Elevations in blood lactate by exogenous infusion blunts exercise-stimulated whole-body lipolysis in healthy individuals: a randomized controlled trial

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Running title: Hyperlactatemia attenuates whole-body exercise lipolysis

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ABSTRACT

The purpose of this study is to determine in humans whether lactate inhibits exercise-stimulated lipolysis, fat oxidation, and plasma glucose turnover.

Eight healthy active individuals (3 women) completed 4 trials in a semi-randomized order. In two trials, subjects exercised for 60 min at 65±11% (mean±SD) of $VO_{2\ MAX}$ while receiving isovolumetric infusions of either sodium lactate (MOD+LACT trial) or saline (MOD trial). In another trial, exercise intensity was increased to 82±11% of $VO_{2\ MAX}$ (INT trial) to match lactate concentrations to those in the MOD+LACT trial. A trial without exercise was included. Stable isotopes of glycerol and glucose were infused to assess whole-body lipolysis (Ra Glycerol) and glucose turnover rates (Ra Glucose). Fat and carbohydrate oxidation were measured using indirect calorimetry.

Basal metabolic rate and plasma concentrations of lactate, glucose, insulin, FFA, and glycerol were similar across trials. Plasma lactate was clamped at 4.31±1.68 mM during the MOD+LACT trial, which tripled MOD concentrations (1.42±0.93 mM; p=0.001) and matched INT concentrations (4.39±1.25 mM; p=0.819). MOD+LACT blunted the exercise-induced elevation in Ra Glycerol of MOD (average during exercise, 7.9±2.0 vs 10.5±2.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; p=0.014). However, fat oxidation rates were similar in MOD+LACT and MOD (7.70 ±2.02 vs 7.38±1.47 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; p=0.612) but reduced during INT (5.30±2.93 $\mu mol \cdot kg^{-1} \cdot min^{-1}$; p=0.045). Ra Glucose, plasma glucose, and insulin were elevated during INT but not affected by lactate infusion.

Raising circulating lactate concentrations to levels observed during high-intensity exercise blunts exercise-stimulated whole-body lipolysis. However, in these metabolically healthy individuals, that reduction was not sufficient to compromise fat oxidation during moderate intensity exercise.

Keyword: Hyperlactatemia, Lipolysis, Plasma glycerol turnover, Exercise, Stable isotopes.

70 **NEW & NOTEWORTHY**

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72 We examined the effect of high blood lactate levels on exercise-induced lipolysis.
73 Lactate may contribute to the reduction in fat oxidation observed with increasing
74 exercise intensity. It could also be responsible for the reduced exercise lipolysis
75 observed in individuals with metabolic diseases. We found that raising blood lactate
76 during exercise reduces exercise lipolysis by 25%. Thus, lactate blunts exercise-
77 induced stimulation of lipolysis in humans, likely contributing to impaired fat metabolism
78 in metabolically diseased individuals.
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INTRODUCTION.

Exercise physiologists measure the increase in blood lactate concentration during incremental-intensity exercise to determine the workload at the lactate threshold (1). According to these measures, training zones are recommended to enhance athletic performance. Measurement of blood lactate is not only relevant to athletic performance but also serves as a key biomarker in emergency care for monitoring clinical conditions characterized by hypoperfusion (e.g., sepsis, shock, severe infection). Lactate is not merely an inert by-product of accelerated glycolysis, as it appears to participate in metabolism actively (2). During prolonged fasting or exercise, lactate helps maintain blood glucose by serving as a precursor for hepatic glucose production (Cori Cycle; (3)). Also, by shuttling lactate via specific transporters (MCTs), skeletal muscles can supply energy to vital organs such as the heart and brain (4). Lactate, in addition to serving as an energy substrate in carbohydrate metabolism, appears to influence lipid metabolism.

Issekutz and Miller were the first to report, in running dogs, that the rise in blood lactate levels at the onset of exercise was associated with a reduction in plasma free fatty acids (FFA) concentration. To establish a cause-and-effect relationship, they elevated resting plasma lactate to 10 mM and observed a concomitant decrease in plasma FFA that could not be explained by a plasma dilution effect. At the time, they speculated that the lactate anion might compete with albumin for FFA transport or that lactate might directly inhibit lipolysis in adipose tissue (5). Only recently have researchers demonstrated that lactate potentiates insulin's antilipolytic effect in mouse adipocytes in vitro (6).

It is debatable whether lactate also blunts adipose tissue lipolysis in humans. Elevations in plasma lactate from resting levels below 1 mM to 3.5 mM by intravenous lactate infusion do not seem to affect lipolysis, as estimated from glycerol and plasma free fatty acid concentrations (7, 8). Using microdialysis probes in abdominal subcutaneous tissue during moderately intense exercise, Trudeau and colleagues found similar elevations of glycerol in dialysate from probes perfused with lactate compared with those perfused with saline (9). During exercise, increasing plasma lactate to 4.5 mM does not reduce plasma glycerol or FFA levels in either the untrained or the trained state (10). In contrast to these data, Boyd and coworkers (11) showed that during low-intensity exercise, raising blood lactate to 8.8 mM significantly inhibited the increase in plasma FFA and glycerol. Thus, available data are controversial regarding the role of lactate as a potential inhibitor of lipolysis and fat oxidation at rest as well as during exercise in humans.

The cited studies assessed lipolysis based on changes in plasma FFA and glycerol concentrations. Although FFA and glycerol are the molecules resulting from triglyceride hydrolysis (i.e., lipolysis), their blood concentrations are determined by the balance between their entry into plasma and their disposal via oxidation or resynthesis. Thus, these ‘static’ measures of lipolysis are influenced by variations in the disposal of FFA and glycerol. However, the measure of glycerol rate of appearance in plasma using stable isotopes accurately reflects whole-body lipolysis. To our knowledge, only two human studies have examined the effects of hyperlactatemia on lipolysis using stable isotopes (12, 13), yielding contradictory results, and none have focused on exercise-induced lipolysis. However, it is of interest to examine the role of lactate in regulating exercise lipolysis since lactate may contribute to the reduction in fat oxidation observed as exercise intensity increases from moderate to intense. In addition, elevated blood lactate could be behind the reduced lipolysis during exercise in unfit individuals with metabolic diseases (i.e., diabetes, obesity, and metabolic syndrome (14)).

In this study of healthy humans, we raised blood lactate during moderate-intensity exercise to assess whether lactate blunts exercise lipolysis (e.g., assessed by Ra Glycerol). We included a high-intensity trial to endogenously raise blood lactate levels to those observed during the intravenous lactate infusion in the moderate-intensity trial. This trial helped determine whether elevations in blood lactate or increased glycolytic rates explain the reduced fat oxidation observed as exercise intensity increases. Combining indirect calorimetry and Ra Glycerol, we calculated fat oxidation and reesterification in the postabsorptive state at rest, during exercise, and during recovery. Finally, we assessed glucose turnover rates to provide a more comprehensive analysis of lactate's effects on exercise metabolism. The hypothesis was that hyperlactatemia could reduce exercise-induced lipolysis and potentially compromise fat oxidation rates.

MATERIAL AND METHODS

Ethical approval. All subjects provided written, witnessed, and informed consent in accordance with a protocol approved by the Bioethics Commission of the University of Murcia (M10/2026/115), complying with the latest version of the Declaration of Helsinki. The study is part of a larger project exploring the effects of blood lactate in individuals with metabolic syndrome, registered on ClinicalTrials.gov as NCT07426172.

Participants. Eight healthy, physically active adults (5 men and 3 women) who exercised for at least 30 minutes three times per week were recruited from students and staff at the University of Murcia and Castilla-La Mancha. Participant characteristics are shown in Table 1. Exclusion criteria included having a clinical history of cardiovascular, musculoskeletal, or other diseases in which intense exercise would pose a health risk. Female participants were not taking contraceptive medication and were eumenorrheic, defined by a self-reported menstrual cycle length (15). Trials were scheduled during the early-to-mid follicular phase (days 3–11 of the menstrual cycle), as determined by participants' reported menses onset.

Preliminary testing. Maximal aerobic power ($VO_{2\ MAX}$) was assessed on an electronically braked cycle ergometer (Ergoselect 200, Ergoline, Germany) during graded exercise testing (GXT) followed by a verification test (16) using indirect calorimetry (Moxus, AEI, USA) with simultaneous 12-lead ECG monitoring (Quark T12, Cosmed, Italy) to discard ischemic heart diseases. Maximal heart rate (HR_{MAX}) was recorded at the later stages of this preliminary test. An incremental lactate test with 4-minute stages was conducted on a different date to assess the workload to be set during the INT trial. After measuring blood lactate at rest using a portable analyzer (Lactate Plus, Nova Biomedics GmbH, Waldorf, DE), men pedaled at 80 watts and women at 60 watts for 4 minutes while blood was collected by fingerprick during the last minute. Workload was increased every 4 min by 15-20 W (men-women), and lactate was measured at each stage. The test was discontinued 3 stages after the blood lactate concentration had increased above baseline. The workload during INT was set at 10% above the intensity that raises blood lactate 1 mM above baseline (17).

Experimental Design. The study employed a quasi-randomized crossover design, including a non-exercise control trial (NO EXER). On four separate occasions, at least 1 week apart, participants arrived at the laboratory in the morning (7-8 AM) after an 8- to 10-hour overnight fast, having avoided strenuous exercise, alcohol, and tobacco for 48 hours before each trial. The night before each trial, participants ate dinner between 19:00 and 21:30, consisting of 325 g of precooked meat lasagna (4 g of fat, 9.5 g of carbohydrate, and 6 g of protein per 100 g), 120 g of white bread, and a medium-sized apple, amounting to 550 kcal. The following morning, subjects arrived at the laboratory fasted, having consumed 500 mL of mineral water upon waking. Participants were asked to keep a log of their meals (including timing, amounts, and types of food), physical activity, and sleep duration during the 48 hours preceding each trial to ensure replication across all trials.

Experimental trials. Upon arrival at the laboratory, body weight, body fat measured by bioimpedance (Tanita BC-418-MA, Japan), and hydration status assessed by urine-specific gravity (Usg; Uricon-NE, Atago, Japan) were recorded. Subsequently, a 20-G intravenous catheter (BD Insyte, Becton Dickinson, Spain) was inserted into an antecubital vein for the infusion of stable isotopes of glucose and glycerol. The catheter was connected through a 3-way stopcock (Luer-lock, CPK IV, Farmaban, Spain) to a 0.22 µm filter (Filter-lab, Spain) and, via extension tubing, to a calibrated dual-channel programmable infusion pump (NE-4000, PumpSystems Inc., USA). The pump contained two 60 cc syringes (Luer-lock, BD Plastipak, Spain) filled with the isotopic

tracers dissolved in sterile saline (0.9%, Laboratorios ERN, Spain). Another catheter was inserted into a dorsal hand vein after the skin was warmed for 15 min in a heat box set at 50 °C. This catheter was used to sample arterialized venous blood after 10 min of hand heating prior to each sampling. Finally, a third catheter was placed in the contralateral arm for infusion of sodium lactate solution (MOD+LACT trial) or an equal volume of isotonic saline (MOD trial). The MOD+LACT trial always preceded the MOD trial, whereas the remaining trials were conducted in a randomized order.

After lying on a gurney for 15 minutes, two baseline blood samples were drawn. Then, an infusion of a saline solution containing deuterated glucose and glycerol (i.e., 6,6-$^{2}H_2$ glucose and 1,1,2,3,3-$^{5}H_2$ glycerol, 99% purity; Cambridge Isotope Laboratories, Eurisotop, France) was initiated with a prime bolus of 18 μmol kg^{-1} followed by continuous infusion at 0.30 μmol kg^{-1}·min^{-1} until the end of the experiment. After 110 minutes of infusion to reach isotopic equilibrium, a blood sample was collected and 10 minutes later (i.e., at 120 minutes after the start of the infusion), another blood sample was taken to determine the resting plasma glycerol and glucose turnover rates. Resting metabolic rate was measured during the final 20 minutes of the 120-minute isotopic equilibrium period, with subjects resting on a gurney, using indirect calorimetry (Moxus, AEI, USA).

Then, lactate infusion commenced in the MOD+LACT trial during a 40 min rest period, while a similar volume of saline was delivered in the MOD trial. A 50% sodium lactate stock solution suitable for pharmaceutical use (Emprove®, Merk, Germany) was diluted 6-fold in sterile saline to a final concentration of 744 mM sodium lactate. After the initial 40 minutes of resting infusion to elevate blood lactate to the desired concentration, participants exercised continuously for 60 minutes on a cycle ergometer at 65±11% of their individual $VO_{2\ MAX}$. The MOD trial was conducted using the same intensity and exercise duration. In the INT trial, the workload was set at 82±11% of $VO_{2\ MAX}$ for 40 minutes, matching the total work performed in the other two exercise trials.

During exercise, indirect calorimetry data were collected at two sample times (15-to-25 min in all trials and 50-to-60 min during MOD and MOD+LACT and 30-to-40 min during INT) to calculate fat and carbohydrate oxidation and energy expenditure. We discarded the first 2 minutes of each 10 min breath collection period to avoid the initial hyperventilation response affecting RER measurements. Heart rate (WearLink®, Polar, Kempele, Finland) was also assessed at those same sample times. Blood was collected every 10 minutes during exercise (Figure 1). After exercise, participants returned to the gurney and rested for 15 minutes, after which blood was collected to assess plasma glycerol and glucose turnover during recovery. VO_2 and VCO_2 were measured at rest, during exercise, and during recovery to calculate substrate utilization. Upon trial completion, the infusion set (i.e., pump, extension lines, filter, 3-way stopcock, and catheter) was tested for accuracy by measuring the time elapsed to fill 5 mL in a graduated cylinder (Labbox, Glassco, Spain) when the pump was set at 0.5 mL·min^{-1}. Aliquots of the infused glycerol-glucose solution were collected for concentration and enrichment assessments. The exact infusion rate in each experiment was determined by measuring the glucose and glycerol concentration in the infusates.

Blood analysis. Eight-milliliter blood samples were collected at each extraction point. We measured whole-blood lactate concentration by placing 0.1 mL of whole blood on a lactate strip of a valid and reliable portable lactate analyzer (Lactate Plus, Nova Biomedics GmbH, Waldorf, DE; (18)). The remaining blood was divided into two tubes (i.e., EDTA K2, BD Vacutainer, USA), centrifuged for 15 minutes at 3000 rcf at 4ºC, and the resulting plasma was frozen at -70ºC until analysis. Plasma was analyzed for glucose and insulin using the glucose oxidase-peroxidase method (Enzymatic Glucose

Reagent, Thermo Scientific, Germany) and chemiluminescence (Architect System Insulin, Abbott Diagnostics Division, Germany; intra- and inter-assay CV of 0.9-1.2%), respectively. Plasma-free fatty acids were analyzed using an enzymatic colorimetric method (Fujifilm Wako Chemicals Europe, GmbH, Neuss, Germany; intra- and interassay CV 8.2%–9.4%). Plasma-free glycerol was analyzed using an enzymatic commercial kit (Sigma TR100, Saint Louis, MI, USA; intra- and inter-assay CV 7.5%-8.2%) with glycerol kinase, glycerol phosphate oxidase, and peroxidase to produce quinoneimine dye. The remaining portion of the plasma samples was prepared for analysis of glycerol and glucose isotopic enrichment.

Plasma isotopic tracer methods. To measure plasma isotopic enrichment, plasma proteins were precipitated by adding 3 mL of ice-cold acetone to 250 µL of plasma, followed by centrifugation at 4,000 rcf for 20 minutes. Then, lipid separation was performed by adding 3 ml of hexane and 3 ml of pure water to the transferred supernatant. After 15 minutes of gentle shaking and a second centrifugation, the lower aqueous phase was collected using disposable glass Pasteur pipettes. The aqueous phase containing the glycerol and glucose was dried using a concentrator (Thermo-Savant SPD Speed-Vac, Thermo Fisher Scientific, USA).

Glucose samples were derivatized by adding 2% hydroxylamine in pyridine to the dried plasma, heating for 45 min at 100ºC, then adding acetic anhydride-pyridine (2:1) and shaking for 20 min at room temperature. Tracer-to-tracee ratio (TTR) for 6,6-$^{2}H_2$ glucose was measured by monitoring ions at mass-to-charge ratios 189.1, and 219.1 for M+2 and dividing those from their M+0 ions.

Glycerol samples were derivatized with acetic anhydride-pyridine (2:1) and shaken for 20 min at room temperature. Tracer-to-tracee ratios for 1,1,2,3,3-$^{5}H_2$ glycerol were calculated from the areas of ions 106.1, 120.1, and 148.1 (i.e., M+3) divided by the areas of ions at M+0. The analysis was performed using gas chromatography-mass spectrometry (GC-MS-QP2020, Shimadzu, Kyoto, Japan) with automatic sample injection on a 30 m, 0.25 mm ID, 0.25 µm df chromatography column (Shimadzu, Kyoto, Japan). Baseline TTR was subtracted from all samples to express units as molecular percent excess (MPE). TTR data were curve-fitted as previously described (19).

Plasma turnover rate calculations. A plasma isotopic steady state was reached after 120 minutes of primed-constant tracer infusion, and plasma glycerol and glucose kinetics were calculated using one-compartment, non-steady-state Steele's equations (20). The glycerol and glucose rate of appearance (Ra) and disappearance (Rd) were calculated as follows:

$$Ra = F - [Vd \cdot [(C_2 + C_1) / 2] \cdot [(E_2 - E_1) / (t_2 - t_1)]] / [(E_2 + E_1) / 2]$$
$$Rd = Ra - [Vd \cdot [(C_2 + C_1) / (t_2 - t_1)]]$$

where F is the isotope infusion rate, Vd is the volume of distribution, estimated to be 145 ml·kg^{-1} for glucose and 230 ml·kg^{-1} for glycerol (20). C_1 and C_2 are plasma concentrations of the glycerol-glucose tracer at times 1 (t_1) and 2 (t_2), respectively, and E_2 and E_1 are isotopic enrichment of glucose (in mol percent excess; MPE) at t_1 and t_2, respectively.

Fat and carbohydrate oxidation rates were calculated using nonprotein Péronnet and Massicotte's equations (21):

$$\text{Fat oxidation } (\mu mol \cdot kg^{-1} \cdot min^{-1}) = [(1.695 \cdot VO_2) - (1.701 \cdot VCO_2)] * 1162.8 / \text{body weight}$$

Carbohydrate oxidation ($\mu mol \cdot kg^{-1} \cdot min^{-1}$) = [(4.585 · VCO_2) – (3.226 · VO_2)] * 5550.7 / body weight

where VO_2 and VCO_2 are in $L \cdot min^{-1}$. We used 860 as the molecular weight for a standard triacylglycerol (22) and 180 for the molecular weight of glucose (23).

Statistical analysis. All statistical analyses were performed in GraphPad Prism v 8.0.1 (GraphPad Prism Software, Inc, LLC, Boston, MA). Data were checked for normality using the Shapiro-Wilk test and for homogeneity of variance using Q-Q plots of predicted vs. actual residuals. The primary endpoint was a change in whole-body lipolytic rate between trials, measured as plasma glycerol turnover during exercise. A pre-study power calculation was performed with $\alpha = 0.05$ and $\beta = 0.8$, assuming an expected reduction in lipolysis of $\Delta = 15\%$ and a standard deviation (SD) of 0.17. This resulted in a power calculation of $n = 8$. Data were analyzed using two-factor (trial x time) repeated-measures ANOVA based on GLM. In the event of a significant main effect or interaction, Tukey's Honestly Significant Difference (HSD) post-hoc tests were performed to identify pairwise differences. Correction for multiple comparisons was performed using the Sidak method. Time, intervention, and their interaction were included as fixed effects, and participants were included as random effects. P values in the text represent those of the post hoc pairwise comparisons at the given sample times (basal, exercise, recovery). Variables measured once per trial were compared across trials using a one-way ANOVA. Data are presented as means ± SD to reflect the spread of individual data points within the sample. Comparisons between trials are further described by providing mean differences with 95% confidence intervals (CIs). P values <0.05 were considered significant.

RESULTS

Resting and recovery responses and lactate clamp. All participants completed all trials, and there were no missing data. As shown in Table 1, participants were lean, moderately fit, and insulin sensitive. Participants arrived at the laboratory, reporting that they had consumed the standardized dinner before 21:30 h the night before. First-morning urine specific gravity indicated that participants arrived euhydrated in all four trials (mean Usg 1.013±0.005). Body weight did not differ among trials (70.8±11, 71.1±10, 70.3±9, and 71.5±11 kg for CONT, MOD, MOD+LACT, and INT, respectively). There were no differences among trials in pre-exercise VO_2 (i.e., resting metabolic rate), respiratory exchange rate (RER), or heart rate (HR; all in Table 2). Fifteen minutes of recovery after exercise returned RER to pre-exercise values, whereas HR remained elevated, particularly after the intense exercise trial (INT; Table 2).

To elevate blood lactate to the target concentration during the MOD+LACT trial, the sodium-lactate solution (calculated osmolality 505 mOsmoles; pH 7.4) was infused at a rate of 0.039±0.001 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during the 40 min of rest and at 0.046±0.001 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ during the 60 min of exercise. A similar volume of sterile saline (calculated osmolality 308 mOsmoles; pH 6.75) was delivered during the MOD trial (i.e., 70±4 ml during the first 40 min of rest and 125±7 mL during exercise). During exercise in the MOD+LACT trial, blood lactate concentration was clamped at 4.31±1.68 mM, which was threefold higher than during MOD (1.42±0.93 mM; p=0.001) and matched the endogenously achieved blood lactate concentration during INT (4.39±1.25 mM; p=0.819; Figure 1).

Effects of lactate on fat metabolism during exercise. During moderate exercise, infusion of lactate (i.e., MOD+LACT) did not significantly alter oxygen consumption (VO_2) or VCO_2 production (i.e., similar RER; Table 2) compared with the MOD trial. Due to the higher workload (147±45 vs 91±24 watts, Table 2), the INT trial elevated VO_2 above the MOD trials. INT also elevated VCO_2, along with VO_2, resulting in higher carbohydrate oxidation and, conversely, reduced fat oxidation compared with the other trials (Figure 4A-4B). During exercise, the average fat oxidation rate was lower during INT than during MOD by 2.15; 95%CI -0.41 to 4.72 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (p=0.045) and lower than during MOD+LACT by 2.48; 95%CI -0.19 to 5.14 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (p=0.040; Figure 4A). Whole-body lipolysis (Ra Glycerol) was similar before exercise in all four trials (Figure 2B). However, during exercise, there was a time × trial interaction (F = 3.321, P = 0.046). MOD had higher Ra Glycerol than MOD+LACT with a mean difference of 2.68; 95%CI 0.81 to 4.54 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (p=0.026). INT also had higher Ra Glycerol than MOD+LACT with a mean difference of 1.80; 95%CI 0.84 to 2.75 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (p=0.012). As a result, the difference in the final 10 minutes of exercise between Ra Glycerol and Fat Oxidation (i.e., reesterification) was larger during INT and almost nil during MOD+LACT (Figure 3). The rates of plasma glycerol disappearance (Rd Glycerol) paralleled the Ra Glycerol response to the treatments (Figure 2C). The traceer-to-tracee ratio of glycerol (Figure 2A) showed the dilution of the tracer from unlabeled glycerol incorporation at the onset of exercise and the lesser dilution in the MOD+LACT trial.

Plasma FFA concentrations were similar before and during exercise and rose above exercise levels during recovery (Figure 5A). Plasma concentrations of glycerol were similar before exercise across all trials and increased progressively during exercise, with higher levels at the end of INT in comparison to the MOD and MOD+LACT trials (0.397±0.003 vs 0.311±0.018 and 0.294±0.039 mM, respectively, p<0.039; Figure 5B). Plasma concentrations of β-hydroxybutyrate did not change above rest with exercise in any of the trials (Figure 5C).

Effects of lactate on exercise carbohydrate metabolism. Ra Glucose was similar at rest across trials (i.e., 11.5±1.55 μmol·kg^{-1}·min^{-1}). During exercise, Ra Glucose increased more rapidly during INT, remaining higher than during MOD and MOD+LACT (exercise averages 28.6±2.9 vs 21.9±2.6 and 21.1±2.2 μmol·kg^{-1}·min^{-1}, respectively, $p<0.019$; Figure 6A). Plasma glucose was maintained at resting levels during MOD and MOD+LACT (4.8±0.2 and 4.8±0.2 mM, respectively) but increased during INT to 5.8±0.5 mM ($p=0.003$; Figure 6B). Lastly, plasma insulin was maintained at resting values during exercise in all trials but increased during INT in the recovery phase above MOD and MOD+LACT (88±39 vs 35±7 and 38±8 ρmol·L^{-1}), respectively, $p<0.033$; Figure 6C).

Effects of lactate on VCO_2 production. Hematocrit increased during the intense exercise trial (INT) and tended to decrease during MOD+LACT, suggesting plasma volume expansion. Ventilation rate was elevated during exercise in INT compared with the other two trials (Table 3). When ventilation rate was divided by the CO_2 production rate (VCO_2) to estimate ventilation drive, the drive was higher in MOD and INT than in MOD+LACT at the end of exercise (Table 3).

DISCUSSION

The key finding of the study was that raising blood lactate during moderate-intensity exercise to levels observed during high-intensity exercise (Figure 1) reduces whole-body lipolysis (i.e., Ra Glycerol; Figure 2B). However, it does not lower fat oxidation in healthy, active individuals. A reduction in lipolysis without a parallel reduction in fat oxidation is possible because lipolysis generally exceeds fat oxidation. Lipolysis exceeds fat oxidation by 70% at rest and by 10-30% during exercise in the fasted state, depending on exercise intensity and aerobic fitness level (24). The participants in this study were healthy and active but not endurance-trained, and their lipolytic rates exceeded fat oxidation by 27% in the MOD trial (Figure 3). The difference between lipolysis and fat oxidation has been attributed to reesterification, a process that requires energy and thus creates a futile cycle (19). Thus, in healthy individuals, hyperlactatemia reduced the excess of lipolysis relative to fat oxidation, approaching levels at which fat supply could compromise fat oxidation (Figure 3).

Our data show that, in healthy individuals, lactate blunting of exercise-stimulated lipolysis is not accompanied by a measurable reduction in fat oxidation during 60 minutes of moderate-intensity exercise. However, it remains unclear whether protracted hyperlactatemia, lasting hours or days, would compromise fat oxidation. Elevated blood lactate levels are associated with chronic metabolic diseases, including diabetes, obesity, and the metabolic syndrome (25). Our group and others have reported that individuals with metabolic dysfunction (26) and metabolic syndrome have elevated blood lactate (27) and a reduced rate of lipolysis during exercise compared with healthy young counterparts (14). It is therefore possible that the persistent elevation in blood lactate characteristic of some metabolic diseases contributes to their reduced ability to oxidize fat compared with healthy individuals. To our knowledge, clinical trials that manipulate lactate concentrations in individuals with metabolic diseases have not been reported.

Two other human studies have examined the effects of elevated blood lactate on lipolysis using stable isotopes, and both used FFA, not glycerol, as the tracer. Pedersen and colleagues used labeled palmitate and observed that, when lactate was increased from 0.6 to 3 mM, Ra FFA decreased by 30% in resting individuals (13). In contrast, Ahlborg and colleagues observed that the appearance of isotopically labeled oleate from adipose tissue was unaffected by raising lactate levels fivefold above resting levels (12). Both studies were conducted at rest. Unlike at rest, lipolysis is markedly stimulated during exercise, not only in adipose tissue but also in intramuscular triglycerides (28, 29), by neural and hormonal stimulation of β-adrenergic receptors. It is possible that lactate's interference with lipolytic stimulation may differ across fat depots, as they differ in the density and sensitivity of their β-adrenergic receptors (30, 31). Combining the isotopic tracer technique with muscle and adipose tissue biopsies may be needed to assess the origin of the inhibition.

While data from running dogs (5, 32) and mouse adipocytes (6) support lactate’s antilipolytic effect, human data are inconclusive and rely primarily on changes in blood FFA and glycerol concentrations as surrogates for lipolysis. Most studies in humans at rest (7, 8) as well as during exercise (9, 10) find that hyperlactatemia induced by intravenous lactate infusion does not reduce blood FFA or glycerol concentrations. Interestingly, the only exercise study to report reductions in plasma FFA and glycerol concentrations also reports elevated blood lactate levels beyond those observed in the other experiments (i.e., 8.8 mM vs 3-4 mM (11)). In vitro experiments using mouse (6) or human (33) adipose tissue show that lactate at 16 mM is required to reduce lipolytic stimulation by isoproterenol, a non-selective β-adrenergic agonist. Thus, it seems that high lactate concentrations may be necessary to counteract the

exercise stimulation of lipolysis via catecholamine activation of β-adrenergic receptors (34). Accordingly, high lactate levels could biologically reduce exercise-induced lipolysis. However, the lactate concentrations required for this effect are observed only when individuals exercise to exhaustion at intensities above their lactate threshold (35). At those exercise intensities, it is unclear whether elevated lactate, elevated glycolytic rate, or blood flow redistribution away from adipose tissue is responsible for the reduced fat metabolism (see discussion below).

Perhaps, the blunting of lipolysis by lactate infusion in the present data was mediated by reduced plasma catecholamine levels. Miller and colleagues have shown that clamping blood lactate at 4 mM during exercise at 55% of $VO_{2\,MAX}$ lowers plasma catecholamine concentrations almost by one half (36). It has been hypothesized that this effect takes place via a metabolic feedback control mechanism (36). Lactate infusion, by providing a readily available fuel source, reduces energy imbalance, which in turn lowers sympathetic nervous system stimulation of catecholamine production. However, in contrast to the study by Miller and colleagues (37), the lactate infusion of the present experiment, despite elevating plasma lactate to 4 mM, did not reduce Ra glucose or Rd glucose (Figure 6A). Thus, it is unclear whether, in the present situation, the metabolic feedback control mechanism acted to reduce sympathetic nervous stimulation of catecholamine production. Heart rate during exercise (a surrogate of sympathetic nerve activity) tended to be higher during MOD+LACT than during MOD at the end of exercise (Table 2). In summary, plasma catecholamine levels may have contributed to the lactate-induced blunting of Ra Glycerol during exercise (Figure 2A), an aspect not addressed in the current experiment.

As in other studies (7, 8, 10), we found that plasma FFA and glycerol concentrations, as static indices of lipolysis, did not change in response to hyperlactatemia (Figure 5). Measuring the plasma glycerol turnover rate (Ra Glycerol) was required to reveal that lipolysis was blunted by hyperlactatemia. Although plasma glycerol concentration usually tracks Ra Glycerol, glycerol is an energy substrate that, after cleavage from the triglyceride molecule, can be reutilized in the liver for glucose formation (i.e., gluconeogenesis) or by the liver and adipose tissue for triglyceride reesterification (19). Thus, plasma glycerol concentrations are affected by changes in glycerol reutilization rates. In contrast, after thorough distribution of the stable glycerol isotope across all fluid compartments (i.e., after 120 min of isotopic equilibrium), all glycerol cleaved out during triglyceride lipolysis that reaches the plasma, regardless of the tissue producing it or its final metabolic fate, will be accounted for by the stable isotope tracer technique. Thus, glycerol reutilization from plasma will not affect lipolysis measured by Ra Glycerol but will lower plasma glycerol concentration.

Glycerol may not reach the circulation due to phosphorylation within the cell and/or by incomplete triglyceride hydrolysis (38). In such cases, Ra Glycerol would underestimate whole-body lipolysis. Some authors report that neither of these events occurs to a significant extent in skeletal muscle or adipose tissue (39, 40) and that nearly all glycerol liberated via lipolysis enters the circulation after triglyceride hydrolysis. However, those experiments were not conducted with exogenous increases in circulating lactate. Thus, lactate may increase intracellular reesterification of glycerol, preventing glycerol from reaching the circulation. However, this would have resulted in a parallel reduction in plasma glycerol concentration during MOD+LACT, which did not occur (Figure 5B). The unchanged plasma glycerol concentration despite blunted Ra Glycerol in MOD+LACT could only be explained by a parallel decrease in the rates of disappearance of glycerol from plasma (i.e., decreased Rd glycerol; Figure 2C). The excess circulating availability of lactate during the MOD+LACT trial probably shifted the liver's preference for gluconeogenic precursor toward lactate (41) at the expense of glycerol (42), thereby reducing Rd glycerol.

Because blood lactate accumulation at intensities above the lactate threshold (i.e., 85% $VO_{2\,MAX}$) coincides with decreased fat oxidation and fatty acid turnover rates (29, 43), a direct effect of lactate on blunting adipocyte lipolysis has been suggested (11, 32). However, it remains uncertain whether this association between blood lactate and lipolysis is causal. Immediately after stopping intense exercise, plasma FFA concentration markedly increases, suggesting that rather than a reduction in lipolysis, a decrease in subcutaneous adipose tissue blood flow during intense exercise (44) may have temporarily sequestered the FFA. Additionally, reductions in fat oxidation at high exercise intensities could be driven by a high rate of glycogenolysis, which, in turn, increases malonyl-CoA, thereby inhibiting CPT1 and blunting long-chain fatty acid transport into the mitochondria (45, 46). The comparison of MOD+LACT to INT partially addressed this question. At a similar blood lactate concentration in both trials, lipolysis was reduced only in MOD+LACT, whereas fat oxidation was reduced in INT. The reduced fat oxidation during INT occurred despite sufficient lipolysis to provide FFA for oxidation (Figure 3). Our data suggest that the high glycolytic rate, rather than the elevated lactate concentration, reduces fat oxidation when exercise intensity is raised above the lactate threshold.

The Brooks lab has pioneered the lactate clamp technique for studying metabolism using stable isotopes. They have reported that lactate infusion during moderate-intensity exercise increases blood lactate oxidation and concurrently decreases blood glucose oxidation (37). The increased contribution of lactate to carbohydrate oxidation spares blood glucose and decreases hepatic glucose production, as assessed using stable isotopes (37). They found that blood lactate elevations could exert a glycogen-sparing effect in the exercising muscle of trained individuals (47). However, not all lactate clamp studies report a reduction in liver glucose output, measured using stable isotopes of glucose, during rest (7) or exercise (10). This sparing may occur only in endurance-trained individuals, as they exhibit enhanced capacity for lactate clearance and oxidation (48). Accordingly, in our healthy-active volunteers, we do not find a decrease in Ra Glucose during exercise or recovery when lactate is elevated to 4.5 millimolar. Hyperlactatemia did not affect blood glucose, insulin concentrations (Figure 6), or the rate of carbohydrate oxidation (Figure 4B).

Some reports have documented a moderate increase in blood buffering capacity following sodium lactate infusion. The rapid facilitated diffusion of lactate into tissues relative to its associated Na^+ results in extracellular strong base cation accumulation (i.e., Na^+; (49)). Probably because of mild alkalosis, pulmonary ventilation drive was reduced during MOD+LACT as evidenced by a lower V_E/VCO_2 ratio (Table 3). Blood alkalosis can lead to CO_2 retention and an overestimation of fat oxidation when using the respiratory exchange ratio (i.e., RER = VCO_2/VO_2; (49)). In addition, a report finds that lactate infusion at rest has a thermogenic effect, increasing oxygen consumption (7), which could further reduce RER. However, our lactate infusion trial did not yield a measurable increase in VO_2 (Table 2). Furthermore, during exercise, CO_2 storage is negligible relative to the high CO_2 production rates. In addition, studies inducing metabolic alkalosis during exercise report no effect on plasma FFA and glycerol concentrations, leg blood flow, or RQ (50). Thus, we argue that during exercise with lactate infusion, fat oxidation rates, as determined by RER, are valid (49). Finally, lactate infusion during MOD+LACT tended to reduce hematocrit, suggesting plasma volume expansion. However, we prevented hematocrit differences between trials by infusing a volume of saline equal to the lactate solution volume of the MOD+LACT trial.

Strengths of the present study include its crossover design, which reduces interindividual variability. Limitations include that although lactate infusion did not alter the main antilipolytic hormone (i.e., insulin), we did not measure catecholamines, the

chief lipolytic hormones during exercise. Lastly, while our study provides novel insight into the role of elevated blood lactate in exercise-induced lipolysis in healthy individuals, it remains unclear whether lactate has similar effects in individuals with obesity or diabetes.

In summary, there is no consensus in the literature on whether hyperlactatemia blunts fat metabolism in humans. In this study, for the first time, the effects of hyperlactatemia on whole-body lipolytic rates during moderate-intensity exercise (i.e., 65±11% $VO_{2\ MAX}$) were investigated. Exercise at that intensity markedly stimulated lipolysis above resting levels, increasing the likelihood of observing lactate's effects on lipolysis. We used Ra Glycerol as an index of whole-body lipolysis, which reliably detects all glycerol that reaches the circulation, regardless of its ultimate metabolic fate (i.e., oxidation, reesterification, or gluconeogenesis). We found that elevations of plasma lactate blunts whole-body lipolysis, but not to the extent of reducing fat oxidation in our sample of metabolically healthy individuals. In turn, hyperlactatemia did not affect plasma glucose turnover. Lastly, a similar elevation in plasma lactate levels, achieved by increasing exercise intensity (i.e., to 82±11% $VO_{2\ MAX}$), did not blunt lipolysis, suggesting that another factor is responsible for the reductions in fat oxidation reported during exercise above the lactate threshold.

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AUTHOR CONTRIBUTIONS. RM-R, DM-G, AM-C, conceived and designed the research. FM-P, LG-G, LM-G, CO-A, MM-G and JG-P, analyzed the data. RM-R, DM-G, AM-C, FM-P, LG-G, LM-G, CO-A, MM-G, and JG-P, performed the experiments. RM-R, DM-G, AM-C, FM-P, LG-G, LM-G, CO-A, MM-G, and JG-P, interpreted the results of the experiments. LG-G, LM-G, CO-A, MM-G, and JG-P, prepared the figures. RM-R, DM-G, AM-C, FM-P, drafted the manuscript. RM-R, DM-G, AM-C, FM-P, LG-G, LM-G, CO-A, MM-G, and JG-P edited and revised the manuscript, and all authors approved the final version of the manuscript.

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764

FIGURE CAPTIONS

Figure 1. Blood lactate concentration at rest, during exercise, and subsequent recovery during a non-exercise trial (NO EXER), a moderate intensity trial (MOD), a similar trial with exogenous lactate infusion (MOD+LACT), and an intense exercise trial to match lactate levels from endogenous production (INT). Data are mean ± SD for 8 healthy recreationally active volunteers (3 women). * Different than the MOD trial, all $p<0.05$.

Figure 2. A) Plasma glycerol tracer-to-tracee ratio, B) Ra Glycerol and C) Rd Glycerol, at rest, during exercise, and subsequent recovery during a non-exercise trial (NO EXER), a moderate intensity trial (MOD), a similar trial with exogenous lactate infusion (MOD+LACT), and an intense exercise trial to match lactate levels from endogenous production (INT). Data are mean ± SD for 8 healthy recreationally active volunteers (3 women). * Different than the MOD trial. # Different than the INT trial, all $p<0.05$.

Figure 3. Lipolysis (i.e., Ra Glycerol) compared to Fat Oxidation rate during the last 10 minutes of exercise in a moderate intensity trial (MOD), a similar trial with exogenous lactate infusion (MOD+LACT), and an intense exercise trial to match lactate levels from endogenous production (INT). Data are mean ± SD for 8 healthy, recreationally active volunteers (3 women). * Different than the MOD trial. † Different than the MOD+LACT trial, all $p<0.05$.

Figure 4. A) Fat Oxidation and B) Carbohydrate Oxidation rates, at rest, during exercise, and subsequent recovery during a non-exercise trial (NO EXER), a moderate intensity trial (MOD), a similar trial with exogenous lactate infusion (MOD+LACT), and an intense exercise trial to match lactate levels from endogenous production (INT). Data are mean ± SD for 8 healthy recreationally active volunteers (3 women). * Different than the MOD trial. † Different than the MOD+LACT trial, all $p<0.05$.

Figure 5. A) Plasma Free Fatty Acid, B) Plasma Free Glycerol, and C) Plasma β-Hydroxybutyrate concentrations at rest, during exercise, and subsequent recovery during a non-exercise trial (NO EXER), a moderate intensity trial (MOD), a similar trial with exogenous lactate infusion (MOD+LACT), and an intense exercise trial to match lactate levels from endogenous production (INT). Data are mean ± SD for 8 healthy, recreationally active volunteers (3 women). * Different than the MOD trial. † Different than the MOD+LACT trial. ‡ Different than the NO EXER trial, all $p<0.05$.

Figure 6. A) Ra Glucose and B) Plasma Glucose, and C) Plasma Insulin concentrations, at rest, during exercise, and subsequent recovery during a non-exercise trial (NO EXER), a moderate intensity trial (MOD), a similar trial with exogenous lactate infusion (MOD+LACT), and an intense exercise trial to match lactate levels from endogenous production (INT). Data are mean ± SD for 8 healthy

808 recreationally active volunteers (3 women). * Different than the MOD trial. † Different
809 than the MOD+LACT trial, all $p<0.05$.

810

811

812 **Table 1.** Participants' characteristics.

813

	means ± SD	(range)
Age (years)	29 ±13	(20 - 59)
Stature (cm)	176 ± 9	(162 - 184)
Body mass (kg)	72 ± 10	(55 - 84)
Body mass index ($kg \cdot m^{-2}$)	23 ± 1	(21 - 25)
Fat mass (kg)	12 ± 6	(6 - 25)
Body fat (%)	17 ± 9	(8 - 31)
Fat-free mass (kg)	60 ± 12	(41 - 76)
Fasting plasma glucose ($mmol \cdot L^{-1}$)	4.7 ± 0.3	(4.2 - 5.5)
Fasting plasma insulin ($pmol \cdot L^{-1}$)	26.2 ± 12.9	(11.1 - 62.5)
HOMA-IR	0.79 ± 0.40	(0.31 – 1.96)
$VO_{2\,MAX}$ ($L \cdot min^{-1}$)	2.94 ± 0.66	(1.89 - 3.61)
$VO_{2\,MAX}$ ($mL \cdot kg^{-1} \cdot min^{-1}$)	41 ± 6	(31 - 48)
Peak power output (W)	241 ± 66	(135 - 300)
HR_{MAX} ($beats \cdot min^{-1}$)	180 ± 14	(151 - 189)

814

815 Data are presented as means ± standard deviation for 8 healthy-active participants (3
816 females). HOMA-IR, homeostatic model of insulin resistance; HR_{MAX}, maximal heart
817 rate; $VO_{2\,MAX}$, maximal oxygen uptake. Body composition values are derived from
818 bioimpedance analysis.

819

820 **Table 2.** Physiological responses at rest, during exercise, and recovery.

	Rest	Exercise		Recovery	Exer AVG	Exer % MAX
		initial	final			
Workload (W)						
MOD+LACT	---	91±24	91±24	---	91±24	39±5%
MOD	---	91±24	91±24	---	91±24	39±5%
INT	---	145±36 *†	139±40 *†	---	147±42 *†	63±13% *†
NO EXER	---	---	---	---	---	---
VO_2 (mL·min^{-1})						
MOD+LACT	359±75	1863±404	1958±465	479±98 #	1902±426	66±11%
MOD	374±64	1829±445	1876±495	442±80 #	1849±464	64±11%
INT	392±84	2311±343 *†	2373±422 *†	481±75 #	2353±384 *†	82±11% *†
NO EXER	352±80	---	---	361±74	---	---
RER						
MOD+LACT	0.81±0.04	0.86±0.05	0.82±0.05	0.77±0.03	0.84±0.05	---
MOD	0.81±0.05	0.85±0.05	0.83±0.04	0.82±0.05	0.84±0.05	---
INT	0.80±0.06	0.93±0.07 *†	0.91±0.04 *†	0.78±0.03	0.92±0.04 *†	---
NO EXER	0.83±0.05	---	---	0.82±0.04	---	---
HR (beats·min^{-1})						
MOD+LACT	61±12	131±15	137±13	73±14 #	133±14	75±2%
MOD	58±10	129±16 †	131±16	72±10 #	130±15	72±3%
INT	57±10	147±16 *†	156±15 *†	78±14 #*†	153±14 *†	90±5% *†
NO EXER	59±6	---	---	57±5	---	---

821

822 Data are presented as means ± SD for 8 subjects (3 females). RER stands for respiratory exchange ratio. HR stands for heart rate. *
823 Different from MOD. † Different from MOD+LACT. # Difference between REST and RECOVERY. All $p<0.05$.

824 **Table 3.** Blood hematocrit, ventilation rate (V_E), and ventilatory equivalent for CO_2 (V_E/VCO_2).

	Rest	Exercise		Recovery
		initial	final	
Hematocrit (%)				
MOD+LACT	45.5±4.7	---	43.0±3.8	42.9±3.9
MOD	44.5±4.1	---	45.6±3.3	43.6±4.1
INT	44.3±4.7	---	47.5±3.8 *†	45.2±4.5
NO EXER	45.0±4.4	---	---	---
V_E ($L \cdot min^{-1}$)				
MOD+LACT	13±4	54±12	54±11	16±4
MOD	14±4	57±8	58±7	17±3
INT	14±4	79±13 *†	81±9 *†	19±3
NO EXER	13±3	---	---	---
V_E/VCO_2				
MOD+LACT	45±6	34±6	34±6	44±7
MOD	46±7	38±7	39±7 †	48±6
INT	45±6	39±6 †	38±6 †	52±9 #
NO EXER	46±8	---	---	---

825

826 Data are presented as means ± SD for 8 subjects (3 females). * Different from MOD. † Different from MOD+LACT. # Difference between
827 rest and recovery, All $p<0.05$.

828

Figure 1

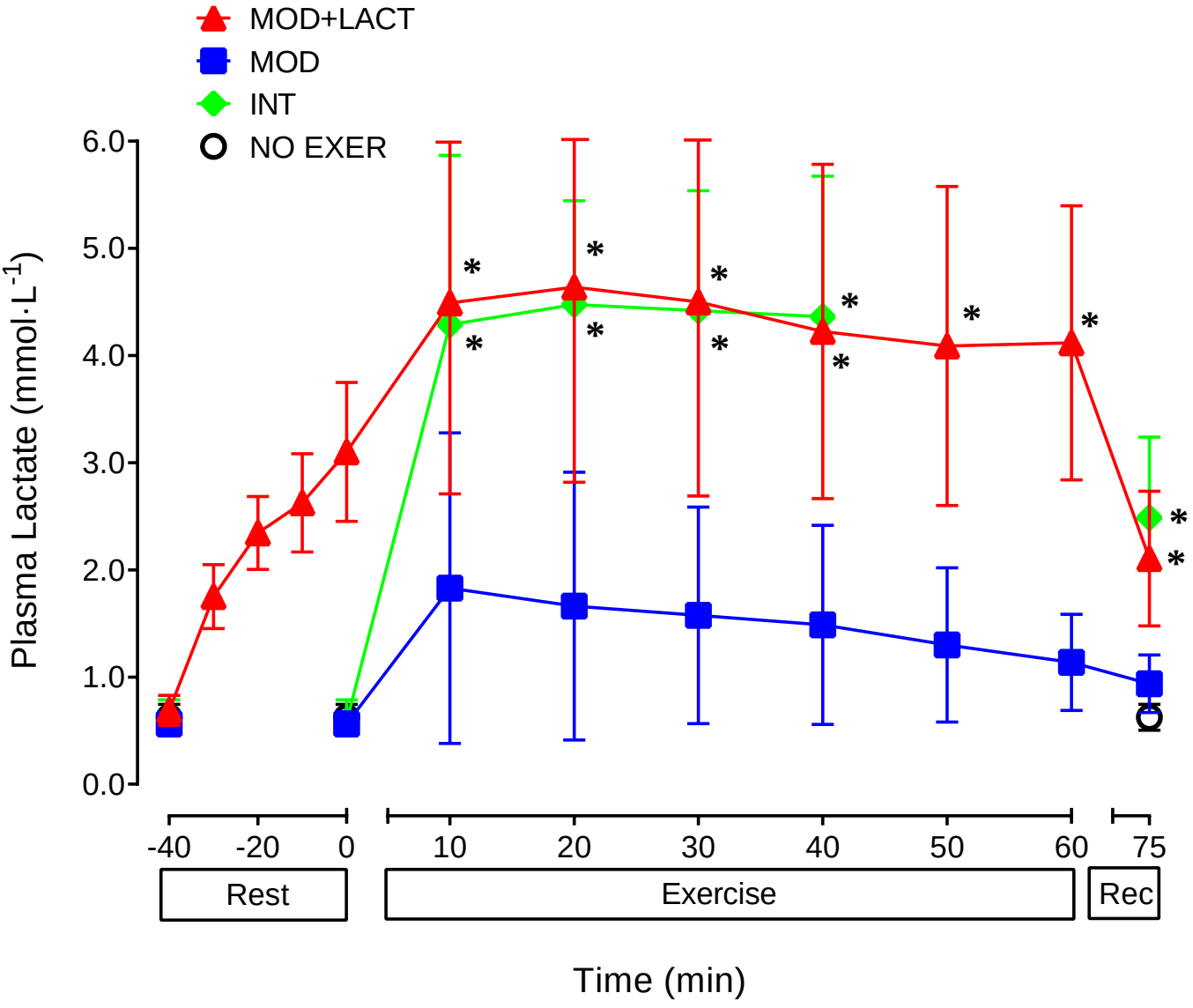


Figure 2

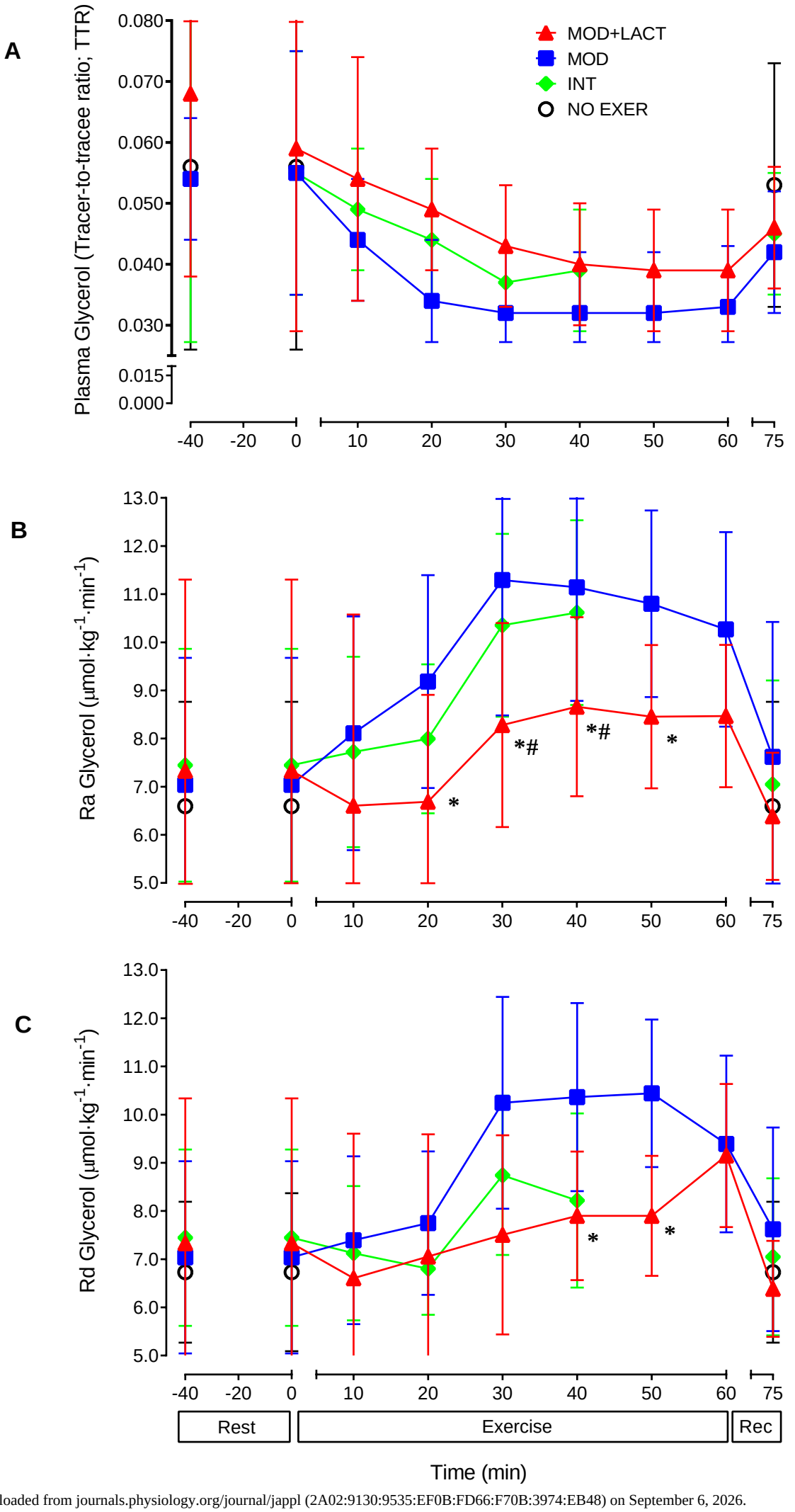


Figure 3

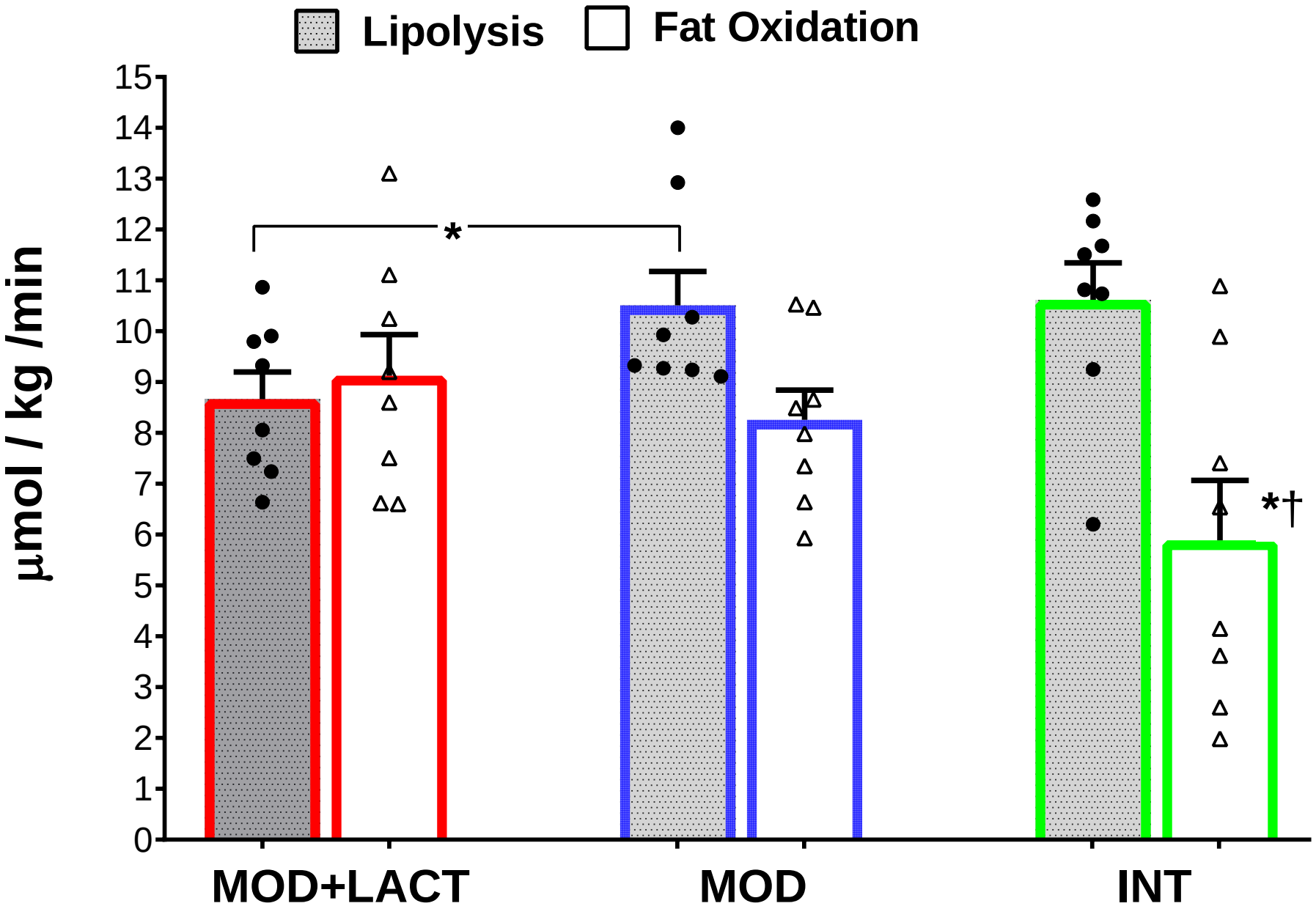


Figure 4

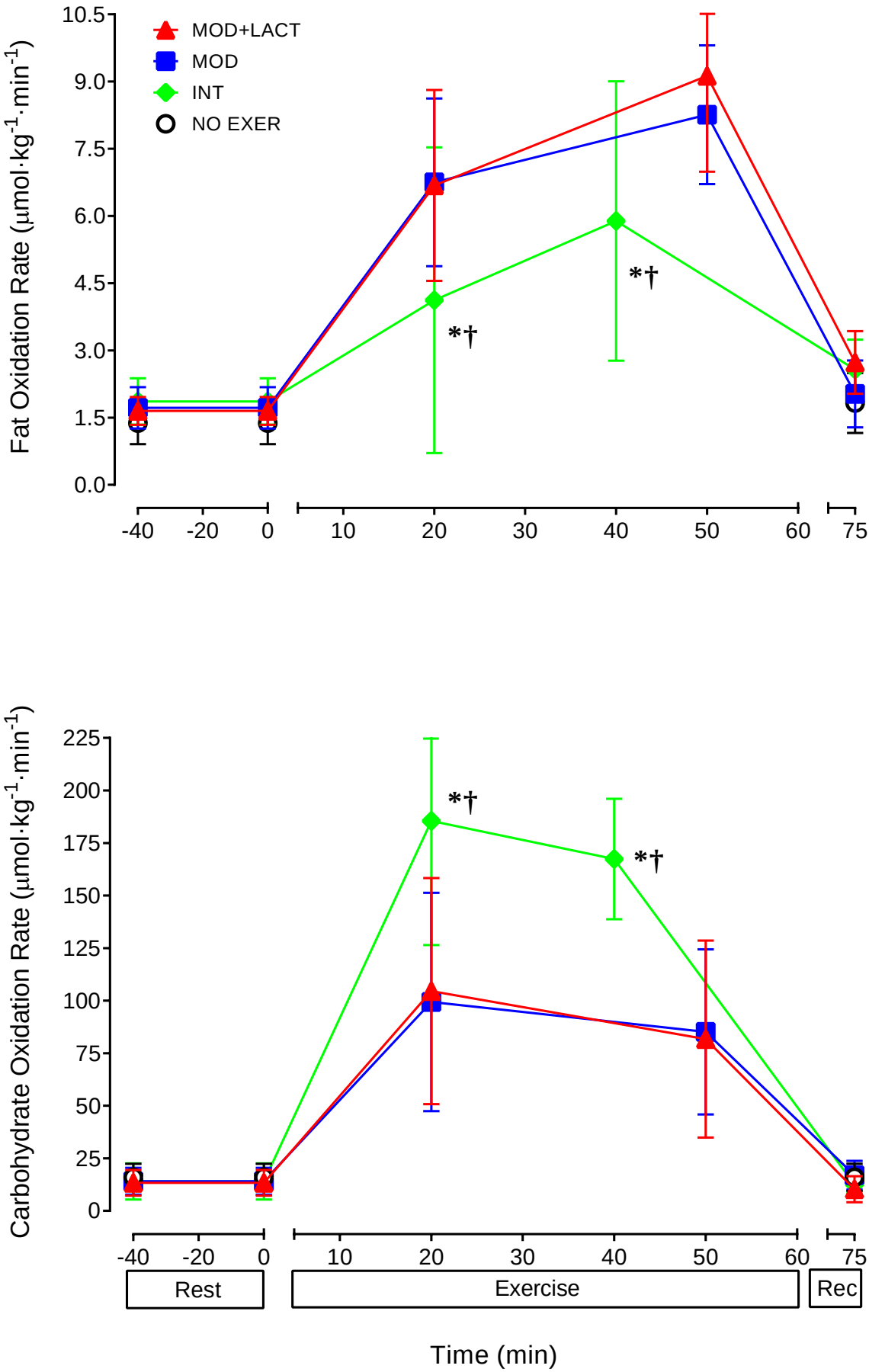


Figure 5

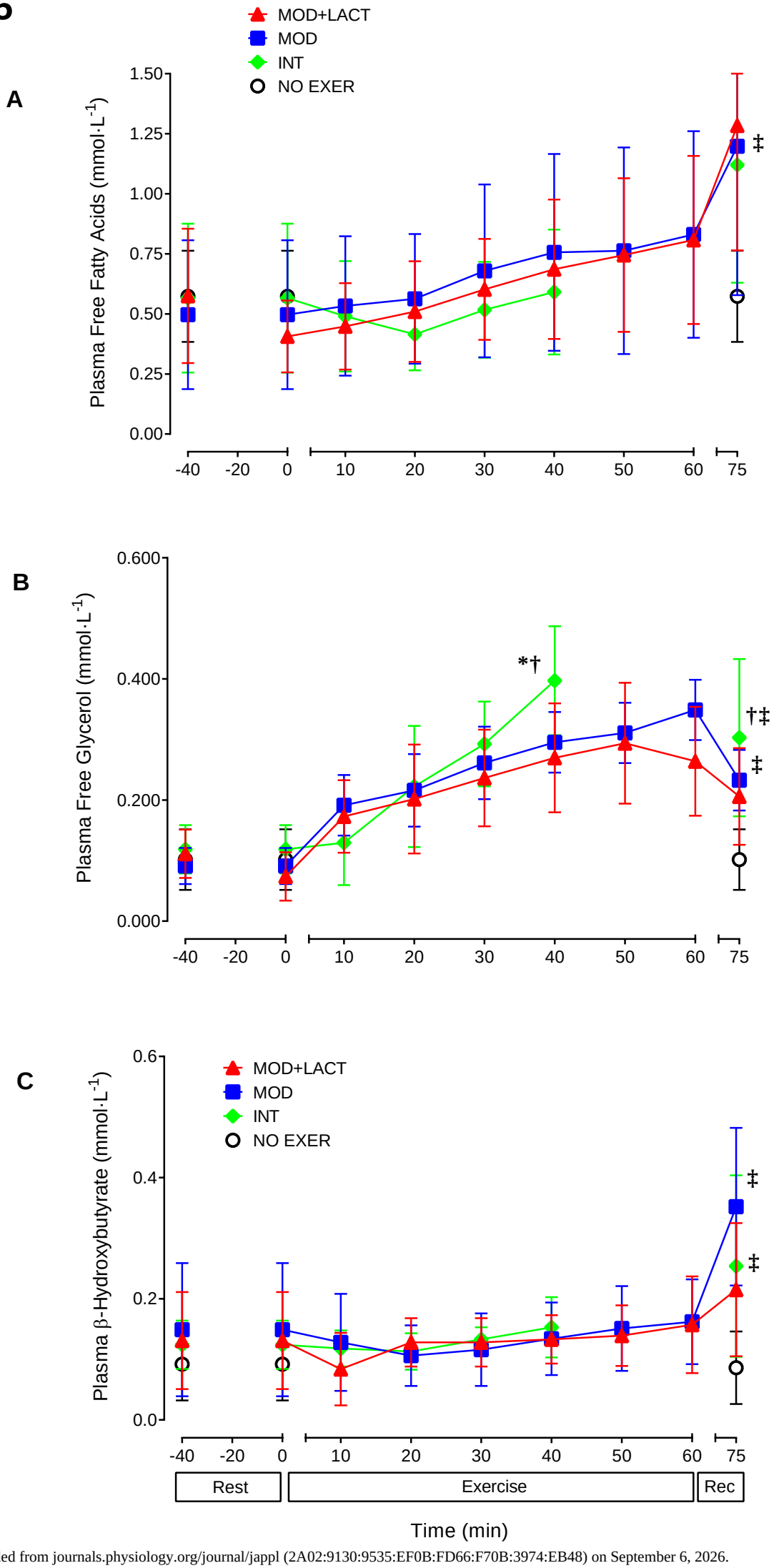
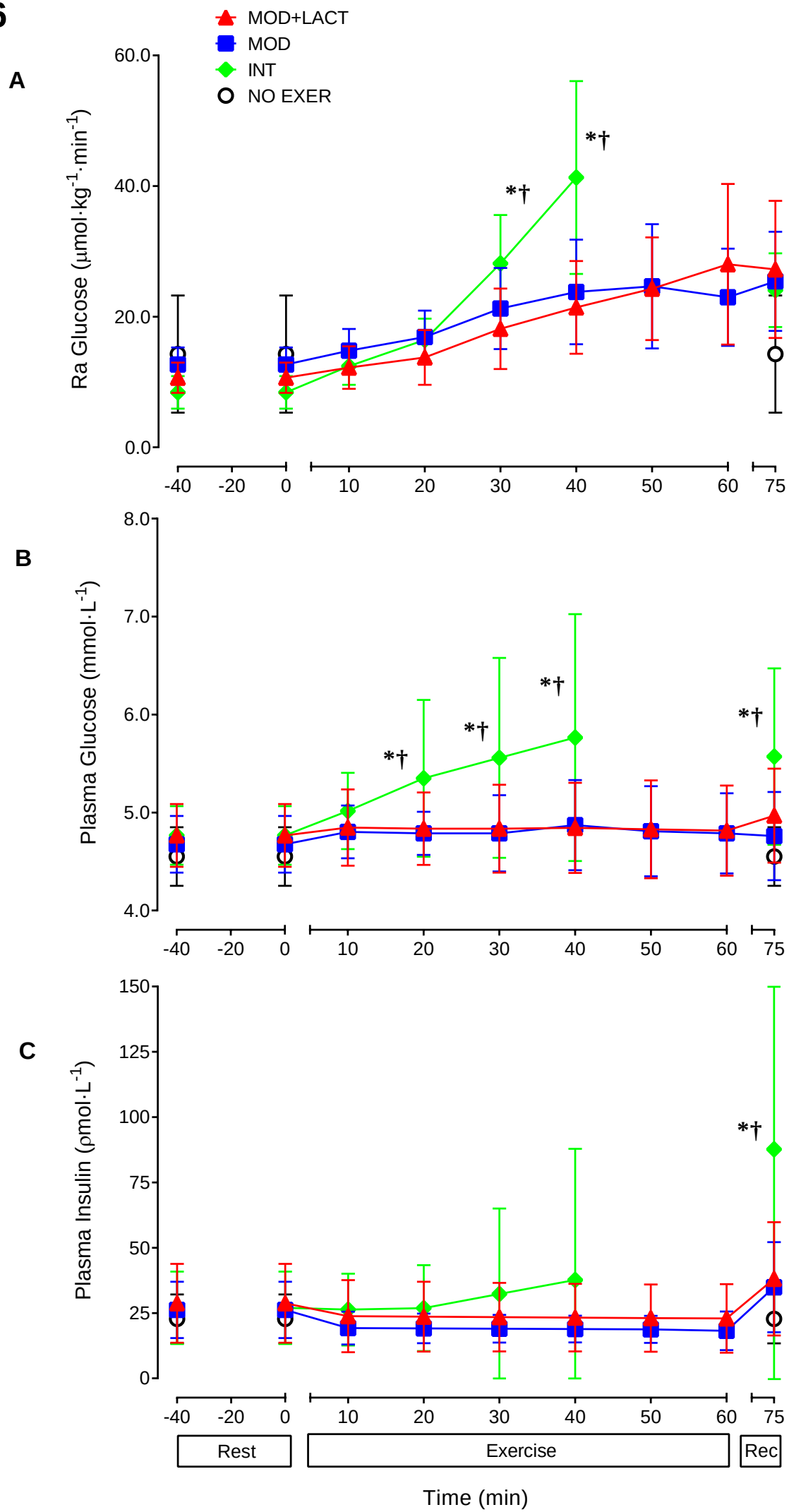
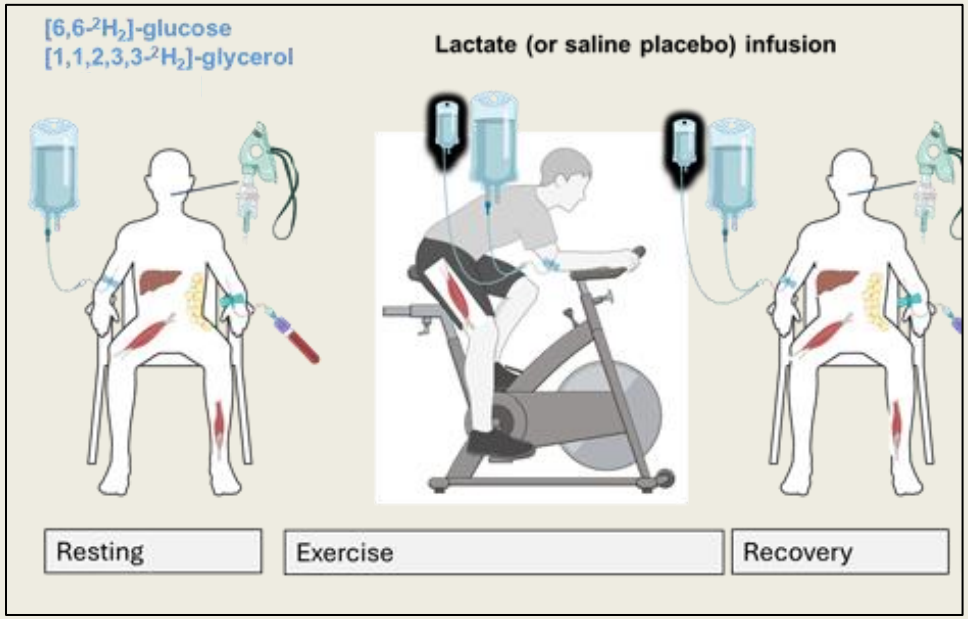


Figure 6



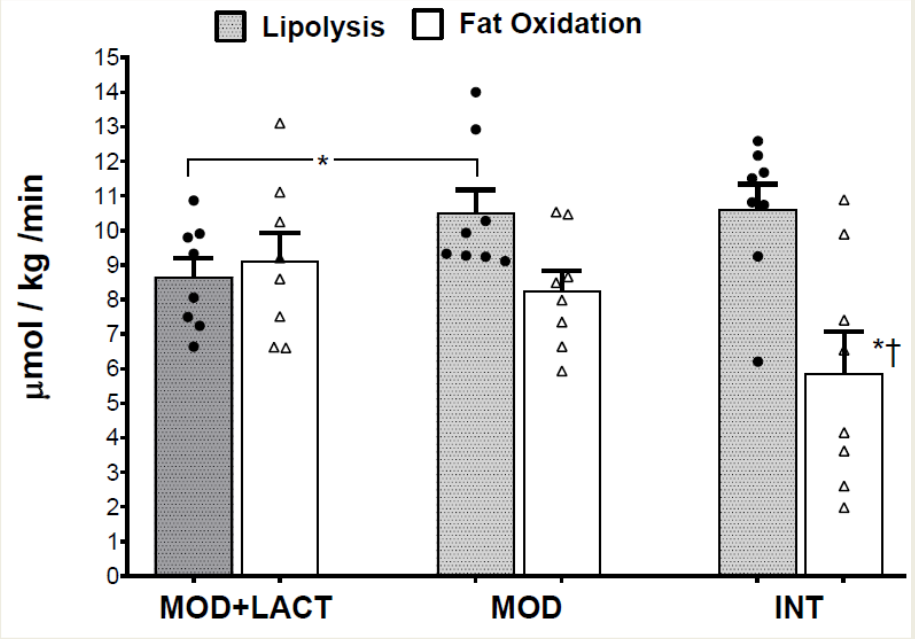
Effects of blood lactate elevations on whole-body lipolysis: A randomized control trial in humans

METHODS



3 exercise trials:
MOD at 65% VO_2max
MOD+LACT at 65% VO_2max with lactate infusion
INT at 82% VO_2max (same lactate than trial above)

RESULTS



Ra Glycerol (lipolysis) reduced during MOD+LACT.
Fat Oxidation reduced during INT.

CONCLUSION

Raising circulating lactate concentrations to levels observed during high-intensity exercise blunts exercise-stimulated whole-body lipolysis