Effects of β-adrenergic receptor stimulation and blockade on substrate metabolism during submaximal exercise

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Mora-Rodriguez, Ricardo, Bradley J. Hodgkinson, Lauri O. Byerley, and Edward F. Coyle. Effects of β-adrenergic receptor stimulation and blockade on substrate metabolism during submaximal exercise. Am J Physiol Endocrinol Metab 280: E752–E760, 2001.—We used β-adrenergic receptor stimulation and blockade as a tool to study substrate metabolism during exercise. Eight moderately trained subjects cycled for 60 min at 45% of V˙O2 peak (1) during a control trial (CON); 2) while epinephrine was intravenously infused at 0.015 μg·kg⁻¹·min⁻¹ (β-STIM); 3) after ingesting 80 mg of propranolol (β-BLOCK); and 4) combining β-BLOCK with intravenous infusion of Intralipid-heparin to restore plasma fatty acid (FFA) levels (β-BLOCK+LIPID). β-BLOCK suppressed lipolysis (i.e., glycerol rate of appearance) and fat oxidation while elevating carbohydrate oxidation above CON (135 ± 11 vs. 113 ± 10 μmol·kg⁻¹·min⁻¹; P < 0.05) primarily by increasing rate of disappearance (Rd) of glucose (36 ± 2 vs. 22 ± 2 μmol·kg⁻¹·min⁻¹; P < 0.05). Plasma FFA restoration (β-BLOCK+LIPID) attenuated the increase in Rd glucose by more than one-half (28 ± 3 μmol·kg⁻¹·min⁻¹; P < 0.05), suggesting that part of the compensatory increase in muscle glucose uptake is due to reduced energy from fatty acids. On the other hand, β-STIM markedly increased glycerogen oxidation and reduced glucose clearance and fat oxidation despite elevating plasma FFA. Therefore, reduced plasma FFA availability with β-BLOCK increased Rd glucose, whereas β-STIM increased glycerogen oxidation, which reduced fat oxidation and glucose clearance. In summary, compared with control exercise at 45% V˙O2 peak (CON), both β-BLOCK and β-STIM reduced fat and increased carbohydrate oxidation, albeit through different mechanisms.

THE ROLE OF β-ADRENERGIC RECEPTOR (β-AR) ACTIVITY FOR BALANCING CARBOHYDRATE AND FAT METABOLISM DURING EXERCISE IS NOT FULLY UNDERSTOOD. β-AR BLOCKADE LARGELY REDUCES FAT AVAILABILITY AND OXIDATION, WHICH REQUIRES A COMPENSATORY INCREASE IN CARBOHYDRATE OXIDATION TO MAINTAIN ENERGY PRODUCTION. INTERESTINGLY, DURING MODERATE-INTENSITY EXERCISE, THIS COMPENSATORY INCREASE IN CARBOHYDRATE METABOLISM INVOLVES INCREASES IN GLUCOSE UPTAKE (2, 3, 32, 38). THE MECHANISMS THAT GOVERN THE INCREASED PLASMA GLUCOSE UTILIZATION WITH β-AR BLOCKADE DURING EXERCISE HAVE NOT BEEN DETERMINED.

Administration of nicotinic acid to dogs during exercise reduces plasma free fatty acid (FFA) concentration and increases the rate of disappearance (Rd) of glucose, whereas plasma FFA replacement progressively lowers Rd glucose (5). These animal data suggest that the increases in Rd glucose are not an action exclusive to β-AR blockade but are possibly a compensatory response to an energy deficit created by reduced fat availability. A way to determine whether the reduction in fat availability is responsible for the increase in plasma glucose utilization is to intravenously infuse an Intralipid-heparin solution to restore intravascular lipolysis and plasma FFA during β-AR blockade. If the reduced fat availability is responsible for the increased Rd glucose during β-AR blockade, the infusion of Intralipid-heparin should increase fat oxidation and reverse the increase in Rd glucose.

However, in one report (41), when Intralipid-heparin was infused into β-blocked humans during intense exercise (70% Wmax ≈ 85% V˙O2 peak), fat oxidation did not increase. β-AR blockade does not impair plasma fatty acid uptake (35); thus the lack of increase in fat oxidation did not seem to be due to insufficient fatty acid availability. It is possible that high exercise intensity increases glycolysis to a level high enough to impair fatty acid oxidation. It has recently been reported that an increase in glycolytic flux associated with high exercise intensity (36) or high carbohydrate availability (12) may actively impair the entry of FFA into the mitochondria for oxidation. During moderate-intensity exercise (i.e., 45% V˙O2 peak), elevations in Rd glucose elicited by a preexercise carbohydrate meal (i.e., insulin mediated) are not reduced by Intralipid-heparin infusion that raises plasma FFA (21). Likewise, during intense exercise (85% V˙O2 max), Intralipid-heparin infusion does not reduce Rd glucose level (34). Despite these previous reports, we hypothesize that, during moderate-intensity exercise (45% V˙O2 peak) with β-AR blockade, restoring fat availability with In-
tralipid-heparin infusion may reduce R_d glucose. We think that this may be the case because, during moderate-intensity exercise with β-AR blockade, glycolytic flux is not stimulated from high glycogenolysis or hyperinsulinemia.

β-AR stimulation by epinephrine infusion in humans during exercise increases lipolysis and plasma fatty acid concentration. However, this increase in fat availability does not result in increased fat oxidation; rather, it is reduced (27). Recent studies performed at moderate and high exercise intensities (45–85% VO_2_max) have shown that carbohydrate use is reduced in the presence of high FFA from Intralipid-heparin infusion. In those studies, fat oxidation increased through a reduction in glycogenolysis (21, 29, 34) without affecting glycogen uptake, which does not support the Randle effect (31). Not surprisingly, epinephrine infusion that stimulates glycogenolysis (14, 22) does not allow for fat oxidation to increase despite elevated plasma FFA concentration. Apparently, the stimulation of glycogenolysis limits the entry of fatty acids into the mitochondria for oxidation (12, 36), despite having high circulating FFA levels.

Epinephrine infusion appears to determine the source of carbohydrate used for substrate during exercise. The powerful increase in glycogenolysis with epinephrine infusion reduces plasma glucose clearance in the contracting muscle of rodents (9). To our knowledge the effects of both β-AR blockade and stimulation in the same individuals during exercise have not been reported.

The main purpose of this study was to determine whether the reductions in fat availability are responsible for the increases in plasma glucose turnover during β-AR blockade. To achieve this goal, we restored plasma fatty acid levels during β-AR blockade. Second, we increased β-AR stimulation during exercise via intravenous epinephrine infusion to determine whether the stimulation of glycogenolysis and fat availability (lipolysis) affects plasma glucose kinetics and substrate oxidation during moderate intensity exercise in humans.

METHODS

Subjects. Eight moderately trained men (n = 4) and women (n = 4) participated in this experiment. Subjects were healthy and were not taking any medication. Women were premenopausal, were not taking oral contraceptives, and were tested during the follicular phase. Subjects’ (mean ± SD) age, peak oxygen consumption (VO_2_peak), peak heart rate, body weight, and percent body fat were 26 ± 7 yr, 55 ± 8 ml·kg⁻¹·min⁻¹, 185 ± 10 beats/min, 65 ± 10 kg, and 17 ± 6%, respectively. Before participation in the testing, subjects were informed of the possible risks involved and signed a consent form approved by the Internal Review Board of the University of Texas at Austin.

Experimental protocol. On four different occasions, subjects arrived at the laboratory in the morning, after an overnight fast (12 h). After 60 min of rest, subjects pedaled the cycle-ergometer (Jaeger-Ergotest) for 60 min at a constant work rate that elicited 45% of their VO_2_peak (Fig. 1). In one trial, β-ARs were stimulated by infusion of epinephrine (Adrenalin Chloride Solution, Parke-Davis, NJ) at a constant rate (0.015 μg·kg⁻¹·min⁻¹) from the 15- to 60-min period of exercise (β-STIM). In another trial, β-AR blockade was produced by ingesting 80 mg of propranolol 2 h before exercise (β-BLOCK). In another trial, the β-blockade treatment was combined with intravenous infusion of a 20% triglyceride emulsion (Intralipid; Clintec Nutrition, Deerfield, IL) with sodium heparin (Elkins-Sinn, Cherry Hill, NJ) to restore lipolysis and plasma FFA concentration (β-BLOCK+LIPID). One hour before exercise, a sodium heparin bolus (7.1 U/kg) was infused, followed by a constant-rate infusion of Intralipid (0.46 ml·kg⁻¹·h⁻¹) with sodium heparin (5.5 U·kg⁻¹·h⁻¹) throughout rest and exercise. Finally, the normal responses to 60 min of 45% VO_2_peak exercise were measured during the control trial (CON). During all four trials, electrocardiogram tracing was monitored throughout the testing period to confirm that normal sinus rhythm was maintained. The order of the trials was randomized, and they were separated by ≥48 h.

Isotope infusion. When subjects arrived at the laboratory, Teflon catheters were inserted into an antecubital vein in each arm for infusion and blood sampling, respectively. A heating pad was affixed to the sampling forearm to obtain arterialized blood. A blood sample (4 ml) was withdrawn for determination of background isotopic enrichment. Then, a primed, constant-rate infusion of [3H_2]glucose (prime = 20 μmol/kg; 0.25 μmol·kg⁻¹·min⁻¹), [3H_2]glycerol (prime = 3.7 g·l⁻¹·min⁻¹) with [3H_2]glucose (prime = 2.0 μmol/kg; 0.25 μmol·kg⁻¹·min⁻¹) and [3H_2]glycerol (prime = 0.5 μmol/kg; 0.25 μmol·kg⁻¹·min⁻¹) was initiated into each arm. Blood samples (4 ml) were withdrawn at 10, 30, and 60 min after the initiation of isotope infusion.
μmol/kg: 0.25 μmol·kg⁻¹·min⁻¹; Isotec, Miamisburg, OH), and [1-¹³C]palmitate (no prime; 0.04 25 μmol·kg⁻¹·min⁻¹; Cambridge Isotope Laboratories, Andover, MA), bound to 5% human albumin (Bayel, Elkhart, IN), was started using calibrated syringe pumps (Harvard Apparatus, South Natick, MA). Subjects were infused for 1 h before the start of exercise to allow attainment of isotopic equilibrium. The rates of infusion were maintained during the 60 min of exercise. Before being infused into subjects, each isotope was diluted in sterile saline, tested for pyrogenicity, and passed through a 0.2- to 0.45-μm syringe filter (Acrodisc, Gelman Scientific, Ann Harbor, MI).

Blood sampling and analysis. For determination of resting glucose, glycerol, and palmitate kinetics, blood samples were withdrawn 5 min and immediately before exercise. During exercise and recovery, blood samples (~14 ml) were collected every 10 min. After collection, blood samples were divided into four different, prechilled tubes according to the constituents to be analyzed. For each tube, plasma was separated by centrifugation (1,000 g for 20 min at 4°C) and immediately frozen at −70°C until analysis. The blood collected during the β-BLOCK+LIPID trials was immediately centrifuged and processed to prevent in vitro lipolysis. Four milliliters of each blood sample were placed into tubes containing 0.2 ml of EDTA solution (25 mg/ml) and analyzed for isotopic enrichment of the heptafluorobutyric anhydride derivative of glucose and glycerol (16) and the methyl ester derivative of palmitate (18) by means of gas chromatography-mass spectrometry (Hewlett-Packard 5989). Five milliliters of plasma were placed in tubes containing 0.25 ml of EDTA (25 mg/ml) for determination of plasma glycerol [fluorometric assay (15)], plasma free fatty acids [FFA, colorimetric assay (28)], glucose (glucose auto-analyzer, YSI, 23A), and lactate [spectrophotometric assay (17)]. Three milliliters of blood were mixed in a tube containing 0.3 ml of a solution of reduced glutathione (4.5 mg), sodium heparin (50 IU), and 20 μl of 0.24 EGTA for determination of epinephrine and norepinephrine concentration (HPLC with electrochemical detection (29)). The final 2 ml of each blood sample were placed into a test tube containing 0.2 ml of an EDTA (24 mg/ml)-aprotinin (0.5 TIU/ml) solution and analyzed for plasma insulin concentration (radioimmunnoassay, Linco Research, St. Charles, MO).

Preliminary testing, diet, and training. VO₂ peak was determined while subjects cycled an ergometer (Monark-819, Varberg, Sweden) by use of an incremental protocol lasting 7–10 min. Two days before the first experimental trial, subjects performed the experimental exercise protocol (60 min at 45% VO₂ peak) to ensure homogeneity of the last exercise bout. Subjects refrained from training during the 24 h before the experimental trials. Subjects were asked to exactly replicate the last meal at the same time of day before each of the four trials.

Measurement of gas exchange. Periodically during exercise, subjects inhaled through a two-way Daniels valve while inspired air volume was measured with a Parkinson-Cowan CD4 dry gas meter (Rayfield Equipment, Waitsfield, VT). The expired gases were continuously sampled from a mixing chamber and analyzed for oxygen (Applied Electrochemistry, SA3, Ametek, Pittsburgh, PA) and carbon dioxide (Beckman LB2; Schiller Park, IL). These instruments were interfaced to a computer for calculation of VO₂ and carbon dioxide production (VCO₂).

Calculations. Plasma glucose, glycerol, and palmitate kinetics were calculated using the one-pool model non-steady-state equations of Steele (39), modified for use with stable isotopes

\[
R_\alpha = \frac{F - V_\alpha \cdot \{(C(1 + [(E_1 + E_2)/2]) \cdot [(E_2 - E_1)/(t_2 - t_1)]\}}{(E_1 + E_2)/2}
\]

where F is the isotope infusion rate, Vα is the effective volume of distribution, C is the plasma concentration of the tracee, and (E₂ − E₁)/(t₂ − t₁) is the change in enrichment (i.e., E = tracer-to-tracee ratio) between two consecutive samples (i.e., t₂ − t₁, −10 min). Vα was assumed to be 100 ml/kg for glucose, 230 ml/kg for glycerol, and 40 ml/kg for palmitate, based on previous reports (33). Fatty acid and carbohydrate oxidations were calculated from VO₂ and VCO₂ (30). The rate of appearance of glycerol in blood (Ra glycerol) was measured to quantify whole body lipolysis (21, 33). Ra FFA were calculated by dividing Ra palmitate by the fractional contribution of palmitate to total FFA concentration as determined by gas chromatography (GC-FID; Varian 3400). Ra FFA was calculated by following the formulas presented above. Ra glucose was quantified as an index of liver glucose output (4) and Rd glucose as the index of whole body plasma glucose uptake. The following was also calculated

\[
\text{minimal glycogen oxidation} = \frac{\text{total carbohydrate oxidation} - \text{Ra glucose}}{\text{VO₂ peak}}
\]

This last calculation assumes that, at this exercise intensity, all of the plasma Ra glucose is directed to the exercising muscle (26) and is oxidized (23). This assumption seems to be generally correct during moderately intense exercise with or without β-AR blockade (32). The relative contributions by plasma glucose (Ra glucose), muscle glycogen, and fatty acids to total energy expenditure were estimated by using standard caloric equivalents.

Statistical analysis. SPSS for Windows software was used for statistical analysis. Statistical differences among treatments and over time were identified by using analysis of variance with repeated measures in a complete within-subjects design. Time points were specifically examined for significance by use of contrasts solved by univariate repeated measures. Statistical significance was defined as P < 0.05. The results are presented as means ± SE for eight subjects.

RESULTS

Plasma catecholamine concentrations. Resting plasma epinephrine concentration was similar among trials (0.6 ± 0.2 nM). β-STIM increased plasma epinephrine concentration above CON from 0.8 ± 0.1 to 2.2 ± 0.4 nM at 60 min of exercise (P < 0.05). β-BLOCK also increased epinephrine above CON to 3.0 ± 0.8 nM at 60 min of exercise (P < 0.05). β-BLOCK+LIPID increased epinephrine levels above CON (2.1 ± 0.9 nM; P < 0.05) but less than with β-BLOCK alone. Plasma norepinephrine was similar among trials at rest (1.4 ± 0.3 nM). β-STIM did not affect the normal increase in norepinephrine observed during CON (2.2 ± 0.2 nM at 60 min). However, β-BLOCK and β-BLOCK+LIPID increased plasma norepinephrine throughout exercise (3.3 ± 0.5 and 2.9 ± 0.4 nM, respectively, P < 0.05).
Plasma glucose and insulin concentrations. During the 20- to 60-min period of exercise, plasma glucose concentration (Fig. 2A) increased above CON (4.8 ± 0.1 mM) during β-STIM (5.4 ± 0.1 mM; P < 0.05). In contrast, plasma glucose concentration decreased during the first 30 min of exercise with β-BLOCK (4.5 ± 0.1 mM; P < 0.05), returning to CON values thereafter. Plasma glucose levels were maintained similar to CON during β-BLOCK+LIPID (4.8 ± 0.2 mM; P < 0.05). After 15 min of exercise, plasma insulin (Fig. 2B) declined in all trials by ~3.0 ± 0.3 μU/ml. After 30 min of exercise, plasma insulin increased with β-STIM above CON values (6.7 ± 0.6 vs. 5.4 ± 0.4 μU/ml, respectively; P < 0.05). However, during β-BLOCK and β-BLOCK+LIPID, insulin concentrations were reduced below CON (3.5 ± 0.3 and 3.7 ± 0.5 μU/ml, respectively; P < 0.05).

Plasma glucose kinetics. During the 20- to 55-min period of exercise, β-STIM did not elevate Ra glucose above CON (22 ± 2 vs. 22 ± 2 μmol·kg⁻¹·min⁻¹). However, β-BLOCK markedly raised Ra glucose above CON (37 ± 2 vs. 22 ± 2 μmol·kg⁻¹·min⁻¹; P < 0.05; Fig. 3A). β-BLOCK+LIPID attenuated the increase in Ra glucose during β-BLOCK by approximately one-half, but Ra glucose still remained higher than CON (28 ± 3 vs. 22 ± 2 μmol·kg⁻¹·min⁻¹; P < 0.05). Rd glucose (index of glucose uptake) was similar to Ra glucose during CON and β-BLOCK+LIPID; thus plasma glucose levels were maintained. β-STIM tended to reduce Rd glucose below Ra glucose [20 ± 2 vs. 22 ± 2 μmol·kg⁻¹·min⁻¹; not significant (NS)], explaining the mild hyperglycemia (i.e., 5.4 mM). During the 20- to 60-min period of exercise, plasma glucose concentration (Fig. 2A) increased above CON (4.8 ± 0.1 mM) during β-STIM (5.4 ± 0.1 mM; P < 0.05). In contrast, plasma glucose concentration decreased during the first 30 min of exercise with β-BLOCK (4.5 ± 0.1 mM; P < 0.05), returning to CON values thereafter. Plasma glucose levels were maintained similar to CON during β-BLOCK+LIPID (4.8 ± 0.2 mM; P < 0.05). After 15 min of exercise, plasma insulin (Fig. 2B) declined in all trials by ~3.0 ± 0.3 μU/ml. After 30 min of exercise, plasma insulin increased with β-STIM above CON values (6.7 ± 0.6 vs. 5.4 ± 0.4 μU/ml, respectively; P < 0.05). However, during β-BLOCK and β-BLOCK+LIPID, insulin concentrations were reduced below CON (3.5 ± 0.3 and 3.7 ± 0.5 μU/ml, respectively; P < 0.05).
During the first 15 min of exercise with β-BLOCK, R_d glucose exceeded R_a glucose (28 ± 3 vs. 21 ± 3 μmol·kg⁻¹·min⁻¹), which was responsible for the mild hypoglycemia. Plasma glucose clearance (Fig. 3B) was significantly reduced below CON during β-STIM (4.5 ± 0.3 vs. 3.6 ± 0.3 ml·kg⁻¹·min⁻¹, respectively; P < 0.05) and increased above CON during β-BLOCK (8.0 ± 0.5 ml·kg⁻¹·min⁻¹; P < 0.05) and β-BLOCK+LIPID (6.0 ± 0.8 ml·kg⁻¹·min⁻¹; P < 0.05).

Calculated minimal glycogen oxidation and plasma lactate. During the 20- to 55-min period of exercise, β-STIM increased the calculated minimal glycogen oxidation above CON (108 ± 11 vs. 92 ± 9 μmol·kg⁻¹·min⁻¹, respectively; P < 0.05), peaking 10 min after the onset of the epinephrine infusion (i.e., 25 min of exercise; Fig. 4A). β-BLOCK also increased the calculated minimal glycogen oxidation above CON, but the increases were smaller and not statistically significant (99 ± 9 vs. 92 ± 9 μmol·kg⁻¹·min⁻¹; NS). During the 20- to 55-min period of exercise, β-BLOCK+LIPID returned calculated glycogen oxidation to CON values (92 ± 9 vs. 92 ± 9 μmol·kg⁻¹·min⁻¹, respectively). However, during the first 25 min of exercise, β-BLOCK+LIPID tended to reduce glycogen oxidation below CON (92 ± 11 vs. 100 ± 10 μmol·kg⁻¹·min⁻¹; NS) and significantly reduced it below β-BLOCK levels (107 ± 10 μmol·kg⁻¹·min⁻¹; P < 0.05). During the 20- to 60-min period of exercise, plasma lactate concentrations followed a pattern similar to the calculated minimal glycogen oxidation. Compared with CON (1.4 ± 0.2 mM), β-STIM increased plasma lactate concentration (2.2 ± 0.4 mM; P < 0.05), whereas β-BLOCK+LIPID tended to decrease plasma lactate levels (1.2 ± 0.2 mM; NS). Furthermore, β-BLOCK did not change plasma lactate concentration compared with CON (Fig. 4B).

R_a glycerol and R_d FFA. During the 20- to 55-min period of exercise, β-STIM tended to raise R_a glycerol (Fig. 5A) above CON (7.8 ± 1.2 vs. 7.1 ± 0.8 μmol·kg⁻¹·min⁻¹; NS). In contrast, β-BLOCK totally prevented R_a glycerol from increasing above rest, and it remained lower than CON throughout exercise (2.6 ± 0.4 μmol·kg⁻¹·min⁻¹; P < 0.05). β-BLOCK+LIPID resulted in higher R_a glycerol than all the other trials (9.6 ± 1 μmol·kg⁻¹·min⁻¹; all P < 0.05) due to the Intralipid-heparin infusion. A response pattern similar to the one reported for R_a glycerol was observed for plasma glycerol...
concentrations. $R_d$ FFA (Fig. 5B) is an index of fatty acid availability from plasma for oxidation or reesterification. β-STIM increased $R_d$ FFA above CON; however, the increases did not reach significance until the 35- to 45-min period (18 ± 3 vs. 12 ± 1 μmol·kg$^{-1}$·min$^{-1}$; $P < 0.05$). β-BLOCK prevented $R_d$ FFA from increasing above rest, and it was lower than CON throughout exercise (6 ± 0.8 μmol·kg$^{-1}$·min$^{-1}$; $P < 0.05$). β-BLOCK+LIPID increased $R_d$ FFA above CON to levels equal to β-STIM during the 45- to 55-min period (17 ± 3 μmol·kg$^{-1}$·min$^{-1}$; $P < 0.05$).

Plasma FFA concentration and FFA oxidation. A response pattern similar to $R_d$ FFA was observed for plasma FFA concentrations (Fig. 6A). During the 20- to 60-min period of exercise, β-STIM raised plasma FFA concentration above CON (0.42 ± 0.1 vs. 0.32 ± 0.06 mM, respectively; $P < 0.05$). β-BLOCK suppressed the increase in plasma FFA concentration (0.15 ± 0.01 mM; $P < 0.05$), remaining at resting levels during exercise. β-BLOCK+LIPID elevated plasma FFA to levels similar to β-STIM during the 40- to 60-min period of exercise (0.44 ± 0.05 mM; $P < 0.05$). Fatty acid oxidation (Fig. 6B) increased throughout exercise during CON (13.2 ± 3 vs. 19.2 ± 1 μmol·kg$^{-1}$·min$^{-1}$; for 7 vs. 55 min of exercise; $P < 0.05$). β-STIM reduced fatty acid oxidation below CON soon after the onset of exercise. The total rate of energy expenditure was constant-rate Intralipid infusion (16.2 ± 2 μmol·kg$^{-1}$·min$^{-1}$ at 60 min of exercise). β-BLOCK greatly reduced fatty acid oxidation throughout exercise. β-BLOCK+LIPID increased fatty acid oxidation at the onset of exercise above all other trials (16.2 ± 2 μmol·kg$^{-1}$·min$^{-1}$; $P < 0.05$ at 7 min of exercise). However, fatty acid oxidation did not increase with exercise duration during the constant-rate Intralipid infusion (16.2 ± 2 vs. 16.2 ± 1 μmol·kg$^{-1}$·min$^{-1}$ for 7 vs. 55 min of exercise). Because CON increased fat oxidation throughout exercise whereas β-BLOCK+LIPID did not, fat oxidation was higher at 60 min of exercise during CON vs. β-BLOCK+LIPID (19.2 ± 1 vs. 16.2 ± 1 μmol·kg$^{-1}$·min$^{-1}$; $P < 0.05$).

**DISCUSSION**

This study altered β-AR activity, through either blockade or epinephrine stimulation, as a means to perturb substrate metabolism during exercise to systematically study the mechanisms of substrate regulation and energy compensation. The observation that both β-BLOCK and β-STIM reduced fat oxidation during exercise at 45% $\dot{V}O_2_{peak}$ suggests that the plasma epinephrine concentration and β-AR activity that normally occur (i.e., CON) are optimal for maximizing fat oxidation (27). The first new finding of this study is that, during exercise at 45% $\dot{V}O_2_{peak}$, moderately trained subjects accelerate plasma $R_d$ glucose to com-

![Image](E757SUBSTRATE REGULATION WITH REDUCED FAT OXIDATION)
pensate for reduced lipolysis and fatty acid availability with β-BLOCK, and this appears to be in response to a deficit in energy from fat oxidation. More than one-half of the increase in \( R_d \) glucose was reversed to CON levels when plasma fatty acid levels were restored (i.e., β-BLOCK + LIPID) and fat oxidation was increased. This agrees with the concept that plasma fatty acid availability and oxidation can regulate plasma glucose utilization [Randle-like effect (31)], in this case, when fat availability and oxidation are low due to β-BLOCK. In contrast, β-STIM increased glycogen oxidation, which greatly reduced fat oxidation despite increased plasma FFA levels, indicating that carbohydrate metabolism can also regulate fat metabolism.

Previous reports show that β-AR blockade increases plasma glucose turnover in healthy people (2, 32) and in type 1 diabetic patients (3, 38) during submaximal exercise (~50% \( \dot{V}_O_2 \max \)). However, to our knowledge, this is the first study in humans to directly test the hypothesis that it is related to fatty acid availability. Our data suggest that β-BLOCK increased \( R_d \) glucose during exercise to compensate for the energy deficit created by the reduction in lipolysis and fatty acid oxidation. Elevating plasma fatty acid levels during β-BLOCK + LIPID increased fat oxidation while simultaneously reducing \( R_d \) glucose compared with β-BLOCK. However, we cannot exclude the possibility that the reductions in \( R_d \) glucose with β-BLOCK + LIPID could have been due to a direct effect of the elevated plasma FFA concentration per se, impairing glucose uptake, rather than to an energy compensatory effect of elevating fat oxidation. Hargreaves et al. (19) observed that raising plasma fatty acids from 0.5 to 1.4 mM with a triglyceride-heparin infusion reduced glucose uptake without increasing FFA uptake or fat oxidation (nonenergy compensatory effect), prompting the idea that the high plasma fatty acid levels impaired glucose transport across the cell membrane. The exercise model of Hargreaves et al. was knee extension at 80% of work capacity, and plasma FFA concentrations were two-fold higher than presently observed, making conditions quite different compared with the present experiment. In contrast, elevations in plasma FFA by Intralipid-heparin have not reduced glucose uptake during cycling exercise at intensities ranging from 40 to 85% \( \dot{V}_O_2 \text{peak} \) (21, 29, 33). Thus it is unlikely that the presently observed reductions in \( R_d \) glucose during β-BLOCK + LIPID compared with β-BLOCK were due to a direct effect of the elevated plasma FFA concentration.

Although the cellular mechanisms behind the increase in glucose uptake with β-BLOCK are not clear, the present observations, together with previous reports of cellular energy disturbance, suggest it to be a compensation for reduced lipolysis and thus fatty acid availability (5, 32). Insulin did not stimulate the elevated \( R_d \) glucose, in that the highest \( R_d \) glucose occurred at the lower plasma insulin level (i.e., β-BLOCK), and β-BLOCK + LIPID reduced \( R_d \) glucose whereas plasma insulin remained unchanged (Fig. 2B). Not only does β-AR blockade reduce fatty acid availability, but it also has the potential to attenuate the normal rate of glycogenolysis that might occur under control conditions due to inhibition of cAMP-induced activation of phosphorylase (8). However, β-BLOCK did not significantly alter the calculated glycogen oxidation, probably because the severe reductions in FFA availability activated other intracellular stimulators of glycogen phosphorylase (Ca\(^{++}\), Pi, ADP, AMP) that counteracted the inhibitory effects of β-BLOCK. Likewise, other investigators have found that β-AR blockade does not reduce muscle glycogen utilization as assessed by muscle biopsies during submaximal, prolonged exercise (10, 24, 40). In that β-AR blockade reduces lipolysis as well as cAMP activation of glycogen phosphorylase, it makes sense that the main compensatory pathway for maintained substrate flux would be increased glucose uptake (Table 1).

In support of our hypothesis that \( R_d \) glucose increased in response to disturbed cellular homeostasis, the elevation of plasma fatty acid availability with β-BLOCK + LIPID decreased the calculated minimal glycogen oxidation below β-BLOCK levels during the 5- to 25-min period of exercise, suggesting that the disturbance of cellular homeostasis was lessened (Fig. 4A). However, β-BLOCK + LIPID did not completely return \( R_d \) glucose to CON levels, despite elevating plasma fatty acids and lipolysis above CON levels. During moderate-intensity exercise (50% \( \dot{V}_O_2 \max \)), both plasma fatty acid and intramyocellular triglyceride are oxidized (12). Although β-BLOCK + LIPID increased lipolysis in the intravascular space from the infused Intralipid-heparin, it likely did not restore the intramuscular triglyceride lipolysis that was also reduced by β-BLOCK (10). Thus deficient intramuscular fat availability may still have limited fat oxidation, thus disturbing cellular homeostasis and increasing \( R_d \) glucose above control. Sufficient plasma FFA was disposed into cells of the whole body (\( R_d \) FFA; Fig. 5B) that, if oxidized, should have returned fat oxidation to CON levels. However, it is unlikely that all of the \( R_d \) FFA was disposed into the exercising muscle (1) and completely oxidized (12). It is likely that not enough fatty acids were delivered to the mitochondria of exercising myocytes to allow for complete restoration of fat oxidation, thus necessitating the elevation of \( R_d \) glucose above control during β-BLOCK + LIPID. Other possible explanations for the maintained elevation in \( R_d \) glucose above control during β-BLOCK + LIPID could involve reduced blood flow (15) and oxygen delivery to the exercising muscles. It has been hypothesized that compromising oxygen delivery during exercise favors the utilization of oxygen-efficient fuels such as glucose instead of fatty acids (11). Contraction when hypoxic increases glucose uptake (7), and superimposition of β-AR blockade further increases glucose uptake (32). β-STIM tended to lower \( R_d \) glucose (i.e., %; NS), whereas it significantly reduced plasma glucose clearance by 20 ± 0.8% (\( P < 0.05 \); Fig. 3B). Because β-BLOCK increased whereas β-STIM reduced glucose clearance, it is logical to first consider a common mechanism that might govern both responses. β-STIM significantly increased muscle glycogen oxidation and plasma lactate, and it probably increased glucose
6-phosphate in muscle (9, 22). In contrast, β-BLOCK reduces glucose 6-phosphate levels (6, 8). Therefore, it is possible that plasma glucose clearance was inversely related to glucose 6-phosphate levels via the hexokinase activity. On the other hand, plasma Ra FFA and concentration were increased with β-STIM but reduced with β-BLOCK. However, it is unlikely that the small increase in fatty acid level with β-STIM (0.2 mM) was responsible for the reduced glucose clearance. High plasma FFA levels during β-BLOCK+LIPID were associated with plasma glucose clearance above control, whereas equally high FFA levels during β-STIM caused plasma glucose clearance to be significantly reduced below control. In line with our previous study of graded intravenous epinephrine infusion at 25% Vo2 peak, it appears that β-STIM results in a rapid reduction in fat oxidation that is associated with increased glycogenolysis despite a high concentration of plasma fatty acids (27). Interestingly, β-BLOCK also reduced fat oxidation, but via reduced lipolysis and fatty acid availability, whereas the compensatory increase in carbohydrate oxidation was derived from increased glucose uptake. Therefore, it appears that control levels of epinephrine and β-AR activation are optimal for fat oxidation at 45% Vo2 peak.

General theories of substrate regulation during exercise have usually argued in favor either of the premise that fat metabolism regulates carbohydrate oxidation (e.g., the Randle effect; (31)) or of the converse, that carbohydrate metabolism regulates fat oxidation (12, 37). Our present observations suggest that either of these theories of regulation can appear dominant, depending upon the nature of the cellular disturbance and the availability of substrates for compensation. For example, in agreement with the idea that fat regulates carbohydrate, it was observed that increased fatty acid availability during β-BLOCK+LIPID reduced glucose uptake compared with β-BLOCK. Furthermore, the restoration of plasma fatty acid during β-BLOCK+LIPID reduced muscle glycogenolysis below β-BLOCK during the 5- to 25-min period of exercise (Fig. 4A). On the other hand, in agreement with the idea that carbohydrate metabolism regulates fat oxidation, it was observed that increases in muscle glycogen oxidation during β-STIM were associated with reduced fat oxidation. Previous studies have indicated that increased glycolytic flux directly reduces fat oxidation, possibly by attenuating the transport of fatty acid across the mitochondrial membrane (12, 36). Given the myriad of responses, a hierarchy should be sought on the basis of potency for regulation of substrate oxidation. Muscle glycogen normally dominates oxidation, even during control exercise at 45% Vo2 peak as observed in the present study (see Table 1). Furthermore, glycogen oxidation can be readily increased, as occurred in the present study with β-STIM, or by other common factors such as increased exercise intensity (33). As aforementioned, increased glycolytic flux appears to actively reduce fat oxidation even when plasma free fatty acids are elevated, as presently observed. Therefore, when glycogen oxidation and plasma FFA appearance were stimulated via β-STIM in the present study, carbohydrate oxidation predominated and fat oxidation decreased. In light of the large ability to increase glycolytic flux in muscle during exercise, it seems that carbohydrate metabolism has great potential to dominate fat metabolism. However, when the potential for increased glycogenolysis and lipolysis was limited, as in the present study during β-BLOCK, plasma FFA availability regulated both glucose uptake and reduced glycogen oxidation during the 5- to 25-min period of β-BLOCK+LIPID. Therefore, it seems that fat metabolism regulated carbohydrate metabolism against the background of restrained glycogenolysis from β-BLOCK. Other situations where fatty acid availability regulates carbohydrate oxidation (the Randle effect) are rest, low-intensity exercise, and elevated glucose uptake induced by euglycemic insulinemic clamp (25).

Little is known regarding the regulation of intramuscular lipolysis during exercise and the extent to which it is influenced by muscle carbohydrate metabolism. β-STIM clearly increased Ra and Rd FFA by ~50%, indicating that lipolysis of adipose tissue triglyceride was markedly elevated. It is interesting to note, however, that whole body lipolysis was only slightly and nonsignificantly elevated above control during β-STIM. This could suggest that lipolysis of intramuscular triglyceride was reduced by β-STIM. We have recently reported that epinephrine stimulation of lipolysis, assessed by clamping plasma epinephrine concentration via intravenous infusion, is attenuated as exercise intensity and glycogenolysis are increased from 25 to 45% Vo2 peak (27). The notion put forth by these observations is that the stimulation of muscle glycogenolysis during exercise might reduce lipolytic sensitivity to epinephrine in muscle during exercise.

In summary, reducing energy availability from fatty acids when muscle glycogenolysis sensitivity is attenuated with β-BLOCK permits a large increase in plasma glucose utilization (Rd glucose) to compensate for the substrate deficit. In this particular situation, fatty acid availability controls glucose uptake (Randle-like effect; β-BLOCK+LIPID). However, when muscle glycogenolysis is stimulated by epinephrine infusion (β-STIM) and plasma fatty acid concentration is also high, carbohydrate metabolism dominated fat oxidation. It appears that carbohydrate oxidation can be robustly increased during exercise via either glycogenolysis or glucose uptake when fat oxidation is reduced by disturbing the normal stimulation of β-adrenergic receptors during exercise.

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