High-intensity exercise attenuates postprandial lipaemia and markers of oxidative stress

Brendan GABRIEL, Aivaras RATKEVICIUS, Patrick GRAY, Michael P. FRENNEAUX and Stuart R. GRAY
Institute of Medical Sciences, University of Aberdeen, Aberdeen AB25 2ZD, U.K.

ABSTRACT

Regular exercise can reduce the risk of CVD (cardiovascular disease). Although moderate-intensity exercise can attenuate postprandial TAG (triacylglycerol), high-intensity intermittent exercise might be a more effective method to improve health. We compared the effects of high-intensity intermittent exercise and 30 min of brisk walking on postprandial TAG, soluble adhesion molecules and markers of oxidative stress. Nine men each completed three 2-day trials. On day 1, subjects rested (control), walked briskly for 30 min (walking) or performed 5 × 30 s maximal sprints (high-intensity). On day 2, subjects consumed a high-fat meal for breakfast and 3 h later for lunch. Blood samples were taken at various times and analysed for TAG, glucose, insulin, ICAM-1 (intracellular adhesion molecule-1), VCAM-1 (vascular adhesion molecule-1), TBARS (thiobarbituric acid-reactive substances), protein carbonyls and β-hydroxybutyrate. On day 2 of the high-intensity trial, there was a lower (P < 0.05) incremental TAG AUC (area under the curve; 6.42 ± 2.24 mmol/l per 7 h) compared with the control trial (9.68 ± 4.77 mmol/l per 7 h) with no differences during day 2 of the walking trial (8.98 ± 2.84 mmol/l per 7 h). A trend (P = 0.056) for a reduced total TAG AUC was also seen during the high-intensity trial (14.13 ± 2.83 mmol/l per 7 h) compared with control (17.18 ± 3.92 mmol/l per 7 h), walking showed no difference (16.33 ± 3.51 mmol/l per 7 h). On day 2 of the high-intensity trial plasma TBARS and protein carbonyls were also reduced (P < 0.05) when compared with the control and walking trials. In conclusion, high-intensity intermittent exercise attenuates postprandial TAG and markers of oxidative stress after the consumption of a high-fat meal.

INTRODUCTION

CVD (cardiovascular disease) is a major cause of mortality and is becoming more prevalent [1]. The most common CVD is CAD (coronary artery disease) a condition with atherosclerosis at the centre of the pathology [2]. Atherosclerosis is often linked to high fasting serum levels of lipids and LDLs (low-density lipoproteins). However, fasting levels of TAG (triacylglycerol) are not a good predictor of atherosclerosis [3] and, as people spend the majority of the day in a postprandial state; it has been suggested that atherogenesis is a postprandial phenomenon [4]. This contention has recently been supported by several studies demonstrating that postprandial TAG concentration is a strong independent risk factor for CVD [5,6].

A single high-fat meal can induce endothelial dysfunction, thought to be due to oxidative stress and...
activation of leucocytes, one of the early stages in the development of atherosclerosis [7–10]. The importance of leucocyte activation is indicated by the early research demonstrating that leucocyte counts are a predictor of future myocardial infarction [11]. However, it should be noted that, although current data suggest that leucocytes are involved in the process of endothelial dysfunction, their precise role has yet to be established. A recent study by Azekoshi et al. [12] has provided some evidence of a definite role for leucocytes in endothelial dysfunction via activation of the RAS (renin–angiotensin system). Moreover, the immune reaction appears to couple dyslipidaemia to atherosclerotic plaque formation partially through the induction of the endothelium to express adhesion molecules, such as ICAM-1 (intracellular adhesion molecule-1) and VCAM-1 (vascular adhesion molecule-1) on their surface [13]. This induction of ICAM-1 and VCAM-1 expression can be induced by an increase in oxidative stress [14].

In support of a role for oxidative stress in endothelial dysfunction it has been demonstrated that the consumption of antioxidants can attenuate the deleterious effects of a high-fat meal [15]. Exercise is also proposed to be a mechanism to reduce postprandial TAG, and adults are advised to accumulate 30 min of moderate-intensity exercise on 5 days a week [16]. Some studies have shown that such moderate-intensity exercise attenuates postprandial TAG levels after a high-fat meal [17,18]. However, the current number of people meeting these recommendations remains low [19], with lack of time frequently cited as the greatest barrier to performing exercise [20]. Short-duration high-intensity exercise has therefore been proposed to be a time-efficient method of improving cardiovascular health [21,22].

Previous studies have shown that a time-efficient exercise protocol, involving four to six 30 s maximal sprints per session, can lead to improvements in endurance performance and muscle oxidative capacity [23]. Further work by this group has also demonstrated that this exercise protocol can reduce hyperglycaemia and improve mitochondrial capacity in Type 2 diabetics [24], and improve peripheral arterial stiffness and flow-mediated dilation in healthy untrained individuals [21]. Similar improvements in flow-mediated dilation have also been shown postprandially by Tyldum et al. [25]. Furthermore, in a recent study, it has been shown that high-intensity intermittent exercise can reduce postprandial TAG for 3 h after a high-fat breakfast [22]. What remains to be determined is whether these effects remain over a longer time period (i.e. 7–8 h), which more closely reflects daily food intake (i.e. breakfast and lunch meals) where endothelial dysfunction can still be observed (e.g. [26]) and whether the beneficial effects extend to improvements in markers of oxidative stress and/or adhesion molecules.

The primary aim of the present study, therefore, was to determine the effect of high-intensity intermittent exercise on postprandial plasma levels of TAG, soluble adhesion molecules and markers of oxidative stress in healthy young men. The second aim was to investigate whether changes in postprandial TAG could be associated with a decrease in hepatic VLDL (very-low-density lipoprotein) secretion, indicated by measures of β-hydroxybutyrate.

**MATERIALS AND METHODS**

**Subjects**

The study conformed to current local guidelines, the Declaration of Helsinki and was approved by the local ethics committee. Nine healthy male volunteers took part in this study (age, 24 ± 3 years; body fat%, 14.9 ± 4.1; weight, 81.5 ± 8.5 kg; height, 1.81 ± 0.10 m). All participants were regularly physically active but none were specifically trained. Exclusion criteria for volunteers included a history of CVD, smokers, hypertension (systolic/diastolic blood pressure >140/90 mmHg), diabetes, obese (body mass index >30 kg/m²) or subjects with any form of musculoskeletal injury. All participants were fully informed of the aims, risks and discomfort associated with the investigation before providing written informed consent.

**Anthropometric measurements**

Height was measured to the nearest 0.5 cm using a stadiometer (Holtain Ltd). Weight was measured to the nearest 0.1 kg using a weighing scale (Ohaus Champ 2). Skinfold thickness was measured on the right side of the body with calipers (Idass) at four sites (biceps, triceps, subscapula and suprailiac) to the nearest 0.1 mm. The percentage body fat was calculated using standard methods [27].

**Experimental protocol**

Subjects completed three 2-day trials in a randomized order. On day 1 (14.00 hours) the subjects rested for 30 min (control), walked briskly for 30 min (walking) or performed 5 × 30 s maximal sprints with 4 min recovery between each sprint (high-intensity). On day 2 (beginning at 08.45 hours) subjects arrived after an overnight fast and consumed a high-fat meal for breakfast and 3 h later for lunch. Each trial was separated by at least 7 days. Subjects were instructed not to ingest alcohol or caffeine in the 24 h period prior to day 1 up until the end of day 2. During this time they were also asked to refrain from exercise or strenuous physical activity other than that of the trials. Subjects were also asked to record their diet on day 1 of the trial and replicate this on day 1 of the two subsequent visits.
Day 1 trials

Walking
The walking trial took place on a treadmill (Cybex International). Subjects were started at a speed of 7 km/h and asked to walk at an intensity similar to that of the current recommendations for 30 min [16], i.e. a brisk walk (out of breath, but still able to talk).

Pulmonary gas exchange $\dot{V_{O_2}}$ (oxygen consumption) and $\dot{V_{CO_2}}$ (carbon dioxide output) were monitored breath-by-breath during several time intervals, i.e. 8–10, 18–20 and 28–30 min respectively using an online gas analysis system (CPX Ultima; Medgraphics).

High-intensity
The high-intensity intermittent exercise was performed on a cycle ergometer (Monark 894 Wingate cycle ergometer). Subjects performed a 4 min warm-up with no load and then performed a 30 s maximal sprint against a load of 7.5 % body weight, followed by unloaded cycling for 4 min. This sprint exercise was repeated a further four times, with 4 min rest in between each sprint. During each 30 s sprint average power (in W), peak power (in W) and peak pedal rate (rev./min) were recorded.

Control
During the control trial participants sat and rested for 30 min.

Day 2
Subjects arrived at 08.45 hours and rested for 15 min before a cannula (20 guage) was inserted into a vein in the antecubital fossa, and a baseline blood sample was collected. The cannula was flushed regularly with saline throughout the day. A standardized high-fat meal was then consumed for breakfast. This consisted of white bread, mayonnaise, butter, whole milk, cheddar cheese and potato crisps. This meal provided approximately 3.4 ± 0.4 MJ of energy with 56 % of energy from fat, 33 % from carbohydrate and 11 % from protein. The meal contained 0.7 g of fat, 1 g of carbohydrate, 0.3 g of protein and 11 kcal/kg of body weight (1 kcal = 4.184 kJ). The mean macronutrient content of the meal was 56.8 ± 6.1 g of fat, 75.7 ± 8.2 g of carbohydrate and 25.8 ± 2.8 of protein. The mean time taken to consume the meal was 13:28 ± 4:43 min.

Further blood samples were collected at 0.5, 1, 2 and 3 h after the breakfast meal. A second identical meal was then consumed for lunch with subsequent blood samples taken at 3.5, 4, 6 and 7 h after the consumption of the first meal. Water was provided ad libitum throughout the day of the first trial and this volume of water was consumed during subsequent trials.

Measurements

Blood handling and analysis
Blood samples were collected with sterile 6 ml K+ EDTA non-ridged vacutainers (Vacuette; Greiner Bio-one) and were centrifuged (Eppendorf Centrifuge 5702/R) at 1500 g at 4 °C for 10 min. Then plasma was removed and frozen at –20 °C until analysis. Blood TAG and glucose concentrations were assessed using manual enzymatic colorimetric assay kits (Randox; Crumlin) using a spectrophotometer (Camspec M330B). Insulin was measured by ELISA (Mercodia Insulin ELISA) using a spectrophotometric plate reader (Synerg HT Multi-mode microplate reader; BioTek). sICAM-1 (soluble ICAM-1) and sVCAM-1 (soluble VCAM-1) concentrations were determined using ELISA kits (R&D Systems) and absorbance was measured using a spectrophotometric plate reader (Synerg HT Multi-mode microplate reader; BioTek). Concentrations from the ELISA were calculated through the interpolation of sample absorbance values compared with generated standard curves.

In order to investigate the effect of the exercise interventions on postprandial oxidative stress, we measured protein carbonyls and TBARS (thiobarbituric acid-reactive substances) in blood samples collected at baseline, 2, 5 and 7 h.

To assess protein oxidation, a fluorometric assay kit for protein carbonyls was used (Cayman Chemical). Protein content was also measured in each sample to express data per mg of protein. To assess lipid peroxidation, a fluorometric TBARS assay kit was used (Cayman Chemical). In both assays, fluorescence was measured using a plate reader (Synerg HT Multi-mode microplate reader; BioTek).

To estimate hepatic lipid oxidation [28], $\beta$-hydroxybutyrate concentration was assessed using an enzyme assay kit (Cayman Chemical). Absorbance was measured using a spectrophotometric plate reader (Synerg HT Multi-mode microplate reader; BioTek).

Concentrations from the assays were calculated through the interpolation of sample absorbance values compared with generated standard curves. The coefficient of variation for each assay was: TAG, 2.02 %; glucose, 4.3 %; insulin, 7.2 %; sICAM-1, 3.38 %; sVCAM-1, 6.3 %; $\beta$-hydroxybutyrate, 4.5 %; TBARS, 6.67 %; protein carbonyls, 3.8 %.

Estimation of energy expenditure
Energy expenditure was estimated during the walking trials from the measurement of $\dot{V_{O_2}}$ and the expiratory exchange ratio [29]. To estimate energy expenditure during the high-intensity trials using the mean power output during the 30 s sprint and an estimate of mechanical efficiency of 18.5 % [30].
Figure 1  Plasma glucose (A), insulin (B) and TAG (C) concentrations in response to the prior walking, high-intensity exercise and control trials. Values are means ± S.D.

Statistical analysis
Data were analysed using the GraphPad Prism 5 software. Both total and incremental (taking into account changes in baseline concentrations) AUC (area under the curve) values for plasma TAG concentration were calculated using the trapezium rule. The AUC values were calculated to provide a summary of the TAG response during the 7 h test period. Fasting plasma concentrations and calculated incremental and total AUC values were compared between trials using a one-way ANOVA. To compare differences between the three trials over time a two-way ANOVA with repeated measures was performed. Where a significant effect was observed post-hoc Tukey’s tests were performed to locate differences. Significance was taken at $P < 0.05$. Results are presented as means ± S.D.

RESULTS

Exercise
Volunteers did not perform any exercise in the control experiment. Subjects walked at an average of 6.7 ± 0.2 km/h and the average $V_o_2$ was 20.03 ± 2.3 ml/kg of body weight per min an estimated energy expenditure of 240.9 ± 35.2 kcal (where 1 kcal = 4.184 kJ) during the walking exercise. The maximum power output during the high-intensity trial was 869.1 ± 198.4 W and mean power output was 632.6 ± 102.2 W. This corresponded to an average energy expenditure of 103.2 ± 5.1 kcal during the high-intensity trial, which was lower ($P < 0.001$) than energy expended during the walking trial.

Insulin and glucose
There was no difference between the three trials in either insulin or glucose concentration in the plasma samples collected. ANOVA did reveal a significant effect of time for both insulin and glucose in response to the meals ($P < 0.0001$) (Figure 1).

TAG
The initial ANOVA revealed no differences between the three trials in plasma TAG concentration, although there was a trend. However, there was with a significant effect of time in response to the meal ($P < 0.0001$) (Figure 1).

The data were analysed in the AUC form. This showed that there was a lower ($P < 0.05$) incremental AUC in high-intensity exercise compared with the control trial (Figure 2). There was also a trend ($P = 0.056$) for a lower total AUC in high-intensity compared with the control trial (Figure 2). There were no differences between the control and walking trials.

Soluble adhesion molecules
There was no difference between the three trials in soluble adhesion molecule concentration (Figure 3). However, sICAM-1 did show a significant effect of time with values increasing throughout the day ($P < 0.0001$).

© The Authors Journal compilation © 2012 Biochemical Society
Figure 2  TAG total (A) and incremental (B) AUC over the 7 h experimental period in response to the prior walking, high-intensity exercise and control trials

Values are means ± S.D. †A trend (P = 0.056) between high-intensity exercise and control trials, *P < 0.05 between the high-intensity exercise and control trials.

Figure 3  Plasma sVCAM-1 (A) and sICAM-1 (B) concentrations in response to the prior walking, high-intensity exercise and control trials

Values are means ± S.D.

Figure 4  Plasma protein carbonyls (A) and TBARS (B) concentrations in response to the prior walking, high-intensity exercise and control trials

Values are means ± S.D. *P < 0.01 between the high-intensity and control/walking trials. ‡P < 0.05 and †P < 0.01 between the high-intensity and walking trials.

Markers of oxidative stress

Protein carbonyl levels (Figure 4) increased (P < 0.05) at 2 and 5 h above baseline in both walking and control trials. However, high-intensity completely prevented this effect, with no change in protein carbonyl levels. TBARS (Figure 4) were raised compared with baseline in all three trials; however, high-intensity reduced the magnitude of this effect. There were no differences in either protein carbonyls or TBARS between walking and control trials.

β-Hydroxybutyrate

β-Hydroxybutyrate levels (Figure 5) increased between baseline and 5 h in all three trials (P < 0.0001), with no difference between the three trials.

DISCUSSION

The main finding of the present study was that a prior bout of high-intensity intermittent exercise
attenuated the postprandial rise in TAG, compared with a control trial, with no differences in glucose or insulin between trials. A total of 30 min of brisk walking, which is equivalent to the current physical activity recommendations, had no effect on postprandial TAG concentrations. These findings agree with the recent study by Freese et al. [22], who employed a similar exercise protocol, although in their work TAG concentrations were only monitored for 3 h postprandially. The present study has extended these findings by also demonstrating that these effects are present for a 7-h period (after breakfast and lunch) and that high-intensity exercise also results in a decrease in markers of oxidative stress, with no change in soluble adhesion molecules. These findings have important clinical implications as high-intensity exercise may be of greater benefit than the currently recommended forms of physical activity. The importance of a reduction in exercise time is highlighted by the consistent findings that a lack of time is frequently cited as the major barrier to participation in exercise [20].

Further to a reduced exercise time, the high-intensity exercise protocol employed also has an approximately 57 % lower energy expenditure. Although this may be seen as a negative observation if one was aiming to reduce body fat, high-intensity intermittent exercise has, in fact, been found to be more effective than traditional steady-state endurance exercise in reducing body fat [31]. The greater TAG-reducing effect of high-intensity exercise may also be surprising, as energy expenditure is known to be a crucial determinant of this response [32]. Both these observations may be due to an elevation in resting metabolic rate and/or a more prolonged/greater elevation in post-exercise V̇O₂ after high-intensity exercise [33,34]. Further work is needed to clarify these assertions. It is often assumed that high-intensity intermittent exercise would not be suitable or enjoyable for patient populations, yet, in patients with CAD, high-intensity intermittent exercise was noted as their preferred mode of exercise, when compared with constant load endurance exercise [35].

It is well established that, in healthy individuals, exercise can reduce postprandial TAG concentrations. Early work demonstrated that a 90 min brisk walk can reduce postprandial TAG concentrations by approximately 20 % [32] and these findings have been supported by several further studies (for a review, see [36]). This duration of exercise is, however, three-fold higher than the current recommendations and is unlikely to be achieved by the general population. Recent work has demonstrated that 30 min of brisk walking results can also reduce postprandial TAG concentrations, by approximately 15 %, in healthy young men [17]. A similar finding was not observed in the present study with 30 min of brisk walking, with no clear reason for the differences in findings. It may be that the present study did not have sufficient number of participants to detect differences between walking and control trials. However, the main aim of the study was to determine the effect of high-intensity intermittent exercise, which had a clear positive effect in reducing plasma TAG.

The mechanisms responsible for an exercise induced decrease in plasma TAG concentrations remain to be elucidated (for review, see [36]). The proposed mechanisms are that either, or a combination of both, the uptake of TAG in peripheral tissues is elevated or the production and release of TAG, packaged in VLDL, from the liver is decreased. With regard to augmented TAG clearance after prior intense exercise it appears likely that this is mediated by an increase in LPL (lipoprotein lipase) activity [37]. However, after prior moderate-intensity exercise there also appears to be a role for decreased secretion of VLDL from the liver. Indeed, Gill et al. showed increased circulating levels of β-hydroxybutyrate alongside reduced postprandial TAG clearance after prior intense exercise it appears likely that this is mediated by an increase in LPL (lipoprotein lipase) activity [37]. However, after prior moderate-intensity exercise there also appears to be a role for decreased secretion of VLDL from the liver.

It is well established that, in healthy individuals, exercise can reduce postprandial TAG concentrations. Early work demonstrated that a 90 min brisk walk can reduce postprandial TAG concentrations by approximately 20 % [32] and these findings have been supported by several further studies (for a review, see [36]). This duration of exercise is, however, three-fold higher than the current recommendations and is unlikely to be achieved by the general population. Recent work has demonstrated that 30 min of brisk walking results can also reduce postprandial TAG concentrations, by approximately 15 %, in healthy young men [17]. A similar finding was not observed in the present study with 30 min of brisk walking, with no clear reason for the differences in findings. It may be that the present study did not have sufficient number of participants to detect differences between walking and control trials. However, the main aim of the study was to determine the effect of high-intensity intermittent exercise, which had a clear positive effect in reducing plasma TAG.

The mechanisms responsible for an exercise induced decrease in plasma TAG concentrations remain to be elucidated (for review, see [36]). The proposed mechanisms are that either, or a combination of both, the uptake of TAG in peripheral tissues is elevated or the production and release of TAG, packaged in VLDL, from the liver is decreased. With regard to augmented TAG clearance after prior intense exercise it appears likely that this is mediated by an increase in LPL (lipoprotein lipase) activity [37]. However, after prior moderate-intensity exercise there also appears to be a role for decreased secretion of VLDL from the liver. Indeed, Gill et al. showed increased circulating levels of β-hydroxybutyrate alongside reduced postprandial TAG concentrations following prolonged exercise [38]. This increase in β-hydroxybutyrate is suggested to be indicative of an increase in hepatic fatty-acid oxidation which would shift hepatic fatty-acid partitioning away from VLDL synthesis. Early evidence has shown that there is an inverse relationship between ketogenesis and VLDL production [39], supporting the contention that increases in β-hydroxybutyrate would be reflected in a decrease in VLDL production. However, further work has demonstrated that this relationship does not hold under all situations [40] and so caution should be employed when interpreting these results. The present investigation found no difference between exercise trials in β-hydroxybutyrate levels, with all three trials showing a rise between 2 and 5 h after the first meal. It is therefore likely that the attenuation of postprandial TAG seen after high-intensity intermittent exercise comes about solely as a result of an increased LPL activity, although further work is required to elucidate the effects of...
high-intensity intermittent exercise on hepatic VLDL secretion.

The magnitude of postprandial TAG concentration has previously been shown to correlate with the magnitude of endothelial dysfunction and intima-media thickness of the carotid artery [41]. Furthermore, both total and incremental TAG AUC measures also correlating with intima-media thickness [42], highlighting the physiological and clinical relevance of these measures of the TAG response during the 7-h test period. Although the present study did not make any measure of endothelial function, previous work [25] has demonstrated that high-intensity intermittent exercise, albeit of a longer duration than in the present study, completely abolished the reduction in brachial artery flow-mediated dilation normally observed after a high fat meal. These authors found that this effect was associated with an increase in total antioxidant status in the blood, with no reductions in plasma TAG. Although this supports the assertion that the deleterious effects of a high-fat meal are associated with an increase in ROS (reactive oxygen species) production (e.g. [9]), a single measure of total antioxidant status gives a limited view of redox status [43]. The present study has shown that while oxidative stress was increased for up to 5 h in the control trial, high-intensity intermittent exercise attenuated the rise in oxidative stress, as measured by plasma protein carbonyls and TBARS. During the walking trial, there was no significant reduction in markers of oxidative stress when compared with the control trial.

Under normal conditions, the state of oxidative stress will reduce the amount of bioactive NO (nitric oxide), via chemical inactivation to form peroxynitrite, and may also make eNOS (endothelial nitric oxide synthase) dysfunctional, producing $\text{O}_2^-$ rather than NO [44]. These conditions will ultimately result in endothelial dysfunction (for a review, see [45]), which is an independent predictor of the progression of atherosclerosis and CVD [46]. The generation of a state of oxidative stress has many other effects in processes such as intracellular signalling and also the induction of adhesion molecule expression [13]. Previous work in Type 2 diabetics has demonstrated that a high-fat meal can result in an increase in circulating levels of the soluble adhesion molecules sICAM-1 and sVCAM-1, and that this increase can be attenuated by the consumption of vitamin E and ascorbic acid [47]. However, others have found no rise in both sICAM-1 and sVCAM-1 after a high-fat meal in healthy subjects [48]. The present study has demonstrated that while sICAM-1 increased throughout the day sVCAM-1 remained constant and that prior exercise has no effect on postprandial sICAM-1 and sVCAM-1 levels. Similar results have been found previously in overweight adolescent boys [49], but not to our knowledge in healthy adults.

One limitation of this study is a relatively low number of participants compared with other similar studies. This may explain the differences in postprandial TAG results after 30 min brisk walking between Miyashita et al. [17] and the present study. Nevertheless, the results from the present study still show a beneficial effect from a single bout of high-intensity intermittent exercise and this would suggest that high-intensity intermittent exercise is more efficacious compared with 30 min walking in attenuating postprandial TAG. Secondly, the present study only used healthy males and it remains to be elucidated whether similar adaptations will be seen in patients with CVD.

In conclusion, the results of the present study have demonstrated that prior high-intensity intermittent exercise reduces postprandial TAG, with no effect of 30 min brisk walking. This decrease in TAG was not associated with an increase in $\beta$-hydroxybutyrate, indicating that this effect is not due to a reduction in hepatic VLDL secretion. The reduction in postprandial TAG was associated with an almost complete abolition of the postprandial increase in markers of oxidative stress. High-intensity intermittent exercise may therefore be a useful tool in the prevention of atherosclerosis and reduction in the development of CVD, although further work is required to confirm this.

**AUTHOR CONTRIBUTION**

Stuart Gray and Michael Frenneaux designed the research; Stuart Gray, Aivaras Ratkevicius, Brendan Gabriel and Patrick Gray conducted the research; Brendan Gabriel and Stuart Gray analysed the data and wrote the paper; Stuart Gray had primary responsibility for final content. All authors read and approved the final manuscript.

**FUNDING**

This study was supported by the National Health Service [endowment grant [project 10/25 (to S.R.G. and M.P.F.)]. B.G. was supported by a Saltire Energy Studentship.

**REFERENCES**


© The Authors Journal compilation © 2012 Biochemical Society
28 Fritz, J. B. (1961) Factors influencing the rates of long-chain fatty acid oxidation and synthesis in mammalian systems. Physiol. Rev. 41, 52–129

Received 24 November 2011/20 February 2012; accepted 21 March 2012
Published as Immediate Publication 21 March 2012, doi:10.1042/CS20110600