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Coffee polyphenol caffeic acid but not chlorogenic acid increases 5′AMP-activated protein kinase and insulin-independent glucose transport in rat skeletal muscle☆,☆☆

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Abstract

Chlorogenic acid is an ester of caffeic and quinic acids, and is one of the most widely consumed polyphenols because it is abundant in foods, especially coffee. We explored whether chlorogenic acid and its metabolite, caffeic acid, act directly on skeletal muscle to stimulate 5′-adenosine monophosphate-activated protein kinase (AMPK). Incubation of rat epitrochlearis muscles with Krebs buffer containing caffeic acid (≥0.1 mM, ≥30 min) but not chlorogenic acid increased the phosphorylation of AMPKα Thr^{172}, an essential step for kinase activation, and acetyl CoA carboxylase Ser^{79}, a downstream target of AMPK, in a dose- and time-dependent manner. Analysis of isoform-specific AMPK activity revealed that AMPKα2 activity increased significantly, whereas AMPKα1 activity did not change. This enzyme activation was associated with a reduction in phosphocreatine content and an increased rate of 3-*O*-methyl-D-glucose transport activity in the absence of insulin. These results suggest that caffeic acid but not chlorogenic acid acutely stimulates skeletal muscle AMPK activity and insulin-independent glucose transport with a reduction of the intracellular energy status.

Keywords: Acetyl CoA carboxylase; Akt; Energy deprivation; Phosphocreatine; Diabetes

1. Introduction

Skeletal muscle plays an important role in glucose metabolism and homeostasis in humans. Skeletal muscle relies on glucose for much of its energy requirements, and as such, it is the major site of glucose uptake in the body — about 75% of plasma glucose is cleared by the skeletal muscles [1]. In subjects with type 2 diabetes mellitus (T2DM), insulin-stimulated glucose uptake is reduced by about 50% [1]. This study supports the notion that the primary defect in insulin action in patients with T2DM resides in the skeletal muscle. Like insulin stimulation, exercise acutely increases the rate of glucose transport into contracting skeletal muscle by the translocation of glucose transporter 4 (GLUT4) to the plasma membrane and transverse tubules (reviewed in Refs. [2,3]). This phenomenon is considered to be responsible for the acute hypoglycemic effect of exercise, with glucose in the blood being taken up by contracting skeletal muscles. Indeed, exercise-stimulated GLUT4 translocation is not impaired in insulin-resistant conditions such as T2DM and obesity [4]. Thus, the insulin-independent mechanisms of exercise have been widely used to decrease blood glucose in patients with T2DM.

It has been demonstrated that exercise and insulin use distinct signaling pathways in skeletal muscle, and 5′-adenosine monophosphate-activated protein kinase (AMPK) has been identified to be involved in the mechanisms leading to exercise-stimulated glucose transport (reviewed in Ref. [5]). AMPK is a heterotrimeric kinase, consisting of a catalytic α-subunit and two regulatory subunits, β and γ. Two distinct α-isoforms (α1 and α2) exist in skeletal muscle [6], and both isoforms can be activated in response to muscle contraction, which stimulates glucose transport in the absence of insulin [7,8]. AMPK is a member of a metabolite-sensing protein kinase family and acts as an energy-sensing and signaling molecule in muscle cells by monitoring cellular energy levels, such as the AMP–adenosine triphosphate (ATP) ratio. AMPK in skeletal muscle is also implicated in a variety of antidiabetic properties of exercise, including GLUT4 expression [9,10], glycogen regulation [11,12], fatty acid oxidation [13,14], activation of peroxisome proliferator-activated receptor γ coactivator 1α and mitochondrial biogenesis [15], activation of SIRT1 [16] and enhanced insulin sensitivity [10,17]. In addition, skeletal muscle AMPK partially mediates glucose and lipid homeostasis by adipokines, including leptin and adiponectin, and the hypoglycemic effect of metformin (reviewed in Ref. [18]). Thus, through these effects in skeletal muscle, AMPK serves as a metabolic activator that reduces risk for T2DM.

Coffee is one of the most commonly consumed beverages in the world. Many epidemiological studies have indicated that long-term

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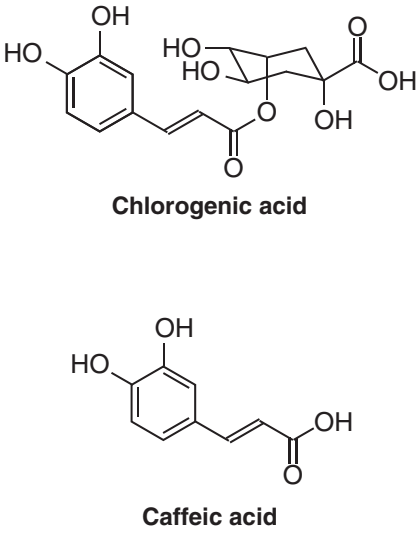

Fig. 1. Structures of chlorogenic acid and caffeic acid.

coffee consumption is associated with a reduced risk of developing T2DM [19,20]. Our recent studies [21,22] reported that caffeine, the major constituent of coffee, acutely increases AMPKα Thr^{172} phosphorylation, an essential step for kinase activation [23] and insulin-independent glucose transport in rat skeletal muscles. Although our results suggest that caffeine is an active compound responsible for the antidiabetic effect of coffee, coffee is a complex mixture of hundreds of chemicals that occur naturally or are formed during the roasting process. In fact, a reduced risk of developing T2DM has been associated with the consumption of decaffeinated coffee [24]. Thus, it is reasonable to speculate that coffee components other than caffeine have beneficial effects on glucose homeostasis in humans.

Phenolic compounds of plant origin, particularly chlorogenic acid (5-*O*-caffeoylquinic acid) and caffeic acid (3,4-dihydroxycinnamic acid) (Fig. 1), have been investigated for their antihyperglycemic properties [25–29]. Coffee is a major dietary source of chlorogenic acid, which reduces blood glucose concentrations in animal models [25–27]. Chlorogenic acid is an ester of caffeic and quinic acids, and caffeic acid has also been reported to decrease blood glucose in animals [28,29].

We have hypothesized that, like caffeine stimulation [21,22], chlorogenic acid and its major metabolite caffeic acid have antidiabetic properties by acting directly on skeletal muscle to stimulate AMPK. To test this hypothesis, we evaluated the effects of chlorogenic acid and caffeic acid on AMPKα Thr^{172} phosphorylation and α1- and α2-isoform-specific AMPK activities in isolated rat skeletal muscles incubated *in vitro*. We found that caffeic acid but not chlorogenic acid increased AMPK phosphorylation and its activity in a dose- and time-dependent manner with a corresponding decrease in muscle energy status and increase in glucose transport activity.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 100 to 120 g were obtained from Shimizu Breeding Laboratories (Kyoto, Japan). Animals were housed in an animal room maintained at 22°C–24°C with a 12:12-h light–dark cycle and fed a standard laboratory diet (Certified Diet MF; Oriental Koubo, Tokyo, Japan) and water *ad libitum*. Rats were fasted overnight before the experiments and were randomly assigned to the experimental groups.

All protocols for animal use and euthanasia followed the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (Physiological Society of Japan) in accordance with international guidelines, and were reviewed and approved by the Kyoto University Graduate School of Human and Environmental Studies and Kyoto University Radioisotope Research Center.

2.2. Muscle incubation

Muscles were treated as we described previously [7,30]. Rats were killed by cervical dislocation without anesthesia, and the epitrochlearis muscles were rapidly and gently removed. Both ends of each muscle were tied with sutures (silk 3-0; Nitcho Kogyo, Tokyo, Japan), and the muscles were mounted on an incubation apparatus with a tension set to 0.5 g. The buffers were continuously gassed with 95% O_2–5% CO_2 and maintained at 37°C. Muscles were preincubated in 7 ml of Krebs-Ringer bicarbonate buffer (KRB) (117 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 24.6 mM $NaHCO_3$) containing 2 mM pyruvate (KRBP) for 40 min.

For the time- and dose-dependent effects, muscles were then randomly assigned to incubation in 7 ml of fresh buffer in the presence of 1 mM chlorogenic acid or 1 mM caffeic acid for up to 60 min, or in 7 ml of fresh buffer in the absence or presence of 0.01 to 1 mM chlorogenic acid or caffeic acid for 30 min, respectively. Immediately after incubation, muscles were either used for the measurement of glucose transport or immediately frozen in liquid nitrogen and subsequently subjected to Western blot analysis. Some frozen muscles were also assayed for ATP and phosphocreatine (PCr) and isoform-specific AMPK activity.

2.3. Western blot analysis

Sample preparation and Western blot analysis for detection of phosphorylated AMPKα, total AMPKα and phosphorylated acetyl CoA carboxylase (ACC), total ACC, phosphorylated Akt and total Akt were performed as described previously [30,31]. Muscles were homogenized in ice-cold lysis buffer (1:40 wt/vol) containing 20 mM Tris-HCl (pH 7.4), 1% Triton X, 50 mM NaCl, 250 mM sucrose, 50 mM NaF, 5 mM sodium pyrophosphate, 2 mM dithiothreitol, 4 mg/L leupeptin, 50 mg/L trypsin inhibitor, 0.1 mM benzamidine and 0.5 mM phenylmethylsulfonyl fluoride (buffer A) and centrifuged at 16,000*g* for 40 min at 4°C. Denatured lysates (10 μg of protein) were separated on either 10% polyacrylamide gel for AMPK and Akt or 7.5% gel for ACC. Proteins were then transferred to polyvinylidene difluoride membranes (PolyScreen; PerkinElmer, Wellesley, MA, USA) at 100 V for 1 h. Membranes were blocked for 1 h at room temperature in TBS-T (TBS with 0.1% Tween 20) containing 5% nonfat dry milk and were then incubated overnight at 4°C with appropriate antibody [phosphospecific AMPKα Thr^{172} (#2531; Cell Signaling Technology, Beverly, MA, USA) diluted 1:1000, AMPKα (#2532; Cell Signaling Technology) diluted 1:1000, phosphospecific ACC Ser^{79} (#07-303; Upstate Biotechnology, Lake Placid, NY, USA) diluted 1:1000, ACC (#3662; Cell Signaling Technology) diluted 1:1000, phosphospecific Akt Ser^{473} (#9271; Cell Signaling Technology) diluted 1:1000 and Akt (#9272; Cell Signaling Technology) diluted 1:1000]. The membranes were then washed, reacted with anti-rabbit IgG coupled to peroxidase and developed with enhanced chemiluminescence reagents according to the manufacturer's instructions (GE Healthcare, Buckinghamshire, UK). The protein signals were detected with ImageCapture G3 (Liponics, Tokyo, Japan) and quantified using ImageJ [32]. The mean intensity of control samples in each membrane was used as a reference for controlling gel-to-gel variation. Equal protein loading and transfer were confirmed by Coomassie brilliant blue staining of the membranes.

2.4. Isoform-specific AMPK activity assay

The AMPK activity assay was performed as described previously [30,33]. Muscles were homogenized as described in Western Blot Analysis, and resultant supernatants (100 μg of protein) were immunoprecipitated with isoform-specific antibodies directed against the α1 or α2 catalytic subunits of AMPK [30] and protein A-Sepharose beads (GE Healthcare). Immunoprecipitates were washed twice both in buffer A and in wash buffer (240 mM HEPES and 480 mM NaCl). Kinase reactions were performed in 40 mM HEPES (pH 7.0), 0.1 mM SAMS peptide [7,30], 0.2 mM AMP, 80 mM NaCl, 0.8 mM dithiothreitol, 5 mM $MgCl_2$ and 0.2 mM ATP (2 μCi of [γ-^{32}P] ATP/sample) (PerkinElmer, Wellesley, MA, USA) in a final volume of 40 μl for 20 min at 30°C. At the end of the reaction, a 15-μl aliquot was removed and spotted onto Whatman P81 paper (Whatman International, Maidstone, UK). The papers were washed six times in 1% phosphoric acid and once in acetone. ^{32}P incorporation was quantitated with a scintillation counter, and kinase activity was expressed as fold increases relative to the basal samples.

2.5. ATP and PCr assay

Frozen muscles were homogenized in 0.2 M $HClO_4$ (3:25 w/v) in an ethanol–dry ice bath (−20°C to −30°C) and centrifuged at 16,000*g* for 2 min at −9°C. The supernatant of the homogenate was neutralized with a solution of 2 M KOH, 0.4 M KCl and 0.4 M imidazole, centrifuged at 16,000*g* for 2 min at −9°C and then subjected to enzymatic analysis [34]. ATP and PCr content was expressed as nanomoles per milligram wet weight of muscle.

2.6. 3-O-methyl-D-glucose (3MG) transport

3MG transport assay was performed as described previously [7,30]. Muscles were transferred to 2 ml of KRB containing 1 mM [^{3}H]3-MG (1.5 μCi/ml) (American Radiolabeled Chemicals, St. Louis, MO, USA) and 7 mM D-[1-^{14}C]mannitol (0.3 μCi/ml) (American Radiolabeled Chemicals) at 30°C and further incubated for 10 min. The muscles were then blotted onto filter paper, trimmed, frozen in liquid nitrogen and stored at −80°C. Frozen muscles were weighed and processed by incubating them in 300 μl of 1 M NaOH at 80°C for 10 min. Digestates were neutralized with 300 μl of 1 M HCl, and particulates were precipitated by centrifugation at 20,000*g* for 2 min. Radioactivity in aliquots of the digested protein was determined by liquid scintillation counting for dual labels, and the extracellular and intracellular spaces were calculated [35].

2.7. Statistical analysis

Results are presented as means±S.E. Means were compared by one-way analysis of variance followed by post hoc comparison with Tukey's and Dunnett's test as appropriate. Two means were compared with Student's *t* test. Differences between groups were considered statistically significant at $P<.05$.

3. Results

3.1. Caffeic acid but not chlorogenic acid increases the phosphorylation of muscle AMPKα Thr^{172} and ACC Ser^{79} in a dose- and time-dependent manner

In the AMPKα1 and α2 catalytic subunits, the primary site responsible for AMPK activation is the Thr^{172} residue [23]. To determine whether caffeic acid stimulation activates AMPK, we measured the degree of phosphorylation of AMPKα Thr^{172} by Western blot analysis using a phosphospecific antibody in muscle homogenates that had been stimulated with chlorogenic (0, 0.01, 0.1 or 1 mM) and caffeic (0, 0.01, 0.1 or 1 mM) acid at various concentrations for 30 min and with 1 mM concentrations for various times (0, 5, 15, 30 or 60 min). The dose–response study showed that significant phosphorylation of AMPKα Thr^{172} occurred at concentrations of 0.1 mM or higher of caffeic acid (Fig. 2A). Phosphorylation of ACC Ser^{79} displayed similar patterns to AMPK phosphorylation (Fig. 2B). ACC is a downstream target of AMPK in skeletal muscle, and phosphorylation of the Ser^{79} site of ACC reflects the total AMPK activity [36–38]. The time-course study revealed that phosphorylation of AMPKα Thr^{172} and ACC Ser^{79} increased rapidly after exposure to 1 mM caffeic acid within 30 and 15 min, respectively (Fig. 3A–B). Chlorogenic acid stimulation did not increase phosphorylation of AMPK Thr^{172} and ACC Ser^{79} (Figs. 2C–D and3C–D). The total AMPK and ACC content did not change throughout the study.

3.2. Caffeic acid activates AMPKα2 but not AMPKα1 in skeletal muscle

To identify which catalytic subunit was activated by caffeic acid, isoform-specific AMPK activity was determined in anti-α1 and anti-α2 immunoprecipitates from rat epitrochlearis muscles after treatment with caffeic acid (1 mM, 30 min). Caffeic acid clearly increased AMPKα2 activity, whereas it did not affect the activation of AMPKα1 (Fig. 4).

3.3. Caffeic acid acutely increases insulin-independent glucose transport activity in skeletal muscle

We investigated whether the activation of AMPK in skeletal muscle by caffeic acid affected insulin-independent glucose transport

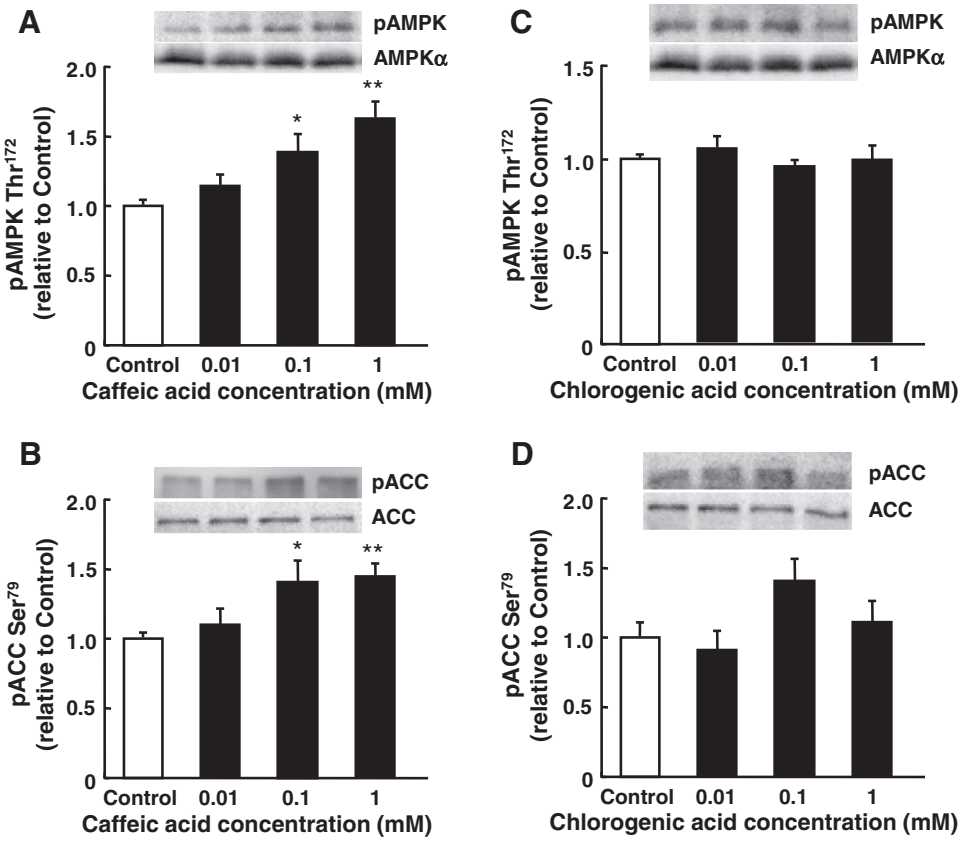

Fig. 2. Caffeic acid but not chlorogenic acid stimulation increases phosphorylation of AMPKα Thr^{172} and ACC Ser^{79} in a dose-dependent manner in rat skeletal muscles. Isolated epitrochlearis muscles were incubated with caffeic acid for 30 min at indicated concentrations. Muscle lysates were then analyzed for phosphorylation of AMPKα Thr^{172} (pAMPK; A and C) and ACC Ser^{79} (pACC; B and D) by Western blot analysis. Fold increases are expressed relative to the level of muscles in the control group. Representative immunoblots are shown. Values are mean±S.E.; $n=4$–10 per group. *$P<.05$; **$P<.01$ vs. control.

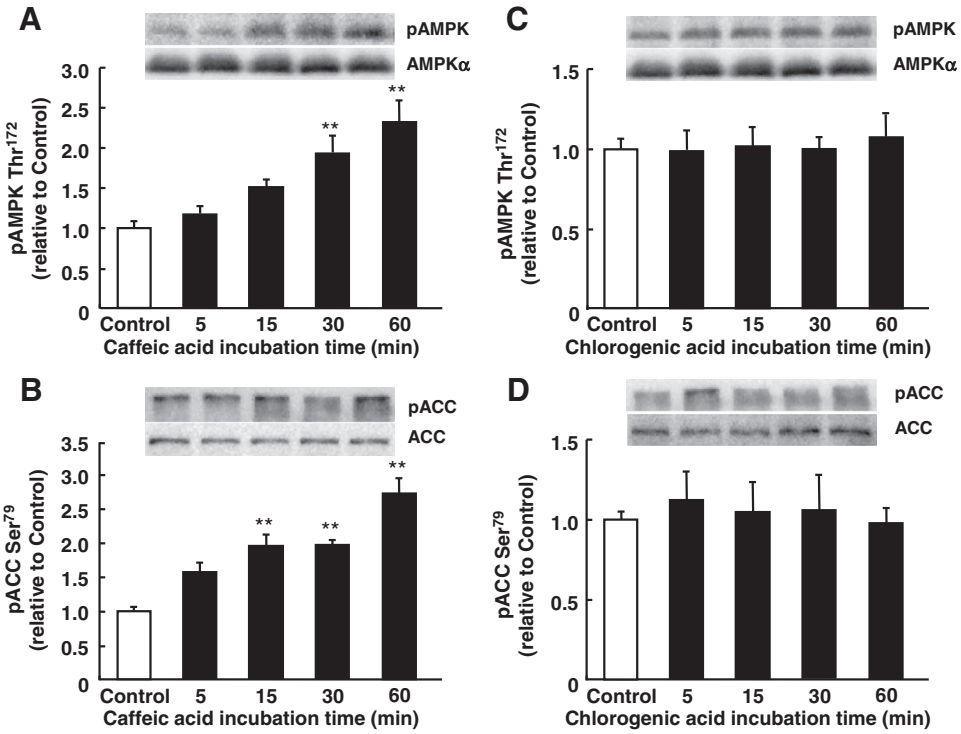

Fig. 3. Caffeic acid but not chlorogenic acid stimulation increases phosphorylation of AMPKα Thr^{172} and ACC Ser^{79} in a time-dependent manner in rat skeletal muscles. Isolated epitrochlearis muscles were incubated with 1 mM caffeic acid for indicated times. Muscle lysates were then analyzed for phosphorylation of AMPKα Thr^{172} (pAMPK; A and C) and ACC Ser^{79} (pACC; B and D) by Western blot analysis. Fold increases are expressed relative to the level of muscles in the control group. Representative immunoblots are shown. Values are mean±S.E.; n=4–12 per group. *P<.05; **P<.01 vs. control.

activity. Incubation with 1 mM caffeic acid for 30 min increased the rate of 3MG transport 1.3-fold above basal levels (Fig. 5).

3.4. Caffeic acid decreases energy status in skeletal muscle

AMPK is activated in response to energy-depleting stresses such as muscle contraction, hypoxia and inhibition of oxidative phosphorylation by pharmacological agents (such as 2,4-dinitrophenol), all of which are characterized by a decrease in PCr level [33]. To clarify whether caffeic acid increased AMPK activity by energy deprivation, we measured the muscle content of ATP and PCr. ATP content after stimulation with caffeic acid (1 mM, 30 min) did not change; however, PCr content was significantly lower than that of the basal samples in epitrochlearis muscles (Fig. 6A–B).

3.5. Chlorogenic acid and caffeic acid do not change phosphorylation of Akt Ser^{473} in skeletal muscle

We next investigated the effect of chlorogenic acid and caffeic acid on Ser^{473} phosphorylation of Akt, a key molecule involved in mediating the metabolic effects of insulin signaling in skeletal muscle. We found that chlorogenic acid (1 mM, 30 min) and caffeic

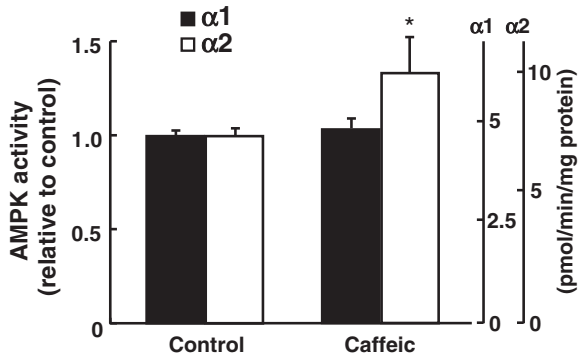

Fig. 4. Caffeic acid stimulation activates AMPKα2 but not AMPKα1 activity in rat skeletal muscles. Isolated epitrochlearis muscles were incubated with 1 mM caffeic acid for 30 min. Isoform-specific AMPK activity was determined in anti-AMPKα1 and -AMPKα2 immunoprecipitates. Fold increases are expressed relative to the activity of muscles in the control group. Values are mean±S.E.; n=4–5 per group. *P<.05 vs. control.

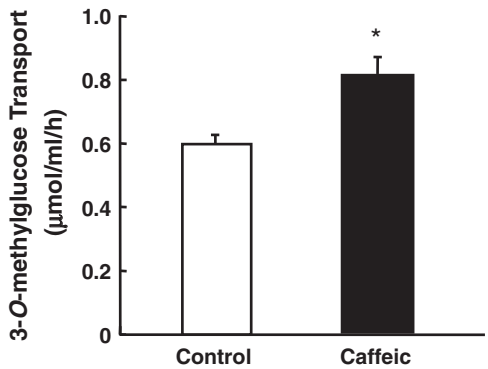

Fig. 5. Caffeic acid stimulation increases 3MG transport in rat skeletal muscles. Isolated epitrochlearis muscles were incubated with 1 mM caffeic acid for 30 min, and then 3MG transport activity was determined. Values are mean±S.E.; n=7–8 per group. *P<.05 vs. control.

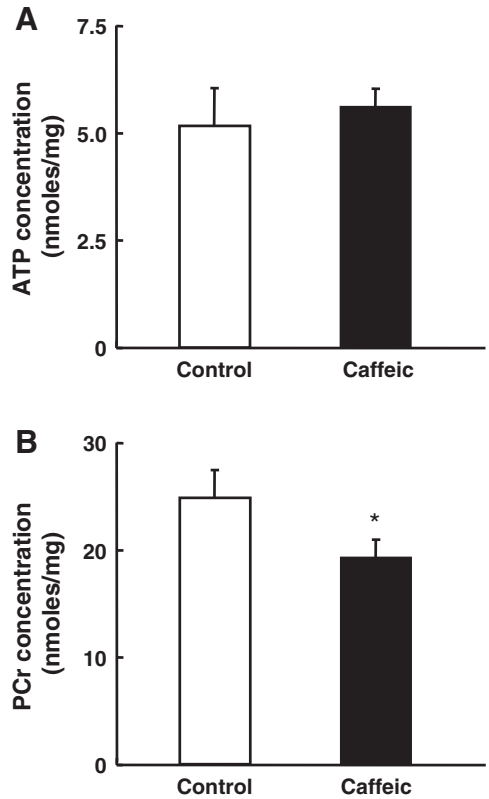

Fig. 6. Caffeic acid stimulation decreases PCr content but not ATP content in rat skeletal muscles. Isolated epitrochlearis muscles were incubated with 1 mM caffeic acid for 30 min, and then ATP (A) and PCr (B) content was determined. Values are mean±S.E.; n=8 per group. *P<.05 vs. control.

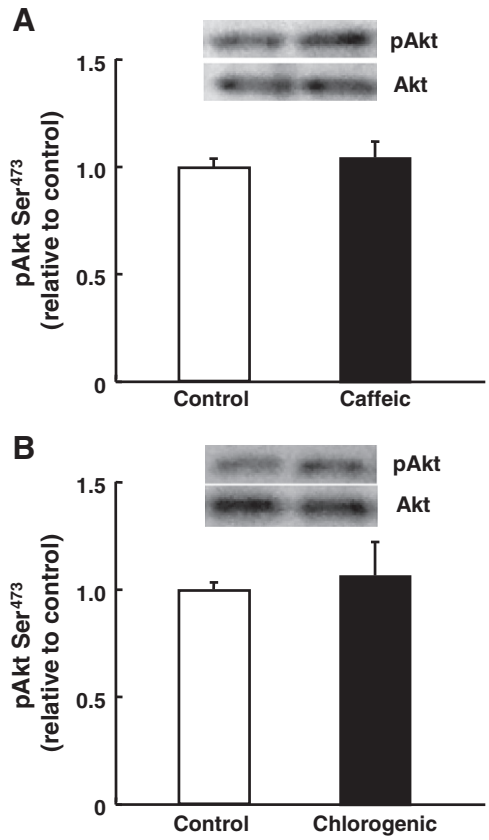

Fig. 7. Caffeic acid and chlorogenic acid stimulation does not change phosphorylation of Akt Ser^{473} in rat skeletal muscles. Isolated epitrochlearis muscles were incubated with 1 mM caffeic acid and 1 mM chlorogenic acid for 30 min. Muscle lysates were then analyzed for phosphorylation of Akt Ser^{473} (pAkt; A and B) by Western blot analysis. Fold increases are expressed relative to the level of muscles in the control group. Representative immunoblots are shown. Values are mean±S.E.; n=4–9 per group.

acid (1 mM, 30 min) had no effect on the Ser^{473} phosphorylation of Akt (Fig. 7).

4. Discussion

Our data provide new findings relating to the metabolic effect of caffeic acid in skeletal muscle. Caffeic acid acutely increased AMPKα Thr^{172} phosphorylation and AMPKα2 activity while reducing the energy status of skeletal muscle. Caffeic-acid-induced activation of AMPK was accompanied by increased glucose transport in the absence of insulin and increased phosphorylation of ACC, suggesting that caffeic acid increases AMPK activity *in vivo*.

Caffeic acid has antidiabetic properties in animal models of T2DM [28,39] and in insulin-deficient animal models [28,29,40]. These studies represent possible mechanisms for the antidiabetic effects of caffeic acid, such as attenuation of glucose output from the liver, protection of β-cell dysfunction, anti-inflammatory action in cardiac muscle or antioxidant action; however, little is known about the precise mechanism responsible for the metabolically beneficial effects of caffeic acid in skeletal muscle. A study by Cheng et al. [29] has shown that activation of the α1-adrenoceptor causes caffeic-acid-induced glucose transport using cultured myotubes, suggesting that AMPK is involved in the stimulatory effect on glucose uptake in skeletal muscle because stimulation of the α1-adrenoceptor in muscle leads to AMPK activation [41]. Our results propose a metabolically important mechanism via which caffeic acid acts directly on skeletal muscle and acutely stimulates AMPK and glucose transport in the absence of insulin.

We reported previously that caffeine treatment at 1 mM for 15 min activated AMPKα1 but not AMPKα2, and that caffeine treatment at 3 mM for 15 min stimulated AMPKα1 and AMPKα2 in incubated rat epitrochlearis muscle [22]. The activation of AMPKα1 by 1 mM caffeine occurred in the absence of an apparent reduction in PCr content, and activation of both isoforms by 3 mM caffeine was associated with a significant decrease in PCr content [22]. These results suggest that caffeine stimulates AMPKα1 in the absence of energy deprivation, and AMPKα1 and AMPKα2 in the presence of energy deprivation. By contrast, the present study showed that caffeic acid activated AMPKα2 predominantly in association with a reduction in PCr content but did not affect AMPKα1. Thus, the isoform specificity of caffeic acid in AMPK activation seems to be distinct from that of caffeine, although both compounds robustly stimulate skeletal muscle AMPK.

The finding that caffeic acid increases AMPKα Thr^{172} phosphorylation provides evidence that caffeic acid induces covalent modification via upstream kinases. The LKB1 complex is constitutively active and is not activated directly by AMP, although the binding of AMP to AMPK facilitates the phosphorylation of AMPK by the LKB1 complex [42]. Thus, LKB1 is believed to be a crucial AMPK kinase in response to energy deprivation in skeletal muscle. In the present study, AMPK activation was accompanied by a decrease in PCr content, a critical indicator of muscle energy status. PCr is produced when the ATP level is high. When ATP is depleted, phosphate is transferred from PCr to adenosine diphosphate (ADP) to replenish ATP. A decrease in PCr concentration results in an increase in free ADP concentration and shifts the equilibrium of the adenylate kinase reaction to accumulate AMP. Therefore, LKB1 may be involved in the caffeic-acid-induced

AMPKα Thr^{172} phosphorylation. An earlier study indicated that PCr allosterically inhibits AMPK activity [43], but recent evidence has shown that PCr does not affect the activation of recombinant and chromatographically purified AMPK by LKB1 [44], suggesting that PCr is not a direct inhibitor of LKB1 or AMPK.

In addition to caffeic acid, many researchers have shown that chlorogenic acid exerts potential antihyperglycemic properties [25–27]. It is notable that chlorogenic acid enhances glucose transport accompanied by an increase in GLUT4 gene expression via an insulin-independent pathway in L6 myotubes [45], indicating that chlorogenic acid regulates these effects through AMPK activation. In the present study, however, chlorogenic acid stimulation did not activate AMPK in skeletal muscle. In accordance with our results, a recent study by Murase et al. [46] reported that chlorogenic acid did not stimulate AMPK and ACC phosphorylation in Hepa 1-6 cells. Chlorogenic acid is hydrolyzed by intestinal microflora into various aromatic acid metabolites including caffeic and quinic acids [47], raising the possibility that the health-promoting effects of chlorogenic acid previously reported *in vivo* might depend on the effects of caffeic acid derived from chlorogenic acid. In this context, further studies are needed to clarify whether oral administration of chlorogenic acid and/or caffeic acid at physiologic doses results in AMPK activation and induces AMPK-related metabolic events, including glucose transport, in skeletal muscle.

The famous polyphenolic compound, resveratrol, which is found in the skin of red grapes and is a constituent of red wine, has been shown to extend life span [48] and provide protection against several metabolic disorders including T2DM [48,49]. It has been proposed that these benefits of resveratrol are associated with increased glucose transport [50,51] and AMPK activity [50–52]. Furthermore, resveratrol can reduce ATP levels by inhibiting ATP synthase [53], suggesting that resveratrol activates AMPK with a reduction of energy status and increases insulin-independent glucose transport. Considering that caffeic acid has resveratrol- and exercise-like effects, such as acutely stimulating AMPK in skeletal muscle, it might be responsible for the preventive effect of coffee on the development of T2DM.

In summary, we have demonstrated for the first time that caffeic acid stimulates AMPK activity (predominantly AMPKα2) in isolated rat skeletal muscle and that this activation is accompanied by insulin-independent glucose transport and a reduction of muscle energy status. We propose that caffeic acid is an active compound responsible for the antidiabetic effects of coffee, at least in part, by stimulating skeletal muscle AMPK.

Acknowledgments

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