Eur J Appl Physiol (1985) 53: 343–347

European Journal of
Applied
Physiology
and Occupational Physiology

Plasma FFA responses to prolonged walking in untrained men and women

Frederick K. Blatchford, Ronald G. Knowlton, and Donald A. Schneider

Physical Education Research Laboratory, Southern Illinois University, Carbondale, IL 62901, USA

Summary. Gender differences in plasma FFA responses to 90 min of treadmill walking at 35% $\dot{V}_{O_{2max}}$ were investigated in six men and six women following an overnight fast. The subjects represented average values for maximal oxygen uptake and body fat percentage for age and gender. Mean plasma FFA concentration at 45 and 90 min of exercise were significantly ($P < 0.05$) higher for women (0.82 mmol · l^{-1}, 0.88 mmol · l^{-1}) than men (0.42 mmol · l^{-1}, 0.59 mmol · l^{-1}). Lower R values for women throughout the exercise period indicated a greater percentage fat in total metabolism than for men while the FFA/glycerol results supported greater lipolytic activity for women. The uniformity of percent fat in metabolism for women from rest to exercise showed that FFA release from adipose tissue increased rapidly with the onset of exercise which was not the case for men. Comparison of metabolic data as well as a statistical analysis (ANCOVA) controlling for the influence of $\dot{V}_{O_{2max}}$ and percentage body fat on FFA plasma concentration suggested that gender differences in FFA responses to prolonged submaximal exercise can be expected to occur in untrained subjects.

Key words: Gender differences – Walking – FFA – Glycerol

Introduction

It is well known that endurance trained athletes have an increased ability to use fat as an energy substrate during muscular exercise. This has been associated with an increased capacity of the oxygen transport system (Astrand and Rodahl 1969; Hermansen et al. 1971; Hickson et al. 1977; Mackie and Terjung 1983; Saltin et al. 1976) and of the skeletal muscle to oxidize fat (Costill et al. 1976; Gollnick et al. 1972; Holloszy and Booth 1976; Jacobs 1981; Rennie and Holloszy 1977; Saltin et al. 1977). Training has also been shown to minimize differences in FFA utilization that might otherwise be attributed to gender. This has been demonstrated by the use of trained men and women who have shown a similarity in FFA utilization when workloads were standardized for differences in maximal aerobic capacity (Costill et al. 1976; Powers et al. 1980). It remains equivocal, however, as to whether FFA mobilization and utilization are differentiated by gender in persons who have not experienced a history of endurance training.

The aim of the present study was to compare free fatty acid responses with respect to plasma concentration and with reference to utilization in men and women during prolonged work of moderate intensity. It was the intention to select untrained subjects so that skeletal muscle adaptation to overload would not mask differences in FFA responses that could be attributable to gender.

Materials and methods

Subjects. This study was performed on 12 healthy volunteer subjects (six men and six women) who were not engaged in regular exercise training. Their general descriptive data are presented in Table 1. A minimum period of 12 h was required without food ingestion before the prolonged submaximal test and all subjects were asked to refrain from smoking and the consumption of beverages containing caffeine during the 24 h preceding the tests. None of the women were taking oral contraceptives. The procedures used in this study had been approved by the institutional Committee for Research Involving Human Subjects.

Measurements. All testing was conducted in a comfortable air-conditioned laboratory under uniform environmental conditions. Percentage body fat was determined by hydrostatic weighing using the equation of Siri (1956). Oxygen uptake and carbon dioxide production were determined by open circuit spirometry with gas samples analyzed by a paramagnetic oxygen analyzer (Beckman OM-11) and infra-red carbon dioxide analyzer (Godart Capnograph) calibrated regularly with standardized mixtures as deter-

Offprint requests to: R. G. Knowlton at the above address

Table 1. Descriptive data for subjects

	Men (n = 6)	Women (n = 6)
Age (years)	33.7 ± 1.9	30.7 ± 0.80
Body weight (kg)	81.9 ± 4.7	65.8 ± 4.5 s
Height (cm)	175.6 ± 1.8	168.5 ± 1.4 s
Body fat (%)	21.4 ± 1.8	29.9 ± 2.0 s
Fat free weight (kg)	64.0 ± 2.6	45.7 ± 2.0 s
$\dot{V}_{O_{2max}}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	44.2 ± 3.3	36.4 ± 3.0
$\dot{V}_{O_{2max}}$ ($ml \cdot kg$ fat free $wt^{-1} \cdot min^{-1}$)	56.1 ± 3.6	52.0 ± 4.3
HR max ($b \cdot min^{-1}$)	179.3 ± 2.1	177.0 ± 2.6

Values are means ± SE
s = significantly different ($P < 0.05$) between men and women

mined by chemical analysis. Ventilatory volumes were obtained from a dry gas meter (Parkinson-Cowans CD-4) which was in line with a recorder (Beckman Type R Dynograph) with the system calibrated against a Tissot gasometer. The electrocardiogram and blood pressure were monitored throughout all treadmill testing.

Blood samples were obtained by venipuncture of the antecubital vein, 3.0 ml was collected in a heparinized tube and 7.0 ml in a nonheparinized container. Triplicates of hematocrit and duplicates of lactate (Gutman and Wahlefeld 1974) and hemoglobin were obtained from the heparinized samples. Hemoglobin and hematocrit were used to determine plasma shifts (Costill and Fink 1974). Duplicate serum samples from the nonheparinized container were used to determine protein concentration (hand protometer), glycerol (BMC assay kit), and FFA concentration (Falholt et al. 1973). The protometer was zeroed against distilled water while glycerol and fatty acids were determined against regularly established standard curves made from known concentrations.

Protocol. Before metabolic data were obtained, percent body fat was determined. This was followed approximately 1 week later by a multi-stage treadmill test for the determination of $\dot{V}_{O_{2max}}$ at a constant speed of 88.5 $m \cdot min^{-1}$ with elevation increases of 4% at 3-min intervals. Gas collections were made each minute after a heart rate of 150 $b \cdot min^{-1}$ was achieved and until the subject voluntarily terminated the test.

The 90-min walk took place following a minimum interval of 7 days from the time of the maximal test. The treadmill speed and elevation were adjusted to approximate 35% of each subject's $\dot{V}_{O_{2max}}$. After a suitable period of time to allow the subject to adjust to the breathing valve (Collins "Triple-J") as determined by a steady state recording of ventilation, expiratory air samples were collected for the final minute of each 15-min interval throughout the walking test and during the recovery period. The expiratory exchange ratio (R) was assumed to be a nonprotein respiratory quotient (RQ) and was used to estimate the fraction of energy expenditure due to carbohydrate and fat oxidation.

Blood samples were obtained after a 20-min pre-exercise rest period, at 45 and 90 min of walking and following 1 h of post exercise recovery. For the safety of the subject it was necessary to stop the treadmill for approximately 1 min while the blood samples were obtained.

Statistical analysis. Descriptive data for the two groups were compared using a SPSS sub-program for the appropriate t-test (Nie et al. 1975). An analysis of covariance (ANCOVA) was used to control for group bias due to differences in percent body fat or $\dot{V}_{O_{2max}}$ acting as independent variables affecting FFA responses to exercise. Any differences were considered to be significant if an alpha level of 0.05 was achieved.

Table 2. Mean oxygen uptake (STPD $l \cdot min^{-1}$) and R values (± SE)

Time period (min)	$\dot{V}_{O_2}$		R	
	Men	Women	Men	Women
Rest	0.250 ± 0.015	0.21 ± 0.017	0.82 ± 0.01	0.80 ± 0.01
Exercise				
15	1.259 ± 0.040	0.926 ± 0.051 s	0.88 ± 0.02	0.82 ± 0.01 s
30	1.276 ± 0.040	0.892 ± 0.042 s	0.86 ± 0.02	0.82 ± 0.01
45	1.245 ± 0.038	0.902 ± 0.045	0.85 ± 0.02	0.80 ± 0.01 s
60	1.248 ± 0.040	0.902 ± 0.047 s	0.84 ± 0.02	0.80 ± 0.01
75	1.268 ± 0.059	0.894 ± 0.045 s	0.84 ± 0.02	0.81 ± 0.01
90	1.259 ± 0.037	0.887 ± 0.052 s	0.82 ± 0.01	0.78 ± 0.01 s
Post exercise				
15	0.296 ± 0.013	0.244 ± 0.005 s	0.74 ± 0.02	0.72 ± 0.01
30	0.283 ± 0.025	0.251 ± 0.018	0.74 ± 0.01	0.75 ± 0.01
45	0.292 ± 0.033	0.220 ± 0.019	0.76 ± 0.02	0.74 ± 0.02
60	0.315 ± 0.034	0.223 ± 0.020 s	0.77 ± 0.01	0.78 ± 0.03

s = $P < 0.05$ between men and women

Results

At each of the six sampling periods for metabolic data during the prolonged walk significant differences between groups existed for oxygen consumption (Table 2). However, the relative intensity of exercise during this same period of time was 35.5% and 38.7% of $\dot{V}_{O_{2max}}$ for men and women respectively. The differences between men and women in these percentages were not statistically significant. The values for the respiratory exchange ratio were consistently lower for the women during exercise as was the case in recovery but in the latter period the differences were reduced. At the onset of exercise the men demonstrated a marked decrease in the percent fat in metabolism while the women maintained values close to those measured during the preexercise period (Fig. 1a). Both groups gradually increased the percent fat in metabolism during exercise with the 90-min values being 59% for the men and 73% for the women. Peak values for both grous in percent fat in

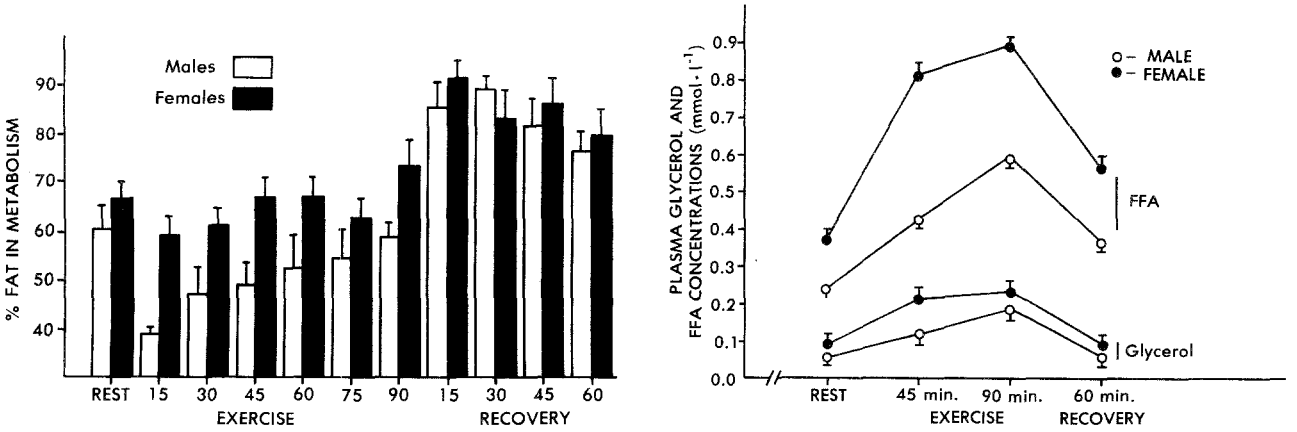

Fig. 1a. Mean percent fat (±SE) in total metabolism for men and women during 90 min walk and 60 min recovery. **b.** Mean plasma FFA and glycerol (±SE) for men and women during 90 min rest, exercise, and post exercise

metabolism were achieved during the recovery period and exceeded exercise values even 1 h after the exercise had been terminated.

Both groups showed significant elevations from rest to 90 min of exercise in serum FFA and glycerol concentrations (Fig. 1b). FFA concentrations were always higher for women than for men as was the case with glycerol concentrations. The glycerol concentrations increased somewhat faster for the women during the first 45 min of exercise whereas the men maintained a more consistent rate of increase throughout the 90-min exercise period.

During the period of exercise only minimal changes occurred for blood lactate, serum protein, and plasma volume for both of the groups.

Table 3. Mean plasma FFA, glycerol (mmol · l^{-1}), lactate (mmol · l^{-1}) concentrations (±SE) for men and women

	Rest	45 min exercise	90 min exercise	60 min post exercise
Men FFA	0.23 ± 0.04	0.42 ± 0.05	0.59 ± 0.07	0.36 ± 0.04
Women FFA	0.36 ± 0.08	0.82 ± 0.12 s	0.88 ± 0.09 s	0.57 ± 0.04 s
Men glycerol	0.052 ± 0.005	0.122 ± 0.019	0.176 ± 0.019	0.061 ± 0.006
Women glycerol	0.089 ± 0.012 s	0.218 ± 0.022 s	0.233 ± 0.018	0.097 ± 0.009 s
Men lactate	0.54 ± 0.12	0.48 ± 0.20	0.46 ± 0.19	0.48 ± 0.020
Women lactate	0.66 ± 0.19	0.77 ± 0.31	0.51 ± 0.20	0.55 ± 0.22

s = $P < 0.05$ between men and women

Discussion

The findings point to gender specific differences in FFA concentrations resulting from prolonged walking at about one third of $\dot{V}_{O_{2max}}$. Although the data for maximal aerobic power and percentage body fat support the contention that these were untrained subjects, it was still necessary to determine if group differences in these variables affected the plasma FFA concentration. An analysis of covariance (ANCOVA) revealed that gender differences accounted for a significant portion of the variance in exercise FFA concentrations when statistical control was given to group differences in aerobic power and percentage body fat (Table 4). Shifts in fluid volume can be ruled out as a factor in this finding due to the fact that there were small gains in plasma volume which averaged 3.8% for the women and 0.75% for the men.

There is little doubt that at the energy expenditure required of the present subjects during the prolonged walk, FFA was available in adequate supply. It is generally known that during prolonged exercise FFA turnover increases rapidly and that the FFA uptake by the working muscle depends largely on an increased blood flow rather than upon an enhanced fractional extraction (Gollnick 1977). With

Table 4. Analysis of covariance

Source of variation	SS	DF	Mean square	F	*P*
Covariates	0.132	2	0.066	1.818	0.223
% Body fat	0.130	1	0.130	3.597	0.094
$\dot{V}_{O_{2max}}$	0.040	1	0.040	1.095	0.326
Main effects	0.198	1	0.198	5.485	0.047
Gender	0.198	1	0.198	5.485	0.047
Explained	0.330	3	0.110	3.040	0.093
Residual	0.290	8	0.036		
Total	0.620	11	0.056		

the exception of the early adjustment portion of the walk the responses of both groups were consistent with the known relationship between FFA plasma concentration and its oxidation by the muscle (Fig. 1a, b). However, the absolute ability of the two groups to use fat as a substrate cannot be directly compared for several reasons. First, even though the relative work performed by the two groups was similar the absolute work required of the men was much greater so it would be expected that they would metabolize a greater quantity of fat to complete their task. Secondly, tissue biopsies were not performed so a measurement of the total oxidative capacity of the muscle was not conducted. However, the plasma glycerol responses (Fig. 1b) during exercise can be used as an indication of fat metabolism as it has been shown that the fractional extraction does not increase with elevations in arterial concentrations (Ahlborg et al. 1974) and that during prolonged exercise glycerol does not serve as a significant glyconeogenic substrate (Miller et al. 1983). As the glycerol levels were elevated to a greater extent with the women than with the men (Fig. 1b) support is given to the conclusion that the higher plasma FFA concentrations observed for these subjects was influenced only secondarily to exercise induced changes in fat oxidation by the active muscle. Consistent with this is the finding that the men used only 14% more lipid by weight during the exercise despite the fact that they possessed 40% more lean body mass than the women. It is also of interest that the percent fat in metabolism remained nearly constant for the women from rest through exercise while the men showed an immediate drop followed by a gradual increase as the exercise continued (Fig. 1a). Despite the fact that women may have a lower mitochondrial density than men (Costill et al. 1978) this suggests that the women were able to rapidly adjust fat metabolism to the caloric needs of the work while the men required the immediate use of alternative substrates.

In view of the finding that women showed an enhanced ability to mobilize fat, speculation as to the reason merits consideration. It is evident that sex hormones play a part in the observed differences between men and women (Kim and Kalkoff 1975; Krotiewski and Bjorntorp 1976; Bonen et al. 1983) but it is also possible that the response of the adipose cell to neurological and hormonal stimuli may have played a part in differentiating the groups. Evidence exists to suggest that women possess more adipocytes and larger adipocytes in the gluteal region than do men (Sjostrom et al. 1972) and that gluteal subcutaneous fat deposits have a greater sensitivity to lipolytic agents than do adipose cells at other sites (Rognum et al. 1982). In the present investigation fat patterning was not considered but the differences in total fat content between the two groups was in the order of that expected for untrained subjects (Durnin and Womersley 1974). This suggests that a resemblance to typical contrast in fat patterning between the groups may have existed. As the present data can only lead to conjecture, the suggestion is given to study gender and fat patterning as related to FFA activity during prolonged exercise.

In conclusion, this study has demonstrated that gender differences occur in FFA responses to prolonged submaximal exercise in untrained subjects. Women were more efficient in responding to exercise with an increased lipolytic rate in adipose tissue and they demonstrated a greater enhancement of FFA metabolism in muscle tissue than was shown for the men. The findings of this study are of potential value to subsequent investigators who may wish to study FFA responses to exercise in mixed groups of untrained men and women subjects.

References

Ahlborg G, Felig P, Hagenfeldt L, Handler R, Wahren J (1974) Substrate turnover during prolonged exercise in man. J Clin Invest 53: 1080–1090

American College of Sports Medicine (1980) Guidelines for graded exercise testing and exercise prescription (2nd ed). Lea and Febiger, Philadelphia

Astrand PO. Rodahl K (1969) Textbook of work physiology. McGraw-Hill, New York

Bonen A, Haynes FJ, Watson-Wright W, Sopper MM, Pierce GN, Low MP, Graham TE (1983) Effects of menstrual cycle on metabolic responses to exercise. J Appl Physiol 55: 1506–1513

Chien S, Peng MT, Chen KP, Huang TF, Chang C, Fang HS (1975) Longitudinal studies on adipose tissue and its distribution in human subjects. J Appl Physiol 39: 825–830

Costill DL, Daniels J, Evans W, Fink W, Krahenbuhl G, Saltin B (1976) Skeletal muscle enzymes and fiber composition in male and female track athletes. J Appl Physiol 40: 149–154

Costill DL, Fink W (1974) Plasma volume changes following exercise and thermal dehydration. J Appl Physiol 37: 521–525

Costill DL, Fink WJ, Getchell LH, Ivy JL, Witzmann FA (1979) Lipid metabolism in skeletal muscles of endurance-trained males and females. J Appl Physiol 47: 787–791

Durnin JVGA, Womersly J (1974) Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. Br J Nutr 32: 77–97

Falholt K, Lund B, Falholt W (1973) An easy colorimetric micromethod for routine determination of free fatty acids in plasma. Clin Chim Acta 46: 105–111

Gollnick PD, Karlsson J, Piehl K, Saltin B (1972) Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. J Appl Physiol 45: 852–857

Gutman I, Wahlefeld AW (1974) L (+) lactate determination with lactate dehydrogenase and NAD. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Academic Press, New York, pp 54–93

Hermansen L, Wachtlova M (1971) Capillary density of skeletal muscle in well trained and untrained men. J Appl Physiol 30: 860–863

Hickson RC, Bomze HA, Holloszy JO (1977) Linear increase in aerobic power induced by a strenuous program of endurance exercise. J Appl Physiol 42: 372–376

Holloszy JO, Booth FW (1976) Biochemical adaptations to endurance exercise in muscle. Ann Rev Physiol 38: 273–291

Jacobs I (1981) Lactate, muscle glycogen and exercise performance in man. Acta Physiol Scand [Suppl] 495: 1–35

Jansson E (1980) Diet and muscle metabolism in man with reference to fat and carbohydrate utilization and its regulation. Acta Physiol Scand [Suppl] 487: 5–24

Kim HJ, Kalkhoff RK (1975) Sex steroid influence on triglyceride metabolism. J Clin Invest 56: 888–896

Krotkiewski M, Bjorntorp P (1976) The effect of progesterone and of insulin administration in regional adipose cellularity in the rat. Acta Physiol Scand 96: 122–126

Laubach LL (1976) Comparative muscular strength of men and women: A review of the literature. Aviat Space Environ Med 47: 534–542

Mackie BG, Terjung RL (1983) Influence of training on blood flow to different skeletal muscle fiber types. J Appl Physiol 55: 1072–1078

Miller JM, Coyle EF, Sherman WM, Hagberg JM, Costill DL, Fink WJ, Terblanche SE, Holloszy JO (1983) Effect of glycerol feeding on endurance and metabolism during prolonged exercise in man. Med Sci Sports Exerc 15: 237–242

Nie NH, Hull CH, Jenkins JG, Steinbrenner K, Bent DH (1975) Statistical package for the social sciences (2nd ed). McGraw-Hill, Inc, New York

Powers SK, Riley W, Howley ET (1980) Comparison of fat metabolism between trained men and women during prolonged aerobic work. Res Q Exerc Sport 51: 427–431

Rennie EM, Holloszy JO (1977) Inhibition of glucose uptake and glycogenolysis by availability of oleate in well-oxygenated perfused skeletal muscle. Biochem J 168: 161–170

Rognum TO, Rodahl K, Opstad PK (1982) Regional differences in the lipolytic response of the subcutaneous fat depots to prolonged exercise and severe energy deficiency. Eur J Appl Physiol 49: 401–408

Saltin B, Nazar K, Costill DL, Stein E, Jansson E, Essen B, Gollnick PD (1976) The nature of the training response: Peripheral and central adaptations to one-legged exercise. Acta Physiol Scand 96: 289–305

Siri WE (1956) Gross composition of the body. In: Lawrence JH, Tobias CA (eds) Advances in biological and medical physics, vol 4. Academic Press, New York, pp 239–280

Sjostrom L, Smith U, Krotkiewski M, Bjorntorp P (1972) Cellularity in different regions of adipose tissue in young men and women. Metabolism 21: 1143–1147

Accepted August 10, 1984