

# Insulin Secretion and Glucose Kinetics During Exercise With and Without Pharmacological $\alpha_1$ - and $\alpha_2$ -Receptor Blockade

Pauliina Aarnio,<sup>1,2</sup> Torsten Lauritsen,<sup>3</sup> and Flemming Dela<sup>1,2,4</sup>

The mechanism behind exercise-induced decreases in plasma insulin concentrations was examined in eight healthy young men. In addition, the influence of specific  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor blockade on glucose kinetics during exercise was studied. To test the hypothesis that exercise-induced decreases in insulin secretion are mediated via  $\alpha_2$ -adrenoceptors, all subjects exercised for 60 min on separate occasions under four conditions: with and without  $\alpha_1$ -receptor blockade (1 mg prazosin) and with and without or  $\alpha_2$ -receptor blockade (15 mg yohimbine). Glucose kinetics were measured using [ $3$ - $^3$ H]glucose. During exercise with  $\alpha_2$ -receptor blockade, the insulin concentration initially increased (first 20 min) then decreased, whereas it continually decreased in the corresponding control experiment. The C-peptide concentration did not change during exercise with  $\alpha_2$ -receptor blockade but decreased in the control experiment. During exercise with  $\alpha_1$ -receptor blockade and corresponding control experiments, insulin and C-peptide levels always decreased. With  $\alpha_1$ -receptor blockade, the glucose concentration increased (first 30 min) and then decreased, whereas it slightly decreased in all other experiments. In addition, with  $\alpha_1$ -receptor blockade, the glucose rate of appearance (Ra) increased rapidly (because of higher catecholamine concentrations in  $\alpha_1$ -receptor blockade versus control) and the glucose rate of disappearance (Rd) was higher compared with control. During exercise with  $\alpha_2$ -receptor blockade, the Ra and Rd were always lower compared with control. Therefore, we conclude that exercise-induced decreases in insulin secretion are mediated via  $\alpha_2$ -adrenoceptors and that blockade of  $\alpha_1$ - and  $\alpha_2$ -adrenoceptors during exercise elicits opposite responses in glucose Ra and Rd. *Diabetes* 50:1834–1843, 2001

Insulin is produced and secreted from the  $\beta$ -cells in the pancreas. The secretion of insulin is regulated by a complex interplay among neurohumoral stimuli originating from food entering the gastrointestinal tract, circulating substrates, and hormones.

During exercise, plasma insulin concentrations decrease with a magnitude closely linked to the intensity and duration of the exercise. This decrease is primarily attributable to a decrease in insulin secretion (1). However, the precise mechanism behind the exercise-induced decrease in insulin secretion is not known. Most likely, it comes from increases in sympathoadrenal activity that accompany exercise. Thus, it has been found that the decrease in plasma insulin during exercise can be partly inhibited by prior administration of phentolamine, a nonselective blocker of  $\alpha$ -adrenergic receptors (2,3). Later animal studies have shown that the decrease in insulin secretion is mediated via postsynaptic  $\alpha_2$ -receptors (4,5), the existence of which has now been demonstrated in human  $\beta$ -cells (6). The exercise-induced inhibition of insulin secretion seen in humans may therefore be attributable to the exercise-induced increase in sympathetic activity, which, by the release of norepinephrine in the synaptic cleft, activates postsynaptic  $\alpha_2$ -receptors.

Results from previous studies using  $\alpha_2$  antagonists during physical exercise have been contradictory regarding plasma concentrations of insulin. In humans, plasma insulin concentrations decreased during 30 min of ergometer cycle exercise at 60% of  $V_{O_{2max}}$  after the administration of yohimbine (a selective  $\alpha_2$  antagonist); however, in that study, the concentration of C-peptide was not measured (7). In swimming mice, however, yohimbine was shown to inhibit the exercise-induced decrease in plasma insulin (8).

It cannot be a priori excluded that  $\alpha_1$ -receptors also play a role in the exercise-induced inhibition of insulin secretion in humans, but to our knowledge, no studies on this have been carried out. To elucidate the mechanism behind the exercise-induced inhibition of insulin secretion in humans, we studied insulin secretion and concentrations before, during, and in the recovery phase of a 60-min ergometer bicycle exercise bout with and without selective pharmacological blockade of the  $\alpha_1$ - and  $\alpha_2$ -receptors.

In contrast to insulin secretion, hepatic glucose production increases with exercise. Feedback signals from the contracting muscles, mediated both neurally and via the blood stream, adjust the stimulus for hepatic glucose

From the <sup>1</sup>Department of Medical Physiology, the Panum Institute, University of Copenhagen, Copenhagen; the <sup>2</sup>Copenhagen Muscle Research Centre, Rigshospitalet, Copenhagen; the <sup>3</sup>Department of Anesthesia, Rigshospitalet, Copenhagen; and the <sup>4</sup>Department of Clinical Physiology and Nuclear Medicine, Herlev Hospital, Herlev, Denmark.

Address correspondence and reprint requests to Flemming Dela, MD, Associate Professor, Department of Medical Physiology, the Panum Institute, Bldg. 12.4.7., University of Copenhagen, Blegdamsvej 3, 2200 Copenhagen N, Denmark. E-mail: f.dela@mfi.ku.dk.

Received for publication 14 November 2000 and accepted in revised form 24 April 2001.

ANOVA, analysis of variance; FFA, free fatty acid; MCR, metabolic clearance rate; Ra, rate of glucose appearance; Rd, rate of glucose disappearance.

production to maintain euglycemia in the blood (9). A rise in blood glucose directly inhibits glucose production during exercise, whereas a drop in blood glucose, via stimulation of counterregulatory hormones, increases hepatic glucose production (9). Also, central mechanisms, coupled with the degree of motor center activity, stimulate epinephrine release, which in turn enhances hepatic glucose production (9). Sympathetic nerves to the liver do not, however, play a role in the exercise-induced increase in hepatic glucose production, and glucagon in humans is also of minor importance (9). In the present study, the use of selective  $\alpha$ -adrenergic antagonists made it possible to evaluate the role of  $\alpha_1$ - versus  $\alpha_2$ -receptors in hepatic glucose production and glucose disappearance from plasma; to our knowledge, this has not been done previously.

## RESEARCH DESIGN AND METHODS

**Subjects.** The study subjects, eight healthy young men (age [means  $\pm$  SE]  $25 \pm 0.9$  years, height  $180 \pm 2$  cm, weight  $73 \pm 2$  kg), gave their informed consent to participate in this study, which was approved by the Ethical Committee for Copenhagen and Frederiksberg, Denmark. The subjects were recruited through announcements in a student magazine. None of the subjects had any family history of diabetes, and none was taking medication.

Within the 4–30 days before the start of the study, each subject's  $VO_{2max}$  was measured during exercise on a bicycle ergometer (Ergoline 900 I; Siemens, Denmark), with the subject seated in a semirecumbent position. The  $VO_{2max}$  measured in this position corresponds to 91–93% of the  $VO_{2max}$  measured during upright ergometer bicycling (10).

**Experimental protocol.** Two independent crossover studies were performed: bicycling exercise with and without pharmacological  $\alpha_1$ -receptor blockade and with and without pharmacological  $\alpha_2$ -receptor blockade. The four experiments were performed during a 3-month period, with at least 7 days between each experimental day. The experiments were carried out in a random fashion, apart from the trial with the  $\alpha_1$ -receptor blockade, which was always performed before its control study. Each subject participated in all four experimental trials.

On each experiment day, the subjects arrived at the hospital at 9:00 A.M. in the fasting state, having abstained from smoking and physical activity during the foregoing 10 h. The subject was placed on an ergometer bicycle (Ergoline 900 I; Siemens), which could be tilted, enabling the subject to perform bicycling in a position anywhere from an upright to a supine position. In the study, the semisupine position was used to prevent possible hypotensive episodes. A catheter was inserted in a brachial artery for continuous measurement of blood pressure. One intravenous catheter was inserted in the retrograde direction in a dorsal hand vein for collection of arterialized blood samples. This hand was kept in a heating pad ( $42^\circ\text{C}$ ) throughout the experiment. A second intravenous catheter was inserted either contralaterally to or more proximally than the first for the infusion of radioactive-labeled glucose.

Each experiment lasted 4 h (from  $-120$  to  $120$  min). The subject remained awake and resting in the first 2 h, then performed 1 h of bicycling exercise followed by 1 h of recovery. The subject's heart rate was continuously monitored by a Polar heart rate monitor (Pulstester PE3000; Polar Instruments, Oulu, Finland).

**Infusion of glucose tracer.** [ $3\text{-}^3\text{H}$ ]glucose (Amersham), pharmaceutically prepared and dissolved in isotonic saline ( $91.1 \pm 1.5$  kBq/ml; Isotopapoteket, Copenhagen) was given as a primed ( $1,138 \pm 21$  kBq) constant infusion ( $11.2 \pm 0.2$  kBq/min) throughout the entire experiment ( $t = -120$  to  $120$  min).

**Exercise and oxygen uptake.** After 2 h of rest, at  $t = 0$  min, the subjects performed bicycle exercise for 60 min in a semisupine position at 60 rpm. Individual workloads were targeted at 60% ( $\alpha_1$ -receptor blockade study) and 70% ( $\alpha_2$ -receptor blockade study) of the previously measured  $VO_{2max}$ . During each experiment,  $VO_2$  was measured by a cardiopulmonary test system (Medical Graphics, St. Paul, MN) before and three times during the exercise (15–19, 35–39, and 55–59 min). The first measurement (15–19 min) was used to adjust the workload, which then was not changed during the last 30 min of exercise. If the workload was adjusted during the first 30 min of exercise, exactly the same adjustment was carried out in the corresponding control or blockade experiment. In two of the  $\alpha_1$ -receptor blockade experiments, the workload had to be diminished because of signs of fatigue (plasma lactate 10 and 15 mmol/l) that would have made the subjects unable to complete the exercise.

**Blockade of  $\alpha$ -receptors.** In the  $\alpha_1$ -receptor blockade experiment, the subjects were given selective  $\alpha_1$ -receptor antagonist tablets orally (1 mg prazosin [Hexapress]; Durascan, Odense, Denmark) 2 h before exercise. In the  $\alpha_2$ -receptor blockade experiment, the subjects were given selective  $\alpha_2$ -receptor antagonist tablets orally (15 mg yohimbine [Yohimbine UNP]; United Nordic Pharma, Rungsted, Denmark) 45 min before exercise. The corresponding control experiments were performed without medication.

**Analytical techniques.** Blood was sampled at the indicated intervals shown on the figures. All blood samples were kept at  $-20^\circ\text{C}$  until analysis, except for samples for free fatty acids (FFAs) and catecholamines, which were kept at  $-80^\circ\text{C}$ . The procedures, stabilization of blood samples, and analysis of all hormones and metabolites have been described previously (11). Blood samples for the determination of glucose and lactate were immediately centrifuged, and the plasma was analyzed by an automatic glucose analyzer (YSI 23AM; Yellow Springs Instruments, Yellow Springs, OH). Concentrations of lactate in plasma were converted to whole-blood values, as previously described (12). From measurements of plasma glucose concentrations and specific activity of the radiolabeled glucose in the last 30 min of the resting period, the individual distribution volume of glucose was calculated (13). The rates of glucose appearance (Ra) and disappearance (Rd) were calculated by using the formulas for non-steady-state conditions (14,15) and the consecutive sliding-fit technique (16). The metabolic clearance rate (MCR) for glucose was calculated as the glucose Rd divided by the corresponding plasma glucose concentration.

One-way analysis of variance (ANOVA) for repeated measures was used to detect significant changes with time. Two-way ANOVA for repeated measures was used to detect significant differences between blockade and corresponding control studies. Single differences were determined using the paired Student's *t* test. The level of significance was set at  $P < 0.05$  in two-tailed testing. All data are reported as means  $\pm$  SE.

## RESULTS

**$VO_2$  and workload.**  $VO_{2max}$  was  $3.6 \pm 0.1$  l/min. During exercise, the absolute workload was  $151 \pm 7$  ( $\alpha_1$ ) and  $188 \pm 8$  ( $\alpha_2$ ) W ( $P < 0.05$ ). Oxygen uptake rates were  $2.3 \pm 0.1$  and  $2.0 \pm 0.1$  l/min for  $\alpha_1$ -receptor blockade and control, respectively ( $P < 0.05$ ), and  $2.4 \pm 0.1$  and  $2.5 \pm 0.1$  l/min for  $\alpha_2$ -receptor blockade and control, respectively ( $P > 0.05$ ). Thus, the relative workload was  $63 \pm 1$  and  $55 \pm 2\%$  of  $VO_{2max}$  for  $\alpha_1$ -receptor blockade and control, respectively ( $P < 0.05$ ), and  $68 \pm 2$  and  $69 \pm 1\%$  of  $VO_{2max}$  for  $\alpha_2$ -receptor blockade and control, respectively ( $P > 0.05$ ).

**Hemodynamic parameters.** In the  $\alpha_1$ -receptor blockade study, heart rates were higher ( $P < 0.05$ ) during rest, exercise, and recovery compared with control (Fig. 1). In contrast, the  $\alpha_2$ -receptor blockade did not influence the heart rate in any phase of the experiments (Fig. 1). Mean arterial blood pressure was not different in the blockade experiments compared with the corresponding control experiments in any of the phases (rest, exercise, or recovery) (Fig. 1).

**Glucose kinetics.** In the  $\alpha_1$ -receptor blockade and control experiments, plasma glucose concentrations were similar during rest and recovery ( $P > 0.05$ ) (Fig. 2). In the blockade experiment, plasma glucose concentrations increased during the first 30 min of exercise then decreased, whereas there was a continuous slight decrease in the control experiment. During exercise, plasma glucose concentrations were significantly higher in the blockade compared with the control experiment ( $P < 0.01$ ) (Fig. 2).

In the  $\alpha_2$ -receptor blockade and control experiments, plasma glucose concentrations decreased ( $P < 0.05$ ) in response to exercise (Fig. 2). During exercise, there was a tendency for the plasma glucose concentration to be lower during the blockade than during the control experiment ( $P = 0.0505$ ) (Fig. 2).

The Ra and Rd were always similar between blockade

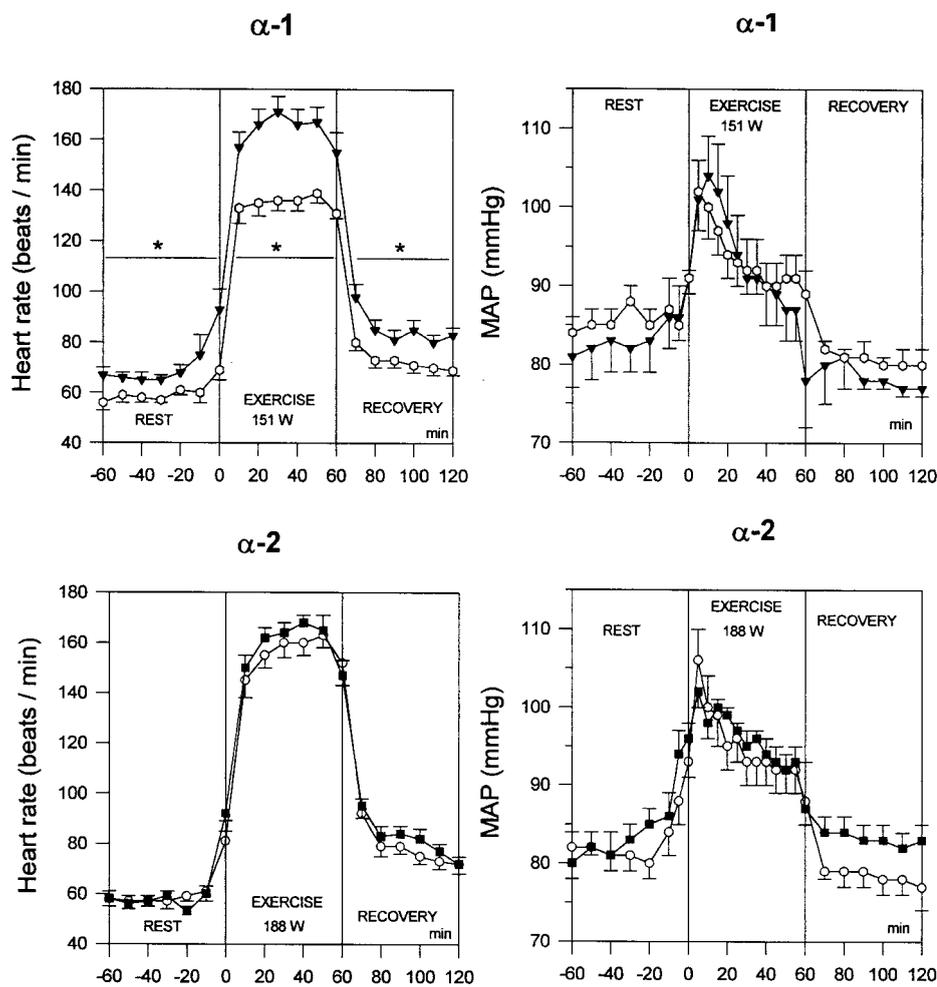


FIG. 1. Heart rate and blood pressure before, during, and after 60-min ergometer bicycle exercise in eight men with  $\alpha_1$ -receptor blockade (prazosin; 1 mg at  $t = -60$  min;  $\blacktriangledown$ ) and without (control;  $\circ$ ), and with  $\alpha_2$ -receptor blockade (yohimbine; 15 mg at  $t = -45$  min;  $\blacksquare$ ) and without (control;  $\circ$ ). Data are means  $\pm$  SE. \*Significant difference ( $P < 0.05$ ) between blockade and corresponding control study.

and control experiments during rest and recovery. However, during exercise with  $\alpha_1$ -receptor blockade, the Ra tended to be higher compared with the control experiment ( $P = 0.052$ ), and at one time point ( $t = 52.5$  min), a significant difference was seen ( $P < 0.01$ ; paired  $t$  test) (Fig. 2). The Rd continued to increase throughout the exercise and generally tended ( $P = 0.08$ ) to be higher compared with during the control experiment (Fig. 2). In the  $\alpha_1$  control experiments, the Ra and Rd leveled off after 20 min of exercise. A significant difference in the Rd between  $\alpha_1$ -receptor blockade and control was seen at the end of exercise ( $P < 0.02$ , paired  $t$  tests) (Fig. 2). In the  $\alpha_2$ -receptor blockade experiments, the Ra and Rd were always lower during exercise compared with the control experiments ( $P < 0.002$ ) (Fig. 2).

#### Insulin, C-peptide, and glucagon concentrations.

Plasma insulin concentrations were similar at rest in all experiments (Fig. 3). However, resting values during  $\alpha_1$ -receptor blockade tended to be higher ( $P < 0.1$ ) compared with all other experiments. In the  $\alpha_1$ -receptor blockade and corresponding control experiments, plasma insulin concentrations decreased during exercise ( $P < 0.05$ ), but the values were significantly higher during exercise and recovery in the blockade compared with the control experiment ( $P < 0.05$ ). However, the relative decrease in plasma insulin concentrations in response to exercise was not different during blockade compared with control ( $P > 0.05$ ) (Fig. 3). During exercise with  $\alpha_2$ -receptor blockade,

an increase in plasma insulin concentrations was seen in the first 20 min of the exercise, followed by a decrease. In the corresponding control experiment, a decrease was seen throughout the exercise period ( $P < 0.05$ ) (Fig. 3). During exercise, plasma insulin concentrations were significantly higher with  $\alpha_2$ -receptor blockade compared with control ( $P < 0.05$ ), whereas no difference was seen in the recovery period (Fig. 3).

At rest, plasma C-peptide concentrations were similar in all four experiments (Fig. 4). During exercise, plasma C-peptide decreased with and without  $\alpha_1$ -receptor blockade ( $P < 0.05$ ). The decrease in C-peptide concentrations from resting values was less with  $\alpha_1$ -receptor blockade compared with control values ( $P < 0.05$ ). During exercise with  $\alpha_2$ -receptor blockade, plasma C-peptide concentrations were significantly higher compared with control values ( $P < 0.05$ ), and the decrease of C-peptide seen in the control experiment was not seen in the  $\alpha_2$ -receptor blockade experiment ( $P < 0.05$ ) (Fig. 4).

Plasma concentrations of glucagon always increased during and immediately after exercise ( $P < 0.05$ ), but no difference between the experiments was seen during rest, exercise, or recovery (Fig. 5).

**Catecholamines.** Plasma epinephrine concentrations were similar at rest in all four experiments; in response to exercise, epinephrine always increased ( $P < 0.05$ ) (Fig. 6). During exercise with  $\alpha_1$ -receptor blockade, epinephrine concentrations were higher compared with control values

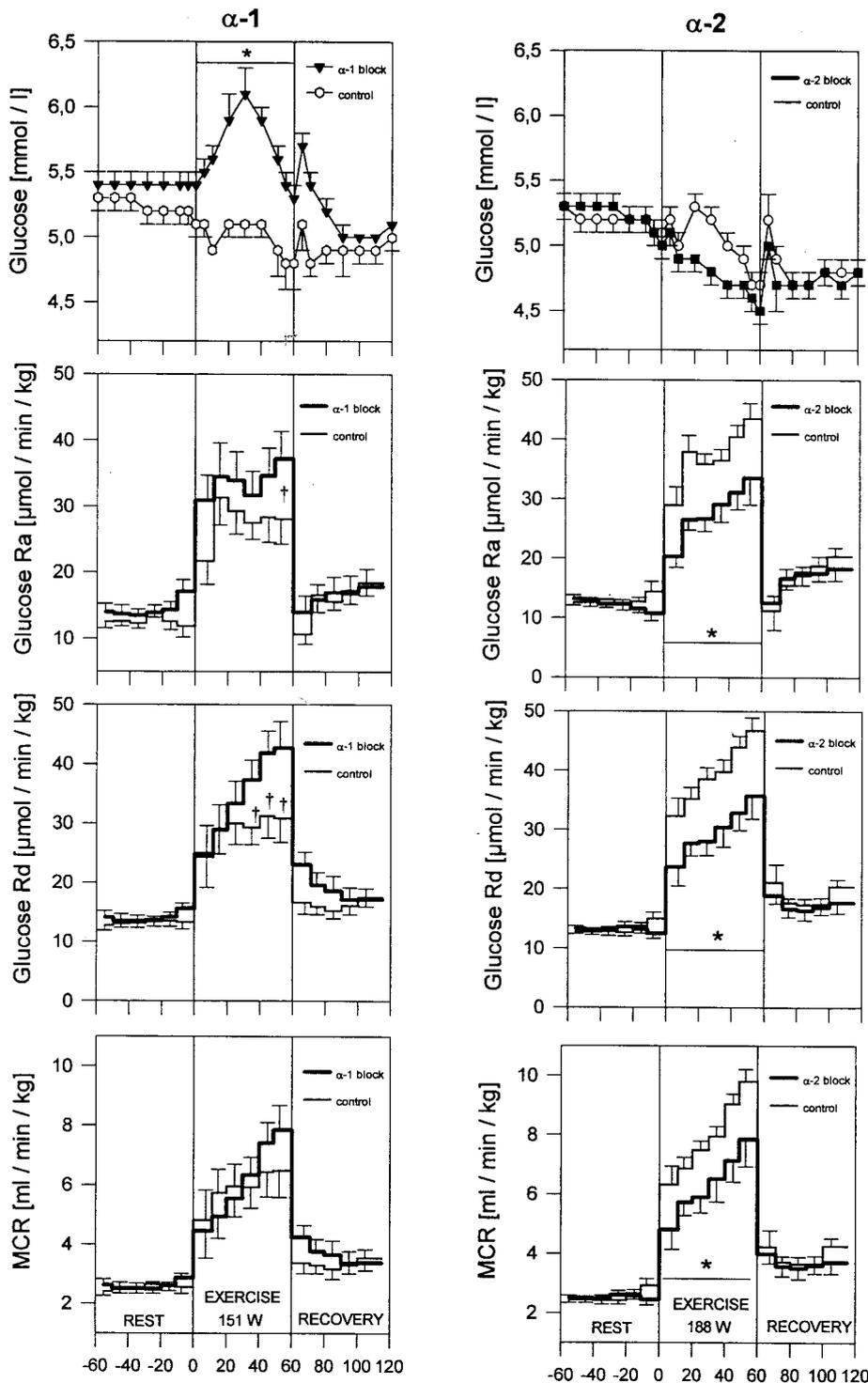


FIG. 2. Plasma glucose, Ra, Rd, and MCR before, during, and after 60-min ergometer bicycle exercise in eight men with  $\alpha_1$ -receptor blockade (prazosin; 1 mg at  $t = -60$  min;) and without (control), and with  $\alpha_2$ -receptor blockade (yohimbine; 15 mg at  $t = -45$  min) and without (control). Data are means  $\pm$  SE. \*Significant difference ( $P < 0.05$ ) between blockade and corresponding control study; †significant difference ( $P < 0.05$ ) at single time points.

( $P < 0.05$ ), whereas no general differences were seen between  $\alpha_2$ -receptor blockade and control values (Fig. 6).

Plasma norepinephrine concentrations were always higher during  $\alpha_1$ -receptor blockade compared with control values, at rest and during exercise and recovery ( $P < 0.02$ ) (Fig. 6). In the  $\alpha_2$  experiments, norepinephrine concentrations were similar at rest and during recovery, but during exercise tended to be higher with  $\alpha_2$ -receptor blockade compared with control values ( $P < 0.1$ ) (Fig. 6).

**Metabolites.** The lactate concentrations in blood were similar at rest in all four experiments (Fig. 7). During  $\alpha_2$ -receptor blockade and corresponding control studies,

there was no difference in lactate concentrations at any time. During exercise and recovery with  $\alpha_1$ -receptor blockade, lactate concentrations were higher compared with ( $P < 0.05$ ) control values (Fig. 7).

Plasma concentrations of D- $\beta$ -hydroxybutyrate (representing ketone bodies) did not change in response to exercise during  $\alpha_1$ -receptor blockade compared with control ( $0.15 \pm 0.05$  vs.  $0.10 \pm 0.04$  mmol/l and  $0.18 \pm 0.05$  vs.  $0.15 \pm 0.06$  mmol/l for rest and exercise, respectively) or during  $\alpha_2$ -receptor blockade compared with control ( $0.14 \pm 0.03$  vs.  $0.15 \pm 0.07$  mmol/l and  $0.17 \pm 0.04$  vs.  $0.13 \pm 0.04$  mmol/l for rest and exercise, respectively).

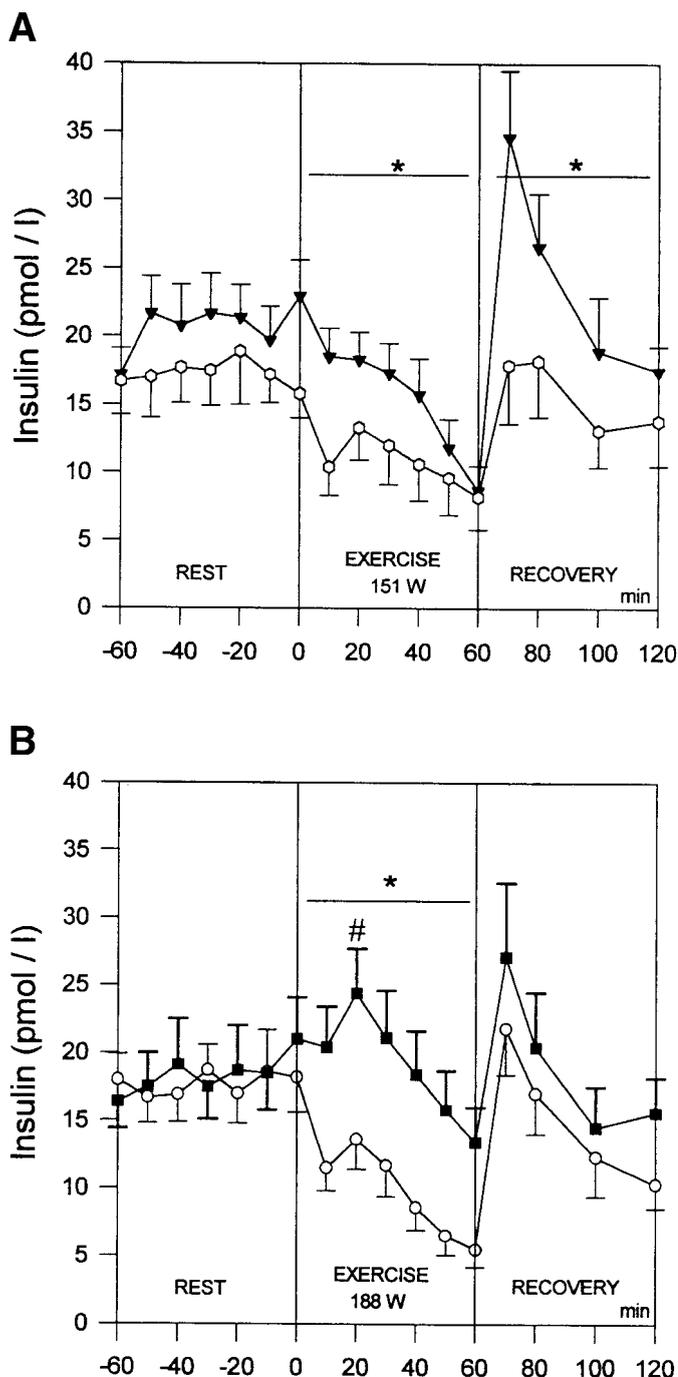


FIG. 3. Plasma insulin concentrations before, during, and after 60-min ergometer bicycle exercise in eight men with (A)  $\alpha_1$ -receptor blockade (prazosin; 1 mg at  $t = -60$  min;  $\blacktriangledown$ ) and without (control;  $\circ$ ), and (B) with  $\alpha_2$ -receptor blockade (yohimbine; 15 mg at  $t = -45$  min;  $\blacksquare$ ) and without (control;  $\circ$ ). Data are means  $\pm$  SE. \*Significant difference ( $P < 0.05$ ) between blockade and corresponding control study; #significantly increased ( $P < 0.05$ ) compared with values at rest and with preceding value.

However, during recovery, the concentration was always increased ( $P < 0.05$ ), and was similar in all four experiments ( $\alpha_1$ -receptor blockade,  $0.41 \pm 0.11$ ;  $\alpha_1$  control,  $0.61 \pm 0.19$ ;  $\alpha_2$ -receptor blockade,  $0.63 \pm 0.09$ ;  $\alpha_2$  control,  $0.53 \pm 0.15$ ).

Plasma concentrations of FFAs (Fig. 8) were similar during rest in all experiments. No changes were seen with exercise, except for a slight increase during  $\alpha_2$ -receptor

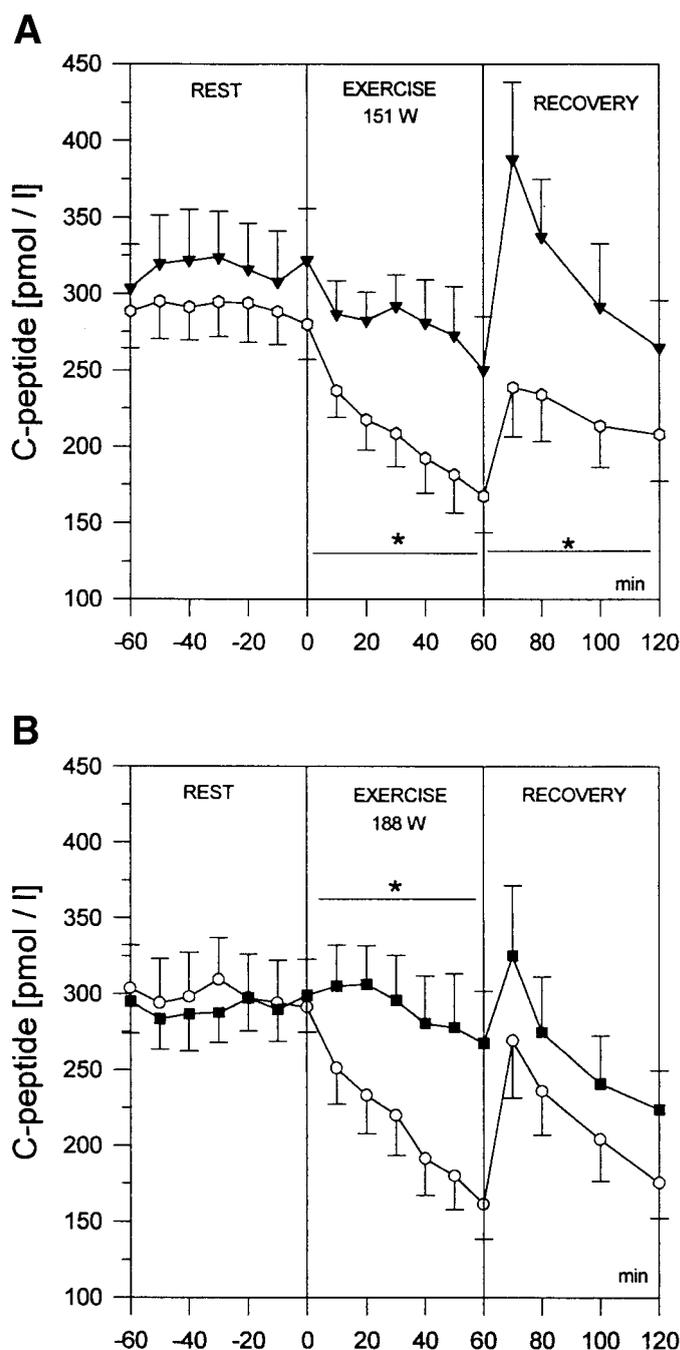


FIG. 4. Plasma C-peptide concentrations before, during, and after 60-min ergometer bicycle exercise in eight men with (A)  $\alpha_1$ -receptor blockade (prazosin; 1 mg at  $t = -60$  min;  $\blacktriangledown$ ) and without (control;  $\circ$ ), and with (B)  $\alpha_2$ -receptor blockade (yohimbine; 15 mg at  $t = -45$  min;  $\blacksquare$ ) and without (control;  $\circ$ ). Data are means  $\pm$  SE. \*Significant difference ( $P < 0.05$ ) between blockade and corresponding control study.

blockade ( $P < 0.05$ ). FFA concentrations always peaked in the early recovery phase, then quickly decreased toward resting values (Fig. 8).

Plasma concentrations of glycerol were similar in both  $\alpha_1$ - and  $\alpha_2$ -receptor blockade and corresponding control studies in all three phases of the experiment. Glycerol always peaked at the end of exercise (Fig. 8).

**DISCUSSION**

The main conclusion of the present study is that the exercise-induced decrease in plasma insulin concentra-

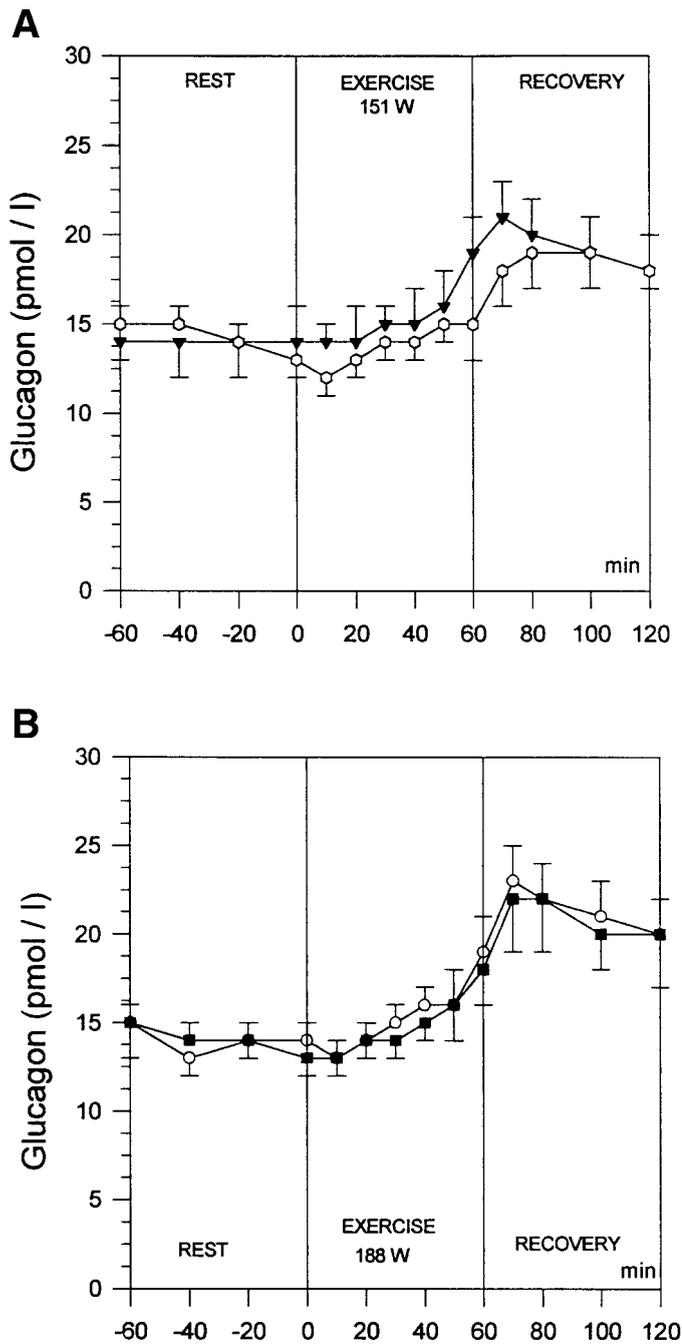


FIG. 5. Plasma glucagon concentrations before, during, and after 60-min ergometer bicycle exercise in eight men with (A)  $\alpha_1$ -receptor blockade (prazosin; 1 mg at  $t = -60$  min;  $\blacktriangledown$ ) and without (control;  $\circ$ ), and with (B)  $\alpha_2$ -receptor blockade (yohimbine; 15 mg at  $t = -45$  min;  $\blacksquare$ ) and without (control;  $\circ$ ). Data are means  $\pm$  SE.

tions is mediated via  $\alpha_2$ -receptors. When yohimbine, a specific blocker of  $\alpha_2$ -receptors, was given, plasma insulin concentrations during exercise were significantly higher compared with control values (Fig. 3). In fact, with  $\alpha_2$ -receptor blockade, plasma insulin concentrations increased during the first part of the exercise, then decreased. An increase in plasma insulin concentrations during exercise is remarkable and can only be attributed to blockade of the  $\alpha_2$ -receptors. The fact that plasma insulin concentrations decreased slightly in the latter part of the exercise may have been because of a fading effect of yohimbine, but other studies using the same dosage, time

frame, and route of administration of yohimbine have shown an effect on lipolysis (7) and plasma norepinephrine (17) for  $>105$  min. However, it is noteworthy that  $\alpha_2$ -receptor blockade did not have an effect on plasma insulin concentrations at rest and especially not during recovery. Thus, the rebound increase in plasma insulin concentrations seen immediately after exercise was not diminished by  $\alpha_2$ -receptor blockade, which, taken together with the fact that plasma insulin concentrations did decrease slightly in the latter part of the exercise with  $\alpha_2$ -receptor blockade, may indicate that the blockade was not complete throughout the whole exercise period. In support of the view that  $\alpha_2$ -receptor blockade abolished the normal exercise-induced decrease in plasma insulin secretion was the finding that C-peptide concentrations changed only minimally (Fig. 4). However, because of a considerably lower metabolic clearance rate than insulin, peripheral C-peptide concentrations may not accurately reflect insulin secretion rates when these change rapidly (18).

In contrast to the experiments with  $\alpha_2$ -receptor blockade, selective  $\alpha_1$ -receptor blockade with prazosin did not abolish the normal exercise-induced decrease in plasma insulin or C-peptide concentrations (Figs. 3 and 4). Although a significant difference in plasma insulin and C-peptide concentrations existed during exercise between the  $\alpha_1$ -receptor blockade and the corresponding control experiments, this was to some extent attributable to differences in concentrations at the onset of exercise (Figs. 3 and 4). The reason for the increased ( $\alpha_1$ -receptor blockade versus control) insulin and C-peptide concentrations in the resting pre-exercise period and, more importantly, during exercise can be explained by the fact that  $\alpha_1$ -receptor blockade inhibits vasoconstriction in the splanchnic vascular bed (see the discussion of workloads below). Thus, splanchnic blood flow was most likely higher and its drainage into the peripheral circulation markedly greater during  $\alpha_1$ -receptor blockade compared with the corresponding control study. This means that delivery of insulin and C-peptide to the sampling site (arterialized venous blood) was greater during  $\alpha_1$ -receptor blockade versus control, causing the higher plasma concentrations (Figs. 3 and 4). Because of the relatively short half-life of insulin ( $\sim 5$  min), we found only a minor impact of the higher insulin concentrations on the exercise-induced decrease. In contrast, a less pronounced (compared with control) decrease of C-peptide concentrations during exercise would be expected in a situation with increased delivery (as during the exercise period with  $\alpha_1$ -receptor blockade) and a relatively long half-life ( $\sim 30$  min). In the recovery period, rebound increases were more pronounced during the  $\alpha_1$ -receptor blockade compared with the corresponding control experiment (Figs. 4 and 5). This was most likely attributable to the fact that plasma norepinephrine concentrations were considerably higher during exercise with  $\alpha_1$ -receptor blockade compared with during control experiments, then quickly declined with the termination of exercise (Fig. 7); therefore, the relief of inhibition had a greater impact on insulin secretion in the  $\alpha_1$ -receptor blockade study compared with in the corresponding control study.

The present results are in accordance with previous

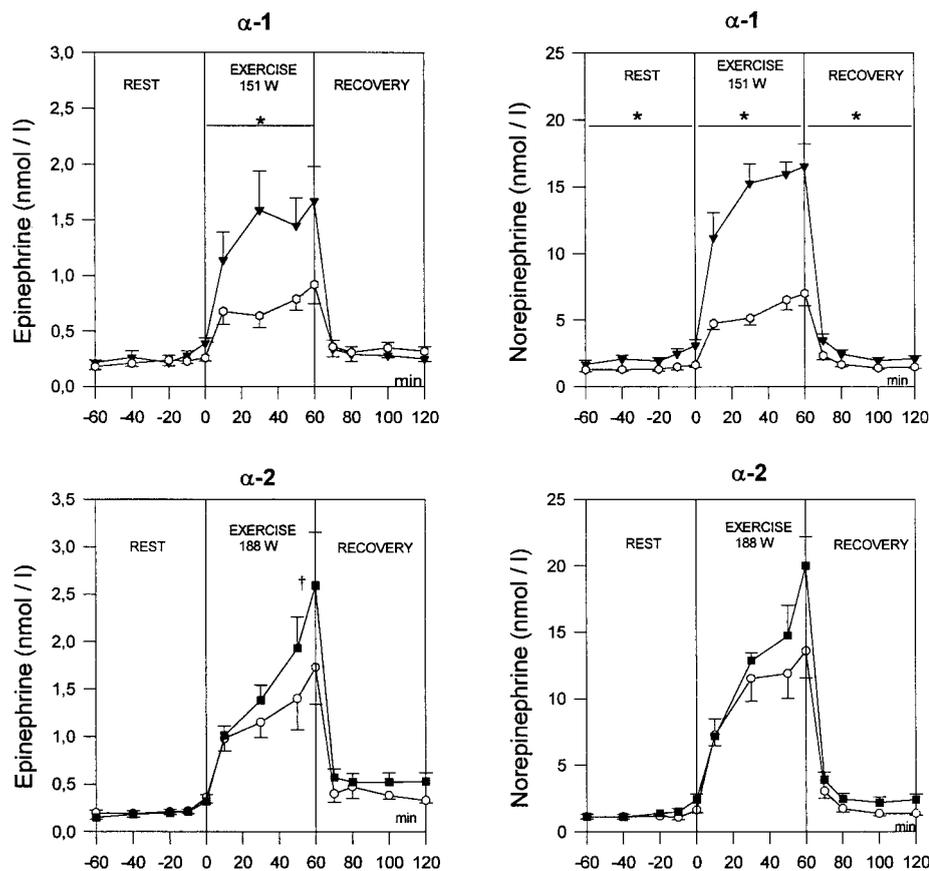


FIG. 6. Plasma epinephrine and norepinephrine concentrations before, during, and after 60-min ergometer bicycle exercise in eight men with  $\alpha_1$ -receptor blockade (prazosin; 1 mg at  $t = -60$  min;  $\blacktriangledown$ ) and without (control;  $\circ$ ), and with  $\alpha_2$ -receptor blockade (yohimbine; 15 mg at  $t = -45$  min;  $\blacksquare$ ) and without (control;  $\circ$ ). Data are means  $\pm$  SE. \*Significant difference ( $P < 0.05$ ) between blockade and corresponding control study; †significant difference at single time points ( $P < 0.05$ ).

results obtained in swimming mice (8) but are in contrast to one study in humans (7), in which it was found that yohimbine (0.2 mg/kg p.o., given 45 min before exercise at 60% of maximal aerobic capacity) had no effect on the exercise-induced decrease in plasma insulin concentrations. However, the focus of that study (7) was not primarily to study the effect on plasma insulin secretion (for example, plasma C-peptide concentrations were not measured). It is difficult to compare the present with the past (7) study, as exercise-related details (e.g., maximal aerobic capacity, heart rate, and oxygen consumption) were not reported (7). Therefore, we are unable to offer an explanation for the divergence in the effect of  $\alpha_2$ -receptor blockade on insulin secretion during exercise. The data on plasma insulin concentrations in the present study are in line with those from another study (19), in which a peripherally selective  $\alpha_2$ -receptor antagonist (MK-467) was infused before and during exercise. However, in that study (19), an exercise-induced decrease in insulin during placebo treatment could not be demonstrated, probably because of an inadequate exercise protocol. Finally, the importance of  $\alpha_2$ -receptors in the exercise-induced inhibition of insulin secretion is supported by the study from Natali et al. (20), in which selective  $\alpha_2$ -receptor blockade with deriglidole prevented epinephrine-induced inhibition of insulin secretion.

In the current study, we used different absolute workloads in the  $\alpha_1$  and  $\alpha_2$  experiments. When choosing the workloads, we aimed at near maximal workloads that could be maintained for 1 h, given that the inhibition of insulin secretion during exercise is directly related to the intensity of exercise. However, during exercise with  $\alpha_1$ -receptor blockade, shunting of blood from the gastrointes-

tinal compartment to the working muscles is inhibited. Normally, the exercise-induced reduction of splanchnic blood volume and flow may be as much as 40–50% of resting values (21), but some of this reduction does not occur with  $\alpha_1$ -receptor blockade. The effect of  $\alpha_1$ -receptor blockade on splanchnic blood flow was not measured in the present study, but the exaggerated heart rate response to exercise did indicate that the effect was not trivial (Fig. 1). The consequences of a lesser reduction in splanchnic blood volume and flow are that a relative lack of blood—and thereby oxygen—supply to the working muscles becomes apparent, nonoxidative glycolysis increases with subsequent considerable lactate release, and the subjects become fatigued. Therefore, the workload had to be diminished during  $\alpha_1$ -receptor blockade and the corresponding control experiment; comparisons between the responses during  $\alpha_1$ - and  $\alpha_2$ -receptor blockade (and corresponding control experiments) should take this into account. The described effect of exercise with  $\alpha_1$ -receptor blockade on the normal shunting of blood was also reflected by the higher oxygen uptake rates, and thus relative intensity, during  $\alpha_1$ -receptor blockade compared with the control study.

Plasma glucose kinetics during exercise were quite influenced by  $\alpha_1$ -receptor blockade (Fig. 2). Normally, during exercise, a slight decrease of plasma glucose concentrations is seen, but with  $\alpha_1$ -receptor blockade, plasma glucose concentrations increased markedly during the first 30 min of exercise, then clearly decreased toward the pre-exercise level. Calculations of glucose Ra and Rd revealed that the immediate increase was attributable to an enhanced Ra (compared with control) and not to changes in the Rd (Fig. 2). The rapid increase in the Ra,

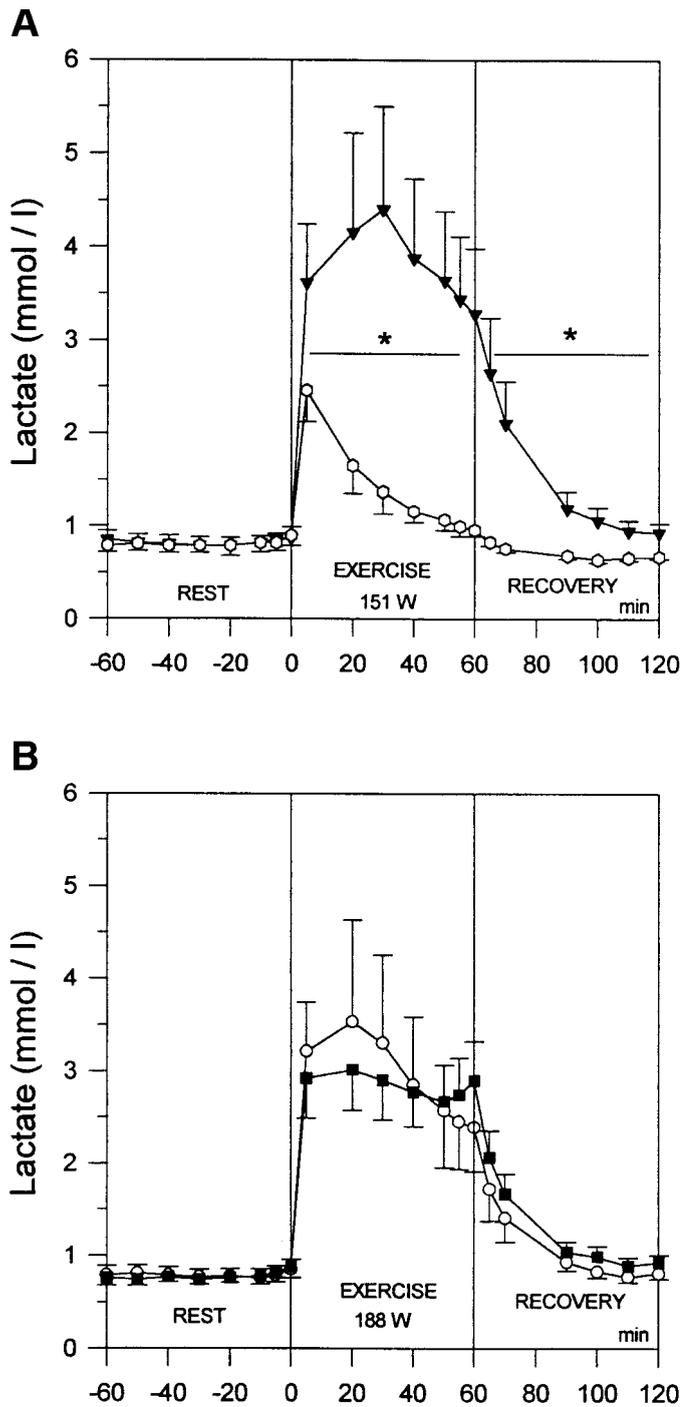


FIG. 7. Blood lactate concentrations before, during, and after 60-min ergometer bicycle exercise in eight men with (A)  $\alpha_1$ -receptor blockade (prazosin; 1 mg at  $t = -60$  min;  $\blacktriangledown$ ) and without (control;  $\circ$ ), and with (B)  $\alpha_2$ -receptor blockade (yohimbine; 15 mg at  $t = -45$  min;  $\blacksquare$ ) and without (control;  $\circ$ ). Data are means  $\pm$  SE. \*Significant difference ( $P < 0.05$ ) between blockade and corresponding control study.

with the subsequent increase in glucose concentrations (Fig. 2), can be attributed to the initial effect of the increased catecholamine concentrations (Fig. 6). The enhanced glucose Ra seen during exercise with  $\alpha_1$ -receptor blockade was most likely attributable to hepatic gluconeogenesis, and lactate may have served as substrate (Fig. 7), but the tracer used in the present study did not allow a definite conclusion about this. The decrease in plasma glucose concentrations during the latter part of exercise

with  $\alpha_1$ -receptor blockade (Fig. 2) was because of continuing increases in the glucose Rd, with only a tendency to steady state at the end of the exercise period (Fig. 2). From the data, it must be concluded that during  $\alpha_1$ -receptor blockade, the disappearance of glucose from plasma during exercise is enhanced (most likely representing increased skeletal muscle glucose uptake). The mechanism is unclear, but it may be attributable to inhibition of the sympathoadrenal vasoconstrictor component in the skeletal muscle, thus allowing increased glucose supply to the working muscles. In addition, during exercise with  $\alpha_1$ -receptor blockade, the relative workload was higher compared with the corresponding control study, which may have resulted in an amplification of motor unit recruitment, especially considering the fact that the subjects were not highly trained athletes. Furthermore, the differences in the glucose Rd between exercise with and without  $\alpha_1$ -receptor blockade seemed to be the consequence of differences in the prevailing plasma glucose concentrations, given that there was no difference in the MCR (Fig. 2).

During exercise with and without  $\alpha_2$ -receptor blockade, plasma glucose concentrations decreased to a similar extent with exercise. There was, however, a tendency ( $P = 0.0505$ ) to lower plasma glucose concentrations during  $\alpha_2$ -receptor blockade versus during the control study (Fig. 2), which could be expected, considering the higher plasma insulin concentrations (Fig. 3). During exercise, the glucose Ra was reduced by the  $\alpha_2$ -receptor blockade (Fig. 2). This may have been a direct effect on hepatic  $\alpha_2$ -receptors, and/or may have been attributable to the fact that plasma insulin concentrations were significantly higher with than without  $\alpha_2$ -receptor blockade (Fig. 3). In the resting nonexercise situation, there was no effect of  $\alpha_2$ -receptor blockade on glucose Ra, as has been also shown by others (20). Surprisingly, the glucose Rd was significantly lower with  $\alpha_2$ -receptor blockade compared with the corresponding control study (Fig. 2). A decreased peripheral glucose uptake could be attributable to a decrease in glucose delivery (i.e., blood flow). Thus,  $\alpha_2$ -receptor blockade should result in a pronounced vasoconstriction because of blockade of presynaptic  $\alpha_2$ -receptors. However, the similar blood lactate concentrations (Fig. 7) during exercise with and without  $\alpha_2$ -receptor blockade speak against such a mechanism. Hence, we speculated that  $\alpha_2$ -receptor blockade partially inhibits skeletal muscle glucose uptake during exercise via an unknown mechanism. This was an unexpected finding, in particular because of the well-known additive effect of exercise and insulin on skeletal muscle glucose uptake.

Our use of labeled glucose, with the subsequent calculations and approximations to estimate glucose Ra and Rd during a non-steady-state condition, such as exercise, can be criticized (22). However, in a recent study, this method was compared with the direct arterial-hepatic vein balance technique, and it was concluded that during exercise in humans, determination of hepatic glucose production can be performed equally well with the two techniques (23).

In human fat cells, the lipolytic activity is subject to regulation via activation of both  $\beta$ - and  $\alpha_2$ -adrenoceptors, with an increase and decrease in lipolytic activity with  $\beta$ - and  $\alpha_2$ -receptor stimulation, respectively. Thus, treatment with an  $\alpha_2$ -antagonist would be expected to increase

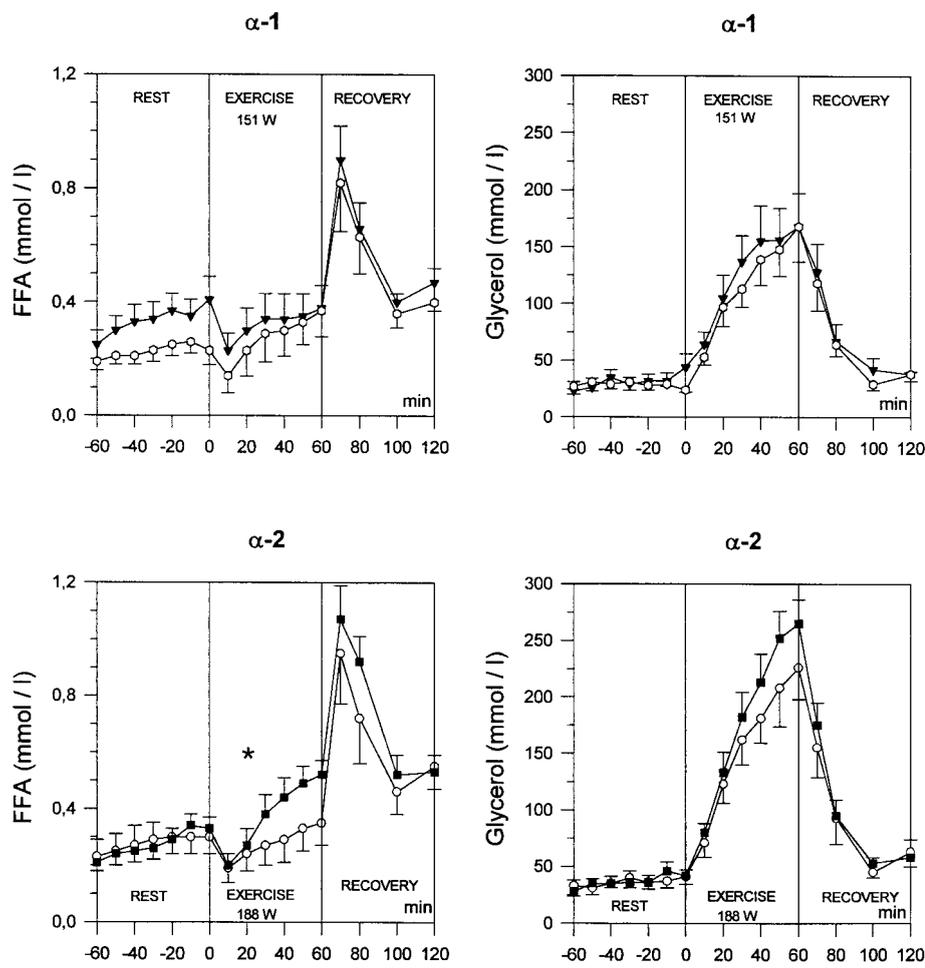


FIG. 8. Plasma FFAs and glycerol concentration before, during, and after 60-min ergometer bicycle exercise in eight men with  $\alpha_1$ -receptor blockade (prazosin; 1 mg at  $t = -60$  min;  $\blacktriangledown$ ) and without (control;  $\circ$ ), and with  $\alpha_2$ -receptor blockade (yohimbine; 15 mg at  $t = -45$  min;  $\blacksquare$ ) and without (control;  $\circ$ ). Data are means  $\pm$  SE. \*Significant increase ( $P < 0.05$ ) with time in blockade study.

lipolytic activity, resulting in increases in plasma FFAs and glycerol concentrations. Such an effect of  $\alpha_2$ -antagonism has also been shown in the past in resting humans (7). Furthermore, during combined exercise and  $\alpha_2$ -receptor blockade, an additive effect on FFAs and glycerol was demonstrated (7). This additive effect was probably attributable to the combined effect of exercise-induced increase in sympathoadrenal activity stimulating fat cell  $\beta$ -receptors and blockade of  $\alpha_2$ -receptors. However, in that study (7), plasma insulin concentrations were unaffected by  $\alpha_2$ -receptor blockade; that is, they decreased with exercise. In the present study, we found that the exercise-induced decreased insulin secretion was abolished by  $\alpha_2$ -receptor blockade, and therefore the antilipolytic effect of insulin was preserved. Thus, in the present study, only a marginal increase in FFA concentrations during exercise was seen with  $\alpha_2$ -receptor blockade, and no difference in the glycerol concentration with or without  $\alpha_2$ -receptor blockade was seen (Fig. 8).

In summary, we have now shown that the exercise-induced decrease in insulin secretion is mediated via  $\alpha_2$ -adrenoceptors. Blockade of  $\alpha_1$ -adrenoceptors did not affect the normal decrease in plasma insulin concentrations during exercise. Glucose kinetics during exercise were differently influenced by  $\alpha_1$ - and  $\alpha_2$ -adrenoceptor blockade. With  $\alpha_1$ -receptor blockade, plasma glucose concentrations initially increased, then decreased as the exercise continued because of the enhanced glucose Rd. In contrast, during a 60-min exercise bout with  $\alpha_2$ -receptor blockade, plasma glucose

concentrations continuously decreased, and the glucose Ra and Rd were diminished. A decreased glucose Ra during exercise with  $\alpha_2$ -adrenoceptor blockade can be attributed to the accompanying elevation in plasma insulin concentrations; the reason for a diminished glucose Rd, however, remains obscured.

#### ACKNOWLEDGMENTS

This study was supported by grant from the Danish National Research Foundation (no. 504-14), the Novo-Nordic Foundation, the Danish Sports Research Council, the P. Carl Petersen Foundation, the Fondation of 1870, the Direktør Jacob Madsen og hustru Olga Madsens Fondation, and the Direktør Ib Henriksens Fondation.

We thank Regitze Kraunsøe and Jeppe Bach for excellent technical assistance.

#### REFERENCES

- Galbo H: *Hormonal and Metabolic Adaptation to Exercise*. New York, Thieme-Stratton, 1983
- Brisson GR, Malaisse-Lagae F, Malaisse WJ: Effect of phentolamine upon insulin secretion during exercise. *Diabetologia* 7:223-226, 1971
- Galbo H, Christensen NJ, Holst JJ: Catecholamines and pancreatic hormones during autonomic blockade in exercising man. *Acta Physiol Scand* 101:428-437, 1977
- Nakaki T, Nakadate T, Kato R:  $\alpha_2$ -Adrenoceptors modulating insulin release from isolated pancreatic islets. *Naunyn Schmiedeberg's Arch Pharmacol* 313:151-153, 1980

5. Morgan NG, Montague W: Studies on the mechanism of inhibition of glucose-stimulated insulin secretion by noradrenaline in rat islets of Langerhans. *Biochem J* 226:571–576, 1985
6. Lacey RJ, Chan SL, Cable HC, James RF, Perrett CW, Scarpello JH, Morgan NG: Expression of alpha 2- and beta-adrenoceptor subtypes in human islets of Langerhans. *J Endocrinol* 148:531–543, 1996
7. Galitzky J, Taouis M, Berlan M, Rivière D, Garrigues M, Lafontan M:  $\alpha$ 2-Antagonist compounds and lipid mobilization: evidence for a lipid mobilizing effect of oral yohimbine in healthy male volunteers. *Eur J Clin Invest* 18:587–594, 1988
8. Karlsson S, Ahrén B: Insulin and glucagon secretion in swimming mice: effects of autonomic-receptor antagonism. *Metabolism* 39:724–732, 1990
9. Kjaer M: Hepatic fuel metabolism during exercise. In *Exercise Metabolism*. Hargreaves M, Ed. Champaign, IL, Human Kinetics, 1995, p. 73–97
10. Kjaer M, Secher NH, Bach FW, Galbo H: Role of motor center activity for hormonal changes and substrate mobilization in humans. *Am J Physiol* 253:R687–R695, 1987
11. Dela F, Mikines KJ, Linstow vM, Secher NH, Galbo H: Effect of training on insulin mediated glucose uptake in human skeletal muscle. *Am J Physiol* 263:E1134–E1143, 1992
12. Wandrup J, Tvede K, Grinsted J, Jordening H: “Stat” measurements of L-lactate in whole blood and cerebrospinal fluid assessed. *Clin Chem* 35:1740–1743, 1989
13. Hetenyi GJ, Norwich KH: Validity of the rates of production and utilization of metabolites as determined by tracer methods in intact animals. *Fed Proc* 33:1841–1848, 1974
14. Radziuk J, Norwich KH, Vranic M: Experimental validation of measurements of glucose turnover in nonsteady state. *Am J Physiol* 234:E84–E93, 1978
15. Steele R, Wall JS, De Bodo RC, Altszuler N: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 187:15–24, 1956
16. Cherrington AD, Vranic M: Effect of arginine on glucose turnover and plasma free fatty acids in normal dogs. *Diabetes* 22:537–543, 1973
17. Galitzky J, Rivière D, Tran MA, Montastruc JL, Berlan M: Pharmacodynamic effects of chronic yohimbine treatment in healthy volunteers. *Eur J Clin Pharmacol* 39:447–451, 1990
18. Polonsky KS, Frank B, Pugh W, Addis A, Karrison T, Meier P, Tager H, Rubenstein AH: The limitations to and valid use of C-peptide as a marker of the secretion of insulin. *Diabetes* 35:379–386, 1986
19. Sciberras DG, Reed JW, Elliott C, Blain PG, Goldberg MR: The effects of a peripherally selective alpha 2-adrenoceptor antagonist, MK-467, on the metabolic and cardiovascular response to exercise in healthy man. *Br J Clin Pharmacol* 37:39–44, 1994
20. Natali A, Gastaldelli A, Galvan AQ, Sironi AM, Ciociaro D, Sanna G, Rosenzweig P, Ferrannini E: Effects of acute alpha 2-blockade on insulin action and secretion in humans. *Am J Physiol* 274:E57–E64, 1998
21. Rowell LB: *Human Circulation Regulation During Physical Stress*. N York/Oxford, Oxford University Press, 1986
22. Cobelli C, Mari A, Ferrannini E: Non-steady state: error analysis of Steele’s model and developments for glucose kinetics. *Am J Physiol* 252:E679–E689, 1987
23. Bergeron R, Kjaer M, Simonsen L, Bulow J, Galbo H: Glucose production during exercise in humans: a-hv balance and isotopic-tracer measurements compared. *J Appl Physiol* 87:111–115, 1999