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**Title:** Fructose modifies the hormonal response and modulates lipid metabolism in aerobic exercise after glucose supplementation

**Short title:** Metabolic effects of fructose in exercise

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**ABSTRACT:**

The metabolic response, when aerobic exercise is performed after the ingestion of glucose plus fructose, is unclear. To compare the hormonal and lipid responses provoked by the ingestion of glucose plus fructose in relation to glucose alone, during aerobic exercise and the recovery phase, we administered two beverages containing glucose plus fructose or glucose in a randomised crossover design, to twenty healthy, aerobically trained volunteers. After a 15-minute resting period they performed 30 minutes of moderate aerobic exercise. Urinary and blood samples were taken at baseline ( $t_{-15}$ ), during the exercise ( $t_0$ ;  $t_{15}$ ;  $t_{30}$ ) and during the recovery phase ( $t_{45}$ ;  $t_{75}$ ;  $t_{105}$ ). Plasma insulin concentrations were higher half-way through the exercise period and during acute recuperation ( $t_{15}$  and  $t_{75}$ ;  $p < 0.05$ ) following ingestion of glucose plus fructose than after glucose alone, without any differences between the effects of either intervention on plasma glucose concentrations. Towards the end of the exercise, urinary catecholamine concentrations were lower following glucose plus fructose ( $t_{45}$ ;  $p < 0.05$ ). Plasma triglyceride concentrations were higher after the ingestion of glucose plus fructose than after glucose ( $t_{15}$ ;  $t_{30}$ ;  $t_{45}$ ;  $t_{105}$ ;  $p < 0.05$ ). Furthermore, with glucose plus fructose we found higher levels of lipoperoxides ( $t_{15}$ ;  $t_{30}$ ;  $t_{45}$ ;  $t_{105}$ ;  $p < 0.05$ ) and oxidised LDL ( $t_{30}$ ;  $p < 0.05$ ) than after the ingestion of glucose alone. In conclusion, hormonal and lipid alterations are provoked during aerobic exercise and recovery, by the addition of a dose of fructose to the pre-exercise ingestion of glucose.

**Keywords:** glucose plus fructose, fructose and hypertriglyceridemia, carbohydrate supplementation, exercise and lipidic metabolism.

**Abbreviations:** Glu, glucose; GluF, glucose plus fructose; DNL, de novo lipogenesis; VLDL, very low density lipoprotein; HR, Heart-rate; VE, ventilation;  $VO_2$ , oxygen consumption;  $VCO_2$ , carbon dioxide consumption;  $PETO_2$ , partial pressure of oxygen;  $PECO_2$ , partial pressure of carbon dioxide; VT1, ventilatory thresholds 1; VT2, ventilatory thresholds 2; W, work-loads; TAG, plasma triacylglyceride; FFA, free fatty acids; LPO, lipoperoxide; Ox-LDL, oxidised low density lipoprotein; E, epinephrine; NE, norepinephrine; RPE, rating of perceived exertion; BMI, body mass index.

## INTRODUCTION

The pre-exercise ingestion of carbohydrates is a common dietary supplement that delays fatigue and this is frequently consumed between sportsmen or recreational practitioners [1]. However, differences in the mechanisms of absorption and the metabolic pathways taken by the ingested carbohydrate provoke a range of metabolic and hormonal responses that are still partially understood.

Fructose is a monosaccharide that has often been studied as an ergogenic supplement in exercise conditions. In comparison with glucose, pre-exercise ingestion of fructose did not lead to reactive hypoglycaemia and improved performance [2]. This improvement also has been reported when the fructose one was combined with glucose and ingested during an aerobic exercise [3,4]. Nevertheless, in the studies mentioned before, the authors extensively studied some mechanisms related to improved performance, e.g. exogenous and endogenous rate of carbohydrates oxidation, without a directly analysis of the metabolic effects of glucose plus fructose on insulinaemic and glycaemic response. Moreover, at least during rest condition, has been reported a reduction in the insulinaemic and glycaemic response following the combination of different doses of fructose with high glycaemic index carbohydrates [5,6,7]; a finding that has not been studied in exercise conditions. Such information would be of great importance, since one of the key objectives in aerobic exercise is a low insulinaemia and the reduction of the vagal alpha-adrenergic stimulus to favour the selective use of substrates [8,9,10] and beta-adrenergic stimulation for lipolysis [8].

On the other hand, there is growing interest in studying the effects on lipid metabolism and insulin resistance following the ingestion of fructose [11,12]. Stimulation of de novo lipogenesis (DNL) [13], a defect in the clearance of very low density lipoprotein (VLDL) particles [14], or even the esterification of non-oxidized free fatty acids in the liver [15] are among the explanations of the hypertriglyceridemia induced by fructose in healthy subjects at rest conditions. Meanwhile, the reduction in insulin sensitivity appears to be dependent on fructose-induced lipid modification [16]. Nevertheless, a high variability have been observed by the studies carried out to date, depending in part on the quantity of fructose administered [17] and the type and quantity of carbohydrate that accompanied the fructose [6]. Furthermore, other factors that have been studied less, such as the training status of the subjects [18] and exercise performed during the post-absorption phase, might also influence glycaemic and lipid metabolism after fructose ingestion. For this reason, there is no consensus regarding the ingestion of fructose, either alone or in combination with high glycaemic

index carbohydrates; despite such fact, its use as a low glycaemic index sweetener is still being recommended, and fructose is even employed in sports drinks [19].

To the best of our knowledge, to date no studies exist that evaluate, in trained adults, the acute hormonal and metabolic effects of the ingestion of a combination of glucose plus fructose before aerobic exercise. The aim of this study was therefore to compare the acute hormonal response induced by the pre-exercise ingestion of glucose or glucose plus fructose, and its effects on lipid metabolism, in the course of a moderate aerobic exercise and the acute recovery phase. The influence of pre-exercise ingestion of these supplements on the oxidative state of circulating lipids was also studied.

## EXPERIMENTAL METHODS

### Subjects

Twenty healthy adult men (mean age,  $26 \pm 4.8$  years) volunteered to participate in the study. All subjects were normoglycaemic and normolipemic, and none had undergone pharmacological treatment or had taken vitamin or mineral supplements in the two months prior to the study. The subjects were aerobically trained, participating in physical exercise sessions more than three times a week. Table 1 shows their anthropometric and biological characteristics and training background. The aims of the study and the possible risks involved were explained and an informed consent formed was signed by each subject before the start of the study. The ethics committee of the Reina Sofia Hospital in Cordova approved all the procedures employed in the study.

\*\*\* TABLE 1 NEAR HERE\*\*\*

### Study design

As a preliminary, the participants were subjected to an anthropometric study, an evaluation of their nutritional state and a pretesting session in order to determine suitable work-loads during the experimental exercise sessions (tests of  $\text{VO}_2$  max. and 10RM). The subjects were thereafter randomly assigned to perform two experimental trials, in consecutive order, which consisted of pre-exercise ingestion of glucose (Glu) or glucose plus fructose (GluF), and a session of moderate aerobic exercise (Figure 1). A one-week washout period followed each experimental trial and the subjects were also instructed to avoid moderate to severe physical exercise during the 24 hours before each intervention. This was controlled by means of an exercise auto-self recorder and by analysing basal concentrations of Srm-creatinine kinase, with levels higher than  $200 \text{ U/l}^{-1}$  qualifying as an exclusion criterion. The consumption of caffeine, alcohol and carbohydrates with a high glycaemic index was also avoided during the day before each trial.

\*\*\* FIGURE 1 NEAR HERE \*\*\*

### Nutritional status and diet

A qualitative-quantitative assessment of the frequency of food intake retrospective to the four weeks before the preliminary evaluations was obtained from each subject by a nutritionist. The composition of the normal diet and consumption of alimentary antioxidants were calculated with the aid of tables of the chemical composition of food. This nutritional information was used to prescribe an isocaloric diet, with a moderate glycaemic load and glycaemic index [20,21]. The daily

ration of vegetables and fruit was set in accordance with the recommended dietary intakes of ascorbic acid. This diet was followed by the subjects for two weeks before the start of the experimental trials and during the washout periods between the interventions. A 24-hour food consumption diary was completed by the subjects during the week before each experimental trial on days 3 (half week) and 6 (the day before the intervention). The composition of the diet followed by the subjects was calculated and is presented in Table 2.

\*\*\* TABLE 2 NEAR HERE\*\*\*

### Pretesting and determination of workload

All the participants performed a progressive endurance test on an ergometer cycle (Ergometrics 800, Ergoline, Barcelona, Spain), in order to calculate the load of the endurance exercise, at the same time of day (10.00 – 13.00) and under identical ambient conditions (21 – 24° C, 45 – 55% relative humidity). The protocol comprised pedalling for 2 min at 25 W, and for other 2 min at 50 W as a warm-up; afterwards, the load was increased at a rate of 25 W min<sup>-1</sup>. Subjects were required to maintain a constant pedalling rate of between 60 and 70 revolutions min<sup>-1</sup>. The test came to an end when: 1) the subject decided on his own to end it; 2) he could no longer maintain the minimum pedalling rate of 60 RPM; 3) or the criteria for completion of the exercise suggested by the American College of Sports Medicine had been fulfilled [22]. Heart-rate (HR) (beats min<sup>-1</sup>) was recorded continuously throughout the tests and for three minutes post-exercise by means of a 12-channel ECG (Viasys™, Pulse Biomedical Inc., PA, USA). Gas exchange data were continuously obtained by means of an automatic breath by breath system (Oxycon Delta, Jaeger™, Höchberg, Germany), which was calibrated before each test to the appropriate ambient conditions. The following parameters were acquired (average of each 15-sec interval): oxygen and carbon dioxide consumption (VO<sub>2</sub> and VCO<sub>2</sub>, in 1 min<sup>-1</sup> STP); ventilation (VE, in 1 min<sup>-1</sup> BPTS), VE/VO<sub>2</sub>, VE/VCO<sub>2</sub>, and end-tidal partial pressure of oxygen and carbon dioxide (PETO<sub>2</sub> and PECO<sub>2</sub>). Ventilatory thresholds 1 and 2 (VT1 and VT2) and the work-loads (W) corresponding to them were calculated using the methodology suggested by Davis [23]. In brief, VT1 was determined by means of the criteria of an increase in both VE/VO<sub>2</sub> and PETO<sub>2</sub> without a concomitant increase in VE/VCO<sub>2</sub>. VT2 was determined by means of the criteria of an increase in both VE/VO<sub>2</sub> and PETO<sub>2</sub> and a drop in PETCO<sub>2</sub>. VT1 and VT2 were independently calculated by two observers. In the event of failure to agree, a third observer was consulted.

### Supplement and exercise protocol

The subjects met up at the laboratory between 08.00 and 09.00, after 10 to 12 hours of nocturnal fasting. Fifteen minutes before the start of the exercise period, they consumed, in accordance with a cross-randomised protocol, a water-based solution (400 ml) of 50 g of glucose (glucose anhydride  $C_6H_{12}O_6$  99.5%) or 50 g glucose plus 15 g monosaccharide fructose. The 50g of glucose, which requires no hydrolysis for absorption, has been established as the minimal amount of glycaemic carbohydrate in the two trials, with the purpose that this may provide the same threshold for detecting changes in the glycaemic response when fructose was given simultaneously in the GluF trial. For such reason, we intentionally used two non isocaloric supplementations like others studies have done previously [5,6]. The Glu and GluF solutions had a total concentration of 12.5% and 16.5% respectively. The fructose provided 23.07% of the carbohydrates in the combined supplement, and this amount was determined on the basis of earlier reports of its digestibility and absorption in combination with glucose under conditions of rest and exercise [24,25]. The specific concentration of fructose (3.75%) was lower than the 10% maximum reported as being capable of being absorbed without risk of gastrointestinal symptoms [26]. Under both trial conditions (Glu or GluF), the aerobic exercise was performed on the ergometer cycle utilised in the preliminary tests. Each subject exercised at an intensity that corresponded to the value of W attained at a point equidistant between VT1 and VT2 during the preliminary aerobic tests. For example, an individual who had reached VT1 and VT2 with loads of 100 W and 200 W respectively would perform the exercise session against a load of 150 W. In addition, for warm-up purposes, during the first and second minute of the exercise sessions we employed loads of 25 W and 50 W respectively; after three minutes we utilised the load calculated during the preliminary test until a total of 30 min of exercise had been accomplished. The pedalling rate was the same as used in the preliminary tests.

### Blood and urine samples, and analytical assays

On the morning of each experimental trial, a cannula (Vennflon, 16 G, London, UK) was inserted into the antecubital vein in order to extract blood samples. The samples were drawn immediately before the ingestion of the carbohydrates ( $t_{15}$ ) and during the two hours of the post-prandial period at intervals of 15, 30 and 60 min, including the exercise period ( $t_0$ ;  $t_{15}$ ;  $t_{30}$ ) and during the recovery phase ( $t_{45}$ ;  $t_{75}$ ;  $t_{105}$ ). A very small volume of sterile saline was infused immediately after the extraction of each sample in order to keep the cannula clear. The blood samples were collected into tubes containing  $1 \text{ g l}^{-1}$  EDTA, and were always protected from the light. The tubes were stored on ice and plasma was separated within 30 min of extraction by low-speed centrifugation at 1500 g for 15 min at  $4^\circ\text{C}$ .

Glucose concentrations were determined by spectrophotometric methods, using a modular analyzer (ISE-4-DDPPEPP, Hoffmann-La Roche<sup>®</sup>, Basel, Switzerland). Plasma insulin levels were measured by chemiluminiscent microparticle immunoassay (CMIA) using an analyzer (Architect i-4000, Abbott<sup>®</sup>, Chicago, IL, USA). Plasma triacylglyceride (TAG) levels were measured by spectrophotometry using a modular analyzer (ISE-4-DDPPEPP, Hoffman-La Roche<sup>®</sup>, Basel, Switzerland). Serum Free Fatty Acids (FFA) concentrations were determined using an enzymatic colorimetric assay (NEFA Kit, Roche<sup>®</sup> Mannheim, Germany). The levels of lipoperoxide (LPO) were determined using a commercially available kit (LPO-586, Oxis International<sup>®</sup>, Portland, OR, USA). Quantitative determination of oxidised-LDL (ox-LDL) in plasma was performed by enzyme immunoassay using a commercially available kit (Biomedica<sup>®</sup>, Vienna Austria). Plasma lactic acid was measured by enzymatic colorimetric assay using an analyzer (Cobas 400, Hoffman-La Roche<sup>®</sup>, Basel, Switzerland).

The total urine production was also collected into sterile containers before the ingestion of the carbohydrates ( $t_{-15}$ ), at the end of the exercise period ( $t_{30}$ ) and the end of the recovery period ( $t_{105}$ ). The subjects were recommended to drink water *ad libitum* during the exercise and recovery periods in order to encourage urine production. Urinary concentrations of epinephrine (E), norepinephrine (NE) and creatinine were determined by means of high-performance liquid chromatography (HPLC), using a chromatograph (BioRad<sup>®</sup>, Hercules, California, USA) with a reverse-phase column (flow rate of  $1 \text{ ml} \cdot \text{min}^{-1}$ ) and an electrochemical detector (at 500 mV and 10 nA). Urinary concentrations of E and NE were expressed relative to urinary creatinine (nmol/mmol creatinine). In addition, one day prior to collecting the urine we encouraged the subjects they abstain from bananas, coffee, pineapple and walnuts. We also assured they did not take any of the following drugs: pheothiazin, paracetamol, salsinol, isoproterenol or alpha-methyldopa.

### **Perceived exertion and heart rate**

The rating of perceived exertion (RPE) was recorded according to the Borg perceived exertion scale (CR10) at three different times: mid-exercise, after completion of the exercise [27], and half-hour after completion of the exercise (Figure 5). This last recording was used to indicate the subjects' overall perception of the session [28]. The scale had been used by the subjects during their last training year; so there was a good reproducibility and a period for such purpose was not deemed necessary. Although, the HR was continuously recorded during the study (Polar<sup>®</sup> 810; Kempele, Finland)

### Statistical analysis

Traditional statistical methods were used to calculate means and standard deviations (SD). The normality of the samples was tested using the Shapiro-Wilk test. The effect of the different interventions (Glu and GluF, independent variables) on glycaemia, insulinaemia, E, NE, TAG, FFA, ox-LDL and LPO (dependent variables) was analyzed by analysis of variance (ANOVA) with repeated [2 (group) x 7 (time)] measures. A Sidak correction was used to adjust the p-value in relation to the number of contrasts that were performed. A  $p < 0.05$  criterion was adopted for statistical significance; for all the statistical tests we used the SPSS 11.5 package for Microsoft Windows.

## RESULTS

### Plasma glucose, insulin and urinary catecholamine

Plasma insulin concentrations were higher in the GluF than in the Glu trials during the exercise and recovery phases ( $t_{15}$  and  $t_{75}$ ;  $p < 0.05$ ), without differences being found between the two interventions in the glucose concentrations during these phases (Figure 2A-B). Lower urinary levels of E and NE (Figure 3A-B) were found at the end of the exercise phase following GluF than after Glu ( $t_{45}$ ;  $p < 0.05$ ).

\*\*\* FIGURE 2 AND 3 NEAR HERE\*\*\*

### Plasma triacylglyceride and free fatty acid concentrations

Following the ingestion of GluF, TAG levels were significantly higher during the exercise phase ( $t_{15}$  and  $t_{30}$ ;  $p < 0.05$ ) and acute recovery phase ( $t_{45}$  and  $t_{75}$ ;  $p < 0.05$ ) than following Glu intake (Figure 4A). FFA levels after GluF (Figure 4B) were lower half-way through the exercise phase ( $t_{15}$ ;  $p < 0.05$ ) and during the recovery phase ( $t_{75}$ ;  $p < 0.05$ ), but were higher at the end of the exercise phase ( $t_{30}$ ;  $p < 0.05$ ) in comparison with Glu intake.

\*\*\* FIGURE 4 NEAR HERE\*\*\*

### Liperoxide and oxidized LDL concentrations

Following the ingestion of GluF, plasma levels of LPO were significantly higher during the exercise and recovery periods ( $t_{15}$ ,  $t_{30}$ ,  $t_{45}$  and  $t_{105}$ ;  $p < 0.05$ ), as were those of ox-LDL at the end of exercise ( $t_{30}$ ;  $p < 0.05$ ) than following consumption of Glu alone (Table 3).

\*\*\* TABLE 3 NEAR HERE\*\*\*

### Blood lactate concentrations, perceived exertion and heart rate

No differences between the supplements were observed in the levels of blood lactate during either the exercise or recovery periods. The RPE and HR was significantly less after GluF than after Glu ( $t_{15}$ ,  $t_{30}$  and  $t_{60}$ ;  $p < 0.05$ ) (Figure 5).

\*\*\* FIGURE 5 NEAR HERE\*\*\*

## DISCUSSION

To the best of our knowledge, this is the first study of healthy sportsmen to demonstrate that the addition of a dose of fructose to the pre-exercise consumption of a glucose supplement produces a cluster of acute metabolic changes during a moderate aerobic exercise and the subsequent recovery period. These fructose-induced metabolic dysregulations consist of a higher level of insulinaemia than following the ingestion of glucose alone, a greater rise in TAG and markers of lipid oxidation, and a lower urinary concentration of catecholamine and plasma FFA during and after the aerobic exercise.

Higher levels of insulin are required for a given glucose concentration when the metabolic clearance of glucose has been reduced [29]. In our study, insulinaemia was 37.2% and 25.8% higher after GluF than after Glu alone during both the exercise and recovery periods ( $t_{15}$  and  $t_{75}$  respectively), without this being accompanied by differences in glycaemic behaviour between the interventions. These findings may reflect both: an improved tolerance to a higher carbohydrate intake, when an extra dose of carbohydrate was administered like fructose; but also, a lower systemic insulin sensitivity following to that fructose ingestion. Dirlwanger et al. [30] have demonstrated that the acute infusion of fructose in healthy volunteers induces hepatic and extrahepatic insulin resistance by doubling the need for insulin required to maintain a glucose steady state. Has been suggested that an overload of lipid metabolites derived from fructose and the subsequent over-production of reactive oxygen species, able to spread outside the liver, could interfere with the insulin signalling [31]. Mainly in animal models of insulin resistance fructose-induced, has been observed that an impaired oxidative state was accompanied by an inflammatory gene response mediated by activated nuclear factor kappa -B (NF $\kappa$ B); and the participation of inflammatory cytokines such as muscle tumor necrosis factor-alpha (TNF- $\alpha$ ) [32,33,34]. In the present study, both postprandial phenomena, a higher rise in TAG and the oxidative damage on lipids, as shown by LPO and ox-LDL in GluF trial, would suggest a higher insulinaemia mediated by inflammatory mechanisms.

Another metabolic change that has been described after fructose consumption is a rise in TAG levels [35,36]. Elevated levels of FFA and TAG have been reported in human, even 45 min after the ingestion of 50 g of fructose [37]. In our study, 30 and 45 min after the ingestion of GluF (half-way through and at the end of the exercise), we observed TAG concentrations that were 38.7 and 34.2% higher than after Glu in the same times. Moreover, the TAG rapidly returned to basal level 45 minutes after its peak level ( $t_{75}$ ) following to GluF, discarding the hypothesis of a delay in the TAG clearance as one of the possible causes of the hypertriacylglycerolemia since it has been proposed

by other authors [15]. Furthermore, the higher level of insulin found with GluF leads us to believe that the mechanism that could have made the greatest contribution to the rise in TAG, was not a reduced activation of lipoprotein lipase in adipose tissue and the subsequent delay in lipid clearance [38], but rather DNL, a mechanism widely explained after fructose intake [13,39].

In these lines, several studies has been reported that the fast arrival of fructose to the liver it could cause an overload of the pentose phosphate pathway leads to the acute expression of lipogenic genes [40, 41]; to the rapid activation of hepatic lipogenesis and the secretion of VLDL via sterol regulatory element-binding protein-1c (SREBP-1c) [42]. In those conditions, the mechanisms of reduction of the glycaemic response (e.g. the hepatic uptake of glucose and glycogen synthesis) possibly are inhibited, while the gluconeogenic and lipogenic pathways are activated [40]. The present study was not designed to investigate the molecular mechanisms responsible for the increase of TAG after GluF ingestion. However, the glucose intake, with an extra dose of fructose (a carbohydrate shown like lipogenesis inducer) and the performance of an aerobic exercise, that may have accelerated the arrival of fructose to the portal system, likely they are the key to explain the shown lipidic behaviour.

On the other hand, it is known that the utilization of substrates during aerobic exercise is modulated by autonomic and endocrine control in response to the type and amount of carbohydrate ingested [43]. Although this study does not provide data that describe substrate oxidation, it has been sufficiently reported that fructose by itself [44], as well as, an elevated insulinaemia during exercise [45] increase carbohydrate oxidation, to the detriment of the lipid contribution to energy consumption. Additionally, in agreement with McLaren et al. [46], we have found that the elevated insulinaemia was accompanied by both; a less concentration of catecholamine in the urinary output from  $t_{-15}$  to  $t_{30}$  of exercise period and a low availability of FFA in the GluF trial. If a lower activation of the lipolysis followed GluF, as it is suggested by these findings, the addition of a dose of fructose, would avoid an important part of the benefits for the health derived of 30min of aerobic exercise. Conversely, if GluF increased the carbohydrate oxidation rate, as reported by other studies with glucose plus fructose [3,4], the use of this faster available fuel, would explain a minor RPE and HR, without a change on blood lactate. Thus, the improvement in these variables during and after the exercise with GluF intake could increase the capacity to perform the exercise more comfortably, but it would not favour the metabolic benefits aimed with an aerobic exercise.

In conclusion, the addition of fructose to the pre-exercise ingestion of a glucose supplement triggers a greater insulin secretion and a lower adrenergic stimulation than the ingestion of glucose alone. This combination of carbohydrates also induces a larger rise in plasma triglycerides, a deterioration of the oxidative state of circulating lipids and the suppression of circulating free fatty acids during the performance of moderate intensity aerobic exercise and the post-exercise recovery phase. Future studies in risk populations (e.g. diabetics, obese) are required, in order to analyse the metabolic effect of supplements containing different doses of fructose in mixed pre-exercise meals or beverages.

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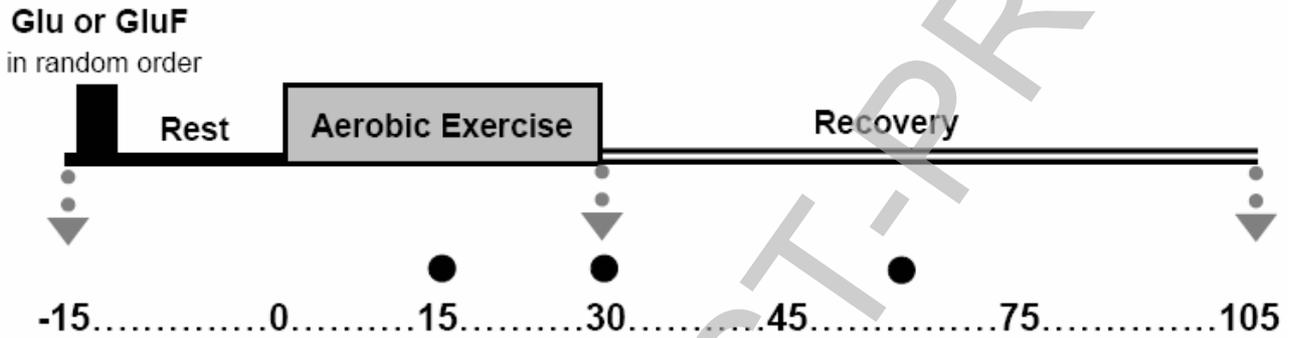
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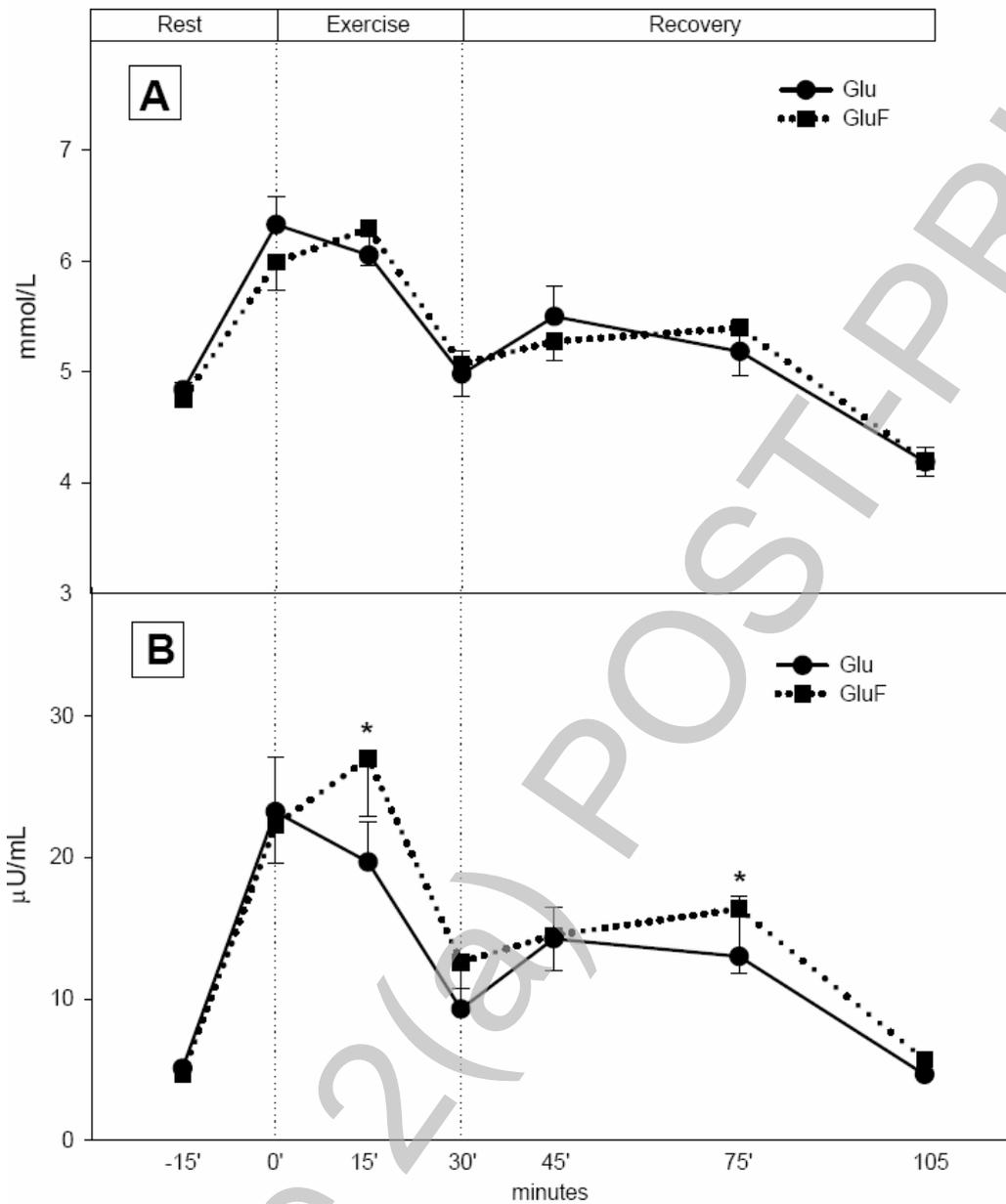
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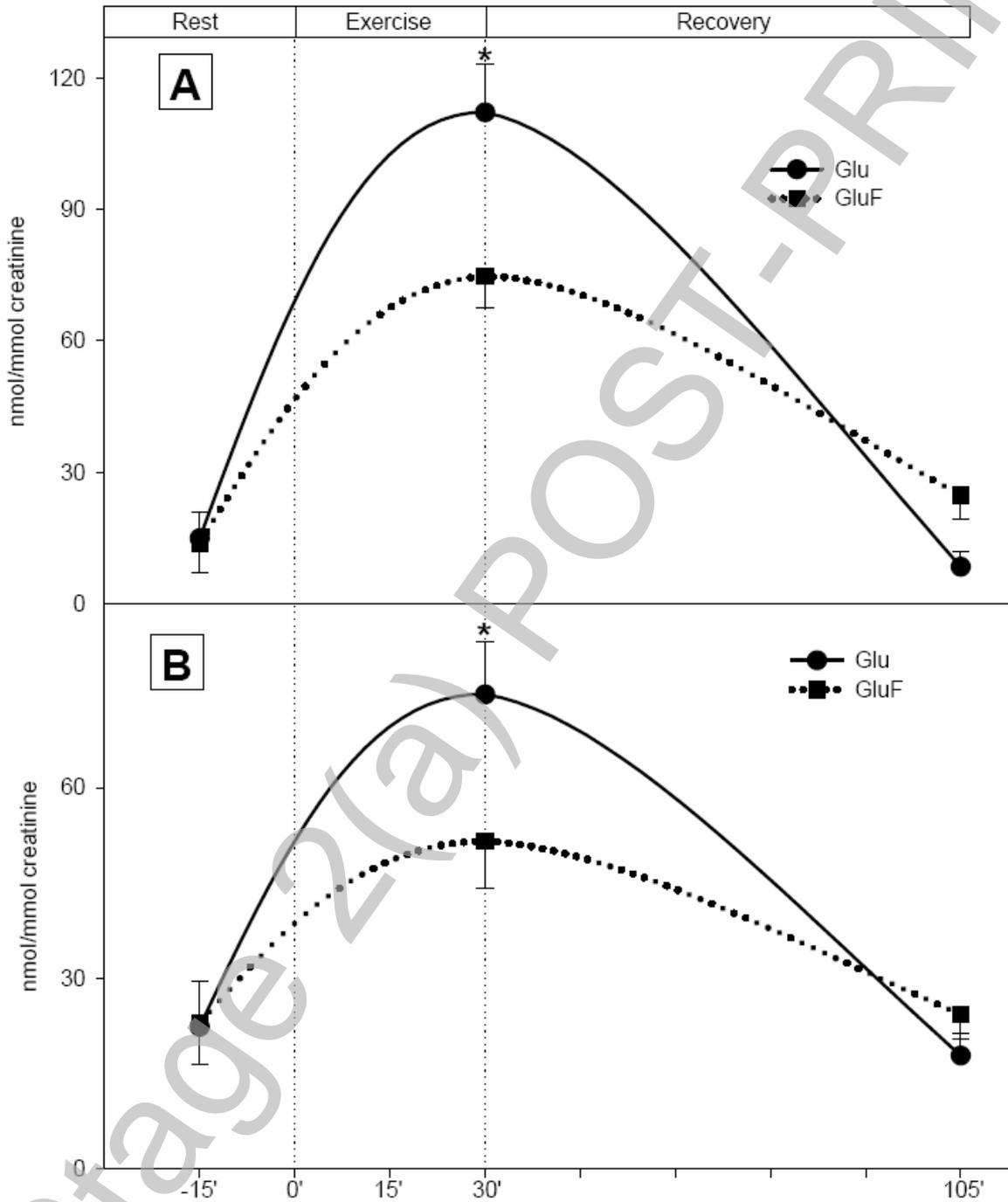
**Figure 1** Schematic diagram of experimental protocol used in glucose (Glu) or glucose plus fructose (GluF) trial. The timing of consumption of carbohydrate (■) in relation to exercise and recovery period is given. Blood samples were taken at the times shown. Urine production (▼) and rate of perceived exertion test (•) are also shown.



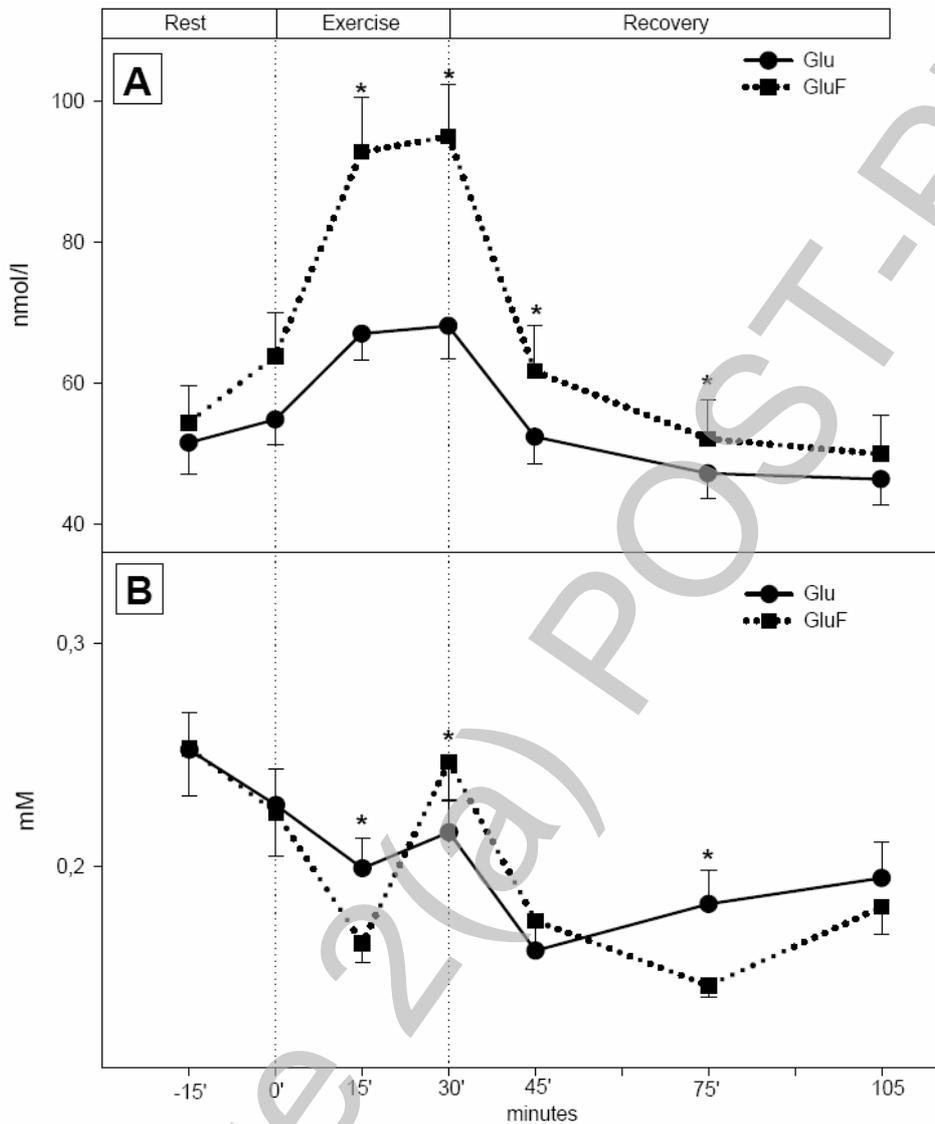
**Figure 2** Plasma glucose (A) and insulin (B) concentrations at rest, throughout exercise and recovery period with the pre-exercise ingestion Glu or GluF. Values are means  $\pm$  SE; n=20 subjects. There were significant carbohydrate x time interactions for plasma insulin ( $p=0.003$ ) by repeated-measures ANOVA. \*Significantly different in Glu compared with GluF,  $p<0.05$ .



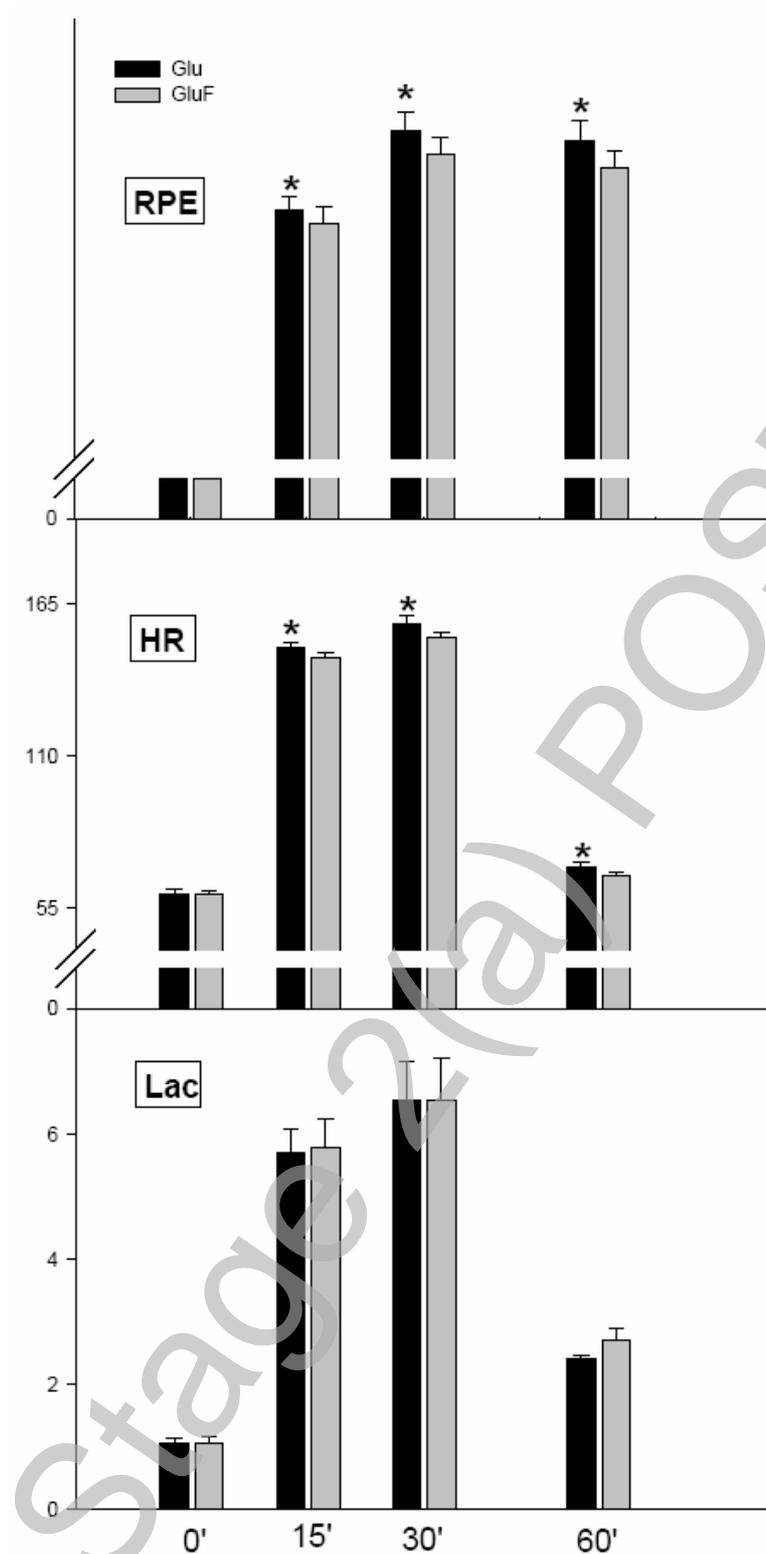
**Figure 3** Urinary epinephrine (A) and norepinephrine (B) concentrations at baseline, end of exercise and recovery period with the pre-exercise ingestion Glu or GluF. Values are means  $\pm$  SE;  $n=20$  subjects. There were significant carbohydrate x time interactions for urinary epinephrine and norepinephrine ( $p=0.001$  and  $p=0.02$  respectively) by repeated-measures ANOVA. \*Significantly different in Glu compared with GluF,  $p<0.05$ .



**Figure 4** Plasma triglyceride (A) and free fatty acid (B) concentrations at rest and during exercise and recovery period following the pre-exercise ingestion of Glu or GluF. Values are means  $\pm$  SE;  $n=20$  subjects. There were significant carbohydrate  $\times$  time interactions for plasma triglyceride and FFA ( $p=0.001$  and  $p=0.03$  respectively) by repeated-measures ANOVA. \*Significantly different in Glu compared with GluF,  $p<0.05$ .



**Figure 5** Rate of perceived exertion, heart rate and blood lactate at rest, middle and end of exercise and its recovery period, for Glu or GluF interventions. Values are means  $\pm$  SE; n=20 subjects. There were significant carbohydrate x time interactions for RPE ( $p=0.032$ ) by repeated-measures ANOVA. \*Significantly different in Glu compared with GluF,  $p<0.05$ .



Tables

**Table 1** Characteristics and training background of the study subjects. Values are means  $\pm$  SD.

	<b>Variables</b>	<b>Mean</b>	<b>SD</b>
Biological	Age (years)	26	4,85
	Weight (Kg)	75	10,5
	BMI (Kg/m <sup>2</sup> )	23,45	1,93
	% Body fat	16,96	3,44
	Glycaemia (mmol/L)	4,19	0,43
Biochemical	Total Cholesterol (mmol/L)	3,60	0,68
	Triglycerides (mmol/L)	0,70	0,21
	High Density Lipoprotein (mmol/L)	1,37	0,34
	Low Density Lipoprotein (mmol/L)	1,88	0,48
Total Training	Years	2,2	1,3
	Day/week	5,7	0,5
	Sessions/week	6,3	1,2
Endurance Training	Min/week	349,7	69,2
	Sesion/week	5,4	0,8
	Min/week	246,3	60,6

**Table 2** Dietary composition during the week before Glu or GluF trial. Values are means  $\pm$  SD.

	<b>Dietary Variables</b>	<b>Mean</b>	<b>SD</b>
	Total Calories (kcal/day)	1790,74	263,56
Caloric Distribution	% Carbohydrate	44,56	5,89
	% Protein	23,85	3,59
	% Fat	31,25	5,54
	Energy Density (kcal/g)	1,36	0,21
Dietary Carbohydrate	Total Carbohydrate (g)	200,01	38,64
	Dietary Glycaemic Load	124,41	16,43
	Dietary Glycaemic index	63,51	6,85
	Total Fiber (g)	23,24	5,51
Ascorbic acid	Intake (mg/day)	65,54	26,94
	% RDIs	109,2	-

**Table 3** Lipoperoxide and Oxidised LDL measured immediately before Glu or GluF supplementation ( $t_{-15}$ ) and at their respective exercise ( $t_0$ ,  $t_{15}$  and  $t_{30}$ ) and recovery phases ( $t_{45}$  and  $t_{105}$ ). Values are means  $\pm$  SE. \*Mean values were significantly different ( $p < 0.05$ ) between the groups.

Treatment	Time, min					
	-15	0	15	30	45	105
	<i>LPO</i> (nmol/dl)					
<b>Glu</b>	0.36 $\pm$ 0.01	0.36 $\pm$ 0.01	0.36 $\pm$ 0.02	0.35 $\pm$ 0.02	0.35 $\pm$ 0.02	0.39 $\pm$ 0.01
<b>GluF</b>	0.36 $\pm$ 0.01	0.38 $\pm$ 0.02	0.40 $\pm$ 0.01*	0.40 $\pm$ 0.02*	0.40 $\pm$ 0.02*	0.42 $\pm$ 0.01*
	<i>Ox-LDL</i> (ng/ml) †					
<b>Glu</b>	83.21 $\pm$ 13.38	-	89.24 $\pm$ 14.57	96.29 $\pm$ 15.96	94.46 $\pm$ 14.06	89.59 $\pm$ 13.93
<b>GluF</b>	82.35 $\pm$ 15.12	-	90.00 $\pm$ 15.98	105.26 $\pm$ 16.15*	97.17 $\pm$ 16.01	89.55 $\pm$ 14.15

† Values adjusted for body mass index (BMI).

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