INTRACELLULAR AND EXTRACELLULAR WATER CONTENT WITH CARBOHYDRATE-LOADING DIETS

CONTEÚDO DE ÁGUA INTRACELULAR E EXTRACELULAR COM DIETAS DE CARREGAMENTO DE CARBOIDRATOS

CONTENIDO DE AGUA INTRACELULAR Y EXTRACELULAR CON DIETAS DE CARGA DE CARBOHIDRATOS

ORIGINAL ARTICLE
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ABSTRACT

Introduction: In prolonged physical activities, water replacement and muscle glycogen content are limiting factors in marathon runners. Carbohydrate-loading (CHO) in the days prior to endurance competition is a commonly employed method to optimise muscle glycogen stores and optimise exercise performance. Since each gram of muscle glycogen binds ~2.7-4 grams of water, water retention may occur during carbohydrate-loading diets. Objective: To evaluate differences between CHO loading strategies (Bergström and Sherman) on intracellular (ICW) and extracellular (ECW) water content. Methods: Twenty-three runners were randomly allocated to two interventions (Bergström and Sherman) in a crossover design. Participants underwent a baseline evaluation before 3 days of glycogen depletion followed by 3 days of carbohydrate loading with a washout of 30 days consisting of normal diet and training. Multifrequency bioimpedance (BIS) was used to assess ICW and ECW at Baseline, Post-depletion and Post-CHO to determine any differences between Bergström and Sherman protocols. Blood samples were also obtained to assess potassium levels. Associations between ICW and ECW and muscle glycogen were determined. Results: There were no differences in ICW or ECW content between the two interventions at any moment. There was an effect of time for ICW, with an increase from Post-depletion to Post-CHO without any difference between interventions. Plasma potassium decreased from Baseline to Post-depletion in both conditions. There was no difference in muscle glycogen content between interventions or moments. Conclusion: There were no differences in ICW and ECW content between the Bergström and Sherman interventions at any moment. ***Level of Evidence I; Tests of Previously Developed Diagnostic Criteria.***

Descriptors: Body Composition; Glycogen; Fluid Therapy; Exercise; Diet.

RESUMO

Introdução: Em atividades físicas prolongadas a reposição hídrica e o conteúdo de glicogênio muscular são fatores limitantes em corredores de maratonas. O carregamento de carboidrato (CHO) nos dias anteriores à competição de resistência é um método comumente empregado para otimizar os estoques de glicogênio muscular e o desempenho no exercício. Uma vez que cada grama de glicogênio muscular liga-se a ≈2,7 a 4 gramas de água, a retenção hídrica pode ocorrer durante dietas de carregamento de carboidrato. Objetivo: Avaliar diferenças entre as estratégias de carregamento de carboidratos (Bergström e Sherman) no teor de água intracelular (AIC) ou água extracelular (AEC). Métodos: Vinte e três corredores foram alocados aleatoriamente para duas intervenções (Bergström e Sherman) num delineamento em "crossover". Os participantes foram submetidos a uma avaliação inicial antes dos 3 dias de depleção de glicogênio, seguidos por 3 dias de carga de carboidratos com tempo de "washout" de 30 dias consistindo em dieta e treinamento normais. Utilizou-se a bioimpedância multifrequencial (BIS) para avaliar AIC e AEC na Etapa Inicial, Pós-depleção e Pós-CHO para determinar quaisquer diferenças entre os protocolos de Bersgstrom e Sherman. Também foram obtidas coletas de sangue para avaliar o potássio. Foram determinadas associações entre AIC, AEC e glicogênio muscular. Resultados: Não houve diferenças no conteúdo de AIC ou AEC entre as duas intervenções em qualquer momento. Houve um efeito do tempo para AIC, com aumento da etapa Pós-depleção para Pós-CHO sem qualquer diferença entre as intervenções. O potássio plasmático diminuiu entre a Linha de base e Pós-depleção em ambas condições. Não houve diferença no conteúdo de glicogênio muscular entre intervenções ou momentos. Conclusão: Não houve diferenças no conteúdo de AIC e AEC entre as intervenções de Bergström e Sherman em qualquer momento. ***Nível de Evidência I; Testes de Critérios Diagnósticos Desenvolvidos Anteriormente.***

Descritores: *Composição Corporal; Glicogênio; Hidratação; Exercício Físico; Dieta.*

RESUMEN

Introducción: En actividades físicas prolongadas, la reposición de agua y el glucógeno muscular son factores limitantes en los corredores de maratón. La carga de carbohidratos (CHO) en los días previos a la competencia de resistencia es un método empleado para optimizar las reservas de glucógeno muscular y el rendimiento del ejercicio. Cómo cada gramo de glucógeno muscular se une a ≈ 2,7 a 4 gramos de agua, puede producirse retención de agua durante las dietas ricas en carbohidratos. Objetivo: Evaluar las diferencias entre las estrategias de carga de carbohidratos (Bergström y Sherman) en el contenido de agua intracelular (AIC) o extracelular (AEC). Métodos: Veintitrés

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corredores fueron asignados aleatoriamente a dos intervenciones (Bergström y Sherman) en un diseño cruzado. Los participantes se sometieron a una evaluación inicial antes de los 3 días de agotamiento del glucógeno, seguido de 3 días de carga de carbohidratos con un tiempo de "washout" de 30 días que consistía en una dieta y entrenamiento normales. Se utilizó bioimpedancia multifrecuencia (BIS) para evaluar AIC y AEC al inicio, después del agotamiento y después de CHO para determinar cualquier diferencia entre las dos intervenciones. También se obtuvieron muestras de sangre para evaluar el potasio. Se determinaron asociaciones entre AIC, AEC y glucógeno muscular. Resultados: No hubo diferencias en el contenido de AIC o AEC entre las dos intervenciones en ningún momento. Hubo un efecto de tiempo para AIC, con un aumento desde Post-agotamiento hasta Post-CHO sin ninguna diferencia entre las intervenciones. El potasio plasmático disminuyó entre el inicio y el post-agotamiento en ambas condiciones. No hubo diferencia en el contenido de glucógeno muscular entre las intervenciones o momentos. Conclusión: No hubo diferencias en el contenido de AIC y AEC entre las dos intervenciones en ningún momento. ***Nivel de Evidencia I; Pruebas de Criterios Diagnóstico Desarrollados Previamente.***

Descriptores: *Composición Corporal; Glucógeno; Fluidoterapia; Ejercicio Físico; Dieta.*

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INTRODUCTION

Races such as marathons have thermal injuries,[1] dehydration or hyponatremia[2] when the speed of sweating and dehydration is greater than water replacement, causing losses of 5 to 6% of water in relation to body weight.[3] It was believed that a loss of 1% of body weight raised core temperature,[4] 3 to 4% of body weight increased heart rate and reduced cardiac output[5] and 4 to 5% of body weight reduced heat dissipation, compromising cardiovascular function and the ability to perform work.[6] However, long-distance runners finish races with weight loss percentages of more than 5% compared to the start, without significant changes in thermal homeostasis.[7]

Bergström et al.[8] and Ahlborg et al.[9] investigated glycogen in skeletal muscle as a determinant of endurance exercise performance and time to exhaustion; subsequently, Bergström et al.[10] described the consumption of a diet rich in carbohydrates and the increase in muscle glycogen, known as "carbohydrate loading" for loading muscle glycogen with better endurance performance. Karlsson and Saltin[11] demonstrated better results obtained between 114 and 163 minutes in 30-kilometer runs when preceded by a special three-day 2,500 kcal/day diet without heavy exercise, preceded by three days of a CHO-free diet with heavy exercise. Sherman et al.[12] linked muscle glycogen depletion with fatigue during prolonged exercise. Hawley et al.[13] and Burke et al.[14] concluded that in prolonged submaximal exercise (75% of VO_{2max}) over 90 minutes, muscle glycogen minimizes muscle fatigue.

Olsson and Saltin[15] demonstrated with labeled water (^{3}H-tritium) an association of 3-4 grams of water for every gram of body glycogen. Maughan et al.[16] suggested errors in the assumptions of hydration status, due to changes in tissue osmolarity and the release of water bound to muscle glycogen, as metabolic oxidation results in carbon dioxide and water. Thus, body content is probably influenced by the carbohydrate content of the diet.

As athletes are routinely suggested to employ low and high carbohydrate diets, depending on their goals, this is likely to influence their intracellular and extracellular body water content. Thus, it would be interesting to determine how much these factors change and whether different glycogen depletion and replacement diets lead to differential effects on water content. The aim of this study was to determine the effects of two low-carbohydrate diets followed by high-carbohydrate diets (Sherman and Bergström) on intracellular and extracellular water content.

MATERIALS AND METHODS

This was a randomized, double-blind, crossover experimental study, first approved by the local ethics committee (CAAE: 33434720.9.0000.0068).

Sample

Initially, 48 subjects from CORPORE and 31 subjects from Nutroex were contacted, after explaining the eligibility criteria: a) healthy men aged between 25 and 60 years; b) normal muscle mass or above, according to the reference value provided by the BIS analysis; c) height between 160 cm and 190 cm; d) runner with a training volume greater than or equal to 40 km/week; and after a clinical investigation questionnaire: a) occurrence of heart, lung, orthopedic or surgical diseases; b) symptoms in the head, neck, chest, abdomen or locomotor system; c) Metabolic Syndrome; 10 CORPORE volunteers and 13 Nutroex volunteers were enrolled as eligible. Table 1 shows age, weight, height, km/week, marathons completed and the feeling of tiredness after the second half of the marathon, a phenomenon known as "hitting the wall" among marathon runners, with a drop in performance associated with fatigue due to muscle glycogen depletion.

Allocation

All the volunteers were informed about the protocols and risks associated with the study and received a free and informed consent form (ICF), in accordance with the recommendations of Resolution 196 of October 10, 1996 of the National Health Council. After signing an informed consent form, they were randomized using computer software and allocated to access A or B; determining whether they started with the Bergström or Sherman intervention. 11 volunteers were allocated to access A and 12 to access B.

Each volunteer took part in the two interventions with a crossover study between the Bergström and Sherman interventions, 30 days apart. After allocation, there were three withdrawals from access A and four withdrawals from access B, leaving eight from access A and eight from access B who handed in the ICF. During the 30-day washout period, there was one dropout from access A (COVID-19) and one from access B (traveled), seven from access A and seven from access B completed the two crossover interventions, totaling 15 collections for the Bergström intervention and 15 collections for Sherman. (Figure 1)

Table 1. Characteristics of the 16 volunteers: age, weight, height and level of physical fitness.

Age (years)	45 ± 8	
Weight (kg)	82.1 ± 18.1	
Height (m)	1.76 ± 0.08	
Training volume	15 run between 40 and 100 km/week	1 runs > 100 km/week
½ Maratona	6 ran < 10 ½ marathons	8 ran > 10 ½ marathons
Maratona	9 ran < 10 marathons	3 have run > 10 marathons
Marathon time	7 finished with time > 3:30 h	5 finished with time < 3:30 h
"Hitting the wall"	12 reported this feeling	1 didn't feel it

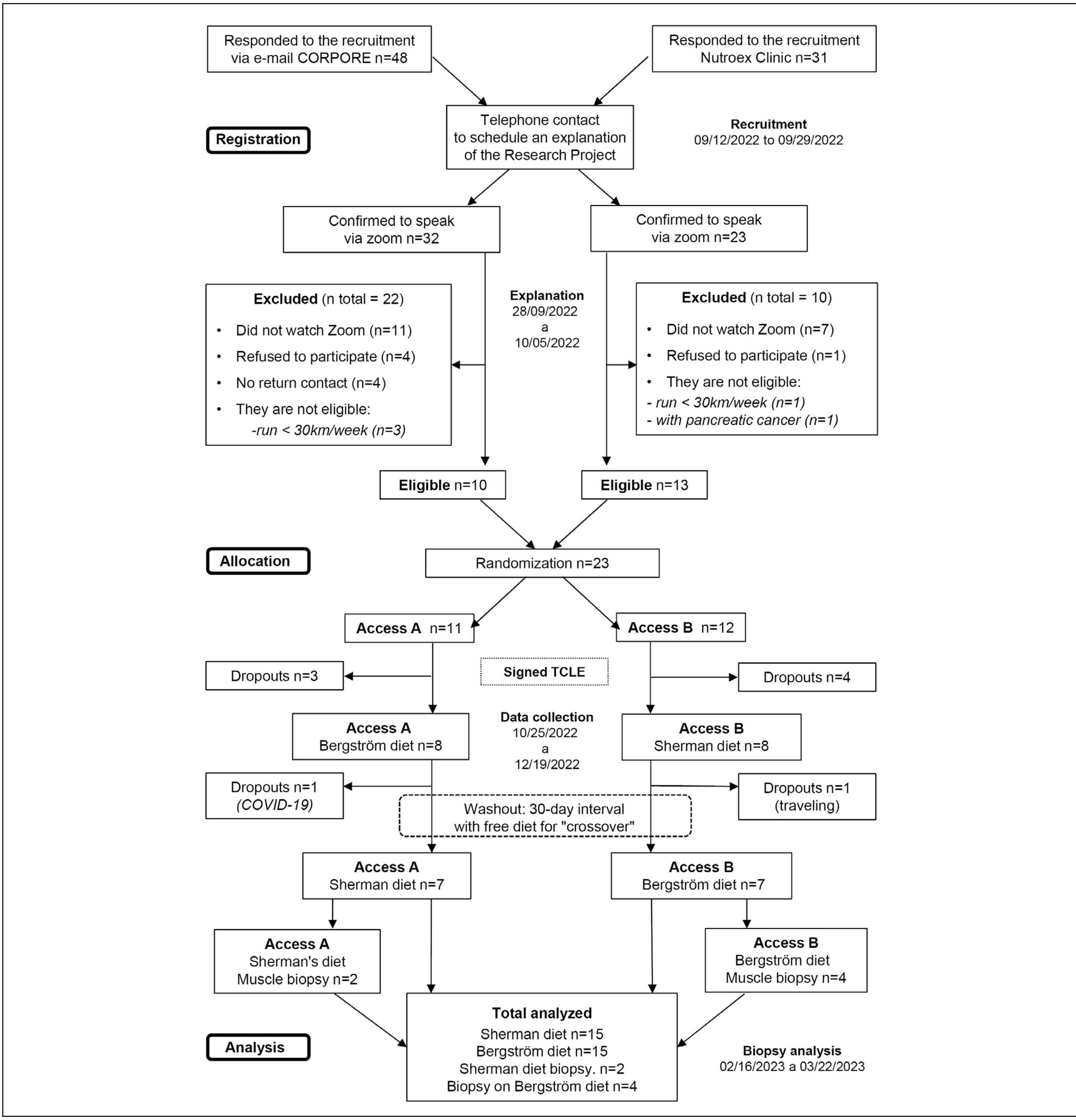

Figure 1. Flowchart of participants.

Breakdown of interventions

ICW and ECW values and muscle and blood samples (Figure 2) were taken on the first day as Baseline (Pre-depletion), after three days of a carbohydrate-restricted diet (day 4, Post-depletion) and then another three days of a carbohydrate-rich diet (day 7, Post-CHO). At Bergström, the restricted diet consisted of 10% carbohydrate of the daily calories and exhaustive exercise (90 minutes of running between 70 and 80% HR_{max}) for three days. On the fourth day, 90% carbohydrates of the daily calorie intake were introduced and physical activity was stopped for three days. In Sherman's case, the restricted diet consisted of 50% carbohydrates and 90 (day 1) and 40 (days 2 and 3) minutes of running at an intensity of 73% of maximum heart rate (HR_{max}) over three days. On the fourth day after collection, 70% carbohydrates and 20 minutes of exercise at an intensity of 73%HR_{max} were introduced for two days and rest from physical activity on the sixth day.

Diet

The quantity and quality of the diet with carbohydrate-rich and carbohydrate-poor or carbohydrate-absent foods was guided according to the individual's daily energy expenditure, by means of color classification, with the carbohydrates in the red group and the others in the green group with their calorie amounts determined for each portion.

Body composition

The participant arrived at the laboratory having fasted for three hours, with an empty bladder, wearing shorts, in a room at 21ºC, and having washed their hands and feet with 70% ethyl alcohol. Weight average of

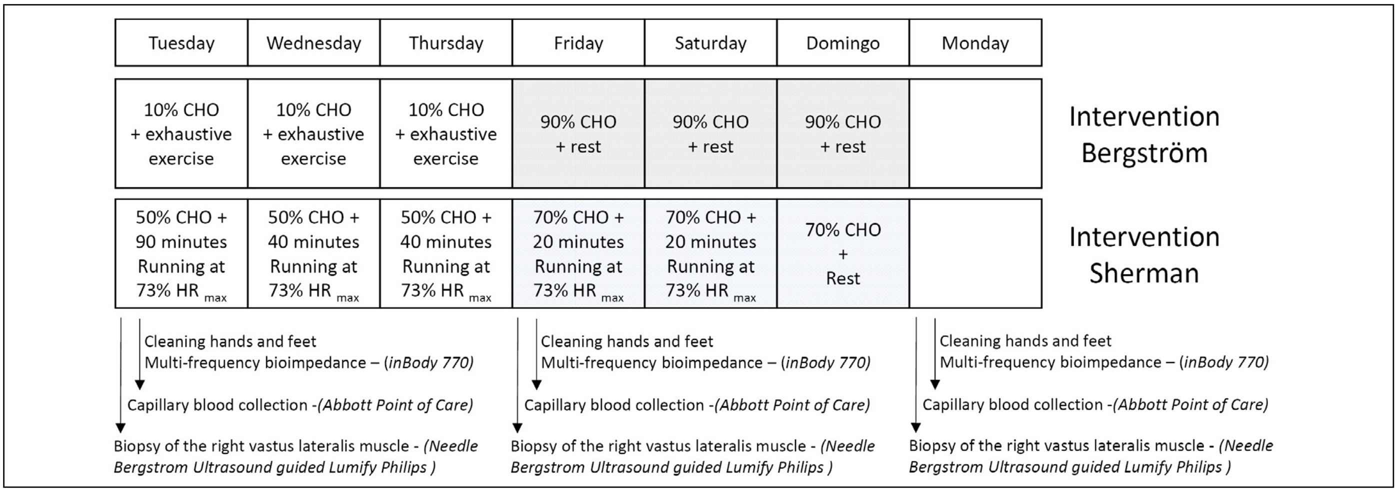

Figure 2. Details of the Bergström and Sherman intervention with bioimpedance, plasma potassium and muscle biopsy samples.

three consecutive weighings calculated for ACT, ICW and ECW measurements in liters. BIS was used with 30 impedance measurements, six electric currents of different frequencies (1 kHz, 5 kHz, 50 kHz, 250 kHz, 500 kHz and 1000 kHz), since electric currents of frequencies below 50 kHz bypass the cells and above 50 kHz pass through the cells. Bioimpedance variables (inBody770®) were evaluated according to Lee et al.[17] and Chen et al.[18]

Potassium

A Point-of-Care analyzer was used to immediately analyze plasma potassium concentration by venipuncture, since potassium binds to glycogen at a ratio of 0.45 mmol K/hydrated glycogen.[7] Measured on the CHEM 8+ cartridge (for potassium: 2.0-9.0 mEq/L) from Point of Care (Abbott i-STAT®).

Muscle biopsy

Biopsy samples were taken from six volunteers, four from volunteers on the Bergström diet and two from volunteers on the Sherman diet. Approximately 100 milligrams were extracted from the vastus lateralis muscle using a Bergström biopsy needle with a technique modified by Shanely et al.[19] stored in liquid nitrogen at -80º C and quantitative glycogen analysis determined using a double enzymatic assay using colorimetric (570 nm) and fluorometric (585/530 nm) methods, product catalog number MAK016 from Sigma-Aldrich® (USA).

Statistics

The variables of the two interventions were analyzed using Repeated Measures Mixed Models using the SAS statistical package (SAS® OnDemand for Academics, SAS Institute Inc., USA) and are presented as means ± 1 standard deviation. Normal distribution was confirmed by the Kolmogorov-Smirnov test. As most of the data was considered normal (P > 0.15), all the analyses were carried out using mixed models considering intervention (2 levels: Sherman and Bergström) and time (3 levels: Pre-, Post-depletion, Post-CHO) as fixed factors and individuals as random factors. Tukey-Kramer adjustments were made when a significant F value was obtained. Pearson correlations were performed to determine any associations between muscle glycogen and a) ECW, b) ICW and c) potassium. Statistical significance was accepted at P≤0.05.

RESULTS

ECW

There was no effect between the Sherman vs. Bergström (P=0.99), or the interaction between the interventions vs. Time (P=0.77), nor for the effect of time on interventions between Baseline, Post-depletion and Post-CHO (P=0.13; Figure 3).

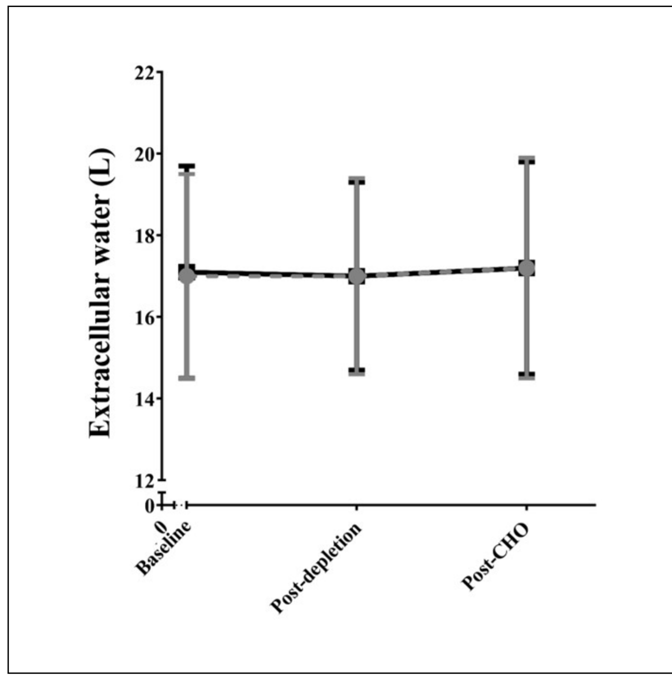

Figure 3. Extracellular water in the Bergstrom (black squares) and Sherman (gray circles) diets at the three time points of the study.

ICW

There was no effect between the Sherman *vs.* Bergström (P=0.91), or the interaction between the interventions *vs.* Time (P=0.37), however, showed an effect of time (P=0.01), with post-hoc showing a difference only between the Post-depletion and Post-CHO condition in both interventions with an upward shift in the CTA content (P=0.01; Figure 4).

Potassium

There was no difference in potassium concentration between the Sherman *vs.* Bergström (P=0.45), or the interaction between the interventions *vs.* Time (P=0.51), however, showed an effect of time (P=0.002) with post-hoc showing a difference between Baseline and Post-depletion in both interventions with downward deviation. (Figure 5)

Muscle glycogen

The statistical analysis of the data obtained between Sherman and Bergström showed that there was no effect between the interventions (P=0.99) for muscle glycogen or the interaction between interventions and time (P=0.64), nor was there an effect of time (P=0.74).

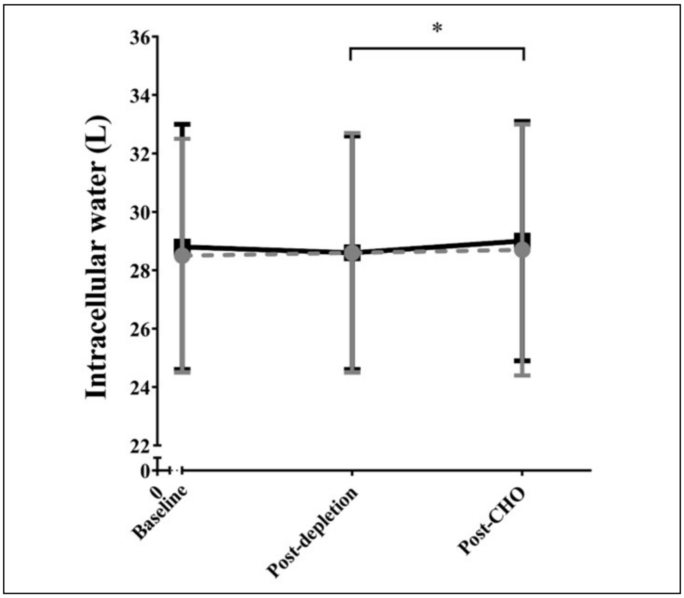

Figure 4. Intracellular water in the Bergstrom (black squares) and Sherman (gray circles) diets at the three time points of the study. *Effect of time between Post-depletion and Post-CHO after post-hoc analysis (P = 0.01).

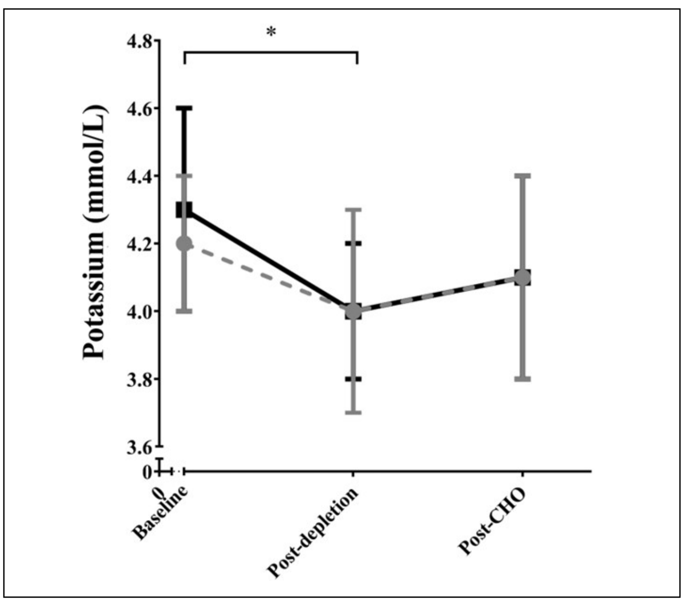

Figure 5. Potassium in the Bergstrom (black squares) and Sherman (gray circles) diets at the three time points of the study. *Effect of time between post-depletion and baseline after post-hoc analysis (P = 0.001).

Muscle glycogen and ECW or ICW

There were no correlations between muscle glycogen and ECW (r=0.033, P=0.90; Figure 6, Panel A) or muscle glycogen and ICW (r=0.087, P=0.73; Figure 6, Panel B).

Muscle glycogen and potassium

There was a significant correlation between muscle glycogen and blood potassium (r=-0.517, P=0.03; Figure 7) with rightward and downward deviation, regardless of whether all the time samples were analyzed together.

DISCUSSION

This study showed that ICW and ECW were not differentially influenced by the two carbohydrate-rich diets, nor were blood potassium or muscle glycogen. However, following a low-carbohydrate diet, high-carbohydrate diets increased ICW. Potassium in the blood decreased with

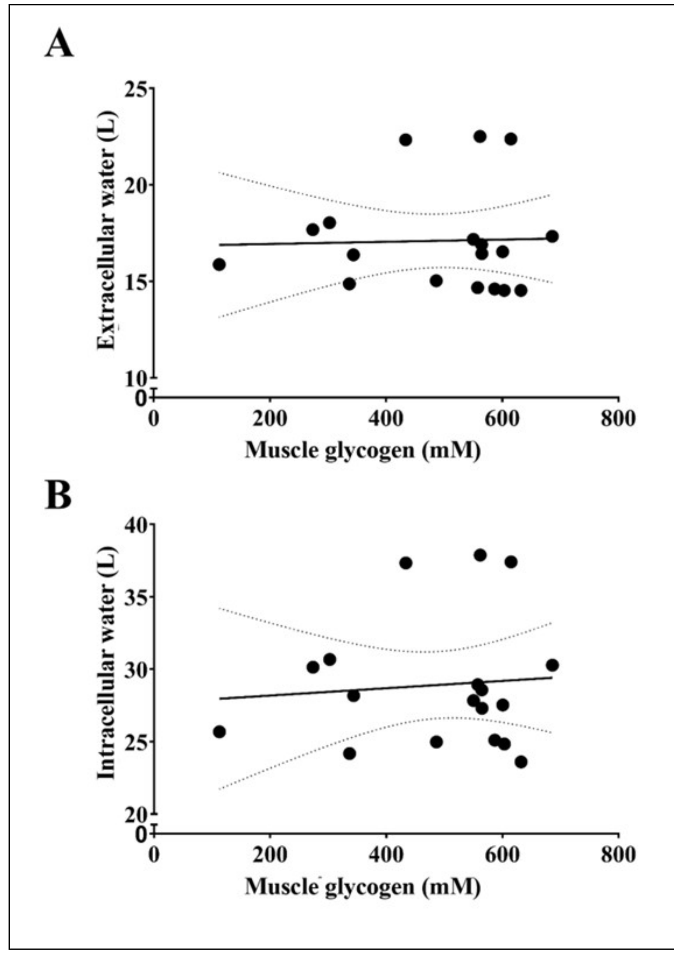

Figure 6. Correlation between extracellular water (Panel A; r = 0.033, P = 0.90) and intracellular water (Panel B; r = 0.087, P = 0.73) and muscle glycogen content.

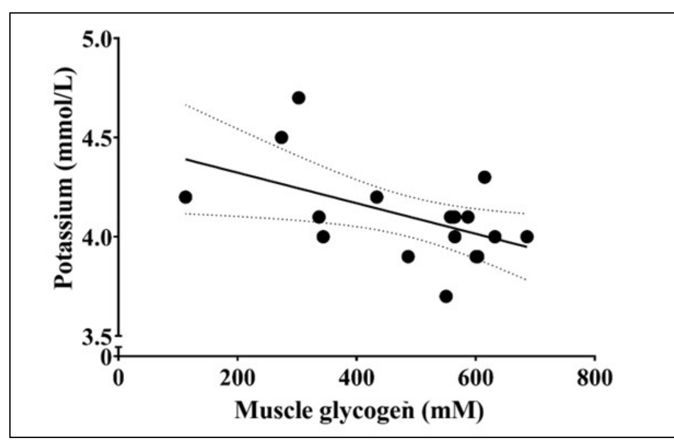

Figure 7. Correlation between plasma potassium and muscle glycogen content (r = -0.517, P = 0.03).

a low-carbohydrate diet, regardless of which specific diet. These data suggest that the Bergströmand Sherman diets do not infer differential effects on these measured parameters.

Neither the low-carbohydrate nor the high-carbohydrate diet affected the ECW; however, there was an increase in the ICW after carbohydrate overload, regardless of the specific diet. This was expected, as each gram of glycogen is stored along with 3-4 grams of water[15] and confirms the work of previous studies.[20] The lack of change in ICW with low-carbohydrate diets is unclear, but previous studies have shown no change in ECW or ICW after glycogen depletion,[21] suggesting that glycogen depletion alone does not alter body water distribution. There was no relationship between ECW or ICW and muscle glycogen content.

Future work should investigate the importance of these changes in the ICW for endurance exercise performance.

Muscle glycogen was not influenced by the diets, which is contrary to expectations. However, this can be explained by the low number of participants who were willing to provide a sample. Only two participants provided samples during the Sherman diet, while four provided muscle samples during the Bergstrom diet. There was substantial variation in these samples with some values increasing after the low carbohydrate diet and decreasing after the carbohydrate loading phase, suggesting that participants may not have strictly adhered to their diets. It is currently difficult to provide solid conclusions on the current data and further studies may wish to determine the influence of carbohydrate-rich or carbohydrate-restricted diets on total muscle glycogen content, within individual fibers and within fiber regions (e.g. subcellular, intrafibrillar, interfibrillar).[22]

Diets rich in carbohydrates usually contain a greater amount of potassium,[20] therefore, in the Post-depletion collection we can consider a drop in plasma potassium to the detriment of the low-carbohydrate diet at this stage, demonstrating the association between dietary carbohydrate intake and blood potassium levels. However, the carbohydrate load did not result in a significant increase in blood potassium and contrasts somewhat with the effects of the low-carbohydrate period. Muscle glycogen content was correlated with blood potassium concentration, regardless of diet and timing, suggesting a relationship between these factors. More work is needed to determine the importance of carbohydrate intake on blood potassium.

Segar et al.[23] reports that evaluation with time domain nuclear magnetic resonance (DT-NMRI) to assess ACT combined with BIS to assess ICW and ECW compartments allow for highly accurate measurements. We used BIS as a method for investigating glycogen-related ICW and ECW, which was initially reported to be a suitable tool in practice;[17,18,20] however, later new research by Shiose et al.[21] showed that changes in muscle glycogen depletion do not alter the ICW and ECW measured by BIS, in agreement with our findings.

Limitations paragraph

Limitations of the study: a) the low number of biopsies and the fact that the biopsies were only taken in one arm of the crossover (two volunteers during Sherman and four volunteers during Bergström) limited the analysis of CTA, CSA and muscle glycogen comparatively between the two interventions and between the Baseline, Post-depletion and Post-CHO collection stages; b) the absence of food recall during the stages made individualized monitoring difficult. Further studies are needed for complementary research.

CONCLUSIONS

This study showed no differences in ICW, ECW, blood potassium or muscle glycogen after glycogen depletion or carbohydrate loading using two diets. There was an increase in ICW with carbohydrate loading, although the relevance of this to exercise performance has yet to be determined.

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All authors declare no potential conflict of interest related to this article

AUTHORS' CONTRIBUTIONS: Each author contributed individually and significantly to the development of the manuscript. MM: writing, revising and preparing the entire research project; BS: data analysis and statistical analysis, writing and revision; BG: orientation, revision and intellectual concept; AJH: guidance, review and intellectual concept, intellectual concept and preparation of the entire research project.

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