A single intake of capsiate improves mechanical performance and bioenergetics efficiency in contracting mouse skeletal muscle

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Kazuya Y, Tonson A, Pecchi E, Dalmasso C, Vilmen C, Le Fur Y, Bernard M, Bendahan D, Giannesini B. A single intake of capsiate improves mechanical performance and bioenergetics efficiency in contracting mouse skeletal muscle. Am J Physiol Endocrinol Metab 306: E1110–E1119, 2014. First published March 18, 2014; doi:10.1152/ajpendo.00520.2013.—Capsiate is known to increase whole body oxygen consumption possibly via the activation of uncoupling processes, but its effect at the skeletal muscle level remains poorly documented and conflicting. To clarify this issue, gastrocnemius muscle function and energetics were investigated in mice 2 h after a single intake of either vehicle (control) or purified capsiate (at 10 or 100 mg/kg body wt) through a multidisciplinary approach combining in vivo and in vitro measurements. Mechanical performance and energy pathway fluxes were assessed strictly noninvasively during a standardized electrostimulation-induced exercise, using an original device implementing 31-phosphorus magnetic resonance spectroscopy, and mitochondrial respiration was evaluated in isolated saponin-permeabilized fibers. Compared with control, both capsiate doses produced quantitatively similar effects at the energy metabolism level, including an about twofold decrease of the mitochondrial respiration sensitivity for ADP. Interestingly, they did not alter either oxidative phosphorylation or uncoupling protein 3 gene expression at rest. During 6 min of maximal repeated isometric contractions, both doses reduced the amount of ATP produced from glycolysis and oxidative phosphorylation but increased the relative contribution of oxidative phosphorylation to total energy turnover (+28 and +21% in the 10- and 100-mg groups, respectively). ATP cost of twitch force generation was further reduced in the 10- (−35%) and 100-mg (−45%) groups. Besides, the highest capsiate dose also increased the twitch force-generating capacity. These data present capsiate as a helpful candidate to enhance both muscle performance and oxidative phosphorylation during exercise, which could constitute a nutritional approach for improving health and preventing obesity and associated metabolic disorders.

capsioids; uncoupling protein; vanilloid receptors; muscle fatigue; ergogenic effect

OVERWEIGHT AND OBESITY, defined as abnormal or excessive body fat accumulation, increase the risk factors for a number of disorders, including type 2 diabetes and cardiovascular diseases. On that basis, compounds that might promote fatty acid oxidation are of potential interest in the field of metabolic disorders. Capsiate, a member of the capsinoid family, is a nonpungent compound extracted from the fruit of a sweet pepper named “CH-19 Sweet,” wherein it is highly concentrated (40). It is a chemical analog of capsaicin, with an ester bond replacing the amide bond between the vanillyl moiety and the fatty acid chain (20). On the basis of infrared thermography and indirect calorimetry measurements showing that capsiate intake increases body temperature and oxygen consumption in resting healthy volunteers and animals, it has been proposed that capsiate accelerates mitochondrial fatty acid oxidation, thereby enhancing energy consumption (16, 17, 28, 29). However, considering that these measurements have been performed at the whole body level, the exact mechanism mediated by capsiate remains to be determined. Among others factors, the involvement of mitochondrial uncoupling proteins (UCPs), which facilitate fatty acid oxidation through an uncoupling process between mitochondrial respiratory chain and ATP synthesis (6, 33), has been suggested (25).

UCP3 is highly and preferentially expressed in skeletal muscle mitochondria (36). In vivo 31P-MRS experiments conducted in UCP3-knockout mice have demonstrated a four times increase in the rate of mitochondrial ATP synthesis, whereas the mitochondrial tricarboxylic cycle flux has remained unchanged, hence suggesting that the lack of UCP3 increases the mitochondrial ATP synthesis efficiency (9). However, this increased mitochondrial efficiency was not consistent with the lack of phenotypic changes regarding whole body energy metabolism and thermoregulation (9, 37). The potential uncoupling effect of capsiate on muscle energetics remains poorly documented and controversial. Previous works from our laboratory have shown that a single administration of a high dose of capsiate (100 mg/kg body wt) decreased UCP3 gene expression significantly and concomitantly enhanced aerobic energy production in resting and contracting rat skeletal muscle (10), which is in line with previous observations in UCP3-knockout mice. Nevertheless, the fact that twitch force-generating capacity was unchanged suggests that the capsiate-induced UCP3 downregulation reduced the contractile efficiency (10). On the contrary, administration of a lower dose (10 mg/kg body wt) of capsiate did not affect UCP3 gene expression (25) but paradoxically improved swimming endurance (14) in mice, thereby suggesting that capsiate could have a dose-dependent effect.

In the present study, we aimed at determining throughout a multidisciplinary approach combining in vivo and in vitro experiments (1) whether capsiate intake affects energy efficiency of contraction and oxidative ATP synthesis in exercising muscle and (2) the potential dose-dependent effect of capsiate. For this purpose, we compared the effect of a low (10 mg/kg body wt) and a high (100 mg/kg) dose of capsiate on skeletal muscle function and energetics in mice. Mechanical performance and ATP production from energetic pathways were assessed strictly noninvasively during a standardized electrostimulation protocol using an original device (12) implementing 31-phosphorus magnetic resonance (MR) spectroscopy.
mitochondrial respiration was evaluated in saponin-permeabilized tissue, 22 animals were randomly assigned to three groups: vehicle group, capsaicin group, and capsinoid C group. The effects of capsaicin were investigated 2 h after a single intake. Changes in basal energy metabolism and UCP3 gene expression have already been reported after a similar period of time in mice (25, 28).

Animal Care and Feeding

Forty 3-mo-old C57BL/6 male mice (Charles River Laboratory, L’Arbresle, France) weighting 25.3 ± 0.3 g were used for these experiments. Animals were housed socially as four to six per cage in an environmentally controlled facility (12:12-h light-dark cycle, 22°C) with free access to commercial standard food and water until the time of the experiment. After experiments, animals were euthanized by cervical dislocation following isoflurane anesthesia, and gastrocnemius muscles were removed for in vitro measurements.

Experimental Design

Animals were administered capsaicin orally (obtained from Ajinomoto Laboratories, Tokyo, Japan) at 10 or 100 mg/kg body wt via stomach intubation using a round-ended needle. As a control solution, we used the capsaicin vehicle, i.e., a solution containing 0.9% NaCl, 3% ethanol, and 10% Tween-80, as described elsewhere (28). The effects of capsaicin were investigated 2 h after a single intake. Changes in basal energy metabolism and UCP3 gene expression have already been reported after a similar period of time in mice (25, 28).

Two sets of experiments were done. The first set was designed to investigate gastrocnemius muscle function and energetics in vivo using 31P-MRS throughout a rest-exercise-recovery protocol. For this purpose, 22 animals were randomly assigned to three groups: vehicle (n = 7), capsaicin at 10 mg/kg (n = 7), or capsaicin at 100 mg/kg (n = 8). The effects of capsaicin on UCP3 gene expression, mitochondrial respiration, and intramuscular ATP content were analyzed in vitro in a second set of experiments conducted in gastrocnemius muscles from three additional groups (vehicle, capsaicin at 10 mg/kg, or capsaicin at 100 mg/kg; 6 animals/group).

Experiment 1: In Vivo Investigation of Gastrocnemius Muscle Function and Energetics

Animal preparation. Mice were initially anesthetized in an induction chamber using 1.75% isoflurane in 33% O2 (0.2 l/min) and 66% N2O (0.4 l/min). The left hindlimb was shaved before electrode cream was applied at the knee and heel regions to optimize electrical stimulation. An anesthetized animal was placed supine into a home-built device that was designed especially for the strictly noninvasive MR investigation of gastrocnemius muscle function (12). The device integrated an ergometer consisting of a foot pedal coupled to a force transducer and two rod-shaped transcutaneous surface electrodes (located above the knee and under the heel, respectively) connected to an electrical stimulator (Type 215/T; Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten, Germany). Corneas were protected from drying by applying ophthalmic cream, and the animal’s head was placed in a facemask supplied continuously with 1.75% isoflurane in 33% O2 (0.2 l/min) and 66% N2O (0.4 l/min) throughout the experiment. Body temperature of anesthetized animals was controlled and maintained at a physiological level during anesthesia, using a feedback loop that included an electrical heating blanket (Prang & Partner, Pfungern, Switzerland), a temperature control unit (ref. 507137; Harvard Apparatus, Holliston, MA), and a rectal temperature probe. The foot was positioned on the ergometer pedal, and the lower hindlimb was immobilized so that the belly of the gastrocnemius muscle was located above an elliptic (8 × 12 mm) 31P-MRS surface coil. To produce a maximum isometric twitch tension in response to supramaximal square wave pulses (1.5-ms duration, 15–20 mA), the gastrocnemius muscle was passively stretched at rest by adjusting the pedal position, thereby modifying the angle between the foot and the lower hindlimb.

Muscular electrostimulation protocol and mechanical performance measurement. The electrostimulation protocol started 2 h after the oral administration of the vehicle or the capsaicin solution. It consisted in 6 min of repeated maximal isometric contractions induced with square wave pulses (1.5-ms duration, 15–20 mA) at a frequency of 1.7 Hz. Analog electrical signal coming out from the force transducer was amplified with a home-built amplifier (gain = 70 dB; bandwidth 0–5 kHz, operational amplifier AD620; Analog Devices, Norwood, MA) and coupled to a digital signal (PCI-6220; National Instrument, Austin, TX) that was monitored and recorded using the WinATS software version 6.5 (Sysma, Aix-en-Provence, France). Isometric force production was calculated by integrating the isometric tension (in N) with respect to time and was expressed as force-time integral (in N/s).

MR data acquisition and processing. Explorations were performed in the 4.7 T horizontal magnet of a 47/30 Biospec Avance MR system (Bruker, Karlsruhe, Germany) equipped with a Bruker 120-mm BGA12SL (200 mT/m) gradient insert. 31P-MR spectra (8 kHz sweep width, 2,048 data points) from the gastrocnemius region were acquired continuously throughout a standardized experimental protocol consisting of 6 min of rest, 6 min of electrostimulation, and 15 min of postelectrostimulation recovery. MR data acquisition was gated to muscle electrostimulation to reduce potential motion artifacts due to contraction. A fully relaxed spectrum (12 scans, 20 s of repetition time) was acquired at rest, followed by a total of 768 free induction decays (FIDs; 1.875 s of repetition time). The first 64 FIDs were acquired at rest and summed together. The next 192 FIDs were acquired during the electrostimulation period and were summed by packets of 32, allowing a temporal resolution of ~60 s. The remaining 512 FIDs were obtained during the postelectrostimulation recovery period and were summed as seven packets of 32 FIDs, followed by three packets of 64 FIDs and one packet of 96 FIDs. MR data were processed using a custom-written analysis program developed with the IDL software (Interactive Data Language; Research Systems, Boulder, CO) (23). Relative concentrations of phosphocreatine (PCr), inorganic phosphate (Pi), and β-ATP were obtained by a time domain-fitting routine using the AMARES-MRUI Fortran code and appropriate prior knowledge for the ATP multiplets (35). Absolute amounts of phosphorylated compounds were expressed relative to a resting β-ATP concentration determined in vitro using high-performance liquid chromatography (see above). Intracellular pH (pHi) was calculated from the chemical shift of the Pi signal relative to PCr (5).

Metabolic flux calculation. ATP production from creatine kinase (CK) reaction, oxidative phosphorylation, and glycolysis were calculated as described previously (4, 11, 18). The ATP of contraction referred to ATP production scaled to twitch force generation during the same time period. ATP production from the net breakdown of PCr via the CK reaction (D) was calculated directly using the [PCR] time course throughout the electrostimulation period: D = -d[PCr]/dt.

Oxidative ATP production (Q) was calculated considering that oxidative ATP synthesis is controlled by [ADP] throughout a hyperbolic relationship (13): Q = Qmax/[1 + KADP/ADP], in which KADP is the ADP concentration at half-maximal oxidation rate and Qmax is the maximal oxidative ATP synthesis capacity. ADP concentration was
calculated from [PCr], [ATP], and pH, considering the equilibrium constant (\(k_{\text{CK}} = 1.67 \times 10^{5} \text{M}^{-1}\)) of the CK reaction (31). \(Q_{\text{max}}\) was calculated from the rate of PCr resynthesis at the start of the post-electrostimulation recovery period \(V_{\text{PCr rec}}\) and ADP concentration measured at the end of the electrostimulation period: \(Q_{\text{max}} = V_{\text{PCr rec}} (1 + k_{\text{CK}}/\text{[ADP]_end})\). \(V_{\text{PCr rec}}\) was the product of \(k_{\text{CK}}\) (the pseudo-first-order rate constant of PCr recovery) and \([\text{PCr}]_{\text{cons}}\) (the amount of PCr consumption measured at the end of the electrostimulation period). The PCr time course during the postelectrostimulation recovery period was fitted to a first-order exponential curve for determining \(k_{\text{CK}}\): 
\[ \text{[PCr]_{cons}} = \text{[PCr]_{cons}} \times e^{-k_{\text{CK}} t} \]
where \([\text{PCr}]_{\text{cons}}\) is the PCr concentration measured at rest.

Glycolytic ATP synthesis (L) was inferred from PCr and pH changes considering that, when coupled to ATP hydrolysis, glycolytic ATP production is related to proton synthesis (\(H_{\text{ Gly}}\)) with a stoichiometry of 1.5 mol ATP/proton (L = 1.5 H Gly) (15). The degradation of each mole of glycosyl unit actually generates 3 mol of ATP, whereas the hydrolysis of 3 mol of ATP is coupled to the synthesis of 2 mol of protons (15). This proton synthesis (\(H_{\text{ Gly}}\)) can be calculated from the observed changes in pH, taking into account protons consumed by PCr degradation throughout the CK reaction (\(H_{\text{CK}}\)) passively buffered in the cytosol (\(H_{\text{ CK}}\)), leaving the cell rate of net proton efflux and \(H_{\text{ Gly}}\) produced by oxidative phosphorylation (\(H_{\text{Ox}}\)). \(H_{\text{CK}}\) was calculated from the time-dependent changes in [PCr] and the stoichiometric coefficient \(\epsilon = 1/[1 + 10^{pH - 6.75}]\), which represents the number of protons associated with P1 production (39): 
\[ \text{H}_{\text{CK}} = \epsilon \text{dPCR/dt} \]

Besides, \(H_{\text{CK}}\) was the product of \(K_{\text{total}}\) (in Slykes, millimoles acid added per unit change in pH) and pH changes \((\Delta pH = pH_{\text{observed}} - pH_{\text{rest}})\): 
\[ H_{\text{CK}} = (K_{\text{total}} \times \Delta pH) \]

The apparent buffering capacity (\(\beta_{\text{total}}\)) is taken into account at the balance of each proton by the buffering capacity of muscle tissue (\(\beta_{\text{tissue}}\)): 
\[ \text{\beta}_{\text{tissue}} = \text{\beta}_{\text{total}} \times [\text{PCr}]_{\text{cons}} \times 10^{-pH - 6.75} \]

During muscle acidification, \(H_{\text{Ox}}\) was calculated using the proportionality constant \(\lambda\) (in mmol min^{-1} pH unit^{-1}). At that time, the proton rate is generated throughout the aerobic PCr resynthesis, pH recovers back to basal because of net proton efflux from the cell; \(H_{\text{Ox}}\) can then be calculated, taking into account proton loads associated with CK reaction and mitochondrial ATP synthesis on the one hand and the rate of pH changes on the other hand: 
\[ H_{\text{Ox}} = H_{\text{CK}} + H_{\text{ Gly}} + H_{\text{Ox}} - \text{\beta}_{\text{tissue}} \Delta pH_{\text{rec}} \]

The rate of aerobic proton production coupled to oxidative ATP synthesis was quantified as described previously (39): 
\[ H_{\text{Ox}} = mV_{\text{PCr rec}} \times m = 0.16/[1 + 10^{(6.4 - pH)}] \]

**Experiment 2: In Vitro Assays**

**Gastrocnemius muscle collection.** Two hours after the oral administration of the vehicle or the capsaicin solutions, animals were euthanized by cervical dislocation following isoflurane anesthesia. Gastrocnemius muscles were quickly removed and dissected free of collagen tissue and surrounding fat. Muscles were either placed in an ice-cold isolation solution (Krebs solution containing (in mM): 2.77 CaK2EGTA, 7.23 K2EGTA, 6.56 MgCl2, 5.70 Na2ATP, 15 PCr, 20 imidazole, 20 uracile, 0.50 dithiothreitol, and 50 K-methansulfonate, pH 7.1, at 22°C) for analyzing mitochondrial respiration in permeabilized muscle fibers or freeze-clamped with liquid nitrogen-chilled metal tongs for measuring UCP3 gene expression and ATP content.

**Mitochondrial respiration in permeabilized fibers.** Respiratory variables of the total mitochondrial population were studied in permeabilized saponin-skinned fibers (21). Given that mouse gastrocnemius muscle is a heterogeneous tissue, only the deep red portion was used. This portion can be easily distinguished macroscopically from the superficial white portion of the muscle. Briefly, thin fiber bundles (100–200 μm in diameter) were excised from the red portion under a microscope in isolation solution, and the fibers were manually separated from each other. Dissected fibers were incubated with gentle shaking for 30 min in the same solution containing 50 mM saponin to selectively permeabilize the sarcolemma. Fibers were then transferred into the respiration solution (solution R; 2.77 mM CaK2EGTA, 7.23 mM K2EGTA, 1.38 mM MgCl2, 3 mM K2HPO4, 20 mM imidazole, 20 mM uracile, 0.50 mM dithiothreitol, 90 mM K-methansulfonate, 10 mM Na-methansulfonate, 5 mM glutamate, 2 mM malate, and 2 mg/ml fatty acid-free bovine serum albumin, pH 7.1, at 22°C) for 10 min. This step was repeated twice to wash out saponin and other metabolites that could interfere with the measurements. Respiratory rates were measured at 22°C using a Clark electrode in oxygraph chambers (Hansatech Instruments, Norfolk, UK) containing 0.7–2 mg of fibers in 1.5 ml of solution R with continuous stirring. ADP sensitivity was investigated by cumulative addition of ADP in the respiration medium from 5 to 2.000 μM, with a 3-min delay between each ADP addition so that a steady-state respiration level was obtained for each ADP concentration. Basal respiration rate was measured without ADP (R0), and cumulative ADP concentrations were added until the maximal respiration rate (Rmax) was reached. The maximal oxidative capacity (Vmax) was calculated as the sum between R0 and Rmax. The ADP-stimulated respiration rates (VADP), above the basal oxygen consumption were plotted against the corresponding ADP concentrations, and this relationship was fitted using the nonlinear Michaelis-Menten relationship to calculate the apparent \(K_{\text{m}}\) for [ADP].

**UCP3 gene expression.** Total RNA was extracted from freeze-clamped muscles (Fibrous Tissue Mini Kit; Qiagen). RNA concentration was measured on a Nanodrop spectrophotometer (ThermoscientificFrance), and RNA quality was assessed using a Bioanalyzer (Agilent France). RNA (1 μg) was reverse transcribed with Quantitect Reverse Transcription kit (Qiagen). UCP3 mRNA expression was quantified by real-time PCR on a Light Cycler 480 (Roche Applied Science France). The equivalent of 5 ng of initial RNA was subjected to PCR amplification in a 6-μl final volume, using specific primers at 0.5 μM and LC 480 SYBR Green I Master (Roche Applied Science France). PCR amplification conditions were initial denaturation for 10 min at 95°C, followed by 45 cycles consisting of 10 s at 95°C, 15 s at 60°C, and 15 s at 72°C. The generation of specific PCR products was confirmed by melting-curve analysis. For each PCR assay, cDNAs were run in triplicate in parallel with serial dilutions of a cDNA mixture to generate a standard linear curve that was used to estimate relative mRNA expression of UCP3 normalized with 18S (internal reference gene). The specific primers used were as follows: UCP3 forward 5’-TAC CCA ACC TTG GCT AGA CG-3’ and reverse 5’-GTC CGA GGA GAG AGC TTG C-3’; 18S forward 5’-ACC GCG GTT CTA TTT TGT TG-3’ and reverse 5’-AGT CCG CAT CGT TTA TGG TC-3’. Intramuscular ATP content. Freeze-clamped muscles (10–20 mg) were homogenized in 1.2 ml of ice-cold 0.6 M perchloric acid with a Polytron PT2100 (Kinematica, Luzern, Switzerland). After incubation for 15 min at 4°C, the homogenate was centrifuged (15 min, 2,000 g, 4°C) and the pellet dissolved into 1 ml of NaOH (1 N) for protein determination according to Lowry et al. (24). The supernatant was neutralized with K2CO3, placed for 30 min at 4°C, and used for determination of ATP concentration by ion-pairing reverse-phase high-performance liquid chromatography (HPLC). HPLC was carried on an RP18 column (5-μm particle size, 250 × 4.6 mm ID; Merck, Darmstadt, Germany) according to Alfy and Park (3). The HPLC system consisted of a Merck-Hitachi L-6200A pump (Merck) equipped with a Rheodyne valve and a Merck-Hitachi L-7400 UV-visible detector. Thymidine monophosphate (ref. T7004; Sigma, Poole, UK) was used as an internal standard. DOI:10.1152/ajpendo.00520.2013 • www.ajpendo.org
**Statistical Analysis**

All values are presented as means ± SE. Analyses were performed with JMP 9.0.1 software (SAS Institute, Cary, NC). One-way ANOVA was used to compare measurements in the three groups. Tukey-Kramer post hoc multiple-comparison tests were applied to determine pairwise differences between groups. The level of significance was set at $P < 0.05$.

**RESULTS**

**Mechanical Performance**

Illustrative layouts of muscle response to the 6-min electrostimulation period are presented in Fig. 1, A and B. For each group, peak force (Fig. 1C) and force-time integral (Fig. 1D)

![Graphs showing force-time integral and peak force](image)

**Fig. 1.** A: typical maximal isometric force trace from the gastrocnemius muscle electrically stimulated (6 min at a frequency of 1.7 Hz) in vivo in a mouse treated with capsiate (CAP) at 100 mg/kg body wt. B: maximal twitch force recorded at the start (left) and at the end (right) of the 6-min electrostimulation protocol. C and D: changes in maximal peak force (C) and force-time integral (D) throughout the 6-min electrostimulation protocol. E–G: extent of force reduction (%start on set of stimulation) measured at the end of the electrostimulation protocol (E) and total amount of force (F) and force-time integral (G) produced during the whole protocol (C). The electrostimulation protocol started 2 h after ingestion of vehicle (control) or CAP at 2 different concentrations (10 or 100 mg/kg body wt). Data are means ± SE. *Significantly different from control.
transiently increased in the early stage of the 6-min electrostimulation period up to a maximal value and then progressively decreased until the end of the electrostimulation period as a sign of fatigue development. At this stage, the extent of force reduction did not differ among the groups (Fig. 1E). However, the total peak force (Fig. 1F) and the force-time integral (Fig. 1G) that developed during the whole 6-min electrostimulation period were significantly larger (+22 and +24%, respectively) in animals receiving the higher dose of capsiate. For each group, the relaxation between consecutive twitches was fully achieved throughout the whole 6-min electrostimulation protocol, as illustrated in Fig. 1A.

**Phosphorylated Compounds and Intracellular pH**

At rest, there were no differences between control and capsiate-administered animals for the [PCr]/[ATP] ratio, [PCr], or [ATP] (Table 1). On the contrary, [ADP] was lower in both groups receiving capsiate compared with the control mice, and intracellular acidosis was larger in animals treated with the higher dose of capsiate (Table 1).

At the start of the 6-min electrostimulation period, PCr was rapidly degraded (Fig. 2A) at a similar rate among the three groups (Table 1). In the middle of the electrostimulation period, [PCr] reached a steady state that was stable until the end of the electrostimulation period (Fig. 2A); at that time, the extent of PCr consumption (∆PCrend) did not differ among the three groups (Table 1). The time course of pH_{i} during the electrostimulation period exhibited an initial 3-min phase of rapid acidosis followed by a steady state that was maintained during the remaining electrostimulation period (Fig. 2B). The extent of acidosis at the end of the electrostimulation protocol (∆pH_{end}) was significantly lower in both capsiate-treated groups (Table 1). For each group, the electrostimulation period did not alter the ATP level that remained close to the basal value (Fig. 2C) but caused a progressive accumulation of ADP (Fig. 2D); the ADP level at the end of the electrostimulation period did not differ between the groups (Table 1).

During the postelectrostimulation recovery period, phosphorylated compound levels and pH_{i} progressively reached their respective basal values (Fig. 2, A–D). In particular, the initial rate of PCr resynthesis was not altered by any of the capsiate treatments (Table 1).

**Metabolic Fluxes and ATP Cost of Twitch Force Generation**

The basal rate of oxidative ATP synthesis measured in vivo did not differ among the groups (Table 1). During the electrostimulation period, ATP production from each metabolic pathway was normalized to the corresponding force output. Neither capsiate dose altered ATP production from CK reaction (Fig. 3A), but they decreased the glycolytic ATP production by 44 and 52% in the 10- and 100-mg capsiate groups, respectively (Fig. 3B). Similarly, oxidative ATP production was reduced in both groups treated with the low (−24%) and high (−36%) doses of capsiate (Fig. 3C). In addition, both capsiate treatments altered the relative contribution of each metabolic pathway to the total ATP turnover rate (Fig. 4); compared with control animals, both CK reaction and oxidative contributions were larger in the 10- (+30 and +28%, respectively) and 100-mg (+37 and +21%, respectively) capsiate groups, whereas the glycolytic contribution was 12 and 11% lower in the 10- and 100-mg capsiate groups, respectively. Overall, the average ATP cost of twitch force generation (calculated across the whole 6-min electrostimulation period as the total amount of ATP production from all metabolic pathways scaled to total force generation during the same period) was lower in the 10- (−35%) and 100-mg (−45%) capsiate groups (Fig. 5).

**Mitochondrial Respiration in Permeabilized Fibers**

No difference was found among the groups, considering the basal respiration rate (Fig. 6A) and the maximal oxidative capacity (Fig. 6B). On the contrary, the K_{m} for ADP was reduced in animals treated with the low (−61%) and high (−67%) doses of capsiate compared with controls (Fig. 6C).

**UCP3 Gene Expression**

As shown in Fig. 7, neither capsiate dose altered the expression of UCP3 mRNA in resting gastrocnemius muscle.

**DISCUSSION**

The purpose of the present study was to investigate the physiological effects of a single intake of a low (10 mg/kg body wt) and a high (100 mg/kg) dose of purified capsiate on gastrocnemius muscle function and energetics in mice. We mainly found that, regardless of the dose, capsiate intake

### Table 1. Phosphorylated compounds and pH_{i} assessed in vivo using ^31P-MR spectroscopy in mouse gastrocnemius muscle

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 7)</th>
<th>Capsiate (10 mg/kg; n = 7)</th>
<th>Capsiate (100 mg/kg; n = 8)</th>
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<tbody>
<tr>
<td><strong>Phosphorylated Compounds</strong></td>
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<tr>
<td>[PCr]/[ATP] ratio</td>
<td>2.49 ± 0.11</td>
<td>2.64 ± 0.17</td>
<td>2.56 ± 0.10</td>
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<tr>
<td>[PCr], mM</td>
<td>14.8 ± 0.7</td>
<td>15.0 ± 0.9</td>
<td>15.4 ± 0.6</td>
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<tr>
<td>[ATP], mM</td>
<td>5.9 ± 0.4</td>
<td>5.7 ± 0.2</td>
<td>6.0 ± 0.1</td>
</tr>
<tr>
<td>[ADP], μM</td>
<td>11.6 ± 1.0</td>
<td>9.5 ± 0.6</td>
<td>7.7 ± 0.5*</td>
</tr>
<tr>
<td>pH_{i}</td>
<td>7.26 ± 0.04</td>
<td>7.19 ± 0.03</td>
<td>7.08 ± 0.03*</td>
</tr>
<tr>
<td><strong>Oxidative ATP synthesis rate, mM/min</strong></td>
<td>1.3 ± 0.2</td>
<td>1.6 ± 0.3</td>
<td>1.0 ± 0.2</td>
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<tr>
<td><strong>Electrostimulation period</strong></td>
<td></td>
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<tr>
<td>Initial rate of PCr consumption, mM/min</td>
<td>11.2 ± 1.5</td>
<td>9.8 ± 1.3</td>
<td>8.7 ± 1.2</td>
</tr>
<tr>
<td>∆PCrend (relative to basal), mM</td>
<td>9.9 ± 0.4</td>
<td>9.2 ± 0.4</td>
<td>10.8 ± 0.6</td>
</tr>
<tr>
<td>∆pH_{end} (relative to basal, pH unit)</td>
<td>0.59 ± 0.06</td>
<td>0.39 ± 0.05*</td>
<td>0.30 ± 0.05*</td>
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<tr>
<td>[ADP]_{end}, μM</td>
<td>47 ± 9</td>
<td>53 ± 12</td>
<td>65 ± 9</td>
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<tr>
<td><strong>Postelectrostimulation recovery period</strong></td>
<td></td>
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<tr>
<td>Initial rate of PCr resynthesis, mM/min</td>
<td>5.2 ± 0.7</td>
<td>4.9 ± 0.6</td>
<td>4.2 ± 0.5</td>
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Values are means ± SE. pH_{i}, intracellular pH; PCr, phosphocreatine. *Significantly different from control in the same row.
improved the control exerted by ADP on mitochondrial respiration, enhanced the oxidative ATP contribution in exercising muscle, and reduced the ATP cost of twitch force generation. In addition, the high dose of capsiate also enhanced the twitch force-generating capacity.

Our data clearly support an acute effect of capsiate on muscle energetics. In resting muscle, \([\text{PCr}] / [\text{ATP}]\) ratio, \([\text{PCr}]\), and \([\text{ATP}]\) were similar in the three groups, whereas \([\text{ADP}]\) was lower in both groups receiving capsiate. Given that \([\text{ADP}]\) is considered to stimulate mitochondrial ATP generation through a feedback loop (18), an idea that immediately springs to mind is that this reduction in basal cytoplasmic ADP content should lead to a reduced rate of mitochondrial ATP production. However, neither of the results that we obtained in vitro (permeabilized fibers) and in vivo (\(^{31}\text{P}-\text{MRS}\)) support such a reduction. Interestingly, the measurements we performed in

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**Fig. 2.** Changes in gastrocnemius phosphocreatine concentration ([PCr]; A), intracellular pH (pHi; B), [ATP] (C), and [ADP] (D) during 6 min of in vivo electrostimulation and 15 min of postelectrostimulation recovery periods. The electrostimulation protocol started 2 h after ingestion of vehicle (control) or CAP at 2 different concentrations (10 or 100 mg/kg body wt). The first time point \((t = 0)\) indicates the resting value. Time points were assigned with the midpoints of the acquisition intervals. Data are means ± SE.
isolated saponin-permeabilized fibers clearly indicated an improved mitochondrial respiration sensitivity for ADP \( K_{m,\text{ADP}} \) resulting from the capsiate administration. On that basis, the reduced ADP concentration we measured at rest might be considered as an adaptive mechanism in the context of an apparent increase in mitochondrial sensitivity to ADP. The origin of this increased sensitivity remains to be determined. One could assume that the occurrence of a simultaneous improved sensitivity to ADP and a reduced ADP level could lighten the potent capsiate-induced activation of mitochondrial uncoupling processes, leading to the dissipation of the proton gradient generated throughout the respiratory chain into heat (25). Capsiate has indeed been shown to modulate the basal expression of several UCP genes in various tissues (25). Yet our assumption appears unlikely herein, because we found that whatever the dose, capsiate intake did not affect the basal expression of the UCP3 gene, which is highly and preferentially expressed in skeletal muscle (36). A similar observation has been reported in a study showing that a 2-wk daily administration of capsiate using a 10 mg/kg dose did not change the UCP3 mRNA level in mouse gastrocnemius muscle (25). Overall, our data demonstrated that skeletal muscle is not involved in either energy metabolism accelerations or uncoupling process activations that have previously been reported at the whole body level in response to capsiate intake in resting healthy volunteers and animals (16, 17, 28, 29).

We also measured a lower pH in resting muscle of animals receiving the higher dose of capsiate. Intracellular acidosis is generally observed under hypoxia when the oxygen supply is limited. However, such an issue can be dismissed in the present study, because basal mitochondrial function was similar in both capsiate groups, hence indicating an adequate muscle oxygenation. This lower pH (corresponding to an increased intracellular concentration of \( H^+ \)) could be interpreted as a compensatory mechanism allowing for the maintenance of a “normal” basal PCr level in the face of reduced ADP content, whereas mitochondrial function would be unchanged. Actually, intramuscular PCr concentration is under the control of the CK reaction, which reversely transfers high-energy phosphate from PCr to ADP to form ATP via the following reaction: PCr + ADP + \( H^+ \) ↔ ATP + creatine.

In exercising muscle, both capsiate doses reduced the amounts of anaerobic and oxidative ATP production and also modified the balance between those both components. Actually, the glycolytic contribution to ATP production was decreased (-12% and -11% in 10- and 100-mg capsiate groups, respectively), whereas the oxidative contribution was increased in the 10- (+28%) and 100-mg (+21%) groups. This latter

**Fig. 3.** ATP production from creatine kinase (CK) reaction (A), glycolysis (B), and oxidative phosphorylation (C) during the 6-min in vivo electrostimulation protocol 2 h after ingestion of vehicle (control) or CAP at 2 different concentrations (10 or 100 mg/kg body wt). ATP productions were normalized by force output. Data are means ± SE. *Significantly different from control.

**Fig. 4.** Relative contributions of CK reaction, glycolysis, and oxidative phosphorylation to total ATP production during the 6-min in vivo electrostimulation protocol 2 h after ingestion of vehicle (control) or CAP at 2 different concentrations (10 or 100 mg/kg body wt). Data are means ± SE. *Significantly different from control.

**Fig. 5.** Average ATP cost of twitch force generation during the 6-min in vivo electrostimulation protocol 2 h after ingestion of vehicle (control) or CAP at 2 different concentrations (10 or 100 mg/kg body wt). Data are means ± SE. *Significantly different from control.
result further supports the view that capsiate promotes energy metabolism via an acceleration of mitochondrial phosphorylation (16, 17, 28, 29). Considering that 1) capsiate ingestion was associated with an increased mitochondrial respiration sensitivity for ADP and 2) the end-exercise ADP level was similar among all groups, we expected to measure a faster initial rate of PCr resynthesis during the postelectrostimulation recovery, a well-recognized in vivo index of mitochondrial activity (22). Surprisingly, however, we did not measure such an acceleration. This paradoxical result might indicate either that the improved sensitivity did not translate into a measurable improvement of the aerobic ATP production during the postelectrostimulation session or that the ADP signal in the feedback loop controlling oxidative ATP synthesis was weaker in the capsiate-treated animals during exercise.

Another important finding is that the highest dose of capsiate also improved the twitch force-generating capacity. Previous studies in mice have shown that swimming endurance was increased by 18 and 40% within 2 h after a single oral administration of 10 mg/kg capsiate (14) and capsicain (19), respectively. Nevertheless, any ergogenic effect of capsiate has to our knowledge never been reported before. Muscle performance can be improved when energy supply is increased (2, 38), but this is unlikely to be the case in the present study given that the total amount of ATP produced during the standardized electrostimulation protocol was even lower in both capsiate-treated animals compared with the control group. This ergogenic effect could be due to the larger basal acidosis we measured in this group. Indeed, a lower intracellular pH has been reported to increase maximal isometric tetanic force possibly via an improvement of muscle excitability (27, 30).

Another attractive explanation regarding the improved twitch force-generating capacity could be linked to an enhanced contractile response to Ca$^{2+}$ (30, 34). It has been shown in vitro that capsainoids and analogs bind specifically the transient potential vanilloid 1 (TRPV1) receptors and increase in turn the basal cytosolic concentration of free Ca$^{2+}$ in a concentration-dependent manner (26, 32). Besides, increased basal Ca$^{2+}$ concentration has been shown to enhance contractile response in exercising muscle (34). This phenomenon, known as potentiation or staircase, would thus be linked to an increased Ca$^{2+}$ sensitivity of the contractile apparatus.

We also report for the first time that capsiate intake led to a reduction in ATP cost of twitch force generation. In other words, capsiate administration was responsible for a reduced energy consumption for a given amount of force. Similarly to what has been done previously, ATP cost was calculated using the force-time integral to take into account the time during which force was produced and chemical energy was consumed (8, 11). In exercising muscle, ATP is indeed used for both contractile and noncontractile processes; the latter, which represents 20–50% of the ATP utilized during the contraction, is related mainly to Ca$^{2+}$ transport associated with the activation-relaxation cycle (7). Then, any reduction of the energy consumed by one of these processes would explain the decreased ATP cost. It seems unlikely that the reduced ATP cost would be due to any decrease in noncontractile processes given that we found that muscle relaxation between consecutive twitches was fully achieved during the whole electrostimulation protocol and that twitch relaxation was not altered. On the contrary, peak tension was higher in capsiate-administered animals, probably as the result of the potentially enhanced contractile apparatus sensitivity in response to the increased basal cytosolic Ca$^{2+}$ occurring from the activation of TRPV1 receptors.

Fig. 6. Basal (A) and maximal rates (B) of oxygen consumption and $K_m$ of mitochondrial respiration for ADP (C) measured in isolated permeabilized fibers from gastrocnemius muscle of animals administered with vehicle (control) or CAP at 2 different concentrations (10 or 100 mg/kg body wt). Data are means ± SE. *Significantly different from control.

Fig. 7. Uncoupling protein 3 (UCP3) mRNA in resting gastrocnemius muscle of animals administered with vehicle (control) or CAP at 2 different concentrations (10 or 100 mg/kg body wt). Results are normalized to 18S mRNA used as an internal standard control. Data are means ± SE.
by capsiate. We assume that even if additional energy would be needed to handle the supplementary Ca$^{2+}$ released by TRPV1 receptors, the positive effect of the increased basal Ca$^{2+}$ concentration on muscle force would be higher, thereby ultimately resulting in a decreased ATP cost of contraction. The improved twitch force-generating capacity we recorded in capsiate-treated animals might also have been due to an increased number of cross-bridges during exercise. Before each experiment, we stretched contractile elements at the optimum length by adjusting resting gastrocnemius muscle length to achieve the maximal isometric twitch force in response to supramaximal electrical pulse. Although this experimental context should involve the maximum number of cross-bridges formed between actin and myosin filaments, it cannot be excluded that the kinetics of attachment and detachment of cross-bridges might be altered with repeated activations, thereby resulting in a higher number of cross-bridges attached simultaneously during a twitch in capsiate-treated animals. An unambiguous interpretation of the mechanisms underlying the changes in force output would have been to use tetanic contractions instead of repeated isometric twitches. However, the use of tetanic contractions was not feasible herein given the implementation of vivo $^{31}$P-MRS for dynamically assessing muscle energy metabolism. Given the poor sensitivity of this technique, our nominal time resolution was 60 s, and our electrostimulation protocol lasted 6 min. In that respect, the utilization of closely spaced tetanic contractions for such a period of time would have resulted in muscle damage, and we would have been unable to achieve the time resolution necessary for assessing metabolic changes.

In conclusion, capsiate intake increases the contribution of oxidative ATP production, improves the metabolic efficiency of twitch contraction in exercising muscle in vivo, and protein turnover for Ca$^{2+}$, improving twitch force-generating capacity we recorded in capsiate-treated animals might also have been due to an increased number of cross-bridges during exercise. Before each experiment, we stretched contractile elements at the optimum length by adjusting resting gastrocnemius muscle length to achieve the maximal isometric twitch force in response to supramaximal electrical pulse. Although this experimental context should involve the maximum number of cross-bridges formed between actin and myosin filaments, it cannot be excluded that the kinetics of attachment and detachment of cross-bridges might be altered with repeated activations, thereby resulting in a higher number of cross-bridges attached simultaneously during a twitch in capsiate-treated animals. An unambiguous interpretation of the mechanisms underlying the changes in force output would have been to use tetanic contractions instead of repeated isometric twitches. However, the use of tetanic contractions was not feasible herein given the implementation of vivo $^{31}$P-MRS for dynamically assessing muscle energy metabolism. Given the poor sensitivity of this technique, our nominal time resolution was 60 s, and our electrostimulation protocol lasted 6 min. In that respect, the utilization of closely spaced tetanic contractions for such a period of time would have resulted in muscle damage, and we would have been unable to achieve the time resolution necessary for assessing metabolic changes.

In conclusion, capsiate intake increases the contribution of oxidative ATP production, improves the metabolic efficiency of twitch contraction in exercising muscle in vivo, and produces an ergogenic effect. Our data support the idea that capsiate might be of interest for patients suffering from obesity and associated metabolic disorders and for healthy subjects aiming at improving their muscle performance.

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The authors have no conflicts of interest, financial or otherwise, to declare. Ajinomoto Laboratories did not influence data collection, analysis, interpretation, or the decision to have the present study published.

AUTHOR CONTRIBUTIONS

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