Biochem. J. (1989) 261, 219–225 (Printed in Great Britain)

Enzymic and metabolic adaptations in the gastrocnemius, plantaris and soleus muscles of hypocaloric rats

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1. The effect of hypocaloric feeding (25% of normal food intake for 21 days) of rats on the enzymic and metabolic adaptations in the gastrocnemius, plantaris and soleus muscles was studied. 2. In control and hypocaloric rats the muscle relaxation rates at 100 Hz were 35.76 and 11.38% force loss/10 ms respectively. Control rats exhibited enhanced force of muscle contraction as the frequency of stimulation increased from 10 to 100 Hz, with maximum force being at 100 Hz. Hypocaloric rats exhibited a decrease in the increment of force being exerted at high frequencies, with maintenance of force at lower stimulatory frequencies. 3. In muscles of hypocaloric rats, there were significant decreases in the maximal activities of hexokinase (17.6–37.0%), 6-phosphofructokinase (22.7–34.2%), pyruvate kinase (21.2–36.0%), citrate synthase (34.1–41.5%), oxoglutarate dehydrogenase (29.4–52.4%) and 3-hydroxyacyl-CoA dehydrogenase (26.7–32.1%), whereas the activities of glycogen phosphorylase increased (23.8–43.4%) compared with control values. 4. In soleus-muscle strip preparations of hypocaloric rats, there were significant decreases in the rates of lactate production (28.1%) and glucose oxidation (32.6%) compared with control preparations. 5. Mitochondrial preparations from muscles of hypocaloric rats incubated with various substrates exhibited decreased rates of oxygen uptake compared with control preparations. 6. In muscles of hypocaloric rats (gastrocnemius and soleus), there were significant decreases in the concentrations of glycogen ($P < 0.001$) and phosphocreatine ($P < 0.001$) and increases in those of pyruvate ($P < 0.001$), lactate ($P < 0.001$) and ADP ($P < 0.001$), whereas those of ATP and AMP remained unchanged. 7. Calculated [lactate]/[pyruvate] and [ATP]/[ADP] ratios exhibited significant increases ($P < 0.05$) and decreases ($P < 0.05$) in muscles of hypocaloric rats respectively. 8. The results are discussed in relation to the genesis of muscle dysfunction caused by malnutrition.

INTRODUCTION

Muscle wasting and negative nitrogen balance are the consequence of several catabolic conditions leading to malnutrition, which may result in morphological, biochemical and physiological changes in various organs (for review see Jeejeebhoy, 1986). In an attempt to define the adverse effects of malnutrition and the need for nutritional support, changes in body composition (Blackburn *et al.*, 1977), anthropometry (Buzby *et al.*, 1980), hepatic proteins (see Jeejeebhoy, 1981), and delayed cutaneous hypersensitivity (Meakins *et al.*, 1977) in patients with suspected malnutrition were studied. However, several factors (e.g. trauma, sepsis, drugs, endotoxins) may interfere with the predictive value of these indices in the assessment of malnutrition (see Jeejeebhoy, 1981).

One of the major organ systems of the human body is the musculo-skeletal system, and it is therefore important to determine the effect of malnutrition on this system. Previous studies of muscle function have been largely related to the examination of fatigue, myopathy and endocrine-metabolic abnormalities (e.g. Edwards, 1978; Wiles *et al.*, 1977). Work in humans (Lopes *et al.*, 1982; Russell *et al.*, 1983*a*,*b*) and experimental animals (Russell *et al.*, 1984*a*) has indicated that malnutrition induces specific abnormalities of muscle function, such as altered force-frequency curves, slower relaxation of recovery rates and fatigue when stimulated at low frequency (for review see Jeejeebhoy, 1986). Moreover, such muscular abnormalities occurred earlier than those detected by standard methods of nutritional assessment during hypocaloric feeding (Russell *et al.*, 1983*a*), fasting (Russell *et al.*, 1983*a*, 1984*b*) or oral refeeding of anorexic-ill patients (Russell *et al.*, 1983*b*).

In an extension of these studies (for review see Jeejeebhoy, 1986), we have investigated the effect of feeding a hypocaloric diet on the maximal activities of several enzymes in the gastrocnemius, plantaris and soleus muscles of the rat: metabolite concentrations and muscle function were also examined. Thus the main object of the present work is to provide more information on the adaptive changes of skeletal muscles to malnutrition.

MATERIALS AND METHODS

Animals

Male Wistar albino rats (195–205 g and 120–130 g) were supplied by King Fahd Medical Research Center, College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Saudi Arabia. Animals were maintained on a standard laboratory diet [commerical rat

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cubes containing (w/w) approx. 18% protein, 3% fat, 77% carbohydrate and 2% of an inorganic-salt mixture with a vitamin supplement (Grain Silos and Flour Mills Organization, Jeddah, Saudi Arabia)] and water *ad libitum*. Rats were housed individually in wire-bottom metabolic cages in a controlled environment (constant temperature 24 °C, and a light cycle of 12 h on, 12 h off). Rats were randomly allocated into two groups; hypocalorically fed (hereafter referred to as 'hypocaloric' group) and pair-fed controls (hereafter referred to as 'control' group). For 21 days, hypocaloric and control rats were given powdered diets and water. Each hypocaloric rat received a quantity of food equal to about 25% of the intake of the control animal on the previous day. A third group of normally fed animals with similar body weight to that of hypocaloric rats (after 21 days on hypocaloric diet) was included for comparison as a second control group (control B). Body weights and daily food intakes of hypocaloric and control rats were recorded.

Chemicals and enzymes

All chemicals and enzymes were obtained from the same sources as described previously (Ardawi & Newsholme, 1985; Ardawi, 1986).

Measurement of contractile properties of gastrocnemius muscle

Rats were anaesthetized with pentobarbital (45 mg/kg body wt.). One hindlimb of the animal was shaved, and the hindlimb muscles (biceps femoris, gastrocnemius and underlying muscles) were carefully exposed so that the blood supply was maintained. The common tendon for the gastrocnemius–plantaris muscle complex was isolated. The tendon of the gastrocnemius was separated and detached at the calcaneous, tied to a silk thread, and attached to a linear transducer. The proximal sciatic nerve was dissected and sectioned together with all distal branches except for that of the gastrocnemius muscle. All experiments were performed at 37 °C, and the contractile properties of the gastrocnemius muscle were measured as described previously (Russell *et al.* 1984*a*).

Preparation of homogenates and assay of enzyme activities

Animals were killed by cervical dislocation and muscles (gastrocnemius, plantaris and soleus) of hypocaloric and control rats were rapidly dissected. Each muscle was weighed and homogenized in 5–10 vol. of extraction medium by using a Polytron homogenizer (PCU-2, at position 7) for 2 × 20 s at 0–4 °C. The whole homogenate was used for enzyme assays without further treatment, except for the assays of citrate synthase, oxoglutarate dehydrogenase and 3-hydroxyacyl-CoA dehydrogenase, in which muscle homogenates were treated immediately before assay with 0.05% (v/v) Triton X-100. The extraction media for all enzymes studied were as described previously (Ardawi & Newsholme, 1982).

Maximal enzyme activities were measured as described in the cited references: hexokinase (Crabtree & Newsholme, 1972); 6-phosphofructokinase (Opie & Newsholme, 1967); pyruvate kinase (Zammit *et al.*, 1978); glycogen phosphorylase (Bergmeyer, 1974*a*); citrate synthase (Alp *et al.*, 1976); oxoglutarate dehydrogenase (Cooney *et al.*, 1981); and 3-hydroxyacyl-CoA dehydrogenase (Bass *et al.*, 1979). The final volume of assay mixtures in all cases was 1.0 ml. All spectrophotometric measurements were performed in a Gilford recording spectrophotometer (model 260) at 25 °C.

Incubation of muscles

Soleus-muscle strips were prepared as previously described (see Espinal *et al.*, 1983; Crettaz *et al.*, 1980). All muscle incubations were carried out in Krebs–Ringer bicarbonate buffer containing 5.5 mM-glucose, 1.5% (w/v) defatted bovine serum albumin (Chen, 1967) and 0.05 μCi of D-[U-^{14}C]glucose/ml (for futher details, see Dimitriadis *et al.*, 1988).

Preparation of skeletal-muscle mitochondria

Mitochondria from gastrocnemius muscles of hypocaloric and control rats were prepared as described previously (Davies *et al.*, 1981). After the final centrifugation step, mitochondrial pellets were resuspended in a medium containing 225 mM-mannitol, 75 mM-sucrose, 0.5 mM-EDTA and 10 mM-Tris/HCl (pH 7.4) at a protein concentration of 5–10 mg/ml. O_2 consumption was measured polarographically with a Clark-type oxygen electrode attached to a Spectro-plus-D spectrophotometer as described previously (Morgan-Hughes *et al.*, 1982).

Sampling of blood and muscles

Blood samples were obtained from the abdominal aorta of ether-anaesthetized animals and extracted as described previously (Ardawi, 1987). In separate groups of rats, animals were killed by cervical dislocation, and muscles (gastrocnemius and soleus) were rapidly removed and immediately frozen between two aluminium plates pre-cooled in liquid N_2 (Wollenberger *et al.*, 1960) and stored under liquid N_2 until required for extraction. The frozen muscles were powdered in a mortar and pestle under liquid N_2 and were then homogenized in 0.7 M-$HClO_4$ in a Polytron homogenizer [the ratio of tissue to $HClO_4$ was 1:2 (w/v)]. The precipitate was removed by centrifugation at 13500 ***g*** for 5 min, and the supernatant was neutralized with 3.5 M-K_2CO_3 containing 0.5 M-triethanolamine. Precipitated $KClO_4$ was removed by centrifugation at 13500 ***g*** for 5 min. The supernatant was used for assays of metabolites.

Assay of metabolites

Metabolites in neutralized extracts of muscles or plasma were determined spectrophotometrically (with a Beckman DU-6 recording spectrophotometer) as described in the cited references: glucose (Bergmeyer *et al.*, 1974); pyruvate (Czok & Lamprecht, 1974); lactate (Gawehn & Bergmeyer, 1974); acetoacetate (Mellanby & Williamson, 1974); 3-hydroxybutyrate (Williamson & Mellanby, 1974); phosphocreatine (Bergmeyer, 1974*b*); ADP and AMP (Jaworek *et al.*, 1974); and ATP (Lamprecht & Trautschold, 1974). Plasma albumin was measured by an albumin kit (Sigma Chemical Co.)

Glycogen was determined in a $HClO_4$ (8%, w/v) extract of muscles by the method of Keppler & Decker (1974). Protein was determined by the procedure of Lowry *et al.* (1951).

Expression of results

The maximal muscle-relaxation rates at 100 Hz were measured after 1–2 s tetanic stimulation at 100 Hz, and the gradient of relaxation was measured to calculate the

percentage force loss/10 ms as described by Wiles *et al.* (1977). Muscular fatigue was presented as percentage force loss/5s. All maximal enzyme activities are expressed as μmol of substrate utilized/min per g dry wt. The concentrations of plasma or muscle metabolites are expressed as μmol/ml or μmol/g dry wt. respectively. The respiratory activities of isolated mitochondria are expressed as nmol of O_2/min per mg of mitochondrial protein.

Data are presented as means ± S.D. and where appropriate, comparisons between sets of data were made by Student's *t* test.

RESULTS

The body-weight gain of hypocaloric rats was markedly less than that of matched controls, and decreased by about 53% ($P < 0.001$) (Table 1). The wet weights of muscles are shown in Table 1. Muscles isolated from hypocaloric rats showed a decrease in absolute wet weights: gastrocnemius (50%), plantaris (44%) and soleus (39%). However, muscle weights were not changed when expressed as percentage of body weight (Table 1), and there was no marked change in muscle water contents of control or hypocaloric rats (results not shown).

Muscle function

The muscle-relaxation rates at 100 Hz were 35.76 and 11.38% force loss/10 ms in control and hypocaloric rats respectively (Table 2). When muscles were continuously stimulated at high frequency (100 Hz), fatigue was demonstrated by the inability of muscles to maintain contractions. Hypocaloric and control rats showed a loss of force on sustained stimulus at 43.20 and 21.03% force loss/5s respectively (Table 2). Control rats showed a rise in the force of muscle contraction as the frequency of stimulation increased from 10 to 100 Hz, with the maximum force attained at 100 Hz. Hypocaloric rats exhibited a decrease in the increment of force being exerted at high stimulatory frequencies, with the maintenance of force at lower stimulatory frequencies (Table 2).

Enzyme activities

The maximal activities of the enzymes studied are presented in Table 3. The maximal activities of hexokinase, 6-phosphofructokinase, pyruvate kinase, citrate synthase and oxoglutarate dehydrogenase were decreased (17.6–52.4%) in gastrocnemius and plantaris muscles obtained from hypocaloric rats. This suggests decreased capacities of glycolysis and the tricarboxylic acid cycle in this condition. Such a suggestion is confirmed from the rates of glycolysis and glucose oxidation obtained for soleus muscle strip preparations of control and hypo-

Table 1. Body weight, muscle weights, food consumption and concentrations of plasma glucose, lactate, ketone bodies and albumin in control and hypocaloric rats

Plasma glucose, lactate, ketone bodies and albumin were measured as described in the Materials and methods section. Values are presented as means ± S.D. for *n* animals. Control A represents pair-fed adult animals, whereas control B represents animals with similar body weight to hypocaloric rats after 21 days on hypocaloric diet. Statistical significance was determined by using Student's *t* test and is indicated by: * $P < 0.05$; ** $P < 0.001$ (control A versus hypocaloric)

	Control (A)	Hypocaloric	Control (B)
n...	7	7	8
Initial body wt. (g)	201 ± 4.12	196 ± 2.70	–
Final body wt. (g)	279 ± 14.28	131 ± 8.45**	126 ± 5.93
Food consumption (g/day per 100 g body wt.)	7.32 ± 0.30	3.09 ± 0.16**	11.97 ± 1.24
Gastrocnemius wt. (g/100 g body wt.)	0.534 ± 0.017	0.530 ± 0.033	0.408 ± 0.012
Plantaris wt. (g/100 g body wt.)	0.086 ± 0.005	0.107 ± 0.007	0.075 ± 0.006
Soleus wt. (g/100 g body wt.)	0.045 ± 0.006	0.052 ± 0.007	0.039 ± 0.003
Plasma glucose (mM)	7.54 ± 0.86	5.81 ± 1.02*	8.63 ± 0.90
Plasma lactate (mM)	3.04 ± 0.36	3.67 ± 0.92	2.62 ± 0.45
Plasma ketone bodies (mM)	0.29 ± 0.03	0.37 ± 0.05	0.27 ± 0.04
Plasma albumin (g/dl)	3.46 ± 0.27	3.40 ± 0.76	3.05 ± 0.18

Table 2. Contraction, relaxation and fatigue properties of gastrocnemius muscle of control and hypocaloric rats

Muscular functions were determined in control and hypocalorically fed rats as described in the Materials and methods section. For maximal force produced (N) measurements were obtained at 10, 50, 100 and 200 Hz. Values are presented as means ± S.D. for seven animals. Statistical significance was determined by Student's *t* test and is indicated as * $P < 0.001$.

State of animal	Maximum muscle relaxation rate at 100 Hz (% force loss/10 ms)	Fatigue at 100 Hz (% force loss/5 s)	Maximal force (N) at frequency:			
			10 Hz	50 Hz	100 Hz	200 Hz
Control	35.76 ± 2.78	21.03 ± 2.46	2.15 ± 0.06	6.52 ± 0.26	7.37 ± 0.11	7.64 ± 0.12
Hypocaloric	11.38 ± 2.41*	43.20 ± 3.35*	2.04 ± 0.05	4.97 ± 0.13*	4.36 ± 0.09*	5.20 ± 0.11*

Table 3. Maximal enzyme activities in the gastrocnemius, plantaris and soleus muscles of hypocaloric and pair-fed control rats

Muscles were isolated from hypocaloric and pair-fed control rats, and maximal enzyme activities were measured as described in the Materials and methods section. Values are presented as means ± S.D. for seven animals. Statistical significance was determined by using Student's *t* test and is indicated as: * $P < 0.01$; ** $P < 0.005$; *** $P < 0.001$.

Enzyme	State of animals	Enzyme activity in muscle (μmol/min per g dry wt.)		
		Gastrocnemius	Plantaris	Soleus
Hexokinase	Control	3.08 ± 0.54	3.91 ± 0.33	5.16 ± 0.84
	Hypocaloric	2.53 ± 0.32	2.90 ± 0.31*	3.25 ± 0.26***
6-Phosphofructokinase	Control	176 ± 25	176 ± 27	38 ± 5
	Hypocaloric	130 ± 18*	136 ± 10*	25 ± 3***
Pyruvate kinase	Control	1051 ± 108	990 ± 39	189 ± 32
	Hypocaloric	722 ± 163**	780 ± 74*	121 ± 27*
Phosphorylase	Control	147 ± 16	145 ± 11	21 ± 2
	Hypocaloric	204 ± 15***	208 ± 17	26 ± 2***
Citrate synthase	Control	53 ± 4	85 ± 13	96 ± 10
	Hypocaloric	31 ± 3***	56 ± 7***	60 ± 4***
Oxoglutarate dehydrogenase	Control	4.71 ± 0.64	4.86 ± 0.66	5.02 ± 0.77
	Hypocaloric	2.24 ± 0.38	3.43 ± 0.40**	2.67 ± 0.49***
3-Hydroxyacyl-CoA dehydrogenase	Control	28 ± 2	–	45 ± 3
	Hypocaloric	19 ± 1*	–	33 ± 4*

Table 4. Rates of lactate production (glycolysis) and glucose oxidation by soleus muscle strips of control and hypocaloric rats

Soleus-muscle strips were isolated from control and hypocaloric rats and incubated as described in the Materials and methods section. Values are presented as means ± S.D. for 11 preparations. Statistical significance was determined by using Student's *t* test and is indicated as * $P < 0.05$.

State of animal	Rate (μmol/60 min per g)	
	Lactate production	Glucose oxidation
Control	5.66 ± 1.07	0.43 ± 0.08
Hypocaloric	4.07 ± 0.83*	0.29 ± 0.06*

caloric rats (see Table 4). The activity of glycogen phosphorylase was increased in both the gastrocnemius and plantaris muscles of hypocaloric rats (Table 3). In the soleus muscles, however, the changes in the activities of glycolytic and tricarboxylic acid-cycle enzymes studied were similar to those of the gastrocnemius and plantaris muscles of the same animals (Table 3). The activities of glycogen phosphorylase and hexokinase were increased (23.8 %, $P < 0.01$) and decreased (37.0 %, $P < 0.001$) in soleus muscles of hypocaloric rats respectively (Table 3).

Respiratory activities of isolated mitochondria

The respiratory activities of isolated mitochondria from control and hypocaloric rats are presented in Table 5. The rates of O_2 uptake with various substrates tested were diminished in mitochondria isolated from muscles of hypocaloric rats (Table 5).

Table 5. Respiratory activities in mitochondria isolated from gastrocnemius muscles of control and hypocaloric rats

Mitochondria were prepared from the gastrocnemius muscles of control and hypocaloric (both $n = 3$) rats as described in the Materials and methods section. State-3 respiration was produced by the addition of 250 nmol of ADP and is presented as nmol of O_2/min per mg of mitochondrial protein at 25 °C. The respiratory control ratio (R.C.R.) is the relative State-3/State-4 O_2-consumption rate. Results are presented as means ± S.D. for five separate mitochondrial preparations from control and hypocaloric rats. Statistical significance was determined by using Student's *t* test and is indicated as * $P < 0.05$.

Substrate	Respiratory activities			
	Control		Hypocaloric	
	State-3 rate	R.C.R.	State-3 rate	R.C.R.
Pyruvate (5 mM) + L-malate (2.5 mM)	203 ± 32	6.35 ± 0.61	161 ± 17*	6.87 ± 0.66
2-Oxoglutarate (10 mM) + L-malate (2.5 mM)	230 ± 24	6.71 ± 0.30	180 ± 12*	6.46 ± 0.50
DL-Palmitoylcarnitine (40 μM) + L-malate (2.5 mM)	159 ± 26	5.57 ± 0.35	104 ± 13*	5.01 ± 0.72

Table 6. Metabolite concentrations in soleus and gastrocnemius muscles of hypocalorically fed and pair-fed control rats

Muscles were isolated from hypocaloric and pair-fed control rats, and metabolites were determined as described in the Materials and methods section. Values are presented as means ± S.D. for seven animals. Statistical significance was determined by using Student's *t* test and is indicated as: * $P < 0.05$; ** $P < 0.001$.

Muscle	State of animals	Metabolite concn. (μmol/g dry wt.)									
		Phospho-creatine	ATP	ADP	AMP	ATP + ADP + AMP	Glycogen	Pyruvate	Lactate	[ATP]/[ADP]	[Lactate]/[pyruvate]
Soleus	Control	79.85 ± 1.92	19.82 ± 1.35	3.21 ± 0.12	0.19 ± 0.03	23.21 ± 1.25	106.9 ± 6.9	0.31 ± 0.04	2.69 ± 0.12	6.20 ± 0.64	8.87 ± 1.22
	Hypocaloric	50.10 ± 2.46**	19.60 ± 1.07	4.43 ± 0.49**	0.18 ± 0.02	24.21 ± 1.14	67.1 ± 9.4**	0.82 ± 0.06**	14.74 ± 0.89**	4.48 ± 0.56*	17.84 ± 1.76
Gastrocnemius	Control	97.70 ± 3.37	28.72 ± 0.58	3.71 ± 0.11	0.22 ± 0.03	32.65 ± 0.55	143.7 ± 9.2	0.33 ± 0.02	4.90 ± 0.23	7.75 ± 0.35	14.97 ± 1.14
	Hypocaloric	60.22 ± 7.76**	28.18 ± 1.00	4.78 ± 0.59**	0.21 ± 0.03	33.17 ± 1.41	87.7 ± 6.8**	0.93 ± 0.06**	47.02 ± 4.86**	5.96 ± 0.66*	50.77 ± 6.65

Glycogen and other metabolite concentrations

A significant decrease in the concentrations of glycogen and phosphocreatine was observed in muscles (gastrocnemius and soleus) obtained from hypocaloric rats (Table 6). The concentrations of pyruvate, lactate and ADP were markedly increased in muscles of hypocaloric rats, but there was no apparent change in those of ATP, AMP or total adenine nucleotides (Table 6). A greater increase in the concentration of lactate was observed in fast-twitch muscles (8.6-fold) as compared with that of slow-twitch muscles (4.5-fold) isolated from hypocaloric rats (Table 6). Calculated [lactate]/[pyruvate] and [ATP]/[ADP] ratios showed significant increases and decreases in muscles of hypocaloric rats respectively (Table 6).

DISCUSSION

The findings in the present work indicate that, in animals fed on a low-energy diet, muscle fatigue can occur much more quickly than in normal animals. Thus, in both human and animal studies, there is enhanced muscular fatigue in the underfed state, and this effect can be reversed by refeeding (Lopes *et al.*, 1982; Russell *et al.*, 1983*a*, 1984*b*). Although there is loss of muscle protein on the hypocaloric diet, this does not mean that the diet results in a loss of all proteins from the muscle; thus, although the activities of several key enzymes decrease on the hypocaloric diet (Table 3), those of glycogen phosphorylase actually increase (Table 3).

Muscle fatigue is suggested to be associated with several metabolic factors, including availability of energy substrates (e.g. glycogen, phosphocreatine), changes in muscle proton concentration and changes in Ca^{2+} kinetics (Hermansen *et al.*, 1967; Pruett, 1970; Karlsson & Saltin, 1970; Fabiato & Fabiato, 1978; Newsholme & Leech, 1983; Russell *et al.*, 1984*a*). It is likely that some or all of these mechanisms contribute to the decrease in resistance to fatigue caused by a low-energy diet and demonstrated in Table 2. First, there is a marked depletion of muscle phosphocreatine (approx. 30%): it has been shown, for maximum power output in muscle of man, which demands maximum rates of ATP utilization, that this can only be provided by simultaneous conversion of glycogen into lactate plus breakdown of phosphocreatine (Hultman *et al.*, 1981). Hence the decrease in phosphocreatine reported in Table 6 could contribute to the inability of the hypocaloric rats to maintain a high power output. Second, a substantial decrease in muscle glycogen was observed in the present work: such changes have been shown to decrease the maximum power output in man (Jacobs, 1987). In addition, sustained exercise at a reasonably high power output is not possible when glycogen approaches depletion (Hermansen *et al.*, 1967): when glycogen is depleted, the power output can only be maintained at about 50% of the aerobic capacity of the muscle (Newsholme & Leech, 1983). Third, most physical activity involves the production of protons, via the conversion of glycogen into lactate plus protons (not lactic acid; see Newsholme & Leech, 1983). A marked increase in the concentration of protons in muscle will result in fatigue (see Hermansen, 1981; Newsholme & Leech, 1983). In the present work, it was shown that the concentration of lactate increased in the muscles of hypocaloric rats at rest: a 10-fold increase in the white

muscle, which is a dramatic increase (Table 6). Since it has been reported that there is a strong correlation between the concentrations of lactate and protons in muscle (Sahlin *et al.*, 1978), the elevated concentration of lactate observed in the present work (Table 6) may indicate a decrease in pH even in resting muscle.

Exercise under anaerobic conditions rapidly leads to a marked accumulation of protons, and hence fatigue, since there is no fate for glycolytically produced pyruvate except conversion into lactate. However, even under aerobic conditions, lactate, and hence protons, will be produced by muscle as exercise intensity increases, well before the maximum capacity of the tricarboxylic acid cycle has been achieved: the point at which a very large increase in muscle and blood lactate occurs is sometimes known as the anaerobic threshold or point of onset of blood lactate (see Conconi, 1982). A mechanism to account for this dichotomy between glycolysis and the tricarboxylic acid cycle has been put forward by Katz & Sahlin (1987). This depends on the effect of decreased O_2 concentration on the regulation of electron flux through the electron-transfer chain in the mitochondria. This mechanism indicates that a low capacity of the electron transfer chain will favour a low anaerobic threshold, i.e. an increase in the ease of fatigue even at levels of exercise well below the maximum aerobic capacity. The present work has shown that a hypocaloric diet produces a marked decrease in the capacity of the electron-transfer chain (Table 5), and hence this may result in susceptibility to fatigue via proton accumulation even at sub-maximal work intensities. If a hypocaloric diet resulted in a decrease in capillary density, this would result in poor perfusion of muscle and a greater dependence on anaerobic metabolism, and therefore a greater susceptibility to fatigue. Unfortunately, capillary density was not measured in the present work.

This work was supported by grants to M. S. M. A. by King Fahd Medical Research Center, College of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia. We thank Mrs. Nancy Abdulrazzak for her excellent secretarial assistance.

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Received 13 January 1989/23 February 1989; accepted 3 March 1989