



# Low-Versus High-Carbohydrate Isocaloric Diets on Continuous Glucose Monitoring Metrics in Healthy Trained Cyclists: A Randomized Crossover Trial

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## ABSTRACT

This randomized crossover trial examined the effects of low-carbohydrate high-fat (LCHF) versus high-carbohydrate low-fat (HCLF) isocaloric diets on continuous glucose monitoring (CGM)-derived glucose metrics in trained, healthy male cyclists. Participants ( $n = 15$ ) followed each dietary condition for 7 days, with glucose data continuously collected using CGM technology. The primary outcomes assessed were mean glucose levels, glucose variability, and time spent within specific glycemic ranges across wake, exercise, and nighttime phases. Results indicated that the LCHF diet significantly reduced mean glucose levels and glycemic variability (CV) compared to the HCLF diet. Mean glucose values were significantly higher during exercise compared to wake and night phases, irrespective of dietary conditions. Exercise intensity was positively associated with higher glucose concentrations during training sessions, independent of dietary conditions. Furthermore, nocturnal glycemia was significantly lower after exercise days, suggesting an influence of prior physical activity on overnight glucose regulation. Substantial interindividual variability in glucose responses was observed, highlighting the necessity of personalized dietary strategies for athletes. This study demonstrates that dietary carbohydrate manipulation significantly influences glucose metabolism, with implications for optimizing training, recovery, and metabolic health in endurance athletes. Future research should further explore individualized glucose responses and longer-term adaptations to dietary interventions in athletic populations.

## 1 | Introduction

The optimization of the training process and athletic performance are among the most important applications of sports nutrition research. Various nutritional approaches can be employed to achieve specific goals, aligning the demands of training and competition with the principles of specificity, periodization, and personalization (Burke and Hawley 2018; Jeukendrup 2017). Carbohydrates (CHO) are well established as

essential for sustaining ATP turnover during intense endurance exercise, thereby enhancing performance and reducing the perception of fatigue (Bergstrom et al. 1967; Coyle et al. 1986). This underpins the widespread prescription of high-carbohydrate low-fat (HCLF) diets for athletes, particularly around periods of intense training and/or racing (Burke et al. 2011; Podlogar and Wallis 2022). However, recent studies have investigated the effects of modulating carbohydrate availability to optimize training adaptations and potentially improve

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### Highlights

- Glycemic responses during daily activities vary significantly according to dietary carbohydrate content, emphasizing the potential for tailored nutritional strategies.
- Nocturnal glycemia is sensitive to both daily carbohydrate intake and previous exercise, highlighting the complexity of overnight glucose regulation in athletes.
- Continuous glucose monitoring reveals substantial interindividual variability, underscoring the importance of personalized nutrition in endurance athletes.

performance (Burke 2015; Impey et al. 2018). The “train-low” strategy, which involves training with reduced carbohydrate availability, may amplify the responses of molecular signaling pathways associated with enhanced mitochondrial biogenesis after exercise which could translate into improvements in performance (Bartlett et al. 2015; Impey et al. 2018).

In this context, it is not uncommon for endurance athletes to experience symptoms of hypo- and hyperglycemia, situations that should be monitored for performance optimization and athlete health (Flockhart and Larsen 2024). Hyperglycemia commonly arises during training or after high-intensity exercise and/or following large post-exercise meals for glycogen and energy restoration. Conversely, a blunted glycemic response during intense exercise could be indicative of overtraining situations (Flockhart and Larsen 2024). Although the acute effects of hyperglycemia in athletes are not well documented, elevated glucose levels were associated with an increased risk of cardiovascular diseases and reduced training adaptations (Zignoli et al. 2023). Hypoglycemia also warrants attention: for example, it has been observed that a significant number of endurance athletes develop reactive hypoglycemia after carbohydrate consumption before a race, with the highest risk occurring if the food is consumed 30–90 min before the competition, resulting in reduced performance and sense of well-being (Zignoli et al. 2023). During-exercise hypoglycemia can result from imbalances between training load and nutrition, although carbohydrate ingestion during exercise has been shown to mitigate this effect regardless of dietary background or glycogen availability (Brun et al. 2001; Prins et al. 2025). Additionally, nocturnal hypoglycemia also appears to have indirect adverse effects on performance by disrupting sleep and nighttime recovery (Flockhart and Larsen 2024).

To address these issues, continuous glucose monitoring (CGM) has emerged as a tool for optimizing performance, recovery, and training adaptation. CGMs use subcutaneous sensors to measure the concentration of glucose in the interstitial fluid every 1–15 min, closely approximating blood glucose levels. Currently available CGMs have a lifespan ranging from 3 to 14 days, and the sensor inserted into the subcutaneous layer of the skin consists of an electrode soaked in glucose oxidase, which catalyzes the glucose oxidase reaction and generates an electrical current proportional to the glucose concentration in the interstitial fluid; this current is then translated into a glucose estimate through algorithms and transmitted to the dedicated smartphone application. Unlike traditional capillary or venous

sampling, CGMs allow near real-time glucose tracking (Bowler et al. 2024, 2023).

Initially developed for clinical use in diabetes, CGMs use has expanded and has been promoted to be used also among healthy individuals and especially athletes, helping in supporting the choice of appropriate and tailored pre-, during, and postexercise nutritional strategies, allowing for the detection of abnormal fluctuations in glucose states. Indeed, recent studies have established associations between blood glucose dynamics during exercise and sports performance (Bowler et al. 2023; Holzer et al. 2022) and that glycemic responses to preexercise meals vary greatly between individuals, underscoring the need for personalized dietary strategies (Zignoli et al. 2023). To individualize the food choices based on glycemic responses, it would be essential—first of all—to understand how dietary patterns affect glucose metrics. Although some research suggests rapid adaptation (around 5 days) to low-carbohydrate high-fat (LCHF) or high-carbohydrate low-fat (HCLF) diets (Noakes et al. 2023), other findings indicate that LCHF adaptation may take up to 4 weeks to achieve glycemic and metabolic homeostasis (Prins et al. 2025).

To the researchers' knowledge, most of the studies conducted on the use of CGMs have focused on individuals with pathological conditions, such as diabetes, rather than healthy subjects. Most studies in the literature investigating the use of CGMs in physiological conditions have recruited healthy individuals, whereas the field of glycemic responses in athletes, especially endurance athletes, remains largely unexplored, especially in response to different nutritional approaches. Existing studies in physiological contexts are largely observational (Skroce et al. 2024; Zignoli et al. 2023), and some attempted to provide an interpretation framework for CGM-derived glycemic measurements in both nonathletic healthy subjects (Bowler et al. 2023; Flockhart and Larsen 2024) and endurance athletes (Bowler et al. 2024). Despite the latter ensuring standardized diet and exercise conditions, its small sample size and very short duration (4-day trial) make it challenging to establish reliable reference ranges (Bowler et al. 2024). Few studies have examined differences in dietary approaches using CGM: Shiose et al. (2023) examined a one-day low-to-high carbohydrate diet shift in a relatively small cohort ( $n = 20$ ), whereas Prins et al. (2023) conducted a crossover trial on 10 middle-aged professional athletes to analyze the effects of transitioning from an HCLF to an LCHF diet on performance and CGM-monitored glycemic values. Hiromatsu et al. (2023) employed the CGM technology to assess the postexercise glycemic responses, but their trial lasted only 3 days with a sample of just eight male participants. Lastly, Zignoli and colleagues conducted a substantial observational study, contributing to the existing literature on CGM use in professional athletes by monitoring 26 healthy professional cyclists using CGM during two consecutive annual training camps (Zignoli et al. 2025).

Despite growing interest, few studies have explored how different dietary approaches affect CGM-derived glycemic data in athletes and methodological inconsistencies persist. This randomized crossover study aimed to explore and describe glycemic responses to an LCHF versus an HCLF diet in healthy endurance athletes by monitoring 24-h CGM-derived glucose metrics over a two-week period in a real-world setting.

## 2 | Materials and Methods

### 2.1 | Study Design

The study had a randomized crossover design. All subjects followed a standard 3-day “run-in” diet (see section *Intervention*). At the end of the “run-in” phase, the subjects were fitted with the CGM sensor (Libre Sense Sport, Abbott Laboratories, IL, USA) and instructed on the use of the CGM system. Then, according to a computer-generated allocation schedule, participants were randomly assigned to one of two treatment sequences: HCLF and LCHF or LCHF and HCLF diets. Each treatment period lasted 7 days, after which participants crossed over to the other treatment. The 14 days of intervention were entirely covered by the CGM sensor measuring interstitial glucose minute by minute; the interstitial glucose data were automatically transferred and stored on the associated cloud (Figure 1). No wash-out period was present between the two periods.

### 2.2 | Participants

Trained and highly trained, healthy, competitive male cyclists were recruited (Tier 2 and 3, according to McKay et al. (2022)). The main inclusion criteria were age between 18 and 55 years, training level of 6–15 h/week in the 12 weeks preceding the study, and having a power meter installed on the bike. All subjects had a valid competitive medical certificate. The study was approved by the Ethics Committee for Human Experimentation of the University of Urbino (approval report no. 56\_2022) and was conducted according to the ethical principles contained in the Declaration of Helsinki. The subjects were enrolled by word of mouth or by advertising in the sports clubs of the district. All participants were contacted by the principal investigator, informed of the study design, and approved of participation by signing an informed consent form.

### 2.3 | Sample Size

The sample size calculation for a crossover design was based on an expected difference in glycemic variability between the two treatments, according to previously reported data (Blaychfeld-

Magnazi et al. 2020). With a within-patient standard deviation of the response variable of 23 mg/dL, an effect size of 1.06 (large effect), and an allocation ratio of 1:1, using an ANOVA for repeated measures with a difference between treatments of 24 mg/dL, a total of 16 participants would have been recruited at a standard significance level of 0.05 and a power of 0.80.

### 2.4 | Intervention

The protocol started with a 3-day ‘run-in’ diet, a necessary process to standardize individual cyclists coming from different dietary habits. Participants then independently opened a personal profile in the smartphone app of the CGM producer (<https://dashboard.supersapiens.com>, accessed on 6 September 2024, Supersapiens Inc., Atlanta, GA, USA) for the transfer and storage of data collected by the CGM. Each participant’s profile was linked to a dashboard through which the researcher could view and download raw data in CSV format. The biosensor was mounted independently by the individuals in accordance with the manufacturer’s guidelines in the distal part of the brachial triceps. The CGM biosensor (Libre Sense Sport, Abbott Laboratories, IL, USA) measures interstitial glucose levels within a 55–200 mg/dL range; it was paired with the smartphone app via an initial NFC scan and remained connected autonomously for the duration of the study. The time of sensor fitting corresponded to the start of the first assigned diet. The CGM sensor gathered the interstitial glucose data minute by minute as long as the smartphone was connected; otherwise, the sensor recorded and saved the data every 15 min. Participants were instructed to scan the sensor at most every 8 hours to minimize data loss.

*Diet characteristics.* During the HCLF period, participants followed a diet high in carbohydrates (> 5 g/kg/day), whereas during the LCHF period, they followed a diet low in carbohydrates (< 26% of total daily energy) (Oh et al. 2025; Volek et al. 2024); the two diets were isocaloric. Each participant was provided with a personalized nutritional scheme drawn up using the WinFood software (WinFood, Teramo, Italy) on the individual’s personal needs and preferences, respecting the macronutrient balance described above and a calorie quota as close as possible to the estimated normal calorie intake. Basal metabolism was estimated using the Mifflin–St. Jeor formula (Mifflin et al. 1990), to which a coefficient was applied for the

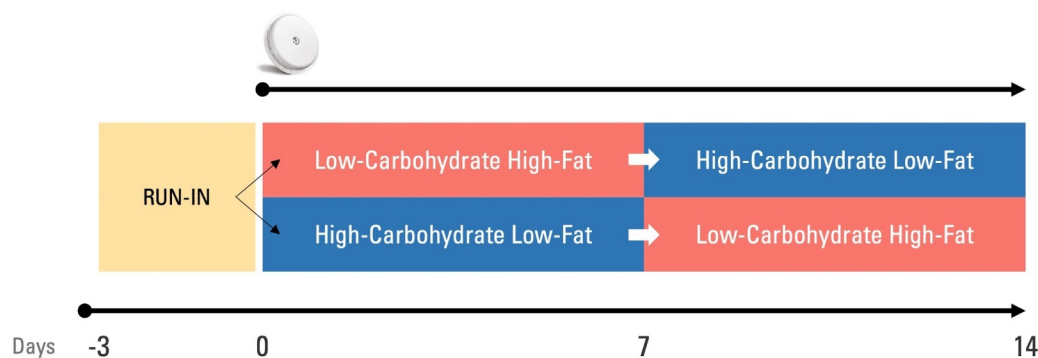


FIGURE 1 | Trial design.

level of physical activity. Examples of dietary plans for the two periods are reported in Table 1.

Each participant had to daily fill in a food diary in which they were asked to write down every food they ate or drank during the day, with the respective quantity, and the time of intake. The food diary was used to highlight any deviations from the prescribed diet. Daily text messages or phone calls were conducted by the researchers to enhance adherence. The supplementation during the training sessions was different for the two dietary approaches: during the HCLF period, the subjects consumed two gels (Keforma, AquaViva Srl, San Marino), each containing 15 g of dextrose; during the LCHF period, the participants consumed a drink (Keforma, AquaViva Srl, San Marino) containing 11.6 g of lipids, 6 g of which were medium chain triglycerides. The two supplement forms (two gels or one drink) were isocaloric. Participants managed the supplementation during the training as needed. At the end of the study, a second contact was made with the researcher to hand over the completed diary. The food diary was then manually uploaded to WinFood software, which allowed the calculation of total calories and macro- and micronutrient intakes.

**Training monitoring.** During the intervention periods, participants were free to train as they were used to. Duration (hours, minutes), distance covered (km), heart rate, and power (via a power meter installed on the bikes) data were collected during each training session with the device mounted on each bike. Data were automatically uploaded on a TrainingPeaks (TrainingPeaks, Louisville, CO, USA) account after each training session for the subsequent analyses. The training log of the 6 months before the study was analyzed for each participant to estimate the critical power (CP) value from training and race power profile (maximum mean powers between 2 and 15 min were used) using a 3-parameter CP model (Leo et al. 2022; Mattioni Maturana et al. 2018). Then, the critical power obtained was used to calculate the standardized intensity for each training session as:  $Intensity\ Factor\ (IF) = Normalized\ Power\ (NP)/Critical\ Power\ (CP)$ .

## 2.5 | Outcomes

Glucose profiles were evaluated based on data collected during the 7 days of each treatment period (HCLF vs. LCHF). Then,

glucose data were categorized into three phases: wake, exercise, and sleep. The nocturnal period was arbitrarily defined as the time between 00:00 and 06:00 h (according to Vu et al. (2019)), as the real sleep time was not monitored. The mean glucose, coefficient of variation (CV), and mean amplitude of glycemic excursions (MAGE) were calculated for each phase of each day and considered as outcome variables. MAGE is the arithmetic mean of the amplitude of glucose excursions greater than the glucose values' standard deviation. The "time in zone" (daily time spent in each glucose range) was calculated for each of the following ranges: < 70 mg/dL (~3.9 mmol/L), 70–120 mg/dL (~3.9–6.7 mmol/L), 120–180 mg/dL (~6.7–10 mmol/L), and > 180 mg/dL (10 mmol/L). Data were analyzed using the Iglu Shiny app (Broll et al. 2021).

## 2.6 | Statistical Analyses

Data were reported as mean  $\pm$  standard deviation or median (2.5 and 97.5 percentiles), where appropriate. This latter solution was necessary as some variables showed a skewed distribution. A descriptive independent samples *t*-test was used to compare the baseline participants' characteristics between the two dietary interventions (LCHF > HCLF vs. HCLF > LCHF). An ROC analysis was used to quantify the accuracy of diet classification (HCLF vs. LCHF) based on the amount of carbohydrates ingested per day for each subject. The area under the curve (AUC) was calculated to assess adherence to the prescribed dietary plan. The optimal cutoff point for categorizing a diet as HCLF or LCHF was determined using Youden's *J* statistic. A linear mixed model was conducted on repeated measures (14 days) to investigate the effect of diet (HCLF vs. LCHF) and phases (exercise, wake, night) on the glucose mean and variability indices (MAGE, CV); diet, phases, and their interaction were included as fixed effects, and subject ID was included as a random effect to account for within-subject variability. The wake phase was considered as the reference for the post hoc analyses. Furthermore, the same linear mixed model analysis was performed considering daily carbohydrate intake (absolute value, grams) as a covariate in the model in order to highlight the relative linear dependence of the response variables. A permutational multivariate analysis of variance (PERMANOVA) was conducted to examine the effect of diet, phases, and their interaction on the "time in zone" (see above for the ranges used), using the Euclidean distance, with strata defined

**TABLE 1** | Examples of meal content in the HCLF and LCHF diets.

	HCLF	LCHF
Breakfast	Bread (150g), baked ham (80g), one apple	White yogurt (150g), dark chocolate (40g), one peach
Snack	One banana	Protein bar
Lunch	Pasta (150g), eggs (80g), grana padano cheese (20g), extra-virgin oil	Wholemeal bread (60g), ham (80g), mozzarella (110g), tomatoes (250g), extra-virgin oil
Snack	Almonds (20g)	Almonds (30g)
Dinner	Bread (150g), mozzarella (130g), tomatoes (200g), extra-virgin oil	Wholemeal bread (60g), Turkey (170g), salad (150g), almonds (30g), extra-virgin oil
Macronutrients (%)	C: 55%, P: 20%, F: 25%	C: 20%, P: 22%, F: 58%

Abbreviations: C, carbohydrates; F, fats; P, protein.

by subject ID. The PERMANOVA approach was chosen because it is distribution-free, and the dependent variable showed a skewed distribution toward zero values (in particular, in the two extreme ranges). Finally, linear mixed model analyses were performed on a subset of data to (a) examine the effects of diet type, exercise duration, and intensity on mean glucose levels during the exercise and (b) explore the effect of an exercise session in the previous day on the nocturnal glycemia. All the analyses were conducted on RStudio (v. 2024.04.02, Posit Software, PBC) at a standard significance level of  $\alpha = 0.05$ .

### 3 | Results

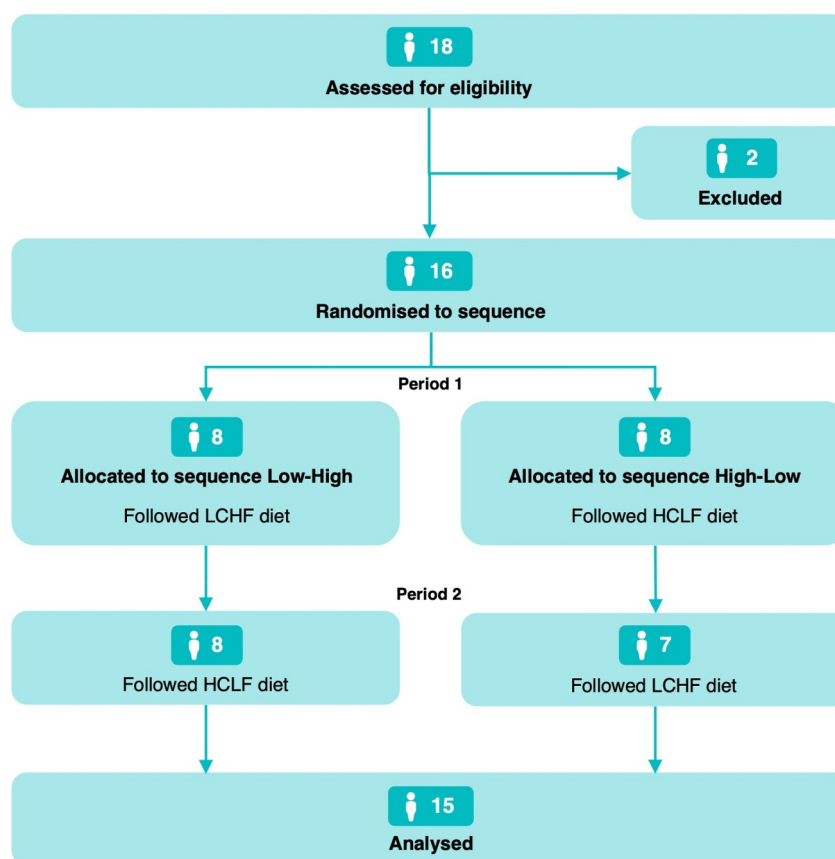
Eighteen participants were initially assessed for eligibility. Two of them were excluded because their phones were not compatible with the CGM sensor, and this would not have allowed the

recording of data. Sixteen participants were then randomized to sequence with a 1:1 allocation ratio; one participant in the HCLF > LCHF arm did not complete the study due to personal reasons. The participants' flow is presented in Figure 2.

Participants' baseline characteristics are reported in Table 2.

#### 3.1 | Dietary Adherence

The adherence to the prescribed diet was monitored using a food diary that participants had to fill out day by day. Daily total calories and carbohydrate intake were then calculated for the two periods (HCLF vs. LCHF). No meaningful differences in the daily caloric intake were reported between the two diets (HCLF:  $2571 \pm 401$  kCal; LCHF:  $2576 \pm 414$  kCal;  $p > 0.05$ ). As expected, significant differences were reported for the absolute



**FIGURE 2** | Participants' flow. HCLF = high-carbohydrate low-fat; LCHF = low-carbohydrate high-fat.

**TABLE 2** | Participants' baseline characteristics.

	LCHF > HCLF (n = 8)	HCLF > LCHF (n = 7)	T (prob)
Age (y)	29.1 ± 9.5	27.3 ± 11	0.346 (0.735)
Height (cm)	172.1 ± 6.5	176.4 ± 6.9	1.240 (0.237)
Body mass (kg)	67.6 ± 6.2	73.4 ± 11.2	1.265 (0.228)
Training volume (h/week)	11.7 ± 3.2	9.5 ± 2.5	1.534 (0.149)
Critical power (W)	305 ± 46	302 ± 37.6	0.106 (0.917)
Critical power (W/kg)	4.51 ± 0.59	4.20 ± 0.81	0.876 (0.397)

(HCLF:  $344 \pm 80$  g; LCHF:  $182 \pm 64$  g;  $p < 0.001$ ) and relative (HCLF:  $5.15 \pm 1.22$  g/kg; LCHF:  $2.71 \pm 0.9$  g/kg;  $p < 0.001$ ) carbohydrate intake between the two periods. An ROC curve was used to evaluate the adherence of the participants to the dietary protocol. It showed an area under the curve of 0.976, indicating excellent discrimination ability (Figure 3). At the optimal cutoff point of 0.466 (corresponding to  $\sim 260$  g CHO), the model demonstrated a sensitivity of 96.0% and a specificity of 96.1%.

### 3.2 | Diet and Phase Effects

A linear mixed model was conducted to investigate the effect of diet (HCLF vs. LCHF) and phases (exercise, wake, night) on the different glucose indices. For the mean glucose, the analysis revealed a significant main effect of diet ( $F_{(1,525)} = 14.68$ ,  $p < 0.001$ ), with lower mean glucose levels observed in the LCHF condition compared to the HCLF condition ( $\beta = -3.77$ ,  $SE = 1.47$ ,  $p = 0.011$ ). There was also a significant main effect of phase ( $F_{(2,525)} = 206.38$ ,  $p < 0.001$ ), where the exercise phases showed the highest glucose levels ( $\beta = 8.89$ ,  $SE = 1.36$ ,  $p < 0.001$ ) compared to the wake phases, and night phases showed the lowest levels ( $\beta = -10.74$ ,  $SE = 1.25$ ,  $p < 0.001$ ). No significant interaction effect between diet and phases was found.

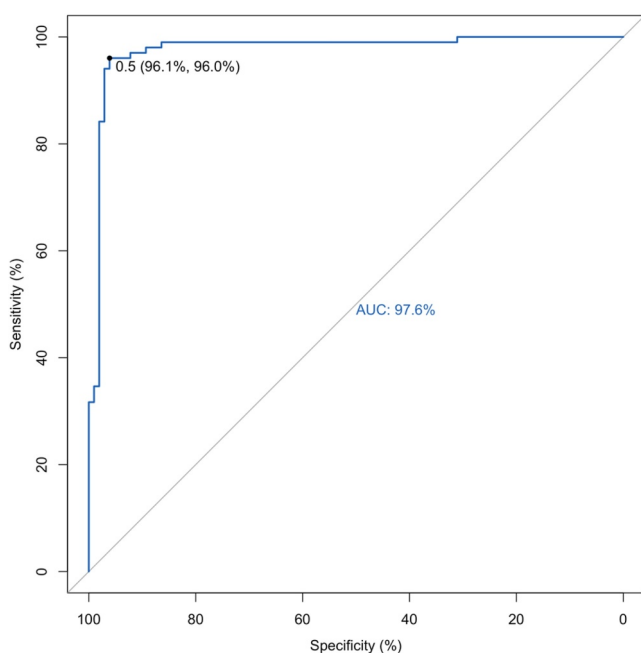
Regarding the MAGE index—which represents the mean amplitude of the glycemic excursions over one standard deviation—the analysis revealed a significant main effect only for the phase ( $F_{(1,516)} = 151.31$ ,  $p < 0.001$ ), where the wake phases showed the highest MAGE levels compared to the exercise phases ( $\beta = -5.69$ ,  $SE = 2.22$ ,  $p = 0.011$ ) and the night

phases ( $\beta = -24.25$ ,  $SE = 2.01$ ,  $p < 0.001$ ). No significant interaction effect between diet and phases was found.

When considering the coefficient of variation (CV) of the glucose measures, the analysis revealed a significant main effect of diet ( $F_{(1,525)} = 4.73$ ,  $p = 0.030$ ), with lower glycemic variations observed in the LCHF condition compared to the HCLF condition ( $\beta = -1.31$ ,  $SE = 0.60$ ,  $p = 0.028$ ). There was also a significant main effect of phases ( $F_{(2,525)} = 159.28$ ,  $p < 0.001$ ), where the wake phases showed the highest CV compared to the exercise phases ( $\beta = -3.28$ ,  $SE = 0.65$ ,  $p < 0.001$ ) and the night phases ( $\beta = -8.18$ ,  $SE = 0.60$ ,  $p < 0.001$ ). No significant interaction effect between diet and phases was found.

Considering the distribution of the glucose data within the different ranges, the results of the PERMANOVA analysis indicated a significant main effect of phases for all the ranges ( $< 70$ ,  $70$ – $120$ ,  $120$ – $180$ , and  $> 180$  mg/dL), suggesting that the glycemic categorized distribution differed significantly across phases. Overall, the time in the  $70$ – $120$  mg/dL zone was maximized during the night phases (medians 98.2% and 100.0% of the total nighttime in the LCHF and HCLF conditions, respectively), whereas the time in the  $120$ – $180$  mg/dL range was significantly higher during the exercise phases, although the median time spent in that zone was low (7.0% and 11.9% in the LCHF and HCLF conditions, respectively). Notably, the ranges (2.5 and 97.5 percentiles) are quite broad, suggesting high individual differences. Significant effects of diet were reported only in the  $121$ – $180$  mg/dL range ( $F_{(1, 539)} = 2.93$ ,  $p = 0.047$ ), with a higher proportion of time spent in this range during the HCLF diet. Lastly, a significant interaction effect between the diet and phase was reported only in the  $> 180$  mg/dL range ( $F_{(2, 539)} = 2.47$ ,  $p = 0.044$ ), with a higher time in this zone during the exercise phases in the HCLF diet.

All the descriptive statistics are reported in Table 3.



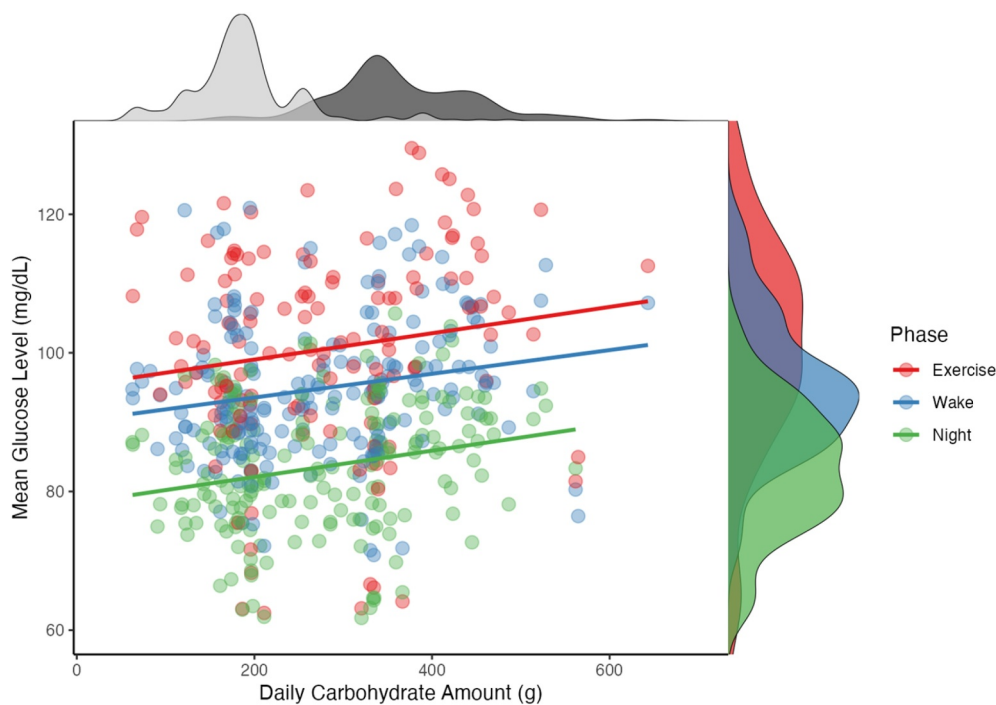
**FIGURE 3** | ROC curve quantifying the adherence of participants to the assigned diet. The AUC value quantifies the global accuracy of classification.

As the adherence to the diets by the subjects was not perfect—as shown by the ROC curve—we decided to also conduct the linear mixed model analysis also considering the daily carbohydrate intake (in grams) as a covariate in the model instead of using the diet as a binary factor. The analysis revealed a significant main effect of carbohydrate intake on the mean glucose ( $\beta = 0.015$ ,  $SE = 0.003$ ,  $p < 0.001$ ). It also confirmed the significant main effect of phases ( $F_{(2,516)} = 21.37$ ,  $p < 0.001$ ), where the exercise phases showed the highest glucose levels ( $\beta = 7.85$ ,  $SE = 0.95$ ,  $p < 0.001$ ) compared to the wake phases, and night phases showed the lowest levels ( $\beta = -11.26$ ,  $SE = 0.98$ ,  $p < 0.001$ ). Again, no significant interaction effect between diet and phases was found. The analysis is graphically shown in Figure 4.

### 3.3 | Interindividual Variability

Subject ID was included in the linear mixed model analysis as a random effect in the model to account for the repeated measures within subjects. The inclusion of the random intercept for subject ID significantly improved model fit compared to a model without this random effect, as indicated by a likelihood ratio test (LRT = 329.12,  $p < 0.001$ ). This suggests substantial variability





**FIGURE 4** | Scatter plot reporting the daily carbohydrate amount (g, x-axis) and the mean glucose level (mg/dL, y-axis), grouped according to the three phases (exercise (red), wake (blue), night (green)). On the top of the plot are reported the distributions (kernel density estimations) of the carbohydrate intake in the two phases (HCLF, dark gray; LCHF, light gray). On the right side are reported the distributions of the mean glucose level in the three phases.

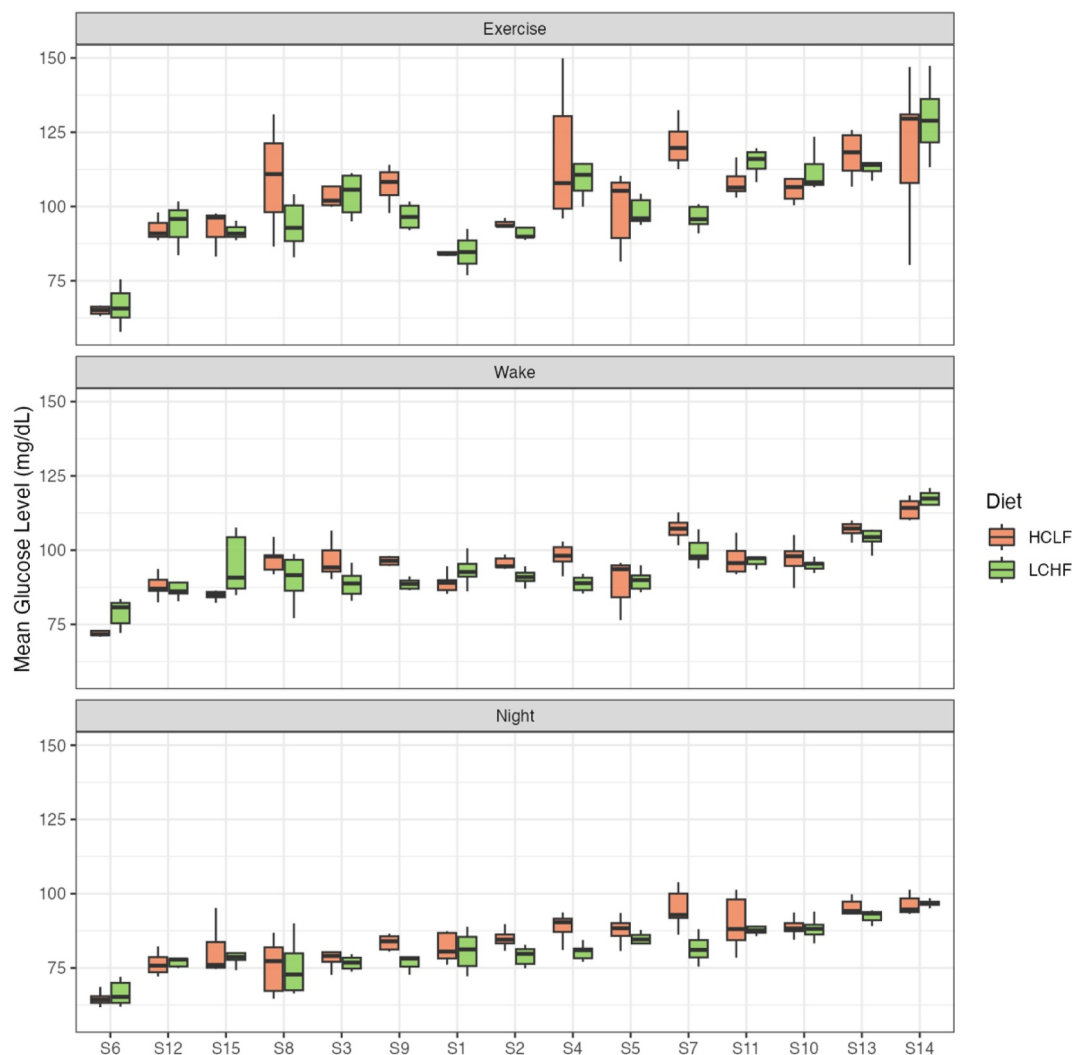
#### 4.1 | Diet Effect

In the present study, significantly lower mean blood glucose levels were observed in the LCHF condition, across all daily phases (wake, exercise and night), with carbohydrate intake emerging as a major determinant of mean glucose concentration. These findings align with prior studies showing that HCLF diets, even when isocaloric, lead to higher mean and peak glucose levels (Shiose et al. 2023). The increase in blood glucose is associated with a delay in fatigue, supporting energy production and preserving muscle glycogen stores (Hargreaves 1997). For instance, Prins et al. (2023) reported in a randomized crossover study that mean blood glucose decreased from day eight of a 31-day LCHF intervention. Notably, our study observed a reduction in mean glucose even with a shorter duration of the dietary intervention. The LCHF diet was also associated with reduced glycemic variability, as indicated by significantly lower coefficients of variation (CV), and less time spent in the 121–180 mg/dL range, especially during wake and exercise phases. The lower availability of glucose in LCHF diets leads to reduced glycogen synthesis as the primary substrate is limited (Podlogar et al. 2023). It is reasonable to assume that, since a significant portion of the ingested glucose is used to replenish glycogen stores, this reduced glucose availability results in lower average blood glucose levels and decreased glycemic variability.

#### 4.2 | Phase Effect

A significant effect of the day phase was observed across all glycemic metrics. Mean glucose levels were significantly higher

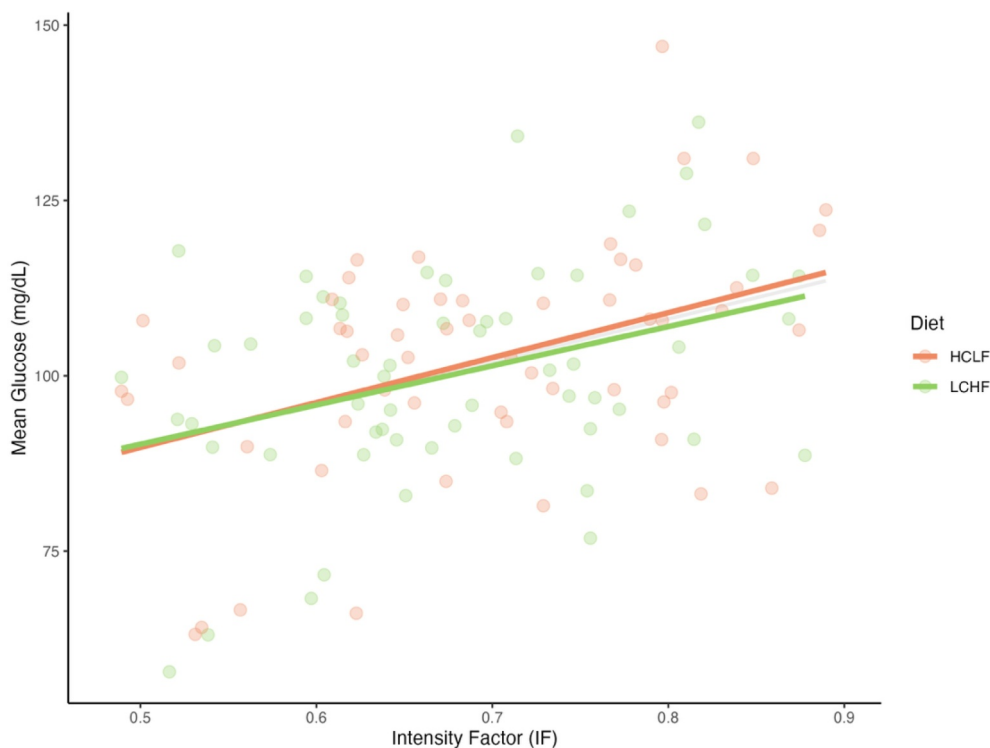
during the exercise, which aligns with the literature indicating that acute physical exercise elevates glucose levels, likely as a mechanism to support the energy demands of active muscles (Noakes et al. 2023; Prins et al. 2023; Skroce et al. 2024). The reason for elevated glucose levels during exercise is biochemical and physiological in nature as hepatic glucose production increases during physical activity due to glycogenolysis, which predominates during intense exercise, and gluconeogenesis, which primarily supports prolonged physical exertion (Kjaer 1998). In this study, exercise intensity was the sole predictor of glucose levels during exercise, confirming the hypothesis that higher training intensity corresponds to higher mean glucose levels. The increase in blood glucose concentration with rising exercise intensity is more evident in trained athletes compared to recreational athletes, likely due to a higher rate of hepatic glycogenolysis in well-trained individuals. Highly trained subjects have demonstrated a greater ability to meet the increasing energy demands of high-intensity exercise through enhanced glucose metabolism, whereas those with lower training levels tend to rely more heavily on muscle glycogen utilization (Aandahl et al. 2021). Additionally, a significant diet  $\times$  phase interaction was observed for the time spent in the  $> 180$  mg/dL range, with the highest values occurring during exercise in the HCLF condition, likely due to the combination of high exercise intensity and increased circulating glucose resulting from the high-carb diet. The time spent in the 120–180 mg/dL range was greater during the exercise phase in both diet conditions (HCLF: 11.9%; LCHF: 7.0%) compared to wake and night phases. Conversely, glycemic variability indices (CV and MAGE) were higher during the wake phase than during exercise or nighttime. This aligns with previous findings showing higher daytime versus nighttime MAGE assessed in 12



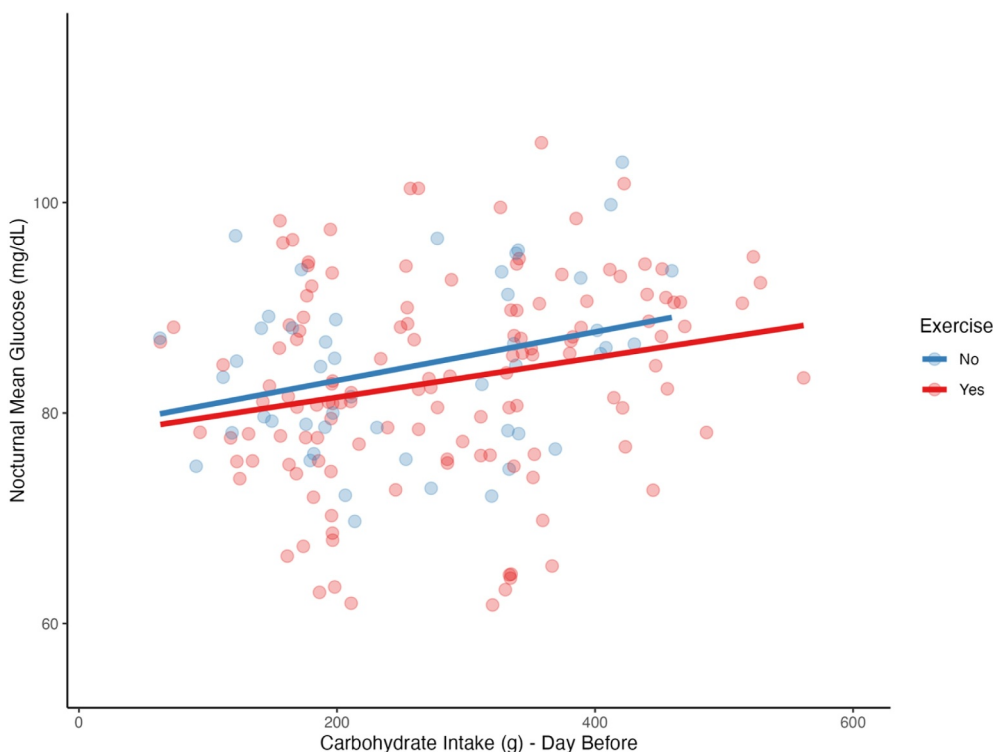
**FIGURE 5** | Box plot of the individual mean glucose level of the participants in the three phases (exercise, wake, night) and the two diets (HCLF, LCHF). Subjects (x-axis) are ordered according to the median glucose level during the night phase.

elite race walkers (Bowler et al. 2024). This increased glycemic variability during the wake phase may be explained by elevated catecholamine levels (e.g., adrenaline, noradrenaline) in response to variations in exercise intensity, which could also influence overall glycemic control throughout the day (Bowler et al. 2024). Furthermore, the adrenaline surge may also explain the significantly higher glucose levels observed during exercise, as it is a primary driver of hepatic glycogen reserve mobilization (Bowler et al. 2024). Endurance athletes tend to exhibit increased glycemic variability as a physiological adaptation to training, and variability itself might even be considered a marker of recovery, reflecting the inflammatory response to endurance exercise (Flockhart and Larsen 2024). Finally, the time spent in the 70–120 mg/dL range was maximized during the night phase. This is particularly relevant as nocturnal hypoglycemia has been shown to disrupt sleep by triggering awakening through epinephrine release, which is induced when glucose levels drop below 50 mg/dL (2.8 mmol/L). This disruption negatively affects sleep quality by increasing nighttime wakefulness and impairing recovery (Flockhart and Larsen 2024). In the present study, the time spent below 70 mg/dL during the nighttime phase was 0% in the HCLF condition and 1.1% in the LCHF condition, lower than the 3.7% of time

below the range observed by Skroce et al. (2024) in a large cohort of physically active men and women. Consistent with the lower mean glucose levels observed in the LCHF condition, carbohydrate intake appears to significantly influence nighttime glucose levels. Prior studies showed that glucose levels during sleep follow a circadian pattern, with a nocturnal nadir occurring around 4 a.m., typically 2–3 h before waking (Skroce et al. 2024). Although data on nocturnal glucose dynamics in healthy populations are limited, DuBose et al. (2021) found a reduction in mean nighttime glucose when exercise had been performed the previous day in a cohort of 153 healthy individuals aged 7–80 years. Interestingly, the present study supports this hypothesis, extending it to highly trained endurance athletes, consistently with the findings by Zignoli et al. (2025). Indeed, our data show that participants who exercised on the previous day had significantly lower nocturnal glucose levels compared to nonexercising days. This observation is probably due to glycogen replenishment time. In humans, liver glycogen stores are critically reduced during endurance training at moderate intensity (Casey et al. 2000), and liver glycogen replenishment takes longer than muscle glycogen replenishment. Considering cycling to exhaustion at 70% of  $\text{VO}_2\text{max}$ , liver glycogen is estimated to be restored in approximately 11 h,



**FIGURE 6** | Linear regression of exercise intensity (intensity factor (IF)) and mean glucose. Different colors represent the two diets (HCLF, LCHF).



**FIGURE 7** | Scatter plot reporting the carbohydrate amount ingested in the previous day (g, x-axis) and the nocturnal mean glucose level (mg/dL, y-axis), divided according to exercise (red) or not exercise (blue) in the previous day.

whereas muscle glycogen takes about 9 h (Gonzalez et al. 2016). This evidence may help explain the lower nocturnal glucose levels observed in individuals who trained the previous day compared to rest days.

### 4.3 | Intra- and Interindividual Variability

The present study highlights strong intraindividual variability in glycemic responses, modulated by both the phase and diet. One

potential source of this variability could be pre-study carbohydrate consumption habits (Shiose et al. 2023). Individuals accustomed to low carbohydrate intake tend to exhibit higher mean glucose levels when switching to a higher-carbohydrate diet, particularly in the afternoon. Conversely, individuals with pre-diabetic glucose levels during a high-carb diet showed greater responsiveness to carbohydrate restriction when transitioning to a low-carb diet, resulting in more pronounced glucose reductions, as observed by Prins et al. (2023). Another important factor in individual variability is training status. Skroce et al. (2024) observed that subjects who self-classified as experienced athletes had glucose levels approximately 10 mg/dL higher during exercise than nonathletes, a trend also evident in our sample, where higher-intensity training sessions corresponded with elevated exercise-phase glucose. Additional individual variability is attributable to intrinsic metabolic and mitochondrial factors. Mitochondrial volume, function, and adaptive capacity decline with age and appear to be associated with insulin resistance, leading to altered glucose metabolism, reduced fat oxidation, decreased ATP synthesis, and a simultaneous increase in reactive oxygen species (Bowler et al. 2024; Noakes et al. 2023). Furthermore, somatotype appears relevant, as individuals with endomorphic characteristics tend to exhibit higher blood glucose levels, whereas mesomorphic profiles are associated with lower levels (Bowler et al. 2023). Extrinsic factors should also be considered. Although current evidence remains limited, it is hypothesized that variations in dietary macronutrient composition, food form (e.g., smoothie vs. whole fruit), and gastrointestinal absorption mechanisms also affect glycemic variability (Flockhart and Larsen 2024). Lastly, measurement-related variability must also be considered. Differences in the correlation between interstitial and capillary glucose, as well as variations in the ability of CGM devices to accurately detect glucose changes between interstitial and circulating pools, may introduce additional individual variability (Flockhart and Larsen 2024; Hutchins et al. 2025).

#### 4.4 | Limitations

This study presents several limitations that should be acknowledged. First, the CGM devices used in this research are limited to a detection range of 50–200 mg/dL, potentially overlooking extreme hypo- or hyperglycemic events. Second, the estimation of critical power was derived from training and racing data rather than standardized laboratory testing, which may have affected the accuracy of exercise intensity classification. Third, the nocturnal phase was uniformly defined as 00:00–06:00, according to the literature but without considering individual sleep patterns, potentially masking intraindividual glycemic variability related to actual sleep timing. Fourth, the analysis focused on aggregated glycemic responses over the 7-day period, without considering day-by-day dynamics that could offer additional insights into acute adaptations to dietary changes. Indeed possible additional limitation is that our study design did not include a wash-out period, and therefore we cannot exclude carry-over effects that may have influenced the results. This choice was made for several reasons: (a) extending the protocol beyond 14 days would have markedly reduced compliance with the training study; (b) related to the previous

point, it would have been difficult to standardize nutrition during a sufficiently long wash-out period to allow glycemic patterns to return to baseline values, which could have required several weeks (Ludwig et al. 2025); and (c) in real-world conditions, cyclists do not usually follow prolonged periods of exclusively HCLF or LCHF diets but, according to recent guidelines, periodize their nutrition based on the training plan. Consequently, assessing whether glycemic adaptations occur when diet is modified over a short-term window (i.e., days) provides more practical and applied information for athletes and coaches who routinely use CGMs. Future studies should investigate the kinetics of changes in glycemic patterns following dietary modifications to determine how many days possible carry-over effects persist in this population, as this may be important when interpreting CGM data. Finally, a limitation that should be acknowledged concerns the diet followed by athletes in the low-CHO phase. Our participants did not achieve a strict LCHF diet (< 25% of energy intake from CHO, as defined by Volek et al. (2024)); on average, they consumed 28% of their calories from CHO, placing them in a moderate-carbohydrate diet regimen. It should also be acknowledged that the Position Stand of the International Society of Sports Nutrition (Aragon et al. 2017) defines low-CHO diets as those providing up to 40% of total kcal from CHO. When considering diet categorization based on absolute carbohydrate intake, our athletes consumed about 180 g of CHO/day on average, whereas the low-CHO limit for a 2600 kcal/day diet would be approximately 165 g (according to the 25% threshold). It should also be noted that 180 g of CHO corresponds to roughly 2.7 g/kg BM/day (for a 70-kg athlete), which is below the usual recommendation of 3 g/kg BM/day for CHO intake during low-intensity or skill-based activities. However, achieving a very low-CHO diet is particularly challenging in this population; because athletes were asked to maintain their training schedule, on some days, they were required to eat slightly more than prescribed due to very long or intense training sessions or races. Moreover, the dietary plan we provided was standardized over the 7 days of each treatment and was not adjusted daily according to individual training loads.

#### 5 | Conclusions

The main findings of this study showed that both the diet (HCLF, LCHF) and the daily phase (wake, exercise, night) significantly influence glycemic parameters, especially LCHF that is associated with a lower average glucose and reduced glycemic variability. It is reasonable to assume that this result derives from the fact that the few carbohydrates consumed are mainly used to restore glycogen reserves, with a consequent lower availability of circulating carbohydrates (reduced average glucose) reduced glycemic variability. Exercise intensity has been shown to be a determinant of glucose levels with significantly very high levels during training, probably due to the combination of glycogenolysis and gluconeogenesis leading to an increase in available glucose. Another effect of exercise is observable during the night: mean glucose is significantly lower in subjects who trained the day before compared to rest days, showing that this phenomenon is not only valid for the healthy population but also for trained endurance athletes. Importantly, the study revealed a substantial

degree of individual variability in glycemic responses, likely reflecting differences in metabolic profiles, training history, and habitual diet. These results underscore the potential value of personalized dietary strategies for optimizing metabolic efficiency and recovery in endurance athletes. Future research should explore long-term adaptations and incorporate individualized sleep-wake patterns and day-by-day glucose trends to better understand the acute and chronic effects of dietary modulation on athletic performance and health.

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## Ethics Statement

The study was approved by the Ethics Committee for Human Experimentation of the University of Urbino (approval report no. 56\_2022).

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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