Effects of Caffeine on Muscle Glycogen Utilization and the Neuroendocrine Axis during Exercise*

DIDIER LAURENT, KEVIN E. SCHNEIDER, WILLIAM K. PRUSACZYK, CAROLE FRANKLIN, SUZANNE M. VOGEL, MARTIN KRSSAK, KITT FALK PETERSEN†, HAROLD W. GOFORTH, AND GERALD I. SHULMAN‡

Department of Internal Medicine and the Howard Hughes Medical Institute (G.I.S.), Yale University School of Medicine, New Haven, Connecticut 06510; and Physiological Performance and Operational Medicine Department, Naval Research Center (K.E.S., W.K.P., H.W.G.), San Diego, California 92186

ABSTRACT

To examine the effect of caffeine ingestion on muscle glycogen utilization and the neuroendocrine axis during exercise, we studied 20 muscle glycogen-loaded subjects who were given placebo or caffeine (6 mg/kg) in a double blinded fashion 90 min before cycling for 2 h at 65% of their maximal oxygen consumption. Exercise-induced glycogen depletion in the thigh muscle was noninvasively measured by means of 13C nuclear magnetic resonance spectroscopy (NMR) spectroscopy, and plasma concentrations of substrates and neuroendocrine hormones, including β-endorphins, were also assessed. Muscle glycogen content was increased 140% above normal values on the caffeine trial day (P < 0.001). After cycling for 2 h, caffeine ingestion was associated with a greater increase in plasma lactate (caffeine: +1.0 ± 0.2 mmol/L; placebo, +0.1 ± 0.2 mmol/L; P < 0.005), epinephrine (caffeine, +223 ± 82 pg/mL; placebo, +56 ± 26 pg/mL; P < 0.05), and cortisol (caffeine, +12 ± 3 mg/mL; placebo, +2 ± 2 mg/mL; P < 0.001) levels. However, plasma free fatty acid concentrations increased (caffeine, +814 ± 133 mmol/L; placebo, +785 ± 85 mmol/L; P = NS), and muscle glycogen content decreased (caffeine, −57 ± 6 mmol/L muscle; placebo, −53 ± 5 mmol/L muscle; P = NS) to the same extent in both groups. At the same time, plasma β-endorphin levels almost doubled (from 30 ± 5 to 53 ± 13 pg/mL; P < 0.05) in the caffeine-treated group, whereas no change occurred in the placebo group. We conclude that caffeine ingestion 90 min before prolonged exercise does not exert a muscle glycogen-sparing effect in athletes with high muscle glycogen content. However, these data suggest that caffeine lowers the threshold for exercise-induced β-endorphin and cortisol release, which may contribute to the reported benefits of caffeine on exercise endurance. (J Clin Endocrinol Metab 85: 2170–2175, 2000)

SINCE THE discovery of tea over 4500 yr ago, caffeine has become commonplace in our diet, and today caffeine is the most commonly consumed drug in the world (1). Athletes often use it as an ergogenic aid during exertion, because experimental studies have shown that caffeine improves endurance when ingested before a prolonged exercise (2–5). In competition, caffeine utilization is permitted by the International Olympic Committee, providing that urinary excretion does not exceed 12 μg/mL, which is roughly equivalent to the ingestion of three cups of coffee. Yet the mechanism by which caffeine potentiates performance in athletes is not completely understood. One hypothesis is that caffeine enhances fat utilization during exercise, thereby sparing muscle glycogen stores (6). This is of importance considering that high concentrations of muscle glycogen contribute effectively to improving performance during long-term aerobic exercise (7). The postulated mechanism has been that caffeine stimulates adipose tissue lipolysis via an increase in catecholamine secretion and consequently promotes free fatty acid oxidation by the active muscle, which, in turn, spares muscle glycogen through substrate competition (8). The ability to influence psychological states and alter pain perception is also an important factor affecting athletic performance. The release of β-endorphins into plasma during high level physical exertion (9, 10) probably plays a major role (11, 12). Because caffeine intake has long been associated with stimulation of the adrenocortical axis (13–18), the question arises as to whether caffeine might also modulate fatigue perception during exercise through promotion of cortisol and β-endorphin release, in addition to its potential ability to alter substrate metabolism.

Therefore, the aim of the present study was to determine whether caffeine ingestion before a prolonged exercise causes muscle glycogen sparing and/or influences the opioid peptide response to exercise. To mimic the physical preparation that endurance athletes usually undergo before a competition event, the study was performed on highly trained subjects whose muscle glycogen concentrations were elevated by means of a supercompensation protocol.

Subjects and Methods

Subjects

Twenty healthy men (age, 26 ± 1 yr; height, 178 ± 2 cm; weight, 81 ± 2 kg; 9.3 ± 0.7% body fat as measured by seven-site skinfold test) without a family history of diabetes mellitus, hypertension, or any major diseases were selected from Naval and Marine Corps as special operations commands in San Diego, CA. Subjects had all been engaged in intense
physical conditioning for at least 1 yr before the study, and none was taking medications. The physical training of about 26 h/wk consisted of about 14 h/wk of aerobic workout (jogging, swimming, biking, walking three or four times per wk each) and approximately 11 h/wk of short intensive resistive (weight lifting) exercise involving mainly quadriceps muscles. One to two weeks before the study, a peak oxygen uptake test was administered on a bicycle ergometer to measure maximal oxygen consumption (VO2 peak) using an incremental load protocol. Their values ranged from 38.9–57.3 mL/kg/min body mass (average, 49.1 ± 1.4 mL/kg/min body mass). Subjects were not permitted to participate in any physical activity other than exercise sessions designed for the study (days 0–7). Experimental procedures were carried out in compliance with the guidelines of the human investigation committee of Yale University School of Medicine as well as the committee for the protection of human subjects, Naval Health Research Center. Both exercise sessions on days 1 and 7 were performed in the morning under fasting conditions. Each subject gave informed consent after the purpose, nature, and potential risks of the study were explained.

Peak oxygen uptake test

The exercise was performed on a mechanically braked bicycle ergometer (Monark Exercise, Varberg, Sweden) at a pedaling rate of 60 rpm, using an incremental load protocol. Power output was set up in a stepwise fashion by 60 watts at the end of each 4 min, starting at 60 watts. Respiratory gas exchange, heart rate, and subjective rating of perceived exertion were determined during the final 30 s of each power output. The test was continued until the subject could not maintain 60 rpm, or volitional exhaustion. During each test, oxygen uptake was measured using the open circuit spirometric method, and the highest value was defined as the VO2 peak.

Glycogen supercompensation protocol

The protocol consisted first in a depletion phase based on performing an exercise session until exhaustion (exhausting exercise), then a repletion phase based on a specific diet highly enriched in carbohydrate (19). During the latter period, subjects daily performed light exercise (training exercise) to minimize the detraining effect of rest.

Dietary regimen

Dietary records were maintained 3 days before the study and were verified through interviews with a nutritionist until completion of the study. Subjects were required to abstain from coffee or any beverage containing caffeine during that entire period. Once participants were admitted to the Yale/New Haven Hospital (day (0), a weight-maintaining diet of 45 Cal/kg BW, prepared by the metabolic kitchen of the General Clinical Research Center, was given. During a preloading period (day 0), the diet consisted of 44.1 ± 0.2% carbohydrate, 18.1 ± 0.1% protein, and 37.8 ± 0.3% fat. During the loading period (days 1–4), carbohydrate intake was augmented to 80.0 ± 0.4% (10.0 ± 0.1% protein, 9.9 ± 0.5% fat), whereas during the postloading period (days 4–7), subjects consumed a 54.5 ± 2.4% carbohydrate–augmented diet (17.8 ± 0.1% protein, 26.6 ± 1.4% fat). During the carbohydrate-loading and postloading periods, subjects were instructed to consume 3 and 2 bottles/day, respectively (17 bottles in total), of a glucose polymer beverage (CarboForce, American Body Builders, Salt Lake City, UT) in addition to their regular diet. Each supplemental beverage contained 105 g carbohydrate (1220 Cal/day). As a result, the daily caloric intake for the subjects averaged 3568 ± 89 Cal during the preloading period, 5724 ± 56 Cal during the loading period, and 5798 ± 103 Cal during the postloading period, of which 44%, 80%, and 56% were estimated to be carbohydrates, respectively.

Exhausting exercise

On day 1, 12 of the 20 subjects underwent a depletion exercise aimed at decreasing glycogen in the vastus lateralis muscle. The exercise consisted of 120 min of pedaling on a cycle ergometer (Monark) at a power output preset to elicit 65% of the VO2 peak (70 rpm). After 5 min of rest, subjects performed 1-min sprints (120% of VO2 peak at 100 rpm) followed by 1-min rest periods. Bouts were repeated until subjects could not maintain the prescribed pedal rate for the full minute of exercise. Instead of the depletion, the 8 remaining subjects performed only 20 min of cycle exercise at 65% of the VO2 peak (70 rpm) on day 1.

Training exercise

From days 2–6, subjects performed 20 min of cycling/day at 65% of the VO2 peak to prevent a detraining effect of rest. Subjects were then instructed to consume one bottle of the glucose polymer beverage within 15 min after this exercise.

Caffeine trial (day 7)

The study was performed in a double blind fashion. Subjects were randomly assigned to receive at time zero 3 capsules containing either caffeine (total dose, 6 mg/kg BW; 10 subjects) or a nonnutritive placebo (10 subjects) with 300 mL water. This dose of caffeine corresponds to about 3 cups of coffee (20). At 90 min, subjects performed 2 h of cycling at 65% VO2 peak (70 rpm).

Plasma sampling

Fasting blood samples were collected before and immediately after the 2-h exercise and the sprint session on day 1. The same procedure was adopted on the caffeine trial day (day 7), e.g. before and 90 min after caffeine was ingested and immediately after the 2-h cycling session.

In vivo NMR spectroscopy

Natural abundance 13C NMR spectroscopy was performed daily to noninvasively examine the time course of muscle glycogen over the carbohydrate loading period. In addition, we measured changes in muscle glycogen concentration induced by the exhausting exercise and the exercise session involved in the caffeine trial. Measurements were conducted in a 2.1-T Bruker (Billericia, MA) model BioSpec Products, Inc., spectrometer with a 1.8-mm diameter magnet bore as previously described (21). During the measurements, subjects remained supine with the stage of a radiofrequency (RF) probe resting above the quadriceps muscle. The probe consisted of a 9-cm diameter inner coil for 13C acquisition and a 13-cm outer butterfly coil for 1H acquisition, image-guided positioning, and decoupling. Proton water line widths were shimmed to less than 50 Hz. Subjects were positioned by an image-guided localization routine and used a TI-weighted gradient-echo image. The subject’s leg was typically positioned so that the observation volume was about 1 cm into the vastus lateralis muscle. A microsphere containing 99% 13C-enriched formic acid as a reference standard was fixed at the center of the double tuned RF coil for calibration of RF pulse widths. By determining the 180° flip angles at the center of the observation coil from the microsphere standard, RF pulse widths were set so that the 90° pulse was sent to the center of the muscle. This setting maximized suppression of the lipid signal that arises from the sc fat layer and optimized signal from the muscle. 13C spectra were obtained with a pulse-acquiring sequence in 10-min blocks consisting of 5500 scans using a 90° pulse at coil center and a repetition time of 120 ms. Decoupling at the 1H frequency at a power of 15 watts was applied at the C1 proton resonance frequency during the 256-s acquisition period. Power deposition, assessed by calculation of the magnetic vector potential specific absorption rate, was less than 4 watts/kg. Intramuscular glycogen concentrations were determined by comparison with an external standard solution (150 mmol/L glycogen and 50 mmol/L KCl). 13C spectra were processed by methods previously described (22, 23). Briefly, Gaussian broadened spectra (30 Hz) were baseline corrected by ±300 Hz on either side of the [1-13C]glucose resonance of both subject spectra and standard spectra. Peak areas were then assessed at ±150 Hz of the resonance. Greater precision in measuring the exercise-induced change in muscle glycogen concentration was gained using differential spectral analysis. The 1H NMR glycogen signal was corrected for the sensitive volume of the 13C coil. An image of the glycogen phantom solution was acquired using a dedicated proton coil of the same size as the 13C coil (fully relaxed gradient-echo sequence with a 90° excitation pulse). The sensitive volume of that image was manually drawn and compared with the set of images previously recorded from each individual by means of the butterfly coil. The corresponding filling coefficient was calculated according to the ratio of the.

CAFFEINE AND EXERCISE 2171
regions of interest defined from the leg muscles and the in vitro dataset (1.18, on the average). In addition, the $^{13}$C NMR signal was corrected for the load of the $^{13}$C coil. A fully relaxed spectrum (four pulses; repetition time, 15 s; pulse length, 100 $\mu$s) of the formic acid sphere was recorded both in vivo and in the presence of the phantom solution of glycogen. The ratio of the formate peak area obtained in both conditions was used as the loading correction factor (0.91, on the average). The $^{13}$C NMR technique for assessing glycogen concentrations has been validated in situ in frozen rabbit muscle (24) and by comparison with human gastrocnemius muscle biopsies (25).

**Analytical procedures**

Plasma glucose was measured by the glucose oxidase method (Beckman Coulter, Inc., glucose analyzer, Fullerton, CA). Plasma immunoreactive insulin, glucagon, cortisol, and human $\beta$-endorphin concentrations were measured using commercially available double antibody RIA kits (insulin: Diagnostics Systems Laboratories, Inc., Webster, TX; glucagon: Linco Research, Inc., St. Charles, MO; cortisol: Diagnostic Products, Los Angeles, CA; $\beta$-endorphin: Nichols Institute Diagnostics, San Juan Capistrano, CA). Plasma lactate concentrations were measured by the lactate dehydrogenase method. Plasma free fatty acids were measured using a microfluorometric assay. Plasma catecholamine concentrations were determined using a three-step procedure involving absorption onto alumina at pH 8.6, elution with a dilute acid, and analysis by high performance liquid chromatography.

**Statistical analyses**

All values are expressed as the mean ± se. A one-way ANOVA with repeated measurements was used to analyze the time course of glycogen supercompensation. When significant changes were obtained over time, post-hoc comparisons were made using a paired $t$ test. Pairwise comparisons for muscle glycogen, plasma substrate, and hormone concentrations to analyze responses to exercise sessions and caffeine intake were made using paired and unpaired $t$ tests when appropriate. Significant differences between groups were accepted at the $P < 0.05$ level.

**Results**

**Muscle glycogen supercompensation time course (Fig. 1)**

On day 1, performing the exhausting exercise caused the glycogen concentration to decrease by 58% in the vastus lateralis muscle. Rapid glycogen resynthesis occurred at about 2.2 mmol/L muscle $\cdot$ h during the first 24-h recovery period, then slowed to approximately 0.6 mmol/L muscle $\cdot$ h ($P < 0.0001$ vs. rapid recovery) from day 2 to day 4. The muscle glycogen concentration remained high over the 3-day postloading period, with peak levels measured on day 6 ($P < 0.0001$ vs. baseline). On the last day of the experiment, elevated muscle glycogen concentrations ($P < 0.05$ vs. baseline) were also measured in subjects who did not perform the exhausting exercise but completed the 7-day high carbohydrate diet ($n = 8$ nondepleted subjects), although muscle glycogen supercompensation was less than that in the predepleted subjects (nondepleted subjects, 107 ± 5 mmol/L muscle; predepleted subjects, 137 ± 8 mmol/L muscle; $P < 0.05$).

**Effect of caffeine on muscle glycogen content (Fig. 2)**

On the trial day (final exercise bout on day 7), there was no difference in exercise-induced muscle glycogen depletion between predepleted and nondepleted subjects regardless of caffeine ingestion [caffeine group, $-51 \pm 6$ mmol/L muscle in nondepleted subjects and $-61 \pm 9$ mmol/L muscle in predepleted subjects ($P = NS$); placebo group, $-57 \pm 11$ mmol/L muscle in nondepleted subjects and $-56 \pm 6$ mmol/L muscle in predepleted subjects].
mmol/L muscle in predepleted subjects (P = NS). Therefore, to increase the statistical power, we pooled values obtained from nondepleted and predepleted subjects (Fig. 2). As a result, muscle glycogen levels were similar in both caffeine and placebo groups before starting the 2-h exercise session. After this exercise, there was again no difference in muscle glycogen depletion between the caffeine and placebo groups.

Effect of caffeine on hormone/substrate concentrations during exercise (Figs. 3 and 4)

Plasma β-endorphin were measured after 2 h of cycling at 65% of the VO₂ peak on day 1 and was found to be unchanged (Fig. 3). However, performing sprints until exhaustion resulted in a 3.3-fold increase in plasma β-endorphin levels (Fig. 3). In addition, stimulation of the corticoadrenal axis occurred in the predepleted group (n = 12) after the 2-h cycling session, as reflected by an increase in plasma catecholamine and cortisol concentrations (epinephrine, 217 ± 34 pg/mL; norepinephrine, 857 ± 120 pg/mL; cortisol, 32 ± 2 μg/mL; P < 0.001 vs. baseline values) with a further increase after the sprint session (epinephrine, 336 ± 83 pg/mL; norepinephrine, 1372 ± 159 pg/mL; cortisol, 39 ± 2 μg/dL; P < 0.001 vs. baseline values). Ninety minutes after caffeine ingestion, there was an increase in plasma epinephrine and cortisol concentrations (P < 0.01 vs. baseline), whereas plasma glucose (~5.5 mmol/L), free fatty acids (280–300 mmol/L), lactate (0.6–0.8 mmol/L), insulin (9–12 μU/mL), glucagon (51–63 pg/mL), and β-endorphin (20–30 pg/mL) concentrations remained unchanged. At the same time, the plasma norepinephrine concentration increased (P < 0.05) in both groups (+242 ± 73 pg/mL for caffeine vs. +109 ± 31 pg/mL for placebo; P = NS). Cycling for 2 h at 65% of the VO₂ peak resulted in a similar increase in plasma free fatty acid concentrations in both groups, whereas there was no

Fig. 3. Changes in plasma β-endorphin concentrations in response to 2 h of cycling at 65% of the VO₂ peak followed by sprints until exhaustion. Data were obtained from subjects who performed both sprints until exhaustion on day 1 and ingested caffeine on day 7 (six subjects). Values are expressed as the mean ± SEM. ***, P < 0.001.

Fig. 4. Effects of caffeine ingestion (■) or placebo (□) on plasma free fatty acids, epinephrine, norepinephrine, cortisol, and β-endorphin concentrations before and after performing 2-h cycling at 65% of the VO₂ peak. Values are expressed as the mean ± SEM. *, P < 0.05; **, P < 0.01; ***, P < 0.001.
substantial change in plasma glucose, triglyceride, and insulin concentrations. By contrast, caffeine ingestion before the exercise resulted in higher increments from baseline in plasma lactate (+1.03 ± 0.19 mmol/L for caffeine vs. +0.14 ± 0.18 mmol/L for placebo; P < 0.005), cortisol (+12 ± 3 mg/mL for caffeine vs. −2 ± 2 mg/mL for placebo; P < 0.005) and epinephrine (+223 ± 82 pg/mL for caffeine vs. +56 ± 26 mmol/L for placebo; P < 0.05) concentrations than in the placebo group (Fig. 4). In addition, a 1.8-fold increase (P < 0.05) in the plasma β-endorphin concentration was observed in the caffeine group, whereas there was no change in the placebo group (Fig. 4).

Discussion

Caffeine is well known to enhance exercise performance (3, 4, 20, 26–28). However, the mechanism by which this occurs is not clear. Previous studies have suggested that caffeine promote lipolysis, which, in turn, spares muscle glycogen and results in greater endurance (4, 6). Contrary to these results, we found that ingestion of caffeine (6 mg/kg) in glycogen-supercompensated subjects did not affect the rate of muscle glycogen utilization or free fatty acid concentrations as previously reported (29, 30). This suggests that caffeine-induced alteration in substrate metabolism is unlikely to be the explanation for its exercise-enhancing effects.

It is unclear why we did not observe a glycogen-sparing effect of caffeine when it was ingested before the exercise, in contrast to other studies (4, 6). It is possible that muscle glycogen supercompensation in our subjects negated the lipolytic and glycogen-sparing effect of caffeine. These differences may also be due to the shorter duration (30–90 min) of the previous studies (2, 31), especially as one report found a glycogen-sparing effect of caffeine confined to only the initial 15 min of exercise (6). However, it is difficult to reconcile this finding with a caffeine-enhancing endurance effect, because prolonging the exercise to 120 min resulted in similar amounts of glycogen depletion regardless of caffeine ingestion. It is also possible that higher concentrations of plasma catecholamines during exercise may have counterbalanced the glycogen-sparing effect of caffeine. Indeed, epinephrine is well known to stimulate muscle glycogenolysis during exercise (19, 32), although not necessarily at rest (33). Finally, needle biopsy techniques, as used in the previous studies, are painful, causing endogenous catecholamine release and are prone to sampling errors due to the small amount of tissue obtained. In this respect, natural abundance 13C NMR estimates of muscle glycogen concentration are probably more precise than those obtained from biopsies. Indeed, the detection threshold for minimal change is approximately 5 mmol glycogen/L muscle, which is about 7% of the baseline concentration. This is almost 30% less than the variation reported previously using biopsies (34).

In contrast to the lack of effect of caffeine on muscle glycogen metabolism, we found that caffeine ingestion before exercise caused an approximately 1.8-fold increase in plasma β-endorphin concentrations and an approximately 1.6-fold increase in plasma cortisol concentration after cycling at 65% of the VO2 peak for 2 h, whereas no change in these hormones were found in the placebo group. The increase in the β-endorphin concentration was in the same range as in previous studies that have demonstrated an exercise-induced increase in β-endorphin release (5, 9, 35, 36). Although we also observed no increase in β-endorphin release in subjects performing the same intensity and duration of exercise on the first day of the supercompensation protocol, we found that plasma β-endorphin concentrations increased about 3.5-fold following sprints after the 2-h exercise at 65% of the VO2 peak. This is consistent with the well known increase in plasma β-endorphin concentrations after intense exercise (11, 37, 38). Taken together, these data suggest that caffeine helps to promote exercise performance by lowering the threshold for exercise-induced β-endorphin release. An increase in β-endorphin is well known to enhance exercise performance through its ability to decrease pain perception and promote euphoria (39). Furthermore, administrating naloxone, an opiate antagonist, during exercise clearly results in decreased exercise performance (40). There is evidence suggesting that caffeine acts as a central nervous system stimulant, primarily through competitive adenosine receptor blockade (41). It is possible that caffeine ingestion promotes corticotropin-releasing factor release from the hypothalamus through this mechanism, which, in turn, increases POMC release, resulting in increased β-endorphin release.

It is noteworthy that our data on hormonal and metabolic changes are consistent with results obtained in a recent study in which subjects responded to caffeine ingestion with an increase in plasma epinephrine at exhaustion and a prolonged exercise time even though plasma insulin and metabolite concentrations were not affected (28).

In summary, caffeine ingestion 90 min before prolonged exercise did not exert a muscle glycogen-sparing effect in glycogen-supercompensated athletes. In contrast, caffeine ingestion before exercise had a marked effect to increase plasma concentrations of epinephrine, cortisol, and β-endorphins in response to exercise. These data suggest that caffeine may lower the threshold for exercise-induced β-endorphin and cortisol release, which may contribute to the reported benefits of caffeine on exercise endurance.

Acknowledgments

We express our gratitude to the nursing staff, the dietary staff, and the Core Laboratory of the Yale-New Haven Hospital General Clinical Research Center for their assistance with the studies. In addition, we thank Dr. Douglas L. Rothman and Mr. Terry Nixon for their help with the NMR spectroscopy.

References