Oral Capsinoids supplementation may not enhance glycogen recovery against exercise-induced loss in exercised human skeletal muscle

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Abstract: We investigated whether post-exercise capsinoids (CSN) supplementation could enhance muscle glycogen resynthesis via GLUT4/Akt expressions in human skeletal muscle. Nine male college students (aged 21.4±0.2 years, BMI 21.9±1.3 kg/m2, VO2max 47.1±1.8 ml/kg/min) participated in this crossover designed study, and completed a 60-min cycling exercise at 70% VO2max. Immediately after exercise, participants consumed high-carbohydrate diet (2 g carb/kg bodyweight) with CSN (12 mg, single dosage) or placebo. Biopsied muscle samples (vastus lateralis) were obtained immediately (0h) and 3h after exercise. Blood and expired gas samples were collected before and after exercise. We found oral CSN supplementation immediately after exercise was unable to enhance glycogen resynthesis in exercised human skeletal muscle. Despite, CSN could alter the energy reliance on fat oxidation during post-exercise recovery, based on gaseous exchange measurement (NEFA and glycerol). We further identified no significant differences in postprandial glucose/insulin area under curve in both trials. Western blot data showed no significant response of p-Akt/Akt ratio with CSN during post-exercise recovery. Inconsistent with glycogen levels, muscle GLUT4 expression was significantly elevated at 3h in CSN trial. Our findings emphasize the necessity of further evidences to confirm the ergogenic properties of CSN in connection with glycogen recovery in exercised human skeletal muscle.

Keywords: ergogenic aid, muscle glycogen, substrate oxidation, GLUT4

1. Introduction

Capsinoids (CSN, Fig. 1) are the novel non-pungent capsaicin analogs, which include capsiate, dihydrocapsiate, and nordihydrocapsiate, extracted from non-pungent type of red pepper [1].
Research reports revealed that capsinoids can increase energy expenditure like capsaicin via activation of cation channel, transient receptor potential cation channel vanilloid subfamily 1 in rodents [2, 3]. Previous literature revealed that capsinoids administration could enhance, as well as suppress the body fat accumulation in rodents [4]. Similar physiological phenomena were also observed in human studies following capsinoids ingestion [5]. Moreover, capsinoids claimed to cause an acute increase of fat metabolism (energy expenditure) and fat oxidation during exercise, or somehow can cause long-term adaptations that promote fat metabolism [6]. However, the vital molecular mechanism remains under investigation, as CSN considering as an ergogenic aid to enhance fat oxidation and boost energy.

Figure 1. The Chemical structures of the Capsinoids

Glycogen concentration plays an important role in skeletal muscle metabolism and endurance performance. The exercise-induced muscle fatigue was brought reduction in the rate of glycogen breakdown occurring in exercising muscle fibers after prolonged intensity exercise. It has been described that overexpression of GLUT4 enhances the capacity of muscle glycogen replenishment in animal studies [7]. GLUT4 is upregulated by the phosphorylation and activation of Akt (serine/threonine specific protein kinase), which directs the GLUT4 protein to plasma, and thus promotes glucose transport [8, 9]. However, the ability of the constitutively active Akt to cause GLUT4 translocation to the plasma membrane of human skeletal muscle during post-exercise recovery need to be determined after capsinoids supplementation. Other studies reported that the capsinoids intake would be able to enhance the VO₂, and tendency for increase resting energy expenditure and fat oxidation [10, 11].

Despite CSN effects on fat oxidation, there is a dearth of reports to demonstrate whether CSN could enhance the muscle glycogen resynthesis in exercised human skeletal muscle. We hypothesized that oral post-exercise capsinoids administration may increase fat oxidation as well as enhance glycogen replenish in exercised human skeletal muscle. Therefore, we conducted this study to explore the evidence that post-exercise capsinoids supplementation could enhance glycogen content in human skeletal muscle. We measured respiratory exchange ratio (RER), glucose response, rate of muscle glycogen synthesis and protein expressions of Akt and GLUT4 following a 60-min cycle exercise with or without oral CSN supplementation in young trained individuals.

2. Materials and Methods

2.1 Participants

Nine male young college students (aged 21.4±0.2 years, height 171.8±1.8 cm, weight 64.9±4.3 kg, BMI 21.9±1.3 kg / m², VO2max 47.1±1.8 ml/kg/min) were recruited from the Department of Physical Education, National Taichung University of Education. As in part of the inclusion criteria, all
participants were non-smokers, non-drinkers and medically stable. Each participant was asked to maintain their regular lifestyle and normal diet during the experimental period. Before participant recruitment, the research protocol was reviewed and approved by the Ethics Committee and Institutional Review Board of Changhua Christian Hospital where this study was conducted. During the experimental period, the participants were asked to maintain their regular lifestyle and normal diet, and the consumption of alcohol, caffeine, tea and tobacco were completely prohibited.

2.2 Experimental procedure

Nine male participants participated in this crossover designed study were equally assigned into CSN (Ajinomoto, Tokyo, Japan) and placebo trials. VO2max was measured at least 1 week before the exercise trial. The same experimental procedures, including VO2 max test was repeated after 2-week washout period to determine the consistency of results. The participants reported to the laboratory at 8.00 hours after a 10-h overnight fast. Thereafter, all participants performed a 60-min cycling exercise at 70% VO2max after a 5-min warm-up exercise on an ergometer. Drinking water was provided ad libitum during and after the exercise. Immediately after a 60-min cycling exercise, subjects ingested a CSN or placebo capsule (12 mg) with a carbohydrate meal within 10 minutes (each meal contains 2 g carbohydrate per kilogram of body weight, 80 % carbohydrate, 8 % fat, 12 % protein; overall GI was 76-6). The carbohydrate meal consisted of Corn Flakes (Kellogg’s Ltd, Manchester, UK), skimmed milk, white bread, strawberry jam, a glucose water and water. Vastus lateralis muscle samples were collected immediately (0h) and 3h after exercise for determination of muscle glycogen, GLUT4, p-Akt and Akt protein contents. Blood and gaseous samples were collected before, immediately after and during 3h post-exercise recovery period for the measurements of circulating glucose, insulin, plasma NEFA and glycerol levels.

2.3 Capsinoids dosage and composition

Safety dosage is very important consideration whether CSN can be used as dietary supplement, irrespective of whether it is physiologically effective. Previous study used a high dose of CSN (>150 mg/d) and showed without any adverse effects on human studies [12]. Therefore, we choose the dose 12mg of CSN as an oral supplement, which is the similar dosage to Josse’s human study [13]. We further found no single adverse effects on all participants in this study. Total four capsules were ingested immediately after a single bout of exercise. The CSN capsules were purchased from the Ajinomoto Co Inc (Ajinomoto, Tokyo, Japan). According to manufacturer details each 3 mg of capsinoids contained capsiate, dihydrocapsiate, and nordihydrocapsiate in a 7:2:1 ratio respectively. Therefore, all participants were instructed to intake either four capsules of CSN (equally 12mg), or capsules of placebo contained rapeseed oil mixture with medium-chain triglycerides.

2.4 Muscle sampling procedure

Human muscle biopsy was performed in accordance with previous studies [14, 15]. Muscle biopsy was performed for all participants under local anaesthesia (2% lidocaine without adrenaline). An incision of 10 mm length and depth was made in the skin and muscle fascia at about 20 cm above the knee using an aseptic technique. V. lateralis biopsies (about 50 mg) from the right quadriceps femoris muscle was obtain using the percutaneous biopsy technique of Bergström [16]. Muscle samples were blotted dry and grossly dissected free of fat and connective tissue, then frozen in liquid nitrogen and stored at −80°C until analyses of muscle glycogen content, GLUT4, p-Akt and Akt protein expression performed.
2.5 Muscle glycogen assay

Approximately 25 mg of skeletal muscle from the deep portion of the V. lateralis was dissolved in 1 M-KOH at 75°C for 30 min. The dissolved homogenate was then neutralised using glacial acetic acid and incubated overnight in acetate buffer (0.3 M-sodium acetate, pH 4.8) containing amyloglucosidase (Boehringer Mannheim), and the reaction mixture was neutralized and the reaction stopped by adding 1M-NaOH after an overnight incubation. The glucosyl units were then measured using a spectrophotometric Trinder reaction (Sigma, St. Louis, MO, USA). The intraassay coefficient of variation calculated from the replicates was less than 8% for glycogen.

2.6 Western blot for GLUT4, p-Akt and Akt protein expressions

Muscle samples were weighed and homogenised in ice-cold homogenisation buffer (20mM-HEPES, 1mM-EDTA and 250mM-sucrose; pH 7.4) using a Polytron tissue homogeniser (Brinkmann Instruments). Protein contents in the homogenate were quantified by the Lowry protein assay, and an equal amount of protein was denatured and separated on 7.5% SDS-polyacrylamide gels and then transferred to polyvinylidene difluoride (PVDF) membranes (New Life Science Product, Inc.). Non-specific binding sites on the membranes were blocked with TRIS buffer (10mM-TRIS-HCl and 100mM-NaCl, pH 7.5) containing 5% non-fat dry milk at 4°C overnight. The blots were incubated sequentially with p-Akt, Akt (1:1000; Cell signaling, Danvers, MA, USA), GLUT4 (1:4000, Chemicon, CA, USA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primary antibodies (1:5000, Cell signaling, Danvers, MA, USA). Antigen-antibody complexes were visualised, detected and quantified with the ECL Western blot detection kit (Amersham Pharmacia Biotech, USA), Luminescent Image Analyser (Fujifilm, Japan) and Zero-Dscan densitometric (Scanalytics, Inc., FairFax, VA, USA), respectively. Total expression of muscle GLUT4 protein content was normalised by the expression of total GAPDH level from the same gel.

2.7 Blood analyses

A 20-G polyethylene catheter (Jelco, Tampa, FL, USA) was placed in an antecubital vein for blood sample collection, and the catheter was kept clean by flushing with a small amount of saline solution containing heparin following each sample collection. During recovery, blood samples were collected in heparin at every 30 min intervals. Plasma was obtained after centrifugation at 4°C for 10 min at 3000 rpm and was stored at −80°C before analysis. Blood glucose concentration was determined by an automated glucose analyser (YSI Life Sciences, Yellow Springs, OH, USA). Plasma concentrations of insulin, non-esterified fatty acids (NEFA) and glycerol, were measured with an automated analyzer (Hitachi 7020, Tokyo, Japan) using commercially available kits (Randox, Antrim, UK).

2.8 Expired gas analysis

Pulmonary respiratory exchange ratio (RER) values (RER = CO2 production/VO2) were calculated online via MetaMax3B indirect calorimetry (Cortex Biophysik, Nonnenstrasse, Leipzig, Germany). The gas samples were analysed at 60 min intervals during the post-exercise recovery period (60, 120 and 180 min after exercise). For each time-point measurement, the 10 min average stable values of VO2 and VCO2 were used to obtain the RER data (VCO2/VO2) and to calculate fuel (fat and carbohydrate) oxidation rates during postexercise recovery [17].
2.9 Statistical analysis

The data obtained in this study were analysed using SPSS software (IBM SPSS Statistics; IBM Corp.). Two-way ANOVA with repeated measures was used to compare the differences in other measured variables. Fisher’s post hoc test, which holds the value of a type I error to 0.05 for each test, was used to distinguish significant differences between pairs of conditions. Data were presented as the mean ± standard error. The α level was set at 0.05 (P<0.05) to indicate a significant difference for all comparisons. Significance levels quoted are two-sided for all comparisons.

3. Results

3.1 Capsinoids alter the energy reliance on fat oxidation during post-exercise recovery

We found RER values at all time points during post-exercise recovery (60, 120 and 180-min) were significantly lower in CSN trial compared with placebo trial. RER above 0.85 in placebo trial indicating energy reliance is primarily from carbohydrate or mix of fat and carbohydrate metabolism during post-exercise recovery. However, lower RER values in CSN trial revealed that fat being used as primary fuel source following exercise (Fig. 2). These findings suggest that capsinoids supplementation immediately after exercise could alter the energy reliance on fat oxidation during post-exercise recovery.

![Figure 2. Respiratory exchange ratio (RER) after a single bout of exercise in CSN (●) and placebo (○) trials. Values are expressed as mean ±SE, N = 9. *Significant difference against placebo (P < 0.05).](image)

3.2 Capsinoids maintained stable blood glucose and insulin levels during post-exercise recovery

Blood glucose and plasma insulin levels were determined before, immediately and every 30-min after exercise in both CSN and placebo trials. Glucose and insulin levels were peaked at 30-min following exercise and subsequently declined in both trials (Fig. 3A and 3B). Noteworthy, blood glucose concentrations in CSN trial during recovery were relatively lower than placebo, however, these values are not statistically significant. Moreover, no significant differences in postprandial glucose and insulin area under curve (GAUC and IAUC) were found after exercise with capsinoids supplementation (Fig 3C and 3D).
3.3 Caspinoids unalter NEFA and glycerol concentrations following exercise

The effect of CSN on circulating concentrations of NEFA and glycerol were measured before and after exercise. The elevated NEFA and glycerol levels immediately after exercise (0 h) were rapidly decreased to pre-exercise level at 30 min, and remained stable during 3h post-exercise recovery in both trials (Fig. 4A and 4B). CSN supplementation (12 mg) immediately after exercise was unable to alter the NEFA and glycerol concentrations during post-exercise recovery in young individuals.

3.4 Capsinoids upregulate muscle GLUT4 expression, but glycogen levels remain same
Changes in skeletal muscle GLUT4 protein with CSN supplementation was determined immediately (0h) and 3h after exercise. Western blot results showed that CSN significantly upregulated muscle GLUT4 protein expression at 3h following exercise, where the similar increase was not found in placebo trial (Fig. 5A). We further determined the glycogen levels in biopsed skeletal muscles. The results showed that the recovery rate of glycogen synthesis in vastus lateralis during 3h post-exercise recovery was not significantly greater with CSN supplementation (Fig. 5B). Noteworthy, the increased muscle GLUT4 protein in CSN trial is inconsistent with glycogen resynthesis in exercised skeletal muscle. Our findings unveiled that oral capsinoids intake immediately after exercise was unable to restore or enhance the skeletal muscle glycogen levels in young men.

![Figure 5. GLUT4 level (A) and Glycogen levels (B) in vastus lateralis of human skeletal muscle after a single bout of exercise in CSN and placebo trials.* Significant difference against placebo at the same time point (P < 0.05).](image)

3.5 Effect of capsinoids on p-Akt and Akt levels in biopsied skeletal muscle

Next we monitored the changes of p-Akt and Akt proteins in skeletal muscle immediately and 3h after exercise, and data presented as p-Akt/Akt ratio (Fig. 6). No significant response of p-Akt/Akt ratio was noticed following an acute bout of exercise and also during 3h recovery period with CSN supplementation.
4. Discussion

In this human study for the first time, we demonstrated the acute effect of capsinoids supplementation on fat oxidation and muscle glycogen resynthesis during post-exercise recovery. We found that capsinoids supplementation immediately following exercise could able to increase the fat oxidation, as we seen the lower level of RER during post-exercise recovery. Western blot data from biopsied muscles showed capsinoids intake failed to promote the p-Akt/Akt ratio, but elevated GLUT4 protein expression during 3h exercise recovery. However, the increased GLUT4 expression with CSN appears not to be contributed to enhance/restore the muscle glycogen content during post-exercise recovery. Increased fat oxidation in CSN trial implies that main energy expenditure of whole body is relied on fat oxidation during post-exercise recovery period.

Nutraceutical supplements have been shown to improve the endurance performance, probably due to the increased metabolic capacity and fat oxidation as a source of energy in skeletal muscle during exercise [18, 19]. Capsinoids increase the energy expenditure like capsaicin via activation channel vanilloid subfamily with increase in the sympathetic nervous system activation in rat and human models [2, 3, 20]. Furthermore, capsinoids are able to enhance the brown lipid metabolism and stimulate glycogen synthesis in humans [21]. Based on the previous evidences, we assume that capsinoids supplementation could increase fat oxidation and promote the glycogen resynthesis in exercised human skeletal muscle. Experimental evidence of respiratory exchange ratio from our study demonstrated that capsinoids are effective in increasing the fat oxidation during recovery following exercise, while muscle glycogen levels remain unchanged. It has been reported that capsinoids can increase the energy expenditure by activating the brown adipose tissue in human study [22]. Despite lack of evidence to support that capsinoids activated brown adipose tissue to enhance fat oxidation in this study, we found increased fat oxidation with acute oral capsinoids supplementation. These findings indicate that capsinoids may influence the brown adipose tissue in young adults, and thereby enhanced the fat oxidation during post-exercise recovery.

Glycogen storage is the most important fuel in muscle cell for contracting skeletal muscle during strenuous prolong exercise. Depletion of muscle glycogen levels have been implicated in muscle fatigue and poor performance [23]. The level of muscle glycogen synthesis substantially depends on nutritional manipulation, training status, fitness condition and glycogen depletion [24]. The
metabolic priority in exercised human skeletal muscle is to strengthen the glycogen resynthesis in turn to increased fat oxidation is associated with increased muscle glycogen synthesis [25]. However, our findings could not demonstrate that capsinoids increased the muscle glycogen resynthesis in parallel with higher response of fat oxidation during post-exercise recovery in young adults. Experimental evidence from our study showed that fat oxidation level is not only an individual factor that influencing the glycogen resynthesis in exercised human skeletal muscle. On the other hand, capsinoids and placebo trials showed no significant differences in muscle glycogen depletion immediately after exercise. These findings emphasized that nutrition manipulations are same in both trials, as we provided the same carbohydrate meal per kg body weight for all participants. We suggest that acute oral capsinoids supplementation might be effective in enhancing the fat metabolism than influencing of metabolic consequence, which in turn affected the glycogen resynthesis in exercised human skeletal muscle.

GLUT4 is a glucose transporter protein expressed in skeletal muscle, which translocate to plasma membrane upon insulin stimulation to facilitate glucose transportation across the plasma membrane [26]. We found elevated muscle GLUT4 protein expression in capsinoids trial at 3h post-exercise recovery period without concurrence of insulin and/or glucose response. Moreover, no significant difference in p-Akt/Akt ratio was found between the trials. These results implied that the elevated GLUT4 protein expression in capsinoids trial was independent of Akt pathway in the skeletal muscle of young adults. Previous study indicated that Akt pathway mediates the effect of insulin, AMPK or contraction on surface recruitment of glucose transport in myocyte [27]. However, our results are inconsistent with the findings of Lee and colleagues, which demonstrated that capsiate, a major capsaicinoids inhibited the activation of phospho-Akt in ultraviolet B induced inflammation [28]. In our study, skeletal muscle p-Akt/Akt ratio was not altered with capsinoids administration. Therefore, it may not be ruled out that acute capsinoids intake could possibly influence the other biomarkers rather than Akt protein that could up-regulate the muscle GLUT4 protein expression.

The total GLUT4 expression was remarkably up-regulated by capsinoids in vivo or in vitro in mice [29]. The response of GLUT4 after capsinoids supplement in our study is consisting with Hong and team study. However, up-regulated GLUT4 didn’t influence the muscle glycogen content during post-exercise recovery, which explain that capsinoids-induced increased GLUT4 may not directly contributed to improve the muscle glycogen resynthesis in young adults. It is also plausible to acknowledge that up-regulated total GLUT4 protein expression did not represent an increased amount of GLUT4 protein on plasma membrane. This finding further suggests that single dose of oral capsinoids supplementation could not enhance the glucose uptake compared to placebo trial based on the blood insulin and/or glucose response curve after a high carbohydrate meal immediately after exercise. Conversely, increased muscle GLUT4 protein at 3h during recovery may not sufficient to promote the glycogen resynthesis. We assume that longer recovery period following exercise or higher dose of CSN could possibly stimulates GLUT4 expression which may subsequently contribute to increase the muscle glycogen levels. However, further studies are necessary to confirm this phenomenon in human skeletal muscle during post-exercise recovery.

5. Conclusions

Our study provided evidence that acute oral capsinoids supplementation could change energy reliance on fat oxidation in exercised young adults. However, these metabolic consequences cannot in turn to increase the muscle glycogen resynthesis during 3h post-exercise recovery period following a carbohydrate meal. Biopsied muscle samples data showed that capsinoids increased the muscle GLUT4 protein expression 3h after exercise without affecting the muscle glycogen levels. Alternatively, no significant difference in p-Akt expression was observed at 0h and 3h between...
capsinoids and placebo trials. The available evidence suggest further investigations are necessary to confirm the ergogenic properties of capsinoids in connections with muscle glycogen resynthesis during post-exercise recovery period in young adults.

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Author Contributions: I-Shiung Cheng and Mallikarjuna Korivi designed the experiments. Jung-Piao Tsao, Su-Fen Liao and I-Shiung analyzed the data, interpreted the results, prepared the figures, and wrote the manuscript. Jung-Piao Tsao, Su-Fen Liao, Duen-Kai Shiau, Mei-Fang Wu, and Chia-Chen Chang carried out the laboratory experiments. I-Shiung Cheng and Mallikarjuna Korivi revised the manuscript. Duen-Kai Shiau, Mei-Fang Wu, and Chia-Chen Chang contributed the reagents. Su-Fen Liao (medical doctor) performed muscle biopsy.

Conflicts of Interest: The authors declare no conflict of interest.

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