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Duckworth, LC and Backhouse, SH and O'Hara, J and Stevenson, EJ (2015) Effect of Galactose Ingestion Before and During Exercise on Substrate Oxidation, Postexercise Satiety, and Subsequent Energy Intake in Females. *Journal of the American College of Nutrition*, 35 (1). 1 - 12. ISSN 0731-5724 DOI: <https://doi.org/10.1080/07315724.2014.994790>

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Title: Effect of galactose ingestion before and during exercise on substrate oxidation, post-exercise satiety and subsequent energy intake in females.

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No conflicts of interest.

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Running Title: Galactose and Exercise Metabolism

Acknowledgements

The authors would like to thank all of the volunteers for their participation. This study was funded by a Leeds Beckett University Promising Researcher Fellowship grant.

Abbreviations

CHO = carbohydrate, GLU = glucose, GAL = galactose, PLA = placebo, GI = glycemic index, FFA = free fatty acid, EE = energy expenditure, TEE = total energy expenditure, AUC = area under the curve.

Abstract

Objective: To examine the effects of consuming a galactose carbohydrate (CHO) drink on substrate oxidation, post-exercise satiety and subsequent energy intake.

Methods: Nine recreationally active eumenorrheic females undertook three trials, each consisting of running for 60-minutes at 65% VO_{2peak} followed immediately by a 90-minute rest period. Prior to (300 ml) and at every 15 minutes during exercise (150 ml), participants consumed either a glucose (GLU: GI 89) or galactose (GAL: GI 20) drink each containing 45g of CHO, or an artificially-sweetened placebo (PLA). Following the rest period, participants were provided with an *ad-libitum* test lunch and asked to record food intake for the remainder of the day.

Results: Plasma glucose was significantly greater throughout exercise and rest following the GLU trial compared with the GAL and PLA trials ($P<0.05$), however there were no differences in CHO oxidation. Hunger was significantly lower ($P<0.05$) throughout the GAL compared to the GLU and PLA trials. There were no significant differences between trials for energy intake during the post-exercise meal. Overall net energy balance for the 24-hours was negative in both the GAL (-162 ± 115 kcal; $P<0.05$ vs. GLU) and PLA trials (-49 ± 160 kcal).

Conclusions: Results demonstrate that ingesting a solution containing galactose before and during exercise can positively impact post-exercise satiety and energy balance throughout the day, compared to a more readily available and widely consumed form of CHO. Despite this, there appears to be no apparent benefit in consuming a CHO beverage on fuel utilization for this moderate exercise intensity and duration.

Key Words

Appetite, galactose, energy intake, substrate oxidation, females.

INTRODUCTION

As the global prevalence of overweight and obesity continues to rise, the prevention of weight gain through restricted energy intake and increased physical activity are continually investigated [1]. It is well known that aerobic exercise is effective at preventing weight gain [2] and altering substrate metabolism during and following exercise. Several studies have reported increases in fat oxidation following exercise [3-5], which is known to protect against long-term weight gain [6]. Studies have also reported that post-exercise energy intake is related to substrate metabolism during exercise, such that increased fat oxidation during exercise has been associated with significantly lower post-exercise energy intake [7, 8]. Despite this, exercise has also been reported to enhance an individual's desire to compensate for the energy expended [9] as well as manipulating the sensitivity of satiety signals [10] whereby hunger and food palatability following exercise are increased [11]. Despite these findings, the influence of nutritional status on appetite regulation and energy intake following exercise is not entirely understood.

The ingestion of CHO before exercise has been shown to modify the relative contributions of substrates as well as post-exercise energy intakes. Studies have shown that consuming a low glycemic index (GI) meal before exercise results in a higher rate of fat metabolism and maintenance of blood glucose levels [12-14]. While it may be intuitive that a low CHO diet (<20g per day) would lead to better glycemic control, studies have reported lower exercise-induced reductions in blood glucose whilst participants consumed a low GI meal. This approach may be safer in terms of reducing hypoglycemic risk during exercise following meal-induced hyperinsulinemia [15]. A number of short-term intervention studies have also shown that low GI meals increase satiety (feeling full) [14] and delay the return of hunger, and/or reduce energy intake at a later meal compared with high GI meals [16]. However, it has been reported

that the GI concept lacks clinical utility because the differences in glycemic indexes of foods are lost once these foods are consumed in a mixed meal [17].

The majority of studies that have investigated the impact of exercise on substrate utilization and post-exercise energy intake have only fed participants before exercise [18, 19]. Indeed, the GI literature has focused on pre-exercise feeding, typically 2-3 hours before exercise [13, 20, 21]. However, the consumption of CHO drinks during exercise has become commonplace not only in elite performers, but also recreational athletes in order to maintain hydration and delay fatigue. In addition, King et al. [22] reported that ingesting a CHO beverage during exercise was compensated for through a subsequent lowering of post-exercise energy intake. The vast majority of research regarding CHOs used in sports drinks has focused on the monosaccharide's glucose and fructose, the disaccharide sucrose and the synthetic polymer maltodextrins (glucose polymers) [23, 24]. A third primary sugar, galactose, is rapidly absorbed at similar rates to glucose by the same sodium co-transport system (SGLT1, [25]) and is unlikely to cause gastrointestinal distress; unlike other low glycemic CHOs such as fructose [26]. Galactose has no primary insulin drive [27] so is unlikely to suppress fat oxidation and much like low GI foods, provides a more stable plasma glucose profile over time [28].

To date, the effects of exercise and ingestion of a CHO solution on substrate metabolism, appetite and subsequent energy intake has received little attention. Whilst it may appear counterintuitive to consume excess calories during exercise as a form of weight maintenance, Melby et al. (2002) reported that consuming a glucose-based CHO drink during moderate-intensity (65% maximal oxygen uptake) exercise resulted in a lower energy intake throughout the remainder of the day[29]. This may have important implications for weight control. However, energy balance was not calculated and therefore further investigation is warranted. In addition, the

consumption of glucose was shown to elevate insulin concentrations thus suppressing fat oxidation during exercise, an undesirable effect for those utilising diet and exercise as a weight-loss tool. Achten and Jeukendrup [30] have outlined that when CHO is ingested before the start of exercise, fat oxidation is significantly lower than during fasting conditions in most studies, but that the magnitude of this is reduced when low glycemic formulations are consumed pre-exercise. Thus, formulations including galactose, which deliver exogenous CHO as well as having the potential to maintain or increase fat oxidation and reduce subsequent food intake, may have important implications for those who wish to exercise to lose weight or maintain weight loss. Given that the majority of research has been carried out with males, little is known about the nature of such responses in females. This is despite the fact that women are more likely to seek a diet or exercise programme for weight loss purposes [31]. Studies have consistently demonstrated that gender differences exist in postprandial glucose metabolism, and the utilization of CHOs and lipids as fuel sources [32, 33]. Therefore, there is a clear need for further research into the impact of supplementation before and during exercise on substrate utilization in females, post-exercise satiety and energy balance.

The purpose of the present study was to investigate the effects of consuming a galactose CHO drink versus a glucose CHO drink or placebo drink before and during exercise on substrate oxidation, post-exercise satiety and subsequent energy intake in recreationally active females.

MATERIALS AND METHODS

Participants

Eleven healthy, moderately active females were recruited to participate in this study; Only 9 participants completed the experimental trials due to cannulation difficulties in one participant and an injury occurring in another. Their mean (\pm SD) age, height,

weight, body mass index (BMI) and VO_{2peak} were 21.8 ± 3.4 years, 170.0 ± 0.6 cm, 63.3 ± 7.6 kg, 22.7 ± 2.31 kg/m² and 50.7 ± 7.0 ml/kg/min respectively. None of the participants were pregnant or lactating or reported any medical conditions, and all had normal resting hemoglobin levels (11.5-16.5 g/dl). All trials were carried out during the follicular phase (days 1-14) of the menstrual cycle (eliminating the influence of hormone interaction on substrate utilization) [34]. A criterion for inclusion in the study was that participants exercised regularly, scored at least 2 on the International Physical Activity Questionnaire (IPAQ [35]) and were able to run for one hour continuously at about 65% VO_{2peak} . Analysis of the IPAQ led to the classification of the sample as recreationally active and none were trained runners. Leeds Beckett University Faculty Ethics Committee approved the protocol and all participants gave their written informed consent.

Preliminary measurements

Following familiarisation with treadmill running and experimental procedures, participants undertook two preliminary tests in order to determine: 1) the relationship between running speed and oxygen uptake using a 16 min incremental test and 2) their VO_{2peak} using an uphill incremental treadmill test to exhaustion. All preliminary tests were conducted according to procedures previously described [36]. Using regression analysis, oxygen uptake, running speed and VO_{2peak} were used to determine the running speed equivalent to 65% of each participant's VO_{2peak} (average: 8.6 ± 1.0 km/h).

Experimental Protocol

All participants completed three experimental trials in a randomised crossover design double-blind procedure separated by at least 5 days. For 2 days prior to the first trial, participants recorded their diet and exercise routine so that it could be repeated before

the following trials to minimise differences in pretesting intramuscular substrate concentrations between experimental trials [21].

After an overnight (12 hour) fast and having refrained from any strenuous activity, alcohol and caffeine consumption in the previous 24 hours, participants were provided with their breakfast to consume at home on the morning of the experiment (at 0800 hours). Participants were asked to complete a check list to ensure these procedures and timings were accounted for. Following this, participants were asked to refrain from eating or drinking (apart from water which was recorded on the first occasions and repeated for subsequent trials) until they arrived at the laboratory at 1000 hours.

On arrival at the laboratory, anthropometric variables and blood pressure were recorded before participants were asked to complete subjective scales for hunger, gut fullness and thirst. Basal blood samples were drawn from an indwelling cannula and participants were then provided with one of the three test drinks (GLU, GAL or PLA). They were asked to consume 300ml of the test drink within 5 minutes. Participants then completed a 5-minute warm up at 60% VO_{2peak} on a motorised treadmill (Model ELG 70, Woodway, Weilam Rhein, Germany) after which the speed was increased to that which represented 65% of their VO_{2peak} . All participants then completed 60 minutes running at this speed. Each exercise session was designed to be equal both in intensity and duration. These exercise sessions have been shown to induce a significant increase in fat oxidation [37, 38] and have been used in previous studies in healthy women [14, 39]. During the exercise period, heart rate was monitored continuously by a radio telemetry monitor (Polar vantage NV, Kempe, Finland); blood samples drawn and subjective scales were taken at 15 minute intervals; and expired air samples were obtained continuously. At the end of the exercise period, participants removed surface sweat and were weighed in minimal clothing. Participants were then asked to rest in the laboratory lounge for a further 90 minutes and blood and expired

air samples were taken at regular intervals (15, 30, 60 and 90 minutes post-exercise). Participants were instructed not to eat or drink anything other than water, which was available *ad-libitum* throughout the first trial, and matched for volume during the following trials.

At the end of the rest period, participants were provided with a standard pasta-based test meal to consume. Following the voluntary termination of the meal, participants were free to leave the laboratory and were asked to record all foods and drinks consumed and activity performed for the remainder of the day in a food and exercise diary provided. An estimated food diary method was chosen as opposed to a weighed inventory in order to reduce participant burden as well as to maintain the quality of the data [40]. Data was analyzed using the dietary analysis software Netwisp (version 8.0, Tinuviel Software, Warrington, UK).

All trials were performed at the same time of day and under similar experimental conditions. The same motorised treadmill was used throughout the study. Ambient temperature (mean \pm SD: 19.0 \pm 0.9 °C) and relative humidity (41.6 \pm 5.2 %) were recorded each morning during the trials.

Blood sampling and analysis

Following baseline anthropometric measures and subjective scales, participants rested on a bed for at least 10 minutes. A cannula (Venflon, 18G, Becton Dickinson Ltd, Helsingborg, Sweden) was inserted in an antecubital vein and a slow running infusion of a sterile saline solution (0.9%) was started to keep the cannula patent. Blood samples were drawn via a vacutainer tube; at least the first 2mL was discarded to avoid contamination with saline'. Blood samples drawn for both plasma glucose and plasma lactate were collected in fluoride oxalate tubes, while those for serum insulin and serum free fatty acids were collected in plain tubes. Serum samples were left for

at least 30 minutes to clot at room temperature, and all other samples were stored on ice until the end of the rest period and spun within three hours of sampling. Whole blood was spun at 3000rpm at 4°C for 10 minutes and plasma/serum was aliquoted into tubes as required for analysis. Aliquots were frozen at -80°C until further analysis. Plasma samples were analyzed enzymatically for glucose and lactate concentration on a semiautomatic analyzer (ILab 2300 stat plus analyzer, Instrumentation Laboratories, Warrington, UK). Serum FFA concentrations were analyzed using a WAKO enzymatic colorimetric kit (Alpha Laboratories, Eastleigh, UK) adapted to an ILab 2300 stat plus analyzer. Serum insulin concentrations were transported to the Department of Chemical Pathology at Leeds General Infirmary and analyzed by an ADVIA sandwich immunoassay using chemiluminescent technology (Siemens ADIVA Centaur, IL, USA). All analyses were made in duplicate. To eliminate inter-assay variation, samples from each participant were analyzed in the same run. The within-run precision (coefficient of variation) for plasma glucose, plasma lactate, serum insulin and serum free fatty acids was 0.5 to 0.6%, 1.0 to 1.9%, 3.2 to 4.6% and 1.1 to 2.7%, respectively.

Expired air samples and substrate oxidation

Samples of expired air were collected continuously throughout the exercise period using an online automated gas analysis system (Meta-Max 3B, Cortex, Leipzig, Germany) to determine oxygen uptake and carbon dioxide production. Samples of expired air were collected continuously throughout the exercise period, but samples were averaged for 5-minute periods at 10-15, 25-30, 40-45 and 55-60 minutes during exercise. During the rest period (R), 5-minute samples of air were collected but participants wore the mask for 5 minutes previous to this collection period for stabilisation of measures. The digital triple V volume transducer was calibrated using a 3-L syringe (Hands Rudolph, Inc., Shawnee KS) and the gas analyzers were calibrated using room air and a mass standard gas mixture (alpha-gravimetric

standard: BOC gases, Guildford, United Kingdom) of oxygen and carbon dioxide in nitrogen equivalent to expired air (15% O₂ and 5% CO₂).

Total fat and CHO oxidation (g/min) were calculated using the following non-protein stoichiometric equations [41], with the assumption that protein oxidation during exercise was negligible:

$$\text{Fat oxidation rate (g/min)} = (1.695 \times \text{VO}_2) - (1.701 \times \text{VCO}_2)$$

$$\text{CHO oxidation rate (g/min)} = (4.585 \times \text{VCO}_2) - (3.226 \times \text{VO}_2)$$

Energy Balance

Each individual's energy expenditure (EE) was calculated using the Schofield equation [42] to estimate basal metabolic rate (BMR) and physical activity levels from the exercise diary. Participants were asked not to exercise outside of the laboratory-controlled condition; however other daily activities such as walking were taken into account. The respective energy potential from CHO (4.1 kcal/g) and fat (9 kcal/g) was calculated to estimate expenditure during the exercise protocol and added to EE to compute total energy expenditure (TEE) throughout the day. Relative energy balance was calculated for the exercise and rest period as the energy intake (test drink + *ad-libitum* test meal) minus the energy expended (during exercise + rest period). In addition, the total 24-hour energy balance was computed, using the total energy intake (breakfast + drink + test meal + self-reported intake) minus the TEE (BMR + during exercise + rest period + self-reported exercise).

Subjective scales

Prior to cannulation and thereafter during each blood sampling period, subjective assessment of hunger, thirst and gut fullness was recorded. These assessments were made using a modified 6-20Borg Scale [43] with revised anchors.

Test meals

Each individual's daily energy requirement (DER) was calculated using the Schofield equation [42] to estimate basal metabolic rate (BMR) and physical activity levels from the IPAQ. The nutritional content of each meal was calculated from information provided by the manufacturer. On the morning of each trial, participants were provided with a standardised breakfast consisting of Rice Krispies (Kellogs, Manchester, UK) and semi-skimmed milk. The cereal:milk ratio was 30g:125ml. This meal was equivalent to 10% of the individuals DER and the proportion of energy from protein, fat and CHO was 14, 14 and 72% respectively. Participants were given 15 min to consume the entire contents of the breakfast meal. The prescription of breakfast for this study, compared to participants arriving in a fasted state, looked to replicate real-world habitual circumstances for recreational exercisers.

A standard pasta-based lunch was provided to participants for all three trials as outlined previously [14]. Participants were initially provided with a dish containing 300g to which 200g was added by the experimenter before the dish became empty and the participant continued to eat. This process was repeated until the participant indicated that they wished to terminate the meal. This ensured that the cue of an empty dish did not prompt the termination of eating.

Test drinks

Participants ingested solutions containing glucose (D-Glucose monohydrate, Thornton and Ross, Huddersfield, UK) galactose (D-galactose, Hollandche, Melk & Suiker, Fabrique, The Netherlands) or an artificially sweetened placebo (distilled water). The CHO drinks provided 45g of CHO for 750ml consumed (approximately 0.71 g/kg/BM/h CHO) at pre (300ml), 15 min (150ml), 30 min (150ml) and 45 min (150ml) during exercise. The GI of the CHOs was approximately 89 and 20 for the GLU and GAL

drinks respectively based on previous calculations [44]. All three solutions contained 1.5g sodium chloride (Premier Foods, Hertfordshire, UK), and 1.0ml of lemon flavoring (Sainsburys Ltd, London, UK) all dissolved in 1000 ml of distilled water. The aim was to provide the same amount of CHO in both the GLU and GAL drinks. As glucose monohydrate contains one extra H₂O molecule, 67.11g of glucose monohydrate was given in the GLU trial and 60.00g of galactose in the GAL trial. The placebo (PLA) solution was indifferent from the CHO solutions in taste and appearance and contained exactly the same ingredients, except that the CHOs were replaced by a non-caloric sweetener (Sweetex, Reckitt Benckiser U.K. Ltd). An independent triangle sensory test revealed 25% correctly identified drinks; less than would be expected to occur purely by chance. Drinks were prepared by an independent researcher.

Statistical analysis

Data were analyzed using PASW software (version 17; IBM SPSS Statistics, Chicago, IL, USA). All data are presented as means with their standard errors unless otherwise stated. Prior to analysis, data were checked for acceptable values of normality (Kolmogorov-Smirnov test) and homogeneity of variance (Levene's test); all data were identified as normally distributed. The alpha was set to 0.05 for all statistical analysis. Paired samples t-tests were used to check for differences in baseline values for all variables. A two- way (time x trial) repeated measures analysis of variance (ANOVA) was used to determine differences in physiological, metabolic and subjective responses between all three drinks trials. Where suitable, a Holm-Bonferroni stepwise post hoc test was utilised to determine differences between conditions and time interactions (alpha level of 0.0166 per test (0.05/3)). Incremental area under the curve (AUC) was calculated according to Wolever and Jenkins [45] using the trapezoidal rule. ANOVA with a within subjects factor of treatment was used to identify any differences in incremental area under the curve (IAUC) for glucose, insulin and total CHO and fat oxidation between trials, calculated for both the exercise and complete trial periods.

Data are reported as mean \pm SEM. ANOVA with a within subjects factor of treatment was used to identify any differences in incremental area under the curve (IAUC) for glucose, insulin and total CHO and fat oxidation between trials, calculated for both the exercise and complete trial periods. Data are reported as mean \pm standard error of the mean (SEM).

RESULTS

Plasma glucose and serum insulin

There was a significant main trial effect for plasma glucose concentrations which were higher in the GLU trial compared to the GAL ($P<0.05$) and PLA ($P<0.05$) trials (**Figure 1A**). This was true for both exercise and resting periods. Main effects of time were apparent for both GLU and GAL trials ($P<0.05$). The incremental area under the curve (IAUC) for plasma glucose was greater for the GLU trial (341.2 ± 10.1 mmol/l/h) compared to the GAL (315.1 ± 6.3 mmol/l/h) and the PLA trials (303.5 ± 7.1 mmol/l/h) ($P<0.05$).

There was a main trial effect for serum insulin concentrations to be higher throughout the GLU trial when compared to GAL ($P<0.05$) and PLA trials ($P<0.0001$) and throughout the GAL trial compared to the PLA trial ($P<0.05$) (**Figure 1B**). Post-hoc analysis revealed significant differences at several time points. The IAUC for serum insulin throughout the trial was significantly greater in the GLU trial (692.9 ± 50.2 mU/l) compared to the GAL (543.2 ± 67.1 mU/l) and PLA (314.0 ± 29.1 mU/l) trials ($P<0.05$) again confirming the main trial effect.

Serum free fatty acid (FFA)

There was a main trial effect for serum FFA concentrations ($P<0.0001$), such that concentrations in the PLA trial were significantly greater than GLU and GAL trials at

several time points ($P<0.05$) (**Figure 1C**). No significant differences were apparent between the GLU and GAL trials. Main effects of time were apparent for all trials ($P<0.05$).

Blood Lactate

Throughout the exercise and subsequent rest period, there were no significant differences between trials (**Figure 1D**). At the end of the rest period, blood lactate had returned to baseline levels or below for all trials.

Respiratory Exchange Ratio (RER) and estimated CHO and fat oxidation rates

There were no significant differences between trials for RER (0.97 ± 0.08 , 0.96 ± 0.07 and 0.96 ± 0.08 for GLU, GAL and PLA trials respectively) during exercise (**Table 1**). During the rest period, RER was significantly greater for the PLA trial compared to both GLU and GAL trials at 15R and 30R min ($P<0.05$). IAUC measures indicated there were no differences between complete trials for CHO oxidation (63.3 ± 4.1 g/h, 62.9 ± 5.5 g/h and 58.7 ± 3.8 g/h for GLU, GAL and PLA trials respectively) (**Figure 2A**). Main effects of time were apparent for all trials ($P<0.05$). IAUC measures indicated that fat oxidation was greater in the PLA (9.8 ± 0.9 g/h) compared to the GLU trial (6.9 ± 0.9 g/h) ($P<0.05$) throughout the duration of the exercise, however there was no main trial effect for the full duration of the trial (**Figure 2B**).

Hunger, gut fullness and thirst scales

Throughout the exercise period following the drinks ingestion, ratings of hunger remained similar to that at baseline in the GLU and PLA trials, and reduced below baseline levels for the GAL trial such that there was a significant main trial effect for hunger values ($P<0.05$) (**Table 1**). During the rest period, hunger increased (time

effect, $P<0.05$) in all three trials. Gut fullness values increased steadily throughout the exercise period for all three trials, after which values declined to below baseline (time effect, $P<0.05$). Thirst values remained within a small range for all three trials throughout the exercise and rest periods (between 7.5 and 10.0) and there were no differences between trials (**Table 1**).

Rating of perceived exertion (RPE) and heart rate

RPE increased throughout the exercise period ($P<0.05$) across all conditions, from average values of 10.4 ± 0.1 to 11.2 ± 0.1 . There were no significant differences between trials overall or at any timepoint.

No differences were observed in heart rate during the 60 min exercise period between trials (161.9 ± 17.5 bpm, 157.1 ± 17.7 bpm and 161.5 ± 11.8 bpm for GLU, GAL and PLA trials respectively).

Energy balance for the exercise and rest period and estimated 24 hour period

Table 2 reports the energy intake and expenditure throughout the course of the exercise and rest periods, whereby intakes and expenditure were controlled, as well as during the self-reported periods, whereby intake and expenditure were estimated. The overall mean energy expenditure during exercise was 583 ± 30 kcal, 607 ± 57 kcal and 624 ± 35 kcal for GLU, GAL and PLA trials respectively, and was not significantly different between trials ($P=0.578$). In addition, the impact of the drinks ingestion on energy intake at the test meal revealed no significant differences between the three trials (838 ± 139 kcal, 818 ± 103 kcal and 847 ± 111 kcal for GLU, GAL and PLA trials respectively). Relative energy balance was calculated for the exercise and rest period as the energy intake (test drink + *ad-libitum* test meal) minus the energy expended (during exercise + rest period) as these were controlled. Results demonstrated a positive energy balance in all three conditions, however there was a significant

difference ($P<0.05$) between energy balance for the GLU (261 ± 143 kcal) and PLA trials (27 ± 65), but not when compared to the GAL trial (196 ± 108 kcal).

In addition, the total 24-hour energy balance was computed, using the total energy intake (breakfast, drink, test meal and self-reported intake) minus the TEE (during exercise + rest period + self-reported exercise). Energy balance results demonstrated the net daily energy balance to be 308 ± 204 kcal, -162 ± 115 kcal and -49 ± 159 kcal for GLU, GAL and PLA trials respectively. The average difference of 468 ± 147 kcal between GLU and GAL/PLA trials was significantly different ($P<0.05$).

DISCUSSION

The aim of the present study was to investigate the effects of consuming a galactose CHO drink versus a glucose or placebo drink before and during exercise on substrate oxidation, post-exercise satiety and subsequent energy intake in recreationally active females. The main findings of the study were that there was a significant improvement in metabolic profile for the GAL and PLA trials compared to the GLU trial (sustained blood glucose and reduced insulin response), however there were no differences in fuel utilization for the duration of the trial (exercise and rest). Throughout the controlled exercise and rest period, there was a positive energy balance for all trials, which was lowest for the PLA trial. It therefore appears that there is no apparent benefit to consuming a CHO beverage for this duration and intensity of exercise in recreational females.

No differences in fuel utilization during exercise were observed between GAL and GLU CHO trials, despite significant differences in plasma glucose concentrations during exercise. These findings are in agreement with previous studies reporting no differences in whole body substrate utilization during exercise at 65% VO_{2peak} , after pre-exercise consumption of glucose, galactose or trehalose [46] or galactose, glucose and fructose combinations [47]. It is therefore likely that the continuous intake of the drinks throughout the exercise period, or the moderate intensity of the exercise protocol, resulted in a reduction in glucose requirements as the main fuel substrate. Previous studies have reported a reduced thermogenic effect after consumption of a sugary beverage. The energy expenditure between conditions during exercise was 583 ± 30 kcal, 607 ± 57 kcal and 624 ± 35 kcal for GLU, GAL and PLA trials respectively, was not significantly different between trials, but does indicate some thermogenic effect between the CHO and non-CHO beverages. There appears to be no previous

studies reporting the thermogenic effect of beverages containing different carbohydrates, which warrants further investigation over a longer period.

In addition, the consumption of GAL prior to and during exercise had no significant effect on fat oxidation during exercise or the subsequent resting period. However, in assessing the total IAUC for all trials, it is evident that ingesting GAL resulted in a 12.1% greater oxidation of fat when compared to ingesting GLU, likely to be due to the reduced insulinemic response. For the purposes of this study, in assessing the effects of these CHO formulations on energy balance, the exercise intensity and duration was kept constant. However, previous studies have reported that ingestion of CHO corresponds with an increase in self-selected exercise intensity [48]. Other studies have consistently demonstrated that ingesting CHO leads participants to feel better during treadmill exercise and report lower rating of RPE [49, 50]. Indeed, we have reported such findings in the same population [51]. Thus, such observations whereby optimal CHO availability and better glycemic control is accompanied with an increased fat oxidation may be particularly pertinent for those wanting to exercise for weight loss or maintenance purposes.

Similar findings are reported in studies when a low GI meal is consumed up 2-3 hours prior to exercise, compared to consuming a high GI meal [12-14, 52, 53]. Given the observed shifts in substrate oxidation in the present study with a relatively small sample size, it would be of interest to investigate the manipulation of CHOs in pre-exercise feeding in addition to supplementation during exercise. It is yet to be determined what effect this would have on self-selected exercise, however the current study looked to replicate recommended habitual submaximal fixed-duration exercise in females [54].

As expected, perceived hunger increased and gut fullness decreased following exercise in all trials. There were differences evident between the trials, with

consumption of the GAL drink suppressing appetite to a greater extent (lower levels of hunger and subsequent reduced *ad-libitum* energy intake). However, these results were not significant. When taking into account energy intake and expenditure, throughout the controlled exercise and subsequent rest periods, there was a positive energy balance in all trials. These energy balance values were significantly greater in the GLU trial when compared to the PLA trial. It is therefore apparent, that the energy intake that was offset by consumption of the PLA drink containing no energy, was not compensated for with an increase in energy intake at the subsequent meal. Thus, the present data imply that when exercise is performed and the energy expended is replaced, energy balance is more favourable for weight loss when a PLA solution is consumed before and during exercise. Although the consumption of a GAL CHO beverage elicited a more favourable metabolic profile in the exercising females, there appears to be no apparent benefit to consuming a CHO beverage for this moderate intensity exercise, as the endogenous supply of CHO energy stores to exercising muscles was not significantly impacted as a result of the exercise task. For physically active adults who exercise for health and fitness, or for those concerned with achieving weight loss, ingestion of a high-energy sports drink appears counterintuitive. It so appears that individual variation exists regarding energy intakes in response to energy expenditure, as previously reported by Gonzalez et al. [55]. This may be due to hedonic processes driving an individual to eat [56]. Therefore, despite the drink consumed and energy expended, some individuals may increase energy intake to a greater extent following exercise (mean range of 1050 kcal for the test meal intake). Yet, there was a small range within individuals' energy intake at the test meal across the three trials (182 kcal). Previous research has demonstrated that moderate intensity exercise does not influence immediate energy intake [57-59] yet others have reported the possibility of the existence of a delay in the compensatory augmentation of energy intake in response to exercise over 3 days [18] or 7 days [60, 61]. Pomerleau et al. [18] observed that daily relative energy intake after low intensity exercise ($40\% \text{VO}_{2\text{peak}}$ for

65 minutes) tended to be lower than no exercise at all. With the addition of breakfast intake improving overall appetite responses to foods consumed later in the day [55], assessing the impact of formations consumed during exercise, a replication of current practice in recreational exercisers appeared warranted. Despite this minimal reduction in the net energy deficit from each exercise bout, these data should be considered alongside the potential of CHO ingestion to increase self-selected intensity and thus overall energy expenditure, better long term adherence to exercise regimes, and potential reductions in energy intakes from subsequent meals [29]

In assessing daily energy balance, taking into account all energy expenditure and intake, the mean net energy balance was negative for both the GAL and PLA trials and positive for the GLU trial, due to the reductions in hunger and post-exercise energy intake as well as a greater overall fat oxidation. These results must be interpreted with caution given that they take into account self-reported energy intakes, the limitations and individual variations of which have been outlined previously [62]. Despite this, these findings could conceivably have implications for long-term regulation of body weight in females, given that the daily difference in energy balance between the consumption of GAL and PLA solutions (357 kcal) is equivalent to completing an additional 36 minutes of exercise (based on a female weighing 65 kg, running at 6 mph jogging pace). Such an increase in activity levels is greater than the current UK recommendations of 30 minutes of physical activity per day [63]. Very few studies have reported findings on energy compensation following CHO ingestion during exercise. Thus, these findings warrant further exploration in a controlled environment to assess energy balance accurately.

CONCLUSION

In conclusion, our findings demonstrate that ingestion of a CHO-containing beverage during moderate intensity exercise could be counterintuitive given that there were no

differences between trials in fuel utilization evident. In addition, a more positive energy balance was associated with the consumption of a CHO beverage when compared to the consumption of a non-CHO beverage; an undesirable effect for those utilising exercise as a weight-loss tool. Despite this, findings indicate that the ingestion of a solution containing galactose before and during exercise can positively impact energy balance throughout the day compared to a more readily available and widely consumed form of CHO, as well as eliciting a more favourable metabolic profile. Thus, the present findings provide insight into a potential mechanism by which consumption of a solution influences appetite and feeding behavior, although further studies are needed to determine whether these observations extend over the longer term.

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TABLE 1 Effects of GLU, GAL and PLA trials on RER, hunger, gut fullness and thirst scale ratings. Values are means±SEM.

Variable	Trial	Time (mins)								
		Pre	15	30	45	60	15R	30R	60R	90R
RER	GLU	0.79±0.05	0.94±0.02	0.94±0.03	0.99±0.03	1.00±0.03	0.94±0.07	0.91±0.07	0.88±0.06	0.95±0.08
	GAL	0.80±0.02	0.93±0.02	0.94±0.03	0.99±0.03	1.01±0.02	0.89±0.04	0.89±0.04	0.84±0.04	0.87±0.08
	PLA	0.82±0.02	0.94±0.02	0.94±0.02	0.99±0.03	1.00±0.04	0.81±0.03 ^{b,c}	0.78±0.03 ^{b,c}	0.79±0.03	0.77±0.02
Hunger	GLU	7.9±0.5	7.7±0.3	7.7±0.3	7.9±0.5	8.2±0.5	9.9±0.8	10.7±0.8	11.8±0.9	13.7±0.5
	GAL	7.6±0.3	7.2±0.2	7.2±0.1	6.8±0.3	7.2±0.3	8.4±0.5	9.2±0.8	11.2±0.7	12.9±0.9
	PLA	7.6±0.4	8.3±0.6	8.6±0.7	7.6±0.4	7.9±0.6	8.9±0.5	10.0±0.6	12.2±0.8	13.4±0.9
Gut fullness	GLU	11.2±0.1	12.1±0.8	13.0±0.7	12.9±0.7	12.4±1.0	11.7±0.7	10.2±0.8	9.3±0.4	8.7±0.6
	GAL	12.4±1.0	11.9±0.8	12.9±0.8	13.6±0.6	13.7±0.6	12.8±0.6	11.8±0.8	9.7±0.6	9.1±0.5
	PLA	12.8±1.4	12.4±1.2	12.4±0.8	12.8±0.7	12.9±0.6	11.1±0.5	10.9±0.6	9.0±0.5	8.2±0.4
Thirst	GLU	8.3±0.7	7.9±0.4	7.7±0.4	8.1±0.4	8.3±0.5	8.9±0.8	9.3±0.6	9.2±0.6	9.6±0.5
	GAL	8.0±0.5	8.4±0.5	8.9±0.7	9.0±0.6	8.2±0.4	9.1±0.5	9.2±0.4	9.3±0.3	8.9±0.4
	PLA	9.2±0.8	7.9±0.4	8.6±0.7	8.3±0.6	8.7±0.8	8.9±0.5	9.6±0.5	9.8±0.5	9.4±0.4

^bsignificant difference between GLU and PLA trials ($P<0.05$). ^csignificant difference between GAL and PLA trials ($P<0.05$).

2 **TABLE 2** Energy balance for the exercise and rest periods, and estimated 24 hour period. Values are means±SEM.

Trial	DER	ENERGY INTAKE					ENERGY EXPENDITURE			ENERGY BALANCE	
		A	B	C	D	E	F	G	H	Controlled	Inc. self-report
		Breakfast (kcal)	Drink (kcal)	Test meal (kcal)	Self reported intake (kcal)	Total energy intake (kcal)	BMR	Exercise + Rest (kcal)	Self- report exercise (kcal)	(B+C)-(G+H)	(A+B+C+D) -(F+G+H)
GLU	2282.69 ± 41.38	221.59 ± 5.86	201.33	837.86 ± 138.82	1399.61 ± 118.71	2660.39 ± 207.72	1426.31 ± 37.60	777.92 ± 35.90	148.44 ± 14.77	261.26 ± 143.24	307.71 ± 203.84
GAL	2282.69 ± 41.38	221.59 ± 5.86	189	818.49 ± 102.52	998.93 ± 87.17	2228.01 ± 131.64	1426.31 ± 37.60	812.02 ± 62.88	152.11 ± 14.89	195.47 ± 108.31	-162.43 ± 115.12
PLA	2282.69 ± 41.38	221.59 ± 5.86	0	846.58 ± 332.53	1307.84 ± 165.32	2376.00 ± 173.56	1426.31 ± 37.60	819.25 ± 34.98	179.89 ± 23.31	27.33 ± 65.12	-49.44 ± 159.82

Figure titles and legends

Figure titles

FIGURE 1 Effects of GLU, GAL and PLA trials on concentrations of blood glucose (A), serum insulin (B), serum FFA (C) and blood lactate (D) during exercise and rest (R). Values are means \pm SEM.

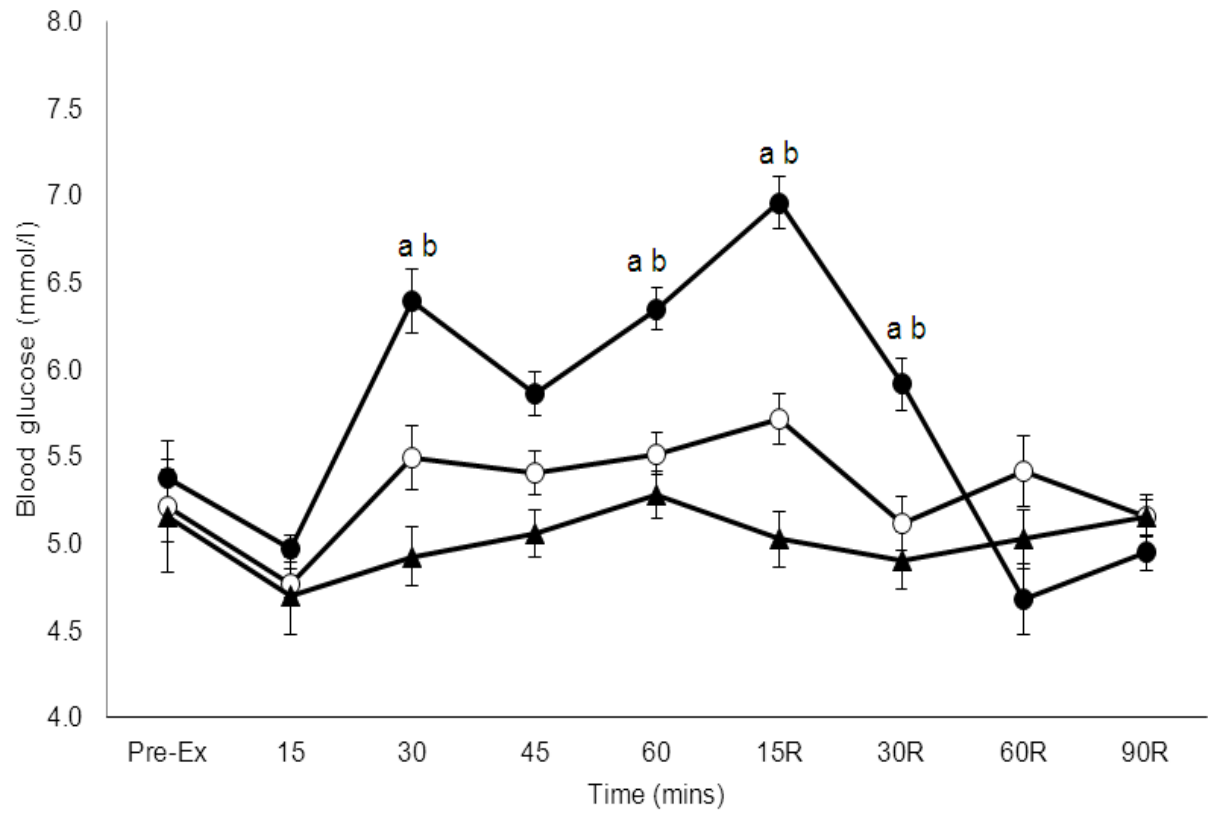
^asignificant difference between GLU and GAL trials ($P<0.05$). ^bsignificant difference between GLU and PLA trials ($P<0.05$). ^csignificant difference between GAL and PLA trials ($P<0.05$).

FIGURE 2 Effects of GLU, GAL and PLA trials on estimated rates of carbohydrate oxidation (A) and fat oxidation (B).

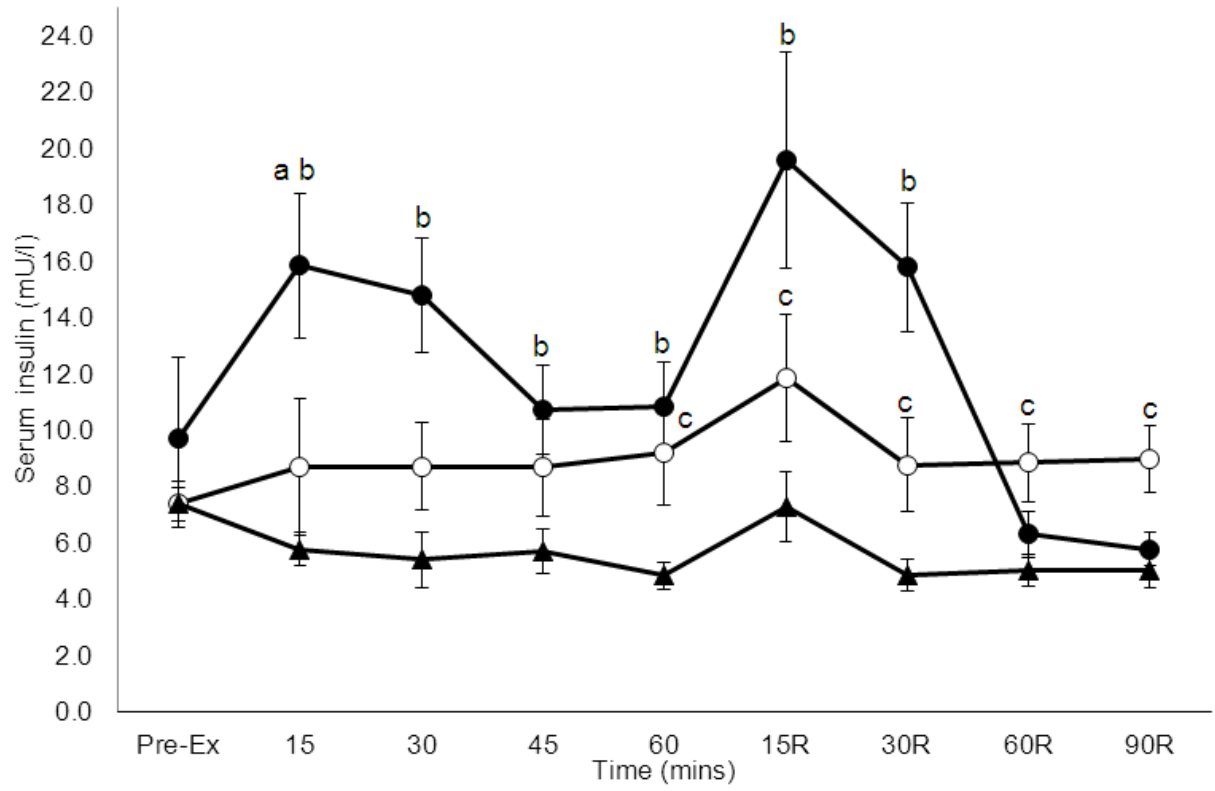
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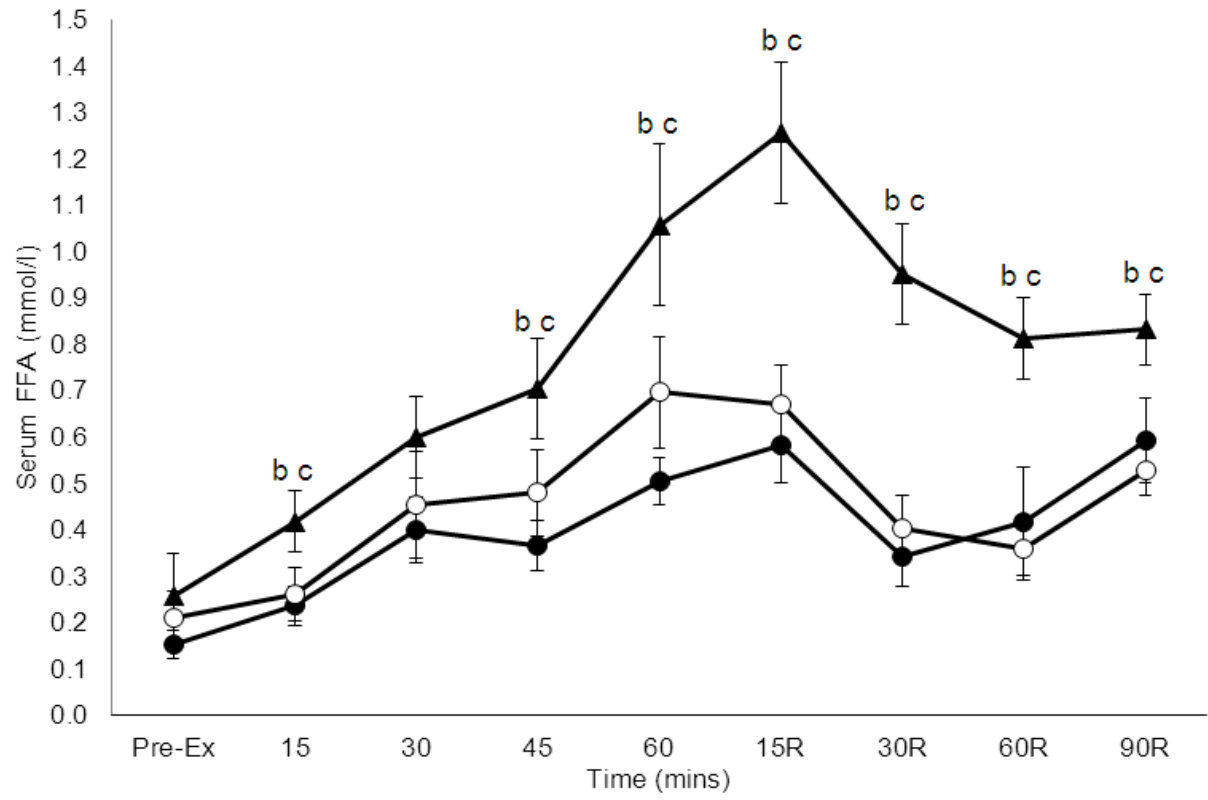
- Glucose
- Galactose
- ▲— Placebo

FIGURE 1



A

**B**

**C**

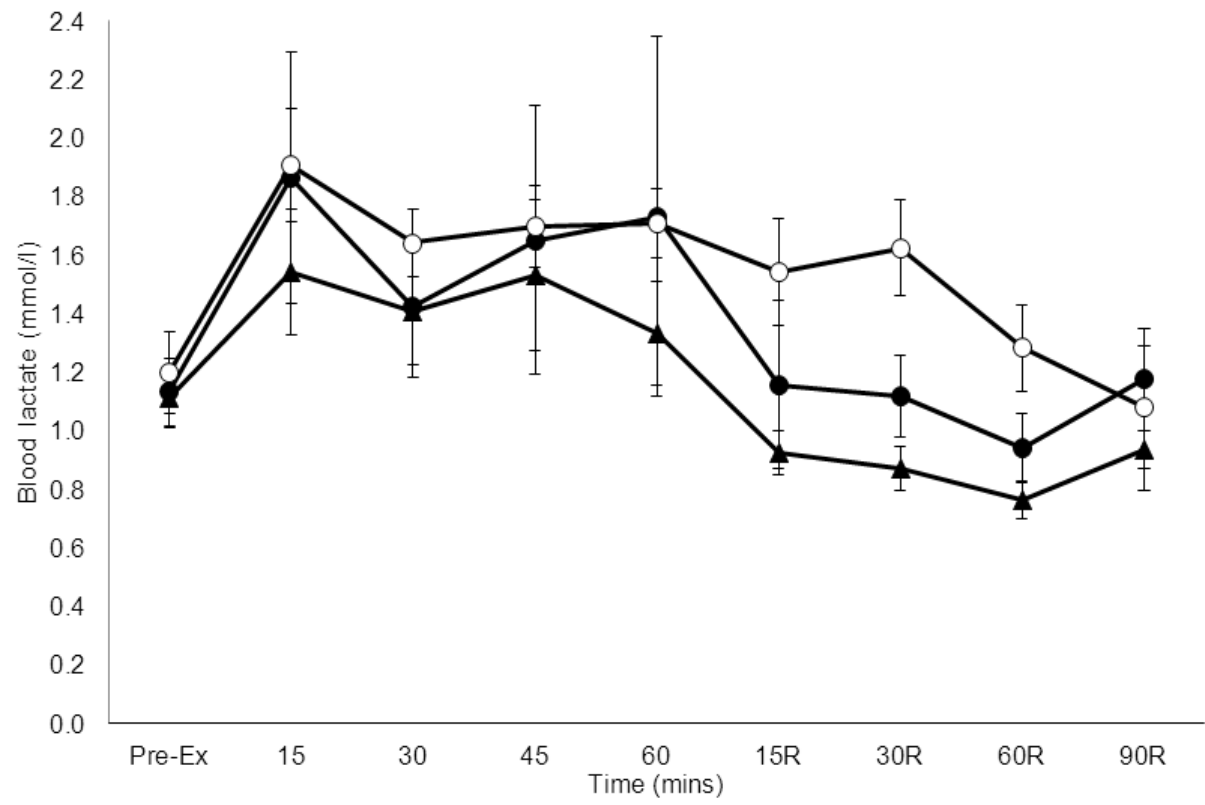
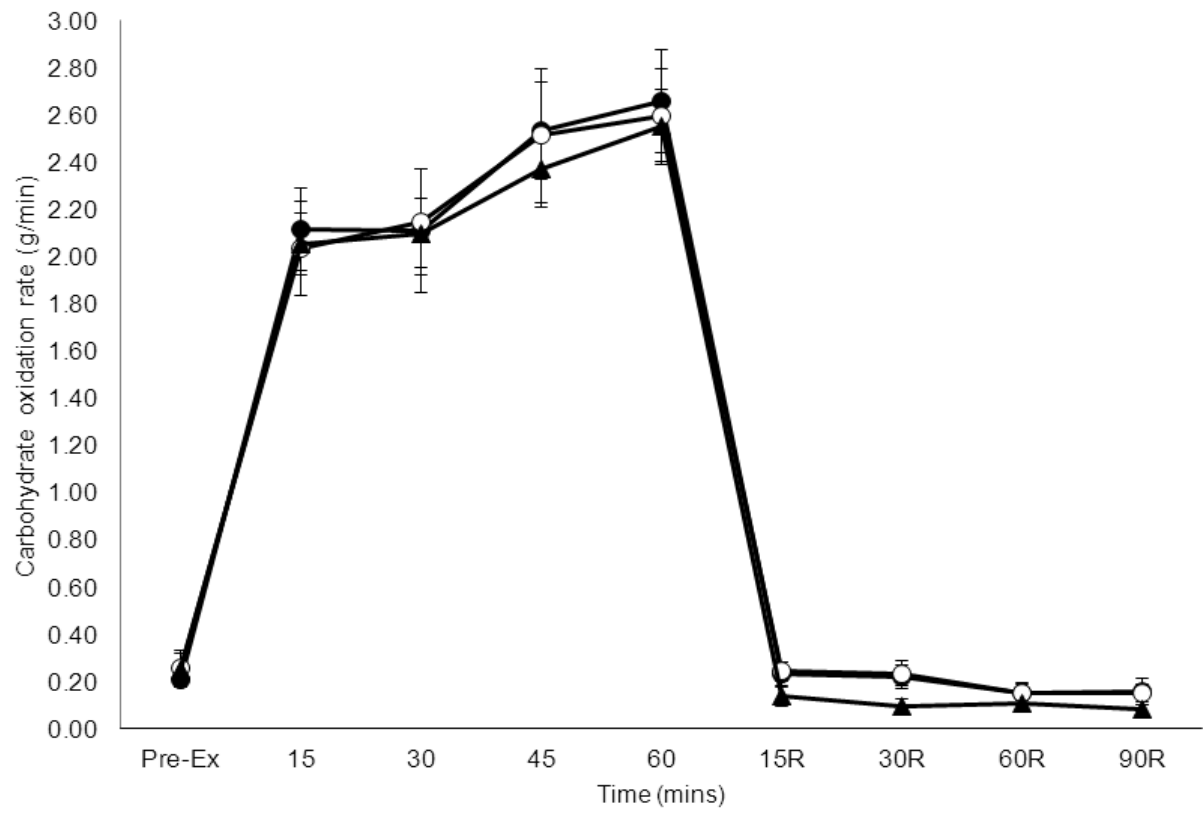
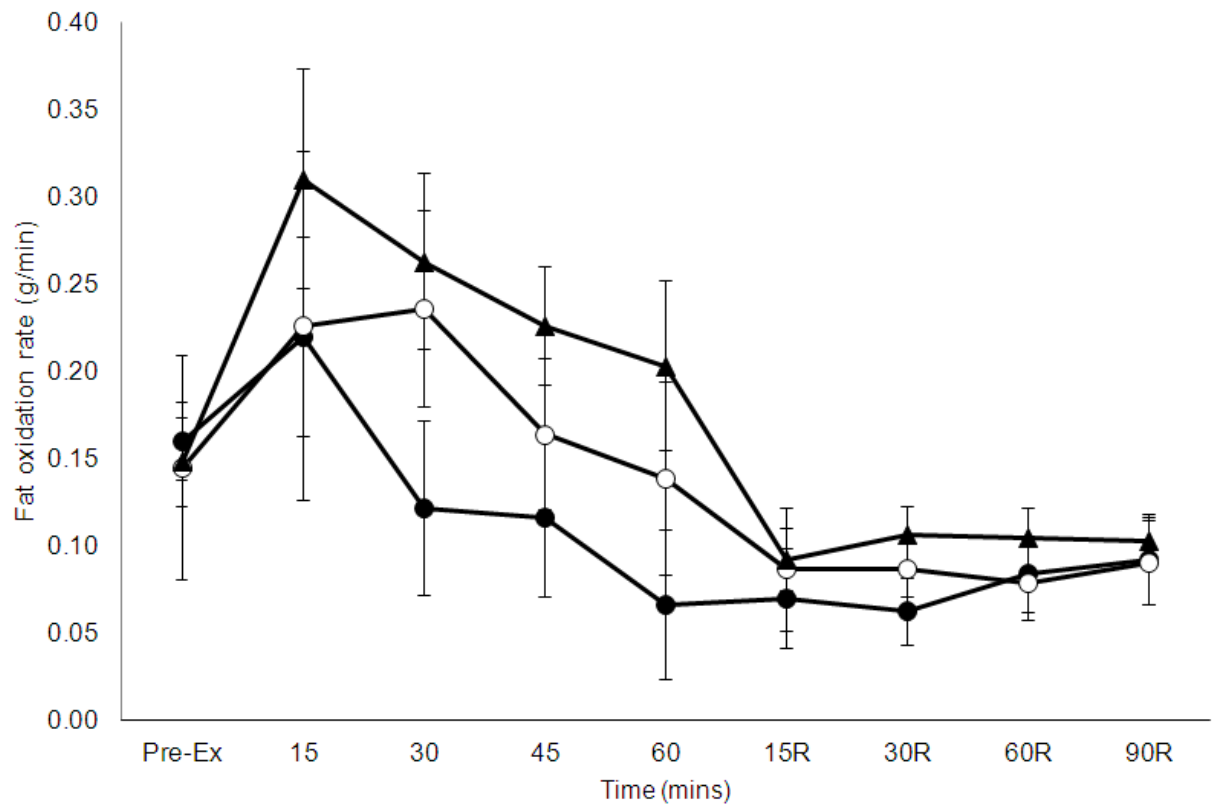
**D**

FIGURE 2



A

**B**