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ORIGINAL ARTICLE

Metabolic and physiological effects of ingesting extracts of bitter orange, green tea and guarana at rest and during treadmill walking in overweight males

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Objective: This study examined the acute effects of ingesting a widely used commercial formula containing extracts of bitter orange, green tea and guarana (Gx) on the metabolic rate and substrate utilisation in overweight, adult males at rest (study 1) and during treadmill walking (study 2).
Subjects: Two different groups of 10 sedentary males with more than 20% body fat participated in studies 1 and 2.
Design: In each study, subjects participated in two experimental trials during which they were given two 500 mg capsules containing either Gx or a placebo (P) in a counterbalanced double-blind manner. Doses of the main active ingredients were 6 mg of synephrine, 150 mg caffeine and 150 mg catechin polyphenols.
Measurements: In study 1, subjects completed 7 h supine rest with baseline measures taken during the first hour, with expired gases, blood pressure, heart rate and venous blood being collected every 30 min for the remaining 6 h following ingestion of Gx or P. In study 2, subjects exercised for 60 min at 60% heart rate reserve following ingestion of Gx or P 1 h previously. Venous blood samples were collected twice at rest and at 5, 10, 15, 20, 30, 40, 50 and 60 min, with expired gas measurements taken at 4, 9, 14, 19, 29, 39, 49 and 59 min. In both studies, venous blood was analysed for NEFA, glycerol, glucose and lactate concentrations, while expired gases were used to calculate ATP production from carbohydrate and NEFA, as well as the total substrate utilised.
Results and conclusion: The results did not show any significant effect of Gx ingestion on total ATP utilisation during 6 h rest or during 60 min treadmill walking. Changes were observed in the relative contributions of CHO and NEFA oxidation to ATP production in both studies, such that there was an increase in ATP production from CHO and a decrease from NEFA. The increase in CHO oxidation was shown to be as high as 30% at rest.
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Keywords: bitter orange; green tea; caffeine; metabolic rate and substrate utilization; dietary supplements

Introduction

A positive energy balance is associated with weight gain and ultimately obesity[1] and can arise from an elevated energy intake, a reduced energy expenditure or a combination of the two. Spraul *et al.*[2] identified a role for reduced sympathetic nervous system activity in bodyweight gain, which increases susceptibility to obesity. Recent data has suggested that obese individuals exhibit significantly lower sympathetic nervous system activity in response to various physiological challenges, such as food intake[3] and capsaicin intake.[4]

These results would suggest that overweight individuals might be treated effectively with the use of sympathomimetic agonists. Indeed, several commercially available supplements, containing ephedrine and ephedra, have been used for this purpose with some success.[5] However, recent reports have identified numerous possible problems with these supplements including an increased risk of psychiatric, autonomic and gastrointestinal symptoms, as well as heart palpitations.[5] These problems have led to the development of non-ephedra-based supplements containing bitter orange (also known as citrus aurantium), green tea-leaf extract and guarana (Gx).

Bitter orange extract contains a combination of five adrenergic amines: synephrine, *N*-methyltyramine, hordenine, octopamine, and tyramine.[6] These amines are thought

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to stimulate β3-adrenoreceptors, which have been implicated in the control of various metabolic processes by catecholamines.[7] Bitter orange ingestion is thought to result in catecholamine release in the vicinity of β3-adrenoreceptors located primarily in the adipose tissue and liver. As a result, bitter orange ingestion is purported to promote weight loss and increased thermogenesis,[5] while having a minimal effect on blood pressure and heart rate.[8]

Green tea-leaf extract contains several polyphenolic compounds that help to prevent the degradation of nor-adrenaline.[9] Green tea and Gx also provide a source of caffeine (a methylxanthine), which is often added to weight loss supplements to enhance plasma catecholamine levels and inhibit transcellular phosphodiesterase. Thus, the combination of these polyphenolic compounds and caffeine are reported to be effective in stimulating thermogenesis by removing the inhibition at different control points along the nor-adrenaline–cAMP axis.[9] Dulloo *et al.*[10] showed that 100 mg of caffeine resulted in a 3–4% increase in metabolic rate over 150 min. In a later study, Dulloo *et al.*[11] showed an increase in energy expenditure (an average of 4% over 24 h) and a 40% increase in urinary nor-adrenaline secretion over the same time period following ingestion of green tea extract.

A shift towards positive energy balance may also reflect reduced physical activity levels.[1] Bell *et al.*[12] reported an attenuated sympathetic nervous system activity in those with a sedentary lifestyle. Indeed, recent data suggest that exercise significantly improves sympathetic nervous system activity in obese subjects.[13] In addition, exercise (particularly at moderate intensities of $\sim$65% $VO_{2\,peak}$) is associated with a higher rate of fat metabolism.[14]

However, stimulation of sympathetic activity by pharmacological means could affect both carbohydrate (CHO) and fat metabolism and it is by no means clear which effect will dominate. This could define the usefulness of commercial formula as an aid to weight loss. The aim of the present investigation was achieved by conducting two separate studies to examine the acute effects of ingesting a widely used commercial formula containing extracts of bitter orange, green tea and guarana on metabolic rate and substrate utilisation in overweight, adult males at rest (study 1) and during treadmill walking at 60% heart rate reserve (study 2).

Methods

Subjects

Ten sedentary, overweight, non-smoking, male subjects participated in study 1 (mean $\pm$ s.d. age 25 $\pm$ 4 years; height: 1.72 $\pm$ 0.07 m and body mass 83.9 $\pm$ 16.2 kg), with a different cohort of 10 sedentary, overweight, male subjects participating in study 2 (mean $\pm$ s.d. age 27 $\pm$ 7 years, height: 1.71 $\pm$ 0.1 m, body mass 87.9 $\pm$ 17.1 kg). Selection criteria for inclusion into both studies were (a) body fat in excess of 20%, (b) sedentary and (c) clinically normal when assessed using 12-lead ECG, blood screening and health history questionnaire. The Ethics Review Committee of University of Chichester approved both studies and all subjects provided their written informed consent before participation.

Experimental design

Study 1. Each subject participated in a preliminary assessment and two experimental trials, with subjects being given either Gx (Xenadrine-EFX™, Cytodyne Technologies Inc., Table 1) or a matching placebo (P) in each trial. The preliminary assessment was always conducted first in order to confirm that subjects met the selection criteria for the study. The effects of Gx and P on metabolic rate and substrate utilisation were examined over 7 h following the ingestion of a light breakfast. Throughout, subjects were tested in pairs, with one administered Gx and the other P, using a counterbalanced, double-blind, experimental design.

Study 2. Each subject participated in one preliminary assessment and two experimental trials (Gx and P). The preliminary assessment was always conducted first in order to confirm that subjects met the selection criteria for the study. In each experimental trial (separated by a minimum of 48 h) subjects exercised for 60 min at 60% of their maximal heart rate reserve (HRR) following the ingestion of either Gx (Table 1) or P. Trials were conducted using a counterbalanced, double–blind, experimental design.

Table 1 Table of ingredients

Ingredient	*Amount per capsule*
Vitamin C	100 mg
Vitamin B6	10 mg
Pantothentic acid	12 mg
Magnesium	10 mg
Thyroplex proprietary blend 1415 mg, containing	
Bitter orange	
Green tea extract	
Gx	
L-Tyrosine	
Acetyl-L-tyrosine	
Theobroma cacao extract	Standardised for synephrine 3 mg, caffeine 75 mg and catechin polyphenols 75 mg
DL-Methionine	
Ginger Root	
Quercetin	
Fisetin	
2-Dimethylaminoethanol	
Grape seed extract	
Pyridoxine Hydrochloride	

Proprietary ingredients were standardised for caffeine, synephrine and catechin polyphenols, which was confirmed by independent analysis from Phoenix Laboratories Ltd, Farmingdale, NY, USA.

Experimental procedures study 1

Baseline resting metabolic rate (BRMR). Before the start of the first experimental trial, subjects reported to the laboratory at 0900 hours for a 1 h baseline assessment following an overnight fast. With the subject resting in the supine position, expired gases were collected into Douglas bags over 5 min, separated by 10 min intervals (between 20–25 and 30–35 min). During this time, the mouthpiece and nose-clip remained in position. A third 5 min expired gas collection was taken (between 40 and 45 min) if the estimates of oxygen consumed (VO_2) from the first two collections differed by more than 5%.[15] BRMR was calculated from the two lowest consecutive values obtained in each case. Heart rate and blood pressure were recorded immediately after each period of gas collection.

Experimental trials. For the two trials, subjects reported to the laboratory at 1100 hours following a standard breakfast taken at 0700 hours (comprising two slices of toast with jam, two boiled eggs, orange juice and water). Subjects were asked to refrain from alcohol, caffeine and exercise for 48 h before each laboratory visit. In addition, subjects were requested to complete a 24 h dietary record on the day before the first laboratory visit and to follow the same dietary pattern before the second visit. Compliance with these experimental controls was verbally confirmed with the subjects before commencing each experimental session.

Height and body mass were recorded, a heart rate monitor (Polar Night Vantage, Oy, Finland) fitted and an indwelling cannula inserted into a forearm vein on each attendance. Subjects underwent 7 h of supine rest in an environmental chamber set at 24°C and 50% relative humidity. During the first hour of supine rest, expired gases were collected between 25–30 and 55–60 min. Following the 60 min gas collection, subjects orally ingested either two 500 mg capsules of Gx or a matching P. Thereafter, expired gases, blood pressure, heart rate and venous blood (10 ml) were collected over the final 5 min in every 0.5 h for the remaining 6 h.

Experimental procedures study 2

Preliminary assessment. Subjects reported to the laboratory at 0900 hours for baseline assessment, following an overnight fast. Subjects were rested in the supine position for 0.5 h, following which ECG and blood pressure measurements were taken. Skinfold thicknesses at four sites (bicep, tricep, sub-scapula and supra-iliac) were measured, using Harpenden skinfold callipers, for the subsequent calculation of body fat percentage.[16] Subjects were also exercised on a treadmill in order to determine the treadmill speed and gradient eliciting 60% of maximal heart rate reserve.

Experimental trials. In conditions Gx and P, subjects reported to the laboratory at 1100 hours following a standard breakfast taken at 0700 hours (comprising two slices of toast with spread and jam, two boiled eggs, orange juice and water). Subjects were asked to refrain from alcohol, caffeine and exercise for 48 h before each laboratory visit. In addition, subjects were requested to complete a 24 h dietary record on the day before the first laboratory visit and to follow the same dietary pattern before the second visit. Compliance with these experimental controls was verbally confirmed with the subjects before commencing each experimental session.

Upon presentation to the laboratory, subjects height and body mass were recorded, a heart rate monitor (Polar Night Vantage, Oy, Finland) fitted and an indwelling cannula inserted into a prominent forearm vein. The cannula was maintained patent by infusion of saline (without the addition of heparin).

Following 30 min rest, a pre-exercise blood sample (10 ml) was drawn for measurement of NEFA, glycerol, glucose and lactate. Subjects then ingested either Gx or P and further blood samples were taken at 30 and 5 min before exercise. Subjects exercised on a treadmill for 60 min, at the predetermined speed and incline sufficient to elicit 60% of maximal heart rate reserve. Further, blood samples (10 ml) were taken at 5, 10, 15, 20, 30, 40, 50 and 60 min and expired air (inspiration to inspiration) was collected between 4–5, 9–10, 14–15, 19–20, 29–30, 39–40, 49–50 and 59–60 min.

General methods

Oxygen consumption, estimation of resting metabolic rate and substrate utilisation. Expired gases were collected using Douglas bags and were analysed by first saturating with water by passing through 50 cm of Nafian tubing (Omnifit Ltd, Cambridge, UK) immersed in distilled water. Thereafter, expired gases were passed through a Bühle condenser at 5.0 ± 0.1°C (Bühler PKE 3; Paterson Instruments, Leighton Buzzard, UK) and analysed as described by Draper *et al*.,[17] using a Servomex series 1400 Paramagnetic Oxygen Analyser (Servomex Plc., Crowborough, UK), a Servomex InfraRed Carbon Dioxide Analyser (Servomex Plc., Crowborough, UK) and a Harvard Dry Gas Meter (Harvard, Edenbridge, UK). Gas analysers were calibrated against 100% nitrogen (0% O_2) and atmospheric air, with a gas sample of known concentration (Linde Gas, Stratford, UK) being used to verify the calibration. Measured volumes of oxygen and carbon dioxide were adjusted to standard temperature and pressure dry. $\dot{V}O_2$ is the oxygen consumed (litres) and RER is the respiratory exchange ratio. The coefficient of variation of $\dot{V}O_2$, VCO_2 and RER, determined in a pilot study, was less than 5%.

Measurements of expired gases were used to calculate metabolic rates and rates of substrate oxidation, according to

$$\%O_2 \text{ utilised for CHO oxidation} = (\text{RER} - 0.696) \times 328.9 \quad (1)$$

mol ATP from oxidation of

$$\text{CHO}\,(ATP_{CHO}) = (\dot{V}O_2/22.415) \times (\%O_{2\,CHO}/100) \times 6.333 \quad (2)$$

$$\text{NEFA}\,(\text{ATP}_{\text{NEFA}}) = (\dot{V}\text{O}_2/22.415)\times(1-(\%\text{O}_{2\,\text{CHO}}/100))\times 5.609 \quad (3)$$

$$\text{Total}\,(\text{ATP}_{\text{TOTAL}}) = (2)+(3) \quad (4)$$

Equations (2) and (3) are based on the oxidation of glucose and palmitate as the sole substrates utilised. Equation (2) assumes 38 molATP and equation (3) 129 mol ATP generated per mol of glucose and palmitate oxidised, respectively. The amounts of CHO (glucose) and fat (palmitate) oxidised over each period were calculated from:

$$\text{g CHO} = (\dot{V}O2/22.415)\times(\%\text{O}_{2\,\text{CHO}}/100)\times 30 \quad (5)$$

$$\text{g NEFA} = (\dot{V}O_2/22.415)\times(1-(\%\text{O}_{2\,\text{CHO}}/100))\times 11.148 \quad (6)$$

The estimates assume that the oxidation of protein was minimal, and that in study 2, the blood lactate increase had minimal effect on blood pH and bicarbonate equilibrium. In the text, it was assumed that the free energy from ATP hydrolysis (ΔG) at circa pH 7 (i.e. metabolisable energy or ME) is 70 kJ/mol[18] assuming a ΔG^0 of -40.2 kJ.[19]

Plasma analyses. Each 10 ml venous blood sample was aliquoted into two separate blood collection tubes coated with EDTA. Samples were immediately centrifuged for 5 min at 5000 r.p.m. at a temperature of 4°C. Plasma was separated and stored at −80°C until required for analysis. Plasma NEFA and glycerol concentrations were determined using enzymic-colourimetric methods from Wako Chemicals (Neuss, Germany) and Randox Laboratories (Crumlin, Co Antrim, UK), respectively. Plasma glucose and lactate were analysed using an automatic analyser (YSI 2300 STAT, Yellow Springs Instruments, OH, USA).

Statistical methods. All data from both studies are presented as means ± s.d., with statistical significance accepted at the $P<0.05$ level, unless otherwise stated.

For study 1, changes in the concentrations of metabolites (NEFA, glycerol, glucose and lactate) over time were analysed by repeated measures ANOVA. Where an effect of treatment was observed (NEFA), the average concentration over the final 3 h of the study was computed for each subject and the mean within-subject difference was compared by Student's *t*-test for paired data.

The mean within-subject differences of the average rate of ATP turnover and total turnover for the stated period were analysed using Student's *t*-test for paired data. For purposes of clarity, rates of ATP turnover measured at the half-hourly times in study 1 have been omitted in Figures 1 and 2 (panel a in each figure). However, the half-hourly rates were used in calculating the total ATP turnover by the end of each hour (panel b in each figure), although ATP turnover by the end of each half-hour is itself again not shown. Similarly in Figures 3 and 4, rates at 5 and 15 min of exercise have been omitted for reasons of clarity (panel a in each figure), although these rates were included when calculating ATP used by the end of each 10th min of exercise (panel b in each figure).

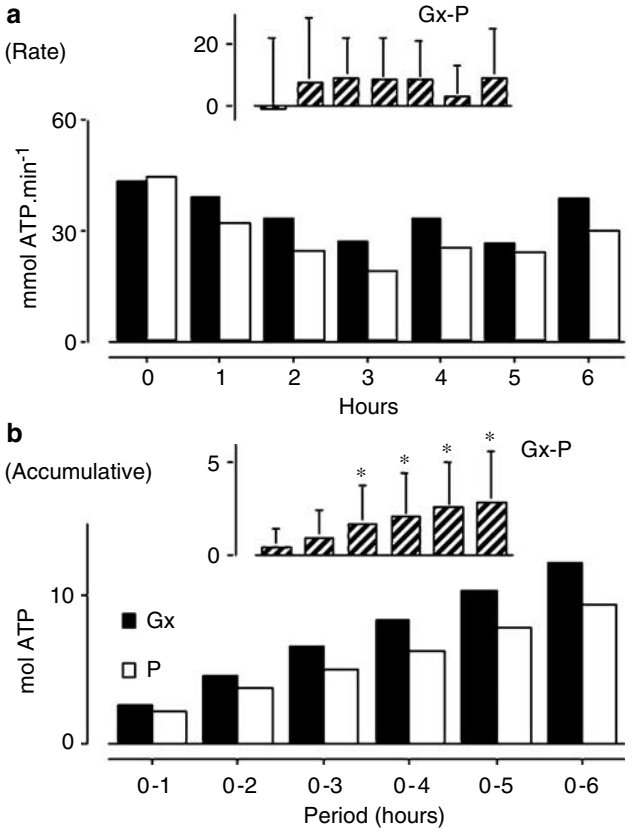

Figure 1 (**a**) The mean rate of ATP (mmol min^{-1}) production from carbohydrate oxidation (ATP_{CHO}) during 6 h of rest following administration of Gx or P at 0 h. The upper inset panel shows the mean (+s.d.) within-subject difference due to treatment at each of the time points. Half-hourly values have been omitted for reasons of clarity. (**b**) The mean total ATP_{CHO} (mol) produced by the end of each hour. The mean difference (+s.d.) between treatments in total ATP_{CHO} is shown in the lower inset panel. The effect of treatment was significant from 3 h onwards. *A Student's *t*-value of >2.306, which was significant ($P<0.05$) with 8 degrees of freedom. Half-hourly values are again not shown although half-hourly rates were used when calculating the total produced by the end of each hour.

Results

The results from one subject who consistently hyperventilated during collection of expired air and whose data for RER was erratic (with some values above 1.00), were omitted from the final analyses in both studies.

Study 1

BRMR following an overnight fast was 73.1 ± 7.9 mmol ATP min^{-1} (corresponding to an ME of 5.12 ± 0.55 kJ min^{-1}), with 56.0% of the ATP produced being from the oxidation of

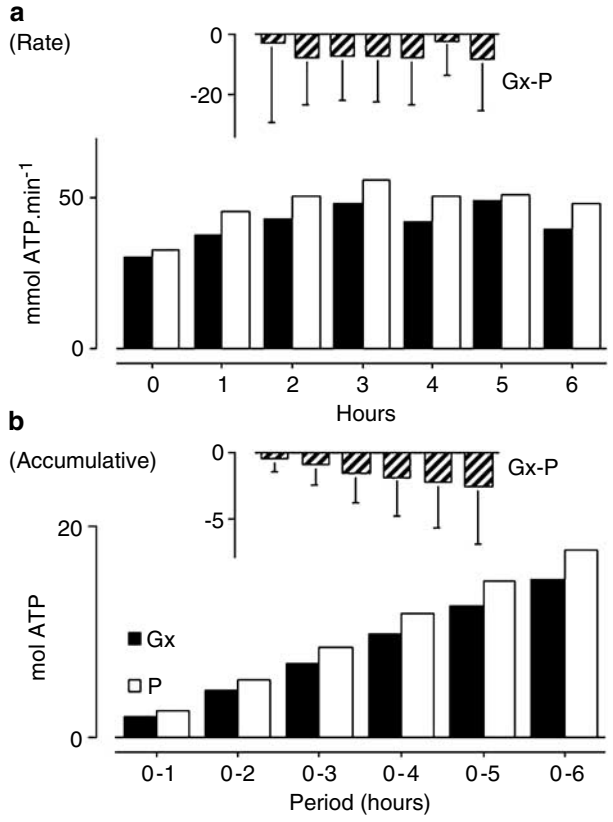

Figure 2 (**a**) The mean rate of ATP (mmol min^{-1}) production from NEFA oxidation (ATP_{NEFA}) during 6 h of rest following administration of Gx or P at 0 h. The upper inset panel shows the mean (−s.d.) within-subject difference due to treatment at each of the time points. Half-hourly values have been omitted for reasons of clarity. (**b**) Mean total ATP_{NEFA} (mol) produced by the end of each hour. The mean difference (−s.d.) between treatments in total ATP_{NEFA} is shown in the lower inset panel. Half-hourly values are again not shown, although half-hourly rates were used when calculating the total produced by the end of each hour.

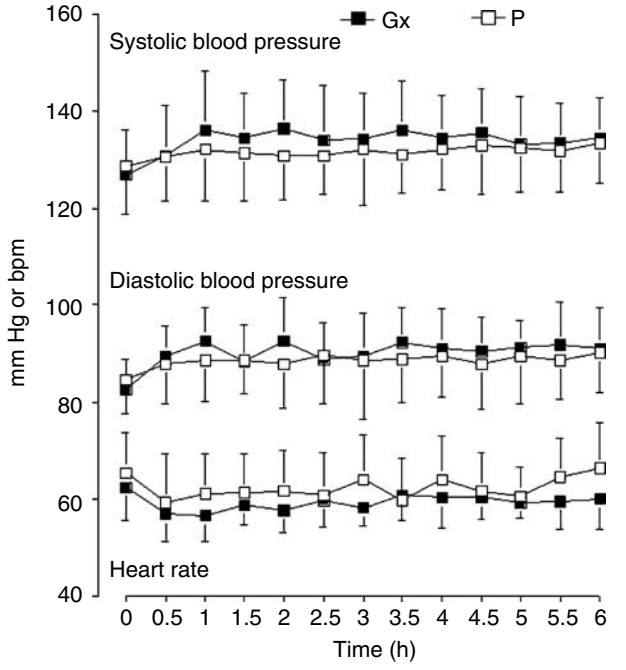

Figure 3 Systolic and diastolic blood pressure, and heart rate, during 6 h rest in the supine position, following administration of Gx or P at 0 h.

CHO (Table 2). Glucose and NEFA oxidation rates were 0.194 ± 0.074 and 0.064 ± 0.028 g min^{-1}. Mean total ATP turnover and CHO and fat oxidation rates at rest at the start of the two experimental sessions were close to the estimates of BRMR despite the intake of a light breakfast four hours earlier.

Mean (±s.d.) $\dot{V}O_2$ (ml min^{-1}) and RER following Gx or P are presented in Table 3. The estimated 1-min rate of ATP production from CHO oxidation ATP_{CHO}, measured every 30 min, decreased reaching a nadir with treatment P at 3 h (-25.5 ± 19.1 mmol ATP min^{-1}, $P<0.01$) (Figure 1, panel a). A similar decrease occurred with Gx but to a lesser extent. The influence of Gx on ATP_{CHO} appeared to be greatest between 1.5 and 3 h; the mean within-subject difference in rates was significant at 1.5 and 2.5 h. Total ATP_{CHO} over the 6 h, and the difference between treatments, are shown in Figure 1, panel b. The effect of Gx was significant ($P<0.05$) from 3 h onwards. Following Gx, an additional 13.3 ± 13.0 g of glucose was oxidised over the 6 h, with the production of an extra 2.80 ± 2.75 mol ATP.

In contrast, the rate of ATP production from NEFA oxidation (ATP_{NEFA}), in both conditions, increased throughout the 6 h period (Figure 2, panel b) and was comparable with the reduction in ATP_{CHO}. In condition P, the increase peaked at 3 h ($P<0.01$ compared to the 0 h rate). With Gx, the increase in ATP_{NEFA} was less, although the difference between treatments was not significant. Accumulative ATP_{NEFA} over the 6 h was not significantly different between Gx and P ($P>0.05$). The mean change in NEFA oxidation with Gx relative to P was -5.8 ± 13.7 g over the 6 h period.

The sum of ATP from CHO and NEFA oxidation (ATP_{TOTAL}) by the end of the 6 h was the same with the two treatments (Gx = 27.29 ± 3.47 and $P = 27.10 \pm 2.38$ mol).

Plasma NEFA concentrations increased following Gx and P over the 6 h (Table 4). Between 3–6 h the increase in plasma NEFA following Gx was as a mean $+76.3 \pm 59.2$ μmol l^{-1} greater than with P ($P<0.01$). Plasma glycerol increased with both treatments but in this case to an equal extent (Table 4). There was no effect of treatment on plasma glucose or lactate concentrations (Table 4). Similarly, there was no effect of treatment on blood pressure or heart rate (Figure 3).

Study 2

Changes in $\dot{V}O_2$ and RER during the 60 min walking exercise are shown in Table 5.

The onset of exercise resulted in a nine-fold increase in ATP_{CHO} from 42.0 ± 29.1 to 371.3 ± 105.1 mmol min^{-1} at

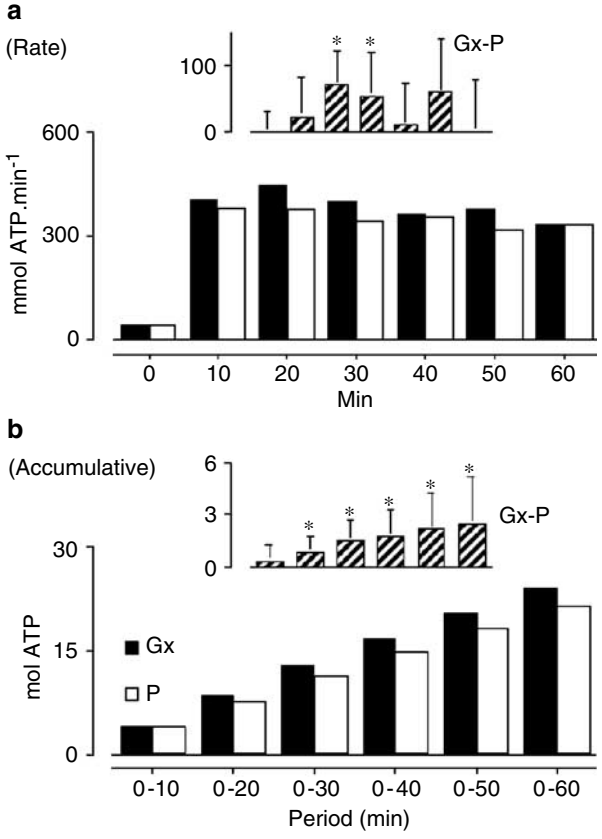

Figure 4 (**a**) The mean rate of ATP (mmol min^{-1}) production from carbohydrate oxidation (ATP_{CHO}) during 60 min of exercise following administration of Gx or P at 0 min. The upper inset panel shows the mean (+s.d.) within-subject difference due to treatment at each of the time points. Values recorded at 5 and 15 min have been omitted for reasons of clarity. The difference between treatments at 20 and 30 min was significant ($P<0.05$). (**b**) The mean total ATP_{CHO} (mol) produced by the end of each period of exercise. The mean difference (+s.d.) between treatments in total ATP_{CHO} is shown in the lower inset panel. The effect of treatment was significant from 20 min onwards. ($P<0.05$). Values at 5 and 15 min are again not shown, although the rates at these times were used when calculating the total produced by the end of 10 and 20 min, respectively.

5 min with treatment P. Thereafter, the ATP_{CHO} rate tended to fall reaching 334.0 ± 90.2 mmol min^{-1} at 60 min (Figure 4, panel a). Following ingestion of Gx, the rate of ATP_{CHO} was higher than with P during the 60 min walking exercise. The difference in treatments was significant ($P<0.05$) at the 20- and 30-min measurements. Total ATP_{CHO} was significantly greater with Gx from 20 min of exercise onwards (Figure 4, panel b), resulting in an additional utilisation of 2.47 ± 2.73 mol by the end of 60 min. This corresponds to an additional oxidation of 11.7 ± 12.9 g of CHO.

At the 5th min of exercise, ATP_{NEFA} was increased 4.5-fold in condition P from 38.3 ± 16.8 to 173.5 ± 61.6 mmol min^{-1}. Thereafter, the rate continued to increase reaching 267.5 ± 90.0 mmol min^{-1} at 60 min (Figure 5, panel a). Following Gx administration, the rate of ATP_{NEFA} was lower at each time point and was significant at 20- and 50-min

Table 2 $\dot{V}O_2$, RER and estimates of ATP from oxidation of carbohydrate (ATP_{CHO}) and NEFA (ATP_{NEFA}), as well as the actual amounts of carbohydrate and NEFA oxidised, per minute in the resting state following an overnight fast (BRMR), or, immediately before administration of Gx or P (0 h RMR)

	BRMR		*0 h RMR*	
	Mean	*±s.d.*	*Mean*	*±s.d.*
$\dot{V}O_2$ (ml min^{-1})	274	29	281	32
RER	0.863	0.061	0.866	0.041
CHO oxidized (g min^{-1})	0.194	0.074	0.208	0.059
NEFA oxidized (g min^{-1})	0.064	0.028	0.063	0.035
ATP_{CHO} (mmol min^{-1})	40.9	15.6	43.9	12.5

BRMR and 0 h RMR are the mean in each case of the average of two measurements made in each subject.

Table 3 $\dot{V}O_2$ and RER at each hour during 6 h rest in the supine position, following administration of Gx or P at 0 h

Time (h)	*0*	*1*	*2*	*3*	*4*	*5*	*6*
$\dot{V}O_2$ (ml min^{-1})							
Gx							
Mean	273	289	289	288	287	291	296
±s.d.	30	40	40	43	49	41	39
P							
Mean	289	297	287	289	291	290	298
±s.d.	44	34	35	25	37	27	36
RER							
Gx							
Mean	0.867	0.842	0.823	0.799	0.82	0.797	0.84
±s.d.	0.055	0.042	0.045	0.024	0.052	0.027	0.068
P							
Mean	0.864	0.814	0.789	0.768	0.792	0.786	0.806
±s.d.	0.072	0.051	0.039	0.045	0.038	0.03	0.034

To simplify the table, half-hourly values have been omitted, although these were used in the calculations of ATP turnover and substrate utilisation.

measurements. The effect was a lower total ATP_{NEFA}, with the difference between treatments (1.92 ± 2.23 mol) approaching significance ($0.06<P<0.08$) between the 30th and the 60th min (Figure 5, panel b). The estimated reduction in fat oxidised by 60 min was 3.8 ± 5.1 g.

Quantitatively, the fall in ATP_{NEFA} with Gx matched the increase in ATP_{CHO} with the result that total ATP utilised by the end of the 60 min was the same for the two treatments ($P = 34.9 \pm 3.9$ and Gx $= 35.4 \pm 3.3$ mol).

There was no effect of Gx on the exercise-induced increases in plasma glycerol or NEFA concentrations, indicating similar lipid mobilisation with Gx and P (Table 6). Similarly, there was no significant effect of Gx ingestion on plasma glucose and lactate concentrations (Table 6) or on heart rate.

Discussion

Gx had no effect on total ATP utilisation in the resting state. These results, based as they are on the use of a proprietary

Table 4 Plasma NEFA, glycerol, glucose and lactate concentrations during 6 h rest in the supine position, following administration of Gx or P at 0 h

Time (h)	*0*	*1*	*2*	*3*	*4*	*5*	*6*
NEFA (μmol l^{-1})							
Gx							
Mean	88	229	284	395	396	374	406
±s.d.	37	93	123	94	78	99	81
P							
Mean	81	171	218	288	282	293	303
±s.d.	37	67	60	73	68	72	84
Glycerol (μmol l^{-1})							
Gx							
Mean	312	551	570	653	701	676	609
±s.d.	129	206	206	182	210	189	165
P							
Mean	294	490	529	585	583	595	611
±s.d.	116	188	174	160	128	141	144
Glucose (mmol l^{-1})							
Gx							
Mean	5.28	5.17	5.11	5.01	4.96	4.85	4.83
±s.d.	0.40	0.25	0.33	0.23	0.26	0.25	0.24
P							
Mean	5.12	5.07	5.06	4.97	4.88	4.85	4.79
±s.d.	0.40	0.34	0.32	0.32	0.25	0.23	0.18
Lactate (mmol l^{-1})							
Gx							
Mean	0.73	0.74	0.79	0.81	0.78	0.86	0.85
±s.d.	0.28	0.29	0.32	0.32	0.30	0.34	0.34
P							
Mean	0.79	0.73	0.78	0.74	0.72	0.71	0.75
±s.d.	0.32	0.30	0.31	0.30	0.28	0.28	0.30

To simplify the table, half-hourly values have been omitted.

Table 5 $\dot{V}O_2$ and RER during 60 min walking exercise at 60% heart rate reserve, following administration of Gx or P at 0 min

Time (min)	*0*	*10*	*20*	*30*	*40*	*50*	*60*
$\dot{V}O_2$ (ml min^{-1})							
Gx							
Mean	273	2073	2129	2105	2068	2071	2132
+s.d.	29	307	297	343	394	380	434
P							
Mean	301	2045	2146	2078	2097	2094	2143
+s.d.	46	317	271	360	381	398	427
RER							
Gx							
Mean	0.862	0.905	0.915	0.886	0.876	0.886	0.858
+s.d.	0.060	0.035	0.032	0.045	0.035	0.035	0.031
P							
Mean	0.847	0.897	0.888	0.872	0.875	0.857	0.860
+s.d.	0.072	0.053	0.047	0.039	0.044	0.037	0.041

To simplify the table, values at 5 and 15 min have been omitted.

product (containing 6 mg of synephrine, 150 mg caffeine and 150 mg catechin polyphenols), differ from those of Dulloo *et al.*,[11] who reported an effect of +4% on 24 h energy expenditure using a combination of green tea extract and caffeine. Caffeine alone (100 mg) increased the metabolic rate by 3–4% over 150 min in lean and post-obese subjects, and by 8–11% over a 12 h period with repeated caffeine administration.[10] Similarly, Koot and Deurenberg[20] reported a 7% increase in RMR over 3 h with 200 mg caffeine. Bracco *et al.*[21] reported increases of 4.8% in obese and 7.6% in lean subjects during 24 h energy expenditure measurement following coffee ingestion. The increases in energy expenditure in these studies were attributed to greater lipid oxidation with or without any increase in CHO oxidation. Acheson *et al.*[22] observed a significantly increased metabolic rate following caffeine ingestion (4 and 8 mg kg^{-1}) in subjects of normal weight, which was accounted for by greater lipid oxidation, although they showed a reduced effect on fat oxidation in obese subjects. Similarly, Colker *et al.*[23] showed an average daily increase of 2–3% in basal

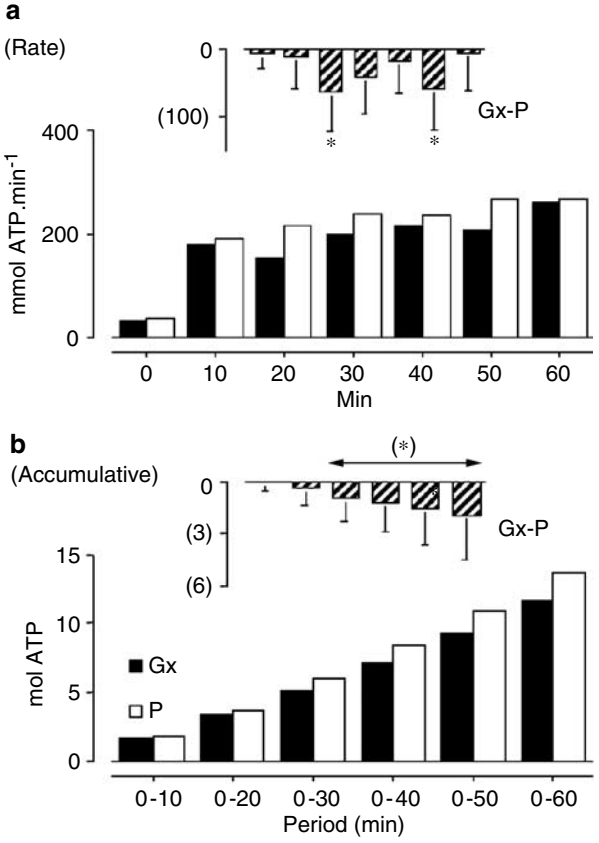

Figure 5 (**a**) The mean rate of ATP (mmol min^{-1}) production from NEFA oxidation (ATP_{NEFA}) during 60 min of exercise following administration of Gx or P at 0 min. The upper inset panel shows the mean (−s.d.) within-subject difference owing to treatment at each of the time points. Values recorded at 5 and 15 min have been omitted for reasons of clarity. The difference between treatments at 20 and 50 min was significant ($P<0.05$). (**b**) The mean total ATP_{NEFA} (mol) produced by the end of each period of exercise. The mean difference (−s.d.) between treatments in total ATP_{NEFA} is shown in the lower inset panel. The effect of treatment was just outside significance from 30 min onwards ($0.06<P$ 0.08). Values at 5 and 15 min are again not shown, although the rates at these times were used when calculating the total produced by the end of 10 and 20 min, respectively.

Table 6 Plasma NEFA, glycerol, glucose and lactate concentrations during 60 min walking exercise at 60% heart rate reserve, following administration of Gx or P at 0 min

Time (min)	*0*	*10*	*20*	*30*	*40*	*50*	*60*
NEFA (μmol l^{-1})							
Gx							
Mean	88.8	72.6	95.7	124.9	159.9	193.1	208.1
+s.d.	49.0	27.1	43.1	59.0	77.5	104.3	114.0
P							
Mean	81.0	68.8	98.8	134.0	160.4	195.0	210.6
+s.d.	57.5	35.9	51.2	66.0	76.5	82.1	85.4
Glycerol (μmol l^{-1})							
Gx							
Mean	38.8	60.9	85.2	111.8	140.6	163.1	172.6
+s.d.	14.3	17.7	32.6	40.5	48.7	59.3	59.5
P							
Mean	35.0	67.4	95.1	130.0	158.0	180.3	199.0
+s.d.	10.2	24.0	46.4	67.3	81.2	88.2	90.3
Glucose (mmol l^{-1})							
Gx							
Mean	5.15	4.58	4.63	4.71	4.71	4.79	4.72
+s.d.	1.14	1.19	1.25	1.36	1.40	1.43	1.46
P							
Mean	4.74	4.56	4.58	4.62	4.58	4.58	4.57
+s.d.	1.48	1.38	1.43	1.51	1.50	1.50	1.48
Lactate (mmol l^{-1})							
Gx							
Mean	1.33	1.98	1.73	1.57	1.44	1.41	1.32
+s.d.	0.50	0.77	0.70	0.72	0.73	0.70	0.61
P							
Mean	1.13	1.58	1.55	1.24	1.07	1.02	1.00
+s.d.	0.53	0.53	0.84	0.47	0.37	0.26	0.22

To simplify the table, values at 5 and 15 min have been omitted.

metabolic rate following the ingestion of 975 mg of citrus aurantium and 528 mg of caffeine when compared to both placebo and control conditions.

Gx again appeared to have no effect on total ATP utilisation during light exercise (study 2). Based on the use of a proprietary product with several active ingredients (6 mg of synephrine, 150 mg caffeine and 150 mg catechin polyphenols), these results differ from those of Engels *et al.*,[24] who reported a significant effect of caffeine ingestion (5 mg kg^{-1}) on the rate of energy expenditure during 60 min exercise at 30% $\dot{V}O_{2max}$, although there was no change in RER. Engels *et al*. used higher doses of caffeine than were administered in this study (150 mg) but exercised subjects at a lower intensity. In respect of the other ingredients present in Gx, the authors are not aware of any studies investigating the effects of green tea or bitter orange ingestion on energy expenditure during exercise.

While Gx appeared to have no effect on total energy expenditure in either condition, it did appear to stimulate ATP production from CHO oxidation relative to treatment P, which in both conditions was balanced by a fall in ATP from fat oxidation. In the resting, state the net gain in ATP_{CHO} over the 6 h corresponded to an additional 13.3 ± 13.0 g of CHO oxidised over and above a total of 44.7 ± 12.1 *g* oxidised during this period with P (a 30% increase). The decline in ATP_{NEFA} over the same 6 h corresponded to a reduction of 5.8 ± 13.7 g of fat oxidised against 35.1 ± 7.4 g oxidised during this period with P (a 13% decrease). The greater increase in the plasma NEFA concentration observed with Gx could be interpreted as resulting from both an increase in lipid mobilisation or a decrease in oxidation. However, given the similar increases in plasma glycerol and the apparent fall in ATP_{NEFA} with Gx, then the latter would seem the most likely explanation. The trend towards higher plasma lactate concentrations following Gx is again indicative of an increase in CHO metabolism, possibly in muscle.

Despite an almost identical metabolic rate with Gx and P in study 2, there was again a significant increase in the contribution of CHO oxidation to total energy expenditure. This coincided with a reduction in the contribution from fat oxidation. Thus, in both resting (study 1) and exercising (study 2) conditions, the ingestion of Gx resulted in an increase in the relative contribution of CHO to energy expenditure, and a decrease in fat oxidation. In study 2, the change in both plasma NEFA and glycerol concentrations were lower following Gx ingestion, possibly indicating, in this instance, a reduced NEFA mobilisation when compared with P ingestion.

The proprietary ingredients of Xenadrine-EFX™ (containing 6 mg of synephrine, 150 mg caffeine and 150 mg catechin polyphenols) have the potential to affect metabolism in several different ways. EGCG, caffeine and the adrenergic amines found in bitter orange extract are likely to increase sympathetic activity by prolonging the half-life in tissues of nor-adrenaline and cAMP. Increased expression of sympathetic activity would be expected to increase NEFA release from adipose tissue by stimulation of lipoprotein lipase.[25,26] However, they also have the potential to stimulate CHO metabolism by activation of glycogen phosphorylase.[27,28] In contrast to this, caffeine may even attenuate glycogenolysis through an inhibitory effect on glycogen phosphorylase transformation.[29,30] The effects of increased cAMP on different enzyme systems, however, will be moderated by allosteric control mechanisms within the metabolising cells in order to balance ATP production with demand. While this still does not establish that increased sympathetic activity would necessarily increase RMR, specific β1-[31] and non-specific (β1- and β2-)[32] antagonists have been shown to reduce the metabolic rate at rest. This does not appear to be mediated through the thermogenic hormone triiodothyronine.[32] Further stimulation of RMR through caffeine-induced release of the effect of adenosine, acting as a prejunctional inhibitor, would be additional to any direct metabolic effects. Surprisingly, there was no effect of Gx on resting heart rate or blood pressure despite the fact that Gx contains approximately 6 mg of synephrine, 150 mg caffeine and 150 mg catechin polyphenols, which might have been expected to have an effect.

It has been reported that the alkaloids in citrus aurantium result in a stimulation of β3-receptors with less of an influence on β1- and β2-receptors.[5] The β3-receptor seems to be the most abundant beta subtype in human brown adipocytes and is thought to play a key role in the lipolytic and thermogenic effects of adrenergic agents,[2,33] without exerting an effect on those receptors responsible for cardiovascular control.[2] These effects might be consistent with the results of study 1 in terms of the observed increase in plasma NEFA minus any significant changes in heart rate or blood pressure. However, the increase in plasma NEFA (observed in study 1 but not in study 2) could equally be the result of a reduced fat oxidation, as mentioned previously. In addition, these effects would not be consistent with the increased contribution of CHO oxidation to the elevated RMR and EMR.

The implications of the present results to the use of Gx for weight loss are not favourable, in that total metabolic rate was unchanged. A pharmacological effect was indicated by the increase in CHO oxidation, matched by a fall in NEFA oxidation, but these are opposite to the effects usually promoted for weight-loss products. However, these effects were recorded following the administration of a single dose of Gx, whereas different effects on the balance of CHO and fat oxidation, and the overall metabolic rate, might occur with repeated ingestion. On the positive side, Gx was not associated with any measurable changes in blood pressure or heart rate as previously reported following the administration of bitter orange extract,[8] although this contrasts with the effects of caffeine.[34]

The treatment formulation, combining the effects of EGCG and caffeine to stimulate β1-adrenoreptor activity seems logical given the link between this and resting metabolic rate.[31] It remains to be seen if chronic administration of Gx is effective in stimulating greater loss of weight, particularly if combined with regular exercise.

Finally, it should be noted that the study was not without limitations. In attempting to estimate directly the resting metabolic rate, certain assumptions were made with respect to substrates utilised. Some glycogen in addition to glucose may have been oxidised and inevitably oxidation of other free fatty acids, such as stearate, will have contributed to overall fat oxidation. However, the effect of such considerations on the calculations is minimal. The assumption is also made that no protein was oxidised and thus did not contribute to energy expenditure. In study 2, it is assumed that loss of bicarbonate through decrease in plasma pH, caused by lactate increase, was minimal and if it occurred was equal in the two sessions. This appears to be supported by the very small changes observed in plasma lactate. Finally, the method of respiratory gas collections was not ideal and the use of a mouthpiece may affect sympathetic activity if this is felt by the subject to increase the resistance to breathing. Although one subject was clearly affected by this, with the result that his data from all sessions were withdrawn, this limitation should have equally affected both treatment conditions, as it did in the case of this subject (but to such a gross level in this case as to render the data incalculable).

We conclude that a single dose of Gx had little or no effect on total ATP utilisation during 6 h at rest or during 60 min of light exercise at 60% HRR. Gx ingestion, however, did result in changes in the relative contributions from CHO and NEFA oxidation to ATP production in both conditions. At rest, the increase in CHO oxidation may have been as high as 30%.

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References

1 Ravussin E, Gautier JF. Metabolic predictors of weight gain. *Int J Obes Relat Metab Disord* 1999; **23**: S37–S41.
2 Spraul M, Ravussin E, Fontvieille AM, Rising R, Larson DE, Anderson EA. Reduced sympathetic nervous system: a potential mechanism predisposing to body weight gain. *J Clin Invest* 1993; **92**: 1730.
3 Matsumoto T, Miyawaki C, Ue H, Kanda T, Yoshitake Y, Moritani T. Comparison of thermogenic sympathetic response to food intake between obese and non-obese young women. *Obesity Research* 2001; **9**: 78–85.
4 Matsumoto T, Miyawaki C, Ue H, Yuasa T, Miyatsuji A, Moritani T. Effects of capsaicin-containing yellow curry sauce on sympathetic nervous system activity and diet induced thermogenesis in lean and obese young women. *J Nutrit Sci Vitaminol* 2000; **46**: 309–315.
5 Shekelle PG, Hardy ML, Morton SC, Maglione M, Mojica WA, Suttorp MJ *et al*. Efficacy and safety of ephedra and ephedrine for weight loss and athletic performance: a meta-analysis. *J Am Med Assoc* 2003; **289**: 1537–1545.
6 Preuss HG, DiFerdinando D, Bagchi M, Bagchi D. Citrus aurantium as a thermogenic, weight reduction replacement for ephedra: an overview. *J Med* 2002; **33**: 247–264.
7 Emorine LJ, Marullo S, Briend-Sutren MM, Patey G, Tate K, Delavier-Klutchko C *et al*. Molecular characterization of the human beta 3-adrenergic receptor. *Science* 1989; **245**: 1118–1121.
8 Penzak SR, Jann MW, Cold JA, Hon YY, Desai HD, Gurley BJ. Seville (sour) orange juice: synephrine content and cardiovascular effects in normotensive adults. *J Clin Pharmacol* 2001; **41**: 1059–1063.
9 Dulloo AG, Seydoux J, Girardier L, Chantre P, Vandermander J. Green tea and thermogenesis: interactions between catechin-polyphenols, caffeine and sympathetic activity. *Int J Obes Relat Metab Disord* 2000; **24**: 252.
10 Dulloo AG, Geissler CA, Horton T, Collins A, Miller DS. Normal caffeine consumption: influence on daily thermogenesis and daily energy expenditure in lean and postobese human volunteers. *Am J Clini Nutri* 1989; **49**: 44.
11 Dulloo AG, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M *et al*. Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. *Am J Clin Nutri* 1999; **70**: 1040–1045.
12 Bell C, Petitt DS, Jones PP, Seals DR. Influence of adiposity on tonic sympathetic support of resting metabolism in healthy adults. *Int J Obes Relat Metab Disord* 2003; **27**: 1315–1318.
13 Amano M, Kanda T, Ue H, Moritani T. Exercise training and autonomic nervous system activity in obese individuals. *Med Sci Sports Exercise* 2001; **33**: 1287–1291.

14 Achten JM, Gleeson M, Jeukendrup AE. Determination of the intensity that elicits maximal fat oxidation. *Med Sci Sports Exercise* 2002; **34**: 92.
15 Smith DA, Dollman J, Withers RT, Brinkman M, Keeves JP, Clark DG. Relationship between maximum aerobic power and resting metabolic rate in young adult women. *J Appl Physiol* 1997; **82**: 156–163.
16 Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness: measurement on 481 men and women aged 16–72 years. *Br J Nutrit* 1974; **32**: 77.
17 Draper SB, Wood DM, Fallowfield JL. The VO_2 response to exhaustive square wave exercise: influence of exercise intensity and mode. *Eur J Appl Physiol* 2003; **90**: 92.
18 Sahlin K. High-energy phosphates and muscle energetics. In: Poortmans JR (ed). *Principles of Exercise Biochemistry, 3rd rev. edn. Medicine and Sports Science*. Basel: Karger, 2004; **46**: 87.
19 Alberty RA. Effect of pH and metal ion concentration on the equilibrium hydrolysis of adenosine triphosphate to adenosine diphosphate. *J Biol Chem* 1968; **243**: 1337.
20 Koot P, Deurenberg P. Comparison of changes in energy expenditure and body temperatures after caffeine consumption. *Annu Nutrit Metab* 1995; **39**: 135.
21 Bracco D, Ferrarra JM, Arnaud MJ, Jequier E, Schutz Y. Effects of caffeine on energy metabolism, heart rate, and methylxanthine metabolism in lean and obese women. *Am J Physiol* 1995; **269**: E671.
22 Acheson KJ, Zahorska-Markiewicz B, Pittet P, Anantharaman K, Jequier E. Caffeine and coffee: their influence on metabolic rate and substrate utilisation in normal weight and obese individuals. *Am J Clin Nutr* 1980; **33**: 987.
23 Colker CM, Kalman DS, Torina GC, Perlis T, Street C. Effects of citrus aurantium extract, caffeine and St John's Wort on body fat loss, lipid levels and mood states in overweight healthy adults. *Curr Ther Res* 1999; **60**: 145–153.
24 Engels HJ, Wirth JC, Celik S, Dorsey JL. Influence of caffeine on metabolic and cardiovascular functions during sustained light intensity cycling and at rest. *Int J Sports Nutr* 1999; **9**: 361–370.
25 Samra JS, Simpson EJ, Clark ML, Forster CD, Humphreys SM, Macdonald IA *et al*. Effects of epinephrine infusion on adipose tissue: interactions between blood flow and lipid metabolism. *Am J Physiol* 1996; **271**: E834–E839.
26 Pedersen SB, Bak JF, Holck P, Schmitz O, Richelsen B. Epinephrine stimulates human muscle lipoprotein lipase activity *in vivo*. *Metabolism* 1999; **48**: 461.
27 Chasiotis D, Sahlin K, Hultman E. Regulation of glycogenolysis in human muscle in response to epinephrine infusion. *J Appl Physiol* 1983; **54**: 45.
28 Danforth WH, Helmreich E, Cori CF. The effect of contraction and epinephrine on the phosphorylase content of frog sartorius muscle. *Proc Nat Acad Sci USA* 1962; **48**: 1191.
29 Kasvinsky PJ, Shechosky S, Fletterick J. Synergistic regulation of phosphorylase *a* by glucose and caffeine. *J Biol Chem* 1978; **253**: 9102.
30 Steiner RF, Greer L, Bhat R, Oton J. Structural changes induced in glycogen phosphorylase b by the binding of glucose and caffeine. *Biochim Biophys Acta* 1980; **611**: 269.
31 Lamont LS, Romito RA, Finkelhor RS, Kalhan SC. β1-adreno-receptors regulate resting metabolic rate. *Med Sci Sports and Exercise* 1997; **29**: 769.
32 Welle S, Schwartz RG, Statt M. Reduced metabolic rate during β-adrenergic blockade in humans. *Metabolism* 1991; **40**: 619.
33 Dupport C, Loeper J, Strosberg AD. Comparitive expression of the human beta(2) and beta(3) adrenergic receptors in *Saccharomyces cerevisiae*. *Biochim Biophys Acta* 2003; **1629**: 34–43.
34 Lane JD, Pieper CF, Phillips-Bute BG, Bryant JE, Kuhn CM. Caffeine affects cardiovascular and neuroendocrine activation at work and home. *Psychosomat Med* 2002; **64**: 595–603.