Exercise-Induced Splanchnic Hypoperfusion Results in Gut Dysfunction in Healthy Men

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Abstract

Background: Splanchnic hypoperfusion is common in various pathophysiological conditions and often considered to lead to gut dysfunction. While it is known that physiological situations such as physical exercise also result in splanchnic hypoperfusion, the consequences of flow redistribution at the expense of abdominal organs remained to be determined. This study focuses on the effects of splanchnic hypoperfusion on the gut, and the relationship between hypoperfusion, intestinal injury and permeability during physical exercise in healthy men.

Methods and Findings: Healthy men cycled for 60 minutes at 70% of maximum workload capacity. Splanchnic hypoperfusion was assessed using gastric tonometry. Blood, sampled every 10 minutes, was analyzed for enterocyte damage parameters (intestinal fatty acid binding protein (I-FABP) and ileal bile acid binding protein (I-BABP)). Changes in intestinal permeability were assessed using sugar probes. Furthermore, liver and renal parameters were assessed. Splanchnic perfusion rapidly decreased during exercise, reflected by increased gap2pCO2 from ~0.85±0.15 to 0.85±0.42 kPa (p<0.001). Hypoperfusion increased plasma I-FABP (615±118 vs. 309±46 pg/ml, p<0.001) and I-BABP (14.30±2.20 vs. 5.06±1.27 ng/ml, p<0.001), and hypoperfusion correlated significantly with this small intestinal damage (r5 = 0.59; p<0.001). Last of all, plasma analysis revealed an increase in small intestinal permeability after exercise (p<0.001), which correlated with intestinal injury (r5 = 0.50; p<0.001). Liver parameters, but not renal parameters were elevated.

Conclusions: Exercise-induced splanchnic hypoperfusion results in quantifiable small intestinal injury. Importantly, the extent of intestinal injury correlates with transiently increased small intestinal permeability, indicating gut barrier dysfunction in healthy individuals. These physiological observations increase our knowledge of splanchnic hypoperfusion sequelae, and may help to understand and prevent these phenomena in patients.

Introduction

Splanchnic hypoperfusion occurs in many pathophysiological conditions, and is often considered to lead to epithelial barrier dysfunction. Vascular disease, trauma, and shock can induce splanchnic hypoperfusion and ischemia [1–2]. In addition, gastrointestinal (GI) dysfunction is one of the more frequent complications in surgical patients, in whom organ perfusion and oxygen delivery are often impaired as a consequence of surgery-induced alterations in cardio-respiratory and metabolic demands [3]. In critically ill patients, inadequate splanchnic blood flow causes intestinal damage, thereby compromising the intestinal mucosal barrier, potentially inducing and aggravating endotoxemia and systemic inflammation [4].

Splanchnic hypoperfusion also occurs in physiological conditions. Young, healthy individuals endure episodes of splanchnic hypoperfusion during strenuous physical exercise [5], whereas older individuals may experience similar events during less exhausting activities. Moreover, there is considerable evidence supporting the theory that splanchnic hypoperfusion plays an important role in the development of GI complications in patients with chronic diseases, such as chronic heart failure or pulmonary disease [6–7]. Interestingly, the consequences of short-term splanchnic hypoperfusion on the organs in the splanchnic area remain to be determined.

In the current study, we focus on the direct effects of splanchnic hypoperfusion on gut, liver, and kidney epithelium in healthy young volunteers who perform moderate-to-high intensity physical exercise. This study is based on the fact that functional splanchnic hypoperfusion occurs during physical exercise, when rapid redistribution of the splanchnic blood flow occurs to secure supply of adequate amounts of oxygen and energy to the active muscle tissue, heart, and lungs [5,8]. In addition, during exercise a reduction of the total circulatory blood volume, caused by
transpiration and inadequate fluid intake, can decrease cardiac output and compromise splanchnic perfusion even more [9]. Especially during prolonged running or cycling, athletes can experience abdominal symptoms such as cramping, nausea, abdominal pain, and (bloody) diarrhea [10]. This points towards compromised GI functioning, but only few studies have looked at exercise-induced intestinal mucosal lesions in man [11–12].

In the current study, we determined the consequences of exercise-induced splanchnic hypoperfusion on gut, liver and kidney epithelium, with particular emphasis on intestinal barrier integrity loss in healthy individuals.

**Results**

**Exercise-induced GI hypoperfusion**

Tonometry revealed a significant increase of gastric-arterIALIZED pCO₂ (gapg-apCO₂) during exercise, from $-0.85 \pm 0.15$ kPa to $0.85 \pm 0.42$ kPa ($p<0.001$) at completion of exercise bout, indicating functional splanchnic hypoperfusion (Figure 1A; test design in Figure S1). The steepness of the gapg-apCO₂ slope was most pronounced during the first 10 minutes of exercise, suggesting that functional splanchnic adaptations occur rapidly after altered perfusion demands in other parts of the body during physical activity. The gapg-apCO₂ approximated baseline within 1 hour after exercise, reflecting fast recovery of splanchnic perfusion following a state of functional GI hypoperfusion. Interestingly, the recovery of GI perfusion was most prominent during the first 10 minutes post exercise, which is in line with the rapid adaptation of abdominal perfusion during the first 10 minutes of cycling.

**Exercise-induced small intestinal damage**

To assess hypoperfusion-induced effects on the small intestinal epithelium, plasma intestinal fatty acid binding protein (I-FABP) levels were determined, which increased rapidly, from $309 \pm 46$ pg/ml at baseline to $615 \pm 118$ pg/ml ($p<0.001$) after cycling (Figure 1B). Similar to the decreased gapg-apCO₂ levels (Figure 1A), circulating I-FABP levels declined substantially in the first 10 minutes post exercise, and gradually decreased further until baseline I-FABP level was reached approximately 50 minutes after cycling (Figure 1B). Interestingly, we did not observe a new peak in plasma I-FABP levels in the 60 minutes after cycling, suggesting that reperfusion injury did not occur in this time period.

The short half-life of I-FABP in plasma (approximately 11 minutes) allows to analyze the correlation of enterocyte damage with splanchnic hypoperfusion [13], calculating the within-person correlation between gapg-apCO₂ and the change in circulating I-FABP. Because this correlation has not yet been described in healthy individuals, we determined the correlation between gapg-apCO₂ and I-FABP 3 times, i.e. with a delay of 10, 20, and 30 minutes. Interestingly, I-FABP levels significantly correlated with gapg-apCO₂ assessed at all measured time points (Spearman correlation coefficient ($r_S$): 0.579 ($p<0.001$); 0.592 ($p<0.001$) and 0.528 ($p<0.001$), respectively). The correlation between gapg-apCO₂ and I-FABP levels determined in samples taken 20 minutes later, is depicted in Figure 1C.

Similar to I-FABP, cycling increased ileal bile acid binding protein (I-BABP) levels (5.06 ± 1.27 to 14.3 ± 2.20 ng/ml ($p<0.001$; Figure 2A), reflecting enterocyte damage in the ileum specifically.

**Exercise-induced GI complaints**

Two individuals reported nausea during the GI perfusion measurements with the nasogastric tube in situ, which resolved after the nasogastric tube was removed upon completion of the experiment. Three additional participants mentioned minor abdominal complaints during the test, including pain in epigastrio, flatulence and belching. The individuals reporting abdominal
complaints did not show higher I-FABP levels during and post exercise than the individuals without abdominal complaints.

Exercise-induced changes in liver parameters

To obtain information on liver injury during exercise, we analyzed a set of four parameters: liver fatty acid binding protein (L-FABP), alanine transaminase (ALT), aspartate transaminase (AST), and alpha-glutathione S-transferase (alpha-GST). Plasma L-FABP levels significantly increased in all individuals upon exercise (4.75 ± 0.30 to 14.2 ± 1.38 ng/ml (p < 0.001; Figure 3A)). Plasma ALT and AST levels increased from baseline 16.5 ± 1.40 to 20.0 ± 1.60 U/l (p < 0.01; Figure 3B) and 18.5 ± 0.93 to 23.8 ± 0.98 U/l (p < 0.001; Figure 3C), respectively. Alpha-GST levels also increased during and after exercise (0.42 ± 0.07 to 0.64 ± 0.10 ng/ml (p = 0.12)), reaching statistical significance at 1 hour post exercise (0.85 ± 0.16 ng/ml (p < 0.01 vs. baseline, p < 0.05 vs. directly post exercise; Figure 3D). Prolonged release of L-FABP and alpha-GST was observed at least for 1 hour post exercise, whereas plasma ALT and AST levels decreased 1 hour post exercise.

Exercise-induced changes in renal parameters

While we assumed that exercise caused renal damage, no significant changes in urinary N-acetyl-beta-D-glucosaminidase (NAG) levels were observed (p > 0.05; Figure 2B). NAG, expressed in tubular epithelial cells [14], which are vulnerable to ischemic injury, is a sensitive marker of renal damage. Our data indicate that exercise, accompanied by enterocyte and hepatocellular injury, does not cause renal damage.

Exercise-induced GI permeability changes without evidence for bacterial translocation

To analyze whether the exercise-induced GI hypoperfusion and consequent enterocyte damage resulted in GI permeability changes, we analyzed small intestinal permeability, which was assessed as lactulose/L-rhamnose (L/R) ratio in urine and plasma. All subjects were able to urinate within 10 minutes of the instructed time point, without using Foley catheters. Overall, a trend towards higher small intestinal permeability after exercise was observed in urine, which was especially pronounced in the first two hours of urinary collection (Figure 4A). However, no statistically significant changes were observed in the urinary L/R ratio or the individual sugars after exercise. Since modest,
Discussion

The redistribution of blood away from the splanchnic area is an adaptation of the body enabling perfusion of critical tissues in both physiological and pathophysiological situations. During physical exercise, reduction of the splanchnic circulation occurs to meet the increased demand for oxygen and substrates in the active muscle, thereby inducing a physiological state of hypoperfusion in the GI system [8]. This study provides for the first time a comprehensive overview of the processes occurring in response to splanchnic hypoperfusion.

In this study, we demonstrate that in healthy individuals, one hour of physical exercise results in splanchnic hypoperfusion and small intestinal damage, reflected by an increase in plasma I-FABP. Interestingly, the extent of intestinal damage significantly correlated with the exercise-induced hypoperfusion and with mild permeability changes in the small intestine. The most pronounced change in splanchnic perfusion, i.e. the most obvious increase in gapg-apCO2, occurred within the first 10 minutes of cycling, which implies that splanchnic perfusion rapidly decreases following the onset of exercise. The speed of adaptation of the splanchnic blood flow is further demonstrated by its fast recovery after cycling, which was also most prominent during the first 10 minutes following cessation of exercise. The rapid decline in splanchnic perfusion during cycling was followed by the development of significant intestinal damage with a time lag of 20 minutes. The data show a correlation between the exercise-induced splanchnic hypoperfusion and enterocyte damage (measured 20 minutes later), which is in line with animal studies and studies performed in critically ill patients demonstrating that intestinal hypoperfusion and ischemia result in intestinal cell damage [4]. The exercise-induced damage to the small intestine was measured using plasma I-FABP and I-BABP, both sensitive plasma parameters of innate inflammation. Plasma MPO levels increased significantly in all individuals upon exercise, from baseline 4.4±0.8 to 33±4.7 ng/ml after cycling (p<0.001; Figure 2C). In line with these results, a significant raise in circulating calprotectin levels was observed (p<0.0001; Figure 2D). Moreover, fecal calprotectin levels increased significantly upon exercise from baseline median 1.07 μg/g (range 0.49–2.20 μg/g) to 1.48 μg/g (range 0.59–40.82 μg/g, p<0.05; data not shown).

Transient changes in intestinal permeability only increase sugar probe excretion transiently, we considered that this increased probe excretion might be overshadowed by normal probe excretion, because all excreted probes accumulate in the bladder before leaving the body. Using our novel approach for sugar probe detection, we could assess the sugar probes in detail by analysis of plasma samples. Plasma analysis revealed an overall increase in small intestinal permeability after exercise compared to rest (p<0.001). Plasma L/R ratios after exercise at individual time points were not significantly different from L/R ratios in rest. Plasma permeability analysis revealed an overall increase in small intestinal permeability after exercise compared to rest (p<0.001). Plasma L/R ratios after exercise at individual time points were not significantly different from L/R ratios in rest. AUC of the plasma L/R ratios determined in the 0–1 h time period were evaluated for similarity, resulting in a RS of 0.42 (p<0.05; data not shown). Finally, the sucralose/erythritol (S/E) ratio was determined in the 5–24 h urinary collections to study large intestinal permeability changes. No changes in large intestinal permeability were observed (Figure 4C).

To assess the translocation of bacterial endotoxin from the intestine into the circulation, EndoCab analysis was performed. The data did not reveal significant changes in endotoxin core antibody levels before and after cycling in the subgroup of participants that underwent permeability analysis (Figure S2A) or in the total group of participants (Figure S2B).

Exercise-induced inflammatory response

Myeloperoxidase (MPO) and calprotectin were analyzed as parameters of innate inflammation. Plasma MPO levels increased significantly in all individuals upon exercise, from baseline 4.4±0.8 to 33±4.7 ng/ml after cycling (p<0.001; Figure 2C). In line with these results, a significant raise in circulating calprotectin levels was observed (p<0.0001; Figure 2D). Moreover, fecal calprotectin levels increased significantly upon exercise from baseline median 1.07 μg/g (range 0.49–2.20 μg/g) to 1.48 μg/g (range 0.59–40.82 μg/g, p<0.05; data not shown).
in small intestinal damage in general, including the ileum. Interestingly, plasma L-FABP levels gradually decreased in the 60-minute ‘reperfusion’ period. The absence of a second peak in plasma L-FABP levels in this 60-minute period after splanchnic hypoperfusion indicates that detectable reperfusion injury did not occur in our healthy individuals. Our observations are in line with recent work on human intestinal ischemia/reperfusion (I/R) studies from our group showing that after 30 minutes of full intestinal ischemia in an isolated part of the jejunum during a Whipple procedure, plasma L-FABP levels rapidly increase, whereas during reperfusion, a gradual decrease in L-FABP levels was observed [20]. During reperfusion, shedding of the damaged enterocytes prevented the jejunum from a massive I/R-induced inflammatory response [21]. Taken together, these results reflect the ability of the gut to withstand episodes of partial or total deprivation of blood flow, with relatively little damage to the intestinal mucosa.

Having obtained strong indications for reduced splanchnic blood flow, we hypothesized that splanchnic outflow may be reduced during strenuous physical exercise, resulting in hepatocyte damage. Plasma levels of all assessed liver parameters (i.e. L-FABP, ALT, AST, and alpha-GST) elevated significantly after exercise. However, two other factors could be responsible for the phenomenon. First, the increase in these plasma parameters could theoretically be partly attributed to an exercise-induced reduction in plasma volume. The increases, however, largely exceeded the 10% decrease in plasma volume that has been described in athletes in more extreme settings than ours [22]. Alternatively, muscle transaminase release is expected in response to (exercise-induced) muscle injury [23,24], while L-FABP release could be due to intestinal damage. Therefore, we additionally analyzed plasma alpha-GST, an early marker of hepatocyte injury unaffected by muscle injury, haemolysis, or intestinal injury [25,26]. In short, the significant rise in the assessed liver parameters after exercise strongly supports the development of mild hepatocyte damage, which is in line with Garcia-Pagán et al. who reported a decrease in hepatic blood flow in patients with liver cirrhosis and portal hypertension after only 10 minutes of cycling at submaximal workload [27].

In contrast to the intestinal and liver data, we did not observe indications for renal damage during exercise. At this stage, it is unclear whether there was no hypoperfusion in the kidneys during cycling, or whether the kidney is more resistant to physiological hypoperfusion. Previous studies support the former, showing relatively unchanged renal flow after short-term splanchnic blood redistribution [28,29].

In conclusion, our study demonstrates that a single, 60-minute bout of moderate-to-high intensity exercise is accompanied by splanchnic hypoperfusion and transient epithelial integrity loss in healthy young males, reflecting the ability of the gut to withstand episodes of physiological stress with minor consequences.

**Methods and Materials**

**Ethical approval**

This study was approved by the medical ethical committee of Maastricht University Medical Centre+, and conducted in accordance with the Declaration of Helsinki (revised version, October 2008, Seoul).
Participant characteristics
Healthy male volunteers were recruited via posters at the University. All volunteers spent 4 to 10 hours per week performing endurance sports as part of their normal life style. The volunteers had no abdominal complaints during daily activities, had not taken any medication for at least one month prior to participation, had no history of GI disease, and had had no abdominal surgery. Volunteers were informed about the nature and risks of the experiments. Written consent was obtained 5 days before the experiments.

Participants had a mean age of 23.6±0.7 years and body mass index of 21.0±0.4 kg/m². Subjects' baseline characteristics and maximum workload capacity did not differ between the study groups (Table 1). All participants were able to complete the 1-hour exercise bout, except for 1 volunteer, who discontinued cycling after 42 minutes due to exhaustion.

Pre-exercise restrictions/arrangements
Prior to the experiments, maximal workload capacity was assessed on a stationary cycle ergometer (Lode Excalibur, Groningen, the Netherlands), during electrocardiographic monitoring (MAC 5500, GE Medical Systems, Freiburg, Germany) to exclude cardiologic abnormalities. A dual energy X-ray absorptiometry scan (DXA²³, Hologic Inc., the Netherlands) was performed to assess body composition.

Test subjects recorded dietary intake 2 days prior to the first experimental day, and maintained dietary intake as recorded for subsequent test days to prevent dietary influence. Participants were not allowed to consume alcohol or caffeine 2 days prior to each test day. Moreover, participants maintained normal activities of daily living, but refrained from strenuous physical activity. The evening before each test day, participants received a standardized meal (1.7 MJ, consisting of 62.6 g carbohydrate, 18.9 g protein and 7.9 g fat).

Study design and sampling
Subjects were tested after an overnight fast. The test design is depicted as Figure S1. In short, a catheter (20 Gauge, Braun, Melsungen, Germany) was placed in the participant’s forearm vein. Every 10 minutes, blood was collected into pre-chilled EDTA tubes (Vacucontainer, Becton Dickinson (BD), Helsingborg, Sweden), and kept on ice. Urine was collected by the participants in 1 l plastic cups before and within 1 hour after exercise. Urinary volume was recorded and 4 ml was directly transferred to pre-chilled tubes. Blood and urine samples were centrifuged within 1 hour at 4°C at 2500 x g for 15 minutes and immediately stored at −80°C until analysis. Following 1 hour rest in supine position, participants started cycling at 150 W. After 3 minutes, workload was increased to 70% of the individual’s pre-assessed maximal workload capacity. Subjects maintained pedal rates of 60 rpm, and workload was decreased by 25 W if the participant was unable to maintain 60 rpm. Participants were only allowed to drink a maximum of 200 ml tap water per 10 minutes. Faeces were collected by 6 participants, before and after cycling, and stored at −4°C until analysis. Subjects were asked to report GI complaints experienced on the test day and the following day.

Assessment of GI perfusion
Gastric air tonometry was performed in 9 participants. The evening before and 1 hour prior to the experiment, participants ingested 150 mg ranitidine orally (GlaxoSmithKline, Zest, the Netherlands) to suppress gastric acid production, which interferes with carbon dioxide, thereby affecting tonometry results [38]. A nasogastric Tonometrics catheter (8 French, Datex Ohmeda, Finland) was introduced and fixed to both nasal flares. Gastric pCO₂ was measured at 10-minute intervals using an automated capnograph (Tonocap TC-200, Datex Ohmeda, Finland). To avoid the use of an arterial catheter, an intravenous catheter (22 Gauge, BD) was placed in a dorsal hand vein and the hand was placed in a hot box heated to 60°C, to arterialize the blood for measuring arterial pCO₂ [39], using a Radiometer ABL 510 (Copenhagen, Denmark). Blood samples were collected simultaneously with the tonometry measurements and gap_{pCO₂} was calculated.

Assessment of intestinal damage
I-FABP, a sensitive marker of intestinal cell damage, was used to determine small intestinal damage [15]. Plasma I-FABP was determined using an ELISA (Hyclut Biotechnology, Uden, the Netherlands).

### Table 1. Baseline characteristics of the healthy male participants.

<table>
<thead>
<tr>
<th>Group size (no. of participants)</th>
<th>GI perfusion</th>
<th>Intestinal damage</th>
<th>GI permeability</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>9</td>
<td>15</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Height (m)</td>
<td>24.2±1.0</td>
<td>23.8±0.8</td>
<td>25.0±0.6</td>
<td>0.67</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>68.4±2.8</td>
<td>68.1±1.7</td>
<td>66.8±3.8</td>
<td>0.92</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>20.3±0.6</td>
<td>21.0±0.5</td>
<td>20.8±0.7</td>
<td>0.68</td>
</tr>
<tr>
<td>Total body: fat percentage (%)</td>
<td>11.6±0.7</td>
<td>11.9±0.8</td>
<td>12.5±0.6</td>
<td>0.81</td>
</tr>
<tr>
<td>fat mass (kg)</td>
<td>8.0±0.6</td>
<td>8.1±0.6</td>
<td>8.4±5.8</td>
<td>0.92</td>
</tr>
<tr>
<td>fat free mass (kg)</td>
<td>61.1±2.6</td>
<td>60.3±1.5</td>
<td>59.4±3.8</td>
<td>0.91</td>
</tr>
<tr>
<td>Both legs: fat percentage (%)</td>
<td>12.6±0.8</td>
<td>12.6±0.9</td>
<td>13.5±0.6</td>
<td>0.77</td>
</tr>
<tr>
<td>fat mass (kg)</td>
<td>3.1±0.3</td>
<td>3.1±0.3</td>
<td>3.3±0.3</td>
<td>0.87</td>
</tr>
<tr>
<td>lean mass (kg)</td>
<td>20.2±1.2</td>
<td>20.5±0.7</td>
<td>20.1±1.5</td>
<td>0.97</td>
</tr>
<tr>
<td>Maximum workload (W/kg)</td>
<td>5.0±0.1</td>
<td>5.2±0.1</td>
<td>5.3±0.1</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SEM.

1 Of all study participants, three subjects participated in all three substudies, and four subjects participated in two substudies. Hence, in total, 20 individuals took part in the study.

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Assessment of GI permeability

GI permeability was determined using a mix of 1 g lactulose (Centrafarm, Etten-Leur, the Netherlands), 1 g sucralose (Brenntag, Sittard, the Netherlands), 1 g erythritol (Danisco, Copenhagen, Denmark), 1 g sucrose (Van Gilse, Dinteloord, the Netherlands), and 0.5 g L-rhamnose (Danisco) dissolved in 150 ml tap water, in a subset of 6 participants.

The study design, depicted in Figure S1, consisted of 2 test days, one to test basal permeability and one for exercise-induced permeability. Participants fasted overnight. The sugar bolus was ingested after 30 minutes of rest in supine position, or after 30 minutes of cycling. Blood was sampled every 20 minutes and urine every hour, up to 5 hours post ingestion. All subjects urinated in plastic cups within 10 minutes of the instructed time point. In addition, subjects collected their 5-24 h urine at home and returned the provided bottles the next morning, when urine was immediately processed.

Combined HPLC (Model PU-1980 pump, Jasco Benelux, Maarsen, the Netherlands) and mass spectrometry (Model LTQ-XL, Thermo Electron, Breda, the Netherlands) were used to determine urinary and plasma sugar concentrations. Lactulose, rhamnose, and L/R ratio were determined in urinary samples to assess small intestinal permeability. Since physiological, transient permeability changes were expected, and novel permeability assay allowed the detection of sugar probes in plasma, we also determined lactulose and rhamnose concentrations, and L/R ratios in plasma. Both lactulose and rhamnose are degraded in the colon. Therefore, the ratio between two inert sugar probes, sucralose and erythritol, was determined in the 5-24 h urine to assess large intestinal permeability. Since we did not draw blood in this time period, we did not perform large intestinal permeability analysis in plasma.

Assessment of inflammatory activation products

Circulating myeloperoxidase (MPO) and calprotectin, antimicrobial proteins that are excreted from neutrophils upon activation, are considered early parameters of systemic inflammatory activation [42,43]. Active MPO is known to elicit secretion of other cytokines including tumor necrosis factor-α and interleukins [44], which were therefore less useful. MPO was measured in plasma before and after cycling using a commercial ELISA (Hycult; detection window 0.40–100 ng/ml). Since evidence is accumulating that inflammatory products are produced in contracting and skeletal muscle [32,45], we determined both circulating and faecal calprotectin. The latter is considered a strong indicator of intestinal inflammation in specific, and has been found to correlate to other parameters of intestinal inflammation in inflammatory bowel disease [46] and enteropathy induced by non-steroidal anti-inflammatory drugs (NSAID) [47]. Calprotectin was measured before and after cycling using a commercial calprotectin ELISA (standard 1.6–100 ng/ml). Faeces were collected by the participants in provided faecal sampling cups. Time of collection was reported by the participants. All faecal samples were collected within 7 hours after cycling. For faecal analysis, faeces were thawed, and 100 mg was added to 4.9 ml of extraction buffer (0.1 M Tris, 0.15 M NaCl, 1.0 M urea, 10 mM CaCl₂ 2H₂O, 0.1 M citric acid, 0.5% BSA, pH 8.0) was added [48]. After 30 minutes shaking, 1 ml of suspension was centrifuged at 10,000 rpm for 20 minutes at 4°C. Fecal calprotectin concentrations were measured in supernatant using the calprotectin ELISA.

Endotoxin core antibodies in plasma were determined using EndoCab ELISA (Hycult Biotechnology, Uden, the Netherlands; detection window 0.13–8.0 IgG median units (GMU)/mL) according to the manufacturer’s instructions in samples taken before and after cycling.

Statistical analysis

Statistical analysis was performed using GraphPad Prism (Version 5.00, GraphPad Software for Windows, San Diego California, USA). Normality of all data was verified by the Kolmogorov-Smirnov test. All normally distributed data are presented as mean ± standard error of the mean (SEM), not normally distributed data as median and range. Outliers identified using Grubbs analysis were excluded from statistical analysis. Continuous data were analyzed using repeated measures analysis of variance with Tukey’s or Dunnett’s post-hoc test for multiple comparisons. Basal and post exercise values were compared using the non-parametric Wilcoxon signed rank test, considering the small number of study participants. Within-person correlations between gap anterior and plasma L-FABP, and plasma L-FABP and plasma L/R ratio were computed by normalizing both data sets, which enables the assessment of the pure association of both variables by calculating the Spearman correlation coefficient rs. Linear regression was used to visualize the correlation. A p<0.05 was considered statistically significant.

Supporting Information

Figure S1 Time frame of GI perfusion, intestinal damage and GI permeability study. (PDF)
Figure S2  One hour of strenuous physical exercise does not lead to bacterial translocation. A) Plasma IgG levels against endotoxins did not change significantly upon physical exercise in the subgroup of six participants that underwent permeability analysis. B) Plasma analysis for IgG against endotoxins in the total group of participants did not reveal significant changes. Data are mean ± SEM. Different from baseline (t = 0) (NS, not significant).

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Author Contributions

Conceived and designed the experiments: KvW KL LlL WP WB CD. Performed the experiments: KvW. Analyzed the data: KvW KL WP. Contributed reagents/materials/analysis tools: LlL WP. Wrote the paper: KvW KL WP. Critical revision manuscript: LlL WP.

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