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Original article

Plasma citrulline concentration, a marker for intestinal functionality, reflects exercise intensity in healthy young men☆

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SUMMARY

Background & aims: Plasma citrulline concentration is considered to be a marker for enterocyte metabolic mass and to reflect its reduction as may occur during intestinal dysfunction. Strenuous exercise can act as a stressor to induce small intestinal injury. Our previous studies suggest that this comprises the intestinal ability to produce citrulline from a glutamine-rich protein bolus. In this study we investigated the effects of different exercise intensities and hydration state on citrulline and iFABP levels following a post-exercise glutamine bolus in healthy young men.

Methods: Fifteen healthy young men (20–35 yrs, VO_2 max 56.9 ± 3.9 ml kg^{-1} min^{-1}) performed in a randomly assigned cross-over design, a rest (protocol 1) and four cycle ergometer protocols. The volunteers cycled submaximal at different percentages of their individual pre-assessed maximum workload (Wmax): 70% Wmax in hydrated (protocol 2) and dehydrated state (protocol 3), 50% Wmax (protocol 4) and intermittent 85/55% Wmax in blocks of 2 min (protocol 5). Immediately after 1 h exercise or rest, subjects were given a glutamine bolus with added alanine as an iso-caloric internal standard (7.5 g of each amino acid). Blood samples were collected before, during and after rest or exercise, up to 24 h post onset of the experiment. Amino acids and urea were analysed as metabolic markers, creatine phosphokinase and iFABP as markers of muscle and intestinal damage, respectively. Data were analysed using a multilevel mixed linear statistical model. p values were corrected for multiple testing.

Results: Citrulline levels already increased before glutamine supplementation during normal hydrated exercise, while this was not observed in the dehydrated and rest protocols. The low intensity exercise protocol (50% Wmax) showed the highest increase in citrulline levels both during exercise (43.83 μmol/L ± 2.63 ($p < 0.001$)) and after glutamine consumption (50.54 μmol/L ± 2.62) compared to the rest protocol (28.97 μmol/L ± 1.503 and 41.65 μmol/L ± 1.96, respectively, $p < 0.05$). However, following strenuous exercise at 70% Wmax in the dehydrated state, citrulline levels did not increase during exercise and less after the glutamine consumption when compared to the resting condition and hydrated protocols. In line with this, serum iFABP levels were the highest with the strenuous dehydrated protocol (1443.72 μmol/L ± 249.9, $p < 0.001$), followed by the high intensity exercise at 70% Wmax in the hydrated condition.

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Conclusions: Exercise induces an increase in plasma citrulline, irrespective of a glutamine bolus. The extent to which this occurs is dependent on exercise intensity and the hydration state of the subjects. The same holds true for both the post-exercise increase in citrulline levels following glutamine supplementation and serum iFABP levels. These data indicate that citrulline release during exercise and after an oral glutamine bolus might be dependent on the intestinal health state and therefore on intestinal functionality. Glutamine is known to play a major role in intestinal physiology and the maintenance of gut health and barrier function. Together, this suggests that in clinical practice, a glutamine bolus to increase citrulline levels after exercise might be preferable compared to supplementing citrulline itself. To our knowledge this is the first time that exercise workload-related effects on plasma citrulline are reported in relation to intestinal damage.

1. Introduction

Citrulline is a non-protein amino acid which is almost exclusively synthesized by the proximal small intestine [1]. Citrulline is formed from ornithine, which can originate from three different amino acid sources; glutamine (the main precursor in adult humans and mice), proline (infants) and arginine (second source in mice) [2]. In humans, depletion of glutamine, the main fuel for enterocytes, results in decreased levels of plasma citrulline [3,4]. The relevance of the non-protein amino acid citrulline in clinical nutrition is multifaceted, viz. it has a role as a biomarker for clinical complications, it is a nutritional amino acid metabolite from glutamine and, finally, it can also be provided as nutritional supplement.

Concerning citrulline as a biomarker for clinical complications, a recent meta-analysis on citrulline in relation to gut function indicates that citrulline is positively correlated with small bowel length and that low levels are indicative of an intestinal insufficiency being present [5]. Studies have shown that a decrease of citrulline formation is proportional to the loss of functional enterocyte metabolic capacity during intestinal diseases [1,6]. For example, Jianfeng et al. [6] have shown that in short bowel syndrome, lower levels of circulating citrulline reflect a reduction in the total enterocyte metabolic mass. Next to that it was reported that citrulline is moderately correlated with intestinal absorption and negatively correlated with the severity of the disease in intestinal enteropathies. Mechanistic background and causal relationships, however, are still largely unclear and need further investigation. Next to that more knowledge is needed on possible confounders that would influence citrulline levels via other pathways. A high and/(or) chronically elevated inflammatory status can for example deplete the nitric oxide donor arginine, resulting in a higher disappearance rate of citrulline, in turn leading to lower citrulline levels.

Luiking et al. [7] showed that sepsis patients display impaired intestinal function and lower citrulline levels. In this situation low citrulline levels might reflect high inflammation rates combined with an impaired intestinal function. This makes citrulline a complex, but interesting biomarker in chronic metabolic diseases. Concerning citrulline as nutritional amino acid metabolite, there are two main routes to consider: citrulline produced from amino acids already in the body and citrulline produced from amino acids provided by nutrition. Recently, we reported that in well-trained men, plasma citrulline levels following a glutamine-containing casein oral bolus were lower after strenuous glycogen-depleted and prolonged endurance exercise compared to the resting condition [8,9]. In addition, we previously showed that high intensity exercise consisting of 1 h cycling at 70% of the maximal workload (Wmax) increases serum intestinal fatty acid binding protein (iFABP) in recreationally trained men [10]. iFABP is a soluble cytosolic protein present within enterocytes which can be measured in blood after injury or inflammation of the small intestine [11,12]. This previous data suggest an exercise-induced decrease of intestinal metabolism and confirms earlier findings of exercise-induced intestinal permeability [13–15]. Based on these findings we hypothesized that different exercise intensities and hydration state are related to the effects of exercise on intestinal function as a consequence of splanchnic hypoperfusion. During physical exercise, splanchnic hypoperfusion is induced in healthy young volunteers [16]. Organ blood flow and oxygen delivery are less impaired by low intensity exercise compared to high intensity or strenuous exercise in dehydrated condition.

The present study was conducted to determine the relationship between citrulline levels and exercise-induced intestinal injury measured as iFABP serum levels. To this end, we performed human exercise studies with healthy volunteers on a bicycle ergometer and measured post-exercise citrulline and serum iFABP levels in volunteers performing various exercise intensities.

2. Subjects and methods

The current study was approved by the medical ethics committee of Wageningen University Research Centre (WUR), The Netherlands, ISRCTN code 13656034, and was conducted according the Declaration of Helsinki (Fortaleza, Brazil, 2013).

2.1. Subjects

Fifteen healthy recreational-active male cyclists were selected for this study. They were recruited by means of flyers distributed at the campus of Wageningen University and regional cycling clubs, by word of mouth and via social media. Exclusion criteria were smoking, records of allergies, gastro-intestinal and immune diseases, use of hard drugs and participation in other clinical studies. The subjects were instructed not to perform intense physical activity and not to consume alcohol, two days prior to the test days. To standardize food-intake during the test period, dinners were provided for the evenings before the test days and at test days themselves. In addition, subjects were requested to keep a diary with training, dietary and illness logs during the whole study period to validate physical activity and food intake and to evaluate subjects health state. Subjects characteristics are presented in Table 1.

Table 1
Characteristics and performance data of all subjects. Data is shown as mean with SD.

Age (yrs)	24.3 ± 2.4
BMI (kg/m^2)	22,5 ± 1,5
Weight (kg)	75.8 ± 6.7
Length (cm)	183.4 ± 3.8
VO_2max (ml/kg/min)	56.9 ± 3.9
Wmax (W)	335.1 ± 39.9

2.2. Preliminary testing

Based on a health questionnaire, subjects were selected for an incremental exercise test. The maximal workload (Wmax) was determined using an electronically braked cycle ergometer (Lode Excalibur, Groningen, The Netherlands). After a short warming-up, the subjects started cycling at 100 W with a pedal frequency of 90–100 rotations per minute (RPM). The power increased every minute with 20 W until the subject was not able to maintain the workload and felled back in pedal frequency to less than 70 RPM.

2.3. Study design

In this study with a cross-over design, all subjects started with protocol 1, the rest protocol without exercise (protocol 1), followed by 4 randomly assigned 1 h cycling protocols with different intensity and hydration status: 70% Wmax hydrated (protocol 2) and dehydrated (protocol 3), 50% Wmax (protocol 4) and intermittent 85/55% Wmax in blocks of 2 min (protocol 5). To standardize the hydration intervention, the 70% Wmax hydrated and dehydrated protocols were assigned as one block and performed consecutively. To attain a dehydrated condition, subjects were asked to restrict their intake of water to 0.5 L the day before the test day. The wash-out-period between the experimental protocols was one week.

Figure 1 shows a schematic overview of the study design. An overview of the experimental protocols is shown in Table 2.

2.4. Test schedule and blood sampling

In the morning, subjects arrived at the laboratory after an overnight fast. Their body weight was measured to control for weight loss and hydration status during exercise. To enable multiple venous blood collection, a cannula (Venflon Pro Safety, Becton Dickinson) was inserted in an antecubital vein. Before obtaining a baseline sample in fasted condition, subjects were asked to sit and relax for 10–15 min. After a light breakfast (2 wholemeal crackers with peanut butter and a cup of tea) subjects started, aside from the rest condition as first experimental protocol, with one of the assigned cycling protocols.

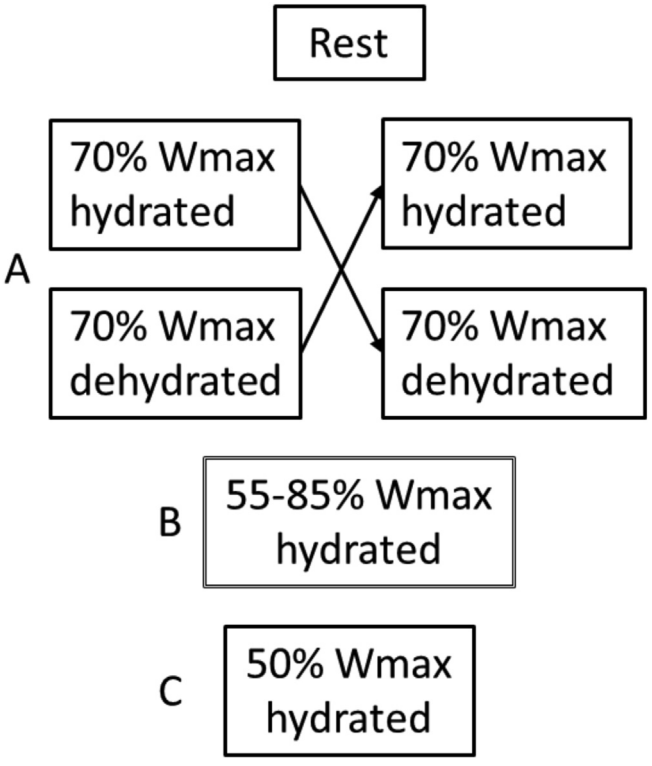

Fig. 1. Following 1 week of rest, each subject underwent 4 different exercise load interventions which were randomly assigned (blocks A, B, and C). The 70% Wmax interventions in hydrated and dehydrated condition (block A) were conducted in sequence. The wash-out-period between the protocols was 1 week.

Table 2
Experimental protocols with exercise intensity and hydration status.

Protocol	Exercise intensity and hydration status
1: Rest condition	No exercise
2: High intensity exercise	1 h cycling 70% Wmax, hydrated
3: Strenuous exercise	1 h cycling 70% Wmax, dehydrated
4: Low intensity exercise	1 h cycling 50% Wmax, hydrated
5: High intensity interval exercise	1 h cycling 55/85% Wmax, hydrated

Directly after 1 h of testing (rest or cycling) body weight was measured to determine post-exercise rehydration corresponding to 150% of body mass loss during exercise. During the remainder of the test day subjects consumed 200 mL of tap water every hour. After the body weight had been measured the volunteers ingested 125 mL tap water with a 7.5 g glutamine (Adamin G, Nutricia/SHS International Ltd) bolus to which 7.5 g of alanine (L-Alanine, Nutricia/SHS International Ltd, England) was added as an isocaloric internal standard. Blood samples were collected during (0.5 h), at the end (1 h), and at several time points after cycling (1.5 h, 2 h, 3 h, 6 h, 24 h) in EDTA plasma tubes as well as in serum separator tubes for analyses of amino acids and iFABP, and urea and cortisol, respectively. Subjects arrived the next morning again fasted at the laboratory for a blood collection of 24 h to analyse recovery. Figure 2 gives an overview of the blood sampling during an experimental protocol.

2.5. Plasma and serum analysis

Plasma levels of citrulline, glutamine and alanine were analysed by ultrafast liquid chromatography (UFLC) (Shimadzu) using a pre-column derivatization with o-phthaldialdehyde and fluorimetric detection [17].

For evaluation of exercise-induced small intestinal damage, serum iFABP levels were measured with a commercial human ELISA Test Kit (HK406, Hycult Biotech, Uden, The Netherlands) and analysed with a multi-detector microplate reader VICTOR™ ×3 (PerkinElmer) using Workout v2.5 software. According to standard procedures, cortisol levels were measured with Immulite 2000 XPI (Siemens), urea and creatine phosphokinase serum levels were measured with Cobas 6000 (Roche) (SHL-group (Etten-Leur, The Netherlands)).

2.6. Statistical analysis

Data was analysed using a multilevel mixed linear model. The model included terms that capture the random variation between the subjects, between the five experimental protocols per subject and within these experimental protocols. The analysis models the effects of overall protocol differences, differences between the time points within a protocol and the protocol by time interaction. The latter models the differences among the protocols of the respective time profiles. The analyses were performed using the statistical software GenStat (version 18) and R [18] packages lme4 [19] and nlme [20]. Prior to analysis, the data were log transformed to ensure compatibility with the assumption of a constant standard deviation of the observations. To focus on statistically significant

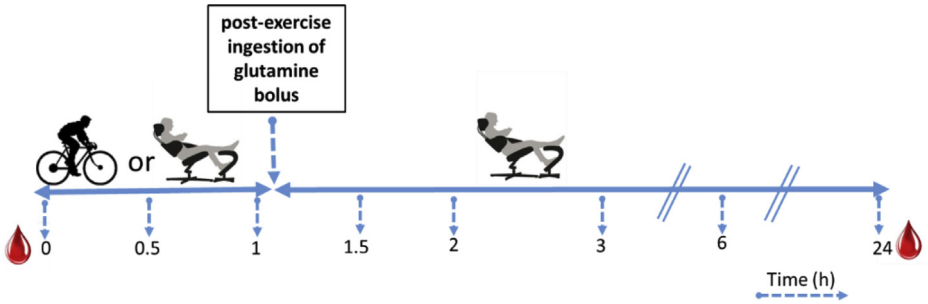

Fig. 2. Schematic overview of blood sampling during an experimental protocol.

effects, we corrected the raw p values for multiple testing [21]. Outcomes of statistical tests with $p < 0.05$ were considered statistically significant.

3. Results

Fourteen out of the fifteen volunteers completed all protocols. Due to personal and practical issues one volunteer did not complete the high intensity exercise protocol in dehydrated condition. The other data of this volunteer was included in the analysis.

The glutamine–alanine bolus was administered immediately after 1 h of exercise or rest. Therefore, the amino acid plasma levels of $0 < T \leq 1$ h represent pre-prandial levels during exercise, and plasma levels >T1h, represent post-prandial levels after exercise.

3.1. Pre-prandial plasma concentrations alanine, glutamine, citrulline and arginine during exercise

Plasma levels of glutamine, alanine, citrulline and arginine were determined at time points indicated in Fig. 3A–D. Already before administration of the glutamine–alanine bolus, levels of alanine and citrulline, but not of glutamine and arginine, changed significantly during some of the exercise protocols.

Alanine levels were almost doubled (from around 350 μmol/L to over 600 μmol/L ($p < 0.001$)) at 30 min of exercise (T0.5) in both hydrated and dehydrated 70% Wmax and the intermittent activity protocols and remained at the same increased level during the exercise. Increase was less (to around 500 μmol/L ($p < 0.01$)) in the low intensity exercise protocol (50% Wmax) and remained at background level during resting condition.

Plasma levels of citrulline were increased in all exercise protocols in hydrated condition (50%, 70% and 55/85% Wmax, ($p < 0.001$, Fig. 3C)). Citrulline levels gradually increased from around 35 μmol/L at start of the exercise to 38–40 μmol/L at 30 min and to 40–45 μmol/L at 60 min of the exercise. Citrulline levels remained at background level or even decreased during the rest and the dehydrated 70% Wmax protocols. Plasma arginine levels increased in all the experimental protocols during exercise. However, the increase in plasma citrulline and arginine levels were most pronounced with the low intensity exercise protocol of 50% Wmax, respectively 45 μmol/L ($p < 0.001$) and 130 μmol/L ($p < 0.05$, (Fig. 3C and D)). The smallest rise in arginine levels ($p < 0.01$) appeared in the high intensity protocol of 70% Wmax in dehydrated condition.

During exercise the pre-prandial levels of glutamine were not significantly different between the conditions ($p \geq 0.05$).

3.2. Exercise-induced post-prandial plasma concentrations alanine, glutamine, citrulline and arginine

After 1 h exercise or rest, (T1) all the subjects ingested a glutamine–alanine bolus containing 7.5 g of each amino acid. Plasma alanine (Fig. 3B) and glutamine (Fig. 3A) levels increased rapidly to peak concentrations 0.5 h post-prandially (T1.5) (1000–1250 μmol/L and 875–975 μmol/L respectively), except with the strenuous exercise at 70% Wmax in dehydrated condition. In the dehydrated condition the time to peak was delayed half an hour (T2) when compared to the protocols in hydrated condition, with lower plasma concentrations of alanine and glutamine (900 μmol/L and 875 μmol/L respectively ($p < 0.001$)). After the ingestion of the glutamine bolus, citrulline levels increased in all protocols and reached a maximum at 1 h post-exercise ((T2), Fig. 3C). This post-prandial increase was, as for the response during exercise, the highest for the mild 50% Wmax exercise protocol (50 μmol/L). The strenuous 70% Wmax exercise protocol in dehydrated condition showed the smallest increase.

Post-prandial plasma arginine (Fig. 3D) levels decreased in time from the post-exercise peak (T1) in all exercise protocols in hydrated condition. In dehydrated condition the plasma arginine concentrations still increased slowly post-exercise to peak 1 h post-prandial ((T2) 115 μmol/L).

3.3. Creatine phosphokinase levels

Creatine phosphokinase levels are used to evaluate exercise-induced muscle damage. CPK serum levels are highest in high intensity exercise in hydrated condition (70% Wmax (268 U/L) and 55/85% Wmax (221 U/L)) (Fig. 3F). The high intensity exercise protocol in dehydrated condition and the mild exercise protocol follow the levels in rest condition and appeared to be the lowest.

3.4. Urea levels

Urea is an end-product metabolite of amino acids breakdown. During the rest condition, pre-prandial urea serum levels remained constant (Fig. 3E), in contrast to a rise in all exercise protocols. After the intake of the glutamine bolus, post-prandial levels increased in all experimental protocols, with the biggest increase in the high intensity 70% Wmax exercise protocol in dehydrated condition ($p < 0.05$).

3.5. Serum iFABP and cortisol levels

To measure exercise-induced intestinal damage of the small intestine, serum levels of iFABP were evaluated [11,12]. Serum cortisol levels were analysed to measure the extent of stress.

The high intensity exercise protocols (70% Wmax (de)hydrated and 55/85% Wmax) resulted in an increase in serum iFABP levels directly from the start of exercise ((T0), (Fig. 4A)). But the 70% Wmax dehydrated protocol appeared to result in the biggest rise which was measured at the end of exercise ((T1), 1750 pg/mL ($p < 0.001$)).

Cortisol levels increased in the three high intensity exercise protocols ($p < 0.001$), but not in the low exercise protocol (Fig. 4B). In the

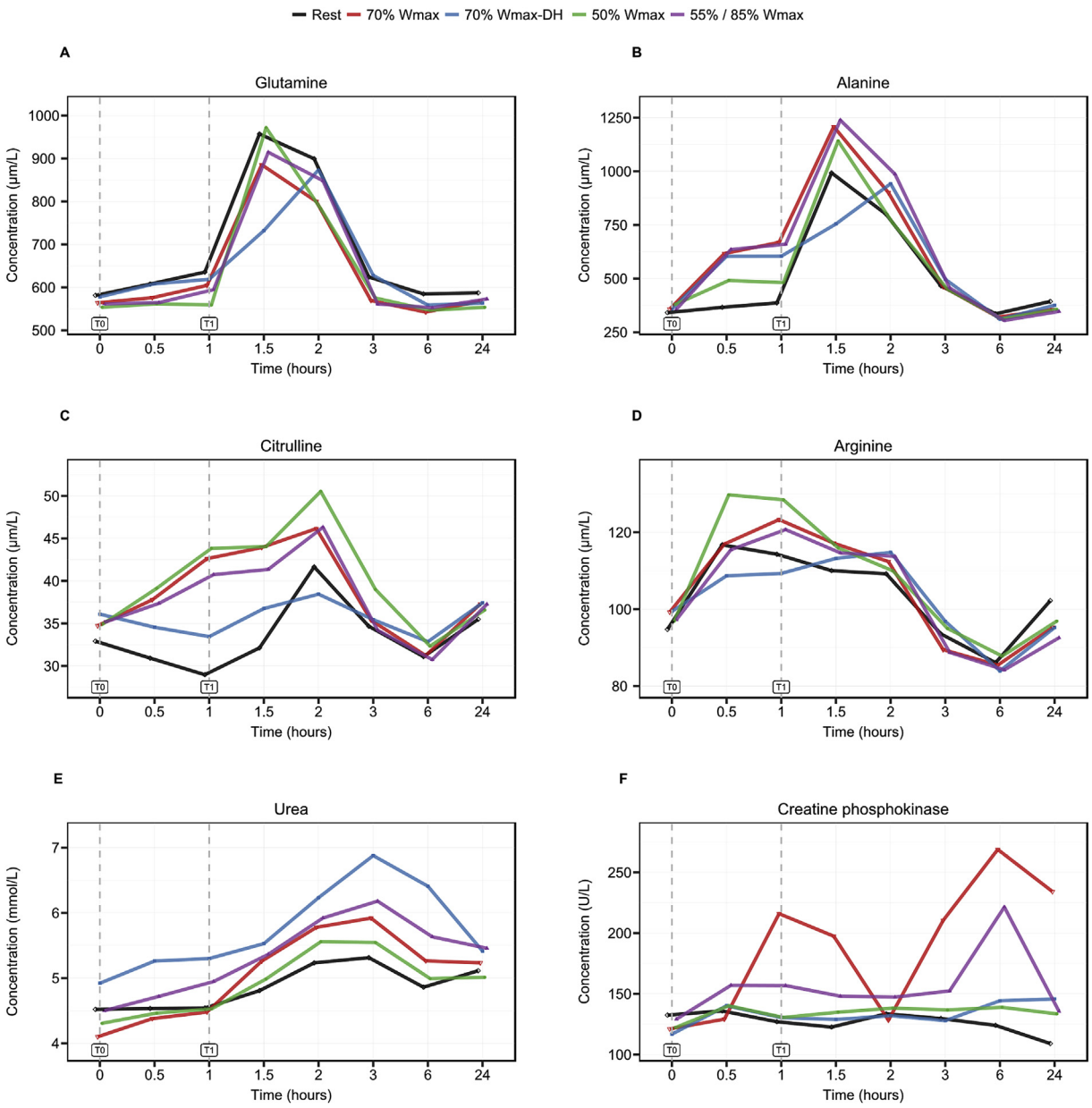

Fig. 3. Effects of the experimental protocols on plasma levels of glutamine (A), alanine (B), citrulline (C), arginine (D) serum levels of urea (E) and creatine phosphokinase (F). The black line represents the rest condition, the red and blue lines are showing the effects of 70% Wmax exercise in hydrated and dehydrated condition respectively and the green and purple lines are representing the exercise effects of 50% Wmax and intermittent 55/85% Wmax. Exercise or rest was performed between T0 and T1. The glutamine bolus was ingested at T1. The effects are shown as mean between the subjects.

low intensity 50% Wmax exercise as well as in rest condition cortisol serum concentration appeared to decrease similar to the iFABP levels.

From the end of exercise (T1), the iFABP levels in the high intensity protocols decreased to the iFABP level similar to the low intensity protocol 2 h post-exercise (T3). At 24 h post exercise, cortisol levels had returned to levels of the rest condition and the low intensity exercise.

4. Discussion

To our knowledge, this is the first study that reports the relationship between exercise intensity and the plasma levels of citrulline. Our data confirm and build on our previous observations in a strenuous exercise model under glycogen-depleted conditions in which we observed an almost complete reduction in post-prandial plasma citrulline [8]. In the present study, the relatively less stressful (reflected by low cortisol levels) exercise protocol (50% Wmax) caused high citrulline levels with corresponding low iFABP levels. The high intensity (reflected by high cortisol levels) protocols (70% and 55/85% Wmax) in hydrated condition, however, produced high levels of both citrulline and iFABP. Importantly, the most exhausting exercise protocol (70% Wmax in dehydrated condition) caused a reduction of the pre-prandial levels of citrulline together with a high iFABP level. The increase of iFABP, known as biomarker of intestinal function and damage [8,13,22], within the same time frame suggests that the reduction of citrulline is due to intestinal damage. The increase of citrulline combined with low iFABP levels is indicative of increased metabolic activity of the intestinal cells. Our conclusion is supported by the knowledge that blood citrulline levels result from one or a combination of the

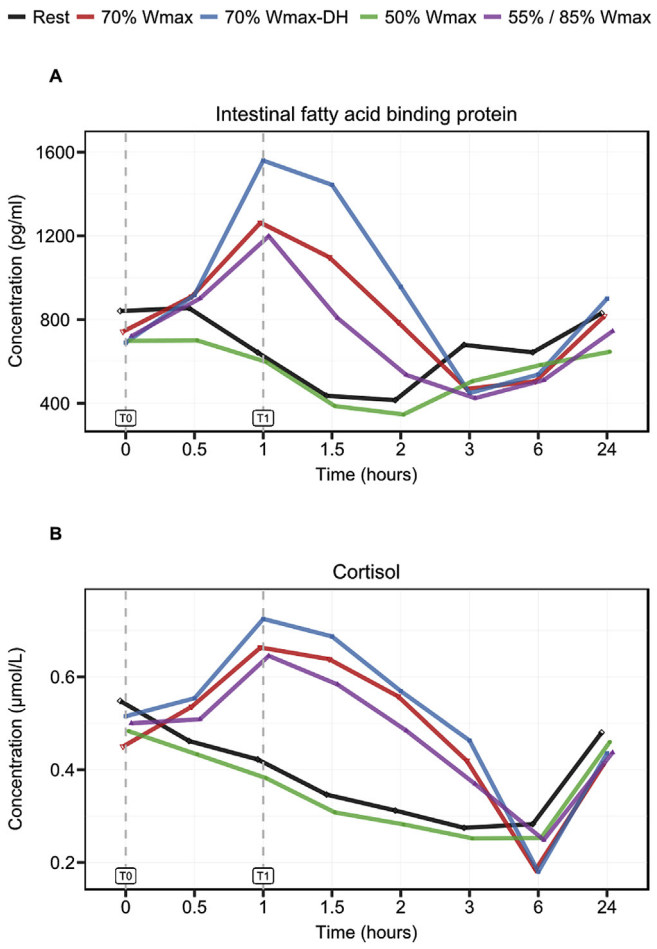

Fig. 4. Effects of the rest protocol (black line) and exercise protocols on serum levels of intestinal Fatty Acid Binding Protein (A) and cortisol (B). The red and blue lines are showing the effects of 70% Wmax exercise in hydrated and dehydrated condition respectively and the green and purple lines are representing the exercise effects of 50% Wmax and intermittent 55/85% Wmax. Exercise was performed between T0 and T1. At T1 the glutamine bolus was ingested. The effects are shown as mean between the subjects.

following processes: 1) release of already available citrulline from tissue, 2) altered disappearance of citrulline by e.g. formation of arginine out of citrulline or tissue uptake of citrulline, or 3) release of de novo synthesized citrulline. With regard to the release during exercise (ad 1), it is possible that citrulline could be released from muscles as a result of damage. Creatine phosphokinase (CPK) is an example of an exercise-induced damage marker for muscle tissue. As a consequence, when the increased levels of citrulline in our study would have resulted from increased muscle cell damage, we would have expected that citrulline followed the same pattern as that of CPK. However, this was not observed. The increase in plasma citrulline concentration was highest for the 50% Wmax protocol and the other two hydrated protocols, and lowest during the 70% Wmax dehydrated protocol and at rest. On the contrary, for CPK the increase was highest during the 70% Wmax and the interval protocols and lowest during rest condition, the 50% and 70% in dehydrated protocols. This difference indicates that increases in levels of citrulline and CPK do not have the same origin, i.e. that citrulline is likely to be gut-derived. In addition, if the changes in citrulline levels would have been the result of only intestinal damage one would expect corresponding patterns of changes for iFABP and citrulline levels in different protocols, which is also not the case. It is also unlikely that the appearance of citrulline is due to a decreased formation to arginine (ad 2). Because, e.g. during the 50% Wmax protocol both citrulline and arginine levels rise in the plasma.

Considering altered citrulline levels due to a change in citrulline synthesis (ad 3), two tissues have been described to be able to synthesize citrulline: the liver and the intestine [2]. Although under pathophysiological conditions such as argininosuccinic aciduria, hepatic synthesis can contribute to plasma citrulline levels, under normal physiological condition it is generally accepted that it is the intestinal tract that produces the major part of the citrulline present in plasma [23]. This is confirmed by a recent meta-analysis including 63 studies that provides evidence to conclude that citrulline present in human plasma is indeed mainly produced by enterocytes of the small intestine [5]. This matches with our finding that at low intensity exercise (50% Wmax) citrulline levels increased (i.e. pre-prandially) whereas levels of iFABP remained at basic level.

Our data are well in line with the concept that intense exercise causes redistribution of the blood flow to the muscles and therefore can induce a temporal situation of intestinal dysfunction, due to ischaemic hypoperfusion [24]. Our findings also indicate that both plasma citrulline and serum iFABP levels are indicative of the degree of exercise-induced intestinal stress, with the 70% Wmax protocol in a dehydrated condition inducing the most pronounced effects. Our iFABP data are also in line with findings of van Wijck et al. [13] who have shown increased iFABP levels during high intensity exercise (1 h cycling at 70% Wmax). In conclusion, at high exercise levels intestinal cells suffer from cellular stress (iFABP release) with concomitant low metabolic activity (citrulline release), and at low exercise levels cells are well capable of metabolic activity. Moreover, the pronounced effect of dehydration might have consequences for exercise protocols applied to patients on fluid restriction (e.g. heart failure patients).

Many chronic diseases, like COPD, cancer, heart failure and IBD are characterised by a reduction in muscle mass accompanied by a reduction in immune function and an increase in intestinal dysfunction [25–27]. The current opinion is that exercise should be included in the treatment protocols of these diseases to increase muscle mass. Not much is known yet about exercise-induced intestinal dysfunction in patients, but interestingly glutamine has been described to stimulate enterocyte proliferation, to attenuate tight junction dysfunction and to improve gut barrier function [28–31]. In addition, glutamine-induced prevention of intestinal-barrier dysfunction has been described in exercise studies [32], to be beneficial in clinical settings [33–35] and to improve immune function [36,37]. In particular, Castell and Newsholme [38] have reported a glutamine-induced reduction in post-running infection rates in marathon runners. These data suggest that glutamine also has a positive effect on recovery of exercise-induced gut-barrier dysfunction. Suzuki et al. [39] have shown that citrulline supplementation enhances cycling time trial performance by modulating muscle fatigue and preventing exercise overload. So, apart from increasing exercise capacity and reducing fatigue [40] increasing citrulline levels via glutamine bolus could also protect against intestinal damage in high intensity protocols. Our findings show that at the end of exercise (T1) iFABP levels decreased rapidly. From our experimental set-up we cannot conclude whether the decrease of iFABP reflects a normal response in time after exercise or is due to either glutamine administration, citrulline formation, or both.

Although alanine was not administered prior to exercise, levels of alanine increased during exercise. This increase probably resulted from enhanced muscle activity of alanine aminotransferase which converts glutamate to alanine in the glucose–alanine cycle. Post-prandial, post-exercise levels of alanine and glutamine show a comparable increase with peak levels already 30 min after glutamine and alanine supplementation (T1.5) in all hydrated conditions. However, in the dehydrated condition peak levels of both

amino acids were lower and appeared 30 min later, at T2, indicating that dehydration delays as well as impairs intestinal uptake of glutamine and alanine and thus not only affects metabolic function of the intestine but also nutrient absorption [41,42].

5. Conclusion

To our knowledge, this is the first time that in one and the same experimental design effects of different exercise protocols on plasma citrulline levels were evaluated in relation to intestinal metabolic capacity and intestinal function. We show that citrulline and iFABP levels both depend on exercise-intensity and that high intensity exercise in dehydrated condition prevents citrulline formation, possibly because of increased intestinal damage. Citrulline, combined with other intestinal markers such as iFABP, can therefore be considered a valuable marker of intestinal functionality in exercise protocols. Our findings contribute to a better understanding of intestinal physiology and adaptations taking place during (strenuous) exercise. Furthermore, the combination of biomarkers and test protocols can be used to evaluate potential nutritional interventions directed at impaired intestinal functionality resulting from stress or disease.

Authors contribution

Conception and design of the experiments: SK, MM, MV, RW, RP, KvN. Study conduction: SK, MM, KvN, LJD. Laboratory analysis: MV, GW, KM. Data and statistical analysis: ES and MT. Interpretation of data: SK, KvN, RP, MT, ES, MV, RW, MM, JG. Manuscript preparation: SK, KvN, RP, RW, JG. All authors contributed to the revision and editing of the manuscript. Approval final version: SKa, KvN, RP.

Conflict of interest

The authors declare that they have no conflict of interest.

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Data and R code for visualizations used in this paper are available from https://github.com/uashogeschoolutrecht/citrulliner. The data and R code in this repository can be installed as an R package: 'citrulliner'. To install the package from github use the 'devtools' package with the command 'devtools::install_github("uashogeschoolutrecht/citrulliner")'.

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