












Glycaemic Response to Acute Aerobic and Anaerobic Exercise Performed in the Morning or Afternoon in Healthy Subjects: A Crossover Trial

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ABSTRACT

Background: The regular practice of physical activity is considered a health promoter and appears to be one of the main contributors to the prevention of chronic diseases. However, the potential effects of exercise on health depending on the time of day at which it is performed have not yet been fully elucidated.

Objectives: To evaluate the effect of physical exercise (aerobic or anaerobic) and chronobiology (morning or afternoon) on the glycaemic metabolism of healthy subjects.

Methods: Healthy subjects participated in aerobic or anaerobic physical exercise sessions, either in the morning or in the afternoon. Blood was drawn from the subjects before, at the end of the exercise and 2 hours after the end of the exercise. Glycaemic parameters were analyzed at these time points. A general linear model test was performed after verifying the normal distribution of the raw data (as assessed by the Shapiro-Wilk test) or after a logarithmic/square root transformation, considering aerobic or anaerobic exercise and morning or afternoon exercise as independent variables.

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Results: Twenty-three subjects (14 women and 9 men) were included in the study. The rate of change in glucose levels was significantly higher at the end of anaerobic exercise compared to aerobic exercise (1.19 ± 0.04 vs. 0.98 ± 0.02 , respectively), with a more pronounced decrease in insulin and C-peptide levels following aerobic exercise. In addition, the increase of glucose was higher after the exercise in the morning compared with the afternoon (1.14 ± 0.03 vs. 1.03 ± 0.03 , respectively).

Conclusions: The type of exercise and chronobiology influence short-term glucose metabolism.

1. Introduction

Physical exercise has been demonstrated to exert significant benefits on health, including the prevention of cardiometabolic diseases and other chronic conditions (e.g. certain types of cancers) [1,2]. It has also been shown to reduce cardiovascular and overall mortality in several population-based cohort studies [3,4]. Importantly, different training types (i.e. aerobic, and anaerobic exercise) are related to distinct metabolic adaptations and responses that may have an influence on these health-related outcomes [5,6]. Moreover, additional factors (i.e. the intensity, frequency, or duration of training) may also impact these results [7,8].

The influence of circadian rhythms on human physiology has been previously described [9]. The disruption of circadian rhythms may play an important role in the pathophysiology of metabolic diseases [10]. In this regard, time-based therapeutic strategies for the management of different metabolic conditions, including dietary and drug interventions, have been developed in the last few years with promising results [11]. Recent research has suggested that time-of-day-based exercise may also elicit different metabolic responses. Accordingly, some studies, mainly conducted in patients with obesity and/or type 2 diabetes, have shown that the performance of physical exercise at different times of day (i.e. morning and afternoon/evening) may be related to different outcomes concerning fasting/postprandial glucose concentrations [12–14]. Therefore, in the context of glycemic outcomes, afternoon/evening exercise appears to demonstrate greater effectiveness when contrasted with morning exercise, as indicated by various studies [14,15]. Nevertheless, it is essential to acknowledge the existence of conflicting findings, as reported in some studies [2,16].

In line with previous reports, it can be hypothesized that exercise-related metabolic benefits might vary depending on the time of day at which it is performed. Specifically, we propose that both acute aerobic and anaerobic exercise will have distinct effects on glycemic response, and that these effects will be time-dependent. Despite the potential significance of exercise timing, this area remains underexplored. Therefore, in this study, we aim to investigate how the timing of exercise (morning versus afternoon) and the type of exercise (aerobic versus anaerobic) influence glycemic response in healthy volunteers.

2. Material and methods

2.1. Study design and participants

This study was a randomized crossover trial (ClinicalTrials.gov identifier NCT05600192) conducted at Virgen de la Victoria University Hospital (Malaga, Spain). Healthy volunteers aged 18–55 years, consisting of non-trained participants who performed less than 20 minutes of moderate physical activity twice per week, were included in this trial. Exclusion criteria included pregnancy, smoking and alcohol abuse (<12 months), the presence of cardiovascular disease, metabolic disease, a body mass index ≥ 30 kg/m², renal/liver disease, acute or chronic inflammatory diseases, infectious diseases, active cancer, blood clotting disorders or mental illnesses.

The final sample consisted of 23 healthy subjects of both sexes, who had given their approval to take part in the study. The subjects completed a structured interview to obtain the following data: sex, age, medical history, exercise habits, drug consumption. In order to evaluate the chronotype of the participants and the link with their glycaemic responses, a Spanish version of the morningness-eveningness questionnaire of Horne JA & Östberg was performed [17]. All subjects underwent a standardized anthropometric examination: weight, height, and biochemical parameters. Baseline peripheral venous blood samples were collected after a 12-hour overnight fast from all subjects for biochemical parameter assessment prior to the exercise intervention. Participants were subjected to a four-week program, with a weekly session of aerobic or anaerobic physical exercise supervised at the University of Malaga facilities. Between each session, a period of six days of washing was respected.

2.2. Ethics approval and consent to participate

This study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of Virgen de la Victoria University Hospital (25 March 2021). All participants gave their signed informed consent to participate in this trial.

2.3. Exercise intervention

Exercise intervention consisted of a morning (8–10 a.m.) or afternoon (3–5 p.m.) single session of anaerobic (high-intensity interval training, HIIT) or aerobic exercise (moderate-intensity cycling) (Figure 1a). Both, aerobic and anaerobic sessions were performed at a cycling ergometer. Workload heart rate was monitored by the chest strapped Polar H10 (Polar Electro Oy, Kempele, Finland) [16] and the maximal heart rate (MHR) was calculated by the formula from Tanaka *et al.* [18]. Anaerobic exercise interventions started with a 10 min warm-up, followed by fifteen intermittent 15 s pulses at >80% of MHR; each pulse was followed by 45 s of active recovery. For aerobic exercise, moderate intensity cycling sessions commenced with a 10 min warm-up, followed by 32 min of cycling at 65–70% of MHR. Measurements of MHR were used to control that aerobic and anaerobic exercises were being performed (Figures 1b and 1c).

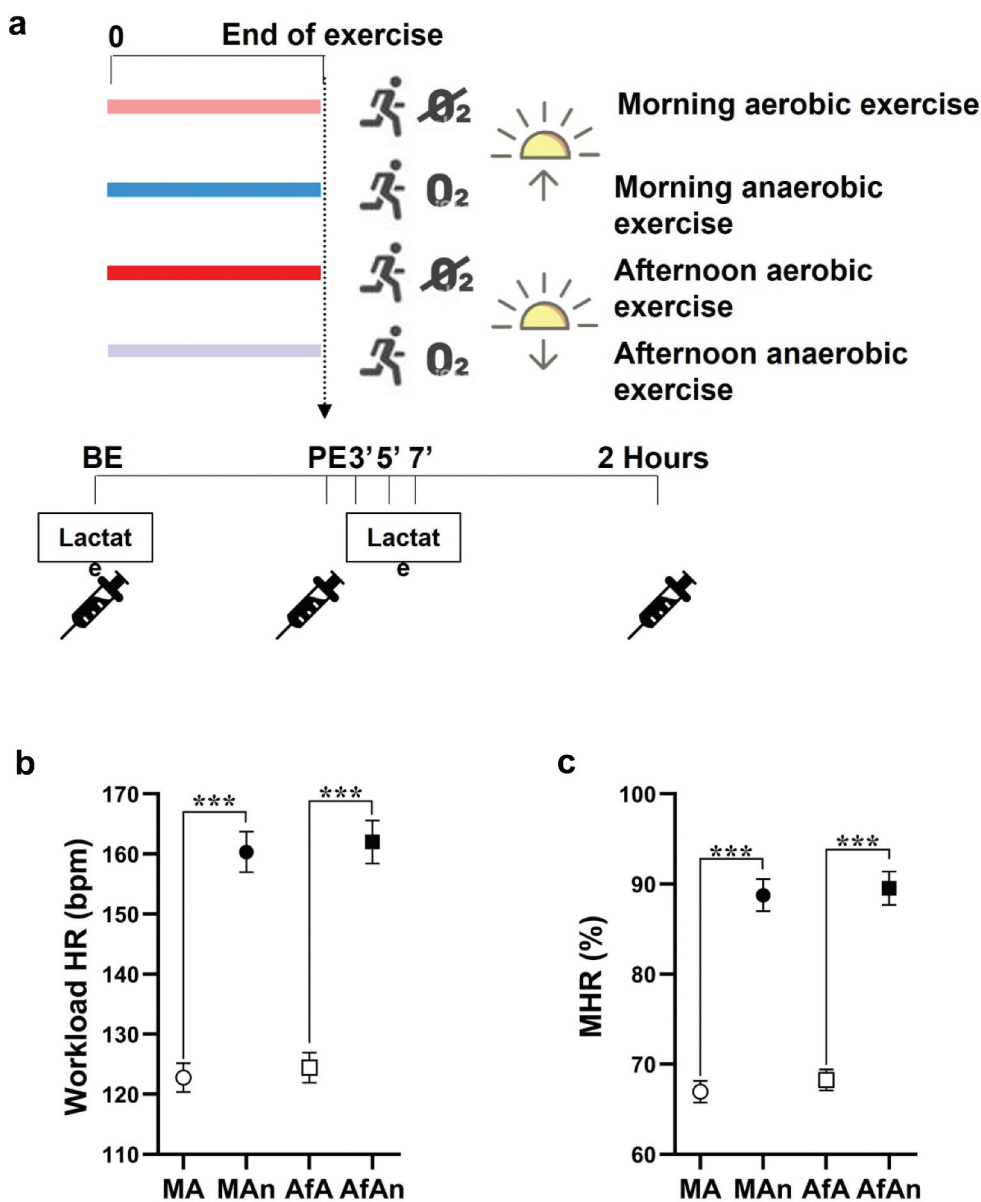


Figure 1. (a) Study design. (b) Workload heart rate in the four types of interventions. (c) Maximum heart rate in the four types of interventions. Mean \pm SEM. ***($p < 0.001$). AfA, afternoon aerobic exercise; AfAn, afternoon anaerobic exercise; BE, before exercise; HR, heart rate; MA, morning aerobic exercise; MAn, morning anaerobic exercise; MHR, maximum heart rate.

Capillary blood lactate concentration was determined by Lactate Pro 2 (AKRAY Europe, Amstelveen, the Netherlands) [19] from the clean fingertip of participants before physical exercise and at 3, 5 and 7-minutes post-exercise.

Participants completed the 4 different training interventions (morning aerobic exercise, morning anaerobic exercise, afternoon aerobic exercise, and afternoon

anaerobic exercise) on the same day of the week during 4 different consecutive weeks. The different possible sequences of the four interventions were randomly assigned using R, where each intervention was independent of the preceding ones, and each participant had a different sequence. Six participants started with morning aerobic exercise, six with morning anaerobic exercise, five with afternoon aerobic exercise, and six with afternoon anaerobic exercise. Participants were requested to eat the same light meal 2 hours before each training session and Mediterranean diet was recommended during the study. The macronutrient content of meals consumed the day before and the day of the exercise was evaluated (Supplementary Tables S1–3).

2.4. Sample collection and biochemical evaluation

Peripheral venous blood samples were collected at three points during each intervention: at the beginning of the session, immediately after physical exercise intervention and two hours after exercise. Biochemical variables were analyzed at the Virgen de la Victoria University Hospital clinical laboratory. The parameters including glucose, insulin, C-peptide, and lactate were evaluated before, at the end, and two hours after each exercise session, with glucose levels being the primary outcome. Lactate was evaluated before and 3, 5 and 7 minutes after the end of the exercise session.

2.5. Statistical analysis

The results are given as the mean \pm standard deviation (SD) or standard error of the mean (SEM). The glycemic metabolism parameter changes after exercise were evaluated using paired analysis within each intervention (Student's t-test or the Wilcoxon test, depending on the normality of the data distribution). Differences in workload heart rate, maximum heart rate, and macronutrient content between the four interventions were evaluated using the Friedman test and post hoc analysis. The change rate of the variables was calculated by dividing the variable after the exercise (PE) or 2 h after the exercise (2hPE) by the variable before exercise (BE) or by dividing 2hPE by PE values. The effect of exercise timing, exercise type, or the interaction between them on the rate of change of biochemical variables (glucose, insulin, C-peptide and lactate) was performed by univariate two-way general linear model (GLM). The exercise timing (morning and afternoon) and the exercise type (aerobic and anaerobic) were introduced as independent variables, and the change of rate of glycemic variables were introduced as dependent variables. In order to study the effect of the exercise on the glycemic variables in each intervention, a univariate repeated-measures GLM was performed. After testing the normal distribution of the continuous variables by the Shapiro-Wilk test, we applied logarithmic transformations as needed to ensure normality of skewed variables in GLM analysis. Relationships between the rate of change of glucose and lactate with the rate of change of insulin or c-peptide, as well as the relation of metabolic parameters with the workload heart rate, MHR were analyzed using Spearman's correlation test. GraphPad Prism and SPSS 28 statistical packages were used. A p value < 0.05 was considered statistically significant.

3. Results

Basal characteristics of the study participants are shown in Table 1. Fourteen females and 9 males participated in the study. Among them, 8 participants were matutine chronotype and 15 were intermediate chronotype.

Glycemic metabolism parameters after physical exercise are shown in Figure 2. Glucose levels exhibited a significant increase immediately following the completion of anaerobic exercise, starting to normalize after 2 hours in both the morning and afternoon.

Table 1. Basal characteristics of the participants.

Parameters	Mean \pm SD
Age (years)	34 \pm 11
BMI (kg/m ²)	24 \pm 4
Glucose (mg/dL)	91 \pm 6
Insulin (μ U/mL)	8.0 \pm 3.6
C-Peptide (ng/mL)	1.13 \pm 0.34
HbA1c (%)	5.1 \pm 0.3
Triglyceride (mg/dL)	70 \pm 25
Cholesterol (mg/dL)	164 \pm 37
HDL-chol (mg/dL)	52 \pm 14

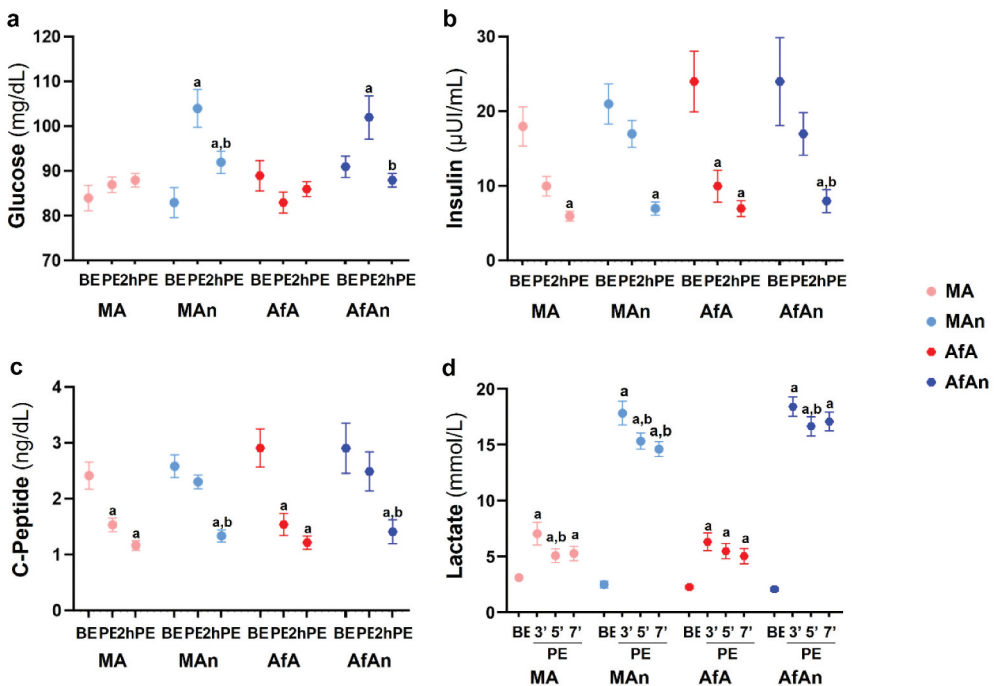


Figure 2. Glucose (a), insulin (b), C-peptide (c) levels before, at the end of the exercise and 2 hours after the end of the exercise. Lactate levels (d) before and 3, 5 and 7 minutes after the end of the exercise. Mean \pm SEM. Significant differences ($p < 0.05$) with basal (a) and at the end of the exercise (b). 2hPE, 2 hours post-exercise; AfA, afternoon aerobic exercise; AfAn, afternoon anaerobic exercise; BE, before exercise; MA, morning aerobic exercise; MAn, morning anaerobic exercise; PE, at the end of exercise.

Conversely, insulin and C-peptide levels displayed a decrease post-exercise, with the most pronounced decline observed at the conclusion of aerobic exercise, while a more substantial reduction was noted 2 hours after the end of the exercise in the anaerobic condition. Levels of lactate, which is a classical byproduct of glucose metabolism, increased following exercise. The highest increment was observed 3 minutes after the end of the exercise, starting to decrease by minute 5 in all the interventions, however a biggest increment was observed in morning and afternoon anaerobic exercises.

We evaluated the effects of exercise timing (morning and afternoon), exercise type (aerobic and anaerobic exercise), and the interaction between both on serum glycemic changes from baseline (prior to exercise) to the end of exercise; from baseline to 2 h after exercise; and from the end of the exercise to 2 h after exercise.

The effect of the type of exercise on glucose, insulin, C-peptide and lactate levels, independently of chronobiology, is represented in Figure 3. Changes of glucose were higher in anaerobic exercise between basal and at the end of the exercise, however, only statistically significant for glucose and C-peptide levels.

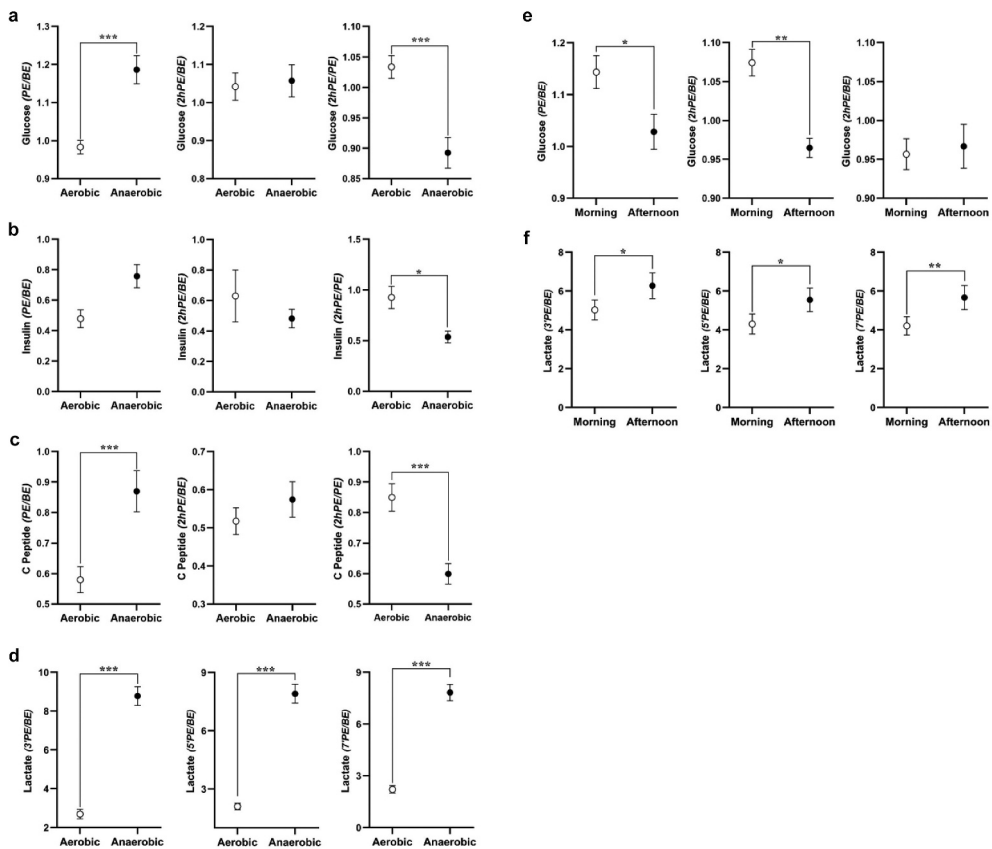


Figure 3. Effect of the type of exercise on the rate of change of glucose (a), insulin (b), C-peptide (c) and lactate (d) levels after physical activity. Effect of chronobiology on the rate of change of glucose (e) and lactate levels (f). Mean ± SEM. * $p < .05$ ** $p < .01$ *** $p < .001$. BE, before exercise; PE, at the end of exercise; 2hPE, 2 hours post-exercise.

Comparing changes after 2 hours of the end of the exercise, we found that glucose levels increased the most at the end of anaerobic exercise, accompanied by a modest decrease in insulin and C-peptide levels, whereas in aerobic exercise glucose levels change slightly. The changes in glucose levels (Figure 3a) were accompanied by a decrease in insulin (Figure 3b) and C-peptide levels (Figure 3c), being more abrupt just after the end of aerobic exercise. However, even though changes in insulin levels showed the same pattern, were only significant for changes observed between 2 hours after the end of exercise and the end of exercise between aerobic and anaerobic exercise types. In addition, Figure 3d shows highly significant increments in lactate levels by anaerobic physical activity compared with aerobic exercise.

The effect of chronobiology, independently of the type of exercise, was also evaluated and only variables that were significantly affected, such as glucose and lactate, were represented in Figure 3. The increase in glucose levels was greater immediately after exercise and 2 hours post-exercise in the morning than in the afternoon (Figure 3e). Moreover, changes in lactate levels according to chronobiology were also significant (Figure 3f). Interestingly, physical activity during the afternoon produced a higher increase of lactate levels 3, 5 and 7 minutes after exercise when compared to mornings.

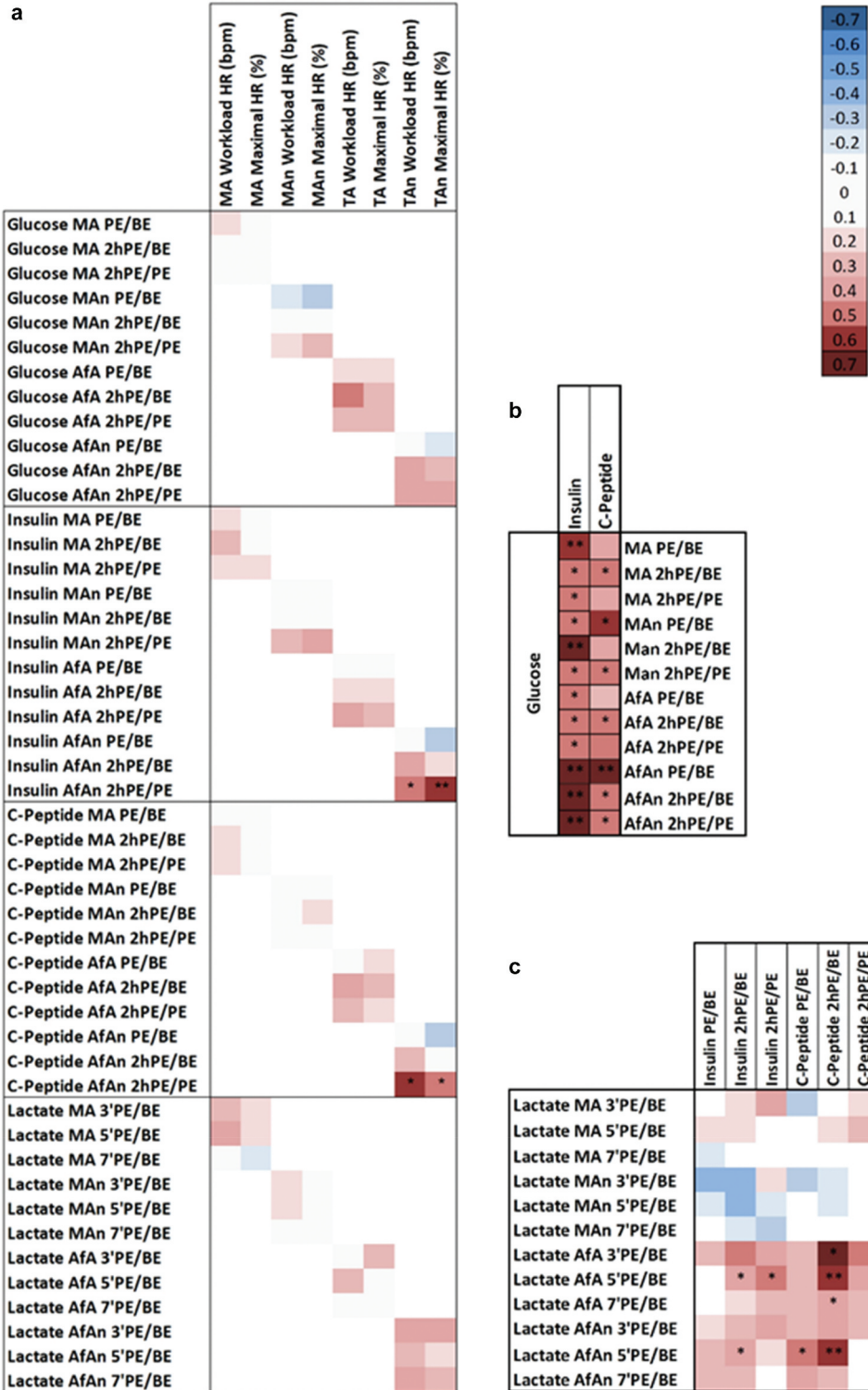
In order to understand the relation among glucose metabolic parameters themselves and with their chronotype and exercise intensity, we performed correlation analysis. The correlation studies between workload HR and MHR with changes in metabolic parameters after exercise are depicted in Figure 4. Just the rate of change of insulin and C-peptide between the end of the exercise and 2 hours after the end of the exercise showed significant positive correlations with higher exercise intensity, workload HR and MHR, during afternoon anaerobic exercise (Figure 4a).

Additionally, positive correlation analyses between the rate of change of glucose with those observed in insulin and C-peptide are depicted in Figure 4b. The stronger correlations were observed with the rate of change of insulin, where insulin exhibited a high correlation with glucose changes after morning aerobic and aerobic exercises, as well as afternoon anaerobic exercise. This corroborates that insulin plays a significant role in glucose regulation across different exercise types and times. Moreover, correlation analyses between the rate of change of lactate with insulin and C-peptide changes are represented in Figure 4c. Significant positive correlations were observed between change of insulin and c-peptide 2 hours after the end of afternoon exercise with those observed in lactate after 3 and 5 minutes the end of the exercise, indicating that post-exercise lactate levels are closely related to changes in insulin and C-peptide.

Moreover, in order to examine the potential link between the chronotype of the participants (morning or intermediate) and their glycemic responses to the 4 interventions, a correlation analysis was conducted, which did not reveal any significant correlations (Supplemental Figure 1).

4. Discussion

Our results show short-term effects of both the type of exercise and the timing of its implementation on glucose metabolism. Performing anaerobic exercise seems to greatly affect glucose level by increasing it at the end of the exercise, regardless of the time of



the day. On the other hand, morning exercise per se increases glucose levels compared with exercise performed in the afternoon.

Physical exercise is recognized for its ability to elicit numerous beneficial effects on whole-body glucose homeostasis and metabolic health [2,20]. Furthermore, ongoing physiological adaptations to exercise, such as increased glycolysis, fat and glucose oxidation, glucose uptake, or mitochondrial oxidative capacity, promote exercise-associated metabolic improvements [21,22]. Despite the growing interest in this field, the potential impact of exercise timing and exercise type on metabolic health in the general population remains poorly explored. To our knowledge, the present study is pioneering in its exploration of the effects of exercise timing (morning and afternoon), exercise type (aerobic and anaerobic exercise), and their interaction on the glycemic metabolism in healthy volunteers.

At the end of the exercise, glucose levels exhibited a marked increase in the morning compared with the afternoon. This result can be explained by the influences of circadian rhythms, which can be traced to several factors such as diurnal rhythms in insulin secretion, clearance or peripheral sensitivity, β -cell responsiveness, and the circadian rhythm of counterregulatory hormones (cortisol, growth hormones, norepinephrine, glucagon, among others). These hormones impact insulin signaling, secretion or action, and can stimulate gluconeogenesis, glycogenolysis, or prevent peripheral glucose uptake, ultimately leading to hyperglycemia. Teo *et al.* [23] reported that after 12 weeks of physical interventions, consisting in 3 sessions per week of 60 minutes of both an aerobic and a resistance exercise component, improved glycemic response of sedentary overweight individuals with or without T2DM independently if it was performed in the morning or evening. Some recent studies, comparing exercise interventions in the morning versus afternoon/evening, demonstrated the influence of chronobiology in glycemic levels in both healthy or altered glycemic metabolism individuals, highlighting that circadian clock and metabolism are tightly intertwined [12,14,15,24]. Some of these studies reported that exercise training performed in the afternoon can induce several more profound benefits in glycemic metabolism. A randomized crossover trial involving HIIT sessions during two weeks of either morning or afternoon, demonstrated that afternoon HIIT improved glucose levels in diabetic individuals [12]. Moreover, a multimodal training program of 12 weeks combining aerobic and resistance exercise performed in the afternoon was associated with improvements in insulin sensitivity and glucose levels in metabolically compromised men [14]. Those results are consistent with Van der Velde *et al.* [24], who demonstrated that daily moderate to vigorous physical activity in the afternoon was associated with greater insulin sensitivity. Furthermore, a randomized crossover trial involving one week of aerobic exercise revealed that late afternoon training, compared to morning sessions, was associated with significantly lower postprandial and 24-hour post-intervention glucose levels [15]. In line with those findings, the present

interventions with glucose change rate (Figure 4b). Correlations between the rate of change of insulin and C-peptide levels with lactate change rate at 3, 5, and 7-minutes post-exercise in the four types of interventions (Figure 4c). AfA, afternoon aerobic exercise; AfAn, afternoon anaerobic exercise; BE, Before exercise; MA, aerobic morning; MAn, anaerobic morning; PE, post-exercise; 2hPE, 2 hours post-exercise. * $p < 0.05$ ** $p < 0.01$.

study identifies improvements in short term glycemic parameters, as metabolic determinations were addressed just after the end or 2 hours after the end of every physical activity intervention in healthy volunteers. We have demonstrated that physical exercise in the afternoon produces an improvement in the glycemic metabolism in healthy subjects, when compared with the exercise in the morning. This gives rise to the notion that exercising in the afternoon, when muscle is ready to meet the energy demand of contraction, might be more beneficial. A possible interpretation of these results may be related to differences in the pattern of energy consumption between morning and afternoon/evening exercise. Therefore, afternoon/evening exercise may lead to a higher oxidative capacity, compared to morning exercise, combined with a more efficient utilization of substrates, including glucose, by skeletal muscle [12]. Furthermore, as glucose tolerance and insulin sensitivity show circadian oscillations, matching circadian clocks with specific exercise sessions according to the time of day may result in greater impacts on glucose metabolism [25,26]. The timing of physical activity could have an important clinical impact, especially on glycemic response, a matter of relevance given the rising rates of insulin resistance/T2DM prevalence nowadays. In fact, the timing of exercise may be crucial in optimizing the management of metabolic disease such as T2DM or obesity. Scheduling exercise at the most appropriate time could enhance glycemic control and improve overall treatment efficacy. Moreover, physiological changes associated with conditions like T2DM or obesity, including insulin resistance and altered hormone secretion, can influence the metabolic response to exercise [11]. This suggests that in patients with metabolic disturbances, exercise interventions may yield different effects compared to healthy individuals, which must be considered when interpreting results and designing exercise programs. Incorporating data from subjects with different metabolic conditions is essential for understanding the heterogeneity in exercise responses. The divergent responses observed in individuals with conditions such as T2DM or obesity highlight the need to personalize exercise interventions to maximize their benefits. Furthermore, most previous studies have investigated the effects on metabolic status in the long term rather than the short term. In contrast, our study evaluates not only the short-term effects of the timing of exercise but also the impact of the type of exercise and their interaction on glycemic metabolism. This comprehensive and holistic analysis contributes to a deeper understanding of the effect of exercise on metabolic responses.

Chronobiology has demonstrated that circadian rhythms significantly impact metabolic and physiological processes, suggesting that the time of the day when an intervention is performed can influence the outcomes observed. Mammalian circadian clocks are internal oscillators that regulate physiological processes over a 24-hour cycle [11]. The central clock, situated in the hypothalamic suprachiasmatic nucleus, serves as a key regulator of metabolism and energy balance through a network of interconnected transcriptional feedback loops and coordinates the synchronization of peripheral clocks in various tissues, which can also operate with their own rhythms. Light and darkness are the primary influencers of circadian rhythms, however factors such as physical activity also significantly affect them [27]. In our recent review [11], we have highlighted recent advances that shed light on the underlying mechanisms connecting exercise, the circadian clock, and metabolic responses. On one hand, it seems that the molecular circadian clock modulates the metabolic response to exercise and exercise performance in a time-dependent manner [28]. And, on the other hand, exercise has been proposed as an

essential synchronizer of the central and peripheral circadian clocks in a time-dependent manner, which has important consequences for metabolic health. Moreover, circadian control of glucose metabolism has also been well documented [29]. Glucose levels per se also display circadian oscillations and peak before the start of the active period. Diurnal variations in glucose tolerance, with higher tolerance in the morning and impairments in the afternoon or evening, are well-established and are linked to circadian rhythms in insulin secretion, β -cell responsiveness, and insulin clearance [29]. Peripheral insulin sensitivity and glucose effectiveness also exhibit circadian variations, being impaired later in day, contributing to the overall diurnal pattern in glycemic control. In our study, while we did not directly measure circadian-related factors, it is plausible that the observed physiological responses could be partially explained by these time-of-day effects. While our study did assess chronotype differences in the study cohort by questionnaire and we did not find any relation between chronotype and glucose metabolism changes, we did not measure chronomarkers which could influence individual responses to exercise timing. It is important to acknowledge that future research should consider these variables. Aligning exercise timing with an individual chronotype may optimize metabolic health outcomes, particularly in populations with circadian misalignment, such as those with type 2 diabetes or obesity.

The present study shows that anaerobic exercise increases glucose levels just at the end of the exercise session, which began to decrease after 2 hours. And, in contrast, aerobic exercise did not significantly affect glucose levels. Furthermore, the great increase in glucose levels at the conclusion of anaerobic exercise was accompanied by a modest decrease in insulin and C-peptide levels, whereas after 2 hours of the end of the exercise, glucose levels decreased, accompanied by a relevant reduction in insulin and C-peptide levels. It appears that insulin and C-peptide levels do not decrease significantly at the end of the exercise as glucose levels are still increased, while at the aerobic exercise, the higher decrease in insulin and C-peptide levels was observed just at the end of the exercises accompanied by modest changes in glucose levels. In order to maintain blood glucose homeostasis during exercise, hepatic glucose output also rises [30]. During aerobic exercise, this process is primarily regulated by a decrease in the secretion of insulin, which alters the ratio of insulin to glucagon levels, thereby affecting the sensitivity of the liver to glucagon. In contrast, during anaerobic exercise, hepatic glycogen breakdown and glucose output are stimulated by the increased levels of circulating catecholamines. As the rate of hepatic glucose output exceeds the rate of glucose uptake, this leads to an elevation in blood glucose concentration. Previous studies have reported that long diverse training types are associated with different metabolic responses and clinical outcomes, irrespective of the time of exercise. Jelleyman *et al.* [31] performed a systematic review to quantify the impact of anaerobic exercise (HIIT) for at least two weeks on glucose and insulin regulation, compared with control conditions or continuous exercise training. They did not find a significant decrease in glucose levels unless the subjects had metabolic syndrome or diabetes, but there was a significant reduction in insulin resistance compared with both control conditions and aerobic groups. Moreover, a recent study [32] showed that one month of either aerobic or anaerobic exercise did not improve glucose metabolism in women with morbid obesity. However, several studies have reported that both aerobic and anaerobic exercises improved glycemic metabolism after 12 [33] or 6 [34] weeks of interventions in men with type 2 diabetes or overweight/

obesity, respectively. In the line, few studies have addressed the short-term effects of exercise on glucose metabolism and have shown disparate results when comparing aerobic versus anaerobic exercises. Parker *et al.* [35] reported that aerobic exercise decreased glucose levels right after the exercise and normalized after 1.5 hours post-exercise in patients with overweight and obesity. While anaerobic exercise did not produce any change in glucose levels right after exercise, it decreased after 1.5 hours post-exercise. Parker *et al.* [35] also reported that the decrease in insulin levels at the end of the exercise was smaller compared to aerobic activity, with the decrease becoming more similar to that observed 1.5 hours after exercise. Schwaab *et al.* [36] showed that postprandial plasma glucose was reduced only by aerobic exercise compared with anaerobic exercise in patients with coronary heart disease and type 2 diabetes mellitus. In contrast, Oliveira Teles *et al.* [37] showed that anaerobic exercise reduced blood glucose levels after the end of the exercise while aerobic exercise did not produce significant changes in type 2 diabetic patients. Furthermore, several studies have found no significant effect on blood glucose levels following both anaerobic and aerobic exercise in untrained individuals [38,39]. In addition, a more basic investigation has recently stated that both types of exercise, aerobic and anaerobic, are very similar in terms of glycemic metabolite response, in healthy subjects. In this study, both exercise types increased glucose-related metabolites (pyruvate and lactate) immediately after exercise [6]. However, they did not show glucose or insulin levels in these subjects. This diversity of results emphasizes the complexity inherent in the effect of exercise on glucose metabolism and highlights the need for further research to control for all factors that could influence the effects of exercise. In this context, our approach, which not only controls the type of exercise but also the timing of its performance, provides a comprehensive perspective of the scenario.

In the present study, lactate values were significantly higher in anaerobic physical exercise compared to those observed in aerobic exercise. Remarkably, exercise performed in the afternoon elicited higher lactate levels than in the morning. Exercise triggers rapid changes in glucose levels due to the heightened demands for glucose by the contracting muscles and changes in counterregulatory hormone secretion. Anaerobic exercise can demand for oxygen and ATP exceeding the cellular supply, promoting lactate production as fuel. Lactate can potentially serve as an alternative substrate for glucose, but it also can promote hepatic glucose production by serving as gluconeogenic precursors. In addition, higher lactate levels may acutely inhibit the action of insulin on peripheral glucose uptake, exhibiting similar effects to those of counterregulatory hormones [40]. Moreover, a recent meta-analysis performed on the levels of lactate and diurnal changes in anaerobic performance reported insignificant results for the role of circadian rhythm on blood lactate levels [41]. However, several studies have reported an influence of chronobiology on lactate levels. Saygin *et al.* [42], evaluated lactate levels after repeated anaerobic sprint test and 20 m shuttle run test and observed that it was significantly increased after tests performed in the afternoon compared with those performed in the morning. Hammouda *et al.* [43] showed that lactate levels of young well-trained soccer players were increased only in the evening after performing Yo-Yo aerobic test. Our results support the idea that the type of exercise and the time when it is performed can influence

substrate utilization and highlight the complexity of the metabolic response. The link to different pathways of glucose utilization underlines the relevance of lactate as a key indicator in the glycemic response. Monitoring lactate levels could offer valuable insights for optimizing physical activity.

Certain limitations of our study, such as the sample size and the acute nature of the exercise, are acknowledged. However, the robust design, which jointly assesses timing and type of exercise, highlights its strength. The need for large-scale, long-term clinical trials is emphasized. These future trials are essential to fully understand the clinical implications and to provide specific guidelines for the prevention of diseases such as diabetes.

5. Conclusion

The type of exercise and the time of the day when it is performed influence glycemic metabolism. Anaerobic exercise highly increases glucose and lactate levels, while aerobic exercise does not affect glucose levels. Afternoon exercise appears to be more favorable in terms of glycemic responses, suggesting circadian variations in insulin sensitivity and glucose utilization during exercise. This observation aligns with previous studies and highlights the importance of considering chronobiology when designing exercise interventions. The present study lays the groundwork for future research exploring the relationship between exercise, chronobiology and glycemic response. The individualization of exercise strategies may be essential, and consideration of both timing and modal factors is imperative when addressing metabolic health issues. The present comprehensive approach, thus, may serve as a stimulus for further research and contribute to the development of personalized medicine through exercise strategies that will benefit the well-being of the general population.

Disclosure statement

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