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Effects of chocolate milk consumption on muscle recovery following rowing exercise: A randomised crossover study

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ABSTRACT

Introduction: For athletes, an excessive increase in blood urea nitrogen (BUN) after multiple endurance exercises indicates muscle glycogen depletion that induces a diminution in performance. Our study aimed to examine the efficacy of chocolate milk (CM) compared with carbohydrate-protein replacement drink (CHOPRO) in suppressing the increase in BUN level following multiple rowing exercises among national male rowing athletes aged 18–23 years. **Methods:** Seven male athletes from the Rowing National Training Centre, Pengalengan, West Java, participated in this single-blind, randomised crossover study. They received CM or CHOPRO during four hours of recovery between two endurance exercises. Before (pre) and after (post) multiple exercises, a venous blood sample was collected to measure the increase in BUN level. The effects of each beverage on BUN level were compared using an independent *t*-test. **Results:** The increase in pre-post BUN level was significantly lower for CM trial compared to CHOPRO trial (164.0±61.3 mmol/L vs 293.5±88.3 mmol/L, p=0.012). **Conclusion:** It was observed that CM reduced rate of increase in BUN level following multiple rowing exercises. Thus, CM can be useful for athletes during intense training regimen with multiple exercise sessions. Future studies should investigate the effect of CM in various types of sports, using convenient, non-invasive, and real-time measurement.

Keywords: athletes, glycogen, milk, muscles, water sports

INTRODUCTION

Rowing is a high-energy demand exercise that involves both aerobic and anaerobic metabolism (Winkert *et al.*, 2022). About 77% of the total energy expenditure yielded during 2000-m rowing comes from the aerobic system, while the remaining 33% comes from anaerobic catabolism (Kim & Kim, 2020). As aerobic and anaerobic system substrates are mainly derived from carbohydrate (CHO), this type of exercise induces muscle glycogen depletion and leads to muscle fatigue (Hargreaves & Spriet, 2020). For elite male rowers, even low-intensity rowing training results in great energy expenditure; and muscle glycogen is likely to be depleted due to

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huge CHO utilisation (Winkert *et al.*, 2022). Therefore, rowers, particularly those who train in consecutive exercise sessions with 4–6 hours recovery periods, need to consume adequate post-exercise intake to promote muscle glycogen recovery (MGR) (Kim & Kim, 2020).

Blood urea nitrogen (BUN) has been shown as an indirect biomarker of muscle fatigue (Wan *et al.*, 2017), as a result of muscle glycogen depletion during prolonged strenuous exercise (Hargreaves & Spriet, 2020). As muscle glycogen is depleted, adenosine triphosphate (ATP) production and supply will be inadequate to accomplish ATP consumption for skeletal muscle contraction (Hargreaves & Spriet, 2020), which leads to peripheral fatigue (Lee *et al.*, 2017). To retain adenosine triphosphate (ATP)/adenosine diphosphate (ADP) ratio, two ADP molecules will be transformed into one ATP and one AMP molecule. AMP is afterwards degenerated into inosine monophosphate (IMP) and ammonia, while ammonia is then transformed into urea nitrogen, thus increasing BUN level (Wan *et al.*, 2017). In addition, the increase in BUN level may also occur when muscle protein, the last energy reserve, is catabolised for energy supply (Howarth *et al.*, 2010) due to depleted muscle glycogen and fat reserves during an intense and long-lasting exercise (Hearris *et al.*, 2018).

Since the post-exercise period is a critical nutrient timing for recovery, a proper nutritional recovery aid is needed immediately after an intense endurance exercise to stimulate MGR (Murray & Rosenbloom, 2018). Studies have found that ingesting carbohydrate and protein (CHOPRO) recovery drinks will result in a higher rate of MGR compared to CHO alone (Alghannam *et al.*, 2018; Nielsen, Lambert & Jeppesen, 2020). Co-ingestion of CHO with protein may directly increase the rate of MGR, independent of protein's ability as an energy source (Burke, Van Loon & Hawley, 2017). A combination of an insulin-tropic protein and/or amino acid may enhance post-meal insulin release that encourages skeletal muscle glucose absorption and glycogen synthase activity (Kleinert *et al.*, 2011), thus accelerating MGR rate.

As an alternative to CHOPRO, there is a growing interest to use chocolate milk (CM) to promote the improvement of MGR during a short-term recovery period, as it contains CHO and protein with an ideal ratio (4:1) (Pritchett & Pritchett, 2013; Molaeikhaletabadi *et al.*, 2022). Previous studies have compared CM and CHO only (Born *et al.*, 2019), as well as CM vs CHO only vs CHO + electrolytes (Ferguson-Stegall *et al.*, 2011). These studies found that CM is either similar or superior compared to the other supplements in promoting MGR and/or performance.

However, since previous studies mainly focused on comparing CM and CHO only supplements among aerobic-dominant exercise athletes (cycling and running) (Amiri *et al.*, 2019), the data cannot be directly adjusted for rowing, as it occupies not only a high-demand aerobic and anaerobic capacity, but also strength and power that lead to injury and unexplained underperformance syndrome (Kim & Kim, 2020). Due to high-risk muscle breakdown, CHO only supplement is inadequate to promote a positive protein balance for muscle repair (Nielsen *et al.*, 2020). The three components of recovery, including refuel (CHO), rehydrate (fluid), and repair (protein), need to be administered (Kim & Kim, 2020). Hence, the effectiveness of CM compared to CHOPRO supplement on MGR needs to be investigated.

During preparation for international competitions, highly trained Indonesian rowing athletes perform multiple exercises a day with short recovery periods. To avoid muscle fatigue during subsequent training sessions, nutrition recovery is required to promote MGR. Therefore, our study aimed to compare the efficacy of recovery drinks (CM vs CHOPRO) in stimulating MGR by using BUN level as a biomarker of metabolic imbalance. Since milk-based drinks give similar results on MGR when compared to CHO replacement drinks (if they contain an adequate amount of CHO) (Loureiro *et al.*, 2021), we hypothesised that our iso-carbohydrate and iso-protein drinks will have similar effects on BUN levels.

MATERIALS AND METHODS

Subject

Seven healthy, non-smoking, highly-trained elite male rowing athletes (defined by a minimum of two years involvement), aged 18–23 years old from the Pengalengan National Training Centre, Indonesia, volunteered to participate in this study. The participants' physical characteristics are listed in Table 1. Using G-Power version 3.1.9.7 (Informer Technologies, Inc., USA) (Faul *et al.*, 2007), the sample size was determined in which a minimum of seven samples yielded a 95% confidence interval (CI) and an effect size of d=0.5, a=0.05, and b=0.85.

Recovery drinks

CM was a readily drink-box package, while CHOPRO was a combination of five sachets of CHO replacement drink and two scoops of formulated protein hydrolysate. CM product consisted of fresh cow's milk, water, sugar, skimmed milk, fat milk, milk identical flavour and stabiliser, whereas CHOPRO consisted of sugar, dextrose, sucralose, flavouring, casein hydrolysate, soya lecithin and citric acid. As a single-blind control, all beverages were poured into unmarked, opaque bottles. In addition, we poured chocolate paste and chocolate flavour into the CHOPRO beverages to create a similar colour and taste perception. Both drinks contained similar amounts of CHO (iso-carbohydrate) and protein (iso-protein), whereas calories was 35% in CM and potassium was almost six times higher in CM. The comparisons of

Table 1. Physical characteristics of subjects (*n*=7)

Subject	*Age (year)*	*Mass (kg)*	*Height (cm)*	*BMI (kg/m²)*	*Body Fat Percentage (%)*	*VO_2 Max ($mL.kg^{-1}.min^{-1}$)*
1	21	72.8	175.5	23.6	17.5	40.3
2	22	69.2	176.5	22.2	18.7	54.4
3	23	71.6	179.2	22.3	15.8	53.1
4	19	72.0	179.2	22.4	15.6	54.3
5	18	72.4	184.6	21.2	18.9	54.3
6	19	70.8	181.7	21.4	15.1	54.6
7	18	83.2	187.8	23.6	19.4	46.2
Mean	20	73.1	180.6	22.4	17.3	51.0
Median	19	72.0	179.2	22.3	17.5	54.3
Standard deviation	2.0	4.6	4.4	0.9	1.7	4.5
Minimum	18	69.2	175.5	21.3	15.1	40.3
Maximum	23	83.2	187.8	23.6	19.4	54.6

BMI: body mass index; VO_2 Max: maximal oxygen uptake the nutrient content of both drinks are listed in Table 2.

Table 2. Comparison of nutrient content of recovery drinks

Content/500 ml	*CM*	*CHOPRO*
Energy (kcal)	450	333
Carbohydrate (g)†	70	70
Protein (g)	12.5	12.5
Fat (g)	12.5	0.3
Sodium (mg)	225	468
Potassium (mg)	1050.0	187.2

CM: chocolate milk; CHOPRO: carbohydrate-protein recovery drink
†Carbohydrate amount was based on 1 g/kg body mass as recommended today (Kerksick *et al.*, 2017)

Study design

This study was a randomised, single-blind experimental study with a crossover design to investigate the effect of iso-carbohydrate and iso-protein post-recovery aid (333-450 kcal) on muscle MGR, marked by BUN level, following muscle glycogen depletion-inducing exercises. Every subject received both beverages (CM or CHOPRO) on two different experimental days separated by a one-week wash-out period. Four subjects received CM on the first experimental day and then received CHOPRO on the second day, and vice versa for the remaining subjects. BUN level was measured four times: before exercise I (pre), after exercise I (before drinking), before exercise II (after drinking), and after exercise II (post).

Experimental procedures

The subjects were asked to refrain from any strenuous activity a day before the experimental day. All subjects discontinued other supplementations three days before and on the experimental day. After fasting for eight hours, the subjects arrived in the morning at the training venue. Height was measured using a wall-mounted stadiometer (OneMed Medicom stature meter, YF.05.05.V.A.1022, Indonesia) to the nearest 0.1 cm, while body mass (to the nearest 0.1 kg) and body fat percentage were measured using the Karada scan body composition monitor HBF-375 (OMRON Healthcare Co., Kyoto, Japan); then BMI was calculated as body mass (kg)/square of height (m). VO_2 max (maximum oxygen uptake during maximal workload) was measured by the researcher and coaches using the rowing ergometer test (Concept II, model C air braked rowing ergometer, Nottingham, UK). The subjects were asked to complete a rowing ergometer test, then total work (watt) was recorded. VO_2 max was estimated using the formula: VO_2 max=[350+(watt x 12)]/body mass (kg).

Exercise I

All subjects performed the first exercise (ED Boat 5 x 15 minutes) in the morning, where they rowed a boat across the waters (Figure 1). Exercise I was performed as a glycogen depletion trial. During the exercise, the subjects were allowed to drink mineral water as much as they needed. Blood samples were collected before and upon completion of exercise I to measure BUN levels. About 3–5 mL blood sample of plasma heparin was taken through the left and right arms. BUN measurement was conducted using the Cobas C111 by Urease GLDH kinetic UV method. Blood samples were poured into a tube to be centrifuged. All procedures of blood sampling were done professionally by the team from P Laboratory. Body mass changes were examined by body mass measurements prior to and upon completion of Exercise I. Extreme body mass changes showed a state of dehydration; subjects had to replace body water as much as their water loss. In addition, body mass changes also showed the exercise intensity.

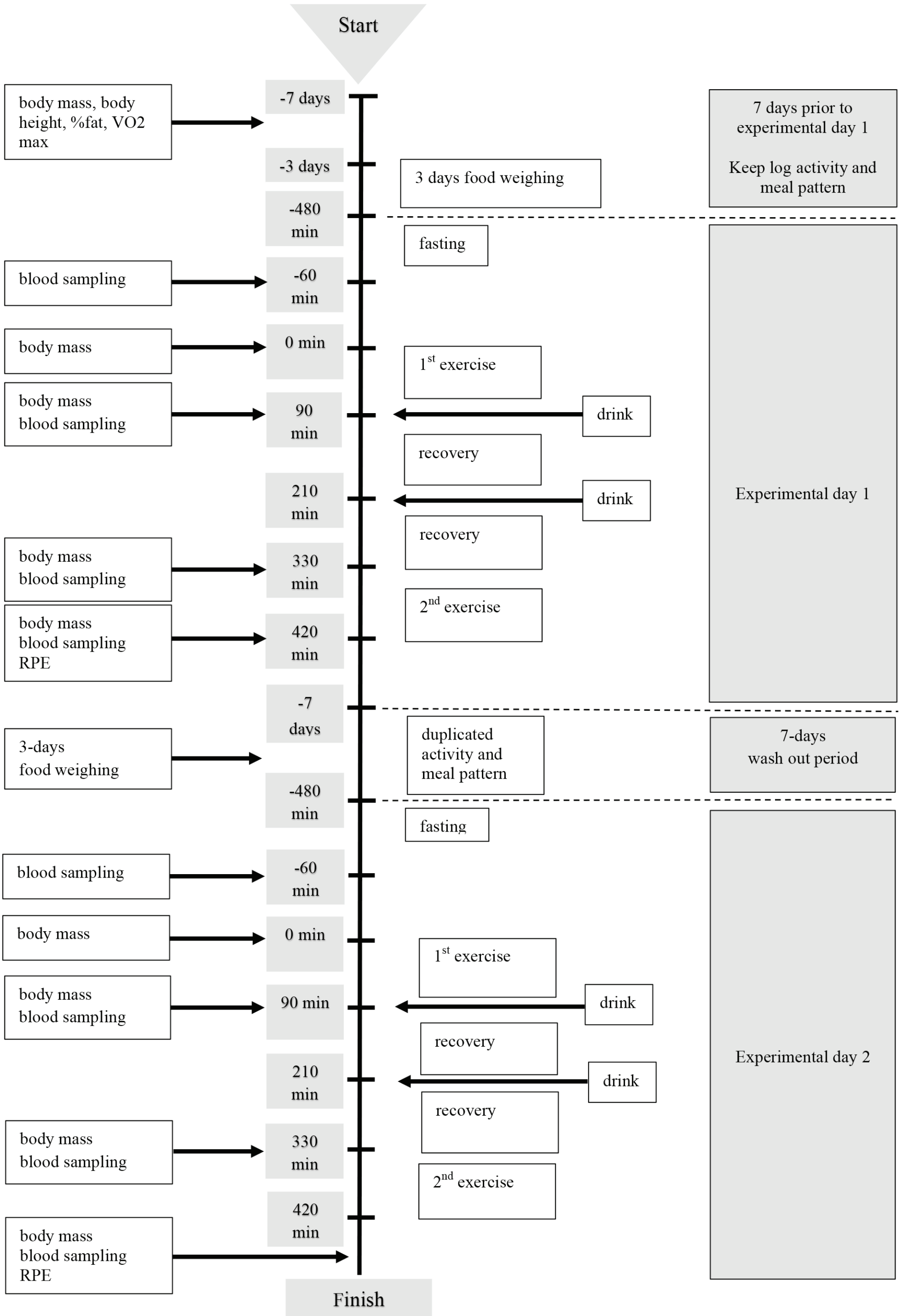

Figure 1. Experimental procedures
*min= minutes
RPE=Rating of Perceived Exertion (Borg, 1982)

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Recovery period
After performing the first exercise, the subjects started their recovery period (four hours) to replenish depleted muscle glycogen, rebuild muscle fibres, and replace water and electrolytes. The subjects received 500 mL of recovery drink (CM or CHOPRO) immediately after the exercise and again two hours into the recovery period, respectively (Figure 1). Provision of recovery drinks immediately after a workout is one of the rules of nutrient timing to hasten the rate of MGR (Kerksick *et al.*, 2017). During the recovery period, no other foods or strenuous activities were allowed. However, subjects were allowed to drink mineral water and engage in simple activities (listening to music, reading, walking around, etc.). All subjects were asked to stay in the rowing venue until all procedures had been completed.

Exercise II
By the end of the recovery period, the subjects were ready for the second exercise (ED Ergo 4 x 20 minutes) using a rowing ergometer (Figure 1). Similar to the first exercise, blood samples were collected, and body mass was determined prior to and upon completion of the exercise. During the exercise, subjects were allowed to drink mineral water as much as they needed. To control for a similar exercise intensity between the two trials, Rating of Perceived Exertion (RPE) was obtained from all the subjects immediately after the exercise. RPE aimed to examine the workout intensity using the Borg Scale (scoring was from 6 to 20, where 6 was very, very light and 20 was very hard) (Borg, 1982).

Wash-out period
After completing the first experimental day, the subjects went into a one-week wash-out period where they duplicated a similar activity and meal pattern prior to the second experimental day (Figure 1). During this period, no trial was given. In order to control for a similar dietary intake during the two experimental periods, the subjects duplicated a similar meal pattern for one week prior to each experimental day. Three days prior to each trial, we conducted three days of food weighing to ensure a similar dietary intake.

Ethical approval and permission
The Committee of Experts for Research and Research Ethics, School of Public Health, Universitas Indonesia approved this study, including the protocols and informed consent forms for athletes (No.186/H2.F10/PPM.00.02/2015). All subjects provided written, informed consent prior to the study.

Data analysis
IBM SPSS Statistic for Windows Version 22.0 (IBM Corp, Armonk, NY, USA) was employed for data analysis. All data from the two trials were compared using independent *t*-test (Mann-Whitney U test for data that were not normally distributed). Statistical significance was set at $p<0.05$. To determine the intervention effect on muscle recovery, Cohen's *d* was applied.

RESULTS

Dietary intake, body mass change, and RPE score
Table 3 showed that there were no significant differences in any of the supporting variables, including dietary intake, body mass change, and RPE score. These findings indicated that both experimental periods took place in similar conditions. Moreover, all subjects who participated in our study were highly trained athletes with relatively stable performances. Hence, their mood changes were less likely to vary, as they were not beginners.

Table 3. Comparison of dietary intake, body mass change, RPE score, and BUN level between CM and CHOPRO trials

Variable	*CM*	*CHOPRO*	*p-value*
Dietary intake			
Calories (kcal)	3857±555	3846±485	0.970
Carbohydrate (g)	544.0±100.7	563.3±88.7	0.738
Protein (g)	145.6±12.1	137.2±22.0	0.062
Fat (g)	121.8±18.3	115.1±14.7	0.676
Changes of body mass			
Exercise I (kg)	- 0.9±0.4	- 0.9±0.6	0.932
Exercise II (kg)	- 1.0±0.4	- 1.0±0.6	0.779
RPE score	15.7±1.0	15.3±1.0	0.416
BUN level			
Before exercise I (mmol/L) (a)	10.9±1.5	11.0±2.6	0.569
After exercise I (mmol/L) (b)	11.4±1.7	11.3±2.5	-
Before exercise II (mmol/L) (c)	11.0±1.7	12.1±2.7	-
After exercise2 (mmol/L) (d)	14.1±2.2	16.8±2.7	-
Δ (b – a)/effect of exercise I	0.4±0.5	0.4±0.9	0.706
Δ (c – b)/effect of drinks	-0.4±0.7	0.8±0.3	0.002*
Δ (d – c)/effect of drinks & exercise II	3.1±1.3	4.6±1.5	0.031*
Δ (d – a)/Pre – Post	3.3±1.2	5.8±1.7	0.012*

CM: chocolate milk; CHOPRO: carbohydrate-protein recovery drink; RPE: rating of perceived exertion; BUN: blood urea nitrogen
*Significant at 5% level

BUN level

Before exercise I (pre)

There were no significant differences in BUN level between the trials before exercise I, suggesting that all subjects started exercise I with the same glycogen reserves (Figure 2).

After exercise I (before drinking)

Exercise I caused a rise in BUN level in

Figure 2. BUN levels (mmol/L) at four points of measurement

all subjects (0.4±0.5 mmol/L increase for CM and 0.4±0.9 mmol/L for CHOPRO, respectively) (Table 3). There were no significant differences in this increase between the trials, suggesting that both trials were exercise-induced ammonia increment that was later converted into BUN (Lin *et al*., 2011).

Before exercise II (after drinking)

At the end of the recovery period (before exercise II), it appeared that CM caused a decrease in BUN level (-0.4±0.7 mmol/L), while CHOPRO caused an increase in BUN level (0.8±0.3 mmol/L) (Table 3). An increase in BUN level following CHOPRO trial indicated a higher level of ATP utilisation to compensate for the need for energy metabolism caused by a higher level of muscle glycogen depletion (Hargreaves & Spriet, 2020).

After exercise II (post)

All subjects performed exercise II to re-examine the effect of the recovery drinks on BUN level. Again, after the exercise, BUN levels increased in all subjects (Figure 2). However, the increase in BUN level was significantly lower in the CM trial (3.1±1.3 mmol/L) than in the CHOPRO trial (4.6±1.5 mmol/L) (Table 3). This showed that although muscle glycogen was re-depleted after exercise II, the increase in BUN level was still much lower after the CM trial.

Effect of recovery drink on BUN level

Overall, the effect of the recovery drinks on BUN level was determined by the increase in pre-post BUN level (before exercise I and after exercise II) (Table 3). Analysis showed that the increase in pre-post BUN level was significantly lower in the CM trial (3.3±1.2 mmol/L) than in the CHOPRO trial (5.8±1.7 mmol/L). It was found that at the end of the recovery period (after drinking/ before exercise II), CM decreased, while CHOPRO increased BUN levels. Also, after exercise II, the increase in BUN level was significantly lower after the CM trial than the CHOPRO trial.

DISCUSSION

The purpose of the present study was to examine the effect of post-workout drinks (CM vs CHOPRO) on the biomarker of MGR (BUN level) after subsequent rowing exercises with a short recovery period. In detail, our main findings were as follows: (1) Before the trial was given, there were no discernible variations in mean BUN levels between the two drinks. However, mean BUN level at the initial stage of both trials (10.9±3.6 mmol/L) was higher than the normal range (1.8–7.2 mmol/L) (Yu, 2011). A higher degree of BUN level may result from an intense rowing exercise in the previous week since BUN recovers slowly after a week of intense-subsequent training (Yun, 2007); (2) After the trials were completed, the total elevation of BUN level was significantly higher in the CHOPRO trial compared to the CM trial. Various physiological indicators other than the rate of MGR, as well as the nutrient substances in both drinks, may influence the change in BUN level.

Besides as an indirect biomarker of muscle glycogen depletion (Hargreaves & Spriet, 2020), BUN level may also be influenced by other conditions such as kidney function, muscle breakdown, and acute kidney injury. When it comes to athletes, conditions such as shock, stress, extreme sunburn or dehydration that decreases blood supply to the kidney may cause BUN to be elevated (Fischbach & Dunning, 2014). Moreover, a high protein diet may also contribute to high BUN levels (Ko *et al*., 2020). Since our athletes had no kidney disease and were controlled for a similar exercise intensity (muscle breakdown rate), dietary protein intake, and fluid consumption between both trials, thus the different BUN levels following sports beverages consumption may have come from the discrepancy in muscle glycogen reserves influenced by recovery drink intake.

This finding was surprising because it was hypothesised that the increase in BUN levels would be similar between the two drinks since they were iso-carbohydrate and iso-protein. However, we need to consider the other nutrients contained in CM since apart from CHO and protein contents, milk-based beverages contain several other nutrients that may help muscle recovery (Loureiro *et al.*, 2021).

Our findings were consistent with previous studies that found a higher MGR after a CM trial compared with a CHOPRO trial (Karp *et al.*, 2006) and a placebo trial (Molaeikhaletabadi *et al.*, 2022). In contrast to our study that measured the increase in BUN level as an indicator, Karp *et al.* (2006) measured performances as an indirect indicator of MGR rate among men cyclists. Karp *et al.* (2006) proved that total work was 57% greater and time to exhaustion was 49% longer after CM trial than CHOPRO drink trial. Meanwhile, Molaeikhaletabadi *et al.*, (2022) measured the delayed onset of muscle soreness (DOMS) along with aerobic and anaerobic performances as an indirect indicator of MGR among female badminton players. They found that aerobic and anaerobic capacities were significantly higher, whereas DOMS was significantly lower after a low-fat CM trial (p<0.05). Since muscle biopsy is invasive and may impair athlete's performance, the need for novel non-invasive techniques to measure MGR rate is inevitable (Greene *et al.*, 2017). Karp *et al.* (2006) and Molaeikhaletabadi *et al.* (2022) suggested that performances and DOMS are closely related to MGR.

In Karp *et al.* (2006)'s study, both drinks had similar amounts of calories, CHO, protein and fat. The different rates in muscle glycogen recovery were caused by the different types of sugar. The CM used by Karp *et al.* (2006) contained disaccharide sucrose that absorbs as easily as glucose (Burke *et al.*, 2017). This explains why during four hours of recovery period, MGR was faster after the CM trial than the CHO-protein replacement drink trial.

In our study, both drinks had the same amount of CHO and protein; however, the number of calories in CM was higher because it contained fat (12.5 g/500 mL), similar to Molaeikhaletabadi *et al.* (2022) who used fat, but in a lower content (7.5 g/500mL). It has been demonstrated that the fat in CM raises free fatty acid circulation in the bloodstream and thereby inhibits muscle glycogen depletion, acting as a fuel that spares glycogen during exercise, as it has a glycogen-sparing effect (Muscella *et al.*, 2020). This explains the higher rate of MGR following CM consumption.

Another explanation is the potassium content that was almost six times higher in CM (1050.0 mg vs 187.2 mg), which may have promoted a higher MGR and thus led to a lower increase in BUN level. Theoretically, potassium stimulates the uptake of glucose by cells, thereby increasing muscle glycogen replenishment. However, potassium requirement after an intense and prolonged endurance exercise will increase due to excessive potassium losses through urine, and to a small degree, through faeces and sweat (DiNuzzo *et al.*, 2014). Thus, an adequate potassium intake is needed immediately after exercise, mostly because of a high rate of potassium uptake by muscle to replenish muscle glycogen (DiNuzzo *et al.*, 2014). It was possible that due to higher potassium content, CM led to a very rapid MGR rate and suppressed the increase in BUN level after four hours of recovery rather than CHOPRO.

A limitation in our study was that there was no control group that received a placebo (plain water) after the exercise since giving plain water after a heavy workout may deteriorate an athlete's recovery and lead to exhaustion. In addition, our study did not examine other indicators of MGR that were used in previous studies, such as post-exercise glucose and insulin level, as well as athlete's performances, which may strengthen our interpretation of BUN level. Thus, the closure that CM was superior compared to CHOPRO in promoting MGR cannot be established. However, in the absence of kidney problems and the same rate of excessive protein intake, exercise intensity and fluid consumption, different increments in BUN levels between the two drinks may result from different muscle glycogen reserves after drinking.

CONCLUSION

We found that the increase in BUN level was lower after the CM trial than the CHOPRO replacement drink trial. Our findings suggest that CM induces a lower increase in BUN level following multiple rowing exercises. Hence, CM may be advantageous for athletes during an intense training regimen with multiple exercise sessions.

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Authors' contributions

Fitriani A, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript, and reviewed the manuscript; Setiarini A, advised on the data analysis and interpretation, and reviewed the manuscript; Ahmad EK, advised on data analysis and interpretation, and reviewed the manuscript; Desiani RP, prepared the draft of methods and results; Fitria, assisted in data collection and data analysis.

Conflict of interest

The authors declare no conflict of interest. This work was supported by DI, Jakarta, Indonesia.

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