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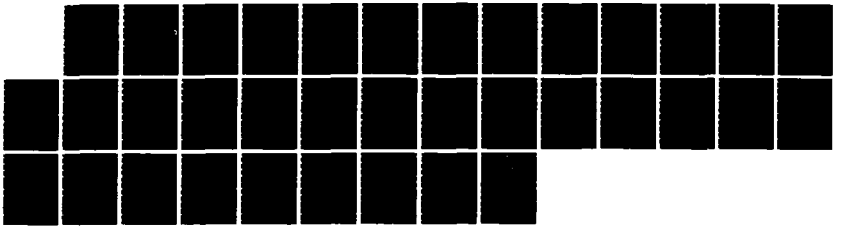

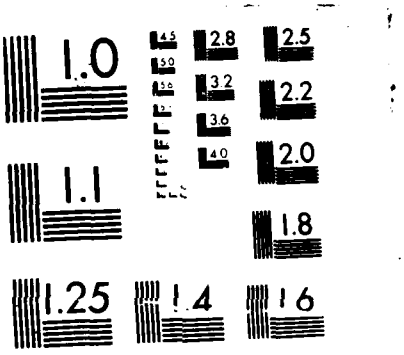

INFLUENCE OF FASTING ON CARBOHYDRATE AND FAT METABOLISM DURING REST AND EXERCISE IN MEN

RUNNING HEAD: CARBOHYDRATE METABOLISM DURING FASTING AND EXERCISE

by

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ABSTRACT

Metabolic effects of an overnight fast (postabsorptive state, PA) or a 3.5-day fast (fasted state, F) were compared in 8 healthy young men at rest and during exercise to exhaustion at 45% VO_2 max. Glucose rate of appearance (Ra) and disappearance (Rd) were calculated from plasma glucose enrichment during a primed, continuous infusion of [6,6-^{2}H]-glucose. Serum substrates and insulin levels were also measured. Glycogen content of the m. vastus lateralis was determined in biopsies taken before and after exercise. At rest, glucose flux and whole body carbohydrate oxidation determined from the respiratory exchange ratio were lower in F than PA but muscle glycogen levels were similar. During exercise, glucose flux, whole body carbohydrate oxidation and the rate of muscle glycogen utilization were significantly lower during the fast. In the PA state, glucose Ra and Rd increased together throughout exercise. However, in the F state Ra exceeded Rd during the first hour of exercise, causing an increase in plasma glucose to levels similar to those of the PA state. The increase in glucose flux was markedly less throughout F exercise. Lower carbohydrate utilization in the F state at rest and during exercise was consistent with higher circulating fatty acids and ketone bodies, lower levels of plasma insulin and the maintenance of physical performance as reflected by similar time to exhaustion.

Index Terms: Glucose flux, muscle glycogen, starvation, glucose, lactate, alanine, free fatty acids, glycerol, beta hydroxybutyrate, respiratory exchange ratio, insulin.

INTRODUCTION

Exercise during a brief or prolonged fast may be undertaken as therapy for obesity, under circumstances of natural or man-made disasters, in military environments, or under other conditions in which physical exertion is necessary despite an absence of food. At rest, a fast of a few days leads to depletion of liver glycogen (27, 35), decreased glucose flux (29, 40), enhanced gluconeogenesis (5, 13, 18), increased fatty acid and ketone body mobilization and utilization (3, 7, 18), and increased release of amino acid from peripheral tissues (42). Similarly, prolonged low-intensity exercise increases rates of gluconeogenesis (1), fatty acid mobilization and oxidation (20, 22), and amino acid release from muscles (15), while glucose flux increases (33), and muscle glycogen decreases (26). Recent studies of metabolic effects of exercise following a 24-hour fast have shown that the lower carbohydrate oxidation in the fasted state is maintained throughout exercise (8) and that the higher levels of free fatty acids increase even further with exercise but the fast has no apparent sparing effect on muscle glycogen (32). The present study was carried out under conditions of more prolonged fasting and less intense exercise, to determine what adaptations occur in the availability and utilization of fuels for muscular energy during a fast of 3.5 days in healthy men.

METHODS

Subjects

Eight male soldiers participated in this study after giving their written, voluntary consent. Their physical characteristics are shown in Table 1. Body fat was estimated from skinfolds (10), muscle mass from 24 hour urinary creatinine (23), muscle fiber type from histochemical analysis of myofibrillar ATPase (6) and VO_2 max using a discontinuous, incremental cycle ergometer protocol.

Study Design

The study was approved by the Human Use Review Committees of the Army Research Institute of Environmental Medicine and the Massachusetts Institute of Technology.

All 8 subjects were tested in both a post absorptive (PA) state (14 hours without food) and a fasted (F) state (3.5 days without food). Four men were studied first in the PA state and 4 were studied first in the F state. Testing in these 2 states were separated by 14 to 35 days. Four days prior to each test subjects consumed a balanced diet consisting of 12% protein calories, 53% carbohydrates calories, and 34% fat calories.

Prior to testing in the PA state subjects spent the night in a metabolic ward and were studied the following morning. Prior to testing in the F state subjects lived in the metabolic ward for 3 days and were studied on the morning of the fourth

day. Subjects were under the constant supervision of the nursing staff and were allowed to consume only distilled water and selected herbal teas. A 24h urine sample was collected in the first 32h of fasting and analyzed for urinary creatinine.

Testing in the PA and F state was identical and the design is shown in Figure 1. Subjects rested in bed for 3h then exercised on a cycle ergometer at 45% VO_2 max until volitional fatigue. Throughout rest and exercise subjects received a continuous intravenous infusion of [6,6-^{2}H]-glucose (Ø.28 umoles·kg^{-1}·min^{-1}) following a bolus dose of 22.4 umoles·kg^{-1} (80:1 ratio between the primer and infusion rate). Samples of expired gas and venous blood were obtained at intervals shown in Figure 1. Near the end of the rest period a muscle biopsy sample was obtained from the m. vastus lateralis (12) and a second biopsy was obtained from the same site 30 minutes after exercise.

Expired gas samples were analyzed for oxygen (Applied Electrochemistry^R Model S-3A) and carbon dioxide concentration (Beckman^R Model LB-2). Gas volumes were measured using a tissot spirometer. Respiratory exchange ratios (RERs) were calculated as VCO_2/VO_2 without correction for urinary nitrogen loss.

Substrates and insulin were analyzed in aliquots of blood, plasma or serum. Lactate was determined on whole blood (Roche^R autoanalyzer). Glucose was determined by the glucose oxidase method (Beckman^R Glucose Analyzer). Serum aliquots were analyzed for glycerol (49), alanine (31), beta-hydroxybutyrate

(B-OHB,50) and free fatty acids (FFA, 9, 31, 36). Insulin was determined by radioimmunoassay (Serono Laboratories kit). Plasma aliquots were derivatized and analyzed for isotopic enrichment of glucose by gas chromatography and mass spectrometry (4).

Muscle biopsy samples were rapidly separated into 3 to 5 smaller samples, and stored in liquid nitrogen until analyzed for glycogen content (39). A sample from the PA session was sectioned and stained for myofibrillar ATPase for muscle fiber type classification (6).

Calculation and Statistical Analysis

Glucose flux was determined using the nonsteady state equations of Steele (47) modified by Radziuk et al. (43) where:

$$Ra = \frac{i - pV\,[(Gt1+Gt2)/2]\,[(IEt2-IEt1)/\Delta t]}{(IEt1+IEt2)/2}$$

where:

$$Rd = Ra - (pV(Gt2-Gt1)/\Delta t)$$

Ra=Rate of appearance (umoles·kg^{-1}·min^{-1})

Rd=Rate of disappearance (umoles·kg^{-1}·min^{-1})

i=Infusion rate (umoles·kg^{-1}·min^{-1})

p=Pool fraction (a constant = 0.65)

V=Volume distribution of glucose (taken at 25% of body weight (43)) (ml·kg^{-1})

Gt1=Serum glucose at time 1 (umoles·ml^{-1})

Gt2=Serum glucose at time 2 (umoles·ml^{-1})

IEt1=Isotopic enrichment of [6,6-^{2}H]-glucose at time 1 (AE)

IEt2=Isotopic enrichment of [6,6-^{2}H]-glucose at time 2 (AE)

Δt=Time over which measurements occurred (min).

Resting and exercising data were analyzed separately using appropriate repeated measures analysis of variance statistics. When significant differences were found in multilevel variables the Tukey test was used to isolate the differences. Values are reported as mean $\pm$ standard error.

RESULTS

Muscle glycogen values are shown in Figure 2. PA and F muscle glycogen levels did not differ before or after exercise. However, there was a difference in the percent change in muscle glycogen from pre to post exercise: there was a 44% decrease in the PA state and a 28% decrease in the F state ($p<0.01$). Average glycogen utilization rates during exercise, calculated as the difference in glycogen before and after exercise divided by the time to volitional fatigue on the cycle ergometer, was 0.31 ± 0.04 umoles$\cdot$gm$^{-1}\cdot$min^{-1} in the PA state and 0.19 ± 0.02 umoles$\cdot$gm$^{-1}\cdot$min^{-1} in the F state ($p<0.01$).

The pattern of glucose enrichment at rest and during exercise is shown in Figure 3. The data show that isotopic steady state was achieved during the hour of rest before exercise in both the PA and F states. During exercise, analysis of

glucose enrichment over time showed that subjects were not at isotopic steady state and non steady state equations were used to calculate glucose kinetics (43, 47).

Glucose flux was consistently lower in the F state compared to the PA state as shown in Figure 4. For statistical purposes exercise was arbitrarily divided into a 0 to 60 minute period and a 60 minute to exhaustion period. Exhaustion occurred at 139±13 min in the PA state and 118±18 min in the F state (28). At rest, the Ra and Rd were about 23% lower in the PA state compared to the F state ($p<0.001$). During exercise in the F state, the rate of increase in glucose flux was lower ($p<0.001$) and the pattern of change in the Ra and Rd was different, compared to the PA state. In the PA state the Ra and Rd changed together at all times. In the F state the Ra and Rd were of similar magnitude at rest and the second hour of exercise; however, during the first hour of exercise the Ra was greater than the Rd ($p<0.01$).

Figure 5 depicts the changes in substrates in the PA and F sessions over time. In the F state, exercise was associated with greater fluctuations in levels of all 6 measured substrates. After the 3.5 day fast, blood glucose at rest was 3.93±0.18 mM which was 18% lower than in the PA state ($p<0.001$). During PA exercise blood glucose remained constant through the first hour then declined at exhaustion to below resting values ($p<0.01$). Conversely, in the first hour of F exercise glucose increased progressively and at 60 minutes blood glucose values were similar in the F and PA states. Glucose had again decreased by exhaustion but not below resting values for the F state.

Blood lactate levels were higher in the F state than in the PA state at rest and throughout exercise ($p<0.01$). This difference became greater within the first 10 to 30 min of exercise. Serum alanine did not differ between the PA and F states at rest or during exercise. However, in the F condition there was a progressive rise in alanine; levels became greater than resting values at 30 min ($p<0.05$) and stayed elevated over resting values for the remainder of the exercise period. There was a significant correlation between serum alanine and blood lactate during exercise ($R=0.85$ ($p<0.01$) in PA state and 0.96 ($p<0.001$) in the F state).

Serum FFA, glycerols and B-OHB values were higher in the F state compared to the PA state both at rest and during exercise ($p<0.01$). For FFA the pattern of change in the 2 conditions was identical although of greater absolute magnitude in the F state. At 10 min of exercise FFA declined from 0.30 ± 0.05 mmoles$\cdot l^{-1}$ to 0.23 ± 0.04 mmoles$\cdot l^{-1}$ in the PA state and from 0.78 ± 0.07 mmoles$\cdot l^{-1}$ to 0.53 ± 0.07 mmoles$\cdot l^{-1}$ in the F state. For B-OHB there was no change in the PA state until exhaustion when values were elevated ($p<0.01$). During F exercise B-OHB declined sharply in the first 10 min ($p<0.001$) and remained depressed throughout exercise.

Serum insulin values were lower in the F state than in the PA state both at rest and during exercise ($p<0.03$) as shown in Figure 6. Respiratory exchange ratios were also consistently lower in the F state both at rest and exercise ($p<0.05$) as shown in Figure 7.

DISCUSSION

The major finding of the present study was that the substantial alterations that have been described in whole body fuel utilization with fasting (3, 7, 18, 29) were maintained during submaximal exercise. There was a greater use of fat as a substrate and decreased production and utilization of carbohydrates during exercise in the F state.

Metabolism of Carbohydrates and Fats at Rest

Carbohydrate utilization at rest was markedly decreased by the 3.5 day fast. Despite this, muscle glycogen levels were unchanged. This contrasts with the 24% muscle glycogen decline reported for a single subject after a 3 day fast (25). Exercise is the main determinant of muscle glycogen utilization whereas food and diet play an important role in its resynthesis (26). In the present study, subjects were confined to a metabolic ward throughout the fast, limiting their opportunities for physical activity and muscle glycogen depletion. In the study of Hultman and Bergstrom (25) the normal daily activity of the subject may have been sufficient to lower muscle glycogen reserves.

Whole body glucose Ra and Rd decreased by about 23% after the 3.5 day fast while circulating glucose decreased 18% and insulin 16%. The glucose Ra in the F state was 434 $\pm$ 26 $umoles \cdot kg^{-1} \cdot h^{-1}$ which agrees with flux values obtained in other fasting studies (29, 40) but is higher than the value of

353 umoles·kg^{-1}·h^{-1} calculated for splanchnic output from arterio-venous difference in 3 day fasted subjects (18). Since liver glycogen stores are essentially depleted after the first day without food (35), glucose appearance in the F state would be the product of hepatic and possibly renal gluconeogenesis (18, 37). In the present study fasting gluconeogenesis supported a glucose flux (Ra) equal to 77% of the flux in the PA state. The decrease in glucose Rd was consistent with the lower rate of whole body carbohydrate oxidation suggested by a decrease in the RER from 0.84 $\pm$ 0.02 to 0.79 $\pm$ 0.02, lower circulating insulin and higher circulating FFA.

It is likely that increased fatty acid availability was the main factor accounting for the lower glucose utilization and oxidation. Circulating glycerol, FFA and B-OHB were elevated about 1.6, 2.6 and 4.6 times, respectively. Studies of arterio-venous differences across peripherial tissues have shown that glucose uptake declines as the availability of these substrates increases (38). Even in a post absorptive state, a 4-week adaptation to a ketogenic diet producing similarly high levels of FFA, ketone bodies and lactate, has been shown to lead to a 29% reduction in the rate of whole body ^{13}C-glucose oxidation (41).

Metabolism of Carbohydrates and Fats During Exercise

Early in F exercise, carbohydrate utilization was reduced when compared to similar exercise in the PA state. The significantly lower muscle glycogen utilization in the F state ($p<0.01$) was the result of 10% lower glycogen stores at rest, 15

higher glycogen after exercise, and a 15% lower time to fatigue compared to the PA state, although none of these changes were individually significant. At the end of exercise, muscle glycogen levels were between 50 and 60 umoles·gm^{-1} for both the fasted and post absorptive state.

One important cause of fatigue during submaximal exercise is depletion of muscle glycogen (26). In exercise at more than 65% VO_2 max, a 24 hour fast significantly reduced time to fatigue without changing glycogen utilization (32). The present study showed that during exercise at 45% VO_2 max similar time to fatigue occurred in both the PA and F states despite a reduced utilization of muscle glycogen in the F state. This has also been seen after adaptation to a eucaloric ketogenic diet in which a similar exercise time to exhaustion at 65% VO_2 max was seen despite pre-exercise muscle glycogen values that were substantially reduced (41). It is also interesting to note that post exercise glycogen values at fatigue were similar to those found with other dietary conditions or training status (19, 32, 41).

Exercise stimulates glucose utilization by muscle, increases hepatic glucose production, and increases the hepatic uptake of gluconeogenic precursors such as lactate, alanine and glycerol (1, 15, 48). In the present study, a 3.5 day fast changed the magnitude and pattern of glucose appearance and disappearance during exercise. In the PA state, glucose Ra and Rd increased by a factor of 2.5 by the end of 90 minutes of exercise, and remained high until fatigue. However, in the F

state, glucose Ra and Rd at fatigue had increased only by a factor of 1.9 compared to already low resting values. Early in F exercise, the increase in Rd did not match the increase in Ra. In the first hour of F exercise, glucose Rd remained low, not significantly different from resting values, while glucose Ra increased 52%, leading to blood glucose levels 20% higher than at rest. Similar increases in circulating glucose in the first 30 minutes of exercise have been reported in the post absorptive state after adaptation to a ketogenic diet (41) or after a 24-hour fast in subjects adapted to a normal diet (32). Tracer studies in men fasted for 3 days have shown that exercise increases the production of ketone bodies and produces an even greater increase in their rate of disappearance and oxidation (3). In these studies, high levels of free fatty acids and ketone bodies may have contributed to lower peripheral uptake and oxidation of glucose. In the present study, during the first hour of F exercise, there was an increase in glycerol, a marked decline in free fatty acids and ketone bodies, with no increase in peripheral glucose uptake, suggesting that the higher energy needs of exercising muscles were met mainly by increased lipid mobilization, uptake and oxidation (3, 20, 22). During exercise, the RER remained lower than in the post absorptive state, supporting an increased reliance on fat oxidation in the F state. However, since gluconeogenesis and ketone body utilization respectively decrease or increase the respiratory exchange ratio (16) the effects of fasting plus exercise on substrate oxidation estimated from respiratory data must be interpreted with caution.

The high levels of blood lactate and alanine observed during F exercise are consistent with increased availability and oxidation of FFA and ketone bodies. In muscles with a large capacity for oxidizing fats, high levels of FFA and ketones can inhibit glucose oxidation (44, 45). Acetyl-COA from fat oxidation inhibits pyruvate oxidation forcing pyruvate carbons into alternate metabolic pathways resulting in production of alanine, lactate and other substances (44). Correlations between alanine and lactate were higher during F exercise than PA exercise indicating a closer relationship between these substrates during the fast.

In the F state, the glucose Ra increased during the first hour of exercise, despite unchanging glucose utilization. This could be due to an increase availability of gluconeogenic precursors coupled with enhanced gluconeogenic capacity. Hepatic and renal gluconeogenesis increase with a greater supply of precursors: intravenous infusion of alanine into fasting men resulted in increased splanchnic glucose output and arterial glucose while glycerol infusion enhanced renal glucose output and arterial glucose (5, 14). Liver gluconeogenesis is accelerated by both short term fasting (18) and exercise (34): activities of the key hepatic enzymes pyruvate carboxylase (17) and phosphoenolpyruvate carboxykinase (46) are increased. Renal gluconeogenesis is stimulated by the metabolic acidosis (2) that can be induced by fasting (21) or acute exercise (24) although exercise alone has not been shown to increase renal glucose output (48).

After the first hour of F exercise, the balance between the glucose Ra and Rd was restored. Despite a further increase in circulating FFA and decrease in insulin, glucose uptake and whole body carbohydrate oxidation tended to increase, for reasons not entirely clear but possibly related to the normalization of blood glucose by the end of the first hour of exercise. In addition, subjects in the present study had an unusually low proportion of Type I muscle fibers. It has been shown that Type I fibers, which have a high capacity for fat oxidation (11), are primarily recruited in the early stages of cycle exercise (19). As their glycogen stores are depleted Type II fibers are recruited. These fibers have a much lower capacity for fat oxidation (11) and use glucose and glycogen as their primary fuel. It is likely that in the subjects of the present study, Type II fibers were recruited early due to the low proportion of Type I fibers. This shift in muscle fiber recruitment may account for the increase in glucose Rd and whole body carbohydrate oxidation in the second hour of F exercise and for the relatively brief duration of the exercise bout in both the PA and F states.

The changes observed in substrate utilization in this study may be specific to the duration of the fast and the intensity of exercise. In obese men exercising at 60% VO_2 max after a 2 week fast, plasma glucose increased very little during 45 minutes of cycling, and Ra exceeded Rd only during the first 15 minutes of exercise (33). In men exercising at 70-75% VO_2 max after a 24 hour fast, the increases in blood glucose and lactate after 60 minutes on a treadmill were greater than observed in this study,

while values for free fatty acids, ketone bodies and respiratory exchange ratio did not indicate any marked increase in lipolysis or fat oxidation (8).

At an exercise intensity of 45% VO_2 max, a 3.5 day fast did not lead to hypoglycemia nor early fatigue. In fact, the reduced utilization of muscle glycogen and blood glucose during exercise, together with the exercise-induced increase in gluconeogenesis, tended to shift circulating glucose levels toward values found in the post absorptive state. This study showed that a 3.5 day fast enhanced fat utilization and had a glycogen-sparing effect during low-intensity exercise.

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TABLE 1. PHYSICAL CHARACTERISTICS OF THE SUBJECTS

	AGE (yrs)	HEIGHT (cm)	WEIGHT (kg)	BODY FAT (%)	MUSCLE MASS (%)	TYPE II MUSCLE FIBERS (%)	VO_2 MAX ($l \cdot min^{-1}$)
M	22.5	172.5	76.8	16.4	43.1	70.3	3.25
SD	2.5	5.4	14.1	4.6	15.8	7.9	0.62

FIGURE LEGENDS

FIGURE 1. Testing design in both the PA and F states. On the vertical axis EXH represents exhaustion.

FIGURE 2. Mean muscle glycogen content in the PA and F states before and after exercise. Vertical bars are SE.

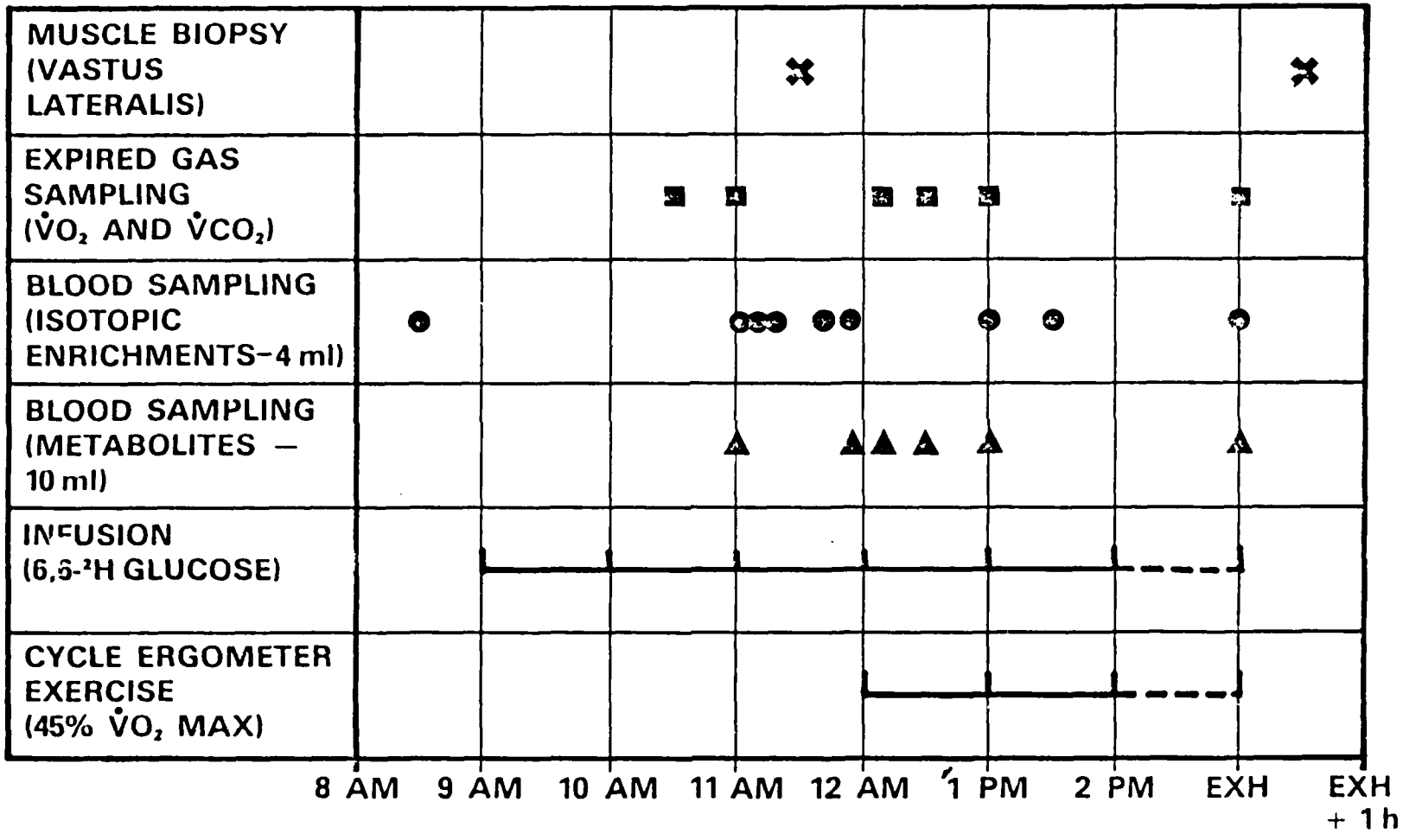
FIGURE 3. Mean isotopic enrichments of [6,6-^{2}H]-glucose. Vertical bars are SE. Horizontal bars are SE of the exercise times to exhaustion. Negative times on the horizontal axis represent time prior to exercise.

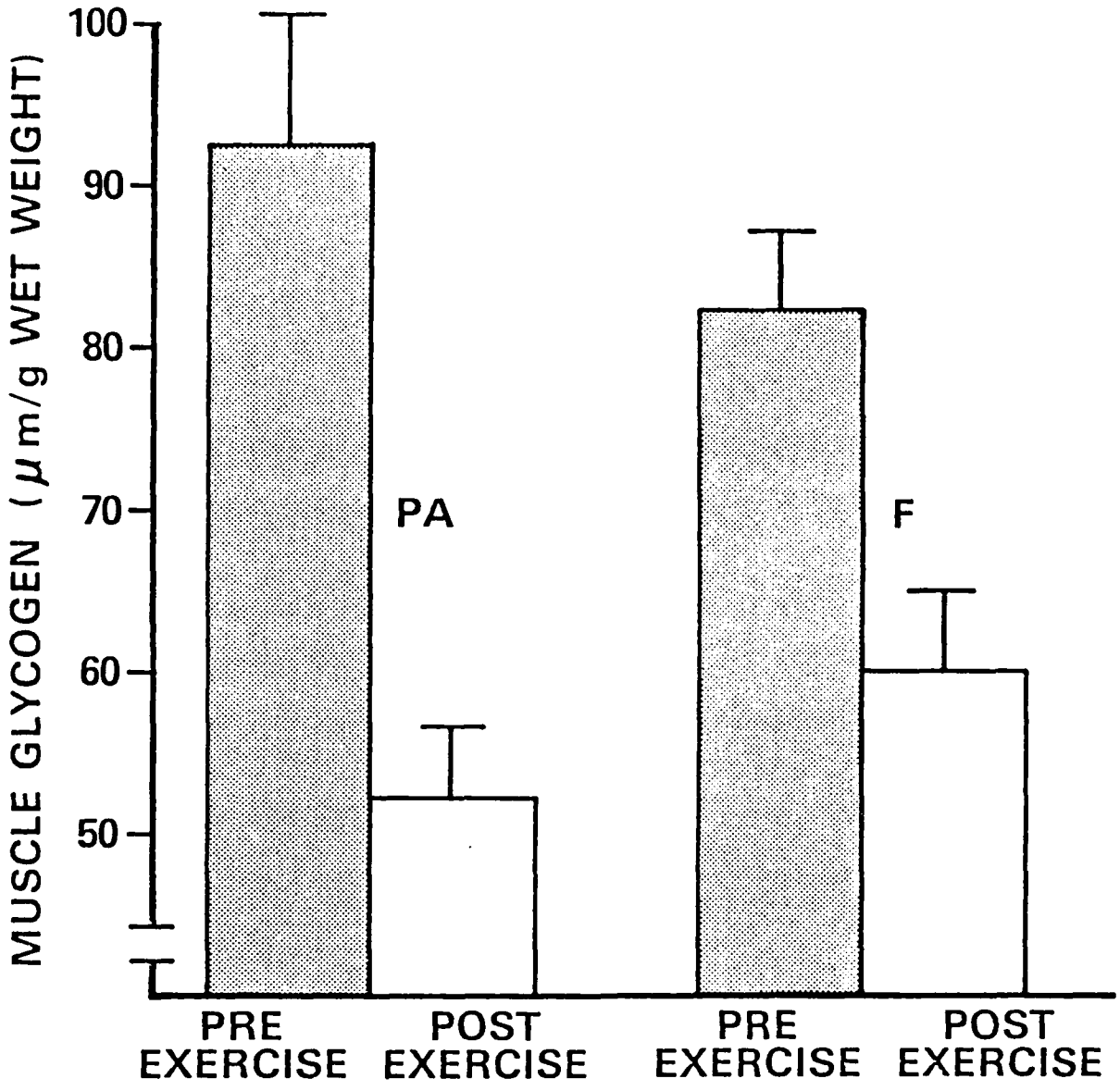
FIGURE 4. Mean glucose flux during rest and exercise in the PA and F states. Vertical bars are SE. Bars on the horizontal axis represent the SE of the exercise times to exhaustion. Negative times on the horizontal axis represent time prior to exercise.

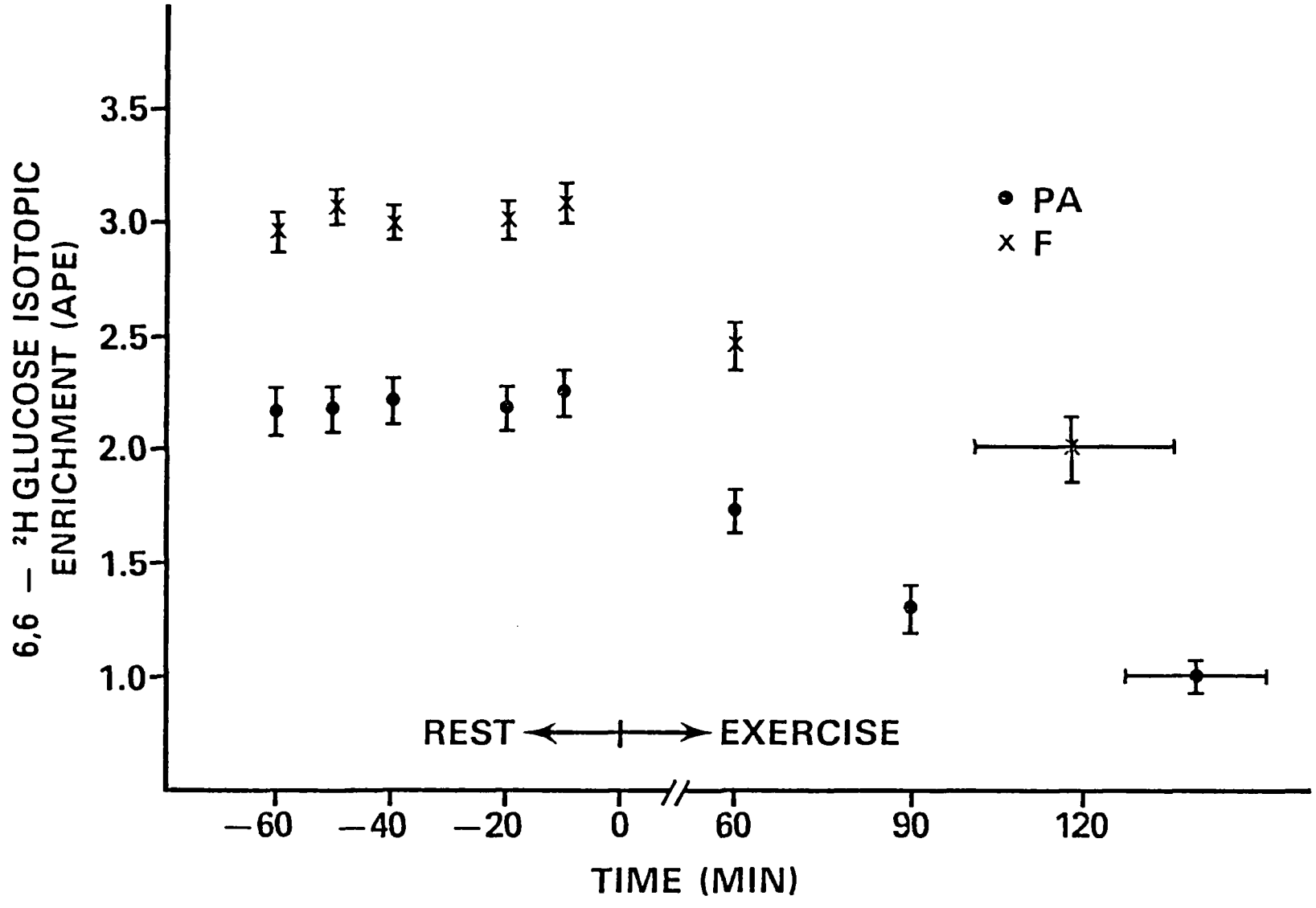
FIGURE 5. Mean changes in serum or blood substrates in the PA and F states at rest and during exercise. Vertical bars are SE. Negative times on the horizontal axis represent time prior to exercise. Broken lines going to final points indicate that time to exhaustion differed for each subject. Symbols: ▲= glycerol, △= FFA, X = B-OHB, □= alanine, ○= lactate, ●= glucose.

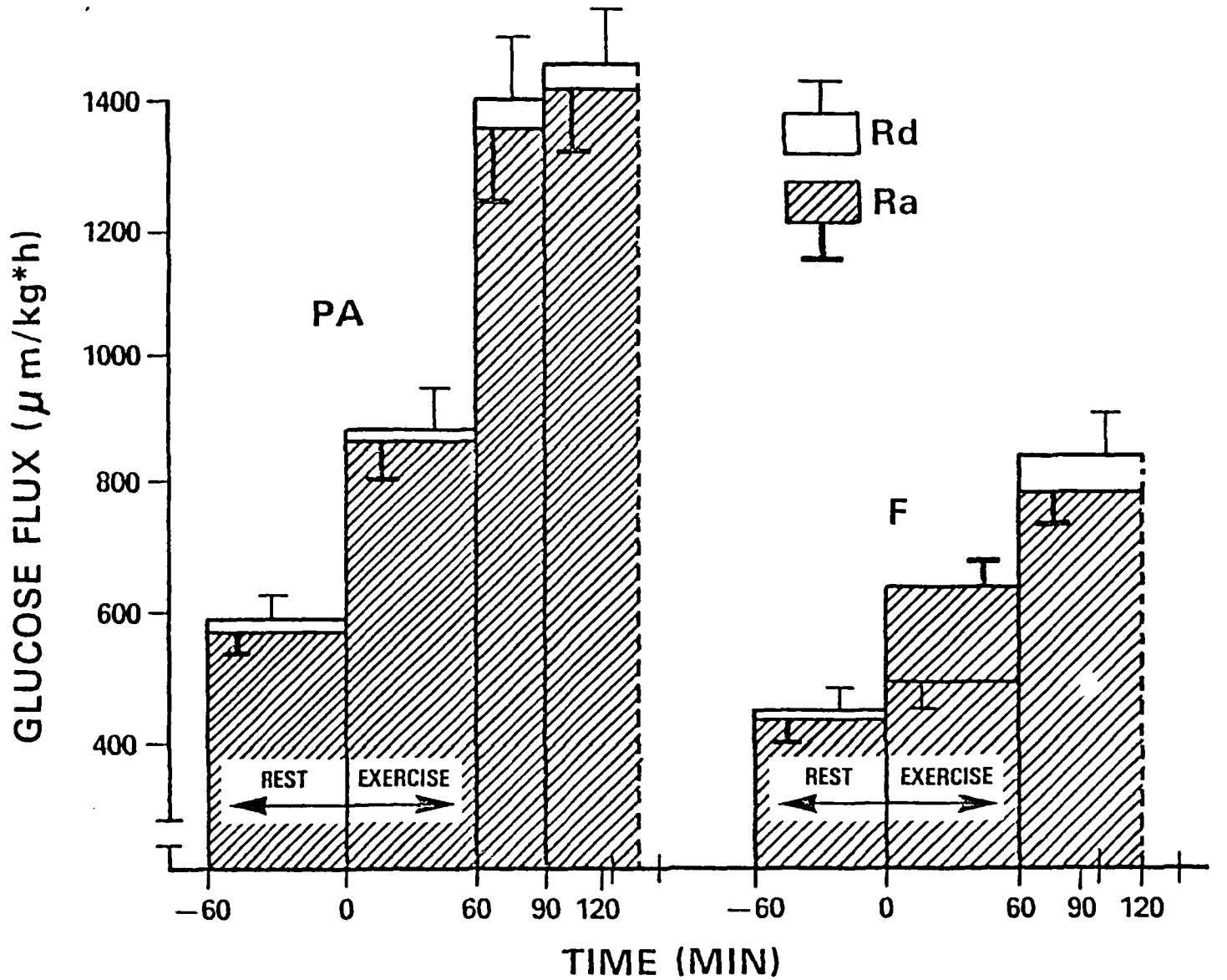
FIGURE 6. Mean changes in insulin in the PA and F states during rest and exercise. Vertical bars are SE. Negative times on horizontal axis represent time prior to exercise. Broken lines going to final points indicate that time to exhaustion differed for each subject.

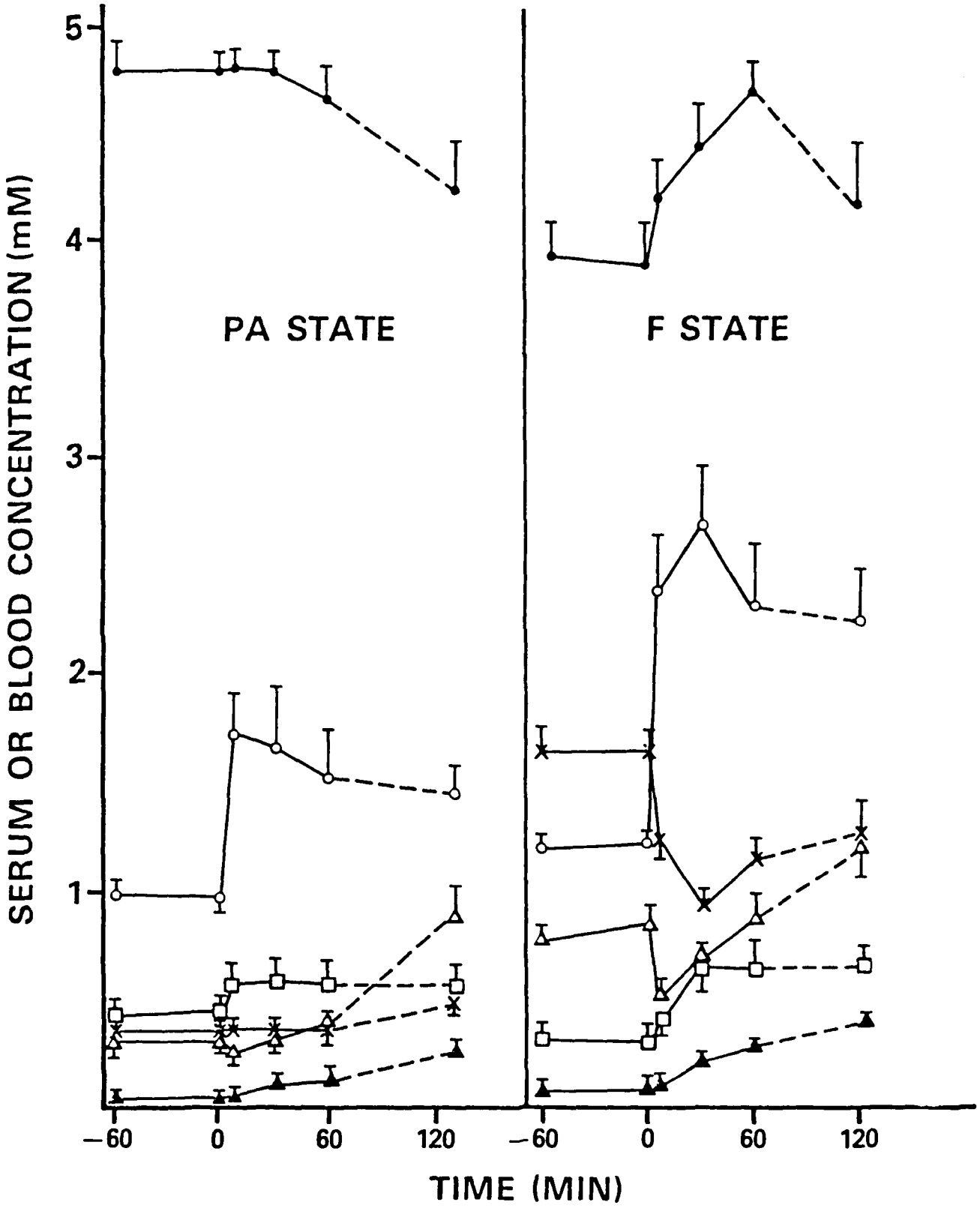
FIGURE 7. Mean changes in respiratory exchange ratios in the PA and F states during rest and exercise. Vertical bars are SE. Negative time on horizontal axis represent time prior to exercise. Broken lines going to final points indicate that time to exhaustion differed for each subject.

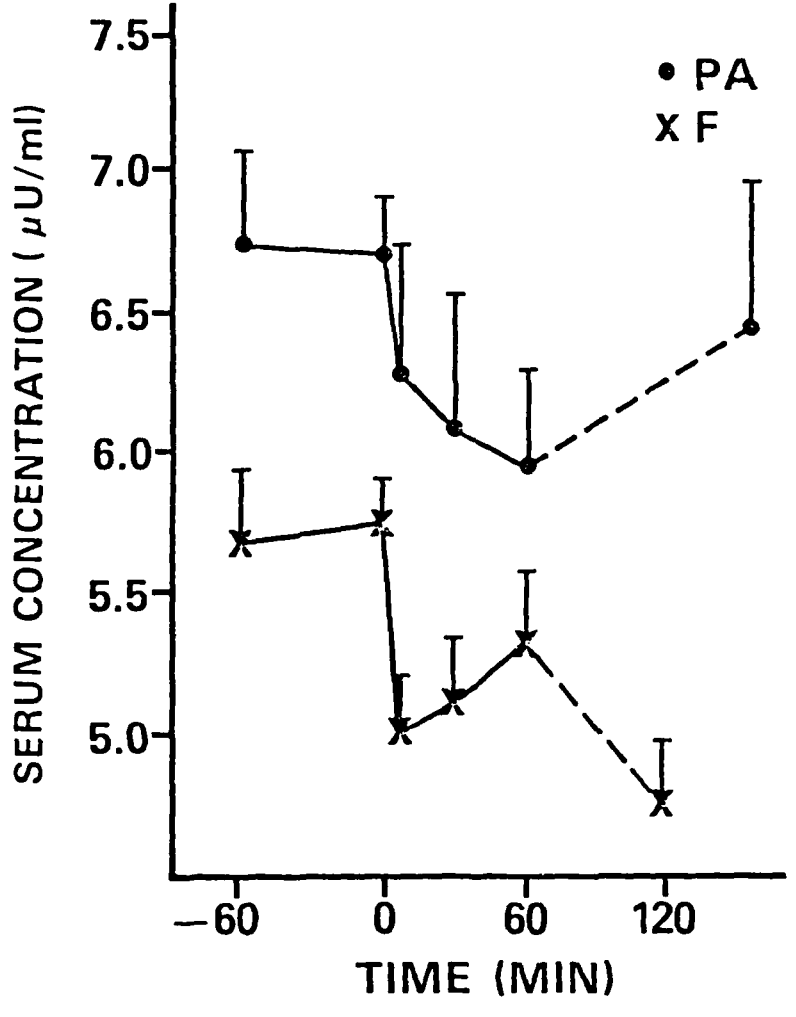

MUSCLE BIOPSY (VASTUS LATERALIS)
EXPIRED GAS SAMPLING ($\dot{V}O_2$ AND $\dot{V}CO_2$)
BLOOD SAMPLING (ISOTOPIC ENRICHMENTS–4 ml)
BLOOD SAMPLING (METABOLITES — 10 ml)
INFUSION (6,6-^{2}H GLUCOSE)
CYCLE ERGOMETER EXERCISE (45% $\dot{V}O_2$ MAX)
8 AM
9 AM
10 AM
11 AM
12 AM
1 PM
2 PM
EXH
EXH + 1 h

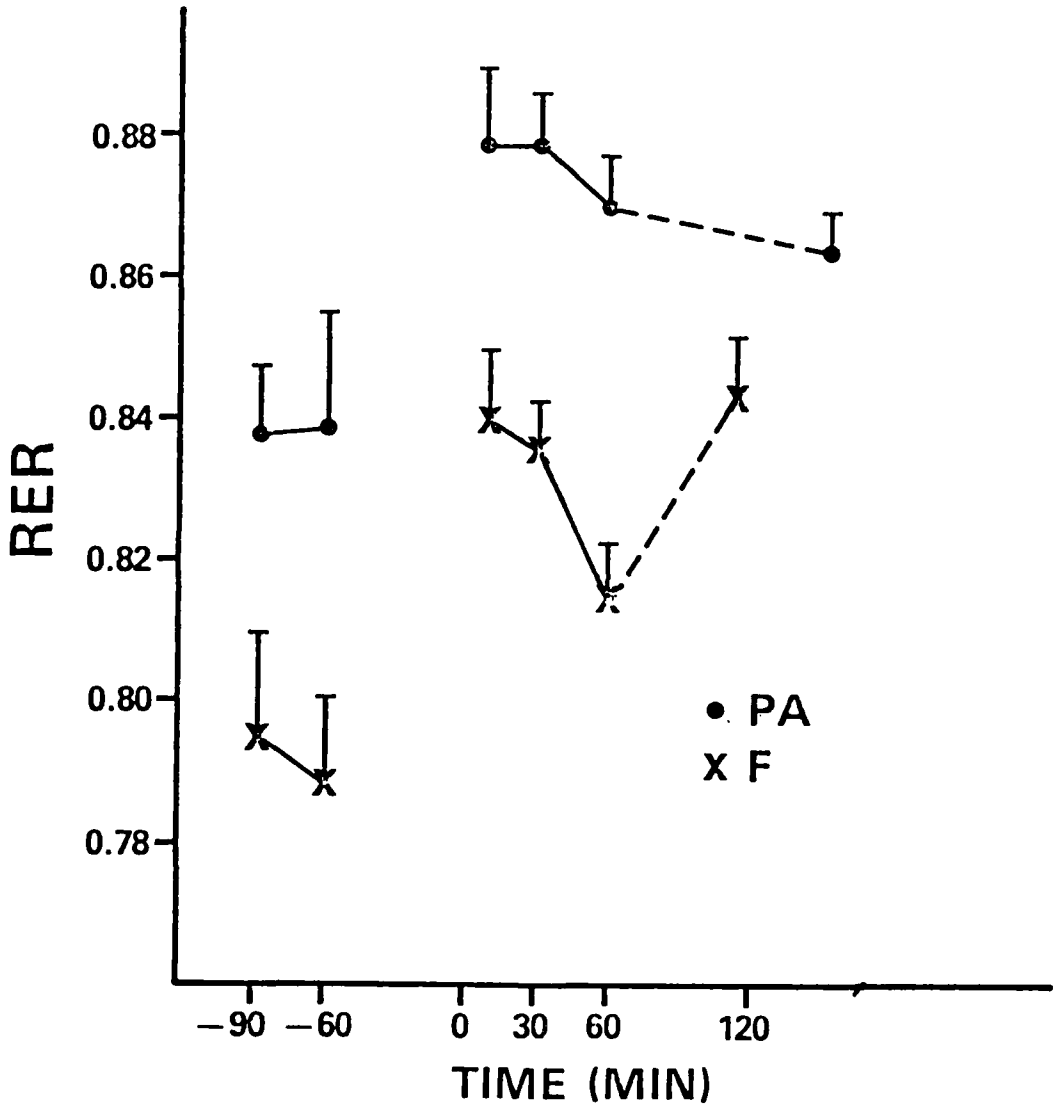

MUSCLE GLYCOGEN (μm/g WET WEIGHT)
100
90
80
70
60
50
PA
F
PRE EXERCISE
POST EXERCISE
PRE EXERCISE
POST EXERCISE


6,6 – ²H GLUCOSE ISOTOPIC ENRICHMENT (APE)
3.5
3.0
2.5
2.0
1.5
1.0
• PA
× F
REST
EXERCISE
–60
–40
–20
0
60
90
120
TIME (MIN)


GLUCOSE FLUX (μm/kg*h)
1400
1200
1000
800
600
400
Rd
Ra
PA
F
REST
EXERCISE
REST
EXERCISE
−60
0
60
90
120
−60
0
60
90
120
TIME (MIN)


SERUM OR BLOOD CONCENTRATION (mM)
5
4
3
2
1
PA STATE
F STATE
−60
0
60
120
−60
0
60
120
TIME (MIN)


7.5
7.0
6.5
6.0
5.5
5.0
SERUM CONCENTRATION (μU/ml)
• PA
X F
−60
0
60
120
TIME (MIN)


RER
0.88
0.86
0.84
0.82
0.80
0.78
• PA
X F
−90
−60
0
30
60
120
TIME (MIN)

END

6-87

DTIC