Carbohydrate Loading Practice in Bodybuilders: Effects on Muscle Thickness, Photo Silhouette Scores, Mood States and Gastrointestinal Symptoms

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Abstract
A common practice among bodybuilders is the use of carbohydrate loading to improve physical appearance during competition, while limited documented data is available about this issue. The aim of the present study was to evaluate muscle thickness, mood states, gastrointestinal symptoms and subjective silhouette assessment following carbohydrate loading in bodybuilders. Twenty-four male bodybuilders were evaluated at the weighing period following three days of carbohydrate depletion (M1), and 24h of carbohydrate loading leading up to the competition (M2), stratified into: no carbohydrate load (NC, n = 9) and carbohydrate loading (CL, n = 15). The silhouette scale, Brunel mood scale (BRUMS), muscle thickness (ultrasound), circumferences, and gastrointestinal symptoms (GIS) were evaluated at M1 and M2. The NC displayed no differences in muscle thickness and circumferences between M1 and M2. Body mass, muscle thickness (elbow flexors, a combination of biceps brachii/brachialis muscle, and triceps brachii) and circumferences (chest, hip, thigh, arm, calves, and forearm) increased significantly (p < 0.05) in the CL at M2. There was a significant increase in photo silhouette scores (p < 0.05) in the CL at M2. There was no significant difference in mood states between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) between groups or time.

Key words: Bodybuilding, athletes, carb loading, mood states, gastrointestinal symptoms.

Introduction
Carbohydrate loading elevates muscular glycogen stores far beyond resting levels, and is achieved through regimens that consist of periods of exhaustive exercise followed by 24–48 h of increased carbohydrate intake (Burke et al., 2017; Bussau et al., 2002; Sherman et al., 1981). Most studies involving carbohydrate loading were performed in endurance athletes (Burke et al., 2017), while bodybuilding athletes have also adopted carbohydrate manipulation as a strategy leading up to a contest because they think that more glycogen stores in their muscles can increase muscle size, and impress judges by allowing participants to appear "more muscular" during the competition since (Balon et al., 1992; Mitchell et al., 2017). Only one study investigated the impact of the carbohydrate loading on muscle girth (Balon et al., 1992), and the authors reported no effect of the carbohydrate manipulation with an isocaloric diet, in which the percentage of carbohydrates was modified without adding calories (Balon et al., 1992). This procedure may have limited the results due to low energy intake (Burke et al., 2017; Tarnopolsky et al., 2001). Indeed, sub-optimal energy intake results in reduced availability of glucose for storage that culminates in impaired glycogen synthesis (Burke et al., 2017; Tarnopolsky et al., 2001).

Another important issue regarding competitive bodybuilding is the psychological alteration during the preparation for a competition (Mettler et al., 2010). Mitchell et al. (2017) reported that bodybuilders using the classic loading, which involved a three-day depletion and then super-compensation during the competition week (Balon et al., 1992), did not perceive significant changes in appearance, and described this method as, "stressful," and "mentally bad". On the other hand, a high carbohydrate diet improves mood states (de Moraes et al., 2018; De Moraes et al., 2017), while severe carbohydrate, and calorie restriction is associated with a decline in mood states during preparation for competition in bodybuilders (de Moraes et al., 2018; Rossow et al., 2013). Therefore, it is reasonable to hypothesize that periods of depletion and carbohydrate loading differentially affect mood states in athletes.

The reasons why athletes consider this manipulation of carbohydrates "stressful" are not well understood, but empiric evidence, and a case-study with a bodybuilder revealed the presence of a strong gastrointestinal discomfort and frequent episodes of diarrhea, followed by moderate-to-intense low abdominal pain (Della Guardia et al., 2015). No studies have investigated gastrointestinal symptoms (GIS) following carbohydrate loading; some findings have demonstrated a clear relationship between GIS and altered mood states in athletic (Pugh et al., 2018) and non-athletic populations (Konturek et al., 2011). To note, it is unknown if there is a correlation between GIS and these adverse psychological effects.

Based on these observations, the aim of the present study was to evaluate muscle thickness, subjective silhouette assessment following carbohydrate loading in bodybuilders, while limited documented data is available about this issue. The aim of the present study was to evaluate muscle thickness, mood states, gastrointestinal symptoms and subjective silhouette assessment following carbohydrate loading in bodybuilders. Twenty-four male bodybuilders were evaluated at the weighing period following three days of carbohydrate depletion (M1), and 24h of carbohydrate loading leading up to the competition (M2), stratified into: no carbohydrate load (NC, n = 9) and carbohydrate loading (CL, n = 15). The silhouette scale, Brunel mood scale (BRUMS), muscle thickness (ultrasound), circumferences, and gastrointestinal symptoms (GIS) were evaluated at M1 and M2. The NC displayed no differences in muscle thickness and circumferences between M1 and M2. Body mass, muscle thickness (elbow flexors, a combination of biceps brachii/brachialis muscle, and triceps brachii) and circumferences (chest, hip, thigh, arm, calves, and forearm) increased significantly (p < 0.05) in the CL at M2. There was a significant increase in photo silhouette scores (p < 0.05) in the CL at M2. There was no significant difference in mood states between groups or time. The most reported GIS was constipation: 7/9 (NC) and 9/15 (CL) during M1 and 6/9 (NC), and 5/15 (CL) at M2 with symptoms described as ‘moderate’ or ‘severe’. Diarrhea was reported by 7/15 CL (4/15 as severe). These data suggest that carbohydrate loading may contribute to an acute increase in muscle volume and physical appearance, however, it needs to be better planned to minimize gastrointestinal symptoms in bodybuilders.
ette, mood states, and GIS following carbohydrate loading in bodybuilders, and ultimately, if there is a correlation between gastrointestinal symptoms and mood states. The hypothesis was that carbohydrate loading would increase muscle thickness, subjective silhouette, mood states, and causes some GIS.

**Methods**

**Participants**

Twenty-four male bodybuilders who participated in either the Amateur South American Arnold Classic sponsored by the International Federation of Bodybuilding and Fitness (IFBB) or the 47th the Brazilian Championship both held in 2016. The competitors were qualified for the competition by reaching at least the top 3 at a regional qualifying event or by winning their respective weight class in one of these two events the previous year. The data were collection on two occasions: at the time of weighing, and at competition (~60-180 minutes prior to competition). The inclusion criteria were: participant of the bodybuilding competitions aged 20-35 years and those able to provide a detailed diet report of the last 4 days before weighing and competition. Subjects reporting the use of diuretics and laxatives were excluded.

After data collection related to estimated energy requirement and dietary intake (see details below), the athletes were stratified into two groups: those who used carbohydrate loading (CL, n = 9) and those who did not use carbohydrate loading between weighing and the day of competition (NC, n = 15). In order to distinguish the strategies used before and after weighing, data were divided in M1 (the weighing day, and the 2 days prior to weighing) and M2 (time period between weighing and the championship, 24 h) (Figure 1). The investigation was approved by the Research Ethics Committee of Ceara State University (process 04463980-5) in accordance with the Helsinki Declaration, and all participants provided informed written consent before participation.

![Study design](image)

**Figure 1. Study design.**

**Anthropometric data**

A scale (Plenna®) with a precision of 1mm and Stadiometer (exata, Brazil), precision of 1mm were utilized for body mass and height measurements, respectively.

Muscle thickness was measured with a 2.5 MHz A-mode transducer portable ultrasound (BodyMetrix, BX2000, IntelaMetrix, Inc., Livermore, CA) using the distance from adipose tissue-muscle interface to the muscle bone interface (Abe et al., 2000). Measurements were taken on the right side of body in two anatomic sites by a single evaluator: elbow flexors (a combination of biceps brachii and brachialis muscle thickness), and triceps brachii. For the anterior and posterior upper arm, measurements were taken at 60% of the distance between the lateral epicondyle of the humerus and the acromial process of the radius in the anterior and posterior portions). Three images were obtained for each site and the coefficients of variation were 0.16 and 0.13 for elbow flexors and triceps brachii, respectively.

Skinfolds were obtained using a scientific skinfold caliper (Lange, EUA) with a precision of 1mm, and constant pressure of 10g/mm². Each skinfold was measured in triplicate, and the mean utilized for calculations, always by the same evaluator. To calculate body density (BD) the Petroski equation developed for Brazilian men (Petroski, 1995) was used as follows: BD = 1.10726862 - 0.00081201 (SI+SE+TR+MC) + 0.00000212 (SI+SE+TR+MC)^2 - 0.00041761 (age, in years) and for the percentage of body fat (% BF), the Siri equation (Siri, 1993) described as: % BF = [(4.95 / BD) - 4.5] x 100 was used, BD: body density in g/ml, and skinfold: suprailiac (SI), subcapular (SE): triceps (TR), medial calf (MC). The coefficient of variation was 0.12, 0.13, 0.08 and 0.10, respectively for SI, SE, TR and MC.

Each girth was measured in triplicate, and the mean utilized for calculations by a single evaluator. Girth measurements were waist, upper arm, forearm, chest, thigh, and calf using a circumference measuring tape (WCS, Brazil). Reliability of the girth measures was determined according to the recommendations of (Uljaszek and Kerr, 1999) and (Perini et al., 2005). The calculation of intra-rater error was performed using two steps: 1) calculation of absolute technical error (ATE) by the equation:

$$ATE = \sqrt{\sum D^2/2N},$$

where D is the difference between measurements and N is the number of individuals; and 2) calculation of relative technical error (RTE) by the equation: $RTE = ATE / VMV \times 100$, WHERE: ATE = absolute technical error and VMV = the average value of the measure. The reliability coefficient (R) was calculated with equation: $R = 1 - (RTE^2 / variation\ coefficient)$.

**Estimated energy requirement and dietary data**

The estimated energy requirement (EER) was calculated by multiplying the resting metabolic rate (RMR) for the physical activity level (PAL). The RMR was based on the Harris Benedict equation (Harris and Benedict, 1918): RMR (kcal/ day) = 66.47+ 13.75 x Weight (kg) + 5 x Height (cm) – 6.76 x Age (yrs) and the PAL assessed through the International Physical Activity Questionnaire (IPAQ) short version (Matsudo et al., 2002). The energy balance was estimated by subtracting energy intake (described below) from EER.

Energy availability (EA) was calculated by the estimation of exercise energy expenditure (EEEx) subtracted from daily total energy intake (TEI), and divided by fat-free mass (FFM): $EA= [(TEI - EEx)/FFM]$ (Fagerberg,
Carbohydrate loading in bodybuilders

The 4-day food diary that included the day of competition was used to evaluate energy and macronutrient intake. Quantities were recorded in serving sizes and converted into grams. Food intake data were processed with assistance of DietWin® software (Porto Alegre, Brazil).

To be included in the CL group, the diet reported had to meet the following requirements: energy availability greater than 25 kcal/FFM/day; not be in a negative energy balance and carbohydrate intake less than 5g/kg body mass in the last three days before weighing, and carbohydrate intake between 8-12g/kg/day after weighing up to the contest day (Burke et al., 2017; Fagerberg, 2018).

Gastrointestinal symptoms
Athletes were encouraged to answer a modified version of the Abdominal and Epigastric Symptoms Questionnaire (Bovenschen et al., 2006) with questions assessing abdominal pain, nausea, vomiting, bloating, regurgitation, loss of appetite, flatulence, abdominal rumbling, belching, heartburn, constipation, and diarrhea. Participants responded to the questionnaire at the weigh-in, and on the day of the contest using a 1-5 Likert scale where; 1 = none; and 5 = unbearable.

Photo silhouette evaluation
The set of photos displayed in Figure 2 was shown to 7 federated bodybuilding judges, who agreed to evaluate the athletes’ photos at the weigh-in and in the day of the contest. The judges were asked to point to the silhouette that most closely resembled the athletes’ current appearance on a scale ranging from 1-7, with 7 being the best silhouette. The judges were blind as to the nutritional manipulations of the athletes.

Mood states
Mood states were measured using the Brunel mood scale (BRUMS) questionnaire, previously translated into Portuguese, and validated in a Brazilian population (Rohlfs, 2006). The BRUMS is a self-report questionnaire consisting of 24 items rated on a 5-point scale (ranging from 0 = “no” to 4 = “extremely”) designed to assess 6 dimensions, each one consisting of 4 items. The total score of each dimension ranges from 0 to 16. Total mood disturbance was determined by the sum score of the negative dimension subtracting the score of the positive dimension vigor, and then adding the value “100”.

Statistical analysis
The Shapiro-Wilk and Levene tests were used to verify the normality and homogeneity of the variances, respectively. The mean, standard deviation (SD) and 90% confidence intervals (CIs) were used after the data normality was assumed. A two-way repeated-measures analysis of variance (group X time) was used to compare the Photo silhouette, nutrient intake, anthropometric, mood states and GIS variables between CL and NC. Compound sphericity was verified by the Mauchley test. When the assumption of sphericity was not met, the significance of F-ratios was adjusted according to the Greenhouse–Geisser procedure.

Differences were considered significant when p ≤ 0.05. Data were analyzed with SPSS-22.0 for Windows (IBM corp., Armonk, NY, USA).

Results
A significant group x time interaction was found for energy intake (F = 15.35, p < 0.001), energy balance (F = 9.64, p = 0.005), and available energy (F = 10.48, p = 0.004). Table 1 demonstrates that in M1, all athletes of both NC and CL groups were in a negative energy balance. In addition, 6/9 (66.7%) in NC and 9/15 (60.0%) in CL were under low energy availability (<25kcal/kg FFM.day⁻¹). In M2, all athletes of the NC group displayed a negative energy balance, while all athletes in the CL group presented a positive energy balance. The energy availability was >30kcal/kg FFM.day⁻¹ in both groups, although it was higher for CL during M2. Moreover, there was no athlete in NC, and only 2/15 (13.3%) in CL who reported being accompanied to the competition by a qualified nutritionist.

There was a significant group x time interaction for protein intake in absolute terms (F = 80.45, p < 0.001) and relative to body weight (F = 45.43, p < 0.001), as well as for absolute carbohydrate intake (F = 29.68, p < 0.001) and relative to body weight (F = 119.59, p < 0.001). As observed in Table 1, there was a high protein intake in both groups, which remained high only in the NC group at M2. Carbohydrate intake was low for both groups in M1, while the increase in M2 for the NC group was below half when compared with CL at M2. The lipid intake did not change between M1 and M2 in the NC participants in absolute terms, while there was an increase in M2 for the CL group. The contribution of lipid consumption for energy intake was higher for both groups at M2 as compared with M1.

Figure 2. Photo silhouette scale (Castro et al., 2011).
Table 1. Energy intake and expenditure, energy balance, macronutrient and fiber intake of bodybuilders before (M1) and after weighing (M2). Values are expressed as mean and standard deviation (SD).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NC (n = 9)</th>
<th>CL (n = 15)</th>
<th>NC (n = 9)</th>
<th>CL (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>26.2 ± 4.9</td>
<td>27.3 ± 5.0</td>
<td>9.2 ± 2.9</td>
<td>6.6 ± 0.9</td>
</tr>
<tr>
<td>Training experience (years)</td>
<td>8.8 ± 2.8</td>
<td>9.2 ± 2.9</td>
<td>8.4 ± 1.2</td>
<td>8.5 ± 1.0</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>6.7 ± 0.7</td>
<td>6.6 ± 0.9</td>
<td>72.2 ± 5.5</td>
<td>70.8 ± 7.8</td>
</tr>
<tr>
<td>Fat mass (g)</td>
<td>8.4 ± 1.2</td>
<td>8.5 ± 1.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat mass (g)</td>
<td>22.3 ± 4.0</td>
<td>23.3 ± 1.8*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake</td>
<td>1897.6 ± 240.2</td>
<td>2983.9 ± 125.8*</td>
<td>2034.8 ± 242.0#</td>
<td>3591.7 ± 259.9*†</td>
</tr>
<tr>
<td>Energy expenditure</td>
<td>3156.5 ± 137.0</td>
<td>3151.0 ± 135.0</td>
<td>3121.3 ± 204.0</td>
<td>3151.0 ± 206.8</td>
</tr>
<tr>
<td>Energy balance</td>
<td>-1258.9 ± 341.6</td>
<td>-193.1 ± 83.2*</td>
<td>-1086.5 ± 243.5</td>
<td>440.6 ± 170.0*‡</td>
</tr>
<tr>
<td>Energy availability</td>
<td>24.6 ± 3.0</td>
<td>41.3 ± 3.1*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein intake</td>
<td>266.3 ± 51.0</td>
<td>205.8 ± 66.5*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbohydrate intake</td>
<td>51.9 ± 6.5</td>
<td>52.9 ± 6.6</td>
<td>28.8 ± 5.2‡</td>
<td>52 ± 3.2*</td>
</tr>
<tr>
<td>Lipid intake</td>
<td>9.0 ± 0.6</td>
<td>5.2 ± 0.9*</td>
<td>16.7 ± 8.1*</td>
<td>9.0 ± 0.7*‡</td>
</tr>
<tr>
<td>Fiber intake</td>
<td>13.2 ± 2.2</td>
<td>14.0 ± 1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tension</td>
<td>2.9 ± 2.1</td>
<td>3.2 ± 2.3</td>
<td>2.9 ± 2.2</td>
<td>3.1 ± 2.4</td>
</tr>
<tr>
<td>Depression</td>
<td>1.9 ± 1.0</td>
<td>1.8 ± 0.9</td>
<td>1.9±1.1</td>
<td>1.8 ± 1.2</td>
</tr>
<tr>
<td>Anger</td>
<td>2.1 ± 2.0</td>
<td>2.2 ± 1.6</td>
<td>2.0±1.7</td>
<td>2.1 ± 1.8</td>
</tr>
<tr>
<td>Vigor</td>
<td>5.5 ± 3.7</td>
<td>5.1 ± 4.1</td>
<td>5.9±4.2</td>
<td>5.6 ± 4.0</td>
</tr>
<tr>
<td>Fatigue</td>
<td>4.5 ± 3.6</td>
<td>5.1 ± 3.2</td>
<td>4.3±3.0</td>
<td>4.9 ± 4.1</td>
</tr>
<tr>
<td>Confusion</td>
<td>2.2 ± 1.8</td>
<td>2.4 ± 2.0</td>
<td>2.4±2.1</td>
<td>2.6 ± 2.0</td>
</tr>
<tr>
<td>Total disturbance mood</td>
<td>105.2 ± 13.0</td>
<td>109.6±15.7</td>
<td>107.6±14.1</td>
<td>108.9 ± 16.6</td>
</tr>
</tbody>
</table>

* Significant difference vs M1. # Significant difference vs NC at M1. † Significant difference vs NC at M2.

Table 2. Anthropometric characteristics of bodybuilders before (M1) and after weighing (M2). Values are expressed as mean and standard deviation (SD).

<table>
<thead>
<tr>
<th>Anthropometric Parameter</th>
<th>NC (n=9)</th>
<th>CL (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.73 ± 0.01</td>
<td>1.73 ± 0.01</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.5 ± 5.6</td>
<td>80.3 ± 7.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.1 ± 1.8</td>
<td>27.0 ± 1.9</td>
</tr>
<tr>
<td>Elbow flexors (mm)</td>
<td>50.9 ± 2.8</td>
<td>50.8 ± 2.7</td>
</tr>
<tr>
<td>Triceps brachii (mm)</td>
<td>52.7 ± 2.9</td>
<td>52.3 ± 3.0</td>
</tr>
<tr>
<td>Chest “expired” (cm)</td>
<td>112.5 ± 5.8</td>
<td>112.0 ± 6.2</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>77.5 ± 3.9</td>
<td>78.0 ± 3.8</td>
</tr>
<tr>
<td>Hip (cm)</td>
<td>100.4 ± 3.0</td>
<td>100.5 ± 2.9</td>
</tr>
<tr>
<td>Thigh (cm)</td>
<td>64.9 ± 4.2</td>
<td>64.8 ± 4.1</td>
</tr>
<tr>
<td>Arm (cm)</td>
<td>42.6 ± 1.5</td>
<td>42.7 ± 1.3</td>
</tr>
<tr>
<td>Calves (cm)</td>
<td>40.4 ± 1.4</td>
<td>40.2 ± 1.4</td>
</tr>
<tr>
<td>Forearm (cm)</td>
<td>32.7 ± 1.9</td>
<td>32.5 ± 1.6</td>
</tr>
<tr>
<td>Silhouette (scores)</td>
<td>4.5 ± 0.9</td>
<td>4.6 ± 0.8</td>
</tr>
</tbody>
</table>

Mood states are presented in Table 3. There were higher scores for negative mood fatigue and lower for positive mood vigor in both groups independent of the measured time point. Moreover, analysis of variance showed no

The average amount of daily meals was 7.6 and 8.0 in NC and CL at M1, and 7.9 and 8.2 in NC and CL at M2, respectively. A total of 60% (9/15) of the athletes in CL reported a meal “Fry up” at contest day, consisting of meat and egg sandwiches, milk shakes and potato chips, contributing with ~28% of daily energy intake.

Table 2 presents anthropometric indicators at moments M1 and M2. There was no significant group x time interaction. However, weight, BMI, muscle thickness and circumferences, with exception of waist, increased in the CL group. The photo silhouette scores were improved only in the CL group according to the judges. The reliability of the girth measures was as follows: the relative technical error for chest, waist, thigh, hip, arm, and forearm were 1.1, 0.9, 1.3, 1.4, 0.7, 0.6 and the reliability coefficient 0.994, 0.996, 0.992, 0.998, 0.997, 0.998, respectively. For skinfold measures the relative technical error for SI, SE, TR, and MC was and the reliability coefficient 1.2, 1.0, 1.4, 0.9, respectively and the reliability coefficient 0.994, 0.996, 0.992, 0.991, 0.996, 0.995. Mood states are presented in Table 3. There were higher scores for negative mood fatigue and lower for positive mood vigor in both groups independent of the measured time point. Moreover, analysis of variance showed no
significant group x time interactions regarding individual mood scores, or for total mood disturbance no major changes between groups, either in different moments.

The most reported GI symptoms (Table 4) were abdominal pain and constipation at M1 for both the groups, with the frequency of these symptoms persisting into M2 for the NC group; abdominal pain, bloating, flatulence, abdominal rumbling, heartburn, constipation, and diarrhea were more often reported for athletes of the CL group at M2. When symptoms were described as 'moderate' or 'severe', constipation was reported for 7/9 and 9/15 of the athletes in NC and CL, respectively at M1, and 6/9 and 5/15 in NC and CL, respectively at M2. In the CL group, diarrhea was reported for 7/15 athletes, with 4/15 reporting as moderate or severe.

### Table 4. Gastrointestinal symptoms of bodybuilders before (M1) and after weighing (M2). Values are expressed as mean and standard deviation (SD).

<table>
<thead>
<tr>
<th>Gastrointestinal Symptoms</th>
<th>M1</th>
<th>M2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>1.44 ± 0.68</td>
<td>1.40 ± 0.49</td>
</tr>
<tr>
<td>Nausea</td>
<td>1.11 ± 0.31</td>
<td>1.07 ± 0.25</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1.00 ± 0.00</td>
<td>1.00 ± 0.00</td>
</tr>
<tr>
<td>Bloating</td>
<td>1.00 ± 0.00</td>
<td>1.11 ± 0.31</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>1.00 ± 0.00</td>
<td>1.11 ± 0.31</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td>1.00 ± 0.00</td>
<td>1.11 ± 0.31</td>
</tr>
<tr>
<td>Flatulence</td>
<td>1.11 ± 0.31</td>
<td>1.33 ± 0.67</td>
</tr>
<tr>
<td>Abdominal rumbling</td>
<td>1.11 ± 0.31</td>
<td>1.22 ± 0.42</td>
</tr>
<tr>
<td>Belching</td>
<td>1.00 ± 0.00</td>
<td>1.22 ± 0.42</td>
</tr>
<tr>
<td>Heartburn</td>
<td>1.00 ± 0.00</td>
<td>1.22 ± 0.42</td>
</tr>
<tr>
<td>Constipation</td>
<td>2.00 ± 0.67</td>
<td>1.89 ± 0.57</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1.11 ± 0.31</td>
<td>1.22 ± 0.42</td>
</tr>
<tr>
<td><strong>Total scores</strong></td>
<td>13.88 ± 0.28</td>
<td>14.9 ± 0.22</td>
</tr>
</tbody>
</table>

* Significant difference vs M1. † Significant difference vs NC at M2.

### Discussion

The results of this present study provide evidence that bodybuilders using carbohydrate loading prior to competition generally increase muscular girth and display improvement in silhouette evaluation by bodybuilding referees. To the best of our knowledge, no previous study has reported this type of judge evaluation with respect to a silhouette measurement. This aspect is particularly interesting because the criterion of judgment in a bodybuilding competition is physical appearance, evaluated subjectively by referees. Conversely, carbohydrate loading did not affect mood states, which were already impaired due to caloric restriction, thus only partially confirming the initial hypothesis. Furthermore, there was a high prevalence of gastrointestinal symptoms, such as constipation and diarrhea, especially in athletes that were on the carbohydrate loading protocol. Putatively, the gastrointestinal symptoms may have hindered improvement of mood states in bodybuilders due to the correlation between GIS and mood states.

Carbohydrate loading consists of three days of carbohydrate depletion with an intake of less than 2 g·kg⁻¹·day⁻¹ and subsequent three days of the of high-carbohydrate intake (8-12 g·kg⁻¹·day⁻¹) in the case of a "classical" loading protocol or 3-day of moderate carbohydrate intake (~5 g·kg⁻¹·day⁻¹) finishing with a period of 24-48 hours of high-carbohydrate intake (8-12 g·kg⁻¹·day⁻¹) with an adapted protocol (Burke et al., 2017). In the present study, M1 was considered as the depletion period, and both NC and CL groups met the necessary carbohydrate intake range. In M2, only the CL athletes consumed sufficient carbohydrate (9.0 ± 0.7 g·kg⁻¹·day⁻¹) amounts to support high carbohydrate loading (Burke et al., 2017). In addition, negative energy balance and low energy availability in M1 for both groups suggests that a hypoenenergetic diet is a marked characteristic of bodybuilders’ diet until weighing day. However, the NC group maintained a caloric deficit in M2, as observed by the negative energy balance. These data suggest that not only insufficient carbohydrate intake, but low energy intake may have contributed to the limited effect on muscular thickness, circumferences and evaluation of silhouette in the NC group at M2.

The major purpose that bodybuilders perform carbohydrate loading is to appear “more muscular” for the referees at competition (Balon et al., 1992; Chappell and Simper, 2018; Mitchell et al., 2017). In accordance to this, body mass increased ~2.8% in CL group, corresponding to the increase typically observed in athletes after carbohydrate loading (Bergström et al., 1972). Moreover, changes observed in muscle thickness and five of the six circumferences may have influenced judges, as observed by subjective assessment of shape with increased scores of the photo silhouettes scale for bodybuilders (Castro et al., 2011). Our findings are supported by Nygren et al. (2001) who reported that carbohydrate loading resulted in increased cross-sectional area of the vastus lateralis muscle (3.5%), and thigh circumference (2.5%).

The CL athletes had the opportunity to refeed following weigh-in, meeting the requirements for high carbohydrate loading protocol. This short term recovery period might have the desired effect of a more muscular look. However, in most bodybuilding competitions, the interval between weigh-in and competition is less than 24 hours and may be insufficient for the athletes load the muscles with supernormal glycogen levels (Burke et al., 2017).
It is interesting to note that the higher energy consumption at M2 does not significantly affect the frequency of meals in both groups. The amount of carbohydrates consumed has been considered more important that the frequency of meals for carbohydrate loading (Burke et al., 1996; 2017). However, from a practical point of view, the frequency of meals can be considered as a strategy to reach an adequate energy intake, especially in athletes with high energy demand. Interestingly, the meal “fry up” reported by CL athletes contributed expressively for total energy intake, while did not result in a lower frequency of meals. Thus, it can be assumed that both: a high frequency of meals and a meal “fry up” were used as a strategy to tailor adequate energy intake, which was also reported previously among bodybuilders (Chappell and Simper, 2018), and reveal a contemporary practice of bodybuilders.

With regards to psychological aspects, a similar profile of mood states in both groups at M1 was likely due to the fact that all athletes were under severe energy restriction. Surprisingly, mood states did not improve at M2, suggesting that a short time change in macronutrient composition does not affect mood states, regardless of energy intake. In fact, short-term manipulations of macronutrients, or refeeding following an energy restriction period have been shown to be incapable of positively affecting mood states (Finn et al., 2004). Thus, changes in mood states may require longer periods to recovery, especially following a bodybuilding competition. In concordance, a case study with a natural bodybuilder revealed a reduction in vigor subscale at competition, and after 6 months the vigor score had not yet been completely restored (Rossow et al., 2013).

Albeit the findings from Finn et al. (2004) and Roslow et al. (2013), another possible reason to explain the impairment in CL athletes, even with high energy intake is the high frequency of gastrointestinal symptoms. While a hypercaloric diet can positively impact mood states in bodybuilders (de Moraes et al., 2017; 2018), studies have demonstrated a clear relationship between the gastrointestinal symptoms and a decrease in mood states (Clark and Mach, 2016; Konturek et al., 2011). In addition, there was a linear correlation between gastrointestinal symptom scores and total mood state in the CL group ($r = 0.71; p = 0.01$). Despite this correlation, the causal relationship is not well established, and deserves future research for a better understanding of the reasons why some bodybuilders consider carbohydrate loading “very stressful” and some of them may avoid this diet strategy (Mitchell et al., 2017).

Notwithstanding, most athletes had high scores related to constipation at M1, remaining high only in the NC group at M2. Constipation may reflect a reduced fiber intake, considering that Recommended Dietary Allowance (RDA) values for dietary fiber intake may be 38g/day for male individuals (Trumbo et al., 2002) and the average intake of athletes did not reach more than 15g/day. Furthermore, especially soluble fibers, add bulk to stools due to its ability to slow transit time of foods through the bowel and draw water into the intestinal space, which leads athletes seeking acute weight loss to restrict the fiber intake (Reale et al., 2017). Therefore, attention to signs of constipation should be directed to avoid symptoms before weighing.

Carbohydrate loading athletes exhibit reduced constipation and increased diarrhea scores. This may reflect a difficulty in food planning organization, because most athletes reported that they did not follow up with a qualified nutrition professional, similarly to what has been reported by other studies (Chappell and Simper, 2018; Della Guardia et al., 2015). Moreover, athletes that were under the supervision of a dietitian showed no GIS. In particular, when athletes progress from a period with very reduced to a very high carbohydrate intake without prior adaptation, gastrointestinal symptoms may increase, supposedly due to an inability to rapidly adjust the amount of intestinal glucose transporters and stomach capacity (Jeukendrup, 2017). Another possible factor is a consumption of foods containing fermentable oligosaccharide, disaccharide, monosaccharide, and polyols (FODMAP’s), which can increase osmotic load in the small intestine and it has been associated with high frequency of diarrhea in athletes (Lis et al., 2018). Further investigation based on nutritional strategies for bodybuilders are needed during the carbohydrate loading in order to avoid gastrointestinal symptoms.

This study is not without limitations. TEI and EEE were self-reported, thus allowing for the potential of errors in the EA equation. Moreover, the use of skinfold is not the optimal method for determining body composition, and is considered an indirect method to estimate FFM (Larson-Meyer et al., 2018). Second, girth and thickness measures do not necessarily reflect the increase in muscle size. Thus, further investigations may incorporate other ultrasonographic parameters (e.g. cross sectional area) in different anatomical sites. To note, errors of measurement in FFM contribute to a relatively small discrepancy to estimate EA compared with other inputs (Burke et al., 2018), and the technical error and the reliability coefficient for anthropometric measurements indicated reproducibility. In contrast, the study has strong points. For example, the difficulty in recruitment of this population, especially at week of the contest, as well as training experience encompassed a considerable time and the withdrawal of those individuals that used diuretics and laxatives are fundamental criteria.

**Conclusion**

The present study provides practical underpinnings regarding carbohydrate loading strategy for bodybuilding competition. The carbohydrate loading was associated with an increase in circumferences and photo silhouette scores in bodybuilders, and potentially explains at least in part, the importance of carbohydrate loading for improving subjective physical appearance. On the other hand, gastrointestinal symptoms were frequent (e.g. constipation and diarrhea) in this scenario, which have not yet been reported previously in the literature. Furthermore, short-term carbohydrate manipulation has no major effects on mood states in bodybuilders. Thus, athletes can seek the assistance of qualified professionals for food planning in order to nutrient adequacy and reduction of gastrointestinal symptoms. Future studies should be conducted to explore the impact of carbohydrate loading among bodybuilding divisions.
Acknowledgements

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Key points

- Carbohydrate loading was associated with an increase in muscle thickness, circumferences and photo silhouette scores in bodybuilders.
- Constipation and diarrhea were Gastrointestinal symptoms more frequent.
- Mood states did not change after short-term carbohydrate manipulation.

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