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The association between running induced gastrointestinal injury and -complaints , and the impact of food intake in 60 km ultramarathon runners

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ABSTRACT

Purpose We aimed to assess the contribution of gastrointestinal injury and food intake to gastrointestinal complaints during a 60-km ultramarathon.

Methods Thirty-three ultramarathon runners provided pre- and post-race blood sample for assessment of GI injury by intestinal fatty acid binding protein (I-FABP), and inflammatory response by IL-6, IL-8, TNF-α, and CRP. GI complaints and nutritional intake were reported by a post-race questionnaire.

Results GI complaints were reported by 73% of the runners, of which 20% reported one or two severe complaints. IL-6, IL8, TNF-α, and CRP increased significantly from pre- to post-race (P<0.001 for all biomarkers), while I-FABP did not (1375 [IQR: 1264-2073] to 1726 [IQR: 985-3287] pg/mL; P=0.330). The 'GI-complaints score', as the integral of the number and severity of GI complaints, did not correlate with ΔI-FABP (rs:-0.050; P=0.790) or energy intake (rs:-0.211; P=0.260). However, there was a significant negative correlation between energy intake and ΔI-FABP (rs:-0.388; P=0.031).

Conclusion GI complaints were neither associated with food intake nor GI injury as assessed by plasma I-FABP response. Energy intake, however, was inversely related to the I-FABP response to exercise. This finding suggests that substantial energy intakes during exercise may prevent exercise-induced GI injury as assessed by the I-FABP response.

Keywords

GI Distress / GI damage / I-FABP / Nutritional intake / Running / Exercise-induced

Highlights

- No association between gastrointestinal complaints and gastrointestinal injury (I-FABP response) or food intake was present.
- There was an inverse correlation between energy intake and plasma I-FABP response, suggesting that higher energy intakes may prevent gastrointestinal injury as assessed by the I-FABP response.

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INTRODUCTION

During exercise, blood from the gastro-intestinal (GI) tract is rapidly redistributed to body parts with higher oxygen demands, such as the heart, lungs, active muscle, and skin, leading to hypoperfusion of the GI tract (ter Steege and Kolkman, 2012, van Wijck et al., 2011). This GI hypoperfusion can cause GI ischemia and subsequent damage of the enterocyte, inducing an inflammatory response, and release of intestinal fatty acid binding protein (I-FABP); a plasma marker of intestinal injury during exercise (Derikx et al., 2008, van Wijck et al., 2011). Severe GI injury may lead to an impaired gut barrier function, thereby potentially impairing exercise performance and recovery due to limited nutrient absorption (Miall et al., 2018, van Wijck et al., 2012).

Strenuous exercise can also lead to exercise-induced GI complaints, which are experienced by ≥60% of (ultra) endurance athletes (Costa et al., 2016, Jeukendrup et al., 2000, Wardenaar et al., 2015). These GI complaints may lead to impaired exercise performance, depending on the type and severity of complaints (Costa et al., 2017a, Hoffman and Fogard, 2011). The highest prevalence and severity of various GI complaints are generally reported during high-intensity and prolonged running (Costa et al., 2020, ter Steege and Kolkman, 2012).

Although an association between plasma I-FABP, as a direct marker for GI injury, and GI complaints during exercise seems self-evident, this has not been clearly established. Laboratory-based work by Costa et al. (2017b) reported a negative correlation between plasma I-FABP levels and GI specific complaints within a 3h treadmill run, at 60%VO2max for the first 2 hours, followed by a 1h distance test. They suggest that a higher degree of GI injury during running is associated with less profound GI complaints. A recent field study in marathon runners (Pugh et al., 2019), did not show a significant correlation between I-FABP response and GI complaints. Additionally, it should be noted that other studies, such as Stuempfle et al. (2016) have identified associations between other GI injury markers and nausea. However, since nausea is not necessarily considered a GI specific complaint (Jeukendrup et al., 2000, Jeukendrup, 2015), this factor is beyond the scope of the current study. Because the I-FABP response, and its association with GI complaints during real life running remains not fully established (Chantler et al., 2020), our first aim was to investigate the association between GI injury as measured by I-FABP levels, and GI complaints during an ultramarathon. Investigating this in an ultramarathon setting seems specifically of interest since it has been speculated that both GI complaints and GI injury are amplified within increased duration of the event (Costa et al., 2017a, Pfeiffer et al., 2012, van Wijck et al., 2011, van Wijck et al., 2012).

Nutritional intake during exercise has been associated with both GI injury and -complaints. In this regard, earlier laboratory work has shown food intake in general, and carbohydrate intake in particular to be effective in increasing GI perfusion (Rehrer et al., 2005, van Wijck et al., 2012), and attenuating the increase in exercise-

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induced GI injury, as indicated by attenuated I-FABP levels (Jonvik et al., 2018, Snipe et al., 2017). In contrast, high intakes of food and carbohydrates have been shown to induce GI complaints (de Oliveira et al., 2014, Costa et al., 2017b, Miall et al., 2018, Rehrer et al., 1992), while other work does not indicate a role of feeding on GI complaints (Glace et al., 2002), or even shown that feeding (higher fluid and fat intakes) were associated with less GI complaints (Stuempfle et al., 2013). Therefore, our second aim was to investigate the role of food intake in relation to both GI complaints and GI injury during a 60 km ultramarathon.

METHODS

Study design

A cross-sectional study in ultramarathon runners was conducted to investigate running induced increases in I-FABP levels, and its association with GI complaints , and the effect of food intake on both factors. This study was part of a larger project, investigating food intake during a 60 and 120 km ultramarathon (Wardenaar et al., 2019, Wardenaar et al., 2018). The study was performed in accordance with the Declaration of Helsinki (2013) and was approved by the HAN University of Applied Sciences ethical advisory board (EACO 63.03/17).

Study population

All 481 participants of the 60 km ultramarathon on the isle of Texel in the Netherlands were approached for participation for the study via the event's newsletter seven days prior to the race. A total of 53 runners volunteered to participate and provided written informed consent before pre-race blood sample collection. After exclusion, as explained in procedures and measurements, 33 runners were included in the study analysis.

Race specifics

The 60 km race took place on 17 April 2017, and started and finished at Den Burg, Texel, the Netherlands, starting at 10:35 AM. The race consisted of 107 meters of elevation gain, with the surface containing a combination of pavement/road (70%), beach/sand (20%), gravel (6%) and other (4%). The average temperature on the race day was 7.0°C (min: 4.3°C and max: 9.6°C, with 1.6 h of rain and 11.1 h of sunshine between sunrise and sunset, a humidity of 67%, and an average wind speed of 5.0 m/s (measured at weather station De Kooy, Den Helder).

Procedures and measurements

A web-based baseline questionnaire was filled in, enquiring about personal characteristics as described by Wardenaar et al. (2018). One person was declared not eligible for inclusion in our study due to a history of GI-related medical issues revealed by the questionnaire, and additional discussion with this person. On race-day, venous blood samples were collected between 7:30-9:30 AM (1 to 2 h pre-race) in a non-fasted state, while post-

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race blood samples were collected within a 5 – 30 min time frame after finishing. When post-race blood samples were not collected within 30 min after finishing (n=11) or not collected at all (n=3), participants were excluded from the analyses. Upon pre- and post-race blood collection, participants were asked about the use of medication before or during the race. One participant reported the use of Non-Steroidal Anti-Inflammatory Drugs and was excluded from the analyses. Body mass and height were obtained after pre-race blood collection. Participants that did not finish the race (n=4) were excluded from the analysis, but were asked about their reasons for abandoning the race. Altogether, 33 participants were included in the statistical analysis.

After finishing, participants were required to complete a Dutch-adapted version of a Food Frequency Exercise Questionnaire (FFEQ) (Pfeiffer et al., 2012) before midnight, as previously described and validated by (Wardenaar et al., 2019). In short, participants were asked to report their food and fluid intake during the race based on a pre-specified list of products that are often used by ultramarathon runners, and a section to report the use of any foods and fluids products missing on the pre-specified list. The questionnaire concluded with a section on GI complaints which has not been validated previously. Runners were queried about the severity of ten GI specific complaints (i.e. belching, heartburn, bloating, vomiting, stomach ache, flatulence, urge to defecate, stomach cramps, intestinal cramps and diarrhea) and six GI related complaints (i.e. nausea, dizziness, headache, urge to urinate, side ache and muscle cramps), experienced during the race (GI classification derived from Jeukendrup et al. (2000) and Jeukendrup (2015). The severity of each complaint was reported on a 10-point scale from 1 (‘’no complaints”) to 10 (‘’never been worse’’). Complaints with a severity <5 were categorized as being mild, and >4 as being serious, as adapted from Pfeiffer et al. (2012).

Blood analysis

After blood collection in EDTA and serum tubes, 1 ml of EDTA blood was transferred to a separate tube and stored at 4°C for hematocrit analysis. Hematocrit was determined by spinning whole blood in a capillary tube, using a Hettich Mikro 220R centrifuge (rotor type 1023) for 10 min on 10000g, whereafter hematocrit was estimated by calculating the ratio of the column of packed cells as part of the total length of the sample in the capillary tube. Within 30 min the remaining EDTA blood was centrifuged in a Hettich EBA 200 centrifuge for 10 min at 20°C and 1000g. Plasma was aliquoted into cryotubes and frozen on dry ice at the research site. The blood in serum tubes, was allowed to clot for 60 min at room temperature. After centrifugation for 10 min at 20°C and 1000g, serum was aliquoted into cryotubes and frozen on dry ice at the research site. Both the plasma and serum were transported to the laboratory the same evening for long-term storage in -80°C freezers.

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To evaluate the extent of small intestinal injury, plasma I-FABP levels were determined by an in-house developed enzyme-linked immunosorbent assay (ELISA), as described by van (van Wijck et al., 2013). As plasma I-FABP levels will vary substantially between different ELISA kits (Peoc'h et al., 2018), I-FABP levels are reported as ΔI-FABP and relative change. For an indication of whole-body inflammation, plasma interleukin-6 (IL-6), IL-8 and tumor necrosis factor-α (TNF-α) were determined by ELISA (V-plex Custom Human Cytokine Proinflammatory Panel 1, MESO QUICKPlex SQ120), and serum C-reactive protein (CRP) was determined by a latex enhanced immunoturbidimetric high-sensitivity assay (ABX Pentra CRP CP).

Statistical analysis

Thirty-three participants were included in the statistical analysis, of which three male runners had missing data on GI complaints and two of those three had missing nutritional data. Raw nutritional data from the FFEQ was processed in Microsoft Excel to calculate energy and nutrient intakes during the race, based on the Dutch food composition database (NEVO, 2016) or product label information.

All data were analysed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). Data distribution was verified by the Kolmogorov-Smirnov test. Normally distributed data are presented as mean±SD and non-normally distributed data as median [IQR].

Blood markers are presented as absolute values, as well as pre- to post race change (Δ). Pre- and post-race data were compared using Wilcoxon signed-rank tests. Correlations were determined using Spearman correlation tests. Statistical significance was set at $P<0.05$.

To enable a meaningful analysis of general GI complaints, the variable 'GI complaint score' has been computed, integrating both the number and severity of GI complaints. I.e. the GI complaint score is the sum of the severity of all individual GI complaints. With a severity rate ranging from 1-10 per GI complaint, and a total of 10 GI complaints, this 'GI-complaints score' ranges from 10-100, with a score of "10" indicating that the participant had no GI complaints at all.

Participants were split in three groups, based om the presence and severity of reported GI complaints (eg (1) no GI complaints, (2) one or more mild GI complaints and (3) at least one severe GI complaint), and were compared for their ΔI-FABP and energy intake by a Kruskal-Wallis test.

RESULTS

Participants characteristics

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Participant characteristics are presented in **Table 1**. The average race speed of the participants (9.7±1.1 km/h) was similar to the average speed of all runners that finished the race (9.8±1.1 km/h, n=367).

GI- and GI-related complaints

The majority of the runners (73%, *n*=22) reported at least one GI specific complaint (**Figure 1**), ranging from 1 to 6 different complaints per participant. GI specific complaints that were reported as being serious (severity rate of >4) were reported by 20% (*n*=6) of the participants, with 1 or 2 different serious complaints per participant (score per complaint ranging from 5-8). The most present GI specific complaints (reported by a severity score of >1) were belching (43%) and flatulence (40%). GI specific complaints that seem most likely to force runners to interrupt their race, i.e. vomiting (2.6%) and diarrhea (0%) were less common, of which none were classified as serious. The median 'GI-complaints score' was 12 [10 to 16], ranging from 10 to 30, on a scale of 10-100.

Of the four runners who were excluded from the analysis for abandoning the race, none reported that this was caused by GI-related issues.

Pre- and post-race blood markers

Median plasma I-FABP levels did not change significantly from pre- to post-race (1375 [1264 to 2073] vs 1726 [985 to 3287] pg/mL; *P*=0.330; **Figure 2A**). Compared to pre-race, 17 of the 33 participants had lower I-FABP levels post-race (ΔI-FABP: -440 [-1008 to -89]) and 16 had higher post-race I-FABP levels (ΔI-FABP: 1160 [400 to 2208]). There was a significant increase from pre- to post-race for median IL-6 (0.2 [0.13 to 0.27] vs 10.41 [7.08 to 15.07] pg/mL; *P*<0.001; **Figure 2B**), IL-8 (7.16 [5.90 to 9.48] vs 15.99 [10.14 to 19.50] pg/mL; *P*<0.001; **Figure 2C**), CRP (0.37 [0.16 to 0.84] vs 0.65 [0.34 to 1.35] mg/L; *P*<0.001; **Figure 2D**), and TNF-α (2.19±0.54 vs 2.80±0.11 pg/mL; *P*<0.001; **Figure 2E**). Hematocrit levels did not change from pre- to post-race (44.1±2.4 vs 44.1±2.5 %; *P*=1.0).

Food and fluid intake

Two male runners did not complete the nutritional questionnaire, resulting in 31 participants with nutritional data. With an overall mean carbohydrate intake of 67±36 [21-149] g/h, protein intake of 1.7±2.3 [0-11.5] g/h, fat intake of 1.0±1.8 [0-8.5] g/h, and fibre intake of 0.7±0,7 [0-2,5] g/h, ~95±8% (265 kcal/h) of the energy intake during the ultramarathon run was obtained via carbohydrate intake. Mean fluid intake was 448±251 [148-1043] ml/h. More detailed information about energy intake, macronutrient intake, and fluid intake during the race are shown in **Table 2**.

The association between GI complaints, I-FABP levels, and food intake

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No correlation was observed between 'GI-complaints score' and ΔI-FABP levels (rs:-0.050; *P*=0.790; **Figure 3A**). 'GI-complaints score' did not correlate with energy intake (r:0.211; *P*=0.260; **Figure 3B**), carbohydrate intake (rs:0.210; *P*=0.260), protein intake (rs:0.072; *P*=0.710), or fat intake (rs:0.029; *P*=0.880). Furthermore, the 'GI-complaints score' did not correlate with ΔIL-6 (rs:-0.010; *P*=0.960), ΔIL-8 (rs:-0.360 *P*=0.850), ΔCRP (rs:0.056; *P*=0.770) or ΔTNF-α (rs:-0.122; *P*=0.520).

Energy intake (kcal/h) during the race was moderately and negatively correlated with both ΔI-FABP levels (rs:-0.388; *P*=0.031; **Figure 3C)**, as well as absolute post-race I-FABP levels (rs:-0.413; *P*=0.021). The intake of individual macronutrients (g/h) was not significantly correlated with with ΔI-FABP levels (carbohydrate: rs:-0.319; *P*=0.080, protein: rs:-0.248; *P*=0.180, and fat: rs:-0.283 *P*=0.120, respectively).

Dividing participants in three groups, based om the presence and severity of reported GI complaints, i.e. no GI complaints (n=8), one or more mild GI complaints (n=16) and at least one severe GI complaint (n=6) did not show differences in ΔIFABP (*P*=0.700) or energy intake (*P*=0.238).

DISCUSSION

This ultramarathon field study aimed to investigate the association between GI complaints and GI injury, and the effect of food intake on both factors. We did not observe any association between GI complaints and GI injury measured by I-FABP response (ΔI-FABP). Although food intake was not associated with GI complaints, we observed a moderate negative correlation between energy intake and GI injury.

GI complaints are known to limit exercise performance, especially in (ultra)endurance events (Costa et al., 2017a, Hoffman and Fogard, 2011). In our study, 73% of the athletes reported GI complaints, which is in line with previous literature, reporting a prevalence of 60 to 96% within ultramarathon events (Costa et al., 2016, Hoffman and Fogard, 2011, Jeukendrup et al., 2000, Stuempfle et al., 2013, Stuempfle and Hoffman, 2015). Although the majority of runners reported at least one GI complaint, the mean severity per individual complaint was relatively low, ranging from 1.0±0.0 (no complaints) to 1.9±1.5 on a 10-point scale (Figure 1). The relatively low severity is consistent with other research on GI complaints during ultra-endurance events. In this regard, Stuempfle and Hoffman (2015) reported GI complaints on a 5-point scale (ranging from 0-4) during an 161km ultramarathon, with an average severity ranging from 0.7±0.4 to 1.1±0.7 per individual complaint. Also, when looking at the 'GI-complaints score' as an integrated measure of the number and severity of GI complaints, the median score was only 12 [10 to 16] out of 100. So, despite the fact that the majority of the runners reported at least one GI complaint, the number and / or severity of the GI complaints was relatively low. It can be argued that a run with a longer distance, exceeding 100 km, as well as warmer and more humid environmental conditions

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might result in more extensive and prolonged GI hypoperfusion, possibly resulting in more profound GI injury and -complaints (Costa et al., 2017a, Hoffman and Fogard, 2011). Nevertheless, the extend of the GI complaints in a small subset of 120 km runners (n=8) participating in the same event as the current study, did not show clear differences compared to 60 km runners (Hoogervorst et al., 2019). Larger studies need to be conducted to clarify this, and we must be carefull with the tranlation of the current results to events with different conditions and duration.

Although GI complaints are known to depend on the type of exercise and its conditions, the underlying mechanism behind these complaints seems to be multifactional (Costa et al., 2017a), yet not fully understood. In pursuit of finding possible mechanisms for GI complaints, earlier work has described that the degree of exercise-induced GI ischemia, and its subsequent GI anomalies (including mucosal injury, increased intestinal permeability and inflammation, and bacterial translocation) (van Wijck et al., 2012, van Wijck et al., 2011) might play a role in the development of GI complaints (de Oliveira et al., 2014, ter Steege and Kolkman, 2012). In the current study, we did not observe an association between GI complaints and GI injury as measured by plasma I-FABP levels. This finding is in line with recent work by Pugh et al. (2019) who did not report a significant correlation between GI complaints and change in I-FABP levels during exercise. A study by Costa et al. (2017b), however, reported an inverse correlation between GI complaints and the change in I-FABP concentrations induced by 3 h of treadmill running. The latter suggests that a higher degree of GI injury is associated with lower degree of GI complaints. Taken together, it can be speculated that exercise-induced GI injury, at least when assessed by I-FABP response, is not a cause of GI complaints during exercise. This view is further reinforced by the link between food intake during exercise and GI complaints. While food intake is normally seen as a main factor driving GI complaints (de Oliveira et al., 2014), recent laboratory studies have shown that the intake of carbohydrate (Jonvik et al., 2018, Snipe et al., 2017) or protein (Snipe et al., 2017) during exercise attenuate or abolish the exercise-induced increase in I-FABP levels. In the current study, representing real-life racing conditions with food being ingested before and during exercise, the I-FABP response was moderate and negatively correlated with the energy intake during exercise (Figure 3C). This suggests that the intake of substantial amounts of energy during exercise may prevent exercise-induced hypoperfusion of the gut and subsequent GI injury. It can therefore be questioned whether GI injury assessed by I-FABP holds any clinical or practical implications, at least within studies with real-life racing conditions where food is being ingested before and during exercise.

Regardless of exercise-induced GI injury, food intake during exercise has been associated with the development of GI complaints (de Oliveira et al., 2014, Costa et al., 2017b, Miall et al., 2018, Rehrer et al., 1992).

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The current study could not confirm this association, as there was no linear correlation between GI complaints and either energy or macronutrient intakes, and no differences in food intake was found between the three complaints groups. Although speculative, visual examination of Figure 3B seems to reveal higher energy intakes within participants with increasing “sums of GI complaints”. One possible explanation could be the ileal brake mechanism; as this mechanism, that slows down gastric emptying when macronutrients enter the ilium, is prone to developing GI complaints (Rehrer et al., 1992, Shin et al., 2013). Also, visual examination seems to show lower energy intakes in runners with the highest 'GI-complaints scores', that could be argued to result from decreased appetite under more profound GI complaints (Hoffman and Fogard, 2011, Wardenaar et al., 2015). Again, the significance of these phenomena could not be demonstrated within the current study based on correlation analysis or comparison of the three complaints groups, but might be of interest for future research.

Significant increases in plasma IL-6, IL-8, CRP, and TNF-α were observed as result of the 60 km ultramarathon. Such increases are known to be caused by exercise-induced muscle damage, internal organ damage, immune suppression and neutrophilia in the systemic circulation, as generally seen after prolonged (ultra) running (Nieman, 1997, Suzuki, 2018). Within the current study, median increases in plasma IL-6, IL-8, and CRP were 52-fold, 2.2-fold, and 1.8-fold, respectively, and a 1.3-fold mean increase for TNF-α respectively. These increases are in line with inflammatory responses as observed after prolonged running in earlier work work (Castell et al., 1996, Nielsen et al., 2016, Ostrowski et al., 1999, Siegel et al., 2001, Snipe et al., 2017). These exercise-induced increases of IL-6, IL-8, CRP and TNF-α indeed seem to point to increased whole-body inflammation, and thus a high degree of exercise strain. Despite this clear inflammatory response to exercise, the severity of GI complaints was relatively low and no significant increase in GI injury as measured by plasma I-FABP levels was seen.

A strength of the current study is the ability to perform research on GI complaints and GI injury as measured by plasma I-FABP levels, and the effect of ab-libitum food intake on both factors during real-life racing conditions. Inherent to this field-based setting, we were unable to collect blood samples immediately upon finishing. Although some studies reported relatively stable I-FABP levels in the first hour(s) following exercise (Snipe et al., 2018a, Snipe et al., 2018b), others have shown that IFABP levels start to decline within 20 min following exercise (van Wijck et al., 2014) and may return to baseline at 1 hour post-exercise (Pugh et al., 2019). Therefore, we excluded participants whose blood samples were not collected within 30 min after finishing, since the practical race setting did not always allow for blood collections directly after finishing. The latter might have introduced some extra variation in post exercise I-FABP concentrations, and potentially caused an underestimation of the magnitude of the I-FABP response as a result of exercise. Another potential limitation of the current study

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is the use of a post-exercise food frequency questionnaire to determine food intake during exercise. Although continuous observations of food intake may provide a more reliable estimate of food and fluid intake in the field (Wardenaar et al., 2019), such prospective methods may draw additional attention to the outcomes of interest, thereby potentially influencing participants' behavior (Larson-Meyer et al., 2018). Similarly, reporting GI complaints in real-time during the race, may lead to slightly different results than retrospective assessment GI complaints (Wardenaar et al., 2018).

Conclusion

The current study found no association between GI complaints and GI injury as assessed by plasma I-FABP response. Moreover, GI complaints were not associated with food intake during real-life racing conditions. Energy intake during exercise, however, was inversely related to the I-FABP response, suggesting that substantial energy intakes may prevent hypoperfusion of the gut and subsequent GI injury.

AUTHOR STATEMENTS

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Competing interests statement

FW reports grants from Eat2Move and Friesland Campina during the conduct of the study. JD has received research grants and speaking honoraria from FrieslandCampina and NIZO. KV has received speaking honoraria from FrieslandCampina. NV, DH and JZ have no relevant financial or non-financial interests to disclose.

Contributors’ statement

This study was designed by FW, KJ and JS; Data were collected and analysed by NV, FW, DH, JS, and KJ; data interpretation and manuscript preparation were undertaken by NV, FW, DH, JS, JD and KJ. All authors approved the final version of the paper. All authors approved the final version of the paper.

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TABLES

Table 1: Participant characteristics, their finish time and race speed during the 60 km race (means±SD).

	Male (n=28)	Female (n=5)	Average (n=33)
Age (y)	46.3±10.6	45.0±8.1	46.1±10.2
Body mass (kg)	77.5±8.1	68.9±11.7	76.2±9.1
Body height (cm)	182.7±6.4	173.4±12.5	181.3±8.1
BMI (kg/m^2)	23.2±1.8	22.8±2.1	23.2±1.8
Finish time (h:min)	6:14±0:45	6:29±0:34	6:16±0:43
Race speed (km/h)	9.8±1.2	9.3±0.8	9.7±1.1

Table 2: The participants' hourly energy, carbohydrate (CHO), protein (PRO), fat (FAT), fibre, and fluid (FLU) intake during the 60 km race (mean±SD and range).

	Male (n=26)*	Female (n=5)	**Total (n=31)***
Energy intake (kcal/h)	291±164 [85-643]	330±86 [232-459]	297±154 [85-643]
Energy intake (kJ/h)	1218±686 [356-2692]	1381±360 [971-1922]	1243±644 [356-2692]
CHO (g/h)	67±39 [21-149]	70±18 [52-97]	67±36 [21-149]
PRO (g/h)	1.3±1.6 [0-7.1]	3.6±4.5 [0-11.5]	1.7±2.3 [0-11.5]
FAT (g/h)	0.9±1.8 [0-8.5]	1.3±2 [0-4.8]	1.0±1.8 [0-8.5]
Fibre (g/h)	0.7±0.8 [0-2.5]	1.0±0.8 [0-2.2]	0.7±0.7 [0-2.5]
FLU (ml/h)	451±263 [148-1043]	430±205 [255-786]	448±251 [148-1043]

Note. * Number of participants differ from Table 1 because 2 male participants had missing nutritional data

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FIGURE LEGENDS

Fig 1 Bars within the chart represent prevalence of GI and GI-related complaints among the participants in percentage (n=30). Missing data from 3 male participants (of the 33 in total) that did not complete the GI complaints questionnaire. A complaint was present when a complaint severity score of >1 was reported. Complaints with a severity score of 5 or higher (out of 10) being categorized as serious, as adapted from Pfeiffer et al. (2012). “Any complaints” represents the presence of any GI- or GI-related complaints. Layout order and GI classification derived from Jeukendrup et al. (2000)-and Jeukendrup (2015). Abbreviations: GI, gastro-intestinal

Fig 2 Blood parameters before and after the 60 km ultramarathon (n=33). I-FABP (A), IL-6 (B), IL-8 (C) and CRP (D) are tested by Wilcoxon signed rank test; black lines represent median [IQR]. TNF-α (E) is tested by paired samples T-test; black lines represent mean±SD; Gray lines represent individual cases.

Fig 3 Scatter plot and Spearman correlations between 'GI-complaints score' and ΔI-FABP levels, n=30 (A), 'GI-complaints score' and energy intake per hour, n=31 (B), and ΔI-FABP levels and energy intake per hour, n=31 (C)

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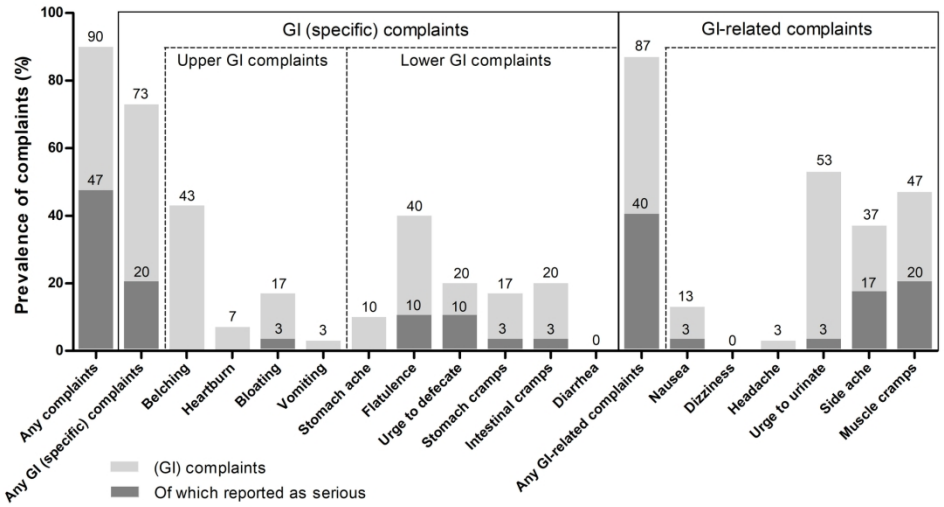

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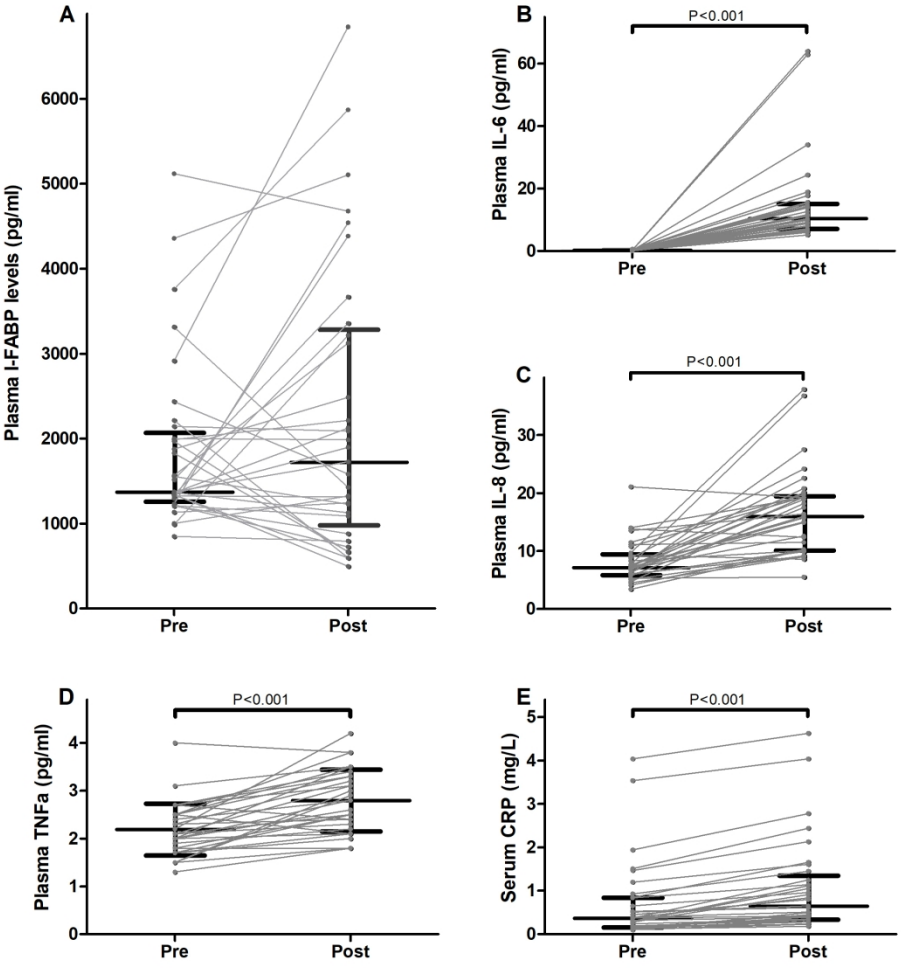

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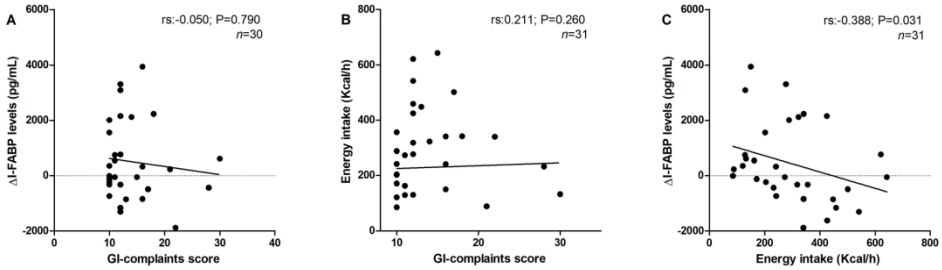

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