学位論文要約

論文題目　Metabolic effects of coffee components on rat skeletal muscle in the resting and contracting states —Evidence for 5'AMP-activated protein kinase activation, glucose metabolism enhancement, and ergogenic effect—
（コーヒー成分が安静時および収縮時のラット骨格筋に及ぼす代謝的効果 —AMPキナーゼ活性化、糖代謝促進および運動機能増進作用の検証—）

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Habitual physical exercise has beneficial effects on glucose, lipid, and energy metabolism, and it reduces the risk of metabolic disorders such as type 2 diabetes mellitus (T2DM). Skeletal muscle is the principal site of whole-body glucose, lipid, and energy metabolism, and several adaptive responses to physical exercise (muscle-contracting stimuli) by skeletal muscle contribute to whole-body health-promoting effects. Much evidence suggests that the acute and/or repeated activation of skeletal muscle 5'AMP-activated protein kinase (AMPK) by physical exercise is involved with several metabolic adaptations, including enhanced insulin-independent glucose transport, insulin sensitivity, glucose transporter 4 expression, fatty acid oxidation via the inhibition of acetyl-CoA carboxylase (ACC), modulation of glycogen synthesis and mitochondrial biogenesis via peroxisome proliferator-activated receptor γ coactivator 1α and sirtuin 1, and fiber type shift towards the slower and more oxidative phenotype. These skeletal muscle adaptations might allow AMPK to serve as a metabolic stimulator, which may reduce the risk of metabolic disorders.

Coffee is one of the most commonly consumed beverages in the world. Many epidemiological studies have indicated that long-term coffee consumption is associated with a reduced risk of developing T2DM. Recent studies have shown that caffeine, the major constituent of coffee, acutely increases AMPKα Thr^{172} phosphorylation and insulin-independent glucose transport in rat skeletal muscle. However, some studies have shown that the consumption of decaffeinated coffee reduces the risk of developing T2DM. In fact, coffee is a major dietary source of chlorogenic acid and its major metabolite, caffeic acid.

First study

In this study, we evaluated whether chlorogenic acid and/or caffeic acid would act directly on skeletal muscle to stimulate AMPK activity, like caffeine.

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 ACC Ser^{79}, a downstream target of AMPK, in dose- and time-dependent manners. Analysis of isoform-specific AMPK activity revealed that AMPK complexes containing the α2 subunit (AMPKα2) activity increased significantly, whereas AMPK complexes containing the α1

subunit (AMPKα1) activity did not change. This enzyme activation was associated with a reduction in phosphocreatine (PCr) content and an increased rate of 3-*O*-methyl-D-glucose (3MG) 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.

AMPK acts as a signaling intermediary by monitoring cellular energy status, and has been identified as a key mediator of contraction-stimulated insulin-independent glucose transport in skeletal muscle. Previous studies showed that caffeine acutely stimulated AMPK and increased insulin-independent glucose transport in resting skeletal muscle. Caffeine-induced AMPK activation was also accompanied by decreased fuel status. Those results indicated that caffeine acts directly on skeletal muscle and has similar actions to those of contraction by acutely promoting AMPK activity with energy deprivation. Moreover, in this ***first study***, caffeic acid had effects like caffeine on skeletal muscle AMPK. However, it is unknown whether caffeine and caffeic acid affect AMPK and glucose transport in contracting skeletal muscle. Many researchers have reported that caffeine increases skeletal muscle force production during contraction by multiple mechanisms, and that it enhances exercise performance and delays fatigue in rodents and humans. These ergogenic actions of caffeine led us to hypothesize that caffeine might activate AMPK and glucose transport in skeletal muscle during contraction by causing profound changes in the cellular energy status.

Second study

Here we aimed to clarify whether caffeine would affect AMPK activity and glucose transport in skeletal muscle during contraction. We also explored the effects of caffeic acid and chlorogenic acid in contracting muscles.

Isolated rat epitrochlearis muscle was preincubated and then incubated in the absence or presence of 3 mM caffeine for 30 or 120 min. Electrical stimulation (ES) was used to evoke tetanic contractions during the last 10 min of incubation. The combination of maximally effective caffeine concentration plus maximally effective contraction had additive effects on AMPKα Thr^{172} phosphorylation, AMPKα1 and AMPKα2 activities, and 3MG transport. In contrast, caffeine inhibited basal and contraction-stimulated Akt Ser^{473} phosphorylation. The intracellular concentration of caffeine reached a maximum by 30 min and was maintained at this level at 120 min after the start of exposure. Contraction did not affect the intracellular caffeine concentration. Caffeine significantly delayed muscle fatigue during contraction, and the combination of caffeine and contraction additively decreased ATP and PCr contents. Consistent with these findings, caffeine significantly increased during the initial peak force phase (30-min caffeine treatment). Caffeine did not affect resting tension. Neither caffeic acid nor chlorogenic acid affected contraction-stimulated AMPKα Thr^{172} or Akt Ser^{473} phosphorylation levels in isolated skeletal muscle. Using *in vivo* experiments, rats were given an intraperitoneal injection of caffeine (60 mg/kg body weight) or saline, and the extensor digitorum longus muscle was dissected out 15 min later. ES of the sciatic nerve was performed to evoke tetanic contractions for 5 min before dissection. Similar to the findings from isolated muscles incubated *in vitro*, the combination of caffeine plus contraction *in vivo* had additive effects on AMPK Thr^{172}

phosphorylation, AMPKα1 and AMPKα2 activities, and 3MG transport. Caffeine also inhibited basal and contraction-stimulated Akt Ser^{473} phosphorylation *in vivo*. These findings suggest that caffeine and contraction synergistically stimulate AMPK activity and insulin-independent glucose transport, at least in part by decreasing muscle fatigue and thereby promoting energy consumption during contraction.

Exercise has beneficial effects on human health by stimulating the metabolic activation of skeletal muscle contraction. Furthermore, in our ***second study***, caffeine mitigated muscle fatigue and increased the initial peak force during contraction in rat skeletal muscle. These findings led us to hypothesize that caffeine might accelerate muscle contraction-induced metabolic activation, thereby contributing to exercise benefits for health promotion.

Third study

Here we aimed to characterize the metabolic signatures of contracting muscles with or without caffeine stimulation, using liquid chromatography-mass spectrometry and capillary electrophoresis coupled to mass spectrometry analyses.

Isolated rat epitrochlearis muscle was incubated in the presence or absence of 3 mM caffeine for 30 min. ES was used to induce tetanic contractions during the final 10 min of incubation. Principal component analysis and hierarchical clustering analysis detected 184 distinct metabolites across three experimental groups—basal, ES, and ES with caffeine (ES + C). Significance Analysis of Microarray identified a total of 50 metabolites with significant changes in expression, and 23 metabolites significantly changed between the ES and ES + C groups. Changes were observed in metabolite levels of various metabolic pathways, including the pentose phosphate, nucleotide synthesis, β-oxidation, tricarboxylic acid (TCA) cycle, and amino acid metabolism. In particular, D-ribose 5-phosphate, inosine monophosphate (IMP), O-acetylcarnitine, butyrylcarnitine, L-leucine, L-valine, and L-aspartate levels were higher in the ES + C group than in the ES group. These metabolic alterations induced by caffeine suggest that caffeine accelerates contraction-induced metabolic activation, thereby contributing to muscle endurance performance and exercise benefits for human health.

Through these three studies using rat skeletal muscle, we investigated the metabolic effects of coffee components on skeletal muscle in the resting and contracting states. In the ***first study***, we found that caffeic acid, like caffeine, acts directly on resting skeletal muscle and increased muscle AMPK activity accompanied by energy deprivation and enhanced glucose transport. In the ***second study***, the combination of the maximally effective caffeine concentration and maximally effective contraction increased AMPK phosphorylation and both AMPKα1 and AMPKα2 activities more than either of the stimuli alone. However, unlike caffeine, the combination of the maximally effective caffeic acid concentration and tetanic contraction was not additive for AMPK phosphorylation. In the ***third study***, we found that, other than glucose metabolism enhancement, caffeine induced various metabolic changes related to energy metabolism during contraction, including the promotion of the pentose phosphate pathway, increased IMP production, stimulation of β-oxidation of fatty acyl-CoA, activation of the TCA cycle, and increases in amino acid levels associated with energy production.

These results clearly indicate that: (1) both caffeine and caffeic acid have exercise-mimicking effects on skeletal muscle AMPK activation; (2) caffeine increases the maximal capacity of contraction-stimulated AMPK activation in skeletal muscle; (3) AMPK-activating agents do not necessarily have an additive effect on contraction-stimulated AMPK activity in skeletal muscle; and (4) metabolic alterations induced by caffeine contribute to its ergogenic actions.

Physical exercise is a powerful tool that promotes good human health and reduces the risk of T2DM. Skeletal muscle AMPK is activated by physical exercise, so AMPK is a potential therapeutic target for improving glucose metabolism. If skeletal muscle AMPK could be activated by alternative approaches other than physical exercise, including phytochemicals, these approaches might benefit people who are unable to engage in physical exercise because of severe musculoskeletal or cardiovascular conditions, or among bedridden elderly people, as well as "couch potatoes." Moreover, if phytochemicals could synergistically enhance exercise-induced skeletal muscle AMPK activity, people might enjoy the beneficial effects of physical exercise more efficiently.

Here we demonstrated that coffee components, especially caffeine, acutely enhanced skeletal muscle metabolism via activation of AMPK. Considering that long-term coffee consumption is associated with a reduced risk of developing T2DM, further research is obviously required to clarify whether coffee components have long-term effects on skeletal muscle metabolism both in resting and contracting conditions. We believe that, similar to exercise training, repeated AMPK activation by phytochemicals is a potentially useful tool for achieving profound metabolic benefits in skeletal muscle.