 12% gradient for 15, 30 or 60 min, and additional animals were exercised for 60 min and were killed 60 min later (post-exercise group). Control rats participated in treadmill familiarization, but were not exercised on the day of the experiment. Rats were killed via decapitation and exsanguinated. Epididymal (male) or parametrial (female) fat pads were separated rapidly, removed and snap-frozen in liquid nitrogen until subsequent processing and analyses (see below).

Adrenaline treatment *in vivo*

Male rats weighing ~150 g were used for the adrenaline studies. At 15 min after the injection of either saline or propranolol (2 mg/100 g of body mass), rats were injected intraperitoneally with either vehicle (1 mg/ml of ascorbic acid) or adrenaline (25 µg/100 g of body mass). At 15 min after the injection of the hormones, animals were killed by decapitation, and epididymal fat pads were removed immediately and frozen in liquid nitrogen.

Exercise training

Female rats initially weighing 75–100 g were purchased from Taconic. Female rats were used for these experiments because they maintain body mass better than males when undergoing exercise training protocols (M. F. Hirshman and L. J. Goodyear, unpublished work). The training protocol used in this study has been described previously [14,15]. Briefly, animals were maintained on a 12 h light/12 h dark cycle and were fed on standard laboratory chow and water *ad libitum*. Rats ($n = 12$) were housed individually in plastic cages and divided into two groups that were counterbalanced by body mass and blood glucose concentrations. Animals were trained on the treadmill twice a day (09:00–10:00 h and 16:00–17:00 h), 6 days/week for 6 weeks. Initial training was at 16 m/min, 15% gradient for 12 min and gradually increased to 32 m/min for 60 min per session twice a day. We separated the training sessions to prevent undue stress on the animals, and because we have found in preliminary studies that this protocol results in optimal training adaptations in skeletal muscle and fat. Food consumption and body mass were measured once a week. In order to differentiate the effects of acute exercise from chronic adaptations to training, rats were killed ~30 h following

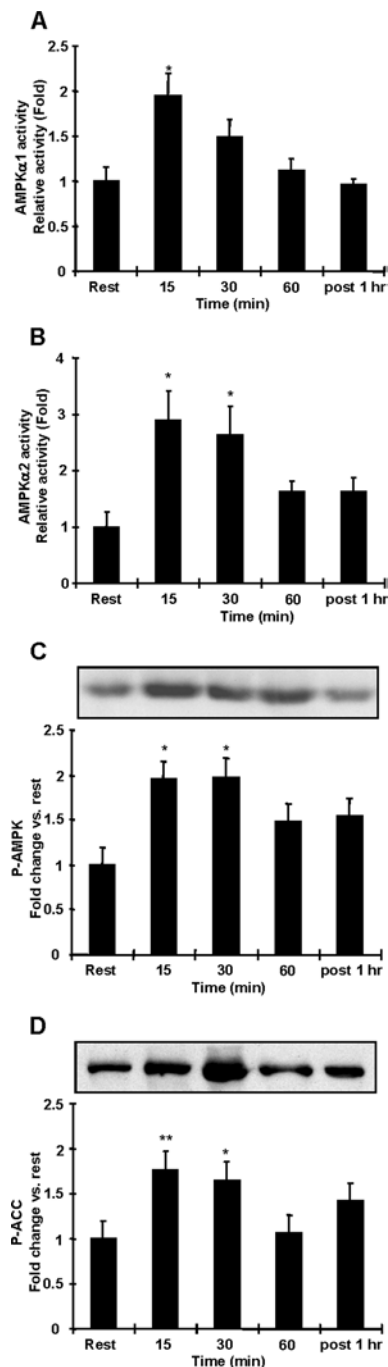


Figure 1 Acute exercise increases AMPK α 1 and AMPK α 2 activities in rat adipocytes

(A and B) Time course of AMPK α 1 (A) and AMPK α 2 (B) activity in epididymal fat from rats exercised on a treadmill (22 m/min, 12% gradient) for 15, 30 or 60 min, or exercised for 60 min followed by 60 min of rest. AMPK α 1 or AMPK α 2 was immunoprecipitated from epididymal fat lysates and used in an *in vitro* kinase assay with SAMS peptide as substrate. Both AMPK α 1 and α 2 activities were increased with 15 and 30 min of exercise with no change with 60 min of exercise. (C) Western blot analysis of epididymal fat lysates show that AMPK Thr¹⁷² phosphorylation was increased with 15 and 30 min of exercise. (D) There were significant increases of ACC Ser⁷⁹ phosphorylation with 15 and 30 min exercise. Results are means \pm S.E.M.; $n = 5$ per group. * $P < 0.05$ and ** $P < 0.01$ compared with the resting group.

the final training session. Parametrial fat pads were snap-frozen in liquid nitrogen and stored at -80°C until being processed as described below.

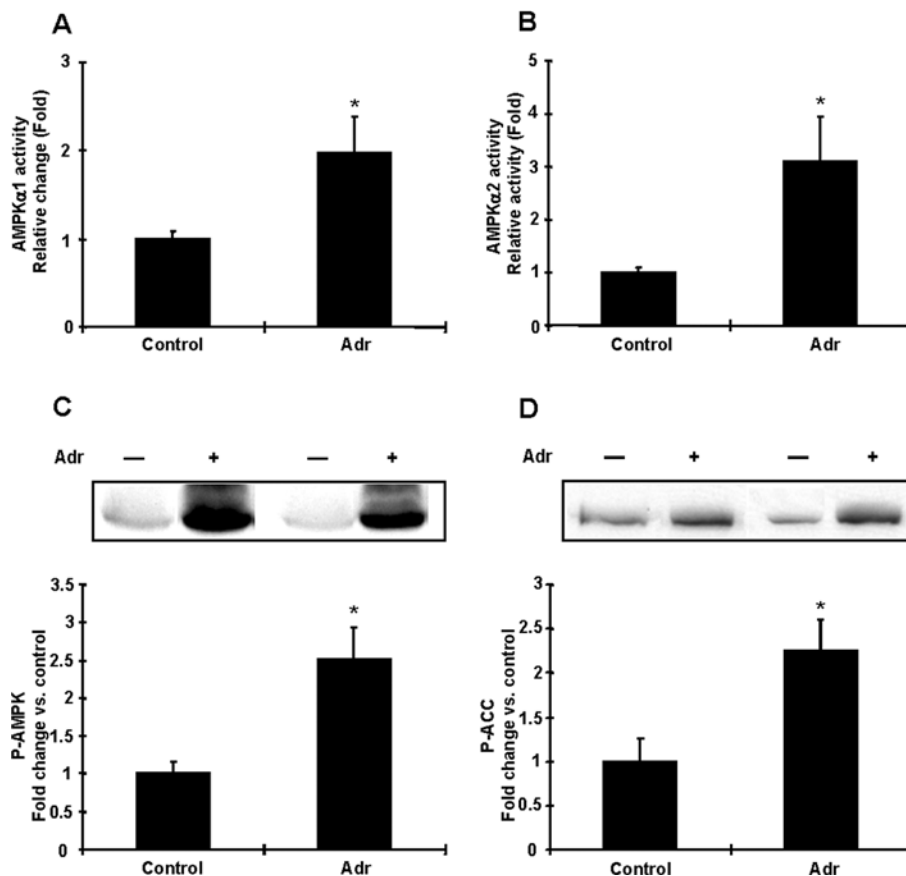


Figure 2 Effects of adrenaline on AMPK activity in rat adipocytes *in vivo*

Animals were injected with vehicle or adrenaline (Adr) ($25 \mu\text{g}/100 \text{g}$ of body mass) and 15 min after injection, epididymal fat pads were removed and assayed for AMPK activity. (**A** and **B**) Both AMPK α 1 (**A**) and AMPK α 2 (**B**) activities were increased by an adrenaline injection. (**C** and **D**) Adrenaline increased phosphorylation of AMPK (**C**) and ACC (**D**) *in vivo*. Results are means \pm S.E.M.; $n = 4\text{--}5$ per group. * $P < 0.05$ compared with control.

Preparation of isolated adipose cells

Male rats weighing $\sim 120 \text{g}$ were used to prepare isolated adipocytes. Immediately after rats were killed, epididymal adipose tissues were removed and adipose cells were isolated by the method of Rodbell [16] as modified by Cushman [17] using crude collagenase (Worthington Biochemical Corp.). All incubations were carried out in KRB (Krebs–Ringer bicarbonate) buffer, pH 7.4, at 37°C . Cells from each group were stabilized by incubation for 1 h before treatment. Isolated fat cells were stimulated with $0.1 \mu\text{g}/\text{ml}$ adrenaline for 10 min. To inhibit α - or β -adrenergic receptors, cells were pre-incubated with $100 \mu\text{M}$ phentolamine (Sigma–Aldrich) or $5 \mu\text{M}$ propranolol for 15 min respectively.

Lipolysis measurements

Adipose cells were distributed among 20 ml plastic incubation vials to provide a final incubation volume of 1.5 ml of KRB–Hepes buffer containing isolated cells and 1 mg/ml glucose in the absence or presence of $0.1 \mu\text{g}/\text{ml}$ adrenaline (Sigma–Aldrich). To inhibit AMPK, cells were pre-incubated with $50 \mu\text{M}$ Compound C (Calbiochem) for 15 min before the addition of adrenaline. The final cell concentration was 10^5 cells/ml. All incubations were carried out in triplicate, and all analyses were corrected for the appropriate blank values obtained from samples of cells incubated for 1 min in an ice bath. At the end of the 30-min incubation, a 0.5 ml sample of cell-free medium was aspirated from each tube

and chilled in an ice bath. Glycerol was assayed enzymatically in 0.2 ml of the cell-free medium [18].

Isoform-specific AMPK activity

Fat pads obtained from rats studied *in vivo* and adipocytes incubated *in vitro* were homogenized as described previously [19]. Lysates ($100 \mu\text{g}$) were immunoprecipitated with specific antibodies against the α 1 and α 2 catalytic subunits of AMPK [20] and Protein A beads (Pierce Biotechnology). Reactions were carried out using the synthetic SAMS peptide (HMRSAMSGHLVKRR) as substrate, as described previously [20]. AMPK activity is expressed as ATP incorporation in pmol/min per mg of protein.

Western blot analysis and antibodies

Standard Western blot analyses were used to assess protein and phosphorylation levels of various molecules. Immunoblots were developed using ECL[®] (enhanced chemiluminescence) reagents (Amersham Biosciences) and quantified using FluorChem version 2.01 (Alpha Innotech). Primary antibodies purchased from commercial sources included those against AMPK–Thr¹⁷², CREB (cAMP-response-element-binding protein)–Ser¹³³ and HSL–Ser⁵⁶³ (Cell Signaling Technology), AMPK α 1, AMPK α 2, ACC and ACC–Ser⁷⁹ (Upstate), CaMKK (Ca²⁺/calmodulin-dependent protein kinase kinase; BD Biosciences), and LKB1 (Santa Cruz Biotechnology). Secondary antibodies used were HRP (horseradish peroxidase)-conjugated anti-rabbit (Amersham Biosciences),

HRP-conjugated anti-mouse (Upstate) and HRP-conjugated anti-goat (Promega).

Nucleotide extraction and analysis

To measure nucleotide concentrations, isolated adipocytes and epididymal fat pads were frozen, pulverized and homogenized with 0.6 M perchloric acid. After neutralization and centrifugation at 5500 *g* for 2 min, the supernatant was filtered and used for measuring ATP, ADP and AMP content. Adenine nucleotide content was determined by HPLC (Waters) and normalized to mg of protein [21].

Statistical analysis

Data are means \pm S.E.M. All data were compared using Student's *t* test or one-way ANOVA. The differences between groups were considered to be significant when $P < 0.05$.

RESULTS

Activation of AMPK in rat adipocytes by acute exercise and adrenaline

To determine the effects of acute exercise on AMPK activity in adipocytes, rats performed treadmill running exercise at 20 m/min for 15, 30 or 60 min, up a 12% gradient. This short-term exercise had no effect on body mass and epididymal fat pad mass (results not shown). AMPK α 1 and α 2 activities were increased with 15 and 30 min of exercise 1.5–2-fold and 2.6–2.9-fold respectively (Figures 1A and 1B). After 60 min of exercise, and 1 h after a 60 min exercise bout, AMPK α 1 and α 2 activities were no longer elevated (Figures 1A and 1B). Consistent with AMPK activity results, AMPK Thr¹⁷² phosphorylation was significantly increased after 15 and 30 min of exercise, with no effect at 60 min of exercise (Figure 1C). Downstream of AMPK, phosphorylation of ACC at the inhibitory Ser⁷⁹ site was also significantly increased with 15 and 30 min of exercise (Figure 1D). These results suggest that a single bout of exercise can increase the activities of both the α 1 and α 2 catalytic isoforms of AMPK in rat adipocytes, but that this effect is diminished with longer periods of exercise.

It is well established that adrenaline concentrations in the blood increase in response to exercise [22] and treadmill exercise for 15 and 30 min increases plasma adrenaline in rats [23,24], and that this in turn activates adrenergic receptor signalling. To begin to address the hypothesis that adrenaline may mediate AMPK activation in adipocytes during exercise, we next determined whether adrenaline activates AMPK *in vivo*, by injecting adrenaline (25 μ g/100 g of body mass) and taking fat pads 15 min later. Adrenaline increased AMPK α 1 and α 2 activities in adipocytes 2–3.1-fold, respectively (Figures 2A and 2B). Likewise, AMPK Thr¹⁷² and ACC Ser⁷⁹ phosphorylation were significantly increased by adrenaline treatment (Figures 2C and 2D). These results demonstrate that adrenaline can also activate AMPK in rat adipocytes *in vivo*.

Exercise-induced AMPK activation is mediated by the β -adrenergic receptor

We next tested the hypothesis that β -adrenergic receptors are required for the effects of exercise on AMPK activation in adipocytes. Since the β -adrenergic receptor is predominantly expressed in adipocytes [25], we injected rats with propranolol, a β -adrenergic blocker, or vehicle, 15 min before the start of a 15 min bout of treadmill exercise. Rats treated with propranolol had no difficulty in completing the exercise bout, and the exercise-induced activation of AMPK was completely blunted by this β -adrenergic

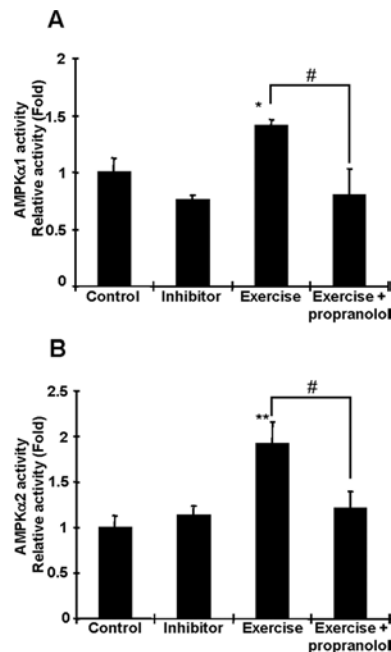


Figure 3 Propranolol abolishes exercise-induced AMPK activation in adipocytes

Rats were injected with propranolol (1 mg/100 g of body mass) or vehicle 15 min before 15 min of treadmill exercise. Exercise-induced AMPK α 1 (A) and AMPK α 2 (B) activations were completely blunted by β -adrenergic blockade injection. Results are means \pm S.E.M.; $n = 4$ –5 per group. * $P < 0.05$ and ** $P < 0.01$ compared with the control. # $P < 0.05$ compared with the exercised group.

blockade (Figures 3A and 3B). These results suggest that adrenaline, which is increased by an acute bout of exercise and activates β -adrenergic receptors, could be a critical candidate for exercise-induced AMPK activation.

Exercise and adrenaline increase AMP concentrations in adipocytes

Increases in AMP concentrations in cells can allosterically activate AMPK, resulting in phosphorylation of the Thr¹⁷² domain of the α subunit by upstream kinases, and inhibiting dephosphorylation of Thr¹⁷² by protein phosphatases [5]. To address whether exercise- and adrenaline-stimulated AMPK activation are associated with altered AMP content, we measured AMP, ADP and ATP concentrations in adipocytes. As shown in Table 1, treadmill exercise for 15 min, which significantly increased AMPK activity, tended to decrease ATP and increase ADP concentrations, and significantly increased AMP content and the AMP/ATP ratio. An intraperitoneal injection of adrenaline *in vivo* significantly decreased the concentration of ATP in adipocytes and increased ADP and ATP concentrations 1.6–4.5-fold, resulting in a 6.2-fold increase in the AMP/ATP ratio.

Adrenaline increases AMPK activity and the AMP/ATP ratio in isolated adipocytes

To understand further the mechanisms by which adrenaline regulates AMPK and the metabolic consequences, we next used incubated adipocytes derived from epididymal fat. At lower doses of adrenaline (0.001–0.005 μ g/ml), similar to what occurs in the resting state [26–29], adrenaline had no effect on AMPK α 1 or α 2 activities (Figures 4A and 4B). Adrenaline at high doses, including the physiological range that would occur during *in vivo* exercise [29,30], increased AMPK α 1 and α 2 activities up to ~4-fold

Table 1 Adenine nucleotides in isolated fat cells and rat epididymal fat pads

Results are means \pm S.E.M., $n = 4$ /group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with control.

	ATP (nmol/mg of protein)	ADP (nmol/mg of protein)	AMP (nmol/mg of protein)	AMP/ATP
Epididymal fat pads				
Control	11.21 \pm 0.70	2.85 \pm 0.17	1.20 \pm 0.14	0.110 \pm 0.018
Propranolol	11.04 \pm 0.42	3.81 \pm 0.64	1.33 \pm 0.34	0.118 \pm 0.027
Adrenaline	07.22 \pm 0.59**	4.67 \pm 0.53*	5.37 \pm 1.45*	0.787 \pm 0.230*
Adrenaline + propranolol	11.38 \pm 1.03	4.79 \pm 0.44**	2.86 \pm 0.46*	0.265 \pm 0.060*
Exercise	10.85 \pm 1.44	3.59 \pm 0.35	1.94 \pm 0.17*	0.186 \pm 0.024*
Isolated fat cells				
Control	9.10 \pm 0.80	1.43 \pm 0.30	0.38 \pm 0.09	0.041 \pm 0.005
Adrenaline	5.48 \pm 0.26**	1.94 \pm 0.12	0.75 \pm 0.04**	0.137 \pm 0.004***

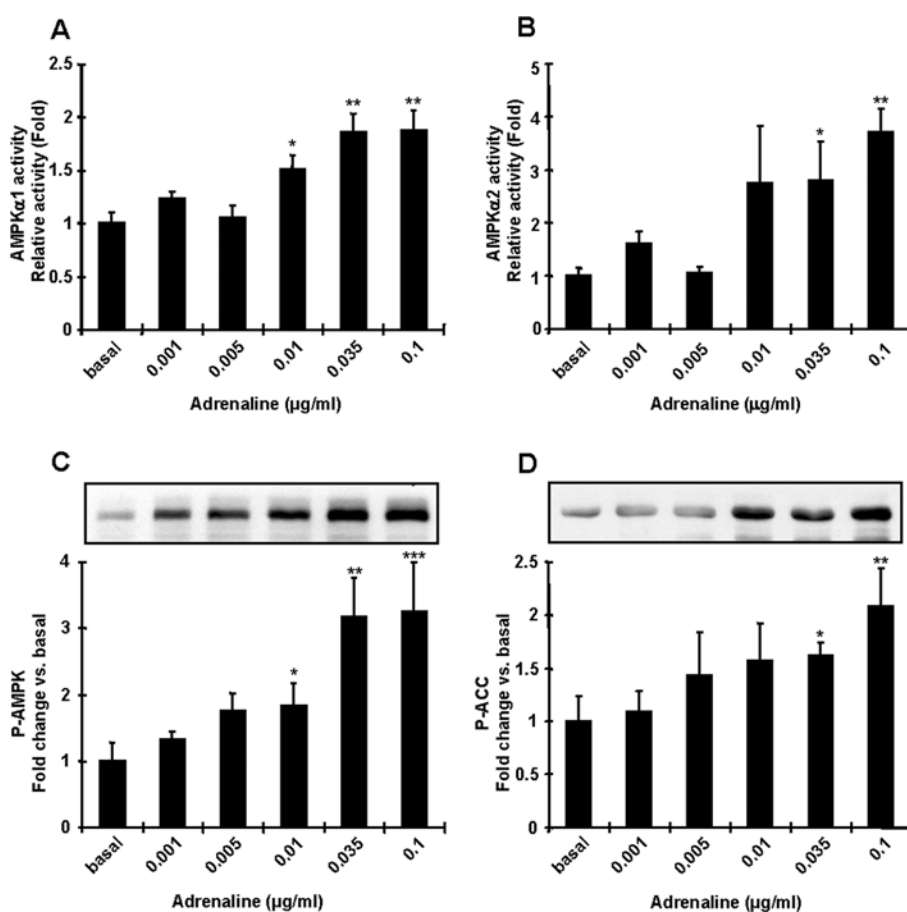
above basal (Figures 4A and 4B). The effect of adrenaline on AMPK activation was dose-dependent, with maximum activation at 0.1 $\mu\text{g/ml}$ (Figures 4A and 4B). AMPK Thr¹⁷² phosphorylation followed a similar pattern to AMPK activity (Figure 4C), and

ACC Ser⁷⁹ phosphorylation was also significantly increased with adrenaline (Figure 4D).

To determine whether adrenaline-stimulated AMPK activation is associated with altered nucleotide content, we measured concentrations of AMP, ADP and ATP in adipocytes incubated with adrenaline. As shown in Table 1, adrenaline decreased ATP concentrations in the isolated fat cells by 40% and increased the concentration of AMP 2-fold, resulting in a 3.3-fold increase in the AMP/ATP ratio (Table 1). These results suggest that adrenaline and exercise alter adenine nucleotide content in adipocytes, making it likely that this is the mechanism for AMPK activation.

Activation of AMPK by adrenaline is mediated by the β -adrenergic receptor

To elucidate whether the effects of adrenaline on AMPK activation are mediated by adrenergic receptor signalling, isolated fat cells were pre-incubated in the presence or absence of α - and β -adrenergic blockades. The α -adrenergic receptor inhibitor had no effect on the ability of adrenaline to stimulate AMPK α 1 and α 2 activities (Figure 5A), whereas β -adrenergic receptor blockade fully inhibited adrenaline-stimulated AMPK α 1 and AMPK α 2 activities (Figure 5B). Increases in ACC Ser⁷⁹ phosphorylation were also completely blunted by β -adrenergic receptor inhibition (Figure 5C).

**Figure 4 Adrenaline activates AMPK and ACC phosphorylation in isolated fat cells**

Isolated fat cells from rats were treated with different doses of adrenaline for 10 min. Extracts were prepared and used in an *in vitro* AMPK activity assay (A and B) or Western blot analyses using anti-AMPK Thr¹⁷² and anti-ACC Ser⁷⁹ antibodies (C and D). Both AMPK α 1 (A) and AMPK α 2 (B) activities were significantly increased by adrenaline treatment. Phosphorylation of AMPK Thr¹⁷² (C) and ACC Ser⁷⁹ (D) were significantly increased with adrenaline treatment. Results are means \pm S.E.M.; $n = 3$ –6 per group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with basal.

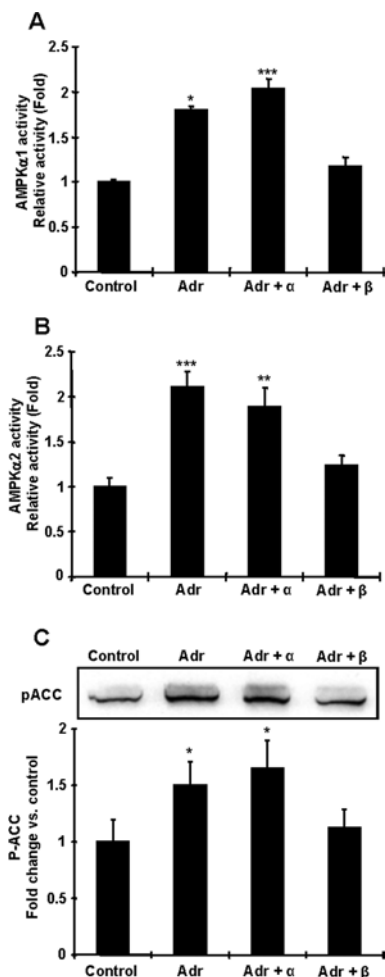


Figure 5 Adrenaline increases AMPK activity in isolated fat cells through the β -adrenergic receptor pathway

Isolated fat cells were stimulated with 0.1 μ g/ml adrenaline (Adr) for 10 min. To inhibit the α - or β -adrenergic receptor pathway, cells were pre-incubated with 100 μ M phentolamine or 5 μ M propranolol for 15 min respectively. (A and B) The effect of adrenaline on AMPK activation was abolished by the pre-incubation with propranolol but not phentolamine. (C) The effects of adrenaline on ACC Ser⁷⁹ phosphorylation were blunted by β -adrenergic blockade, whereas α -adrenergic receptor blockade had no effect. Results are means \pm S.E.M.; $n = 3-6$ per group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with control.

AMPK is necessary for maximal activation of lipolysis induced by adrenaline

To determine whether AMPK activation mediates adrenaline's effects on lipolysis, we examined lipolytic activity in isolated rat adipocytes. For these studies we used Compound C, which is a potent inhibitor of AMPK [31], and was effective in inhibiting adrenaline-stimulated AMPK α 1 and α 2 activities in the incubated adipocytes (Figure 6A). Incubation of the control adipocytes with Compound C did not alter lipolysis (Figure 6B). Incubation of isolated adipocytes with adrenaline increased lipolysis 2.6-fold above control, an effect that was completely abolished by propranolol (Figure 6B). Compound C partially inhibited the adrenaline-induced increase in lipolysis (Figure 6B). Phosphorylation of HSL Ser⁵⁶³, which is stimulated by PKA [32], was increased by adrenaline, while compound C treatment blunted this phosphorylation (Figure 6C). The mechanism for this effect is not known, but since CREB phosphorylation at Ser¹³³ (a PKA substrate) was not altered by Compound C pre-treatment (results not

shown), it is unlikely to be due to decreases in PKA activity. Compound C was effective in inhibiting adrenaline-stimulated ACC Ser⁷⁹ phosphorylation, a key enzyme for the regulation of fatty acid synthesis and a downstream substrate of AMPK (Figure 6D). These data suggest that AMPK activation plays an important role in the stimulation of maximal adrenaline-induced lipolysis.

Exercise training increases AMPK activities in adipocytes

To determine the effects of long-term chronic exercise on AMPK activity and expression in adipocytes, rats were trained using the protocol described in the Materials and methods section. At the end of the 6 weeks, there was no difference in body mass between controls and trained rats, but trained rats had a $\sim 50\%$ decrease in parametrial fat pad mass (Figure 7A). Food consumption during training was not different between the two groups (data not shown). Training increased AMPK α 1 activity 2.3-fold over controls in the adipocytes, with no change in total AMPK α 1 expression (Figure 7B). Similarly, AMPK α 2 activity was increased 1.9-fold in the trained animals, with no change in AMPK α 2 protein (Figure 7C). AMPK Thr¹⁷² phosphorylation was also significantly increased in the trained rats (Figure 7D). Thus both short-term and chronic exercise are major regulators of AMPK activity in adipocytes.

DISCUSSION

The effects of chronic exercise training to reduce adiposity in humans results from an overall increase in energy expenditure, due largely to the increased energy demands of the working skeletal muscles during each individual exercise bout. During aerobic exercise, the contracting skeletal muscles require increased carbohydrate and fat, the latter fuel derived almost exclusively from adipocyte lipolysis. Adrenaline has long been known to be an important mediator of exercise-stimulated adipocyte lipolysis, and the results of the present study suggest that AMPK may be an intermediate for this effect. The β -blocker propranolol fully inhibited the increase in AMPK activity and phosphorylation in adipocytes of exercised rats. Furthermore, injection of adrenaline *in vivo* mimicked the effects of exercise to increase AMPK activity and phosphorylation. Although we cannot fully rule out the possibility that other circulating factors (e.g. hormones and noradrenaline), which are also increased by physical exercise and act through β -adrenergic receptors, are contributing to the effects of exercise on AMPK activity in adipocytes, our data strongly support a primary role for circulating adrenaline.

If adrenaline is mediating the effects of acute exercise to increase AMPK activity, and adrenaline itself increases AMPK activity, what is the mechanism for the adrenaline-induced increases in AMPK activity? Our findings suggest that increases in AMPK activity with exercise and adrenaline *in vivo* and adrenaline in the isolated adipocytes are due, at least in part, to increases in cellular AMP concentrations. Increases in adrenaline-mediated AMP concentrations are consistent with studies in human adipocytes showing that increases in cAMP and adenine nucleotide catabolism by adrenaline or isoprenaline are associated with an enhanced release of purines in the form of inosine and hypoxanthine [33,34]. Collectively, adrenaline seems to decrease ATP and increase AMP due to increased ATP utilization and adenine nucleotide breakdown, which results in increases in the AMP/ATP ratio, a critical regulator for AMPK activation [35].

Exercise and adrenaline increased Ser⁷⁹ phosphorylation of ACC (Figures 1D and 2D), which in turn decreased ACC activity, and adrenaline-induced ACC Ser⁷⁹ phosphorylation was abolished by Compound C treatment, suggesting that adrenaline increased

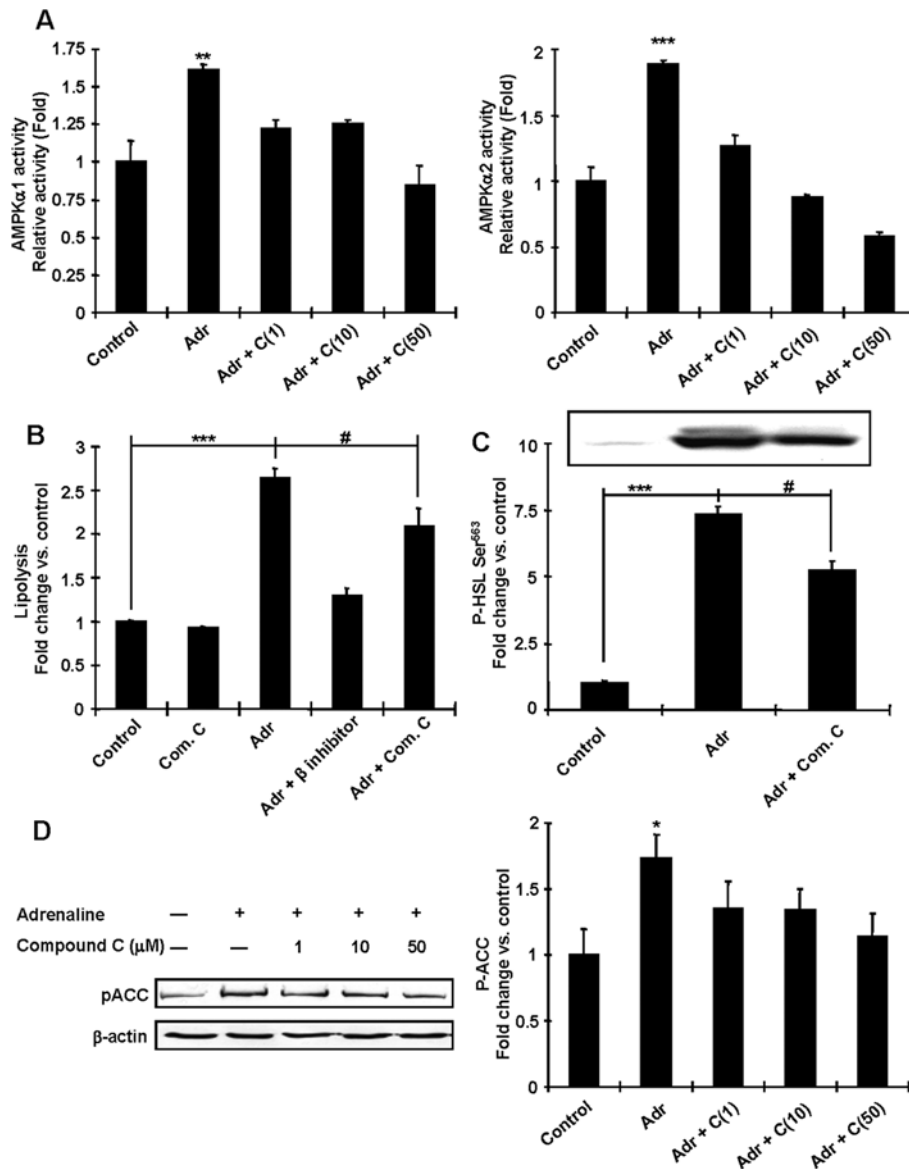


Figure 6 AMPK is necessary for adrenaline-induced lipolysis and inhibition of fatty acid synthesis in isolated adipocytes

Isolated adipocytes were incubated with adrenaline (Adr) for 30 min in the presence or absence of inhibitors as indicated for 15 min. (A) Adrenaline-induced AMPK activation was blunted by Compound C (C), a known AMPK inhibitor. (B) Released glycerol was measured to assess the rate of lipolysis. Adrenaline increased lipolysis 2.6-fold, but inhibition of AMPK achieved by Compound C (Com. C) treatment (50 μ M) blunted this activation. (C) Phosphorylation of HSL Ser⁶⁶³ was increased by adrenaline, whereas pre-treatment with Compound C (Com. C) blunted this phosphorylation. (D) Phosphorylation of ACC Ser⁷⁹ was increased by adrenaline, whereas co-incubation with Compound C (C) decreased this phosphorylation compared with adrenaline treatment alone. Results are means \pm S.E.M., and the blots shown are representative of $n = 3$ per group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with the control. # $P < 0.05$ compared with the indicated control group.

ACC Ser⁷⁹ phosphorylation by AMPK. It has been reported that PKA, which is activated by adrenaline, also phosphorylates ACC, but this phosphorylation is not necessary for ACC activity [36]. Although we cannot exclude the possibility that ACC phosphorylation by PKA contributes to adrenaline-induced ACC activity, it is apparent that AMPK is a major regulator of ACC activity in adrenaline-induced ACC Ser⁷⁹ phosphorylation.

A previous study has reported that 30 min of treadmill exercise increases total AMPK activity 1.5-fold in rat adipocytes [11]. Our findings demonstrate that a single bout of exercise increases the activity of both the AMPK α 1 and AMPK α 2 catalytic subunits, as well as AMPK Thr¹⁷² and ACC Ser⁷⁹ phosphorylation approx. 2–3-fold. We also show that AMPK activation and phosphorylation occur with only 15 min of exercise, but are not maintained with longer periods of exercise or in the post-exercise period.

Interestingly, in the skeletal muscle of these same animals, AMPK α 2 activity and AMPK Thr¹⁷² phosphorylation are increased with 60 min of exercise (H.-J. Koh and L. J. Goodyear, unpublished work). Thus it appears that a single bout of exercise induces a transient activation of AMPK in adipocytes, whereas effects in skeletal muscle are more prolonged. AMPK in adipocytes may be turned on rapidly and transiently because AMPK may function to regulate one or more aspects of the lipolytic machinery in priming fashion. Thus, if AMPK primes adipocytes for lipolysis, i.e. phosphorylates HSL and promotes its translocation into lipid droplets [9], sustained activation of AMPK may not be needed when exercise continues for periods longer than 30 min.

Adipocytes are important organs in which to store excess energy as triacylglycerols and provide energy to skeletal muscles in the form of unbound ('free') fatty acids during conditions

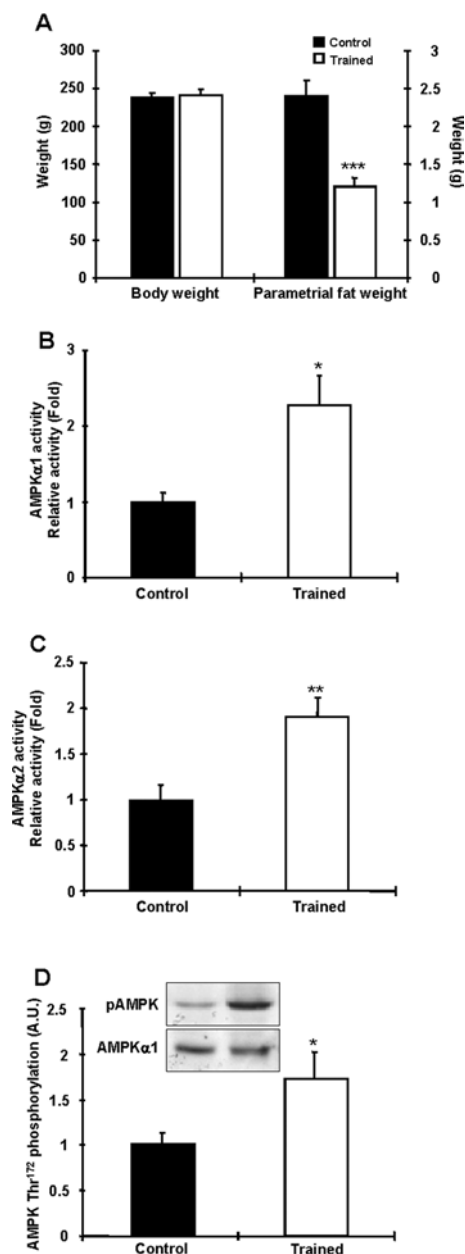


Figure 7 Chronic exercise increases AMPK α 1 and AMPK α 2 activities in rat adipocytes

(A) Exercise training for 6 weeks efficiently reduced fat mass without altering body mass. (B and C) AMPK α 1 (B) and AMPK α 2 (C) activities in epididymal fat from rats trained for 6 weeks and allowed to rest for 36 h before study. Both AMPK α 1 and AMPK α 2 activities were increased in trained rats. (D) AMPK Thr¹⁷² phosphorylation was increased in trained rats. Results are means \pm S.E.M.; $n = 6$ per group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared with the control. A.U., arbitrary units.

of increased energy demand such as exercise [37]. The role of AMPK in adipocytes is poorly understood, most notably whether AMPK acts as a pro- or anti-lipolytic signal. Previous reports suggested that the role of AMPK in adipocytes is to function as an anti-lipolytic signal [6,38,39]. In the present study, we found that modulation of AMPK activity by AICAR or Compound C did not affect lipolysis as well as HSL Ser⁵⁶³ phosphorylation (results not shown and Figure 6B). However, inhibition of AMPK by Compound C partially blunted adrenaline-induced lipolysis and HSL phosphorylation (Figures 6B and 6C), suggesting that

AMPK is necessary for adrenaline-induced lipolysis. The reasons for the differences in the role of AMPK activation on lipolysis in adipocytes between previous studies and the present study are not clear. However, two studies have recently reached the same conclusion for the function of AMPK as a pro-lipolytic signal [8,9]. Furthermore, the effects of AMPK on HSL activity in a tissue-specific manner have been investigated [8]. HSL activity was reduced in L6 myotubes expressing constitutively active AMPK, while AMPK activation did not prevent HSL activity or glycerol release in 3T3-L1 adipocytes.

Exercise training results in enhanced sensitivity to hormones that control adipocyte lipolysis [40,41]. If AMPK is an important mediator of lipolysis with exercise, then one would predict that AMPK activity would be increased with chronic exercise training. In fact, we found a 2-fold increase in the activity of both AMPK isoforms, as well as a significant increase in AMPK Thr¹⁷² phosphorylation in rats trained for 6 weeks. Interestingly, the increase in activity was not accompanied by an increase in the expression of the AMPK catalytic subunits. In addition, training did not result in significant changes in the protein content of LKB1 and CaMKK, known upstream kinases for AMPK ([42–47], and H.-J. Koh and L. J. Goodyear, unpublished work). Determination of the underlying mechanism is currently ongoing in our laboratory. Thus adipocytes adapt to changes in training status by increasing AMPK activity, a factor that may play a role in the effects of training to increase lipolysis. The effect of training to increase adipocyte AMPK activity is different from that which occurs in skeletal muscle, where previous work has shown no change in AMPK activity in response to endurance exercise training [48,49]. Similarly to published reports, AMPK activity in multiple skeletal muscles were not increased in the animals used in the present study (H.-J. Koh and L. J. Goodyear, unpublished work). This demonstrates that adipocytes and skeletal muscle adapt differently to training, and point to differing functions of AMPK in these tissues.

In summary, we show that acute exercise and adrenaline activate AMPK activity in adipocytes. Inhibition of the β -adrenergic receptor abolished the effects of exercise and adrenaline on AMPK activity in adipocytes. We also demonstrate that adrenaline and a single bout of exercise increase the AMP/ATP ratio in adipocytes. Activation of AMPK by adrenaline contributes to maximally activate lipolysis and phosphorylate ACC. All these data suggest that adrenaline is a major molecule for acute exercise-induced AMPK activation, and probably contributes to the benefits of exercise to reduce adiposity. Fully elucidating the molecular signals that mediate the effects of exercise to activate lipolysis has important medical relevance and could provide clues in the development of anti-obesity agents.

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