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Sucrose but Not Nitrate Ingestion Reduces Strenuous Cycling–induced Intestinal Injury

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ABSTRACT

JONVIK, K. L., K. LENAERTS, J. S. J. SMEETS, J. J. KOLKMAN, L. J. C. VAN LOON, and L. B. VERDIJK. Sucrose but Not Nitrate Ingestion Reduces Strenuous Cycling–induced Intestinal Injury. *Med. Sci. Sports Exerc.*, Vol. 51, No. 3, pp. 436–444, 2019. **Purpose**: Strenuous exercise induces intestinal injury, which is likely related to splanchnic hypoperfusion and may be associated with gastrointestinal complaints commonly reported during certain exercise modalities. Increasing circulating nitric oxide (NO) levels or inducing postprandial hyperemia may improve splanchnic perfusion, thereby attenuating intestinal injury during exercise. Therefore, we investigated the effects of both dietary nitrate ingestion and sucrose ingestion on splanchnic perfusion and intestinal injury induced by prolonged strenuous cycling. **Methods**: In a randomized crossover manner, 16 well-trained male athletes (age, 28 ± 7 yr; W_{max}, 5.0 ± 0.3 W·kg^{-1}) cycled 60 min at 70% W_{max} after acute ingestion of sodium nitrate (NIT; 800 mg NO_3), sucrose (SUC; 40 g), or a water placebo (PLA). Splanchnic perfusion was assessed by determining the gap between gastric and arterial pCO_2 ($gap_{g\text{-}a}pCO_2$) using gastric air tonometry. Plasma intestinal fatty acid–binding protein (I-FABP) concentrations, reflecting enterocyte damage, were assessed every 20 min during and up to 60 min of postexercise recovery. **Results**: The exercise protocol resulted in splanchnic hypoperfusion, as $gap_{g\text{-}a}pCO_2$ levels increased during exercise ($P < 0.001$), with no differences between treatments ($P = 0.47$). Although plasma I-FABP concentrations increased during exercise and postexercise recovery for all treatments ($P < 0.0001$), the increase was different between treatments ($P < 0.0001$). *Post hoc* comparisons showed an attenuated increase in I-FABP in SUC versus PLA ($P = 0.020$). In accordance, I-FABP area under the curve ($AUC_{0–120}$) was significantly lower in SUC versus PLA (57,270 ± 77,425 vs 114,907 ± 91,527 pg·mL^{-1} per 120 min, $P = 0.002$). No differences were observed between NIT and PLA ($P = 0.99$). **Conclusion**: Sucrose but not nitrate ingestion lowers intestinal injury evoked during prolonged strenuous cycling. These results suggest that sucrose ingestion, but not nitrate, prevents hypoperfusion-induced gastrointestinal damage during exercise and, as such, may help to lower exercise-related gastrointestinal complaints. **Key Words:** GASTROINTESTINAL DAMAGE, HYPOPERFUSION, ATHLETES, CYCLING, CARBOHYDRATE

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Nutritional strategies implemented around exercise are aimed to support or promote optimal performance and address various factors such as dehydration, glycogen depletion, and gastrointestinal discomfort (1). Gastrointestinal complaints are frequently reported in endurance athletes, with the highest prevalence and severity reported in ultraendurance runners and triathletes (2). During strenuous exercise, sympathetic nervous system activity is massively increased and redistributes blood from the splanchnic (gastrointestinal) organs to serve the working muscle (3). The consequence of this exercise-induced splanchnic vasoconstriction is splanchnic ischemia, which can lead to mucosal injury, impaired nutrient uptake, and loss of gastrointestinal barrier functions (4). Previous work has demonstrated that splanchnic hypoperfusion, measured by

gastric tonometry during strenuous cycling exercise, is associated with small intestinal injury, shown as increased plasma intestinal fatty acid–binding protein (I-FABP) concentrations (5). Although a causal relationship has not yet been established, exercise-induced intestinal injury is likely related to the development of gastrointestinal complaints that can acutely hamper exercise performance (2). Importantly though, even in the absence of acute gastrointestinal symptoms, an exercise-induced reduction in splanchnic blood flow and the development of intestinal injury can induce detrimental effects such as reduced nutrient uptake around exercise (6) or even chronic health complications (2). Therefore, interventional strategies to attenuate intestinal injury could likely benefit exercise performance both directly, i.e., improved nutrient availability to sustain strenuous exercise, and indirectly, i.e., improved muscle repair and nutrient repletion, thus enhancing subsequent exercise performance, as well as prevention of potential health issues on the long term.

It has been suggested that splanchnic blood flow can be manipulated by increasing the local availability of nitric oxide (NO) (7). NO is an important vasodilator, regulating microvascular tone, leukocyte adhesion, thrombocyte aggregation, and microvascular permeability (8). Being a short-lived gas, NO cannot readily be applied to the gut, but several NO donors can be used to increase the bioavailability of NO. One NO donor that has been studied is L-citrulline, which is converted into L-arginine, and further oxidized upon activation of endothelial NO synthase (eNOS) to produce NO (9). Interestingly, van Wijck et al. (10) showed that ingestion of L-citrulline before exercise preserves splanchnic perfusion and attenuates intestinal injury during prolonged strenuous cycling. In line with the recent interest in nitrate as a potent NO donor, it has been suggested that dietary nitrate can also modulate splanchnic perfusion (4). Circulating nitrate is actively taken up by the salivary glands and concentrated in the saliva, where it can be reduced to nitrite by facultative anaerobic bacteria in the oral cavity. Upon swallowing the saliva, nitrite enters the circulation and can be further reduced to NO via various pathways (11). In a study in rats, oral nitrate supplementation was shown to increase gastric mucosal blood flow (12). The role of dietary nitrate for gastrointestinal function during exercise in humans has never been investigated. Therefore, our first aim was to assess the effects of dietary nitrate ingestion on splanchnic perfusion and intestinal injury during prolonged strenuous cycling.

It is generally accepted that oral meal ingestion (13), and glucose ingestion in particular (14), increases splanchnic blood flow, a phenomenon referred to as postprandial splanchnic hyperemia. One possible explanation is the NO and adenosine A1 receptor-mediated microvascular vasodilation during intestinal glucose absorption. A study in rats showed that NOS inhibition significantly blunts the glucose-induced vasodilation of the premucosal arterioles (15). Furthermore, carbohydrate ingestion before and every 20 min during exercise was recently shown to attenuate the increase in I-FABP levels after 2 h of running at moderate intensity in the heat (16). We aimed to extend on these findings by investigating the effects of carbohydrate ingestion on intestinal injury as well as splanchnic perfusion during and after exercise. To this end, we used a strenuous cycling protocol as stationary ergometer cycling allows for the assessment of splanchnic perfusion using gastric tonometry. Although cycling exercise typically results in less gastrointestinal complaints, splanchnic perfusion is clearly reduced, and the relative increase in I-FABP has been reported in the same range as for running (5,10,16).

We hypothesized that both nitrate and carbohydrate ingestion improve splanchnic perfusion and attenuate intestinal injury evoked during prolonged strenuous cycling. Therefore, 16 well-trained male athletes performed a 60-min strenuous cycling protocol (70% W_{max}) after ingestion of sodium nitrate, sucrose, or placebo. During exercise and upon 60 min postexercise recovery, splanchnic perfusion was measured by gastric tonometry and intestinal injury was measured by plasma I-FABP concentrations.

METHODS

Subject's characteristics. Sixteen male cyclists (28 ± 7 yr, body mass index = 23.0 ± 2.2 $kg \cdot m^{-2}$) were included in the present study. Before the experiments, individual maximal workload capacity (W_{max}) was assessed on a stationary cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands), by completing an incremental exercise test starting with a 5-min warm-up at 150 W and increasing with 50 W every 2.5 min until volitional exhaustion; subjects were excluded when W_{max} was <4.5 $W \cdot kg^{-1}$. All subjects were well-trained (9.4 ± 3.3 h of weekly endurance training) and had an average W_{max} of 5.0 ± 0.3 $W \cdot kg^{-1}$. Furthermore, they had no abdominal complaints during daily activities, had no history of gastrointestinal diseases/disorders or abdominal surgery, did not take any medications or dietary supplements (e.g., beetroot juice and probiotics) interfering with test outcomes, and were nonsmokers. After being informed about the purpose and potential risks of the study, all subjects provided written informed consent. The experimental protocol and procedures were approved by the medical ethics committee of Maastricht University Medical Centre+ and conducted in accordance with the Declaration of Helsinki (2013).

Study design and nutritional interventions. In this randomized controlled crossover study, all subjects performed 60 min of cycling exercise at 70% W_{max} on three test days. During these test days, subjects ingested 200 mL of a test beverage before and during exercise. On a test day, the subject was provided sodium nitrate (NIT), sucrose (SUC), or placebo (PLA) interventions. Splanchnic perfusion was assessed by gastric tonometry, and intestinal injury was assessed by the rise in plasma I-FABP concentrations. For each subject, testing was performed on the same day, at the same time, with 1–2 wk between test days.

Test beverages. Each subject ingested one "salty" beverage dissolved in 200 mL tap water 150 min before cycling ($t = -150$). The beverage contained either sodium nitrate in NIT (1.1 g $NaNO_3$, Food grade, F.C.C. Brenntag, the Netherlands) or an equal amount of NaCl in SUC and PLA (ESCO Steen-Consumptiezout, the Netherlands), with similar taste, smell, and appearance. The dose of 800 mg nitrate (in 1.1 g $NaNO_3$) was chosen based on previous studies reporting substantial increases in plasma nitrate and nitrite concentrations and physiological effects such as reduction in blood pressure and/or performance benefits (17,18). Furthermore, subjects ingested one beverage of 200 mL 15 min before ($t = -15$) as well as 30 min into cycling ($t = 30$). The beverages contained either 20 g sucrose in SUC (granulated sugar; Sundale, Breda, the Netherlands) or tap water in NIT and PLA. The test beverages were identical in appearance and smell, but since no additives or sweeteners were used, a difference in taste was unavoidable between sucrose and water placebo. However, the subject's knowledge of the intervention was unlikely to affect the main outcomes.

Physical activity and dietary standardization. Subjects maintained normal activities of daily living and recorded their activity pattern 48 h before a test day but refrained from strenuous physical activity for 24 h before each test day. To ensure that the diet before test days did not differ between interventions, a standard evening meal was provided before each test day and the subjects reported their dietary intake for the 48 h leading into test day 1. Before test days 2 and 3, they received a copy of their food diary and were instructed to repeat the exact intake. Subjects were not allowed to consume alcohol and caffeine 24 and 12 h before testing, respectively. On the evening before each test day, the subjects ingested ranitidine (150 mg; Accord Healthcare Limited, UK) to inhibit gastric acid production and secretion. The latter is necessary to enable gastric tonometry measurements because the presence of acid in the stomach during tonometry can buffer carbon dioxide molecules, thereby interfering with the outcome of the tonometry measurements (19). An additional 150 mg of ranitidine was consumed on the test days at 7:00 AM, 2–3 h before start of tonometry measurements. Furthermore, to prevent any attenuation in the reduction of nitrate to nitrite in the oral cavity by commensal bacteria (11), subjects were asked to refrain from using any antibacterial mouthwash/toothpaste and tongue scraping from 1 wk before the first test until the day after the last test day.

Experiments and sampling. All subjects reported to the laboratory at 8:00 AM by car or public transport in an overnight fasted state. After seated rest for 5 min, a Teflon catheter was inserted in a dorsal hand vein of the subject, and the hand was placed in a hot box set at 60°C to enable collection of arterialized blood for analysis of arterial pCO_2 levels (20). For practical reasons, no hotbox was used during exercise, but blood oxygen saturation never dropped below 95%, confirming sustained arterialized blood (21). To measure gastric pCO_2, an 8-French tonometrics catheter (MEDI-LINE s.a., Liege, Belgium) was introduced via the nose into the stomach of the subject and fixed to the nasal flares. Gastric pCO_2 was measured at 10-min intervals before, during, and up to 60 min postexercise using an automated capnograph (Tonocap TC-200; Datex-Ohmeda Oy, Helsinki, Finland). Arterialized blood samples were collected at baseline ($t = -150$), before exercise ($t = -50$, $t = -30$ and $t = -10$) and every 20 min during and postexercise in heparin syringes (safePICOTM aspirator; Radiometer Benelux BV, Zoetemeer, Netherlands), for determination of arterial pCO_2. By subtracting the gastric and arterial pCO_2 values, the $gap_{g\text{-}a}pCO_2$ per 20 min time point was calculated as a measure of splanchnic perfusion (10). Tonometry data were obtained from 14 subjects because of inability to introduce the nasogastric catheter into the stomach of one of the subjects and measurement errors in one other subject. At each time point, a second blood sample was collected into lithium–heparin tubes and centrifuged immediately at 1000*g* for 5 min, at ±4°C. Aliquots of plasma were stored at −80°C for the subsequent analysis of plasma I-FABP, lipopolysaccharide binding protein (LBP), nitrate, nitrite, and glucose concentrations.

After a 5-min warm-up at 50% of the individual's preassessed W_{max}, subjects continued cycling at 70% W_{max} (262 ± 33 W) for 60 min ($t = 0$–60). Subjects were free to cycle according to their own comfortable pace, with a minimum of 50 rpm. If the subject was unable to maintain >50 rpm, workload was decreased in steps of 25 W. Performance per test day was estimated by calculating the average workload throughout the 60-min exercise. Subjects consumed water *ad libitum*, with a maximum of 200 mL every 20 min during exercise. Intake during the first test day was replicated on the subsequent test days. Including the interventional beverages, all subjects ingested 800–1400 mL water throughout the test day. Because gastric pCO_2 levels drop directly after fluid or meal ingestion due to dilution rather than an actual change in pCO_2 (22), the gastric pCO_2 value directly after ingestion of a beverage was substituted for the previous gastric pCO_2 value (e.g., $t = 30$ was used instead of $t = 40$) when calculating the $gap_{g\text{-}a}pCO_2$. Gastric pCO_2 is shown to stabilize within 15–20 min after fluid ingestion (23), and the values used for analysis are all extending this period after fluid ingestion.

Secondary measurements included heart rate during exercise (Polar Electro Oy, Kempele, Finland), rate of perceived exertion after completion of the exercise (24), and a multiple factor gastrointestinal symptoms questionnaire using a VAS scale. The 16-item questionnaire is adapted from Jeukendrup et al. (25), and >50% on the scale is defined as a severe symptom. We report data as a percentage of possible maximum achievable score for all four moments of registration ($t = -30$, $t = 60$, $t = 120$ and 1 d after).

Plasma analysis. To evaluate the extent of small intestinal injury during and postexercise, plasma concentrations of intestinal I-FABP were determined. Plasma I-FABP levels were measured by an in-house developed enzyme-linked immunosorbent assay (26). The assay is specific for the detection of the I-FABP isoform, with a lower limit of detection of 12.5 $pg \cdot mL^{-1}$. The intra- and interassay coefficients of variation are 4.1% and 6.2%, respectively. To

provide further insight into the nature of exercise-induced intestinal injury, plasma LBP was determined as a marker of endotoxin translocation in response to exercise using an in-house developed ELISA with a lower limit of detection of 200 pg·mL^{-1} (27). Plasma nitrate and nitrite concentrations were analyzed using the chemiluminescence technique (NOA; Sievers NOA 280i; Analytix, Durham, UK) as described previously (17). Plasma glucose was analyzed using Uni Kit III (Roche, Basel, Switzerland).

Statistical analysis. Power calculations were based on previously reported percentage changes in I-FABP area under the curve (AUC) after L-citrulline versus placebo supplementation during high-intensity exercise (10). This resulted in an effect size of 1.19. Taking into account a power of 95% and an alpha level of 0.025 (for preplanned *post hoc* comparisons for PLA vs NIT and PLA vs SUC), a minimum of 14 subjects should be included. Normality of all data was verified by the Kolmogorov–Smirnov test. The primary outcome parameters were $AUC_{0–120}$ for I-FABP (pg·mL^{-1}·120 min) and $AUC_{0–60}$ for $gap_{g\text{-}a}pCO_2$ (kPa·60 min) and were analyzed using repeated-measures ANOVA with "treatment" (NIT, SUC or PLA) as within-subjects factor. Continuous data for absolute I-FABP concentrations, $gap_{g\text{-}a}pCO_2$, plasma nitrate, plasma nitrite, and plasma glucose were analyzed using repeated-measures ANOVA, with "time" and "treatment" as within-subjects factors. Other secondary parameters were analyzed using repeated-measures ANOVA, with "treatment" as within-subjects factor. Statistical significance was set at $P < 0.05$, and any interaction or main effect was subsequently analyzed using a Bonferroni-corrected *post hoc* test for preplanned comparisons (i.e., SUC vs PLA and NIT vs PLA). All data were analyzed using SPSS 22.0 (IBM Corp., Armonk, NY) and are presented as mean ± SD.

RESULTS

Plasma nitrate/nitrite and glucose. There were no baseline differences between treatments for plasma nitrate ($P = 0.144$; Fig. 1A) or nitrite ($P = 0.694$; Fig. 1B) concentrations. A significant time–treatment interaction was observed for both plasma nitrate and nitrite concentrations (both $P < 0.0001$). *Post hoc* analysis showed significantly higher plasma nitrate and nitrite concentrations after NIT versus PLA throughout the test day (both $P < 0.0001$), with no differences between SUC and PLA. After NIT, plasma nitrate and nitrite concentrations were increased at all time points when compared with baseline ($P < 0.0001$). Subtle changes in plasma nitrate and nitrite concentrations were also observed within SUC and PLA (Figs. 1A and 1B).

For plasma glucose concentrations, there were no baseline differences between treatments ($P = 0.181$; Fig. 1C). There was a significant time effect ($P < 0.0001$) and a trend for a time–treatment interaction ($P = 0.088$). Plasma glucose concentrations increased compared with baseline at $t = 20$ and $t = 40$ ($P < 0.01$), tended to be increased at $t = 60$ ($P = 0.063$), and decreased at $t = 120$ ($P < 0.01$). Furthermore, plasma glucose was higher in SUC versus PLA at $t = -10$ ($P = 0.008$) and tended to be higher in SUC versus PLA at $t = 60$ ($P = 0.076$).

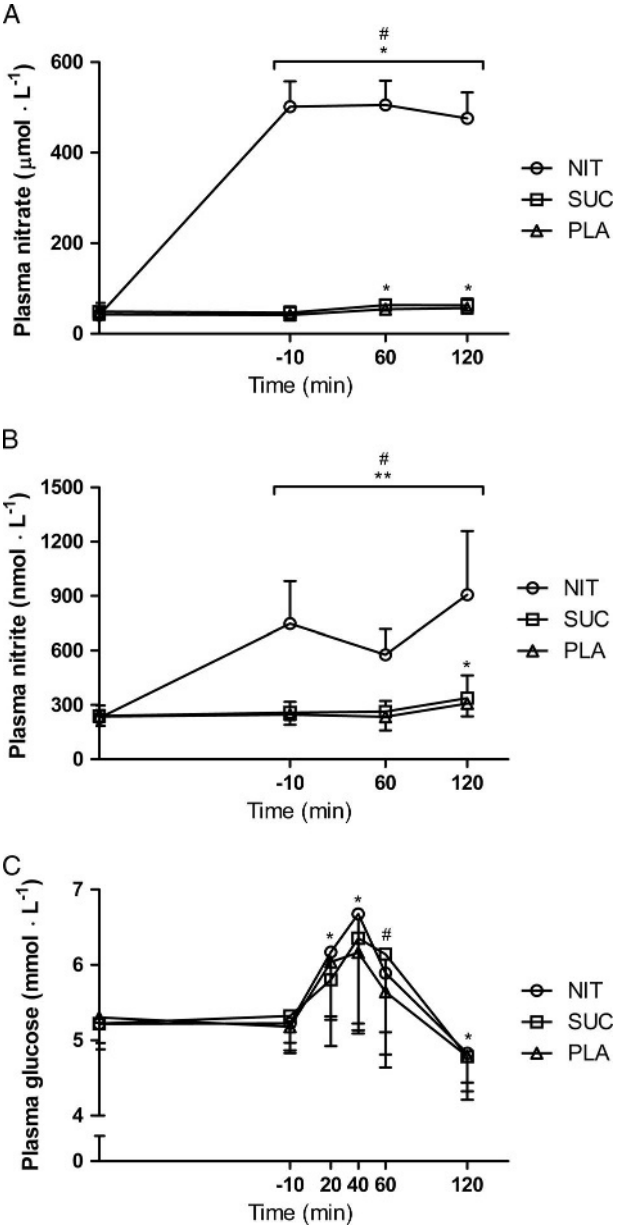

FIGURE 1—Mean ± SD plasma concentrations of nitrate (A), nitrite (B), and glucose (C) after oral bolus of sodium nitrate (NIT), sucrose (SUC), or placebo (PLA) in healthy young men ($n = 16$). Data were analyzed with repeated-measures ANOVA. Plasma nitrate (A): *Significantly different from baseline (main effect for all treatments; all $P < 0.001$). #Significantly different in the NIT vs PLA treatment ($P < 0.0001$). Plasma nitrite (B): *Significantly different from baseline at $P < 0.05$, only in the SUC and PLA treatment, and **$P < 0.0001$ only in the NIT treatment. #Significantly different in the NIT vs PLA treatment ($P < 0.0001$). Plasma glucose (C): *Significantly different from baseline (main effect for all treatments; all $P < 0.01$). #Significantly different in the SUC vs PLA treatment ($P < 0.01$).

Splanchnic perfusion. For $gap_{g\text{-}a}pCO_2$ levels, no differences were observed at baseline, and no time–treatment interaction ($P = 0.47$) or overall effect of treatment ($P = 0.53$) was observed. However, there was a significant time effect ($P < 0.001$), with *post hoc* analyses showing that for

all treatments, $gap_{g\text{-}a}pCO_2$ levels significantly increased compared with baseline at all time points during cycling ($P < 0.01$), returning to baseline levels after exercise cessation (Fig. 2A). In accordance, AUC for $gap_{g\text{-}a}pCO_2$ during exercise was significantly higher than zero, with no differences between treatments ($P = 0.64$; Fig. 2B). Likewise, peak values for $gap_{g\text{-}a}pCO_2$ ($P = 0.87$) were not different between treatments.

Intestinal injury. Baseline plasma I-FABP concentrations (at $t = -150$, before the first supplement) were not different between treatments (NIT, 1037 ± 309; SUC, 1213 ± 385; PLA, 1171 ± 384 pg·mL^{-1}; $P = 0.26$) and remained stable until the onset of exercise. A significant time–treatment interaction was observed for the plasma I-FABP concentrations throughout the exercise and postexercise period ($P < 0.0001$; Fig. 3A). *Post hoc* analysis showed that the increase over time was significantly lower in SUC versus PLA from $t = 60$ through $t = 120$ (all $P < 0.05$). By contrast, no differences were observed between NIT and PLA. After SUC, plasma I-FABP was increased from baseline at $t = 20$, $t = 40$, $t = 60$, and $t = 80$, but not at $t = 100$ and $t = 120$. After NIT and PLA, plasma I-FABP remained elevated above baseline at all time points during and postexercise. In accordance, AUC for the I-FABP concentrations during and postexercise was significantly different between treatments ($P < 0.0001$), with *post hoc* analysis showing lower I-FABP AUC after SUC versus PLA (57,270 ± 77,425 vs 114,907 ± 91,527 pg·mL^{-1}·120 min, $P = 0.002$; Fig. 3B), with no differences between NIT (125,106 ± 83,591 pg·mL^{-1}·120 min) and PLA ($P = 0.99$). Also, when plasma I-FABP was expressed as percentage change from baseline, a difference was observed between treatments, with peak values for the percentage increase in plasma I-FABP from baseline being significantly lower in SUC (179% ± 60%) versus PLA (249% ± 88%, $P = 0.004$), with no differences between NIT and PLA (299% ± 120%, $P = 0.31$). No correlations between $gap_{g\text{-}a}pCO_2$ and I-FABP levels were found.

At baseline, no differences were observed in LBP concentrations between treatments (12.6 ± 3.7, 12.9 ± 3.5, and

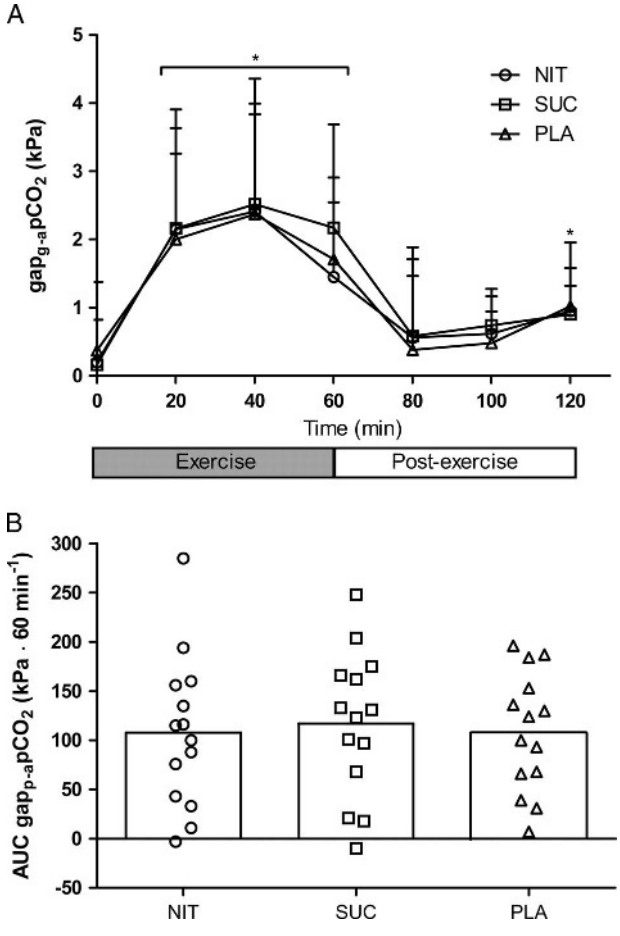

FIGURE 2—Mean ± SD $gap_{g\text{-}a}pCO_2$ levels at baseline, during, and postexercise (A) and AUC of $gap_{g\text{-}a}pCO_2$ levels during exercise (B) after consumption of sodium nitrate (NIT), sucrose (SUC), or placebo (PLA) in healthy young men ($n = 14$). The data over time (A) were analyzed with repeated-measures ANOVA. *Significantly different from baseline (main effect for all treatments; all $P < 0.01$). AUC data (B) were analyzed with a one-way ANOVA; no differences were observed between treatments.

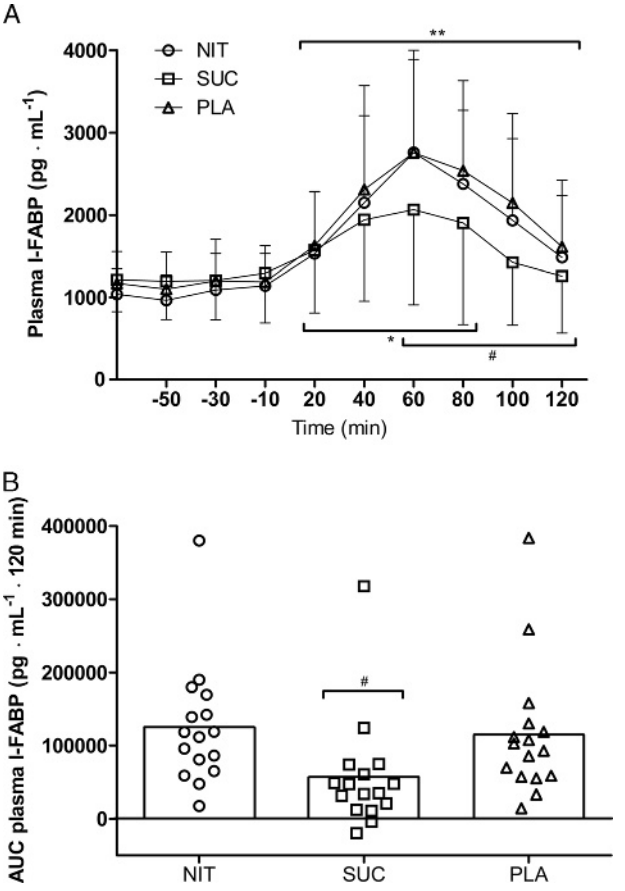

FIGURE 3—Mean ± SD absolute plasma I-FABP concentrations preexercise, during and postexercise (A) and AUC of absolute plasma I-FABP change (B) after consumption of sodium nitrate (NIT), sucrose (SUC), or placebo (PLA) in young healthy men ($n = 16$). The data over time (A) were analyzed with repeated-measures ANOVA. *Significantly different from baseline at $P < 0.05$, only in the SUC treatment, and **$P < 0.01$ only in the NIT and PLA treatment. #Significantly different in the SUC vs PLA treatment ($P < 0.01$). AUC data (B) were analyzed using one-way ANOVA. #Significantly different compared with PLA ($P < 0.01$).

12.6 ± 4.0 $\mu g \cdot mL^{-1}$ in NIT, SUC, and PLA, respectively). LBP concentrations were increased by ~9% after exercise ($P < 0.001$), with no differences between treatments (13.5 ± 3.6, 14.1 ± 3.1, and 14.2 ± 4.3 $\mu g \cdot mL^{-1}$ in NIT, SUC, and PLA, respectively, $P = 0.69$).

Gastrointestinal complaints and exercise performance. There was a low degree of gastrointestinal complaints at all time points, with a small but significant increase immediately after exercise ($t = -30$: 2.9% ± 2.6%, $t = 60$: 4.8% ± 4.7%, $t = 120$: 2.7% ± 3.5%, 1 d after: 2.6% ± 3.5%, $P = 0.002$ for time effect), with no difference between interventions ($P = 0.57$). At $t = 60$, two subjects reported severe gastrointestinal complaints after NIT (one urge to vomit and one flatulence) and three subjects after PLA (one flatulence, one dizziness, and one nausea). One subject experienced gastrointestinal complaints during cycling on all three test days, likely related to the exercise intensity, but was able to complete the test protocol each test day. Average cycling power output did not differ between treatments (NIT, 247 ± 34 W; SUC, 250 ± 35 W; PLA, 249 ± 32 W; $P = 0.22$). A tendency was observed toward reduced rating of perceived exertion in the SUC treatment (NIT, 17.4 ± 1.3; SUC, 16.5 ± 1.9; PLA, 17.4 ± 1.5; $P = 0.082$). The highest 1-min heart rate during cycling did not differ between treatments (NIT, 178 ± 12 bpm; SUC, 178 ± 13 bpm; PLA, 180 ± 13 bpm; $P = 0.28$).

DISCUSSION

Cycling for 60 min at 70% W_{max} resulted in splanchnic hypoperfusion and intestinal injury in well-trained cyclists. We observed no differences in splanchnic perfusion between treatments, and dietary nitrate did not affect intestinal injury. However, sucrose ingestion attenuated the increase in plasma I-FABP levels, indicative of a substantial reduction in cycling-induced intestinal injury.

Strenuous exercise has been associated with splanchnic hypoperfusion and intestinal injury (5), which is likely to hamper gastrointestinal function, exercise performance, and postexercise recovery (6). In line with this, after placebo ingestion, the prolonged strenuous cycling protocol of the current study led to a clear ~2-kPa increase in $gap_{g\text{-}a}pCO_2$ levels within the first 20 min of exercise. This reflects a rapid development of profound splanchnic hypoperfusion, where blood flow is reduced to less than 30%–40% of baseline levels (28,29). Furthermore, a clear increase in plasma I-FABP levels throughout the exercise until 60 min postexercise was seen, reflecting the development of intestinal injury at this intensity and duration of cycling. The plasma I-FABP concentrations presented in the current study appear somewhat higher when compared with previous studies (5,10,16). Although Snipe et al. (16) corrected for plasma volume changes, in the current study as well as in previous work, we did not perform such corrections (5,10). During strenuous exercise, plasma volume can decline with ~10% (30,31), and because we expected changes of I-FABP to be substantially larger (>200%), we chose not to correct for plasma volume. However, as a consequence, and as a limitation of the current study, plasma I-FABP levels during exercise may have been slightly overestimated, and direct comparisons between studies should be performed with caution. Furthermore, in light of the relatively high I-FABP values reported here, it is important to note that baseline I-FABP concentrations tend to vary substantially between individuals and even within individuals between subsequent test days, and I-FABP values are highly variable between different ELISA kits (32). Therefore, we also report I-FABP values as a percentage change from baseline. The increase in plasma I-FABP levels at the end of exercise after placebo ingestion (~250%) is in the same range as previous studies using the same exercise protocol (5,10), and after prolonged running in the heat (16). It should be noted though that because our baseline values were much higher, the absolute change (~1600 $pg \cdot mL^{-1}$) also appears to be higher, which obviously complicates any direct comparison between studies. As opposed to the profound gastrointestinal complaints of running in the heat (16), the current study reports large increases in plasma I-FABP levels while the complaints were negligible. Yet, even in the absence of such complaints, an association between intestinal injury and impaired nutrient uptake has been reported (26), and such impaired nutrient uptake could theoretically negate postexercise recovery. Thus, in an attempt to attenuate the exercise-induced splanchnic hypoperfusion and marked enterocyte damage, we studied the effect of dietary intervention strategies in the form of dietary nitrate and carbohydrate (sucrose) ingestion.

Dietary nitrate ingestion increased plasma nitrate and nitrite concentrations by ~12- and ~3-fold, respectively, in line with a previous study from our group supplementing the same dose of sodium nitrate in well-trained cyclists (33). However, the effective uptake of dietary nitrate in the circulation and its endogenous conversion into nitrite did not result in an improvement in splanchnic perfusion or intestinal injury when compared with the placebo treatment. This contradicts previous results after supplementation of another NO donor L-citrulline. Using the same cycling protocol as in the current study, van Wijck et al. (10) reported that 10 g L-citrulline preserved splanchnic perfusion and attenuated the increase in I-FABP levels during exercise, although these benefits were not maintained in the recovery period. The proposed mechanism was increased arginine-induced intracellular NO production leading to improved perfusion, as demonstrated in a mice model (34) where L-citrulline supplementation indeed reduced intestinal microcirculatory dysfunction and increased intracellular NO production. L-citrulline induces NO production through the L-citrulline–L-arginine–NOS pathway, which is highly oxygen dependent. By contrast, NO production through the nitrate–nitrite–NO pathway is gradually activated as oxygen tension and pH decrease (35). Indeed, because the oxygen-dependent NOS pathway can still be stimulated under conditions of splanchnic

hypoperfusion (10), such conditions may be suboptimal for the nitrate–nitrite–NO pathway to be stimulated. Perhaps a total blockage of the splanchnic blood flow resulting in a highly hypoxic state would have been necessary to see an effect of nitrate. Furthermore, the suppression of gastric acid secretion by ranitidine ingestion increases gastric pH to allow for an accurate gastric pCO_2 measurement (19). As a potential side effect though, the increased gastric pH could have attenuated the gastric reduction of nitrite to NO (35). This may partly explain our findings of a lack of effect of dietary nitrate ingestion on exercise-induced gastrointestinal hypoperfusion and damage.

Splanchnic hypoperfusion during high-intensity exercise is caused by increased activity of the sympathetic nervous system to redistribute blood flow from the splanchnic organs to the working muscle (3). This centrally acting mechanism can be counteracted by providing macronutrients during exercise to stimulate local vasodilation, which could be an effective strategy to prevent intestinal injury (10). It is well known that oral meal ingestion (13), and glucose in particular (14), can improve intestinal blood flow in nonexercise conditions. It has been shown that glucose-induced vasodilation of premucosal jejunal arterioles is mediated through adenosine A1 receptors, and that NO at least partially mediates the adenosine A1 receptor-induced vasodilation (15). Many athletes consume sports drinks containing multiple-transportable carbohydrates during exercise because these have been shown to enhance exogenous carbohydrate oxidation rates, postpone fatigue, and improve endurance performance (36,37). As such, we investigated the effect of sucrose ingestion (i.e., providing both glucose and fructose) on splanchnic blood flow and intestinal injury during prolonged strenuous cycling. Plasma glucose levels were elevated in the 10–30 min after sucrose ingestion compared with placebo, indicating an effect of exogenous ingestion on top of the endogenous glucose production during cycling (Fig. 1C). In contrast to dietary nitrate, sucrose ingestion strongly attenuated the increase in plasma I-FABP levels throughout both the exercise and postexercise period when compared with placebo (Fig. 3A), and I-FABP AUC during and after cycling was decreased by more than half after sucrose ingestion. Peak I-FABP concentrations were only increased from baseline by ~185% after sucrose, compared with ~250% after placebo. The attenuation of the exercise-induced increase in plasma I-FABP levels has previously been seen after glucose, and also whey protein ingestion during 2 h of running at 60% $\dot{V}O_{2max}$ (16). The authors recommended using carbohydrates rather than protein during exercise because carbohydrates can support endotoxin clearance and reduce stress markers, whereas protein seems to increase gastrointestinal complaints. We extend on these findings by showing the effectiveness of carbohydrate ingestion during strenuous cycling (i.e., 70% W_{max} theoretically representing ~80% $\dot{V}O_{2max}$) and by measuring an attenuated rise in I-FABP concentrations throughout exercise and in the postexercise period. Furthermore, we observed splanchnic hypoperfusion, which likely caused the observed exercise-induced intestinal injury, but no clinically relevant response in terms of endotoxin translocation (on average ~9% increase in plasma LBP).

Despite the attenuation of intestinal injury, sucrose ingestion did not preserve splanchnic perfusion during exercise. In line with this, a previous study found that glucose ingestion increased portal vein flow at rest but did not lead to a significant attenuation of the exercise-induced reduction in blood flow (38). However, the expected mechanism for the attenuated intestinal injury after sucrose ingestion would be an improved splanchnic perfusion, potentially through the NO-mediated glucose-induced vasodilation (15). The lack of change in $gap_{g\text{-}a}pCO_2$ levels after the sucrose intervention compared with placebo could be attributed to a local stimulation of perfusion at the site of intestinal villi tips, and that the measured gastric blood flow thereby remained unaffected. Gastric pCO_2 has been shown to correlate well with jejunal pCO_2 at rest (39) but is not a direct marker of total intestinal blood flow. A correlation between gastric pCO_2 and plasma I-FABP levels has been reported using the same cycling protocol as the current study (5). However, the profound exercise-induced hypoperfusion might limit the possibility for the tonometry method to detect perfusion changes corresponding with the reduction in systemic I-FABP appearance as seen for the sucrose intervention in the current study. Moreover, it is recommended to use gastric tonometry in a fasted state, and a rise in gastric pCO_2 after feeding can be caused by a buffer effect of meal-induced gastric acid secretion (22). We aimed to suppress basal gastric acid production by providing ranitidine. Yet, we cannot rule out the possibility that gastric acid secretion evoked by sucrose ingestion was not sufficiently inhibited by ranitidine (22), which is a limitation of this study. As such, gastric pCO_2 values might have been elevated after sucrose ingestion, overshadowing a potential effect of sucrose on the attenuation of the exercise-induced hypoperfusion, thus explaining the absence of a reduction in $gap_{g\text{-}a}pCO_2$ levels after sucrose ingestion.

The current study aimed to investigate splanchnic perfusion and intestinal injury during prolonged strenuous cycling. Intestinal injury has been associated with intestinal inflammation and permeability, bacterial translocation, and impaired nutrient absorption (4), and exercise-induced cytokinemia has been associated with gastrointestinal complaints (40). However, no correlation (41–43) or even an inverse correlation (36) has been shown between I-FABP levels and gastrointestinal complaints during prolonged and/or high-intensity running activities reporting a high degree of gastrointestinal complaints. Importantly, the difficulties of obtaining objective measures of gastrointestinal complaints may to some extent explain the lack of association with intestinal injury. Furthermore, a transient increase in systemic I-FABP levels does not represent a direct marker for gastrointestinal complaints but rather reflects acute enterocyte damage being indicative of temporary loss of

intestinal integrity and absorptive capacity. Indeed, reduced dietary protein digestion and absorption rates have been shown during recovery from a resistance-type exercise session when I-FABP levels were increased during exercise (26). Although a direct causal relationship remains to be established, these findings could support the relevance of this marker for enterocyte damage, even under asymptomatic conditions. In addition to enterocyte damage, systemic cytokine levels are known to increase as a consequence of exercise (5,16). These inflammatory markers could be derived from the working muscle (44) as well as other tissues, including the intestine. To address specifically the role of the intestine in the systemic responses, we determined LBP concentrations as a marker of endotoxin translocation. Although plasma LBP increased slightly (by ~9%) from pre- to postexercise, the increase was not different between the interventions, making it difficult to relate this marker to the observed intestinal injury. Furthermore, such modest increases in plasma protein levels should be interpreted with caution as they could be due to, e.g., a lower plasma volume as a consequence of exercise (45).

Previously, intestinal injury has been associated with splanchnic perfusion (5), and the extent of splanchnic hypoperfusion is likely related to the development of gastrointestinal complaints; however, this has never been established (3). This relationship is difficult to investigate because of the low degree of gastrointestinal complaints seen in cycling exercise models that are used to measure splanchnic perfusion (5,10,38), just like for the current study. To further assess the relation between splanchnic perfusion, intestinal injury, and gastrointestinal complaints, as well as the effect of carbohydrate ingestion on these aspects, future work should recruit athletes that are prone to gastrointestinal symptoms. In addition, our findings should be confirmed in exercise conditions triggering a high degree of gastrointestinal complaints, such as prolonged strenuous running in extreme conditions, although the tools to assess splanchnic perfusion under such conditions remain to be established.

In line with previous work in this area (16), our findings suggest that consuming carbohydrates frequently and consistently during exercise may represent a feasible protective strategy against exercise-induced gastrointestinal disturbance. This is the case for all levels of athletes, as even highly trained athletes may only be highly trained in a specific exercise and do not necessarily have highly trained guts. Two weeks of repetitive gut challenge has been shown to improve gastrointestinal complaints and reduce carbohydrate malabsorption during endurance running, which may have performance implications (36,37). The current study shows that ingesting small amounts of carbohydrates (2×20 g) shortly before and during cycling is sufficient to strongly attenuate exercise-induced intestinal injury. We propose that athletes experiencing ischemia-related gastrointestinal complaints during exercise of high-intensity and/or long duration could benefit from practicing carbohydrate ingestion during exercise. For instance, ingesting 20 g of carbohydrate from sport drinks or gels every 20 min of prolonged endurance exercise, in line with the recommendation of ~60 $g \cdot h^{-1}$ carbohydrate ingestion (1), can be an optimal strategy for athletes to limit intestinal injury. Future work should investigate whether the attenuated increase in I-FABP levels due to ingestion of carbohydrates during exercise in asymptomatic athletes can also improve postexercise nutrient uptake to enhance early recovery and subsequent performance.

CONCLUSION

Sucrose but not nitrate ingestion lowers intestinal injury evoked during prolonged strenuous cycling. These results suggest that sucrose ingestion, but not nitrate, prevents hypoperfusion-induced gastrointestinal damage during exercise and, as such, may help to lower exercise-related gastrointestinal complaints.

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