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ORIGINAL ARTICLE

Metabolic effects of caffeine ingestion and physical work in 75-year old citizens. A randomized, double-blind, placebo-controlled, cross-over study

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Summary

Objective Whereas caffeine has been demonstrated to impact substantially on the metabolic response to exercise in healthy young subjects, this issue remains to be addressed in healthy elderly subjects.

Design and participants The metabolic response to caffeine ingestion (6 mg/kg) and exercise in healthy elderly citizens at 70 years was examined in a randomized, double-blind, placebo-controlled, cross-over study. We included 30 subjects attending for driver license renewal at their general practitioner. Participants abstained from caffeinated drinks and food for 48 h and were randomized to receive placebo-caffeine or caffeine-placebo with 1 week between sessions.

Measurements A cycling endurance test at 65% of the expected maximal heart rate was performed 1 h after intervention. Blood samples were taken before intervention, before cycling, after 5 min of cycling, and at exhaustion. Analysis was by intention-to-treat and $P < 0{\cdot}05$ was regarded as significant.

Results Caffeine significantly increased the concentration of plasma epinephrine (by 42%, 39%, and 49%), serum-free fatty acids (by 53%, 44%, and 50%), and plasma lactate (by 46%, 36%, and 48%), and insulin resistance (homeostasis model assessment-IR) (by 21%, 26%, and 23%) during rest, after 5 min of cycling, and at exhaustion. At exhaustion, the concentration plasma norepinephrine was elevated by 29%. A decrease was seen with caffeine treatment in blood potassium after 5 min of cycling and at exhaustion (by 3% and 2%, respectively).

Conclusions Caffeine treatment increased epinephrine, fatty acids, lactate and norepinephrine at different times during test session and led to insulin-resistance.

Hence, caffeine ingestion elicits a similar metabolic response in elderly participants at 70 years old to that seen in younger subjects.

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Introduction

In young healthy adults, the metabolic changes following caffeine ingestion and physical work are well described. Caffeine ingestion increases the plasma concentration of epinephrine both at rest[1–3] and during exercise,[1,4–7] whereas the plasma concentration of norepinephrine is unaffected in most studies[1,6–8] Caffeine also increases the plasma concentration of free fatty acids (FFA),[3,8–15] glycerol,[1–3] glucose,[11–13,16–18] insulin[2,18] and lactate[3,11–13,16–18] during exercise in some, but not all[1,8,10,19–22] studies. One study compared the metabolic effects of caffeine ingestion in younger men and elderly men and found that older men showed a smaller increase in fatty acid availability after caffeine ingestion without any relation to alterations in norepinephrine kinetics or fat oxidation.[23]

As the population of elderly with a physically active lifestyle is growing rapidly, and as caffeine is found not only in coffee and tea, but also in many carbohydrate-rich energy drinks the metabolic effect of caffeine ingestion and physical work in the elderly is of interest. However, the effect of caffeine on physical performance and metabolic response in the elderly has previously not been examined.

We previously reported the effects of caffeine on physical performance in the elderly.[24] The main physical results found were an increase in cycling endurance and isometric arm flexion endurance with caffeine compared to placebo. Caffeine also reduced the rate of perceived exertion after 5 min of cycling and postural stability with eyes open.

Now, we report the metabolic response to caffeine ingestion (6 mg/kg) and exercise in healthy elderly citizens at 70 years of age.

Materials and methods

Participants

Healthy elderly individuals aged 70 years were included, when coming for a health evaluation at their general practitioner prior to driver license renewal. Recruitment was performed by 15 general practitioners in Herning, Denmark between July 2002 and October 2003. Subjects with minor disabilities such as hypertension treated with angiotensin-converting enzyme inhibitors or diuretics, well-treated asthma, and slight osteoarthritis were also considered suitable for inclusion.[24]

Exclusion criteria were dementia or invalidating psychiatric disease; general debility, angina or other diseases that would render participation in the test program impossible; treatment with beta receptor-blocking drugs, calcium-channel blocking drugs, digitalis, or nitroglycerine; acute disease, e.g. infection, and injury; diabetes; conditions that would contraindicate caffeine ingestion or participation in the test program; treatment with medication that interacts with caffeine, e.g. theophylline; and ingestion of caffeine-containing drinks and foods less than 48 h before each session.[24]

Written informed consent was obtained from all participants in accordance with the Helsinki Declaration II. The study was approved by the National Board of Health and the Regional Ethical Committee, and was monitored by the Good Clinical Practice Unit at Aarhus University Hospital, Denmark.[24]

Procedures

This randomized, double-blind, placebo-controlled, cross-over study was carried out at the Surgical Research Unit, Herning Hospital, Denmark. Participants were included consecutively until 30 subjects had completed the two test sessions.[24]

Randomization was stratified by sex and carried out using sealed envelopes in blocks of four and six to allocate the participants to placebo-caffeine or caffeine-placebo treatment with 1 week between interventions. The pharmacy department of Herning Hospital produced a white capsule containing 6 mg/kg caffeine (Unikem A/S, Copenhagen, Denmark) and a placebo capsule (containing glucose monohydrate). The pharmacy department packed the medication according to randomization. Participants and investigators were unaware of treatment allocation at all times. Code-breaking sheets for emergency use were kept at the pharmacy department, but were never used.[24]

Participants avoided caffeine-containing drinks and food for 48 h before each visit and were recommended a carbohydrate-rich diet on the preceding day and up until 2 h before each session. Capsules were taken with a glass of water and the subject rested for 1 h before a cycling endurance test at 65% of the expected maximal heart rate was performed.[24] Blood samples were taken before intervention, before cycling, after 5 min of cycling and at exhaustion.

Endurance was measured on a cycle ergometer (Ergoline Ergometrics 900, Kivex A/S, Denmark), with an increasing load of 25 W every second minute until 65% of the maximal heart rate (220 minus age) was reached. The load remained at this level and subjects cycled until exhaustion.[24]

Corpuscular haemoglobin concentration (mM) was analysed by photometry (Advia 120, Bayer A/S Diagnostics, Lyngby, Denmark) with an intra- and inter-assay CV of 1·8%.

White blood cells (10^9/l) (intra-assay CV = 2·7% and inter-assay CV = 3·7%) and platelets (10^9/l) (intra assay CV = 2·9% and inter assay CV = 3·4%) were registered optically (Advia 120, Bayer A/S Diagnostics, Lyngby, Denmark).

Plasma albumin (g/l) was analysed using colour-binding reflectometry (intra assay CV = 1·2%, inter-assay CV = 1·7%), plasma creatinine (μM) was analysed by enzymatic reflectometry (intra assay CV = 1·0%, inter assay CV = 2·2%), and sodium-ion concentration in plasma (mM) was analysed by selective ion-electrode with an intra- and inter-assay CV of 0·6% (Vitros 950/250, Johnson & Johnson, Ortho Clinical Diagnostics, Birkerod, Denmark).

Concentrations of plasma potassium-ion (mM) (intra assay CV = 1·5%, inter assay CV = 0·6%), calcium ion (mM) (intra- and inter-assay CV = 1%), lactate-ion (mM) (intra-assay CV = 3%, inter-assay CV = 2%), and glucose (mM) (intra-assay CV = 2·8%, inter-assay CV = 1·5%) were analysed using a selective ion-electrode (ABL 735, Radiometer, Bronshoj, Denmark).

Free fatty acids (mM) were analysed with Wako NEFA *C*-test kit (Waco Chemicals USA, Inc., USA) which utilizes an *in vitro* enzymatic colourimetric method for the quantification of FFAs in serum. The intra-assay CV was 1–2·5% and the inter assay CV < 3% (the samples were analysed in the same test kit).

Plasma insulin (pM) was analysed by a two-site immunoassay ELISA[25] with an intra assay CV of 5–7% and a inter assay CV of 4–9%.

Plasma epinephrine (nM) and plasma norepinephrine (nM) were analysed by high-pressure liquid chromatography (HPLC) with electronically detection (Millipore Ltd, Watford, Herts, UK).[26] The intra-assay CV was 6·4% for norepinephrine and 9·1% for epinephrine concentrations > 0·2 nM and 18·2% for epinephrine concentrations < 0·2 nM. The inter assay CV were 15·6% for norepinephrine and 25% for epinephrine with a detection limit of 0·02 nM norepinephrine and 0·05 nM epinephrine.

The used method for the caffeine (μM) measurements was a slightly modified version of a previously published procedure.[27] In the modified method, the plasma proteins are precipitated by addition of trifluoroacetic acid and spun down. The supernatant is neutralized by addition of buffer before injection onto the HPLC. The intra-assay CV was < 6·5%. The inter-assay CV was for a single analysis < 6·5% and for duplicate analyses < 4·8%. The method has been validated for analysis of caffeine and its metabolites in urine but not in blood.

Data were entered before treatment codes were broken, and analysed on an intent-to-treat basis according to a pre-established analysis plan.

Statistical analysis

Data were $\log_e$ transformed and the distribution was evaluated visually and by Shapiro-Wilk's test for normality. Normally distributed data were analysed by the two sample *t*-test for unpaired data; otherwise we used the Mann–Whitney test for unpaired data.

Participants were separated into two groups; group A (placebo-caffeine) and group B (caffeine-placebo). The presence of a treatment effect was tested by comparing the difference in response (period 1–period 2) between groups A and B. Half of the difference in response for group A plus group B estimated the magnitude of the treatment effect, thus taking the different treatment order into account. Analyses were carried out using STATA software version 7·0 (StataCorp 2001, College Station, Texas, USA), and reported *P* values < 0·05 was regarded as being statistically significant.

Results

The effects of caffeine ingestion on endurance, muscular performance, rate of perceived effort, balance, walking speed and psychomotor function are reported separately.[24]

Table 1. Subject characteristics

	Total	Male	Female	*P* value
Participants	30	15	15	–
Age (years)*	74·7 (5·5)	75·9 (5·8)	73·6 (5·1)	0·26
Height (cm)*	164·3 (9·2)	170·9 (5·8)	158·6 (6·1)	< 0·0001
Weight (kg)*	72·1 (13·4)	77·5 (13·3)	66·8 (11·5)	0·03
Regular coffee intake (cups per day)*	4·5 (3·3)	5·6 (2·5)	3·5 (3·8)	0·08
Physical activity† per week (h)*	3·0 (2·6)	3·1 (2·6)	3·0 (2·7)	0·93
Body mass index (m^2/kg)*	26·7 (4·0)	26·5 (4·0)	26·8 (4·0)	0·83
Systolic blood pressure (mmHg)*	143 (23)	150 (23)	143 (23)	0·22
Diastolic blood pressure (mmHg)*	90 (15)	90 (15)	90 (15)	0·79
Heart rate (min^{-1})*	84 (15)	83 (14)	85 (15)	0·65

*Mean (SD).
†Cycling, walking, swimming, gymnastics.

One hundred thirty-four participants were initially assessed for eligibility; of these, 40 participants were included and 30 were randomized. Baseline values were similar in the two groups and are summarized in Table 1. Mean daily coffee intake was 4·5 cups of coffee corresponding to approximately 486 mg caffeine. Two of the 30 participants were caffeine nonusers.

The median concentration of caffeine was 0·3 μM (95% CI: 0·3–0·3) at rest and increased to 48–57 μM (95% CI: 41–66) during the caffeine trial, whereas the median caffeine concentration remained unchanged during the placebo trial.

In the caffeine trial, mean systolic blood pressure before cycling test was 11% higher and the heart rate was 6% higher than in the placebo trial.

No differences in the metabolic values were found between caffeine and placebo before intervention. The main results are shown in Fig. 1.

For plasma calcium-ion, a decrease of 1% ($P = 0·04$) was seen with caffeine compared to placebo after 5 min of cycling, whereas there were no differences for calcium ion between caffeine and placebo at rest and at exhaustion.

Homeostasis model assessment-insulin resistance (HOMA-IR) was calculated (p-insulin concentration (mU/l) multiplied by glucose concentration (mmol/l) and divided by 22·5)[28] and reported in Fig. 1.

Metabolic values were also analysed adjusted by gender; No significant differences were found between placebo and caffeine trials for insulin, glucose, HOMA-IR, norepinephrine, epinephrine and potassium when adjusted by gender. Differences between male and female participants were found in the placebo trial for FFAs and in the caffeine trial for FFA, caffeine, and calcium-ion. Plasma concentrations of calcium-ion were 3% higher for men than women at exhaustion. Mean values of FFA and lactate for men and women are shown in Table 2.

Discussion

In this study we examined the metabolic response to caffeine (6 mg/kg) ingestion and exercise in healthy elderly citizens aged 70 years.

Analyses of plasma caffeine showed that participants actually did abstain from caffeinated drinks and food before trial.

Caffeine treatment increased plasma epinephrine concentration at rest (by 42%) and during exercise at 5 min cycling and at exhaustion (by 39% and 49%, respectively). This result is in agreement with former studies in younger persons. For example, in a study by Graham *et al.*,[3] circulating epinephrine concentration was significantly increased at rest in young male subjects who ingested caffeine 6 mg/kg and then exercised 1 h at 70% of maximal oxygen consumption (VO_2, max), and Van Soeren *et al.*[5] found that after ingestion of caffeine 5 mg/kg and 1 h of steady-state exercise at 50% maximal VO_2, the plasma epinephrine was elevated in nonusers after 30 min of exercise with caffeine, whereas there was no effect on plasma epinephrine in habitual caffeine users. The mechanism by which caffeine affects plasma epinephrine is most likely by increasing epinephrine secretion directly and also by increasing the sympatic nervous system itself by acting as an adenosine receptor antagonist.[3] A primary role of adenosine in the central nervous system appears to be to inhibit the release of various neurotransmitters through presynaptic receptors, and hence caffeine can be suspected to increase the release of neurotransmitters.[29]

In this study, there were no differences between caffeine and placebo treatment for plasma norepinephrine at rest and after 5 min of cycling, but at the time of exhaustion, plasma norepinephrine concentration was significantly greater (+29%) during the caffeine than the placebo trial, although plasma concentration of norepinephrine did rise throughout the test period for both treatments.

Caffeine significantly increased he concentration of serum FFAs (s-FFA) at rest, after 5 min exercise and at exhaustion (by 53%, 44% and 50%, respectively) with caffeine compared to placebo treatment, although there was a small rise in FFA during the placebo trial. The increase in FFA concentration before and during exercise in the caffeine trial is probably the result of the effect of caffeine as an adenosine receptor antagonist.[11,20] Adenosine receptors are found in most tissues, including lipocytes and skeletal muscles[16] and activation of A_1-receptors leads to inhibition of lipolysis.[30]

HOMA-IR calculated from results of plasma glucose and insulin was found to be significantly elevated before cycling, after 5 min of cycling, and at exhaustion (21%, 26% and 23%, respectively) with caffeine treatment compared to treatment with placebo. As

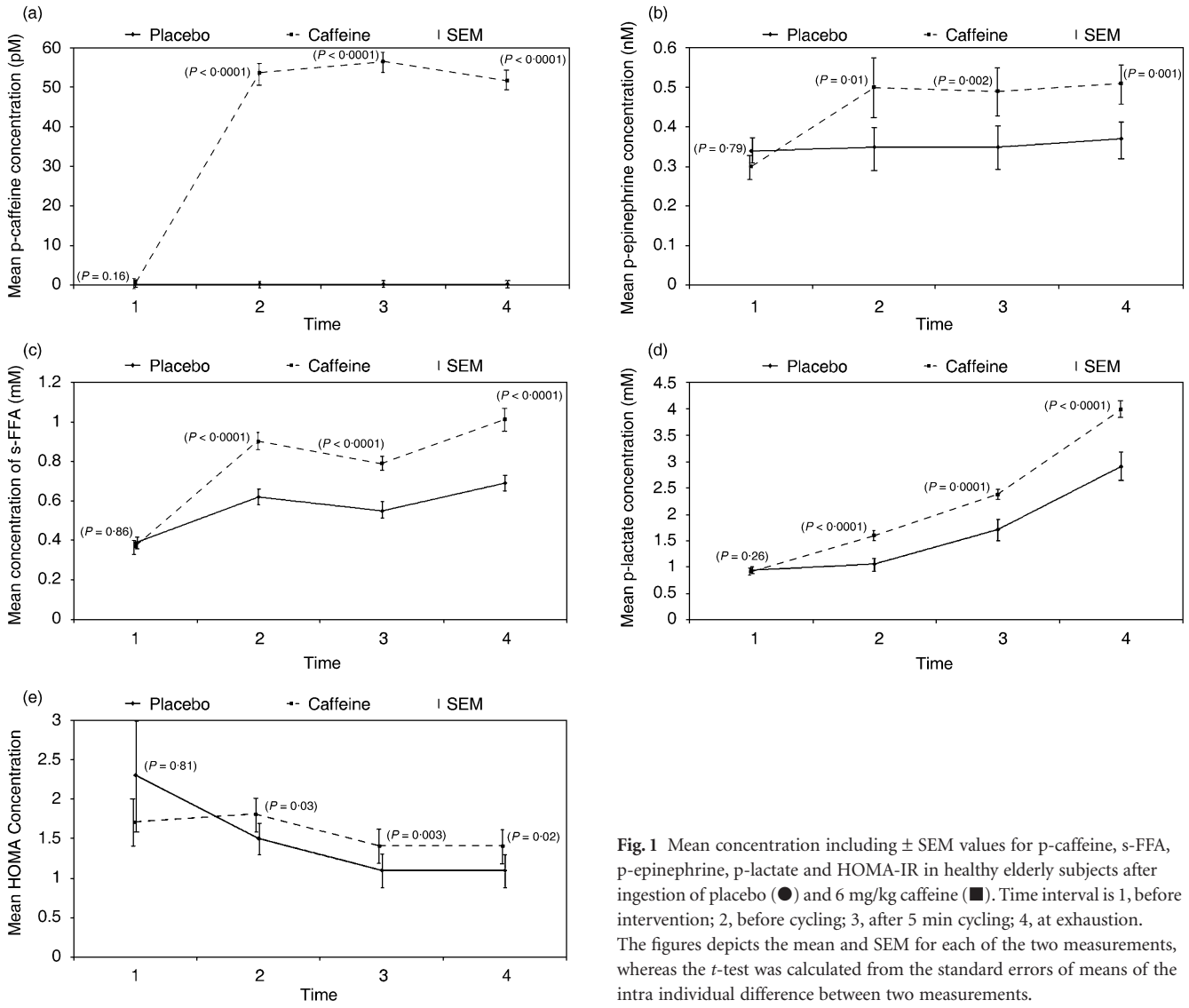

Fig. 1 Mean concentration including ± SEM values for p-caffeine, s-FFA, p-epinephrine, p-lactate and HOMA-IR in healthy elderly subjects after ingestion of placebo (●) and 6 mg/kg caffeine (■). Time interval is 1, before intervention; 2, before cycling; 3, after 5 min cycling; 4, at exhaustion. The figures depicts the mean and SEM for each of the two measurements, whereas the *t*-test was calculated from the standard errors of means of the intra individual difference between two measurements.

Table 2. Main results adjusted by gender

		Placebo Mean (± SEM)			Caffeine		
Time	Variable	M	F	*P* value‡ Mean (± SEM)	M	F	*P* value‡
Before intervention	FFA (mM)	0·30 (0·05)	0·48 (0·05)	0·02	0·30 (0·05)	0·43 (0·05)	0·06
	Lactate (mM)	0·98 (0·10)	0·91 (0·07)	0·54	0·92 (0·08)	0·89 (0·07)	0·81
Before cycling*	FFA (mM)	0·52 (0·05)	0·71 (0·07)	0·04	0·75 (0·05)	1·06 (0·06)	0·0006
	Lactate (mM)	1·01 (0·07)	1·10 (0·08)	0·41	1·46 (0·12)	1·73 (0·23)	0·32
After 5 min cycling	FFA (mM)	0·50 (0·04)	0·60 (0·06)	0·14	0·66 (0·06)	0·92 (0·05)	0·0006
	Lactate (mM)	1·43 (0·11)	2·07 (0·18)	0·005	2·18 (0·26)	2·55 (0·21)	0·28
At exhaustion	FFA (mM)	0·59 (0·05)	0·79 (0·07)	0·04	0·92 (0·12)	1·12 (0·08)	0·20
	Lactate (mM)	2·69 (0·43)	3·10 (0·41)	0·50	3·74 (0·61)	4·21 (0·46)	0·55

*approximately 11/2 hour after intervention.
†*P* values signify the comparison between males and females in the placebo and caffeine group, respectively.
M, male; F, female.

HOMA-IR is an indicator of insulin resistance, it seems that caffeine has an effect on the insulin–glucose pathway. This effect may be related to the effect of caffeine on epinephrine, as caffeine ingestion leads to a reduction in insulin-mediated glucose uptake as a result of insulin resistance[2,31] probably mediated by elevated epinephrine,[32] as caffeine had no effect on plasma epinephrine and plasma insulin concentration during exercise in spinal cord injured patients.[33]

Plasma lactate concentration increased in both the placebo and the caffeine trial although subjects only cycled at 65% of expected maximal heart rate corresponding to approximately 55% VO_2, max. In this study, the range of 95% confidence interval for median has a maximum plasma lactate concentration at exhaustion of 4·0 mM and 4·8 mM for placebo and caffeine, respectively. A p-lactate concentration of 4·0 mM is usually regarded as the anaerobic threshold.[34] Hence, it seems that although subjects exercised in the range of only 55% VO_2 max, some probably did reach their anaerobic threshold, although the threshold of lactic acid in the elderly in a former study by Brubaker *et al.*[35] has been shown to reach 6·6 mM.

During exercise, the concentration of plasma potassium ion increased in both the caffeine and placebo trials, although the rise was significantly dampened with caffeine compared to placebo after 5 min of cycling and at exhaustion (by 3% and 2%, respectively). A lower extracellular potassium concentration, together with a possible higher intracellular potassium concentration may contribute to the known ergogenic effect of caffeine by maintaining membrane potential in contracting muscle,[6] although it is questionable whether a reduction in potassium of 2–3% would be of a physiological relevance in this matter.

When adjusting for gender in the analyses of caffeine and placebo trials, there was a significant difference between men and women in FFA, caffeine, lactate and calcium-ion concentrations. In the caffeine trial, mean calcium-ion concentration at exhaustion was 3% higher for women than for men, although such a small increase may not have any clinical consequences. A more clear difference between female and male participants in the caffeine trial was found for s-FFA before cycling and after 5 min of cycling, where mean FFA concentration was significant higher for women than for men (57% and 29%, respectively). Also in the placebo trial, the female participants have a significantly higher mean FFA concentration than the males (67% before intervention, 40% before cycling, and 33% at exhaustion) (Table 2). This result suggests that the difference in FFA for men and women is basal and not the result of treatment with caffeine. The mean concentration of caffeine after 5 min of exercise was also 23% higher in the female participants, which could be an indicator for a slower degradation of caffeine in women than men during exercise, although there was no difference for gender in mean caffeine concentration at exhaustion.

In this study, we found that after 48 h of caffeine withdrawal, ingestion of 6 mg/kg caffeine led to a similar metabolic response to exercise in the elderly participants to that found in earlier studies in younger subjects, including an increase in epinephrine, FFA, lactate and insulin-resistance. The long-term health consequences of an increased insulin resistance during caffeine treatment is of interest, but cannot be deduced from a short-term experimental study such as this. Furthermore, this study was conducted in persons fasting for caffeine for 48 h. As many people are daily caffeine consumers, it would be of interest to examine whether an overnight caffeine fast would elicit a similar metabolic response.

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References

1 Graham, T.E. & Spriet, L.L. (1991) Performance and metabolic responses to a high caffeine dose during prolonged exercise. *Journal of Applied Physiology*, **71**, 2292–2298.
2 Graham, T.E., Sathasivam, P., Rowland, M., Marko, N., Greer, F. & Battram, D. (2001) Caffeine ingestion elevates plasma insulin response in humans during an oral glucose tolerance test. *Canadian Journal of Physiological Pharmacology*, **79**, 559–565.
3 Graham, T.E., Helge, J.W., MacLean, D.A., Kiens, B. & Richter, E.A. (2000) Caffeine ingestion does not alter carbohydrate or fat metabolism in human skeletal muscle during exercise. *Journal of Physiology*, **529**, 837–847.
4 Bangsbo, J., Jacobsen, K., Nordberg, N., Christensen, N.J. & Graham, T. (1992) Acute and habitual caffeine ingestion and metabolic responses to steady-state exercise. *Journal of Applied Physiology*, **72**, 1297–1303.
5 Van Soeren, M.H., Sathasivam, P., Spriet, L.L. & Graham, T.E. (1993) Caffeine metabolism and epinephrine responses during exercise in users and nonusers. *Journal of Applied Physiology*, **75**, 805–812.
6 Lindinger, M.I., Graham, T.E. & Spriet, L.L. (1993) Caffeine attenuates the exercise-induced increase in plasma [K+] in humans. *Journal of Applied Physiology*, **74**, 1149–1155.
7 Jackman, M., Wendling, P., Friars, D. & Graham, T.E. (1996) Metabolic catecholamine, and endurance responses to caffeine during intense exercise. *Journal of Applied Physiology*, **81**, 1658–1663.
8 Tarnopolsky, M.A., Atkinson, S.A., MacDougall, J.D., Sale, D.G. & Sutton, J.R. (1989) Physiological responses to caffeine during endurance running in habitual caffeine users. *Medicine and Science in Sports and Exercise*, **21**, 418–424.
9 Van Soeren, M.H. & Graham, T.E. (1998) Effect of caffeine on metabolism, exercise endurance, and catecholamine responses after withdrawal. *Journal of Applied Physiology*, **85**, 1493–1501.
10 Poehlman, E.T., LaChance, P., Tremblay, A., Nadeau, A., Dussault, J., Theriault, G., Despres, J.P. & Bouchard, C. (1989) The effect of prior exercise and caffeine ingestion on metabolic rate and hormones in young adult males. *Canadian Journal of Physiological Pharmacology*, **67**, 10–16.
11 Graham, T.E. & Spriet, L.L. (1995) Metabolic, catecholamine, and exercise performance responses to various doses of caffeine. *Journal of Applied Physiology*, **78**, 867–874.

12 Bell, D.G., Jacobs, I. & Zamecnik, J. (1998) Effects of caffeine, ephedrine and their combination on time to exhaustion during high-intensity exercise. *European Journal of Applied Physiology and Occupational Physiology*, **77**, 427–433.
13 Kovacs, E.M., Stegen, J.H.C.H. & Brouns, F. (1998) Effect of caffeinated drinks on substrate metabolism, caffeine excretion, and performance. *Journal of Applied Physiology*, **85**, 709–715.
14 Chesley, A., Howlett, R.A., Heigenhauser, G.J., Hultman, E. & Spriet, L.L. (1998) Regulation of muscle glycogenolytic flux during intense aerobic exercise after caffeine ingestion. *American Journal of Physiology*, **275**, R596–R603.
15 Bruce, C.R., Anderson, M.E., Fraser, S.F., Stepto, N.K., Klein, R., Hopkins, W.G. *et al.* (2000,) Enhancement of 2000-m rowing performance after caffeine ingestion. *Medicine and Science in Sports and Exercise*, **32**, 1958–1963.
16 Graham, T.E. (2001) Caffeine and exercise: metabolism, endurance and performance. *Sports Medicine*, **31**, 785–807.
17 Bell, D.G., Jacobs, I. & Ellerington, K. (2001) Effect of caffeine and ephedrine ingestion on anaerobic exercise performance. *Medicine and Science in Sports and Exercise*, **33**, 1399–1403.
18 Collomp, K., Candau, R., Millet, G., Mucci, P., Borrani, F., Prefaut, C. & De Ceaurriz, J. (2002) Effects of salbutamol and caffeine ingestion on exercise metabolism and performance. *International Journal of Sports Medicine*, **23**, 549–554.
19 Graham, T.E., Sathasivam, P. & MacNaughton, K.W. (1991) Influence of cold, exercise, and caffeine on catecholamines and metabolism in men. *Journal of Applied Physiology*, **70**, 2052–2058.
20 Costill, D.L., Dalsky, G.P. & Fink, W.J. (1978) Effects of caffeine ingestion on metabolism and exercise performance. *Medicine and Science in Sports*, **10**, 155–158.
21 Denadai, B.S. & Denadai, M.L. (1998) Effects of caffeine on time to exhaustion in exercise performed below and above the anaerobic threshold. *Brazilian Journal of Medical and Biological Research*, **31**, 581–585.
22 Greer, F., McLean, C. & Graham, T.E. (1998) Caffeine, performance, and metabolism during repeated Wingate exercise tests. *Journal of Applied Physiology*, **85**, 1502–1508.
23 Arciero, P.J., Gardner, A.W., Calles-Escandon, J., Benowitz, N.L. & Poehlman, E.T. (1995) Effects of caffeine ingestion on NE kinetics, fat oxidation, and energy expenditure in younger and older men. *American Journal of Physiology*, **268**, E1192–E1198.
24 Norager, C.B., Jensen, M.B., Madsen, M.R. & Laurberg, S. (2005) Caffeine improves endurance in 75-year old citizens. A randomized, double-blind, placebo-controlled, cross-over study. *Journal of Applied Physiology*, **99**, 2302–2306.
25 Andersen, L., Dinesen, B., Jorgensen, P.N., Poulsen, F. & Roder, M.E. (1993) Enzyme immunoassay for intact human insulin in serum or plasma. *Clinical Chemistry* **39**, 578–582.
26 Carstensen, E. & Yudkin, J.S. (1994) Platelet catecholamine concentrations after short-term stress in normal subjects. *Clinical Science*, **86**, 35–41.
27 Weinmann, S., Siscovick, D.S., Raghunathan, T.E., Arbogast, P., Smith, H., Bovbjerg, V.E., Cobb, L.A. & Psaty, B.M. (1997) Caffeine intake in relation to the risk of primary cardiac arrest. *Epidemiology*, **8**, 505–508.
28 Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F. & Turner, R.C. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, **28**, 412–419.
29 Nehlig, A., Daval, J.L. & Debry, G. (1992) Caffeine and the central nervous system: mechanisms of action, biochemical, metabolic and psychostimulant effects. *Brain Research. Brain Research Review*, **17**, 139–170.
30 Olah, M.E. & Stiles, G.L. (1995) Adenosine receptor subtypes: characterization and therapeutic regulation. *Annual Review of Pharmacological Toxicology*, **35**, 581–606.
31 Lee, S., Hudson, R., Kilpatrick, K., Graham, T.E. & Ross, R. (2005) Caffeine ingestion is associated with reductions in glucose uptake independent of obesity and type 2 diabetes before and after exercise training. *Diabetes Care*, **28**, 566–572.
32 Thong, F.S. & Graham, T.E. (2002) Caffeine-induced impairment of glucose tolerance is abolished by beta-adrenergic receptor blockade in humans. *Journal of Applied Physiology*, **92**, 2347–2352.
33 Mohr, T., Van Soeren, M., Graham, T.E. & Kjaer, M. (1998) Caffeine ingestion and metabolic responses of tetraplegic humans during electrical cycling. *Journal of Applied Physiology*, **85**, 979–985.
34 Bauer, T. & Weisser, B. (2002) [Effect of aerobic endurance exercise on immune function in elderly athletes]. *Schweiz Rundsch Medical Prax*, **91**, 153–158.
35 Brubaker, P.H., Marburger, C.T., Morgan, T.M., Fray, B. & Kitzman, D.W. (2003) Exercise responses of elderly patients with diastolic versus systolic heart failure. *Medicine and Science in Sports and Exercise*, **35**, 1477–1485.