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RESEARCH PAPER

Reduced glucose tolerance and insulin sensitivity after prolonged exercise in endurance athletes

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Abstract

Aim: The purpose of this study was to 1. investigate if glucose tolerance is affected after one acute bout of different types of exercise; 2. assess if potential differences between two exercise paradigms are related to changes in mitochondrial function; and 3. determine if endurance athletes differ from nonendurance-trained controls in their metabolic responses to the exercise paradigms.

Methods: Nine endurance athletes (END) and eight healthy nonendurance-trained controls (CON) were studied. Oral glucose tolerance tests (OGTT) and mitochondrial function were assessed on three occasions: in the morning, 14 h after an overnight fast without prior exercise (RE), as well as after 3 h of prolonged continuous exercise at 65% of VO_2max (PE) or 5×4 min at ~95% of VO_2max (HIIT) on a cycle ergometer.

Results: Glucose tolerance was markedly reduced in END after PE compared with RE. END also exhibited elevated fasting serum FFA and ketones levels, reduced insulin sensitivity and glucose oxidation, and increased fat oxidation during the OGTT. CON showed insignificant changes in glucose tolerance and the aforementioned measurements compared with RE. HIIT did not alter glucose tolerance in either group. Neither PE nor HIIT affected mitochondrial function in either group. END also exhibited increased activity of 3-hydroxyacyl-CoA dehydrogenase activity in muscle extracts vs. CON.

Conclusion: Prolonged exercise reduces glucose tolerance and increases insulin resistance in endurance athletes the following day. These findings are associated with an increased lipid load, a high capacity to oxidize lipids, and increased fat oxidation.

KEYWORDS
endurance athletes, endurance exercise, glucose tolerance, insulin sensitivity, mitochondria, reactive oxygen species

1 | INTRODUCTION

Exercise acutely enhances glucose uptake in skeletal muscle[1] and this effect seems to peak 12–16 h after exercise and can persist for several days[2] due to sustained positive effects on insulin action.[3] Therefore, exercise training has been extensively studied as a strategy to promote health and as a treatment in many pathologic states, including type 2 diabetes.[4] Different training regimens using moderate intensity exercise[5] as well as high-intensity interval training (HIIT)[6] and sprint interval training[7] have all shown positive effects on glucose control. Endurance athletes have consistently been found to have a high capacity for skeletal muscle glucose uptake, have a high insulin sensitivity[8,9] as well as a high activity of glycogen synthase (GS).[10] The oxidative capacity of skeletal muscles is related to whole-body oxygen uptake,[11,12] and metabolic flexibility[13] and endurance athletes exhibit the highest values for these parameters. In contrast, obese and diabetic subjects show low mitochondrial function[14] and respiratory capacity,[15,16] have elevated ROS production,[16–18] and show dysregulated lipid oxidation and storage,[19] of which all are associated with impaired glucose regulation.

Despite the advantageous physiological characteristics evident in endurance-trained subjects, endurance athletes have sometimes been found to have reduced glucose uptake and insulin sensitivity after demanding exercise sessions.[20–22] This stands in contrast to the overwhelming evidence of positive effects of exercise on postexercise glucose control in less-trained subjects and has been described as an athlete's paradox.[21] The main proposed explanation for these findings is an inhibiting action of fatty acid transport and metabolism on glucose transport and metabolism.[13,23] However, we recently demonstrated that performing excessive amounts of HIIT decreased mitochondrial function and glucose tolerance in recreationally trained subjects.[24] Glycogen and FFA levels were normal, indicating that glucose tolerance after chronic exercise training can be reduced through other mechanisms. Here we investigate if different types of acute exercise have divergent effects on glucose tolerance and mitochondrial function. Further, we assessed whether the responses of endurance athletes differed from those of nonendurance-trained controls.

2 | RESULTS

2.1 | Endurance athletes have superior fat oxidation during prolonged exercise and a different glucoregulatory response to HIIT than controls

During PE, mean power output and contribution of fat to total energy expenditure were ~50% higher in END than in CON (Figure 1A). During HIIT, END had a 24% higher mean power output than CON. Performance and metabolism during training sessions are presented in Table 1. During PE, END exhibited low lactate values throughout the session, whereas lactate was elevated in CON, especially during the initial period of exercise (Figure 1B). During extensive work periods, a drop in blood glucose is associated with the depletion of liver glycogen and the maintenance of glucose uptake is crucial for preserving the glucose oxidation rate in the working muscles.[25] We found that glucose decreased throughout PE in a similar manner for both END and CON (Figure 1C). During the HIIT session, lactate increased slightly during the warm-up in CON but not in END and was similar in both groups during HIIT (Figure 1D). Blood glucose was unaffected in both groups at 100 W but increased during HIIT in END. This response was absent in CON (Figure 1E). During high-intensity exercise, the rise in blood glucose is likely caused by increasing catecholamine levels that stimulate hepatic glycogen breakdown and glucose output at a rate that exceeds muscle glucose uptake. It is well documented that endurance-trained athletes secrete higher levels of catecholamines during intense exercise.[26]

2.2 | Glucose tolerance is reduced in endurance athletes after prolonged exercise

Glucose tolerance was decreased after PE in END compared with RE. The effect of PE was less pronounced in CON and was not significant ($p = 0.16$). HIIT did not affect glucose tolerance in either group. The glucose curves for the full OGTT are presented in Figure 2A,B, and the area under the curve (AUC) is calculated for the first 90 min in Figure 2C. Insulin was unaltered regardless of condition. The insulin curves during the OGTT are presented in Figure 2D,E. AUC for insulin during the first 90 min of the OGTT is presented in Figure 2F. To assess peripheral insulin action, we calculated $\text{glucose}_{AUC}/\text{insulin}_{AUC}$ (Figure 2G). The ratio was markedly increased after PE in END, indicating the development of insulin resistance. In contrast, no noteworthy change occurred in the ratio after HIIT in END. In CON, neither exercise paradigm altered the ratio. No alterations in fasting HOMA-IR (baseline measurements for END 0.79 ± 0.28, CON 1.15 ± 0.54) or the Matsuda IS index,[27] calculated from values during OGTT (baseline measurements for END 14.5 ± 5.6, CON 12.7 ± 8.0), were found in either group. We also measured lactate during the OGTT as it has been found to be associated with obesity and impaired glucose

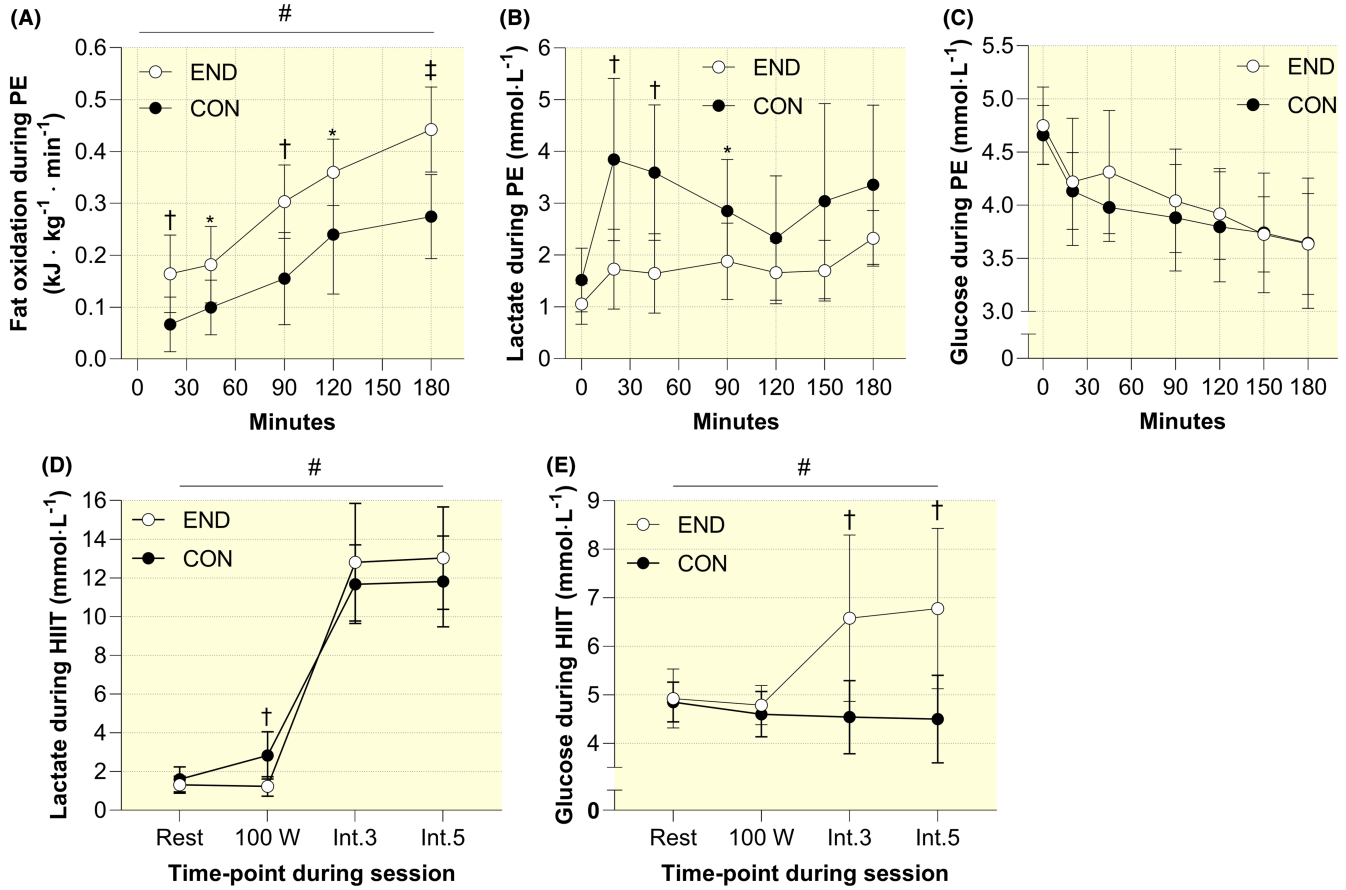


FIGURE 1 Physiological measurements during exercise: (A) fat oxidation during PE, (B) lactate during PE, (C) glucose during PE, (D) lactate during HIIT, (E) glucose during HIIT. Sampling time points during HIIT refer to rest, after warm-up, and after the 3rd and 5th intervals. END = endurance athletes (white circles), CON = controls (black circles). RE = rest, PE = 3 h at 65% of VO_2max, HIIT = 5 × 4 min at ~95% of VO_2max. All values as group means ± SD. In all figures, $n = 9$ in END, $n = 8$ in CON. # denotes a main effect (condition and group) two-way RM-ANOVA. Difference between groups; $^{*}p < 0.05$, $^{\dagger}p < 0.01$, $^{\ddagger}p < 0.001$.

TABLE 1 Performance, external work (measured on the ergometer), energy expenditure (EE), and substrate contribution during PE and HIIT sessions.

		65% of VO_2max (W)	W_{mean}	Total external work (kJ)	Total EE (kJ)	FAT %	CHO %
PE	END	173 ± 46	167 ± 45	1804 ± 481	9617 ± 2055	39 ± 12	61 ± 12
	CON	131 ± 29	111 ± 24	1195 ± 264	7786 ± 1789	31 ± 11	69 ± 11
HIIT	END		260 ± 51	372 ± 61	2135 ± 307	5 ± 2	95 ± 2
	CON		210 ± 38	311 ± 46	1952 ± 318	1 ± 1	99 ± 1

Note: W_{mean} refers to the mean power output during the whole PE session (65% of VO_2max was held as long as the subjects managed) and during high-intensity work periods during the HIIT session (5 × 4 min at ~95% of VO_2max). Measurements of energy expenditure during the HIIT session include warm-up and rest between work periods. END ($n = 9$), CON ($n = 8$). Values are presented as mean ± SD.

Abbreviations: CON, controls; END, endurance athletes.

tolerance and increase[28] or decrease[29] in response to a glucose load. We found that lactate, after glucose ingestion, slowly increased during the first hour of the OGTT and thereafter stayed elevated during the second hour (Figure 2H,I). The calculated AUC for lactate was lower after PE for END whereas CON had increased values (Figure 2J).

2.3 | Intrinsic mitochondrial respiration is not affected by acute training, but prolonged exercise increases ROS production in endurance athletes

A high mitochondrial capacity is associated with efficient glucose handling, and we have previously found that a

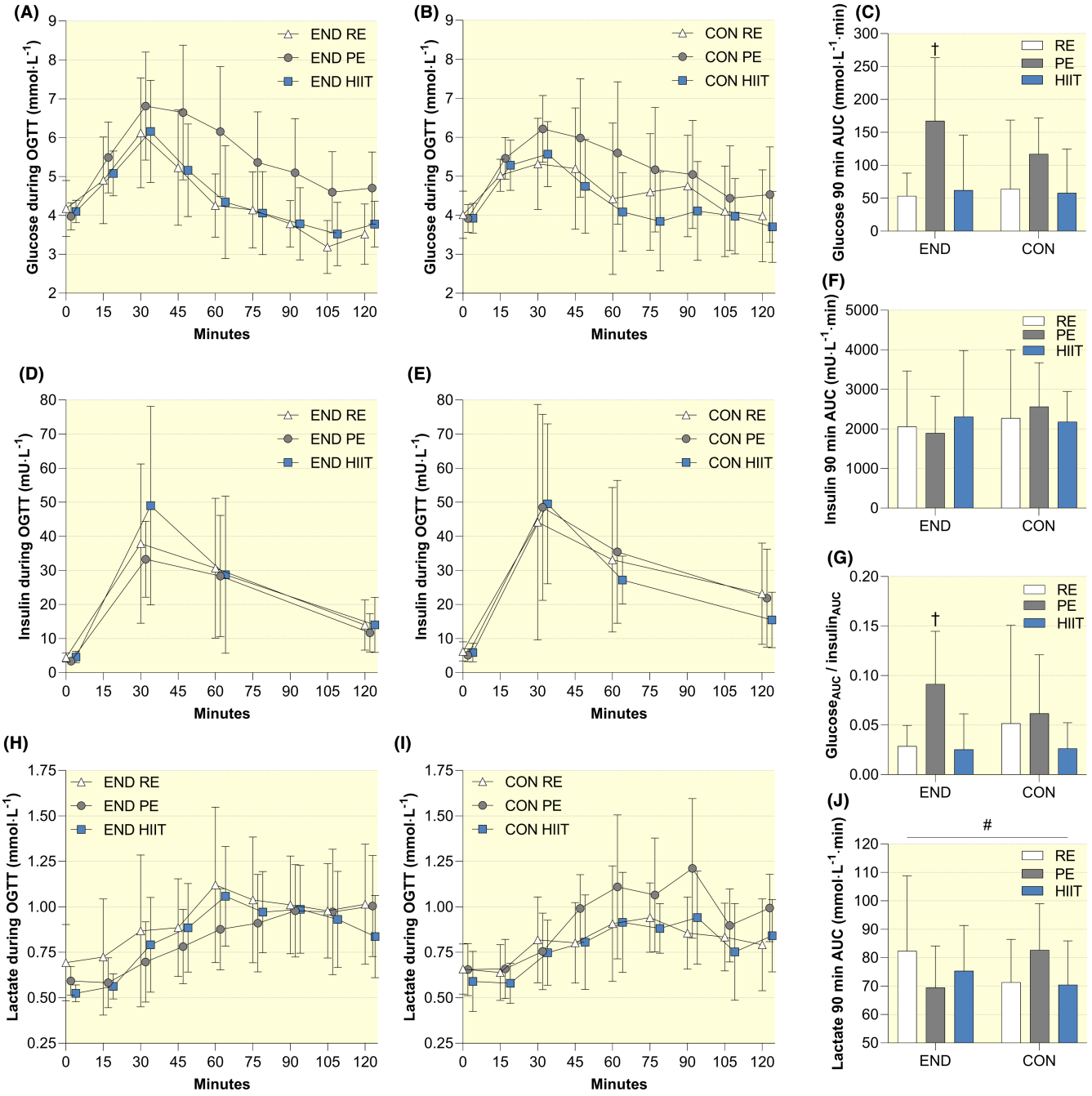


FIGURE 2 Blood measurements ~14 h after exercise: (A) glucose curves during OGTT for END, (B) glucose curves during OGTT for CON, (C) AUC glucose during OGTT, (D) insulin curves during OGTT for END, (E) insulin curves during OGTT for CON, (F) AUC insulin during OGTT, (G) $glucose_{AUC}/insulin_{AUC}$ ratio during OGTT, (H) lactate curves during OGTT for END, (I) lactate curves during OGTT for CON, (J) AUC lactate during OGTT. AUC is calculated from individual baselines for glucose and insulin, and absolute values were used for lactate. In all figures, END = endurance athletes ($n = 9$), CON = controls ($n = 8$). RE = rest, PE = 3 h at 65% of VO_2max, HIIT = 5 × 4 min at ~95% of VO_2max. All values as group means ± SD. # denotes a main effect (condition and group) two-way RM-ANOVA. Differences to RE condition within each group; $^{\dagger}p < 0.01$.

loss in mitochondrial function coincided with a decreased glucose tolerance in moderately trained subjects after excessive HIIT training.[24] We, therefore, investigated if the observed decrease in glucose tolerance in the present investigation was related to alterations in mitochondrial parameters. We found that intrinsic mitochondrial respiration was not different between groups at baseline regardless of respiratory state, and exercise had no significant effects on maximal ADP-stimulated respiration. When using octanoyl carnitine as a substrate for fat metabolism, we found that exercise increased leak respiration in END whereas CON showed the opposite response

(Figure 3A). When ADP was added to fully stimulate fat oxidation (Figure 3B), and with the addition of succinate for complex II respiration (Figure 3C) no effect was evident regardless of the type of exercise. Similarly, leak respiration using pyruvate, glutamate, and malate (PGM) (Figure 3D), with further addition of ADP, activating complex I (Figure 3E), and succinate, activating complex I+II (Figure 3F), were not different between conditions. We also measured mitochondrial H_2O_2 emission and found that it was not affected during leak respiration using octanoyl carnitine (Figure 3G), with PGM (Figure 3H) or when ADP was added for assessing fat oxidation (Figure 3I). However, when activating complex I (Figure 3J), complex II (Figure 3K), and complex I+II (Figure 3L), H_2O_2 emission was highest after PE in END, whereas the opposite response was found in CON. Elevated basal production of mitochondrial ROS is associated with impaired mitochondrial function, glucose tolerance, and insulin sensitivity[30] and increased exposure to ROS has been shown to initiate insulin resistance in mice.[18] In contrast, increases in cytosolic ROS production have been shown to increase during exercise and to be an important regulator for glucose uptake.[31,32]

2.4 | Changes in glycogen concentration are not associated with changes in glucose tolerance

Low levels of glycogen are associated with increased rates of muscle glucose uptake and increased activity of glycogen synthase.[33] We, therefore, measured glycogen in biopsies collected before the OGTT and found that both types of exercise reduced glycogen depots compared to baseline values. After HIIT, glycogen content was reduced to 82% of baseline values in END and 64% in CON.

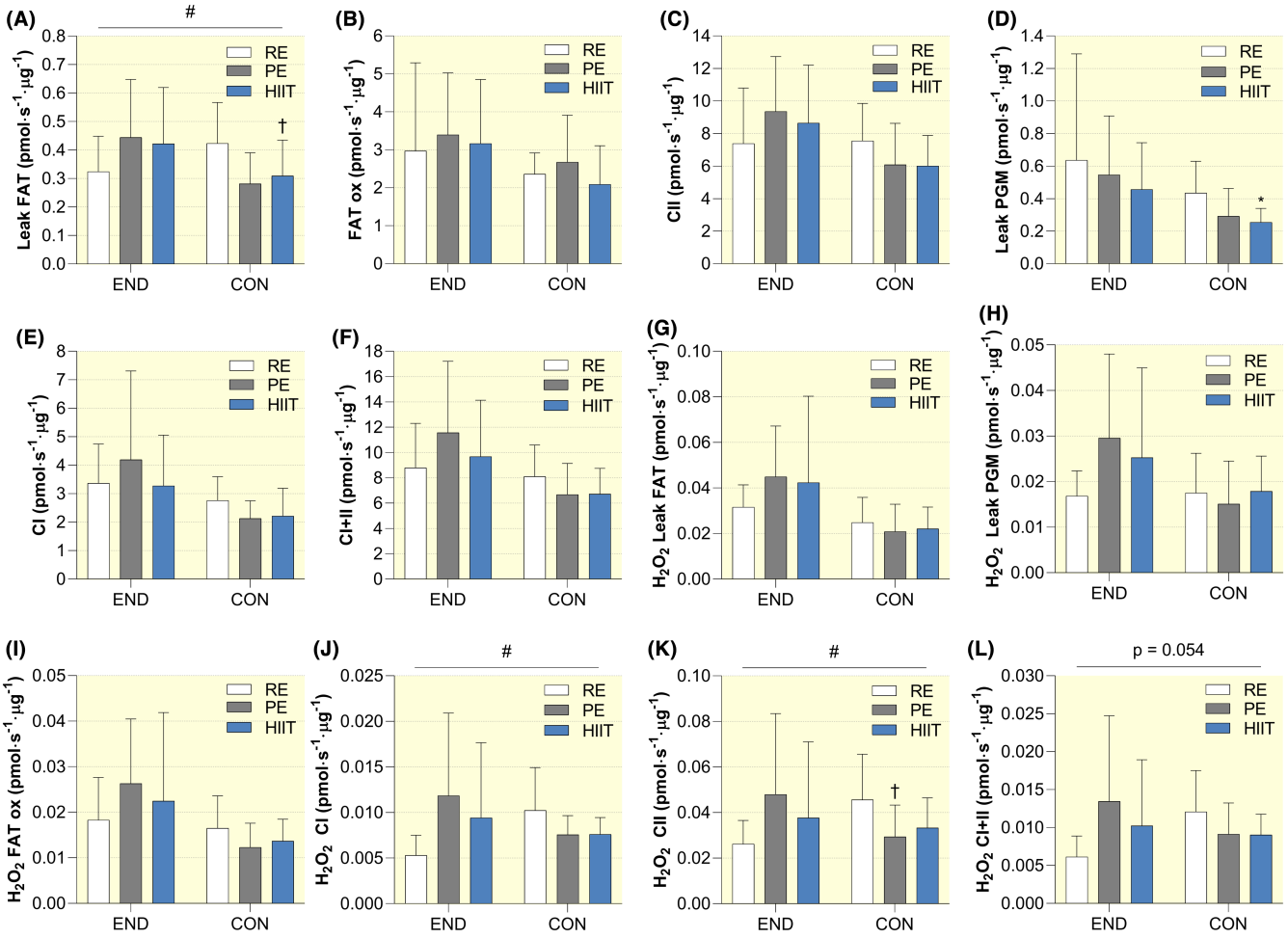


FIGURE 3 Intrinsic mitochondrial respiration ~14 h after exercise: (A) leak fat, (B) fat oxidation, (C) complex II, (D) leak PGM, (E) complex I, (F) complex I+II. Intrinsic mitochondrial H_2O_2 emission: (G) leak fat, (H) leak PGM, (I) fat oxidation, (J) complex I, (K) complex II, (L) complex I+II. END = endurance athletes, CON = controls. RE = rest, PE = 3 h at 65% of VO_2max, HIIT = 5×4 min at ~95% of VO_2max. All values as group means ± SD. In figure (A–F), $n = 9$ in END, $n = 8$ in CON, in (G–L), $n = 6$ in END, $n = 8$ in CON. # denotes a main effect (condition and group) two-way RM-ANOVA. Differences to RE condition within each group; $^{*}p < 0.05$, $^{\dagger}p < 0.01$.

The corresponding values after PE were 52% in END and 60% in CON (Figure 4A). We also found that the phosphorylation status of glycogen synthase decreased after PE in both END and CON (reflecting the activation of the enzyme), whereas this response was less pronounced after HIIT (Figure 4B). No change in the abundance of GLUT4 was found ~14 h after exercise sessions (Figure 4C). Since glycogen and GLUT4 levels, as well as the phosphorylation status of GS, were similar in END and CON prior to the OGTT after PE, it is unlikely that they can account for the marked differences in glucose tolerance between groups.

2.5 | FFA, ketones, and fatty acid oxidation increase in endurance athletes after prolonged exercise

During exercise, fat oxidation is fueled by circulating FFA and intramyocellular triacylglycerols (IMTGs) stored in the muscles, and the relative contribution of the two pools to energy turnover is determined by work rate[34] and duration.[35] Endurance-trained subjects have higher usage of IMTGs during exercise[36] and higher IMTG resynthesis during recovery.[37,38] We measured FFA in serum and found that fasting levels were increased in END after PE, but that CON, who had higher levels of FFA at baseline, did not further increase after PE (Figure 4D). We did not find any differences in energy expenditure during the OGTT between treatments (Figure 4E), but END showed a pronounced shift in substrate oxidation after PE with a decrease in carbohydrate oxidation (Figure 4F) and a corresponding increase in fat oxidation (Figure 4G). CON showed a trend for the same substrate shift, but the differences were not significant. To gain further insight into the metabolic capacities of our subjects, we measured citrate synthase (CS) and 3-hydroxyacyl-CoA dehydrogenase (HAD) activity in muscle samples and found them both to be higher in END than in CON (Figure 4H,I). We also measured fasting levels of ketones in serum. Exogenous ketones (β-hydroxybutyrate, BOH) have been shown to reduce insulin-mediated glucose uptake in isolated murine oxidative muscles.[39] Interestingly, BOH in serum was found to be elevated in END after PE, but to be unaffected in CON (Figure 4J).

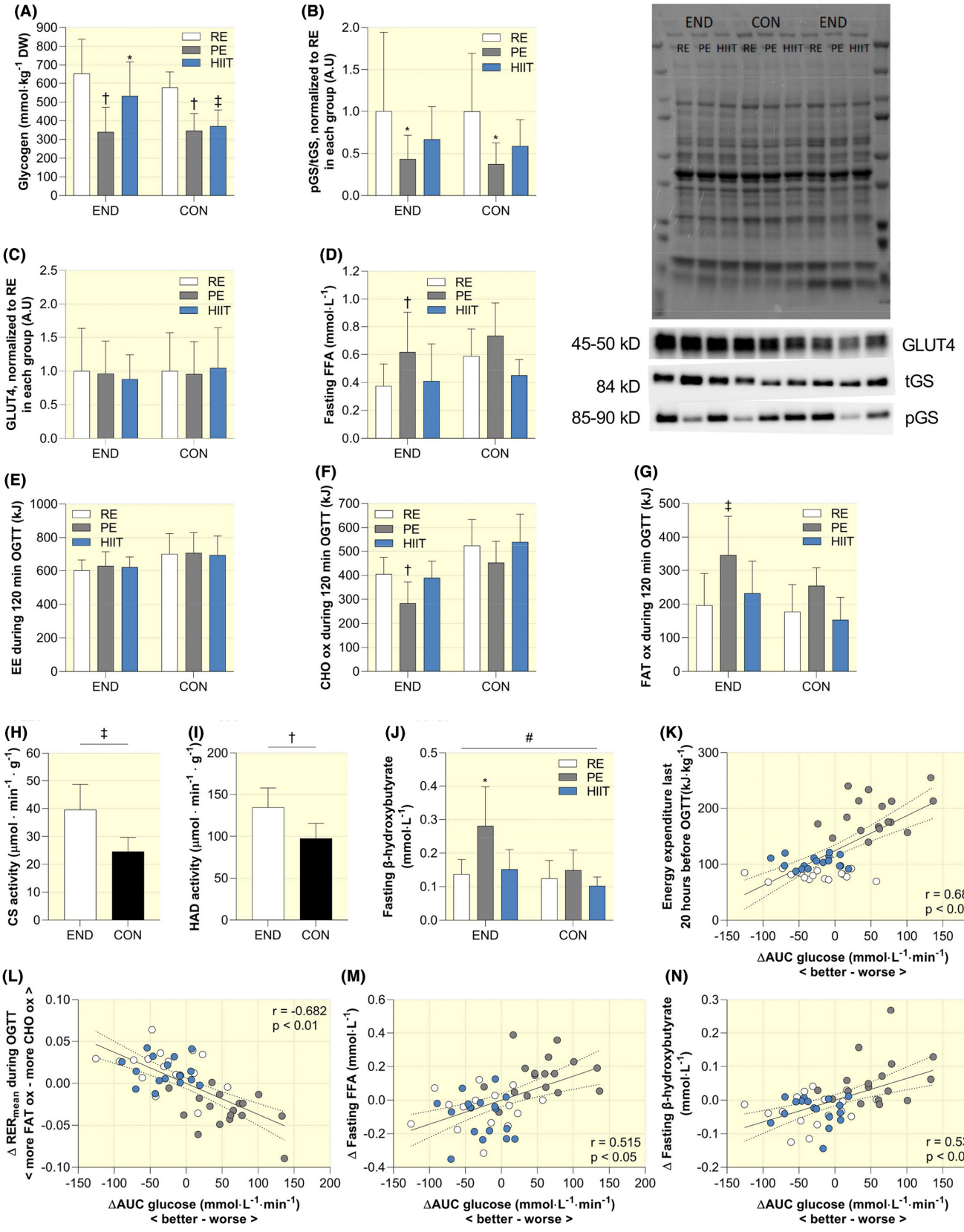
2.6 | The change in glucose tolerance is associated with metabolic alterations and accumulated energy deficit

To assess which physiological parameters were best associated with changes in glucose tolerance, we calculated for each subject, and measurement, the deviation in each condition from the mean values during all three conditions (RE, PE, and HIIT) and correlated these changes with changes in glucose AUC. We calculated the energy expenditure during the last 20 h preceding the OGTT (from the last meal before exercise to OGTT) and found that it was highly associated with changes in glucose tolerance ($r = 0.687$, $p < 0.01$) (Figure 4K). During the OGTT, we found that the respiratory exchange ratio (RER) ($r = -0.682$, $p < 0.01$) (Figure 4L), fasting values of serum FFA ($r = 0.515$, $p = 0.05$) (Figure 4M), and serum BOH ($r = 0.534$, $p < 0.05$) (Figure 4N) were best related to alterations in glucose tolerance.

3 | DISCUSSION

The major findings of the present study are (1) prolonged exercise results in deterioration of glucose tolerance in END but not in CON; (2) the deterioration appears to be explained by the development of insulin resistance; and (3) the deterioration is associated with an increased lipid load, an increased capacity to oxidize lipids and an increase in whole-body fat oxidation. The finding that endurance athletes with high metabolic fitness show a reduction in a parameter strongly associated with metabolic health can be perceived to be paradoxical and unexpected. However, it can also constitute an adaptive response to the metabolic situation that follows prolonged exercise.

FIGURE 4 Metabolism during OGTT, muscle tissue measurements ~14 h after exercise and correlations: (A) glycogen, (B) phosphorylation quotient of glycogen synthase protein, (C) abundance of glucose transporter 4 protein. Pictures of the blots are provided as well as a reference memcode picture showing the loading control with END and CON placed on the same gel. (D) Fasting serum-free fatty acids, (E) energy expenditure during OGTT, (F) carbohydrate oxidation during OGTT, (G) fat oxidation during OGTT, (H) CS activity, (I) HAD activity, (J) fasting serum BOH. Correlations show changes from individual mean values for AUC glucose (RE, PE, and HIIT) for (K) calculated energy expenditure during the last 20 h before OGTT, (L) changes in respiratory exchange ratio during OGTT, (M) changes in fasting free fatty acids and (N) changes in fasting ketones. END = endurance athletes, CON = controls. RE = rest, PE = 3 h at 65% of VO_2max, HIIT = 5 × 4 min at ~95% of VO_2max. All values as group means ± SD. In figure (A–J), $n = 9$ in END, $n = 8$ in CON. In (K–N), END and CON are shown together ($n = 17$). (A-G and J) # denotes a main effect (condition and group) two-way RM-ANOVA. Differences to RE condition within each group; $^{*}p < 0.05$, $^{\dagger}p < 0.01$, $^{\ddagger}p < 0.001$. (H and I) Students paired t test; $^{\dagger}p < 0.01$, $^{\ddagger}p < 0.001$. (K–N) are Pearson's correlations and the 95% confidence intervals are shown. White circles = RE, gray circles = PE, and blue circles = HIIT.



Such findings are, to our knowledge, not highlighted or properly described in the existing literature.

There is a substantial body of evidence supporting that exercise training improves insulin sensitivity and glucose uptake, but these results have often been found when less-trained, diabetic, and obese subjects have been included.[40] Regulation of glucose uptake after acute exercise has been investigated in several different settings. In contrast to

the numerous studies associating exercise training with improved glucose handling (for review see[40]), several studies have found negative effects on glucose uptake the day after exercise.[21,22,24,41] It has been shown that exercise can acutely reduce glucose uptake when measured up to 3 h after cessation of exercise.[20,42–44] This effect of mild postexercise insulin resistance has been attributed to a blunted insulin release and altered secretion of gut peptides and hormones,[45] differences in glucose uptake in exercised and nonexercised muscles,[43] an increase in plasma FFA that interact with glucose metabolism,[20] and increased fat oxidation and ketonuria.[44] Indeed, in early work by Courtice et al., prolonged low-intensity exercise induced a subsequent loss of glucose tolerance during an OGTT and altered substrate metabolism in favor of fatty acid oxidation. When insulin infusion was added in the same setting, glucose uptake was normalized, whereas carbohydrate oxidation was still suppressed.[44] Within the first hours after exercise, muscle glucose uptake and glycogen synthesis are stimulated by an increase in muscle permeability to glucose,[46] splanchnic glucose output,[47] blood flow to the activated muscles,[43] and the activity of glycogen synthase.[48] Although low levels of glycogen lead to enhanced glycogen synthesis,[49] they are also associated with increased levels of plasma FFA,[50] which in turn, have been associated with decreased glucose tolerance.[23] Indeed, increased levels of FFA have repeatedly been put forward as the main cause of reduced glucose tolerance after exercise[20,21] and during fasting.[51] Also, muscle damage caused by eccentric loading during running has previously been suggested as a cause of reduced insulin-mediated glucose uptake after exercise.[22] In contrast to the studies that have used running,[21,22] our investigation employed cycling, which is not associated with muscle damage. We observed normal glucose tolerance after HIIT despite reduced glycogen levels, and we have previously reported alterations in glucose tolerance after exercise to be independent of alterations in glycogen.[24]

Based on previous studies and the results presented here, fatty acids appear to be a prioritized fuel during and after prolonged exercise training, but not during and after HIIT. This phenomenon is more pronounced in endurance-trained subjects than in controls. Endurance-trained subjects have a greater capacity for IMTG storage than less-trained subjects[52] and appear to be well protected against the increase in insulin resistance that occur after lipid infusion.[53] The high mitochondrial oxidative capacity in endurance-trained subjects has been pointed out as a possible explanation for this effect.[54] In a study by Phielix et al., trained athletes had a 32% higher mitochondrial oxidative capacity and a 22% higher insulin sensitivity at baseline compared with controls. During lipid infusion, the insulin-stimulated glucose uptake was reduced by 29% in athletes and 63% in controls. Athletes increased glycogen synthesis and reduced glucose oxidation in favor of lipid oxidation, thereby preserving glucose disposal to a higher degree than controls.[54] Although not measured, a higher capacity for insulin-stimulated glucose disposal would also be assumed in our endurance athletes. Despite this, the 70% higher mitochondrial respiratory capacity (Table 2) observed in END vs. CON did not protect against a lower glucose tolerance after PE. Indeed, previous findings of increased insulin resistance after severe energy deficit[51] have been shown to be attenuated in untrained subjects when exercise was performed at the end of a fasting period.[55] Interestingly, in the latter study, FFA and ketones were even higher after exercise compared with the rested state during fasting. It is likely that in the latter study, there was a greater positive effect of exercise on insulin sensitivity and glucose tolerance (compared with the changes in END after PE), since the exercise period was of shorter duration (about 1 h), and the IVGTT was performed 3 h after exercise. We observed a robust change in substrate metabolism in favor of fatty acid oxidation in END after PE whereas this response was less pronounced in CON. END further showed the greatest relative changes in serum FFA and ketones after PE. In absolute terms, fasting FFA levels were not higher than in CON, which suggests a higher sensitivity for altering the metabolic responses during exposure to increased levels of circulating FFA in END. Indeed, the higher activities of CS and HAD, reflecting higher mitochondrial volume and capacity to oxidize fat in END supports such a possibility. Moreover, the activity of carnitine palmitoyltransferase 1B (CPT1B) (transports medium/long chain acyl-CoAs across the mitochondrial membrane) is well correlated to the activity of CS,[56] which suggests that the capacity to transport fatty acids into mitochondria is increased in END vs. CON. Small increases in substrate availability can therefore induce relatively large increases in oxidation owing to increased transport and oxidative capacity in END. We further found that the calculated energy expenditure for the 20 h prior to OGTT correlated to changes in glucose tolerance. It is possible that the elevated fat metabolism during exercise and sensitivity to changes in substrate availability during recovery initiated a response to spare glucose in END. Interestingly, the duration of exercise has been pointed out as a determinant of improved insulin sensitivity in untrained subjects as two, but not 1 h of low-intensity exercise led to a robust decrease in insulin resistance the day after low-intensity exercise.[57] We did not measure plasma FFA at the end of exercise sessions but assume that all our subjects ended the PE session with substantially increased levels. However, our subjects ingested carbohydrates in both the recovery drink and evening meal after exercise. As demonstrated by Ivy and

TABLE 2 Subject characteristics.

	n	Age (year)	Weight (kg)	Height (cm)	BMI kg/m^2	VO_2max (ml·kg^{-1}·min^{-1})	W_{max} (W·kg^{-1})	LT1 (W·kg^{-1})	LT2 (W·kg^{-1})	Mito respiration pm·s^{-1}·mg^{-1} WW
END Female	5	35 ± 10	66 ± 12	171 ± 5	22.4 ± 2.9	51 ± 12	4.3 ± 1.1	2.1 ± 0.7	3.1 ± 0.8	20.2 ± 4.8
END Male	4	38 ± 5	73 ± 10	177 ± 5	23.5 ± 3.0	57 ± 5	5.0 ± 0.4	2.1 ± 1.0	3.5 ± 0.4	24.9 ± 6.5
END All	9	36 ± 8[†]	69 ± 11	173 ± 6	22.9 ± 2.5	53 ± 9[†]	4.6 ± 0.9[†]	2.1 ± 0.8[‡]	3.3 ± 0.7[‡]	22.3 ± 5.8[‡]
CON Female	2	27 ± 4	62 ± 13	168 ± 14	22.0 ± 1.1	38 ± 4	3.1 ± 0.3	0.9 ± 0.2	2.0 ± 0.1	12.6 ± 0.5
CON Male	6	24 ± 3	85 ± 14	180 ± 7	26.4 ± 4.5	43 ± 5	3.4 ± 0.5	0.5 ± 0.5	2.1 ± 0.4	13.3 ± 3.8
CON All	8	25 ± 3	79 ± 17	177 ± 10	25.3 ± 4.3	42 ± 4	3.3 ± 0.5	0.6 ± 0.4	2.1 ± 0.3	13.1 ± 3.2

Note: W_{max} = maximal power at the VO_2max test, LT1 = the first lactate threshold, LT2 = the second lactate threshold, Mito respiration = mean mitochondrial state III respiration activating CI + II related to muscle tissue wet weight. All values are measured at the inclusion test except for mitochondrial respiration, which is measured in all biopsies collected before OGTT and the mean respiration of each subject is included in the calculation of group mean respiration. Differences between END and CON were assessed using an unpaired students *t* test; Values are presented as mean ± SD.

Abbreviations: CON, controls; END, endurance athletes.

[†] $p < 0.01$;

[‡] $p < 0.001$.

colleagues, this strategy should suppress circulating FFA and increase glycogen synthesis, thereby blocking the negative effects of elevated FFA at the end of the exercise.[46,48] In summary, the deterioration of glucose tolerance after PE in END is likely due to the development of insulin resistance, which is associated with small increases in lipid load, a high mitochondrial capacity to oxidize lipids, and an increase in whole-body fat oxidation during the OGTT. Noteworthy, is that recent studies from this laboratory have shown similar results using a different paradigm. Untrained subjects were divided into two groups, a priori, based on a high vs. low expression of type I oxidative muscle fibers.[58] A large and similar lipid load was induced in both groups by a 3-day fast. The subjects who expressed higher amounts of type I muscle fibers and higher rates of fat oxidation in permeabilized muscle fibers also exhibited a greater loss of whole body-insulin sensitivity after starvation.[58] How increased fat oxidation would lead to insulin resistance under the conditions of the present study is not clear. However, others have demonstrated that increased fat oxidation is associated with the generation of lipid species that result in insulin resistance.[59–64]

We found the increase in circulating lactate during OGTT to be different between END and CON. A regulatory function of increasing lactate during increased glucose availability is that lactate, per se, can inactivate lipolysis, thereby reducing plasma FFA availability and uptake and subsequent oxidation in the muscles.[65] The suppressed lactate response during a glucose load can therefore assist in preserving fat oxidation to spare glucose for glycogen resynthesis, providing that the observed differences in lactate are sufficient to alter lipolysis. In obese subjects, high levels of fasting plasma lactate are used as a marker of non-oxidative glucose metabolism and is associated with impaired glucose handling.[66] In contrast, we observed that CON had increased lactate during OGTT after PE but that glucose tolerance was not affected, whereas END showed a decrease in lactate but had reduced glucose tolerance. We do not know if the alterations in circulating lactate reflected a change in metabolism or an increase in gluconeogenesis. Regardless of the origin, lower levels of circulating lactate can have a functional purpose by maintaining lipolysis, but with regard to the relatively small changes, it more likely reflects a change in substrate metabolism.

We did not find mitochondrial function to be affected by the different training regimens. Instead, we found that PE induced an increased H_2O_2 emission during state III respiration in END, whereas CON showed lower values. Higher production of mitochondrial ROS is often observed in different pathologies and is associated with negative changes in mitochondrial parameters.[30] Also, a diet high in fat has the potential to increase mitochondrial H_2O_2 emission in lean as well as obese subjects.[67] In line with our observation,

a similar effect of chronic training has previously been observed by Konopka et al. in obese females who underwent a training intervention. Twelve weeks of exercise training lowered basal H_2O_2 emission in the fasted state, but when measured 4 h after a high-fat meal, H_2O_2 emission increased and was equally as high as in the fed state before the training intervention.[16] In athletes, mitochondrial H_2O_2 emission has been found to be increased acutely after 24 h of ultra-endurance exercise and to be positively correlated with plasma FFA levels.[68] Our results, therefore, imply that the higher H_2O_2 emission and intact mitochondrial function observed in END are related to the increase in lipid metabolism observed during OGTT after exercise. In CON, substrate metabolism did not significantly change during OGTT after exercise and, in addition, exercise could have an inhibitory effect on the relatively higher basal values of H_2O_2 emission, thereby explaining the divergent response.

3.1 | Practical relevance and guidance

Endurance training increases the capacity for fatty acid oxidation. This metabolic strategy serves, in part, to spare circulating and stored carbohydrates, and prolonged exercise appears to have the potential to initiate a response to preserve glucose homeostasis in endurance athletes. High sensitivity for glycogen and glucose deficits and a shift in substrate priority in favor of fatty acids can have metabolic consequences that initially can be interpreted as maladaptive. However, a reduced glucose uptake can be a protective action to ensure sufficient substrate availability for the brain and other tissues, and it is possible that this metabolic strategy can be advantageous in endurance events where athletes must perform under severe energy deficits. On the downside, a reduced glucose uptake can also have negative effects on glycogen resynthesis if this response is not neutralized or avoided. Thus, recovery and subsequent performance may be negatively affected if glycogen resynthesis is not adequate. Athletes should therefore take this response into account when training and make sure proper nutritional strategies are implemented in preparation for competitions.

3.2 | Limitations in the present study and suggestions for future research

The main limitation of this study is the sample size and characteristics of the studied groups. In all, the collected data suggest that glucose homeostasis after exercise is affected by training status. The nonendurance-trained subjects that took part in this intervention were, in part, physically active sport science students, and some of the subjects regularly performed strength training. Thus, including only endurance athletes and sedentary controls may translate into larger differences in glucose homeostasis and better separation of the groups. The choice of training sessions can also be considered. For nonendurance-trained controls, a 3-h session of continuous cycling can be considered an extreme endurance feat, whereas, for endurance-trained athletes, it can be a medium–long endurance session that can be performed several times during a week. By scaling the exercise sessions to the subjects' capacity, larger differences in glucose tolerance might be expected. Also, the subjects in the two groups differed in age, and although this may affect the results, the differences were not dramatic. Future research should, besides taking this into consideration, investigate if an exercise-induced alteration in glucose tolerance and insulin sensitivity can affect glycogen resynthesis during recovery and if changes in glucose homeostasis can have consequences for athletic performance.

4 | CONCLUSION

Here we show that endurance athletes can exhibit a reduced glucose tolerance the day after prolonged exercise, whereas this response is less pronounced in nonendurance-trained controls. The reduced glucose tolerance observed in athletes was associated with insulin resistance and concomitant increases in circulating lipids and whole-body lipid oxidation. Prolonged exercise appears to induce a robust metabolic response in favor of lipid metabolism and sparing of glucose in athletes that is not as accentuated in nonendurance-trained controls. We believe, therefore, that this response constitutes an adaptation to endurance training that can be beneficial for securing blood glucose homeostasis during conditions when metabolism and substrate availability are challenged.

5 | MATERIALS AND METHODS

5.1 | Study design

The subjects participating in the intervention completed measurements of glucose homeostasis and mitochondrial function under the following three conditions: (1) after 3 days of rest (RE), (2) the day after 3 h of continuous exercise at low to moderate intensity (PE), or (3) the day after high-intensity interval training (HIIT). The three conditions were performed in a randomized order after 2 days of strict standardization of diet and rest. The subjects that exercised regularly also standardized their last exercise session before entering the study period.

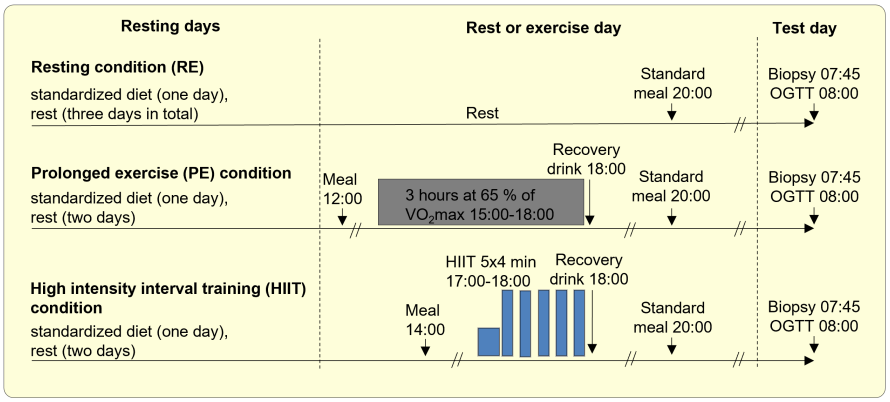


FIGURE 5 Schematics of study design. The subjects refrained from their own exercise for ~48 h before performing the exercise sessions that were studied. All exercise sessions took place 3 h after an individually standardized meal and a recovery drink was consumed immediately after cessation of exercise. A standardized dinner meal was consumed 2 h after cessation of exercise. The subjects arrived fasting to the laboratory the following morning ~14 h after exercise and donated a muscle biopsy before an OGTT was performed.

Biopsy sampling and OGTT were performed after an overnight fast, ~14 h after termination of exercise. The sample size needed for detecting significant differences in the primary outcomes was based on statistical power analysis and experience from previous investigations with similar settings. A schematic of the study design is presented in Figure 5.

5.2 | Subject characteristics

Eighteen subjects were separated into two groups depending on their background in endurance sports. One subject was excluded after having hyperglycemia at the end of the baseline OGTT (>7 mmol · L^{-1} at 120 min). Therefore, the data for 17 subjects are included in this report. The endurance-trained group (END) included 5 female and 4 male subjects, all of whom had several years of long-term acclimatization to training in preparation for competitive endurance events, ranging from hours up to ultra-events lasting several days. The control group (CON) consisted of 2 female and 6 male subjects. They did not engage in any kind of endurance training, but some of the subjects performed strength training regularly. The female subjects completed all trials and measurements during the luteal phase of their menstrual cycle. Subject characteristics are presented in Table 2. All subjects received written and oral information about the study design and risks and gave their written consent to participate. The study was approved by the local Regional Ethical Review Board in Stockholm and was conducted according to the Declaration of Helsinki. All material published in this paper conforms with good publishing practice in physiology according to the Acta Physiologica guidelines.[69]

5.3 | Pretest

A pretest for assessing physiological characteristics and determining VO_2max was performed on a cycle ergometer (Schoberer Rad Messtechnik, SRM, Jülich, Germany) and included measurements of heart rate (Polar Electro OY, Kempele, Finland), VO_2 (Oxycon Pro, Erich Jaeger GmbH, Hoechberg, Germany), and lactate (Biosen C-Line Clinic, EKF-diagnostics, Barleben, Germany). Cycling commenced at a workload of 70–100 W and increased by 15–30 W per stage consisting of 5 min duration, separated by 1 min of rest, which continued until the second lactate threshold was reached (LT2). VO_2 and CO_2 were continuously measured, and lactate was analyzed in capillary blood after each stage. After a few minutes of rest, a short-step incremental test followed (starting at the work rate associated with LT2 and increased with 15–20 W · min^{-1}) for assessing VO_2max, defined as the mean of the highest consecutive 45 s. The first and the second lactate thresholds (LT1 and LT2) were calculated using a modified Dmax-model standard at the lab.[70]

5.4 | Exercise training sessions

HIIT and PE were performed on the SRM ergometer. The HIIT session included a 10-min-long warm-up at 100 W, whereafter five 4-min-long intervals interspersed by 3 min of passive rest was performed. The first interval was paced at a work rate corresponding to 120% of LT2 and the remaining four intervals were self-paced with the highest possible mean power output as a goal. The mean work rate corresponded to ~95% of VO_2max. Blood lactate and glucose were measured after intervals three and five. Power output, VO_2, and CO_2 was measured continually during

the session. The PE session started at a work rate corresponding to 65% of VO_2max. If the subjects could not maintain the pre-set power output, the work rate was lowered, and the session was completed as close to the target work rate as possible. Lactate, glucose, VO_2, and CO_2 were measured throughout the exercise. Energy expenditure and substrate usage were calculated using the Brouwer equation.[71]

5.5 | Nutritional standardization and timing of muscle biopsies

The diet was standardized for each subject for 2 days before the biopsies and OGTT. During their first trial (RE, HIIT, or PE), a diet registration was completed. The registered energy intake was replicated on the following two occasions, and the last meal was always consumed 3 h before exercise. After exercise, the subjects consumed a recovery drink containing 1 g · kg^{-1} bw of carbohydrates and 0.25 g · kg^{-1} bw of protein and received an evening meal and drink consisting of 74 g of carbohydrates, 38 g of protein, and 26 g of fat, which was consumed 2 h after cessation of exercise. Thereafter, they remained fasting for 12 h until biopsy sampling and OGTT the next day.

5.6 | Oral glucose tolerance test and resting metabolic rate

The subjects arrived at the laboratory in the early morning after an overnight fast. First, the resting metabolic rate was measured using the Oxycon Pro with a ventilated hood. Thereafter, a catheter was inserted into an antecubital vein of the forearm and 15 mL of blood was drawn and a muscle biopsy was obtained. After drawing a second blood sample (time point 0), the subjects ingested 75 g of glucose dissolved in 300 mL of water. Additional blood samples were drawn at 15, 30, 45, 60 75, 90, 105, and 120 min after ingestion of glucose, and blood glucose and lactate was immediately analyzed in the Biosen C-line Clinic. Blood samples were drawn at 30, 60, 90, and 120 min and were put on ice and later centrifuged at 2800 rcf. Plasma and serum were transferred to new tubes and stored at −80°C. Additional measurements of resting metabolic rate were made at 40 and 115 min after ingestion of glucose.

5.7 | Biopsy sampling and mitochondrial measurements

Muscle biopsies from m. Vastus Lateralis were collected from alternating legs of each subject and in a randomized order. Local anesthesia (2% Carbocain, AstraZeneca, Södertälje, Sweden) was given at the biopsy site, an incision was made, and ~200 mg of wet tissue was removed using a 5 mm Bergström needle with manually applied suction[72] or a Weil-Blakesley chonchotome.[73] The muscle tissue was divided and put in ice-cold isolation medium for respiratory measurements, or in liquid nitrogen and later transferred to −80°C for storage. Isolation of mitochondria was performed as previously described[74] with modifications.[75] 10 µL of the mitochondrial fraction was injected into each of two wells of an oxygraph-2 k (Oroboros Instruments Corporation, Innsbruck, Austria) with an attached probe to measure H_2O_2 emission. The respiration medium used was MIR05 and all experiments were performed at 37°C after calibration. Two parallel mitochondrial protocols were performed. Protocol A: 1 U/mL Horseradish peroxidase +10 µM amplex ultrared, octanoyl carnitine 0.2 mM + malate 0.5 mM (leak fat), 2.5 mM ADP (fat respiration), rotenone 0.5 µM + succinate 10 mM (Complex II), channel B: 1 U/mL Horseradish peroxidase +10 µM amplex ultrared, pyruvate 5 mM + glutamate 10 mM + malate 0.5 mM (Leak PGM), ADP 0.75 mM (complex I), succinate 10 mM (Complex I + II). Mitochondrial respiration and H_2O_2 emission were related to the protein content in the mitochondrial fraction measured with a Pierce 660 nm protein assay (Thermo Fisher Scientific) except for measurements presented in Table 2. There, state III respiration was related to muscle wet weight, and the individual mean value from three measurements was used to calculate group means. Respiration normalized to the abundance of proteins in the isolated fraction offers the possibility to detect changes in mitochondrial function, that is, respiration per mitochondrial protein, whereas respiration normalized to muscle tissue wet weight reflects the oxidative capacity of the muscle. The composition of the isolation medium, respiration medium, and preservation solution has previously been published.[76] All measurements and analyzes were performed in DatLab 5.2 software (Oroboros, Paar, Graz, Austria).

5.8 | Immunoblotting

Immunoblotting was performed on freeze-dried muscle. Description of the homogenization protocol is found in[77] and immunoblotting in.[78] The antibodies were from Cell-signaling technologies; GS 3893, RRID: AB_2279563, pGS (Ser641) 47043, RRID: AB_2279563 and GLUT4 2213. RRID: AB_823508. The staining for each protein was related to the total memcode staining for each lane.

Visualization and quantification were performed in the Molecular Imager ChemiDoc XRS system with Image Lab software (version 6.0.1; Bio-Rad).

5.9 | Measurements in blood and muscle tissue

Insulin in serum was measured using an ELISA kit (Mercodia, 10-1113-01) according to the manufacturer's instructions.

β-hydroxybutyrate (BOH) concentration in serum was measured enzymatically with a method described elsewhere.[79] Serum and a buffer containing Tris-HCI (20 mM), hydrazine-hydrate (200 mM), and NAD+ (1.5 mM) were added to a 96-well plate and incubated at 25°C. Thereafter, β-hydroxybutyrate dehydrogenase was added and NADH concentration was read in a spectrophotometer (Tecan infinity pro) at 340 nm.

FFA concentration in serum was measured enzymatically based on a method described elsewhere.[80] Briefly, samples were added to a buffer containing Acyl-CoA synthetase and NADH. Coenzyme A was added to start the reaction and the disappearance of NADH was measured at 340 nm.

Citrate synthase (CS) activity was measured in homogenate from freeze-dried muscle. Homogenization procedures are described elsewhere.[77] Cuvettes were loaded with 5 µL of samples and 470 µL of reaction mix: 250 µL 0.4 mM DTNB, 25 µL 0.1 mM acetyl-CoA, 25 µL 1% Triton-X, and 170 µL H_2O. 25 µL oxaloacetate was added and the activity was measured in a spectrophotometer (Beckman Coulter DU800) at 412 nm at room temperature.

3-hydroxyacyl-CoA dehydrogenase (HAD) activity was measured in the same homogenate used for CS. Cuvettes were loaded with 2.5 µL of samples and 450 µL of reaction mix with stock concentrations: imidazole 500 mM, EDTA 20 mM, and NADH 5 mM, and 50 µL of 1 mM acetoacetyl-CoA was added and the activity measured in a spectrophotometer (Beckman Coulter DU800) at 340 nm at room temperature.

Muscle glycogen was measured in freeze-dried samples. The samples were dissolved in KOH (1 M) for 20 min at 70°C, neutralized with HCl, and incubated in acetate buffer (pH 4.9) and amyloglucosidase for 2 h. Thereafter, free glucose was measured following the production of NADPH at 340 nm in a spectrophotometer (Beckman Coulter DU800). The method has previously been described.[81]

5.10 | Data analysis

The area under the curve (AUC) for glucose, insulin, and lactate during the OGTT was calculated above the individual baseline for each subject and during the first 90 min. Values from the last 30 min were thereby excluded as our highly insulin-sensitive subjects displayed reactive hypoglycemia at the end of the OGTT. Curves for the full OGTT are presented for visualization. In Figure 4K, we calculated the energy expenditure (EE) for each subject during the 20 h preceding OGTT by adding the EE during the exercise sessions and the measured resting metabolism for the remainder of the time before the morning test.

5.11 | Statistics

Repeated measures of two-way ANOVA was used to detect an interaction effect (group and condition). If there were missing values, a mixed effects model analysis was used instead. The Geisser–Greenhouse correction was applied to correct for eventual cases of uneven distribution. Differences between groups at each time point during exercise (Figure 1) were assessed using Fischer's LSD test. Differences within groups between RE and in exercised states for all other measurements (Figures 2–4) were assessed using Dunnett's test. A paired Student's *t* test was used in Figure 4H,I and an unpaired t test was used in Table 2. Pearson's correlations were performed in Figure 4, and the p values are adjusted for repeated measures within each subject. All statistical analyses and creation of figures were performed in Graph Pad prism 8.3.1 for Windows (GraphPad Software, San Diego, California USA), and a p value of <0.05 was considered significant. Results are expressed as mean ± standard deviation (SD).

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CONFLICT OF INTEREST STATEMENT

The authors have nothing to disclose.

DATA AVAILABILITY STATEMENT

Some or all data sets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding authors upon reasonable request.

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