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RESEARCH ARTICLE

Probiotic supplementation increases carbohydrate metabolism in trained male cyclists: a randomized, double-blind, placebo-controlled crossover trial

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Pugh JN, Wagenmakers AJM, Doran DA, Fleming SC, Fielding BA, Morton JP, Close GL. Probiotic supplementation increases carbohydrate metabolism in trained male cyclists: a randomized, double-blind, placebo-controlled crossover trial. *Am J Physiol Endocrinol Metab* 318: E504–E513, 2020. First published February 18, 2020; doi:10.1152/ajpendo.00452.2019.—We hypothesized that probiotic supplementation (PRO) increases the absorption and oxidation of orally ingested maltodextrin during 2 h endurance cycling, thereby sparing muscle glycogen for a subsequent time trial (simulating a road race). Measurements were made of lipid and carbohydrate oxidation, plasma metabolites and insulin, gastrointestinal (GI) permeability, and subjective symptoms of discomfort. Seven male cyclists were randomized to PRO (bacterial composition given in METHODS) or placebo for 4 wk, separated by a 14-day washout period. After each period, cyclists consumed a 10% maltodextrin solution (initial 8 mL/kg bolus and 2 mL/kg every 15 min) while exercising for 2 h at 55% maximal aerobic power output, followed by a 100-kJ time trial. PRO resulted in small increases in peak oxidation rates of the ingested maltodextrin (0.84 ± 0.10 vs. 0.77 ± 0.09 g/min; $P = 0.016$) and mean total carbohydrate oxidation (2.20 ± 0.25 vs. 1.87 ± 0.39 g/min; $P = 0.038$), whereas fat oxidation was reduced (0.40 ± 0.11 vs. 0.55 ± 0.10 g/min; $P = 0.021$). During PRO, small but significant increases were seen in glucose absorption, plasma glucose, and insulin concentration and decreases in nonesterified fatty acid and glycerol. Differences between markers of GI damage and permeability and time-trial performance were not significant ($P > 0.05$). In contrast to the hypothesis, PRO led to minimal increases in absorption and oxidation of the ingested maltodextrin and small reductions in fat oxidation, whereas having no effect on subsequent time-trial performance.

exercise metabolism; exogenous carbohydrates; probiotics

INTRODUCTION

Adequate carbohydrate (CHO) availability, as the main fuel for skeletal muscle and the central nervous system during endurance exercise lasting 1–2 h, and the maintenance of high CHO oxidation rates are critical components for optimal performance. Liver and muscle glycogen stores are limited, and oral ingestion of CHO before and during exercise has been reported to improve performance (11) and delay fatigue during cycling and running (9, 51). This performance benefit has since been reported in numerous publications, with exogenous carbohydrate ingestion showing ergogenic effects for endurance performance in most of these studies (39, 49). However, oxidation rates of orally ingested glucose and maltodextrin (glucose polymer) solutions appear to plateau around 1 g/min (or 60 g/h) (53), even with ingestion rates as high as 2.6 g/min (26), a finding that has been shown many times (12). The capacity of the sodium-glucose transporter 1 in the small intestine is generally regarded as the limiting factor for glucose absorption and the oxidation rate of glucose and maltodextrin ingested during endurance exercise (21). Whereas there appears to be a mean maximal rate of exogenous glucose oxidation of 1 g/min, there appears to be variation among individuals in one study (53) and also among studies (23). Environmental factors can also reduce the maximal oxidation of consumed carbohydrates. Reductions in exogenous carbohydrate oxidation have been seen at increased environmental temperatures (20). This has been related to reductions in splanchnic blood flow and compromised intestinal absorption (47). Strategies that may increase the maximal oxidation rate of orally ingested carbohydrates, either above the previously established 1 g/min or above an individual's own maximal oxidation rate under normal or compromised environmental conditions, could be of benefit to endurance athletes.

One such method, proposed to increase the oxidation of ingested CHO during exercise, is supplementation with probiotic bacteria. In vitro research has shown that coincubation of Caco-2 cells (enterocyte model) with as-yet unidentified, heat-labile metabolites from bacterial strains from the *Lactobacilli* species increases glucose uptake (46). Probiotics, which are shown to increase both the abundance and activity of sodium-glucose transporter 1 (50), as well as to increase insulin secretion following CHO ingestion (28), can also modulate luminal short-chain fatty acid production (44). The latter of these could have wider implications on total CHO oxidation, given that insulin suppresses lipolysis and lipid oxidation during exercise (19). There are then potential mechanisms by which probiotics could increase absorption and thus the subsequent oxidation of consumed glucose, as well as alter total carbohydrate oxidation, and these findings could have practical and relevant implications for athletes if replicable during endurance exercise.

As well as the potential to increase CHO absorption and oxidation, probiotics have also been proposed to be beneficial to performance via positive effects on gastrointestinal (GI)

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permeability and damage. It has been shown that probiotic supplementation or inoculation with the metabolites of probiotic bacteria can prevent epithelial apoptosis (57), increase mucin secretion (6), inhibit attachment of pathogenic bacteria (3), as well as increase expression of tight-junction proteins and decrease secretion of proinflammatory cytokines (33). Given that endurance exercise has been shown to increase markers of GI permeability [e.g., serum lactulose/rhamnose (LR) ratio] and damage [e.g., intestinal-fatty acid binding protein (I-FABP)] (52), probiotic supplementation could attenuate such changes in GI physiology. Whereas there is some evidence that probiotic supplementation can attenuate exercise-induced increases in GI permeability and circulatory endotoxin concentrations (45), we have previously shown there to be no effect (43). However, it is difficult to draw definitive conclusions from such field-based studies. Laboratory-controlled investigations should hopefully provide more insight into the effects of probiotics on exercise-induced GI damage.

The aim of the current study was to investigate whether probiotic supplementation (PRO) increases the oxidation of an ingested maltodextrin drink and total CHO oxidation during 2 h of cycling exercise at 55% maximal aerobic power output (W_{max}). It is hypothesized that 4 wk of probiotic supplementation would increase the intestinal digestion and absorption rate of the maltodextrin drink, the percent contribution of the drink to carbohydrate oxidation rates, and total carbohydrate oxidation rates. It is also hypothesized that the ingestion of the probiotic supplement would significantly reduce the LR ratio and intestinal damage (I-FABP) and improve performance during the 2 h of cycling exercise. These hypotheses have been tested using a double-blind placebo (PLC)-controlled crossover design.

METHODS

Participants. Seven trained cyclists participated in this study [means ± SD; age 23 ± 4 yr, body mass 73.4 ± 7.1 kg, peak oxygen consumption ($\dot{V}O_{2peak}$) 64.0 ± 2.2 $mL \cdot kg^{-1} \cdot min^{-1}$]. None of the participants used medication (e.g., NSAIDs, antidepressants, or diuretics) or nutritional supplements or reported a history of GI-related medical issues (e.g., irritable bowel disease or abdominal surgery). After an explanation of the nature and risks of the experimental procedures to the subjects, their informed, written consent was obtained. The study was approved by the Institutional Ethics Committee of Liverpool John Moores University and conformed to the standards set by the Declaration of Helsinki.

Pre-testing. At least 7 days before the first experimental trial, subjects completed preliminary testing. $\dot{V}O_{2peak}$ and maximal aerobic power output (W_{max}) were determined on an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands) during an incremental exhaustive exercise test. Work rate commenced at 95 W for 3 min, followed by incremental steps of 35 W every 3 min until volitional exhaustion. Oxygen uptake ($\dot{V}O_2$) was measured continuously during exercise using an online gas analysis system (Moxus modular metabolic system; AEI Technologies Inc., PA). $\dot{V}O_{2peak}$ was determined from the highest recorded $\dot{V}O_2$ value from 10-s averages. W_{max} was calculated from the last completed work rate, plus the fraction of time spent in the final, noncompleted work rate, multiplied by the work-rate increment (22). After a rest period of 30–60 min, participants then completed 1 h of cycling exercise at 55% W_{max} following the prescribed drinking protocol and followed by a time trial to familiarize themselves to the real testing procedures described in the following paragraphs.

Treatment allocation. In a randomized, double-blind, placebo-controlled crossover design, each subject completed two, 28-day periods of supplementation, as well as consumption of an additional supplement capsule on the morning of the trials at the end of each intervention period, 1 h before commencing exercise. This 28-day supplement period is in line with our previous work showing lower GI symptoms during exercise (43). Each supplement period was separated by a 14-day washout period. This washout period was based on unpublished data showing that the probiotic strains used are undetectable from stool samples after this time. It also has been shown that this period allows for a number of complete epithelial cell turnovers within the small intestine (2). Participants were randomized to consume either a capsule of a commercially available probiotic (PRO) or a visually identical placebo, daily for 28 days. The PRO supplement contained the active strains *Lactobacillus acidophilus* (CUL60), *Lactobacillus acidophilus* (CUL21), *Bifidobacterium bifidum* (CUL20), and *Bifidobacterium animalis* subsp. *lactis* (CUL34; Proven Probiotics, Port Talbot, UK). The minimum concentration was 25 billion colony-forming units. This probiotic supplement has previously been shown to survive the GI tract during consumption (31) and to have beneficial effects for endurance athletes (43, 45). During the supplementation period, participants were informed to avoid consumption of probiotic foods, such as fermented foods and yogurts. The PLC capsules were visually identical and consisted of starch only (Proven Probiotics, Port Talbot, UK). Subjects were instructed to swallow the capsule daily after their first meal. The randomization code was held by a third party (Cultech Ltd.) and unlocked for statistical analyses by the authors upon sample analysis completion.

Experimental trials. Each subject underwent four experimental trials—one before and at the end of each supplementation period. Trials consisted of 120 min of cycling at 55% W_{max}, in line with previous work examining exogenous oxidation during cycling exercise (55, 58). This was followed by a time trial amounting to 100 kJ of work, simulating the final sprint in a competitive, long-distance road race. Subjects were instructed not to perform any strenuous exercise 24 h before testing and to avoid caffeine, alcohol, and any spicy food. Subjects also recorded their food intake in the 24 h before the first trial and repeated this for each subsequent visit.

Subjects reported to the laboratory at the same time (~7:30 AM) for each trial after an overnight fast of at least 12 h. A cannula (Safety Lock 22G; BD Biosciences, West Sussex UK) was inserted into the antecubital vein, and a baseline blood sample was taken. Resting breath samples were collected over a 5-min period (Moxus modular metabolic system; AEI Technologies Inc., PA), and Exetainer tubes were filled directly from the mixing chamber to determine the $^{13}C/^{12}C$ ratio in expired CO_2. Subjects then began cycling at 55% W_{max} for 120 min. Heart rate (Polar FT1 heart rate monitor; Polar Electro, Kempele, Finland) and ratings of perceived exertion (4) were recorded every 15 min. Immediately following the 120 min of steady-state cycling, simulated cycling time trials were undertaken with the ergometer set in a cadence-dependent power output (linear) mode for subjects to complete 100 kJ of work. Power output was therefore a function of cadence, and a fixed factor (alpha value) was used, as described in the following equation: power (W) = L × rpm^2, in which the rpm is the pedaling rate, and L is a linear factor. This factor was chosen in a way that would evoke 100% peak aerobic power output at a pedaling rate of 90 rpm.

Maltodextrin drink. During exercise, subjects consumed a 10% CHO drink enriched with the stable isotope [U-^{13}C]glucose (CK Isotopes, Ibstock, UK). Maltodextrin (176.4 g; Myprotein Inc., Northwich, UK) and 3.6 g [U-^{13}C]glucose were dissolved in water and made up to a total volume of 1,800 mL. For the two participants in which the oxidation rate of the ingested maltodextrin was not measured, the drink consisted of 10% maltodextrin only. Total drink volume was prescribed according to participant body weight, with an 8-mL/kg body weight bolus in the first 3 min of exercise, followed by 2 mL/kg body weight each subsequent 15 min during 120-min cycling

exercise (24). Total fluid volume and carbohydrate intakes prescribed were 1,790 ± 152 mL and 179 ± 15.2 g, respectively. An elemental analyzer-isotope ratio mass spectrometer (Europa Scientific 20-20; Iso-Analytical Ltd., Crewe, UK) was used to measure accurately the ^{13}C enrichment of freeze-dried samples of the maltodextrin/[U-^{13}C]glucose drinks and the natural ^{13}C background enrichment of the maltodextrin powder, expressed as $\delta^{13}C$ ‰ versus Pee Dee belemnite (PDB). The ^{13}C enrichment of the consumed drinks was very high at 1,681 ‰ versus PDB. The drinks also contained 35 mM of sodium chloride, as sodium in the 30- to 50-mM range leads to better fluid delivery and retention in endurance-trained individuals (32).

$^{13}C/^{12}C$ analysis of breath CO_2. Breath samples were analyzed using an isotope ratio mass spectrometer (Delta XP, coupled to a Gas Bench II and GC Pal autosampler; Thermo Electron, Bremen, Germany). The breath tubes were held in a heated sample tray at 26°C. The breath sample was continuously transferred through a Valco sampling port in a flow of helium. Carbon dioxide was separated from the presence of other gases by using a capillary column (PoraPLOTQ; Agilent JW columns) with dimensions of 27.5 m × 0.32 mm × 10 μm. The oven temperature was kept constant at 68°C. Nafion water traps removed H_2O from the sample. Multiple analysis of each sample was achieved by switching the contents of the sample loop to the GC column every 50 s. Each switch corresponded to starting the GC separation of the sample coming from the loop. Ions mass-to-charge ratio (*m/z*) 44 and 45 were monitored for CO_2 and $^{13}CO_2$, respectively. The ^{13}C enrichment results from breath samples expressed as $\delta^{13}C$ ‰ versus PDB. The $\delta^{13}C$ ‰ versus PDB results of the maltodextrin powder, the maltodextrin/[U-^{13}C]glucose drinks, and breath samples were converted to the tracer-to-tracee ratio (TTR) by using the following equation (14):

$$TTR\left(^{13}C : {}^{12}C\right) = \left[\left(\delta^{13}C\,‰ / 1,000\right) + 1\right] \times 0.0112372.$$

Analysis of plasma [U-^{13}C]glucose enrichment. Plasma glucose isotope enrichment was measured as the tracer/tracee ratio (TTR) by gas chromatography-mass spectroscopy using a trimethyl silyl-O-methyloxime derivative, according to methods previously described (48). The peak areas of the ions *m/z* 319.2 and *m/z* 323.2 for natural glucose and [U-^{13}C]glucose, respectively, were measured by GC-MS on an Agilent 5975C Inert XL EC/CI MSD (Agilent Technologies, Wokingham, Berks, UK).

The concentration of [U-^{13}C]glucose in the plasma ([U-^{13}C]Glu_p) was calculated as follows:

$$\left[U - {}^{13}C\right]Glu_p = Glu_p \times TTR,$$

where Glu_p is the plasma glucose concentration in millimoles per liter and TTR of [U-^{13}C]glucose in the plasma.

At any given time point, the concentration of unlabeled glucose ([^{12}C]Glu_p millimoles per liter) in the plasma that had originated from the maltodextrin drink was derived from the known enrichment of the glucose in the drink as follows:

$$\left[{}^{12}C\right]Glu_p = \left(\left[U - {}^{13}C\right]Glu_p / 3.1\right) \times \left(100 - 3.1\right),$$

where 3.1 is the percent isotopic enrichment of the test drink.

The total concentration of glucose in plasma that originated from the maltodextrin drink (Glu_m) was calculated as:

$$Glu_m = \left[{}^{12}C\right]Glu_p + \left[U - {}^{13}C\right]Glu_p,$$

and the difference between this value and total plasma glucose concentration was assumed to have been derived endogenously. This calculation distinguishes between [^{12}C]Glu produced endogenously and [^{12}C]Glu in the test drink.

Indirect calorimetry and calculations. Respiratory gas exchange variables were measured using a mixing chamber (Moxus modular metabolic system; AEI Technologies Inc, PA), with oxygen uptake ($\dot{V}o_2$), carbon dioxide output ($\dot{V}co_2$), and respiratory exchange ratio measured during a 4-min interval and sample frequency of 15 s after every 15 min during the 2 h of cycling exercise. Breath samples were collected in duplicate directly from the mixing chamber of the Moxus system into sealed Vacutainer collection tubes again every 15 min. Total CHO and fat oxidation rates were calculated from indirect calorimetry data assuming negligible protein oxidation (27):

$$\text{Glucose oxidation} = 4.55 \times \dot{V}co_2 - 3.21 \times \dot{V}o_2.$$

$$\text{Fat oxidation} = 1.67 \times \dot{V}o_2 - 1.67 \times \dot{V}co_2.$$

Exogenous glucose oxidation was calculated using the formula (35):

$$= \dot{V}co_2 \times \left(\delta Exp - \delta Expbkg\right) / \left(\delta Ing - \delta Expbkg\right) / k,$$

in which δExp is the ^{13}C enrichment of expired air during exercise at different time points, δIng is the [U-^{13}C]enrichment of the ingested maltodextrin drink, δExpbkg is the ^{13}C enrichment of expired air before exercise (background), and k is the amount of CO_2 in liters (L CO_2) produced by the oxidation of 1 g of glucose (k = 0.7467 L CO_2/g glucose).

Plasma glucose oxidation was calculated using the formula:

$$= \dot{V}co_2 \times \left(\delta Exp - \delta Expbkg\delta / \delta PG - \delta PGbkg\right) / k,$$

in which, δPG is the plasma [U-^{13}C]glucose enrichment during exercise, and δPGbkg is the plasma [U-^{13}C]glucose enrichment before ingestion of the maltodextrin/[U-^{13}C]glucose drink and the start of exercise (background sample). In the above calculations, the ^{13}C enrichments were all expressed as tracer-to-tracee ratio (TTR) for all analyzed samples (breath, enriched maltodextrin drinks, unenriched maltodextrin powder, plasma glucose).

Because plasma glucose oxidation represents the oxidation of both glucose coming from the gut (exogenous glucose) and the contribution of the liver (glycogenolysis and gluconeogenesis), liver-derived glucose oxidation and muscle glycogen oxidation could be calculated by the following formulas:

$$\text{Liver-derived glucose oxidation} = \text{plasma glucose oxidation} - \text{exogenous glucose oxidation}.$$

$$\text{Muscle glycogen oxidation} = \text{total CHO oxidation} - \text{plasma glucose oxidation}.$$

Blood parameter analysis. Plasma glucose, lactate, nonesterified fatty acids (NEFAs), and glycerol were analyzed using a Randox Daytona spectrophotometer and commercially available kits (Randox Laboratories, Ireland). Analysis for lactulose and rhamnose ratio (LR) as a marker of GI permeability and intestinal-fatty acid binding protein (I-FABP) was performed as previously described (42). Cytokine concentrations were measured using cytometric bead array (BD Biosciences, San Diego, CA) for the cytokines IL-1α, IL-6, IL-8, and IL-10, using the manufacturer's instructions with four bead populations with distinct fluorescence intensities coated with capture antibodies specific for IL-1α, IL-6, IL-8, and IL-10 proteins. Following acquisition of sample data using the flow cytometer, the sample results were generated in graphical and tabular format using BD cytometric bead array analysis software. Postexercise and 1-h postexercise sample concentrations were corrected for plasma volume changes, as described by Dill and Costill (13).

Assessment of gastrointestinal damage and symptoms. Intestinal permeability was assessed by analyzing serum samples using a previously published protocol (15), with the modification of using rhamnose instead of mannitol as the monosaccharide probe. Briefly, immediately after the 100-kJ time trial, a 50-mL sugar-probe solution (5 g lactulose, 2 g rhamnose) was consumed, and the ratio of the sugars was measured from serum samples 60 min after ingestion. Concentrations of intestinal-fatty acid binding protein (I-FABP) were measured pre-, post-, and 1 h postexercise from EDTA plasma using an ELISA (Hycult Biotechnology, Uden, The Netherlands; detection window 47–5,000 pg/mL), according to the manufacturer's instruc-

Table 1. *CHO and fat metabolism during 0–60 and 60–120 min*

	0–60 min			60–120 min		
	PLC	PRO	*P* Value	PLC	PRO	*P* Value
RER	0.89 ± 0.03	0.92 ± 0.03*	0.037	0.86 ± 0.03	0.90 ± 0.02*	0.005
CHO oxidation, g/min	2.11 ± 0.45	2.38 ± 0.32	0.087	1.87 ± 0.39	2.20 ± 0.25*	0.038
Fat oxidation, g/min	0.46 ± 0.11	0.34 ± 0.08*	0.041	0.55 ± 0.10	0.40 ± 0.11*	0.021

Data are means ± SD. CHO, carbohydrate; PLC, placebo; PRO, probiotic supplementation; RER, respiratory exchange ratio. *Significantly different from PLC ($P < 0.05$).

tions. Specific GI symptoms were recorded every 30 min during exercise, whereby a visual analog scale was used to assess specific symptoms, such as bloating, nausea, urge to vomit, and urge to defecate. GI symptoms were scored on a 10-point scale (0 = no symptoms, and 9 = very severe symptoms), with a score of >4 regarded as moderate. To ensure understanding, specific symptoms were explained and described to participants.

Statistical analysis. ANOVA for repeated measures was used to compare differences in substrate utilization and in blood-related parameters over time between the trials. A Tukey's post hoc test was applied in the event of a significant F-ratio. Where appropriate, the comparison of variables between the two conditions was conducted by using a Student's *t* test for paired samples. For plasma metabolites, area under the curve (AUC) calculation was completed, and differences between conditions were compared using a Student's *t* test for paired samples. To detect a meaningful increase in exogenous CHO oxidation of 0.1 g/min with a standard deviation (SD) of 0.05 g/min (58) at 80% power, a minimum of five participants would be required. All values are expressed as means ± SD. Statistical significance was set at $P < 0.05$.

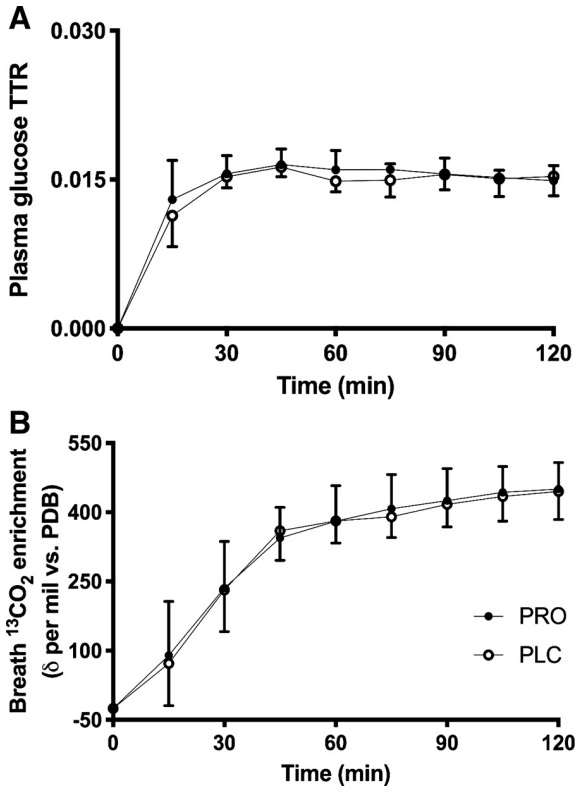

Fig. 1. *A*: plasma glucose tracer/tracee ratio (TTR) and (*B*) breath $^{13}CO_2$ enrichment during exercise. Values are means ± SD. PDB, Pee Dee belemnite; PLC, placebo; PRO, probiotic supplementation.

RESULTS

Physiological response to exercise. Participants cycled for 2 h at 180 ± 20 W across trials corresponding to 55% of their W_{max}. There were no significant differences among mean heart rate (149 ± 18 vs. 146 ± 16 beats/min), $\dot{V}O_2$ (34.5 ± 3.9 vs. 34.2 ± 3.4 mL · kg^{-1} · min^{-1}), or rating of perceived exertion (12 ± 1 vs. 12 ± 1) for PLC and PRO, respectively. CHO and fat oxidation during each hour is presented in Table 1. CHO oxidation was lower during the second hour in both trials. CHO oxidation was higher ($P = 0.019$) in the second hour in PRO compared with PLC. Fat oxidation was lower in PRO during both the first ($P = 0.026$) and the second ($P = 0.004$) hour compared with PLC. Energy expenditure did not differ between PLC (6,292 ± 644 kJ) and PRO (6,232 ± 493 kJ; $P = 0.662$).

Substrate utilization during the 60- to 120-min exercise period. Plasma [U-^{13}C]glucose/^{12}C glucose ratios increased as a result of maltodextrin/[U-^{13}C]glucose drink and were stable during the 60- to 120-min period (Fig. 1*A*). Baseline ^{13}C enrichments from resting breath samples were comparable between PLC (−25.2 ± 3.6 ‰ vs. PDB) and PRO (−25.0 ± 1.8 ‰ vs. PDB; $P > 0.05$). Changes in enrichment after ingestion of the drink at the start of 2 h of endurance exercise at 55% W_{max} are shown in Fig. 1*B*. $^{13}CO_2$ enrichments leveled off from 45 min during both trials, and there were no significant differences at any time point between PLC and PRO.

Mean CHO substrate oxidation during 60–120 min is summarized in Table 2. Mean oxidation of the ingested maltodextrin/[U-^{13}C]glucose drink was higher in PRO compared with PLC (Table 2), as was the maximal oxidation observed (0.84 ± 0.10 vs. 0.77 ± 0.09 g/min; $P = 0.016$), which was achieved at 120 min during both trials (Supplemental Fig. S1; see https://doi.org/10.24377/LJMU.d.00000050). There was no difference in mean liver-derived glucose oxidation, and muscle glycogen oxidation tended to be higher in PRO but did not reach statistical significance.

Blood metabolites. At the start of exercise, plasma glucose, lactate, NEFA, and glycerol concentrations were all similar in both trials (Fig. 2). Plasma glucose increased during the first 30

Table 2. *Mean CHO utilization calculated during 60–120 min*

	PLC	PRO	*P* Value
Ingested maltodextrin	0.75 ± 0.09	0.79 ± 0.10*	0.024
Liver-derived glucose	0.21 ± 0.08	0.19 ± 0.04	0.323
Muscle glycogen	0.99 ± 0.41	1.24 ± 0.28	0.087

Data are means ± SD in g/min. CHO, carbohydrate; PLC, placebo; PRO, probiotic supplementation. *Significantly different from placebo ($P < 0.05$).

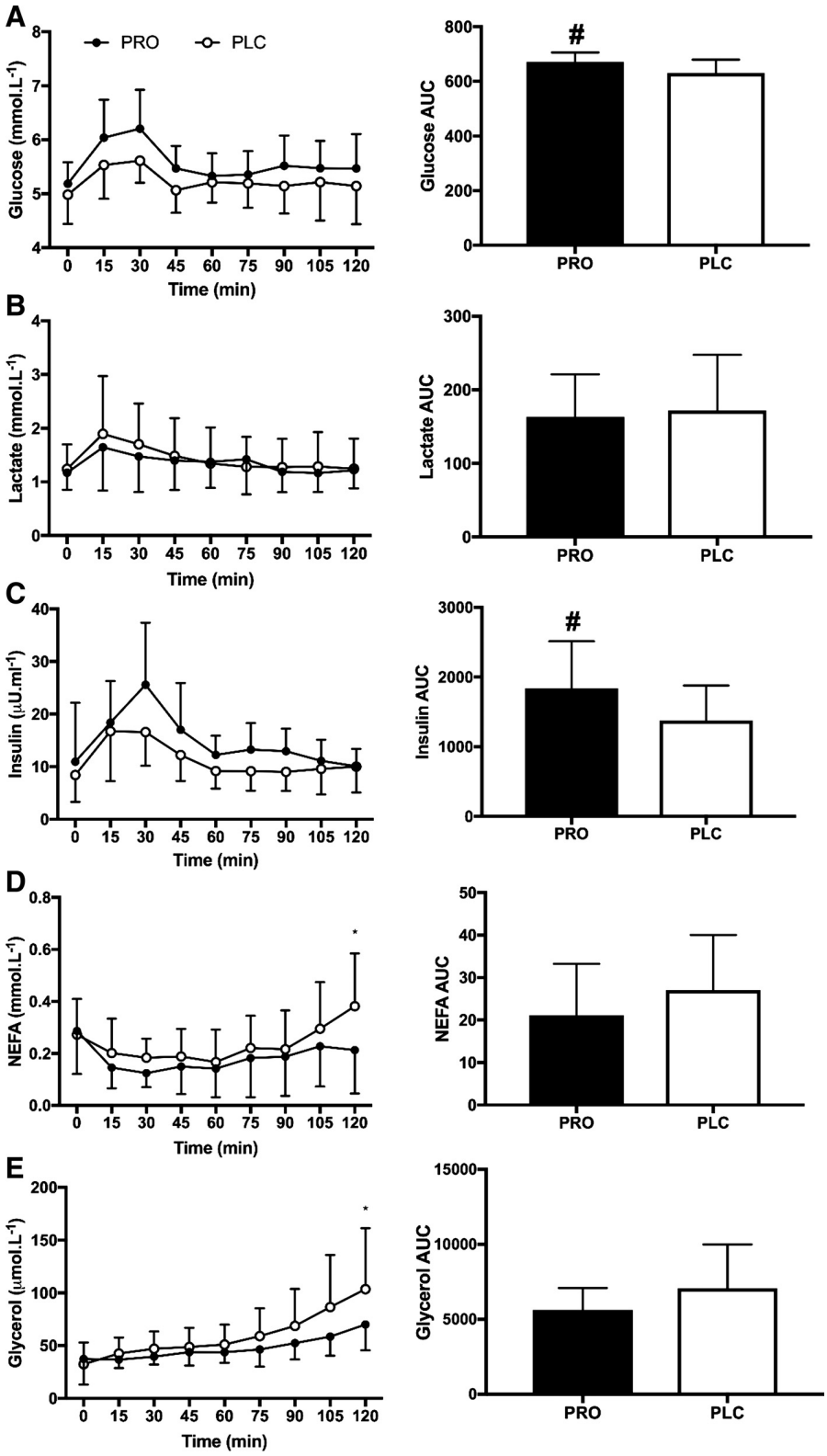

Fig. 2. Plasma glucose (*A*), lactate (*B*), insulin (*C*), nonesterified fatty acid (NEFA; *D*), and glycerol (*E*). *Significant difference between probiotic supplementation (PRO) and placebo (PLC) at the corresponding time point ($P < 0.05$); #significant difference between PRO and PLC. AUC, area under the curve.

min of exercise, before decreasing at 45 min, and remained stable for the rest of the exercise bout. When expressed as AUC, there was a significant difference between PRO and PLC ($P = 0.013$; Fig. 2*A*). After the ingestion of the maltodextrin drink, there were significant and concomitant increases and decreases in exogenously derived and endogenous plasma glucose concentrations, respectively ($P < 0.05$; Fig. 3). Plasma glucose concentrations derived from exogenous glucose were

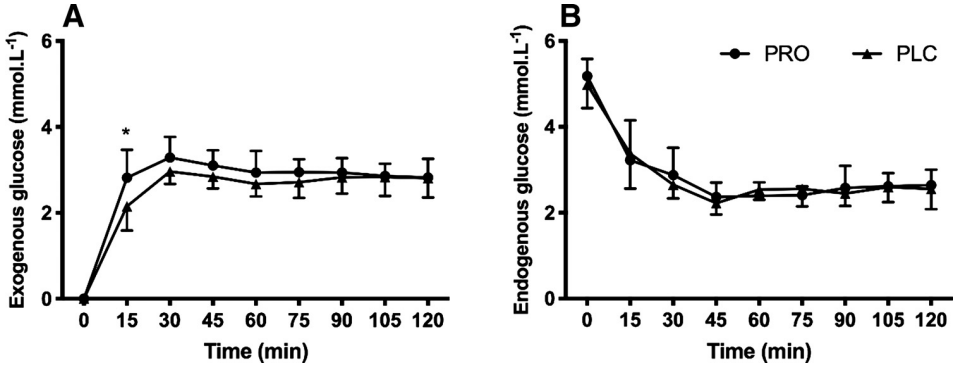

Fig. 3. Plasma glucose concentration with (*A*) glucose derived from ingested maltodextrin and (*B*) endogenously derived glucose sources. *Significant difference between probiotic supplementation (PRO) and placebo (PLC) at the corresponding time point ($P < 0.05$).

significantly greater at 15 min in PRO compared with PLC ($P = 0.01$; Fig. 3*A*). Plasma lactate increased in response to exercise to ~2 mM and then gradually declined during the course of the exercise bout (Fig. 2*B*). Insulin concentrations were higher at 30, 45, and 75 min in PRO compared with PLC ($P < 0.05$), with a significant difference between AUC data ($P = 0.04$). NEFA concentrations reduced at the onset of exercise and subsequently increased from 60 min in both trials, with a significant difference between trials by 120 min ($P = 0.043$; Fig. 2*D*). Plasma glycerol increased during exercise in both trials, with significantly lower concentrations at 1,200 min during PRO compared with PLC ($P > 0.001$; Fig. 2*E*).

Markers of GI permeability, damage, and cytokines. Individual data points for LR during PRO and PLC are presented in Fig. 4*A*. There was no significant difference in LR between PRO (0.045 ± 0.02) and PLC (0.052 ± 0.03; $P = 0.436$). For I-FABP, there was no significant difference between PRO and PLC pre ($P = 0.364$)-, post ($P = 0.374$)-, or 1 h postexercise ($P = 0.393$) for PRO and PLC, whereas there was also no effect of exercise (Fig. 4*B*). Plasma cytokine concentrations for pre- and postexercise are presented in Table 3. For pre-exercise measures, IL-1α and IL-6 concentrations were lower in PRO, whereas IL-6 was also lower postexercise in PRO.

GI symptoms and time-trial performance. During exercise trials, individual GI symptoms assessed were low (<4 on the scale of 0–10), even when using maximum values from each trial. During the 100-kJ time trial, there was no significant difference in the time to complete between placebo (308 ± 69 s) and probiotic (301 ± 74 s; $P = 0.714$).

DISCUSSION

The main aims of this study were to investigate the potential of 4 wk of probiotics supplementation (PRO) compared with placebo (PLC) to alter exercise metabolism and improve performance in trained athletes during 2 h of cycling exercise at moderate intensities. One of our hypotheses was that PRO would exert this effect via positive effects on GI permeability and prevention of GI damage. This was difficult to ascertain, given that there were no increases in exercise-induced damage/permeability, most likely relating to the exercise intensity. The other hypothesis was that PRO would increase the maximal rate of the oxidation of maltodextrins to a value higher than the maximal value of 1 g/min, which we observed in a previous study (53). To the authors' knowledge, this is the first study to investigate whether PRO, compared with PLC, has the potential to increase the oxidation rate of both total carbohydrates and orally ingested maltodextrins during endurance exercise. The use of stable isotope tracer methodologies, in combination with indirect calorimetry, has enabled us to quantitate total CHO and total fat oxidation and the gradual change in the fraction of plasma glucose originating from the ingestion of the maltodextrin drink and estimate muscle glycogen and liver glucose utilization over the 120-min exercise period (Fig. 3).

We have shown for the first time that PRO leads to a small but significant increase in total carbohydrate oxidation in the 60- to 120-min exercise period (Table 1) by increasing both the oxidation of the ingested maltodextrins ($P = 0.024$) and muscle glycogen (not significant) compared with placebo (Table 2). The increase in total CHO oxidation coincided with a decrease in total fat oxidation (Table 1). It is well established that the ingestion of multiple transporter carbohydrates (i.e., glucose and fructose) can improve the oxidation of exogenous CHO well above the values reported here (~1.5 g/min vs. 0.8–0.9 g/min) (11, 25). However, the proof of principle shown here—that PRO may alter substrate utilization and increase the oxidation of glucose derived from ingested malto-

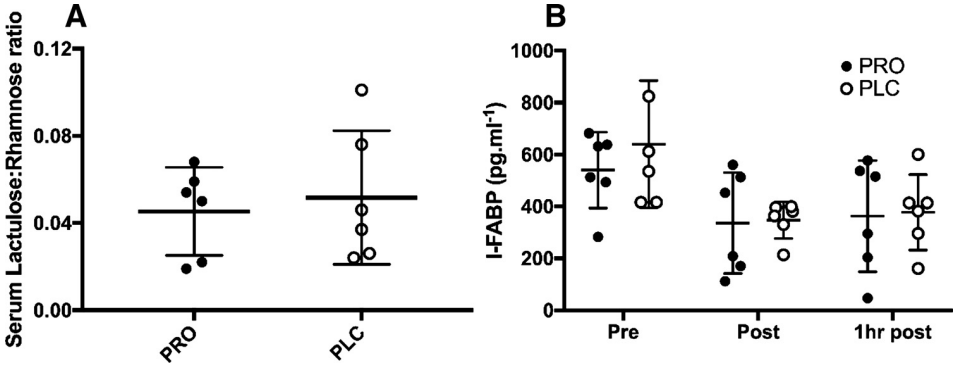

Fig. 4. *A*: serum lactulose/rhamnose ratio for probiotic supplementation (PRO) and placebo (PLC). *B*: intestinal-fatty acid binding protein (I-FABP) pre-, post-, and 1 h postexercise for PRO and PLC.

Table 3. *Pre- and postexercise cytokine concentrations for PLC and PRO*

	Pre-exercise			120 min		
	PLC	PRO	*P* Value	PLC	PRO	*P* Value
IL-1α	1.39 ± 0.82	0.63 ± 0.64	0.031*	1.84 ± 1.23	1.78 ± 1.40	0.450
IL-6	2.32 ± 1.22	1.22 ± 0.90	0.037*	3.69 ± 2.02	2.27 ± 1.19	0.049*
IL-8	2.49 ± 1.18	2.53 ± 1.38	0.481	4.70 ± 1.57	4.49 ± 2.83	0.409
IL-10	1.95 ± 1.37	1.14 ± 0.90	0.093	3.13 ± 2.05	3.25 ± 3.09	0.433

Data are means ± SD in pg/mL. PLC, placebo; PRO, probiotic supplementation. *Significantly different from placebo ($P < 0.05$).

dextrins—warrants further investigation to both replicate this finding and observe if this apparent difference is observed when both glucose and fructose are consumed during exercise. If the results here are replicated, then this would, at the very least, be of interest to those exercising for 1–2.5 h, for which current CHO-intake recommendations are 30–60 g per hour during exercise (5).

We, in this and previous studies (53), have chosen to orally administer a 10% maltodextrin solution, as maltodextrins are rapidly hydrolyzed into free glucose and absorbed into the vena porta. The rapid hydrolysis of maltodextrins in the human GI tract is an important reason for their frequent use in commercial sports drinks (18). In the previous study (53), we used maltodextrins naturally enriched with ^{13}C. In the current study, we mixed the maltodextrin solution with a [U-^{13}C]glucose tracer to thus achieve a high plasma glucose TTR. This was required in this study to facilitate accurate estimates of the rates of the oxidation of the ingested maltodextrins and the estimation of the production and oxidation of glucose by the liver (sum of liver glycogen breakdown and gluconeogenesis). Pilot data in the first two participants revealed that the plasma [U-^{13}C]glucose enrichment reached a plateau in the 30- to 120-min period, therefore excluding a difference in appearance kinetics between the maltodextrin solution and the [U-^{13}C]-glucose tracer. The profile of the ^{13}C enrichment curves of the breath gas samples was similar to the profiles seen in a previous study in which we used naturally enriched maltodextrins (53). For breath gas samples, a plateau was reached toward the end of the second hour in both studies. However, the variation between individuals in plateau enrichment was lower here than with naturally enriched maltodextrin consumption (53). Such variations in plateau enrichments have been shown to be the consequence of variations in the natural ^{13}C enrichment of individuals' endogenous carbohydrate stores (54). In the current study, the [U-^{13}C]glucose enrichment was >100-fold higher than seen in Wagenmakers et al. (53), and the variation among the seven participants for the plasma [U-^{13}C]glucose TTR and breath $^{13}CO_2$ enrichment (δ per mil vs. PDB) was minimal (Fig. 1, *A* and *B*). We believe that the current study is therefore the first to validate the use of a mixture of naturally enriched maltodextrins with a [U-^{13}C]-glucose tracer to estimate the oxidation of the maltodextrin solution during prolonged exercise.

It is clear in the present study that there are small but significant differences in fuel selection between PRO and PLC. The AUC data for plasma glucose and plasma insulin concentrations were higher for PRO than for PLC (Fig. 2), suggesting that the intestinal absorption of glucose, particularly in the first 30 min of exercise, was higher for PRO than for PLC. This is supported by the higher plasma glucose concentration originating from the ingested maltodextrins after 15 min of exercise during PRO compared with PLC (Fig. 3*A*). Higher duodenal glucose absorption rates are known to stimulate insulin production by the pancreatic β-cells (40). Higher systemic insulin concentrations, as are seen during PRO compared with PLC (Fig. 2*C*), have previously been shown to suppress lipolysis in subcutaneous adipose tissue (7). It has been shown that oral ingestion of glucose, before and during exercise, reduced lipolysis both in subcutaneous adipose tissue stores and lipolysis of the intramuscular triglyceride (IMTG) stores in skeletal muscle (10). This led to a substantial reduction in the oxidation rate of plasma fatty acid, IMTG, and total fat during exercise (10). These mechanisms contribute to the lower plasma NEFA and glycerol levels (Fig. 2, *D* and *E*, respectively) and the reduction in total fat oxidation in PRO compared with PLC (Table 1). However, the small difference in intestinal absorption suggested here does not likely fully explain the differences in plasma insulin and glycerol. Other GI-related factors, such as the intestinal incretin response, have been shown to affect the insulin response to an oral glucose load (16). It may then be plausible that differences in other GI-related factors that follow PRO supplementation explain some of the differences in plasma insulin and glycerol observed here.

During the 2 h of exercise, there is a gradual increase in the fraction of plasma glucose originating from the oral ingestion of the maltodextrin drink (Fig. 3*A*), whereas at the same time, there is a reduction in the fraction of glucose produced by the liver (sum of liver glycogen breakdown and gluconeogenesis; Fig. 3*B*). The plasma glucose concentration (sum of the two fractions) remains constant. These data collectively support the concept that the liver acts as a "glucostat" (8, 38, 56). The data in Fig. 3, *A* and *B*, and in previous work (10, 26) clearly show that oral ingestion of carbohydrates reduces endogenous glucose production in proportion to the intestinal absorption rate of glucose and oxidation rate of glucose in the exercising muscles. This role of the liver as a glucostat, keeping the blood glucose concentration during exercise constant, explains the data we obtained in the current study.

Whereas we have presented data that demonstrate differences in exercise metabolism following PRO, we did not observe any difference in the 100-kJ time-trial performance between supplement groups. It was hypothesized that had PRO resulted in greater consumed CHO absorption and had oxidation been substantially increased, then this would have resulted in larger muscle and/or liver glycogen stores. This would then be of benefit during a higher intensity exercise performance test, following an initial endurance exercise, as has been seen previously with exogenous CHO feeding studies (17, 34). A time trial lasting ~240–360 s was chosen, as this has been shown to be a reliable performance indicator in trained cyclists

(30) and suggested to be at the higher end of duration of sustained power-output increases during successful breakaways during professional road cycling (1). The failure to observe an effect of PRO on performance is most likely related to the small increase in the oxidation of exogenous CHO during the preceding exercise. Greater increases in exogenous CHO oxidation following PRO would have likely reduced muscle and/or liver glycogen oxidation and thus would have been spared for use during the higher intensity exercise during the time trial. However, it should also be highlighted that a limitation of the time-trial performance measure here was the potential lack of statistical power. Whereas the primary outcome and thus power calculation performed to ensure sufficient sample size was to detect differences in exercise metabolism, this may have been under powered for a number of secondary measures, including the time-trial performance and GI measures.

The presented GI permeability and damage data did not show an increase in measures for I-FABP or GI symptoms during 120 min of cycling, whereas GI permeability was not different between PRO and PLC, and many values were similar to resting values previously reported in our laboratory (41, 42). GI permeability has previously been reported only to increase significantly compared with resting values at exercise intensity of ≥80% maximal $\dot{V}O_2$ at ambient temperatures (37), whereas increases in plasma I-FABP have also not always been shown during moderate-intensity exercise, particularly when subjects maintain euhydration and consume CHO (29). In the present trial, exercise intensity was 55% W_{max} (~55 $\dot{V}O_{2peak}$). This exercise intensity, in line with previous investigations (55, 58), was chosen, as it mimics exercise intensities experienced by professional road cyclists during competition (36) and the intensity at which they are most likely to consume CHO during exercise. In regard to GI symptoms, these were generally low, again, most likely due to the chosen exercise modality (cycling instead of running), duration, and intensity, which do not appear to have led to a sustained, functional challenge to the GI system. To investigate better the effects of probiotics on exercise-induced GI permeability, damage, and symptoms, particularly in the presence of carbohydrate ingestion, exercise of a greater intensity and duration should be considered.

We conclude that the presented stable isotope tracer data demonstrate for the first time in humans in vivo that after 4 wk of PRO during 2 h of cycling exercise at 55% W_{max}, a small but significant increase in the oxidation rate of glucose, originating from an orally ingested maltodextrin solution, was observed. We also show significant increases in the plasma glucose and insulin concentration, with significant increases occurring in the 2-h area under the curve (AUC) both for plasma glucose and plasma insulin (Fig. 2) following PRO. The higher plasma insulin concentrations during exercise reduce total fat oxidation (Table 2) via inhibition of lipolysis of subcutaneous adipose tissue and IMTG lipolysis, as we have shown previously (10). Although these data show that PRO does lead to small but measurable changes in fuel selection and oxidation during exercise, they do not confirm our prior hypothesis that PRO could lead to larger increases in the absorption and oxidation rate of the ingested maltodextrins or that the maximal maltodextrin oxidation rates reached a plateau to values higher than 1 g/min.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

J.N.P., A.J.M.W., D.A.D., J.P.M., and G.L.C. conceived and designed research; J.N.P. and G.L.C. performed experiments; J.N.P., D.A.D., S.C.F., B.A.F., J.P.M., and G.L.C. analyzed data; J.N.P., A.J.M.W., D.A.D., S.C.F., B.A.F., J.P.M., and G.L.C. interpreted results of experiments; J.N.P., D.A.D., S.C.F., J.P.M., and G.L.C. prepared figures; A.J.M.W., B.A.F., J.P.M., and G.L.C. drafted manuscript; J.N.P., A.J.M.W., B.A.F., J.P.M., and G.L.C. edited and revised manuscript; J.N.P., A.J.M.W., D.A.D., S.C.F., B.A.F., J.P.M., and G.L.C. approved final version of manuscript.

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