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Human Kinetics
ORIGINAL RESEARCH

Metabolism and Whole-Body Fat Oxidation Following Postexercise Carbohydrate or Protein Intake

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Purpose: This study investigated how postexercise intake of placebo (PLA), protein (PRO), or carbohydrate (CHO) affected fat oxidation (FO) and metabolic parameters during recovery and subsequent exercise. ***Methods:*** In a cross-over design, 12 moderately trained women (VO_{2max} 45 ± 6 ml·min^{-1}·kg^{-1}) performed three days of testing. A 23-min control (CON) incremental FO bike test (30–80% VO_{2max}) was followed by 60 min exercise at 75% VO_{2max}. Immediately postexercise, subjects ingested PLA, 20 g PRO, or 40 g CHO followed by a second FO bike test 2 h later. ***Results:*** Maximal fat oxidation (MFO) and the intensity at which MFO occurs (Fat_{max}) increased at the second FO test compared to the first following all three postexercise drinks (MFO for CON = 0.28 ± 0.08, PLA = 0.57 ± 0.13, PRO = 0.52 ± 0.08, CHO = 0.44 ± 0.12 g fat·min^{-1}; Fat_{max} for CON = 41 ± 7, PLA = 54 ± 4, PRO = 55 ± 6, CHO = 50 ± 8 %VO_{2max}, $p < 0.01$ for all values compared to CON). Resting FO, MFO, and Fat_{max} were not significantly different between PLA and PRO, but lower for CHO. PRO and CHO increased insulin levels at 1 h postexercise, though both glucose and insulin were equal with PLA at 2 h postexercise. Increased postexercise ketone levels only occurred with PLA. ***Conclusion:*** Protein supplementation immediately postexercise did not affect the doubling in whole body fat oxidation seen during a subsequent exercise trial 2 h later. Neither did it affect resting fat oxidation during the postexercise period despite increased insulin levels and attenuated ketosis. Carbohydrate intake dampened the increase in fat oxidation during the second test, though a significant increase was still observed compared to the first test.

Keywords: endurance exercise, maximal fat oxidation, post-exercise drinks

In order to maximize the adaptive response to endurance training among athletes and/or to promote healthy living in the general population, there has recently been interest in ways to increase the capacity of fat oxidation during exercise. As glycogen stores are limited, a higher reliance on fat oxidation during long duration endurance events could spare muscle glycogen and be beneficial for performance. Previous research show that periods of training performed with low exogenous or endogenous carbohydrate availability (i.e., with high fat utilization) result in molecular adaptations to a higher fat oxidation capacity and, in some cases, better performance (Cochran et al., 2015; Hansen et al., 2005; Yeo et al., 2008). A nutritional periodization strategy including some training sessions performed with limited carbohydrate availability has been suggested as an approach to maximize both fat and carbohydrate oxidizing pathways, both important for performance (Bartlett, Hawley, & Morton, 2015). From a broader perspective, increased fat oxidation could also have implications for

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weight loss (Dandanell et al., 2017) or in exercise interventions aiming at increasing metabolic flexibility (i.e., the ability to adapt to different fuels) in other subject groups such as type 2 diabetics (Goodpaster & Sparks, 2017). In a recent study, we showed that prior exercise increased whole-body fat oxidation by approximately 75% in a second exercise bout (Andersson Hall et al., 2016). The increase in fat oxidation was significantly higher compared to exercising after overnight fast, indicating that the repeated exercise bout strategy might be superior for maximizing fat oxidation.

Important regulators of fat oxidation identified to date are availability of exogenous and endogenous carbohydrates, mobilization of triglycerides and delivery of free fatty acids (FFA) via the blood stream (Kiens et al., 2011; Spriet, 2011; van Loon et al., 2001). These metabolites are heavily influenced by activity level and diet. Various hormones, including glucagon, cortisol, growth hormone, epinephrine, norepinephrine, and insulin, can rapidly change the amount of fat oxidized in both exercise and resting conditions (McMurray & Hackney, 2005).

Although commencing training with low endogenous carbohydrate availability seems to be an effective approach to increase fat oxidation capacity (Gavin et al., 2015), such strategies might also cause negative outcomes for acute and long term performance and recovery, including negative impact on iron metabolism (Badenhorst et al., 2015), increased effort perception (Yeo et al., 2008), loss of skeletal muscle mass (Mettler et al., 2010; Morton et al., 2010), and exercise-induced immunosuppression (Gleeson, 2016). Ingesting protein in the recovery phase between sessions could potentially be a beneficial strategy to recover immune function (Cruzat et al., 2014) and muscle force capacity (Buckley et al., 2010), and to counteract loss of lean mass (Mettler et al., 2010). Protein, however, stimulates insulin secretion which is known to inhibit fat oxidation. Essential amino acid mixes have been shown to be as potent as glucose in inducing insulin secretion (Floyd et al., 1966). On the other hand, Impey et al. (2015) reported that repeated protein feedings during a 2 h carbohydrate-restricted training did not impair exercise-induced fat oxidation despite elevated insulin levels. No studies have investigated the strategy of protein intake during repeated exercise bouts, and it is therefore an open question whether enhanced fat oxidation rates following prior exercise can be preserved after protein ingestion.

The aim of this study was to investigate postexercise metabolism after intake of protein, carbohydrate, or placebo and to measure effects on fat oxidation during a second bout of exercise.

We hypothesized that there would be a marked increase in fat oxidation after prior exercise and that protein intake would not interfere with the increase whereas carbohydrate intake would.

Methods

Subjects

Twelve recreationally active women (age 25.2 ± 3.6 years, BMI 24.9 ± 3.0 kg·m^{-2}) exercising at least twice weekly were recruited. The subjects gave their written informed consent to participate in the present study. Test procedures were performed in accordance with the Declaration of Helsinki 2008 and approved by the local ethics committee of Gothenburg University (Dnr 121-15). VO_{2max} on an ergometer bike was determined as described in Andersson Hall et al. (2016). Briefly, three steady state work-loads were followed by incremental load increases each minute until exhaustion. Power corresponding to VO_{2max} was extrapolated and used to calculate loads for fat oxidation tests. Average VO_{2max} was 45.4 ± 5.8 $mlO_2 \cdot min^{-1} \cdot kg^{-1}$.

Test Design

The subjects completed three test days separated by a minimum of three days in a randomized cross-over design (Figure 1). The day before tests, participants were sedentary and instructed to consume an identical dinner all three times, containing approximately 2.1 g·kg^{-1} of carbohydrate to ensure replenished glycogen stores.

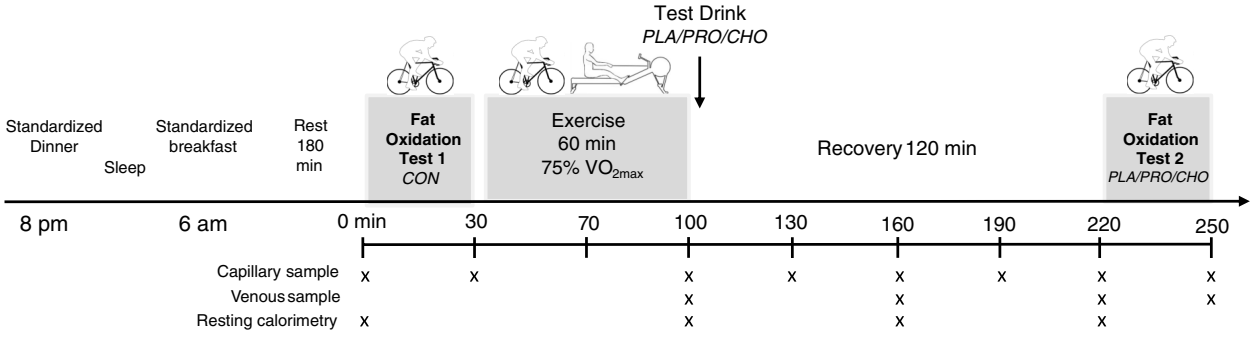

Figure 1 — Experimental design. Subjects performed three days of testing in a cross-over design with different postexercise drink intake (placebo [PLA], protein [PRO], or carbohydrate [CHO]).

Mornings of the test days, subjects ate a standardized breakfast, 1.2 $g \cdot kg^{-1}$ bw carbohydrate, 0.22 $g \cdot kg^{-1}$ bw protein, and 0.15 $g \cdot kg^{-1}$ bw fat, three hours before performing a submaximal incremental fat oxidation (FO) test (control test–CON). After 10 min rest, a 60-min training session followed comprising alternating cycling and rowing (two repetitions of 20 min cycling and 10 min rowing) at a heart rate corresponding to 75% of VO_{2max} determined from the cycle ergometer. Water was consumed ad libitum. Five min after exercise, one of three drinks was consumed: flavored water (PLA), 20 g whey protein in flavored water (PRO), or 40 g maltodextrin in flavored water (CHO). Two hours of recovery followed before a second FO test was performed.

Venous blood samples were collected immediately after exercise, after one and two hours of rest, and immediately after the second FO test. Capillary blood was collected before and after tests and exercise and every 30 min during recovery. Resting metabolism was determined by indirect calorimetry immediately preceding the two FO tests and twice during the recovery period. The subject was sitting still in a calm environment for at least five min prior to measurement, and expired VO_2 and VCO_2 was measured (Jaeger Oxycon Pro, Viasys Healthcare) for five min once the test leader assessed that the ventilator quotient was stable and within resting range. One subject's resting RER was excluded due to hyperventilation. During recovery, subjects were weighed and made to drink water corresponding to weight lost through perspiration.

FO Test

Subjects cycled on an electromagnetic braked bike ergometer (SRM, Jülich); five-min warm-up on 25% of VO_{2max}, followed by load increases every three min corresponding to 30%, 40%, 50%, 60%, 70%, and 80% of VO_{2max} as calculated from pretests. VO_2 and VCO_2 were recorded continuously and fat oxidation calculated from the last 60s of every load using Frayn's equations (Frayn, 1983). Heart rate was monitored (Polar RS300X) and rate of perceived exertion (RPE) for whole body and legs (Borg, 1970) was reported immediately after each FO test.

To ensure that protein oxidation did not contribute to any differences seen in RER, urea nitrogen was measured. Subjects emptied their bladder one hour before the second FO test and urine was collected immediately after the second FO test. Urine volume was measured and 2 ml stored at –20°C until assayed (urea was determined using absorbance method *NPU03930, Roche-Cobas 8000*). There was no significant difference in urea between the different conditions (data not shown).

Biochemical Analysis

Capillary blood was analyzed immediately for glucose and lactate (Biosen-C-line, EKF-diagnostic). Venous blood was drawn in vacutainer EDTA and serum separation tubes. EDTA tubes were centrifuged immediately at 4°C, serum coagulated at room temperature before centrifugation. Plasma and serum were immediately aliquoted and stored at –80°C.

Serum cortisol was assayed using Elisa (Cat. No. MBS043519; MyBioSource, San Diego, CA) and insulin using Human Insulin Kit (Cat. No. KAQ1251; Invitrogen Corporation, Frederick, MD). Other metabolites were measured using NMR metabolomics.

NMR Metabolomics

Samples were prepared by mixing equal amounts (100 µl) of plasma from 1.8 ml cryo vials with septum caps (Sarstedt) with buffer (75 mM sodium phosphate pH 7.4, 20% D_2O, 0.02% NaN_3, 0.5 mM 3-(Trimethylsilyl)-1-propanesulfonic acid-d_6 sodium salt) in 96-well deepwell plates (Sarstedt) using a SamplePro L liquid handling robot (Bruker BioSpin). From the plate, 180 µl of each sample was transferred to a 3mm SampleJet tube with the SamplePro L. All liquid handling was performed at 2°C. NMR data was acquired on a Bruker Avance-III-HD 800 MHz spectrometer equipped with a 3 mm TCI cryoprobe, using a 1D CPMG perfect-echo experiment with excitation sculpting for water suppression ('zgespe' pulse sequence). Total duration of the CPMG pulse train was 193 ms. A sweep width of 20 ppm and 128 scans per experiment were used with a relaxation delay of 1.3 s and a data acquisition period of 2.04 s. NMR samples were kept at 6°C in a cooled SampleJet automatic sample changer before measurement and at 25°C during data acquisition. Spectra were processed using TopSpin 3.5pl6 (Bruker Biospin) before being imported into MatLab (MathWorks Inc.) using in-house written scripts. Peak alignment using icoshift 1.2 (Savorani et al., 2010; Tomasi et al., 2011), integration to a linear baseline and probabilistic quotient normalization, was performed with an in-house MatLab routine (Dieterle et al., 2006). Concentrations are expressed as percentage of the average peak height of the first sample (postexercise) combining all subjects and test days.

Statistical Analysis and Calculations

For each incremental FO test, a third degree polynomial regression was performed using fat oxidation as a function of measured intensity (VO_2) including points for resting fat oxidation and origo according to previously described methods (Stisen et al., 2006). This enabled determination of maximal fat oxidation (MFO) and the intensity at which MFO occurs (Fat_{max}).

Fat oxidation data was compared using two-way analysis of variance for repeated measures (ANOVA). MFO, Fat_{max}, respiratory exchange ratio (RER), mean VO2, RPE, and blood metabolites were compared using one-way ANOVA for repeated measures, using Tukey posthoc analysis when significance was found. There was no difference in fat oxidation, VO_2 or RER during the control test performed each of the three test days.

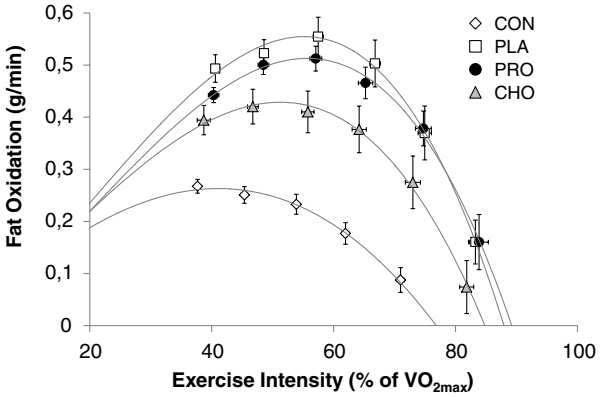

Figure 2 — Whole-body fat oxidation rates during incremental bike tests. CON shows the averages for Test 1 over the three test days. PLA/PRO/CHO shows the respective values of Test 2 of the different test days. Values are plotted as mean ± SE (n = 12). Third degree polynomial regression has been used for curve fitting. Using 2-way ANOVA analysis, $p < 0.01$ for PLA, PRO and CHO compared to CON, and $p < 0.05$ for CHO compared to PLA and PRO. Abbreviations: CON = control; PLA = placebo; PRO = protein; CHO = carbohydrate.

These results have therefore been pooled in figures and tables. A p value < 0.05 was taken to indicate statistical significance. Values are expressed as mean ± SD in text and tables, and mean ± SE in graphs. All $N = 12$ unless otherwise stated.

Results

Compared to the control test performed at the start of each test day, there was a significant increase in fat oxidation during the second test after all three drinks and at all intensities (Figure 2, $p < 0.001$ using 2-way ANOVA for all intensities). There was no significant difference in fat oxidation between PLA and PRO, whereas CHO was significantly lower than both PLA and PRO ($p < 0.01$). This was also confirmed calculating maximal fat oxidation (MFO) for each individual test after polynomial regression (Table 1) resulting in MFOs for PLA, PRO, and CHO all higher than CON ($p < 0.01$), where PLA was also significantly higher than CHO ($p < 0.05$). The intensity at which MFO occurred (Fat_{max}) was also higher for the second test in all three conditions ($p < 0.01$). Fat_{max} for CHO was significantly lower than PLA and PRO ($p < 0.05$).

While using the same work load in each test, average VO_2 was significantly increased during the second test for all three conditions compared to CON ($P < 0.01$). VO_2 for CHO was significantly lower than PLA ($p < 0.05$). Perceived exertion was also significantly ($p < 0.01$) increased at the second test compared to the first test during all three days. No differences in RPE between PLA, PRO, and CHO were observed.

Resting fat oxidation was increased postexercise and remained elevated throughout the two-hour rest period (Figure 3a). After intake of CHO, fat oxidation decreased compared to PLA and PRO ($p < 0.05$ compared to PLA and $p < 0.01$ compared to PRO), but after two hours rest (at the start of the second test) fat oxidation was similar in all three tests.

Capillary glucose and lactate levels measured throughout the test day (Figure 3b) followed the same pattern for PLA and PRO with slowly decreasing glucose concentrations, whereas for CHO glucose levels increased immediately after intake. At the start of the second test, after two hours recovery, glucose levels were at the same level for all three conditions. Interestingly, after CHO but not PLA or PRO intake, MFO during the second test inversely correlated with glucose levels at the onset of the second test ($R = -0.75$, $p < 0.01$).

Venous blood samples were collected during the postexercise period and after the second FO test. Insulin levels stayed low after PLA, but were increased after PRO and CHO with the highest levels after CHO (Figure 4). There was no difference in cortisol concentration between conditions. Postexercise levels of glycerol decreased during rest in all three conditions, and

Table 1 Data From Incremental Fat Oxidation Tests

	CON	PLA	PRO	CHO
	Test 1	Test 2	Test 2	Test 2
Maximal fat oxidation (g·min^{-1})	0.28 ± 0.08	0.57 ± 0.13[1]	0.52 ± 0.08[1]	0.44 ± 0.12[1,2]
Fatmax (% of VO_{2max})	41 ± 7	54 ± 4[1]	55 ± 6[1]	50 ± 8[1,2,3]
RER at fatmax	0.87 ± 0.04	0.80 ± 0.02[1]	0.82 ± 0.02[1]	0.84 ± 0.03[1,2]
VO_2 (% of VO_{2max})	58 ± 3	62 ± 2[1]	62 ± 3[1]	60 ± 3[1]
Heart rate (bpm)	132 ± 15	148 ± 15[1]	145 ± 17[1]	143 ± 14[1,2]
RPE (Borg scale 6–20)	15.8 ± 0.8	17.1 ± 0.8[1]	16.5 ± 0.3[1]	16.6 ± 0.7[1]

Note. CON = control; PLA = placebo; PRO = protein; CHO = carbohydrate; RER = respiratory exchange ratio; RPE = rate of perceived exertion. Data are presented as $M \pm SD$.
[1]Significant vs CON.
[2]Significant vs PLA.
[3]Significant vs PRO using repeated measures ANOVA, $p < 0.05$.

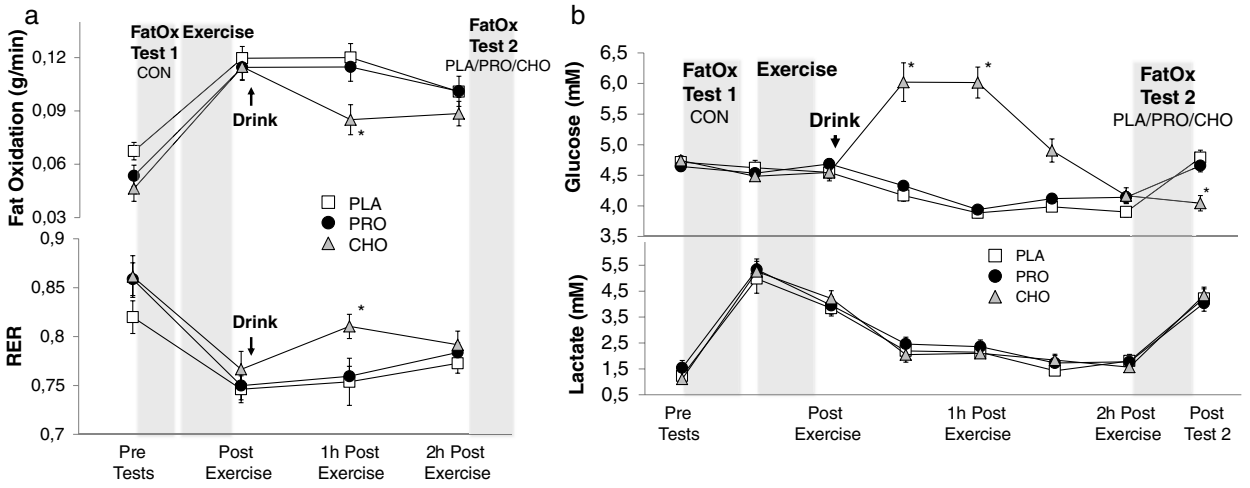

Figure 3 — a) Whole body fat oxidation during rest. Fat oxidation rates and respiratory exchange ratio (RER) measured during rest at four time points throughout the three test days PLA, PRO, and CHO, $n = 11$. b) Capillary blood glucose and lactate levels throughout the three test days PLA, PRO, and CHO, n = 12. Values are plotted as mean ± SE. *$p < 0.05$ compared to PLA and PRO using repeated measures ANOVA. Abbreviations: PLA = placebo; PRO = protein; CHO = carbohydrate.

increased again during the second test (Figure 4). The decrease was, however, slower in PLA with more than double the glycerol concentration at 1h postexercise compared to PRO and CHO ($p < 0.05$). Concentrations of ketones 3-hydroxybutyrate (3-HB), acetoacetate (Acac) and acetone showed large increases during the postexercise period after PLA intake with 3-HB increasing approximately fivefold before decreasing during the second FO test ($p < 0.01$ at one hour and two hours postexercise compared to immediately postexercise, Figure 4). 3-HB and Acac showed similar profiles, whereas acetone had a delayed increase. The postexercise increase in ketones was inhibited by both PRO and CHO intake, with lowest levels after CHO intake.

Plasma levels of branched chain amino acids (BCAAs) increased greatly after PRO intake (Figure 5). All three BCAAs were significantly lower after CHO compared to PLA two hours postexercise ($p < 0.01$). Alanine concentration decreased to the same extent for both PLA and CHO postexercise, whereas after PRO intake it remained at a steady level for one hour before a decrease was observed.

Discussion

This study showed that in moderately trained women, fat oxidation increased by 100% during a second bout of exercise two hours after an initial training session. Postexercise intake of whey protein after the first session did not significantly affect this increase in fat oxidation, whereas maltodextrin intake somewhat attenuated the increase in fat oxidation during the second bout. Both PRO and CHO intake increased blood insulin levels and attenuated the postexercise ketosis seen after placebo intake. Only carbohydrate intake decreased resting fat oxidation during the postexercise rest period. Two hours following CHO intake, when the blood glucose surge had declined, fat oxidation during exercise correlated with blood glucose concentration.

Twice daily training bouts have previously been shown to increase fat oxidation capacity (Hansen et al., 2005; Yeo et al., 2008), and we have shown in the acute setting that repeated bouts are more effective at increasing MFO than training after an overnight fast (Andersson Hall et al., 2016). As this strategy has shown performance benefits and is used by athletes (Close et al., 2016; Cochran et al., 2015; Hansen et al., 2005), modifications to the set-up that could limit potential negative effects would be of interest. Current sports nutrition guidelines (Thomas et al., 2016) suggest that athletes should consume 1.0–1.2 g·kg^{-1} of carbohydrate per hour postexercise or 0.6–1.0 g·kg^{-1} during the first 30 mins (Kerksick et al., 2008). Since small carbohydrate feedings are advised to reduce the risk of gastrointestinal discomfort (i.e., bloated feeling) during limited recovery (Jentjens & Jeukendrup, 2003), athletes in the present study were given 40 g of carbohydrates (corresponding to 0.6 g·kg^{-1}) following the first exercise bout. Furthermore, consumption of approximately 20 g of dietary protein in the immediate postexercise period is recognized as an effective strategy to maximize muscle protein remodeling (van Loon, 2013). Although no fat oxidation studies have investigated carbohydrate intake between exercise sessions, Achten and Jeukendrup (2003) demonstrated that when 75 g glucose was ingested prior to a single fatmax test, MFO rates were found to be suppressed by 28% compared with no carbohydrate ingestion. In our study the carbohydrate intake resulted in a 40% suppression of MFO compared to placebo, but it was still 60% higher during the second FO test compared to the first test. The suppression occurred despite similar

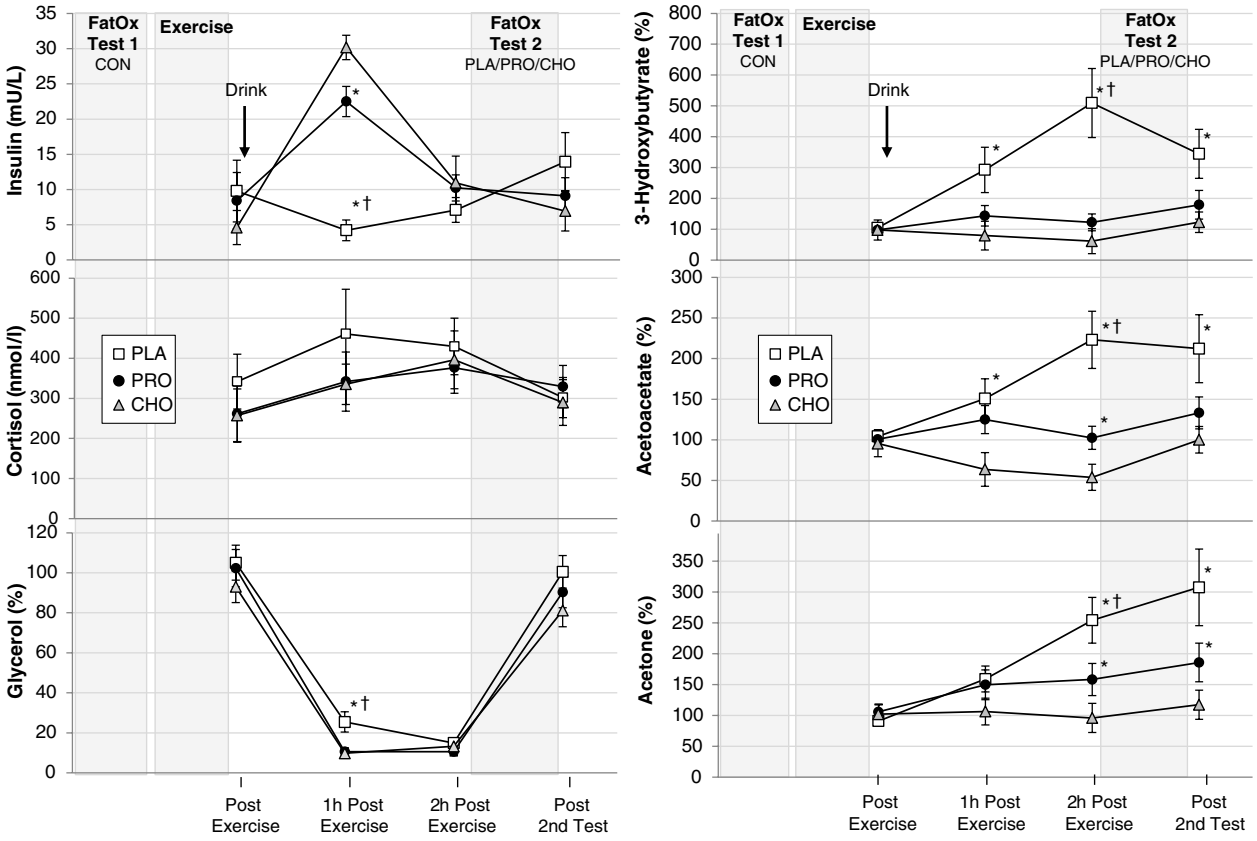

Figure 4 — Blood concentrations of insulin, cortisol, glycerol, 3-hydroxybutyrate, acetoacetate and acetone during the three test days PLA, PRO, and CHO. Values are plotted as mean ± SE (n = 12). *$p < 0.05$ compared to CHO and $^{\dagger}p < 0.05$ compared to PRO using repeated measures ANOVA analysis. Abbreviations: PLA = placebo; PRO = protein; CHO = carbohydrate.

blood glucose and insulin levels and resting fat oxidation prior to the second FO test. The protein ingestion, however, did not affect resting fat oxidation or exercise induced fat oxidation despite temporary increased insulin concentration in the postexercise period. The results during exercise is in line with a two-hour bike study showing that whey protein ingestion did not reduce circulating FFA or fat oxidation during exercise (Impey et al., 2015).

To our knowledge, no previous study have investigated ketone metabolites in conjunction with nutritional strategies aimed at increasing fat oxidation during repeated bouts of exercise. With placebo intake, a clear postexercise ketosis was induced with a five-fold increase in 3-HB levels compared to levels directly after exercise. This correlated well with simultaneous increase of its precursor acetoacetate, and a slightly delayed increase in the breakdown product acetone. Ketosis is known to be reduced by initiation of exercise (Koeslag, 1982), which was seen during the second bike test, a reduction probably resulting from increased peripheral ketone uptake and oxidation during exercise. Previous literature has also suggested that the level of postexercise ketosis depends on training level of the athletes (Koeslag, 1982). Though not published, data from our previous study with identical set-up in elite endurance athletes (Andersson Hall et al., 2016) showed only a 25% increase in 3-HB levels during the postexercise period, supporting this. As expected, CHO intake increased glucose availability which effectively inhibited ketosis. However, PRO also attenuated ketosis almost as efficiently even though fat oxidation was kept at a high level. This can be explained by previous findings showing both glucose and alanine being inhibitors of postexercise ketosis (Koeslag et al., 1982).

In the present study, the concentrations of plasma BCAA increased immediately after PRO as expected, and should prevent potential net muscle protein degradation in the recovery phase (Blomstrand & Saltin, 2001). Interestingly, plasma BCAA levels decreased after CHO intake with significantly lower concentrations than PLA at two hours postexercise. This is in line with recently published results by Rustad et al. (2016), and could be explained by higher insulin levels leading to increased amino acid removal from blood.

From a health perspective, metabolic flexibility (i.e., the ability to switch between different fuels) is linked to insulin resistance (Galgani et al., 2008). Though metabolic flexibility in patients or in at-risk subjects is usually measured at rest, more subtle differences in fuel

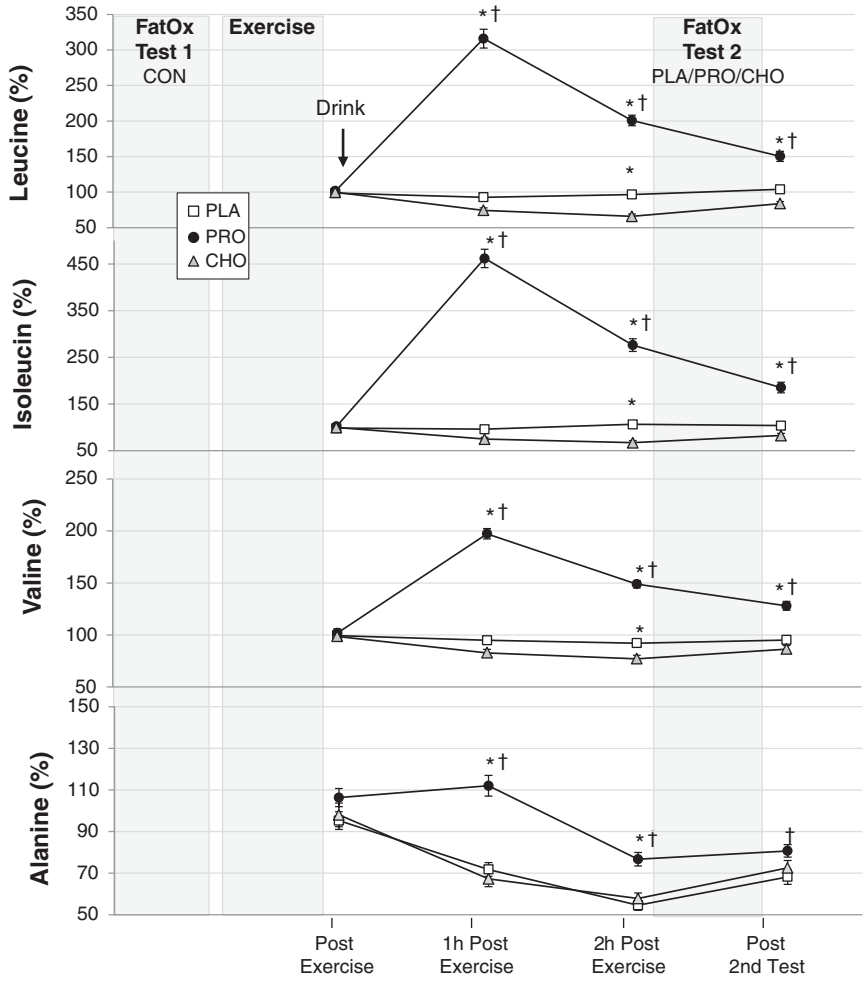

Figure 5 — Blood concentrations of branched chain amino acids (Leucine, Isoleucine, and Valine) and alanine. Values are plotted as $M \pm SE$ ($n = 12$). $^{*}p < 0.05$ compared to CHO and $^{\dagger}p < 0.05$ compared to PRO using repeated measures ANOVA analysis. Abbreviations: PRO = protein; CHO = carbohydrate.

selection might be revealed when the oxidation rates are high during exercise. There has been one report correlating MFO during exercise to insulin sensitivity in normoglyceamic subjects (Robinson et al., 2015). It is therefore highly interesting that after carbohydrate intake in our study, the maximal fat oxidation during exercise correlated to the glucose level immediately before the test (i.e., the women who cleared their glucose to the lowest levels had the highest fat oxidation during that test).

The high fat oxidation during the second test under all three conditions indicate that substrate availability is not solely responsible for the increased fat oxidation, but other exercise induced factors play a role, such as increased circulating hormones promoting fat oxidation (e.g., catecholamines and growth hormone) and enzymatic changes in the muscle (e.g., increased AMPK activity) (Bartlett et al., 2015; Stich et al., 2000). A limitation of the present study is a lack of these hormone measurements and enzymatic and glycogen measurements of muscle biopsies. In addition, as discussed above, we chose 20 g of whey protein, the most commonly recommended dose and frequently used by athletes. We were also interested in how CHO intake would affect fat oxidation in the same set-up, but an isocaloric CHO drink of 20 g would not be a realistic postexercise intake for athletes and therefore chose 40 g (0.6 $g \cdot kg^{-1}$) as discussed above.

Previous research has demonstrated that suppression of lipolysis and lipid oxidation in resting conditions occur at insulin concentrations as low as 13 μU and 44 μU, respectively (Campbell, Carlson, Hill, & Nurjhan, 2006; Groop et al., 1992) and an increment of plasma insulin of only 3–4 μU has been shown to cause a 50% decline in plasma FFA concentration and turnover (Groop et al., 1992). Further research is therefore necessary to determine whether differences in MFO were the result of higher total caloric content thus insulinotropic actions of the CHO beverage or due to specific glucose-mediated mechanisms, as well as the effect of

other carbohydrate sources such as fructose/saccharose or a combination of amino acids and carbohydrate.

In conclusion, postexercise whey protein intake allowed sustained high fat oxidation during subsequent exercise despite increased insulin concentration and attenuated ketosis during the inter-exercise period. 40 g of maltodextrin intake only partly affected the enhanced exercise induced fat oxidation. Whether intake of protein or carbohydrate in the current set-up has advantages such as protective effects for the immune system and sustained net muscle protein synthesis is yet to be determined.

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References

Achten, J., & Jeukendrup, A.E. (2003). The effect of pre-exercise carbohydrate feedings on the intensity that elicits maximal fat oxidation. *Journal of Sports Sciences, 21*(12), 1017–1025. doi:10.1080/0264041031000164140 3

Andersson Hall, U., Edin, F., Pedersen, A., & Madsen, K. (2016). Whole-body fat oxidation increases more by prior exercise than overnight fasting in elite endurance athletes. *Applied Physiology, Nutrition, and Metabolism, 41*(4), 430–437. doi:10.1139/apnm-2015-0452

Badenhorst, C.E., Dawson, B., Cox, G.R., Laarakkers, C.M., Swinkels, D.W., & Peeling, P. (2015). Acute dietary carbohydrate manipulation and the subsequent inflammatory and hepcidin responses to exercise. *European Journal of Applied Physiology, 115*(12), 2521–2530. PubMed doi:10.1007/s00421-015-3252-3

Bartlett, J.D., Hawley, J.A., & Morton, J.P. (2015). Carbohydrate availability and exercise training adaptation: Too much of a good thing? *European Journal of Sport Science, 15*(1), 3–12. doi:10.1080/17461391.2014.920926

Blomstrand, E., & Saltin, B. (2001). BCAA intake affects protein metabolism in muscle after but not during exercise in humans. *American Journal of Physiology, Endocrinology and Metabolism, 281*(2), E365–E374. PubMed

Borg, G. (1970). Perceived exertion as an indicator of somatic stress. *Scandinavian Journal of Rehabilitation Medicine, 2*(2), 92–98. PubMed

Buckley, J.D., Thomson, R.L., Coates, A.M., Howe, P.R., DeNichilo, M.O., & Rowney, M.K. (2010). Supplementation with a whey protein hydrolysate enhances recovery of muscle force-generating capacity following eccentric exercise. *Journal of Science and Medicine in Sport, 13*(1), 178–181. PubMed doi:10.1016/j.jsams.2008.06.007

Campbell, P.J., Carlson, M.G., Hill, J.O., & Nurjhan, N. (2006). Regulation of free fatty acid metabolism by insulin in humans: Role of lipolysis and reesterification. *American Journal of Physiology, 263*(6), E1063–E1069. PubMed

Close, G.L., Hamilton, D.L., Philp, A., Burke, L.M., & Morton, J.P. (2016). New strategies in sport nutrition to increase exercise performance. *Free Radical Biology and Medicine, 98*, 144–158. PubMed doi:10.1016/j.freeradbiomed.2016.01.016

Cochran, A.J., Myslik, F., MacInnis, M.J., Percival, M.E., Bishop, D., Tarnopolsky, M.A., & Gibala, M.J. (2015). Manipulating carbohydrate availability between twice-daily sessions of high-intensity interval training over 2 weeks improves time-trial performance. *International Journal of Sport Nutrition and Exercise Metabolism, 25*(5), 463–470. doi:10.1123/ijsnem.2014-0263

Cruzat, V.F., Krause, M., & Newsholme, P. (2014). Amino acid supplementation and impact on immune function in the context of exercise. *Journal of the international Society of Sports Nutrition, 11*(1), 61. PubMed doi:10.1186/s12970-014-0061-8

Dandanell, S., Husted, K., Amdisen, S., Vigelsø, A., Dela, F., Larsen, S., & Helge, J.W. (2017). Influence of maximal fat oxidation on long-term weight loss maintenance in humans. *Journal of Applied Physiology, 123*(1), 267–274. doi:10.1152/japplphysiol.00270.2017

Dieterle, F., Ross, A., Schlotterbeck, G., & Senn, H. (2006). Probabilistic quotient normalization as robust method to account for dilution of complex biological mixtures. Application in H-1 NMR metabonomics. *Analytical Chemistry, 78*(13), 4281–4290. PubMed doi:10.1021/ac051632c

Floyd, J.C., Jr., Fajans, S.S., Conn, J.W., Knopf, R.F., & Rull, J. (1966). Stimulation of insulin secretion by amino acids. *Journal of Clinical Investigation, 45*(9), 1487–1502. doi:10.1172/JCI105456

Frayn, K.N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of Applied Physiology: Respiratory, Environmental and Exercise Physiology, 55*(2), 628–634.

Galgani, J.E., Moro, C., & Ravussin, E. (2008). Metabolic flexibility and insulin resistance. *American Journal of Physiology: Endocrinology and Metabolism, 295*(5), E1009–E1017. PubMed

Gavin, J.P., Myers, S.D., & Willems, M.E. (2015). The effect of glycogen reduction on cardiorespiratory and metabolic responses during downhill running. *European Journal of Applied Physiology, 115*(5), 1125–1133. PubMed doi:10.1007/s00421-014-3094-4

Gleeson, M. (2016). Immunological aspects of sport nutrition. *Immunology & Cell Biology, 94*(2), 117–123. PubMed doi:10.1038/icb.2015.109

Goodpaster, B.H., & Sparks, L.M. (2017). Metabolic flexibility in health and disease. *Cell Metabolism, 25*(5), 1027–1036. PubMed doi:10.1016/j.cmet.2017.04.015

Groop, L.C., Bonadonna, R.C., Simonson, D.C., Petrides, A.S., Shank, M., & DeFronzo, R.A. (1992). Effect of insulin on oxidative and nonoxidative pathways of free fatty acid metabolism in human obesity. *American Journal of Physiology, 263*(1 Pt 1), E79–E84. PubMed

Hansen, A.K., Fischer, C.P., Plomgaard, P., Andersen, J.L., Saltin, B., & Pedersen, B.K. (2005). Skeletal muscle adaptation: Training twice every second day vs. training

once daily. *Journal of Applied Physiology, 98*(1), 93–99. PubMed doi:10.1152/japplphysiol.00163.2004

Impey, S.G., Smith, D., Robinson, A.L., Owens, D.J., Bartlett, J.D., Smith, K., ... Morton, J.P. (2015). Leucine-enriched protein feeding does not impair exercise-induced free fatty acid availability and lipid oxidation: Beneficial implications for training in carbohydrate-restricted states. *Amino Acids, 47*(2), 407–416. PubMed doi:10.1007/s00726-014-1876-y

Jentjens, R., & Jeukendrup, A. (2003). Determinants of post-exercise glycogen synthesis during short-term recovery. *Sports Medicine, 33*(2), 117–144. PubMed doi:10.2165/00007256-200333020-00004

Kerksick, C., Harvey, T., Stout, J., Campbell, B., Wilborn, C., Kreider, R., ... Antonio, J. (2008). International Society of Sports Nutrition position stand: Nutrient timing. *Journal of the International Society of Sports Nutrition, 5*, 17.

Kiens, B., Alsted, T.J., & Jeppesen, J. (2011). Factors regulating fat oxidation in human skeletal muscle. *Obesity Reviews, 12*(10), 852–858. doi:10.1111/j.1467-789X.2011.00898.x

Koeslag, J.H. (1982). Post-exercise ketosis and the hormone response to exercise: A review. *Medicine & Science in Sports & Exercise, 14*(5), 327–334. PubMed doi:10.1249/00005768-198205000-00002

Koeslag, J.H., Noakes, T.D., & Sloan, A.W. (1982). The effects of alanine, glucose and starch ingestion on the ketosis produced by exercise and by starvation. *The Journal of Physiology, 325*, 363–376. PubMed doi:10.1113/jphysiol.1982.sp014155

McMurray, R.G., & Hackney, A.C. (2005). Interactions of metabolic hormones, adipose tissue and exercise. *Sports Medicine, 35*(5), 393–412. PubMed doi:10.2165/00007256-200535050-00003

Mettler, S., Mitchell, N., & Tipton, K.D. (2010). Increased protein intake reduces lean body mass loss during weight loss in athletes. *Medicine & Science in Sports & Exercise, 42*(2), 326–337. PubMed doi:10.1249/MSS.0b013e3181b2ef8e

Morton, J.P., Robertson, C., Sutton, L., & MacLaren, D.P. (2010). Making the weight: A case study from professional boxing. *International Journal of Sport Nutrition and Exercise Metabolism, 20*(1), 80–85. PubMed doi:10.1123/ijsnem.20.1.80

Robinson, S.L., Hattersley, J., Frost, G.S., Chambers, E.S., & Wallis, G.A. (2015). Maximal fat oxidation during exercise is positively associated with 24-hour fat oxidation and insulin sensitivity in young, healthy men. *Journal of Applied Physiology (1985), 118*(11), 1415–1422. PubMed doi:10.1152/japplphysiol.00058.2015

Rustad, P.I., Sailer, M., Cumming, K.T., Jeppesen, P.B., Kolnes, K.J., Sollie, O., ... Jensen, J. (2016). Intake of protein plus carbohydrate during the first two hours after exhaustive cycling improves performance the following day. *PLoS One, 11*(4), e0153229. PubMed doi:10.1371/journal.pone.0153229

Savorani, F., Tomasi, G., & Engelsen, S.B. (2010). Icoshift: A versatile tool for the rapid alignment of 1D NMR spectra. *Journal of Magnetic Resonance, 202*(2), 190–202. PubMed doi:10.1016/j.jmr.2009.11.012

Spriet, L.L. (2011). Metabolic regulation of fat use during exercise and in recovery. Paper presented at the Sports Nutrition: More Than Just Calories– Triggers for Adaptation. Nestlé Nutrition Institute Workshop, Kailua-Kona, Hawaii.

Stich, V., de Glisezinski, I., Berlan, M., Bulow, J., Galitzky, J., Harant, I., ... Crampes, F. (2000). Adipose tissue lipolysis is increased during a repeated bout of aerobic exercise. *Journal of Applied Physiology (1985), 88*(4), 1277–1283. PubMed

Stisen, A.B., Stougaard, O., Langfort, J., Helge, J.W., Sahlin, K., & Madsen, K. (2006). Maximal fat oxidation rates in endurance trained and untrained women. *European Journal of Applied Physiology, 98*(5), 497–506. PubMed doi:10.1007/s00421-006-0290-x

Thomas, D.T., Erdman, K.A., & Burke, L.M. (2016). Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. *Journal of the Academy of Nutrition and Dietetics, 116*(3), 501–528. PubMed doi:10.1016/j.jand.2015.12.006

Tomasi, G., Savorani, F., & Engelsen, S.B. (2011). Icoshift: An effective tool for the alignment of chromatographic data. *Journal of Chromatography A, 1218*(43), 7832–7840. PubMed doi:10.1016/j.chroma.2011.08.086

van Loon, L.J. (2013). Role of dietary protein in post-exercise muscle reconditioning. *Nestle Nutrition Institute Workshop Series, 75*, 73–83. PubMed

van Loon, L.J., Greenhaff, P.L., Constantin-Teodosiu, D., Saris, W.H., & Wagenmakers, A.J. (2001). The effects of increasing exercise intensity on muscle fuel utilisation in humans. *Journal of Physiology, 536*(Pt 1), 295–304. PubMed doi:10.1111/j.1469-7793.2001.00295.x

Yeo, W.K., Paton, C.D., Garnham, A.P., Burke, L.M., Carey, A.L., & Hawley, J.A. (2008). Skeletal muscle adaptation and performance responses to once a day versus twice every second day endurance training regimens. *Journal of Applied Physiology(1985), 105*(5), 1462–1470. doi:10.1152/japplphysiol.90882.2008