Deficiency of skeletal muscle succinate dehydrogenase and aconitase. Pathophysiology of exercise in a novel human muscle oxidative defect.

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Research Article

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Deficiency of Skeletal Muscle Succinate Dehydrogenase and Aconitase

Pathophysiology of Exercise in a Novel Human Muscle Oxidative Defect

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Abstract

We evaluated a 22-yr-old Swedish man with lifelong exercise intolerance marked by premature exertional muscle fatigue, dyspnea, and cardiac palpitations with superimposed episodes lasting days to weeks of increased muscle fatigability and weakness associated with painful muscle swelling and pigmenturia. Cycle exercise testing revealed low maximal oxygen uptake (12 ml/min per kg; healthy sedentary men = 39±5) with exaggerated increases in venous lactate and pyruvate in relation to oxygen uptake ($\dot{V}O_2$) but low lactate/pyruvate ratios in maximal exercise. The severe oxidative limitation was characterized by impaired muscle oxygen extraction indicated by subnormal systemic arteriovenous oxygen difference (a-v O_2 diff) in maximal exercise (patient = 4.0 ml/dl, normal men = 16.7±2.1) despite normal oxygen carrying capacity and Hgb-O_2 P_{50}. In contrast maximal oxygen delivery (cardiac output, $\dot{Q}$) was high compared to sedentary healthy men ($\dot{Q}_{max}$, patient = 303 ml/min per kg, normal men 238±36) and the slope of increase in $\dot{Q}$ relative to $\dot{V}O_2$ (i.e., $\Delta\dot{Q}/\Delta\dot{V}O_2$) from rest to exercise was exaggerated ($\Delta\dot{Q}/\Delta\dot{V}O_2$, patient = 29, normal men = 4.7±0.6) indicating uncoupling of the normal approximately 1:1 relationship between oxygen delivery and utilization in dynamic exercise. Studies of isolated skeletal muscle mitochondria in our patient revealed markedly impaired succinate oxidation with normal glutamate oxidation implying a metabolic defect at the level of complex II of the mitochondrial respiratory chain. A defect in Complex II in skeletal muscle was confirmed by the finding of deficiency of succinate dehydrogenase as determined histochemically and biochemically. Immunoblot analysis showed low amounts of the 30-kD (iron-sulfur) and 13.5-kD proteins with near normal levels of the 70-kD protein of complex II. Deficiency of succinate dehydrogenase was associated with decreased levels of mitochondrial aconitase assessed enzymatically and immunologically whereas activities of other tricarboxylic acid cycle enzymes were increased compared to normal subjects. The exercise findings are consistent with the hypothesis that this defect impairs muscle oxidative metabolism by limiting the rate of NADH production by the tricarboxylic acid cycle. (*J. Clin. Invest.* 1991. 88:1197–1206.) **Key words: succinate dehydrogenase • aconitase • complex II • exercise**

Introduction

Larsson et al. (1) and Linderholm et al. (2) described 14 patients from five families in northern Sweden with a history of lifelong, severe exercise intolerance punctuated by episodes of increased muscle fatigability, weakness, and muscle swelling in association with myoglobinuria. Investigation of seven of these patients using cardiac and peripheral vascular catheterization during cycle exercise revealed low oxidative capacity and low maximal muscle oxygen extraction as indicated by low systemic and femoral arteriovenous oxygen differences. The cardiac output response to exercise was greatly exaggerated or "hyperkinetic" relative to exercise workload and metabolic rate, as evident clinically by marked tachycardia and a sense of cardiac palpitations induced by trivial exercise. The biochemical basis of this disorder has not been fully characterized but Linderholm et al. recently reported finding reduced muscle succinate dehydrogenase in an affected patient (3). We evaluated a 22-yr-old man from a similar coastal area of northern Sweden with an identical clinical history and similar exercise pathophysiology. Biochemical investigation of skeletal muscle revealed impaired oxidation of succinate, deficiency of succinate dehydrogenase activity, and reduced levels of the 30-kD (iron-sulfur) and 13.5-kD proteins of complex II (succinate ubiquinone oxidoreductase, EC 1.3.5.1). This defect in complex II was associated with deficiency of mitochondrial aconitase assessed enzymatically and immunologically and with abnormal deposition of iron in the patient's mitochondria. We postulate that the abnormal metabolic and circulatory responses to exercise in this condition relate to severely impaired muscle oxidative phosphorylation due to a low rate of generation of NADH by the tricarboxylic acid (TCA)[1] cycle attributable to deficiency of succinate dehydrogenase and aconitase activities.

Methods

Clinical history

The patient was a 22-yr-old man with lifelong exercise intolerance marked by premature fatigue. He recalled that he could never walk as long or as far as other children. Exercise was limited by the rapid devel-

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1. *Abbreviations used in this paper:* a-v O_2 diff, arteriovenous oxygen difference; L/P, ratio of venous lactate to venous pyruvate; P_{50}, oxygen tension at which 50% of hemoglobin is saturated with oxygen; $\dot{Q}$, cardiac output; $\Delta\dot{Q}/\Delta\dot{V}O_2$, increase in cardiac output relative to the increase in oxygen uptake from rest to exercise; RER, respiratory exchange; SDH, succinate dehydrogenase; TCA, tricarboxylic acid; $\dot{V}CO_2$, carbon dioxide production; $\dot{V}E$, ventilation; $\dot{V}O_2$, oxygen uptake.

opment of heaviness, weakness, and sometimes aching of active muscles. In addition, trivial exertion provoked a rapid heartbeat and a sense of dyspnea. Exertional fatigue most prominently involved his legs and he felt that his arms were relatively spared. In the legs, the calves were most prone to aching and weakness and usually there was some degree of persistent calf tenderness to palpation. In recent years he has been aware of prominent calf hypertrophy.

The patient also experienced episodes of significant worsening of his muscle symptoms which occurred about two times per year and typically lasted for 1–2 wk. These periods were characterized by increased muscle fatigability and muscle pain which at times progressed to severe generalized muscle aching and swelling associated with frank muscle weakness. During these spells pigmenturia often was noted. Anorexia, prominent thirst, and sleepiness also were present during these "sick" spells. Severe episodes of muscle pain, swelling, and weakness with pigmenturia had occurred only in the last 6–8 yr. The patient was unaware of consistent triggering events such as infections, fasting, or unusual exertion, but believed that exercise after alcohol consumption at times may have played a role. Diet was not otherwise noted to affect exercise tolerance. The patient was a resident of Sundsvall, Sweden, employed as an engineer in a manufacturing firm. A 16-yr-old sister and both parents were well. There was no other known family history. The patient's mother was from Umeå, Sweden, the same area in which the patients described by Larsson, Linderholm, Wahren, and coworkers resided (1, 2, 4).

Physical examination

Cardiopulmonary examination including spirometry, maximal voluntary ventilation, ECG, and two-dimensional echocardiography were normal. Cranial nerves, sensation, reflexes, and coordination were intact. Resting muscle strength was normal. The calf muscles were notably large (calf circumference 41 cm) but muscle bulk was otherwise normal. Normal laboratory tests included: hematocrit, hemoglobin, thyroid function tests, serum electrolytes, calcium, magnesium, iron, glucose, and urinalysis. Serum creatine kinase and aspartate and alanine transaminases were not elevated. 24-h urinary organic acid screening revealed markedly increased lactate excretion. Hemoglobin-O_2 oxygen tension at which 50% of hemoglobin is saturated with oxygen (P_{50}) was normal.

The experimental protocol was approved by the Institutional Review Board for Human Studies of the University of Texas, Southwestern Medical School, and the Ethical Committee of the University of Linköping, Sweden, and informed consent was obtained for all studies.

Evaluation

Cycle exercise. The patient and nine healthy sedentary men of similar age (26±4 yr, mean±SD) and weight (pt = 77 kg, controls = 78±10 kg) exercised using an electrically braked, pedal-rate independent cycle ergometer (Skylab, National Aeronautics and Space Administration, Cape Canaveral, FL). Submaximal and maximal workloads of 5–6 min duration were performed in ascending order of intensity with a 15-min rest period between each workload. Maximal exercise was regarded as the highest workload at which cycling could be continued for 5 min. At rest and in the last minute of each workload, expired air was collected in Douglas bags and cardiac output ($\dot{Q}$) was determined. Ventilation ($\dot{V}E$), oxygen uptake ($\dot{V}O_2$), and carbon dioxide production ($\dot{V}CO_2$) were measured using a Tissot spirometer and mass spectrometer (1100A; Perkin-Elmer Corp., Norwalk, CT). The respiratory exchange ratio (RER, $\dot{V}CO_2/\dot{V}O_2$), $\dot{V}E/\dot{V}O_2$, and $\dot{V}E/\dot{V}CO_2$ were calculated from gas exchange data. Cardiac output was measured noninvasively using acetylene rebreathing (5, 6). For each subject the increase in cardiac output ($\Delta\dot{Q}$, liters/min) from rest to exercise relative to the increase in $\dot{V}O_2$ (liters/min, i.e., $\Delta\dot{Q}/\Delta\dot{V}O_2$) was determined as the slope of $\dot{Q}$ on $\dot{V}O_2$ calculated by linear regression analysis of resting, submaximal, and maximal exercise data. Systemic arteriovenous oxygen difference was calculated as the ratio $\dot{V}O_2$ (ml)/$\dot{Q}$ (dl). Heart rate was determined from continuous electrocardiographic recordings. Venous (antecubital) blood was collected at rest and in the last minute of each workload in the patient and four control subjects. Lactate and pyruvate were determined in perchloric acid extracts of whole blood (7) and the lactate/pyruvate ratio (L/P) was calculated. In an additional experiment separated by an ~ 1-yr interval from other testing, a femoral venous catheter was placed for sampling of femoral venous P_{O_2} and O_2 saturation, lactate, and pyruvate at rest and during maximal cycle exercise. Results were compared to eight healthy Swedish men (8).

Morphological and biochemical evaluation of muscle. An open biopsy of the rectus femoris muscle was performed under local anesthesia for histopathological and biochemical determinations. Portions of the biopsy were frozen in liquid nitrogen–cooled Freon for histochemistry, fixed in 2% glutaraldehyde, 2% paraformaldehyde for electron microscopy and x-ray microanalysis, frozen in liquid nitrogen for subsequent biochemical assays, and used for preparation of fresh skeletal muscle mitochondrial fractions for oxygen electrode and cytochrome determinations (9, 10). The remainder of the mitochondrial fraction was frozen for assay of selected mitochondrial enzymes. Controls for biochemical and histochemical studies were patients undergoing diagnostic muscle biopsy who ultimately were deemed to be free of neuromuscular disease and normal subjects undergoing hip replacement. X-ray microanalysis was performed using an x-ray analyzer (Northern 5500; Tracor Inc., Austin, TX). Samples were osmium fixed but unstained. Comparison was made with normal controls and a patient with morphologic mitochondrial abnormalities of skeletal muscle attributable to a mitochondrial DNA deletion.

Polarographic determinations of oxygen consumption by freshly isolated mitochondria were performed in a thermostatically closed chamber of 0.6 ml vol (Instech Laboratories, Inc., Plymouth Meeting, PA) at 30°C using a Clark-style oxygen electrode and model 5300 amplifier (Yellow Springs Instrument Co., Yellow Springs, OH). The oxygraph medium contained, at pH 7.5, 25 mM Tris, 150 mM sucrose, and 10 mM potassium phosphate. Oxidative rates were determined using 5 mM glutamate (+2.5 mM malate) and 10 mM succinate (+2.5 μM rotenone) and aliquots of ~ 0.3 mg mitochondrial protein. The respiratory control index was calculated as the ratio of state 3 (+ADP, 82 or 164 nmol) relative to state 4 (after ADP depletion) respiration. ADP:O ratios were calculated according to Estabrook (11). Cytochrome difference (reduced-oxidized) spectra were determined at room temperature on a dual beam spectrophotometer (DW-2a; SLM Instruments, Inc., Urbana, IL) with cytochromes reduced using substrate (glutamate) or sodium dithionite (12). Gassing with carbon monoxide revealed no spectral shift indicating that the mitochondrial preparations were free of contaminating hemoglobin and myoglobin. Cytochrome concentrations were calculated using the extinction coefficients employed by Bookelman et al. (12).

Activities of oxidative enzymes were determined spectrophotometrically in extracts of freeze-thawed mitochondria with optimal mitochondrial disruption monitored by levels of citrate synthase activity. NADH-dehydrogenase (NADH ferricyanide reductase, EC 1.6.5.3) (13), rotenone-sensitive NADH-cytochrome c oxidoreductase (EC 1.6.99.3) (14), fumarase (fumarate hydratase, EC 4.2.1.2.) (15), and citrate synthase (EC 4.1.3.7) (16), were assayed according to published methods. Cytochrome c oxidase (ferrocytochrome c: oxygen oxidoreductase, EC 1.9.3.1) was assayed at 25°C based on the method of Wharton and Tzagoloff (17) using as final concentrations 50 mM potassium phosphate, and 9 μM reduced cytochrome c and calculated employing 20 as the extinction coefficient of reduced cytochrome c. Succinate-cytochrome c reductase (succinate-cytochrome c oxidoreductase, EC 1.3.99.1) was modified from Sottocasa (14) using 1.5 μM rotenone, 1.5 μM KCN, and 34 μM oxidized cytochrome c. Aconitase (aconitate hydratase, EC 4.2.1.3) was assayed according to Rose and O'Connell (citrate → isocitrate) (18) using 0.25% taurodeoxycholate, 2 mM NADP, and 2 U/ml of isocitrate dehydrogenase, and according to Robinson et al. (*cis* aconitate → isocitrate) (19). For both assays, activation of aconitase was according to Morrison (20). Isocitrate dehydrogenase (EC 1.1.1.41) was assayed at 30° according to Plaut et al. (21) using 1 mM MgSO4, 1.34 mM ADP, 10 mM isocitrate, 0.66 mM NAD, 4.5 μM rotenone, and 0.9 mM KCN. Malate dehydrogenase (malate:NAD oxidoreductase, EC 1.1.1.37) was assayed at 25°C based on the method of Wilcock (22) using 50 mM 3-(4-Morpholino) pro-

pane sulfonic acid, 0.24 mM NADH, and 0.3 mM oxaloacetate. Succinate dehydrogenase (succinate:FAD oxidoreductase, EC 1.3.99.1) was assayed with phenazine methosulfate and/or dichlorophenol indophenol as artificial electron acceptors (23) with and without succinate dehydrogenase (SDH) inhibitors, malonate (20 mM) and thenoyltrifluoroacetone (300 μM). Mitochondrial samples were incubated with 15 mM succinate for 20 min at 30°C preceding SDH and succinate-cytochrome c reductase assays (23). Mitochondrial protein was determined colorometrically (24). In freeze-thawed crude muscle homogenates, activities of citrate synthase, aconitase, malate dehydrogenase, fumarase, succinate dehydrogenase, NADH dehydrogenase, succinate-cytochrome c reductase, and cytochrome c oxidase were determined spectrophotometrically using the same assays.

Immunoblot analyses were performed on mitochondria isolated from frozen muscle by the method of Moreadith et al. (13). Samples were dissociated in 10% glycerol, 3% SDS, 50 mM Tris, pH 6.5 at 95°C for 1 min and separated by SDS-PAGE, using 15% polyacrylamide and the Laemmli system (25). The separated proteins were transferred to nitrocellulose as described by Rosenbaum et al. (26), with 0.1% SDS added to the transfer buffer. Gels were incubated with antibodies raised to purified beef heart complex II (kindly provided by Dr. Mark Birch-Machin, Division of Clinical Neuroscience, University of Newcastle-upon-Tyne, England) and to beef heart mitochondrial aconitase (kindly provided by Dr. Paul Srere, Department of Biochemistry, Dallas VA Medical Center). Bound antibodies were visualized with biotinylated protein A and an avidin-linked biotinylated alkaline phosphatase reaction (Vectastain ABC Kit; Vector Laboratories, Inc., Burlingame, CA) according to the manufacturer's instructions.

Results

Physiology. Oxygen uptake was normal or slightly high at rest but maximal oxygen uptake (12 ml/min per kg) and work capacity (0.52 W/kg) were markedly low compared to healthy men ($\dot{V}O_{2\,max}$ = 39.2±5.2 ml/min per kg, mean±SD, and 2.95 W/kg, respectively). In cycle exercise, oxygen uptake relative to work was normal to slightly high compared to normal men (Fig. 1).

Cardiac output and heart rate were normal at rest but during exercise increased dramatically relative to oxygen uptake

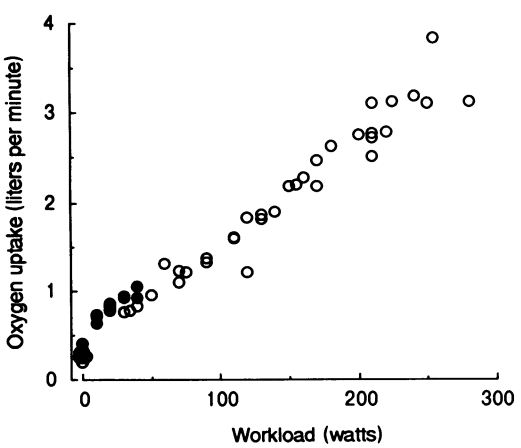

Figure 1. Oxygen uptake ($\dot{V}O_2$) relative to exercise workload as plotted at rest (0 W) and during cycle exercise for normal men (*open circles*) and the patient with SDH/aconitase deficiency (*solid circles*). Normal men (n = 8) were studied at rest and during exercise at three workloads with all testing completed during a single testing session. Results for the patient represent three exercise sessions performed on separate days.

(Table I and Fig. 2 *A*). The slope of increase in cardiac output in relation to oxygen uptake, i.e., $\Delta\dot{Q}/\Delta\dot{V}O_2$, was approximately sixfold greater than normal (patient = 29; controls = 4.7±0.6). Maximal cardiac output was high compared to normal sedentary men (303 ml/min per kg vs. 238±36) consistent with normal cardiac pump performance. In contrast to the threefold increase in systemic arteriovenous oxygen difference (a-v O_2 diff) from 5.1 (rest) to 16.7 ml/dl (maximal exercise) in normal men, a-v O_2 diff actually fell during exercise in the patient (rest = 5.8, max exercise = 4.0) (Table I, Fig. 2 *B*). Correspondingly, in contrast to the normal 50% or greater decline from resting levels of P_{O_2} and oxygen saturation in venous blood from working muscle (27), femoral venous P_{O_2} and oxygen saturation rose in the patient during cycle exercise (femoral venous P_{O_2} rest = 47 mmHg, max exercise = 70; $O_{2\,sat}$ = 80% at rest, 91% in max exercise) (Table II) indicating disruption of the normal coupling between oxygen delivery and utilization in exercise.

Ventilation was normal at rest, but the slope of increase in ventilation was exaggerated relative to $\dot{V}O_2$ (Fig. 3 *A*) during exercise. Ventilation was normal in relation to $\dot{V}CO_2$ (Fig. 3 *B*). Compared to healthy men, ventilation in maximal exercise was subnormal (Table I, Fig. 3) in contrast to the normal to high maximal cardiac output response. RER was normal at rest but rose more steeply than normal relative to workload and oxygen uptake and maximal RER was higher than normal (1.37 vs. 1.15±0.07).

Venous lactate and pyruvate were normal at rest but increased excessively relative to workload and oxygen uptake during cycle exercise (Table I, Fig. 4). Lactate/pyruvate in forearm venous blood during maximal cycle exercise was low in the patient (L/P = 37) compared to normal men (range of maximal L/P = 39–56) (Table I, Fig. 4) and L/P was low relative to the increase in venous lactate at all levels of exercise (Fig. 4 *D*) consistent with attenuated increase in cytoplasmic redox. In femoral venous blood (Table II), L/P during cycle exercise in the patient was virtually unchanged from resting levels, attributable to proportional elevations from rest to maximal exercise in blood pyruvate (0.15 → 0.6 mM) and lactate (2.9 → 9.8 mM). This contrasts with the normal disproportionate increase in lactate with intense exercise in normal subjects attributable to the approximately fivefold increase in muscle cytoplasmic redox (NADH/NAD) which shifts the lactate dehydrogenase equilibrium toward lactate production (27).

Histology. Muscle histology was noteworthy for the absence of staining for succinate dehydrogenase in virtually all muscle fibers though many blood vessels appeared to be normally stained (Fig. 5). NADH tetrazolium reductase and cytochrome c oxidase showed normal staining. No striking subsarcolemmal accumulations indicative of "ragged red" fibers were apparent in the modified Gomori trichrome. Ultrastructurally, glycogen and lipid droplets were abundant in skeletal muscle, in some areas apparently displacing myofibrils. Many mitochondria contained paracrystalline or dark amorphous inclusions (Fig. 6). The dark amorphous mitochondrial inclusions were rich in iron as determined by x-ray analysis. No other areas of the patient's muscle contained iron by x-ray criteria and no iron signals were detected in analysis of muscle from normal subjects or from a patient with a mitochondrial myopathy due to a mitochondrial DNA deletion.

Biochemistry, isolated mitochondria. The rate of oxygen uptake by isolated mitochondria was low with succinate (+ rotenone) as substrate but was normal with glutamate + malate (Table III), consistent with a metabolic block at the level of

Table I. Circulation and Gas Exchange at Rest and in Maximal Exercise

	Work	$\dot{V}E$	$\dot{V}O_2$	$\dot{V}E/\dot{V}O_2$	$\dot{V}E/\dot{V}CO_2$	RER	$\dot{Q}$	HR	Syst a-v O_2 diff	$\Delta\dot{Q}/\Delta\dot{V}O_2$	Venous lactate	Venous pyruvate	L/P
	W	*ml/min per kg*	*ml/min per kg*				*ml/min per kg*	*bpm*	*ml/dl*		*mM*	*mM*	
Rest													
cont mean	—	131	3.6	36.3	43.5	0.77	73	82	5.1	—	0.84	0.06	13
SD		31	0.5	6.9	5.1	0.09	15	9	1.2		0.30	0.01	7
patient	—	146	4.5	32.4	41.9	0.76	78	69	5.8	—	1.06	0.09	13
LL patients	—	131	4.1	32.2			126	91	3.5	—			
SD	—	11	0.5	5.2			41	14	0.8				
Max exercise													
cont mean	229	1750	39.2	44.7	39.0	1.15	238	190	16.7	4.7	9.95	0.22	46
SD	32	317	5.2	6.3	6.6	0.07	36	10	2.1	0.6	2.18	0.05	8
patient	40	630	12.0	52.5	38.1	1.37	303	190	4.0	29.0	9.57	0.26	37
LL patients	23	559	10.4	53.7			269	162	4.0	23.4			
SD	7	119	1.4	8.8			54	9	0.7	4.1			

Data from Larsson, Linderholm (LL) patients (n = 7) were calculated from Table III, Linderholm et al. (2).

complex II. The respiratory control index was low with succinate but normal with glutamate + malate. The ADP:O ratio was low with both substrates compared to the mean of normal subjects (Table III). Consistent with the polarographic findings, antimycin-sensitive succinate-cytochrome c reductase (complex II + III) activity was low while other respiratory chain enzyme activities including NADH dehydrogenase, rotenone-sensitive NADH-cytochrome c reductase (complex I + III), and cytochrome c oxidase (complex IV) levels, and mitochondrial cytochrome concentrations were only slightly reduced (NADH dehydrogenase) or were within the range of normal subjects (Table IV). Consistent with impaired succinate oxidation and deficient succinate-cytochrome c reductase activity, succinate dehydrogenase activity in isolated mitochondria assayed with dichlorophenol indophenol in the presence or absence of phenazine methosulfate was reduced to ~ 20% of control subjects. The percentage of inhibition of SDH activity in the presence of malonate or thenoyltrifluoroacetone was similar in the patient and in control subjects. Mixing experiments using patient and control samples resulted in the arithmetic mean in the assay of SDH implying that the presence of an inhibitor was not responsible for low enzyme activities in the patient. Mitochondrial aconitase activity was similarly reduced to 20–30% of normal control subjects while activities of other TCA cycle enzymes, including citrate synthase, isocitrate dehydrogenase, fumarase and malate dehydrogenase, were approximately twice the level of controls.

Muscle homogenates. In crude muscle homogenates SDH, succinate-cytochrome c reductase, and aconitase activities were low while activities of other TCA cycle or respiratory chain enzymes were within the range of normal (Table IV).

Immunoblots. Western blots of mitochondrial protein revealed deficiency of the 30-kD (iron-sulfur) and 13.5-kD polypeptides of complex II but near normal levels of the 70-kD polypeptide (Fig. 7). Levels of core protein I of complex III and subunit 4 of complex IV visualized on the same gels were normal (Fig. 7). Mitochondrial aconitase immunoblots revealed reduced aconitase cross-reacting material in the patient (data not shown).

Discussion

The patient's symptoms of lifelong exercise intolerance marked by prominent cardiac palpitations and dyspnea with superimposed crises of increased fatigability with muscle swelling, pain, and pigmenturia, and associated with prominent calf hypertrophy, encompass the cardinal clinical features of the disorder described by Larsson, Linderholm, Wahren, and coworkers in patients from the vicinity of Umeå in Northern Sweden (1, 2, 4). The pathophysiology of exercise in our patient also is virtually identical to that defined by Larsson and Linderholm (Tables I and II, Fig. 2). $\dot{V}O_{2\,max}$ in cycle exercise was

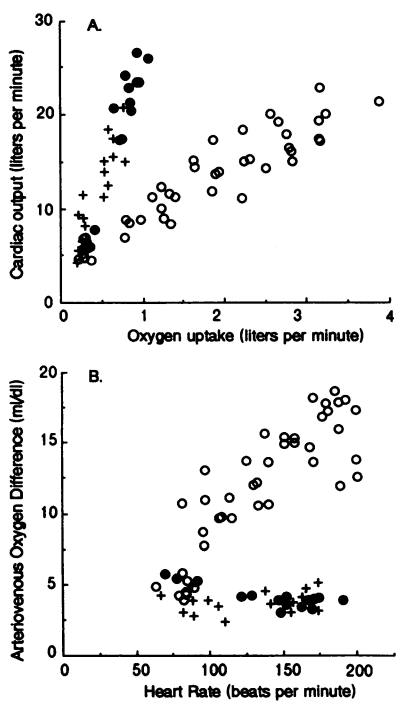

Figure 2. (*A*) Cardiac output relative to oxygen uptake at rest and during exercise. (*B*) Systemic a-v O_2 diff relative to heart rate (as an index of relative workload). Individual values for the patient with SDH/aconitase deficiency were obtained in three separate exercise sessions. The mean slopes, $\Delta\dot{Q}/\Delta\dot{V}O_2$, and mean maximal systemic a-v O_2 diff for normal men and the patient are indicated in Table I. For comparison, data from the patients studied by Linderholm et al. (2) are included (*crosses*). Symbols and exercise conditions for our patient and normal men were as indicated in Fig. 1.

Table II. Oxygen, Metabolites in Femoral Venous Blood in Cycle Exercise

	Work	Heart rate	Po_2	O_2 saturation	Lactate	Pyruvate	L/P
	W	*bpm*	*mmHg*	*%*	*mM*	*mM*	
Rest							
patient	—	80	47	80	2.86	0.15	19
normal men (n = 8)±SD	—	65±7	43±3	79±2	0.48±.1	—	—
LL patients	—	87		78	1.50	0.13	12
Exercise							
patient	30	186	70	91	9.83	0.60	16
normal men (n = 8)±SD	168±29	156±19	20±2	26±6	3.88±2.1	—	—
LL patients	29	170		76	9.50	1.05	9

Data from Larsson, Linderholm (LL) patients were calculated from Table IV, Linderholm et al. (2).

similarly low, apparently limited by a profoundly impaired capacity of skeletal muscle for oxygen extraction. Systemic arteriovenous oxygen difference was remarkably low and femoral venous Po_2 and oxygen saturation actually rose from rest to maximal leg exercise in contrast to the normal marked decline observed in healthy subjects. Maximal oxygen delivery (cardiac output) tended to be high compared to healthy sedentary men. The slope of the relationship between exercise cardiac output and oxygen uptake was increased to a level six times that of normal subjects. Muscle glycogenolysis as indicated by increases in venous lactate and pyruvate was exaggerated relative to work intensity but in maximal exercise the lactate/pyruvate ratio was low.

These findings are consistent with severely impaired skeletal muscle oxidative metabolism. Our study indicates that impaired function of the TCA cycle in skeletal muscle attributable to deficiency of muscle succinate dehydrogenase and mitochondrial aconitase is responsible. SDH activity was low in isolated mitochondria and in crude muscle homogenates. Histochemically determined SDH activity also was markedly reduced in virtually all muscle fibers. The finding of impaired oxidation of succinate but preserved glutamate oxidation in isolated mitochondria is consistent with a block at the level of SDH and indicates that complexes I, III, and IV of the respiratory chain are not rate-limiting to oxidative metabolism. Integrity of complexes I, III, and IV is suggested by findings of normal levels of enzyme activities encompassing these respiratory complexes. In contrast, succinate-cytochrome c reductase (complex II, III) levels in isolated mitochondria and crude muscle homogenates were reduced to ~ 20% of normal compatible quantitatively with the level of muscle SDH deficiency as assessed biochemically and histochemically. Mitochondrial aconitase activity was also reduced to 20–30% of the level of control subjects. Though aconitase does not catalyze a rate-limiting reaction of the TCA cycle, deficiency of mitochondrial aconitase may potentiate the block in TCA cycle flux attributable to SDH deficiency. In contrast to SDH and aconitase, activities of other enzymes of the TCA cycle, including citrate synthase, isocitrate dehydrogenase, fumarase, and malate dehydrogenase were elevated approximately twofold compared to controls. Such an apparently compensatory increase in TCA cycle enzyme activities has been recognized in human and experimental defects impairing mitochondrial respiration (6, 28).

Defects involving respiratory chain complexes I, III, and IV increasingly have been recognized as causes of muscle disease (29) but reports of patients with possible defects involving complex II (30, 31–33) or the TCA cycle (29) are rare. SDH deficiency has only recently been recognized (34–36) and aconitase deficiency has not previously been identified. Rivner and coworkers described a patient with Kearns-Sayre syndrome of ophthalmoplegia, retinal pigmentary degeneration, and heart block (34). In this patient, histochemical staining of muscle for SDH revealed a mixture of intensely and poorly stained muscle fibers, unlike the generalized loss of muscle fiber staining for SDH found in our patient. Desnuelle and coworkers identified a patient with proximal muscle weakness and central nervous system manifestations (35) with multiple respiratory chain defects including deficiency of complex II as determined by enzymatic and immunologic assays and low levels of activity of complexes I and III. Schapira and coworkers described a patient with exercise intolerance and a reversible episode of weak-

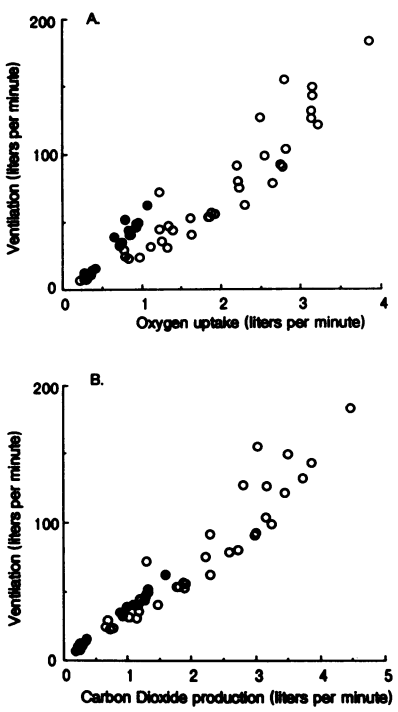

Figure 3. $\dot{V}E$ relative to (*A*) $\dot{V}O_2$ and (*B*) $\dot{V}CO_2$ at rest and during cycle exercise for normal men and the patient with SDH/aconitase deficiency. Symbols and exercise conditions were as indicated in Fig. 1.

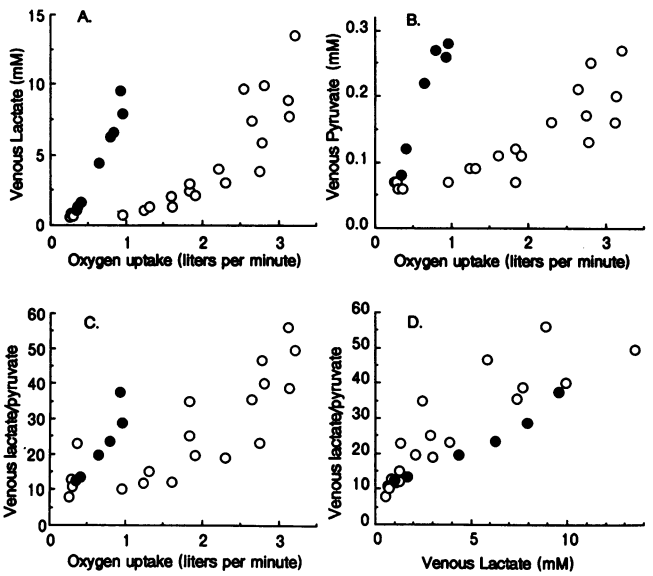

Figure 4. (*A*) Venous lactate, (*B*) venous pyruvate, and (*C*) the ratio of venous lactate to venous pyruvate (L/P) relative to oxygen uptake at rest and during cycle exercise for normal men and the patient with SDH/aconitase deficiency; (*D*) venous L/P relative to venous lactate during cycle exercise. Symbols and exercise conditions as indicated in Fig. 1.

ness in association with elevated serum creatine kinase. SDH deficiency sparing vascular smooth muscle was defined histochemically in association with biochemical defects involving complexes I, III, and IV. Antibody which detected the two large subunits of complex II demonstrated severe deficiency of the 30-kD protein and moderate reduction of the 70-kD protein (36). Recently Linderholm and coworkers have reported a patient clinically identical to ours in whom muscle SDH activity was low compared to literature controls (3).

Complex II consists of two large subunits: a 70-kD flavoprotein and a 30-kD iron-sulfur containing protein which comprise SDH (37, 38), and two smaller subunits believed to be involved in attaching SDH to the inner mitochondrial membrane. Unlike the other respiratory chain complexes, all of the subunits of complex II are coded on the nuclear genome. We are unaware of experimental data indicating the existence of isoenzymes of SDH, but the finding of severe deficiency of skeletal muscle SDH with clinically evident sparing of heart (normal maximal cardiac stroke volume and cardiac output) and central nervous system and histochemically evident sparing of blood vessels in our patient indicates tissue-specific expression of the enzyme defect. The mechanism of muscle SDH deficiency is unclear, but regulatory factors governing the synthesis, mitochondrial import, assembly, or maintenance of the enzyme in the mitochondrial inner membrane could be involved. Both SDH and aconitase contain iron-sulfur centers, so the finding in our patient of low levels of mitochondrial aconitase in addition to selective deficiency of the 30-kD iron-sulfur polypeptide of SDH suggests a common defect related to the iron-sulfur centers present in both enzymes. The presence of electron dense, iron-rich mitochondrial inclusions reinforces the possibility that a defect of iron or sulfur metabolism may be involved. Similar electron dense inclusions also were present in the patients reported by Linderholm et al. (3) and Schapira et al. (36), and in the latter were recognized to contain iron. A

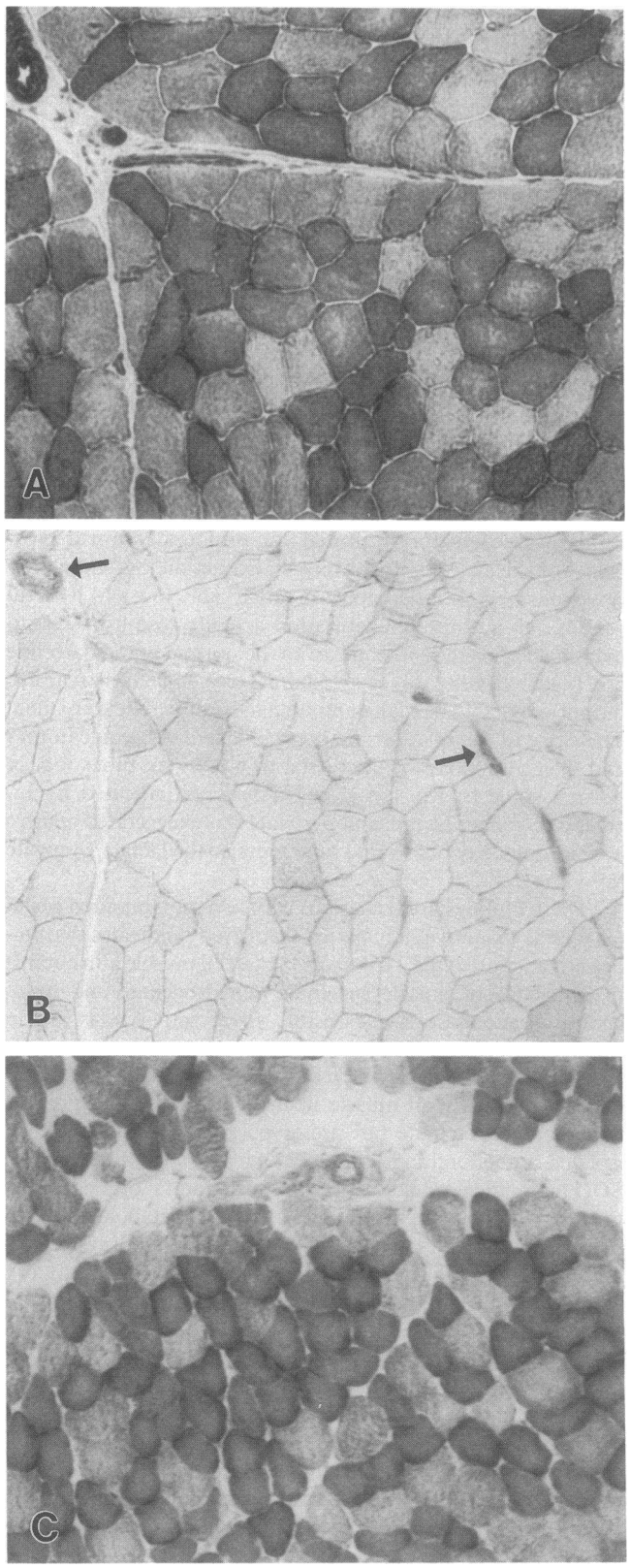

Figure 5. Histochemistry of rectus femoris from the patient (*A, B*) and a control male (*C*). Note the generalized absence of staining for SDH of muscle fibers in the patient's biopsy (*B*). In contrast SDH staining of blood vessels in the patient is normal (*arrows*). NADH tetrazolium reductase staining of the patient's muscle is normal (*A*). Normal staining with SDH is seen in a control subject (*C*). Magnification, 40 for all sections.

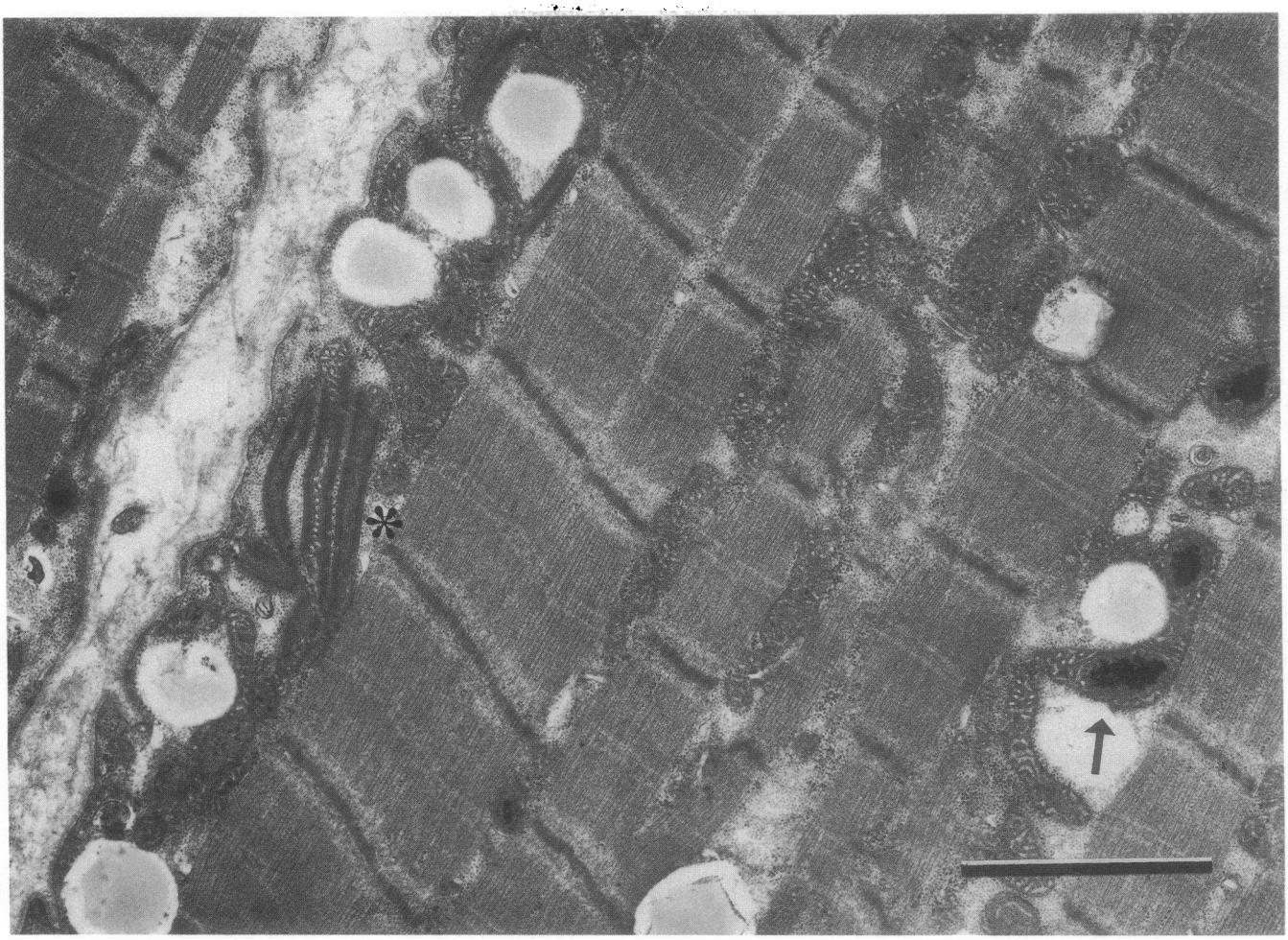

Figure 6. Ultrastructure of the patient's muscle revealing many abnormal mitochondria containing crystalline (*) or electron dense inclusions. The arrow indicates examples of electron dense inclusions which by x-ray analysis contain large amounts of iron. Scale bar is 2 μm.

generalized defect in iron availability is excluded in our patient by normal levels of iron and hemoglobin as well as near normal activities of enzymes encompassing respiratory complexes I and III and normal levels of cytochromes in contrast to the results of experimental iron deficiency (39). While there is no precedent for a tissue-specific defect affecting predominantly the iron-sulfur proteins of the TCA cycle, mitochondrial defects involving predominantly the iron-sulfur proteins in complex I have been reported (13, 40, 41). Although our patient had no known affected family members, apparent autosomal recessive inheritance was documented in previous reports (1, 2), consistent with a nuclear genomic defect.

The syndrome of severe exercise intolerance associated with marked elevations in blood lactate and prominent muscle fatigue, tachycardia, and dyspnea with trivial exercise is similar to that seen in respiratory chain defects involving complexes I, III, and IV (6, 42–46), although the presence of episodic myoglobinuria is atypical of electron transport defects (29, 47). Additional distinct differences in metabolic and physiologic responses to exercise between SDH/aconitase deficiency and intrinsic muscle respiratory chain defects are apparent. The finding of low ratios of blood lactate/pyruvate despite high rates of glycogenolysis as indicated by venous levels of lactate and pyruvate is distinctive and is consistent with a metabolic

Table III. Oxygen Electrode Data

	Succinate/Rotenone			Glutamate/Malate		
	QO_2	RCI	ADP/O	QO_2	RCI	ADP/O
Control (n = 9)						
Mean±SD	191±43	3.28±1.01	1.93±0.24	120±34	5.36±3.09	2.98±0.16
Patient	61	1.63	1.46	98	5.83	2.45

QO_2, natoms O_2/min per mg mitoprotein; RCI, ratio of O_2 uptake in presence relative to absence of ADP; ADP/O = ratio of ADP phosphorylated/nanatom O_2 in state 3 respiration.

Table IV. Mitochondrial Enzymes/Cytochromes

Isolated mitochondria	Patient	Control±SD	Range	*n*
Respiratory chain (nmol/min per mg protein)				
NADH dehydrogenase (Complex I)	2792	3983±995	(2914–5965)	7
NADH-cytochrome c reductase, rotenone sensitive (Complex I + III)	88	131±83	(55–296)	7
Succinate-cytochrome c reductase, antimycin sensitive (Complex II + III)	65	339±115	(157–488)	7
Cytochrome c oxidase (Complex IV)	432	563±229	(223–1025)	7
TCA cycle (nmol/min per mg protein)				
Citrate synthase	1989	819±185	(638–1132)	7
Aconitase (*cis*-aconitate → isocitrate)	113	348±124	(156–467)	5
Aconitase (citrate → isocitrate)	6.9	40.3±20.9	(23–75)	6
NADH-isocitrate dehydrogenase	20.1	11.4±2.6	(8.2–13.8)	6
Succinate dehydrogenase	26	135±27	(105–182)	7
Succinate dehydrogenase (PMS)	66	300±59	(217–411)	7
Fumarase	1099	634±127	(502–852)	7
Malate dehydrogenase	11129	4686±874	(3595–5862)	7
cytochromes (nmol/mg protein)				
b	0.29	0.29±0.08	(0.19–0.42)	7
c + c1	0.47	0.42±0.11	(0.30–0.57)	7
aa3	0.29	0.28±0.06	(0.17–0.35)	7
Muscle homogenates (μmol/min per g tissue)				
Citrate synthase	14.8	10.6±2.0	(7.0–12.7)	7
Aconitase (aconitate → isocitrate)	4.0	5.4±1.6	(3.3–7.6)	7
Aconitase (citrate → isocitrate)	0.16	0.59±0.16	(0.34–0.78)	7
Succinate dehydrogenase (PMS)	1.0	4.2±1.5	(2.6–7.0)	7
Fumarase	9.1	8.9±3.5	(6.2–16.2)	7
Malate dehydrogenase	241	169±55	(114–273)	7
Cytochrome c oxidase	2.1	3.8±2.3	(1.3–7.7)	7
Succinate-cytochrome c reductase (antimycin sensitive)	0.45	1.92±1.1	(.63–4.30)	7

block affecting the TCA cycle. Larsson, Linderholm, and colleagues reported high blood lactate and especially pyruvate with lactate/pyruvate ratios in arterial and femoral or mixed venous blood of only 8–19.5 during intense cycle exercise (heart rates 144–196) compared to normal L/P levels in maximal exercise of ~ 50 (27). We also found a low L/P ratio during maximal cycle exercise in femoral (L/P = 16) and forearm (L/P = 37) venous blood. These findings indicate anomalously low muscle cytoplasmic NADH/NAD in maximal exercise. The normal increase in cytoplasmic NADH in maximal exercise parallels and may be due to the much larger increase in TCA cycle-generated mitochondrial NADH which results in a large concentration gradient against which cytoplasmic NADH must be transported via the malate-aspartate shuttle (48). An expected consequence of deficiency of SDH/aconitase is a diminished maximal rate of NADH production by the TCA cycle thus limiting the normal increase in mitochondrial NADH and facilitating the shuttling of cytoplasmic NADH in maximal exercise such that the cytoplasmic L/P remains low. This formulation implies that the metabolic defect in our patient limits muscle oxidative metabolism primarily by impairing the rate of NADH production by the TCA cycle. In contrast, metabolic defects affecting other respiratory chain complexes limit the rate of oxidation of TCA cycle-generated NADH and are associated with high venous L/P in exercise, compatible with high mitochondrial NADH/NAD (42, 44, 49).

Disruption of the normal relationship between oxygen uptake and oxygen delivery in exercise in SDH/aconitase deficiency is similar to that found in other severe muscle oxidative defects (6, 50). An approximately 1:1 relationship between oxygen delivery ($\dot{Q}$) and utilization ($\dot{V}O_2$) typifies normal dynamic exercise as indicated by the usual ratio of increase in $\dot{Q}$ relative to increased $\dot{V}O_2$. Since oxygenated blood normally contains ~ 200 ml O_2/liter, cardiac output must increase ~ 5 liters to provide 1 liter of increased oxygen uptake, i.e., $\Delta\dot{Q}/\Delta\dot{V}O_2 \approx 5$. This ratio, $\Delta\dot{Q}/\Delta\dot{V}O_2 \approx 5$, is found in normal subjects irrespective of level of physical conditioning and is preserved in patients with neuromuscular disorders without defects in muscle oxidative metabolism (49, 50). A regulatory role for muscle oxidative phosphorylation in the control of $\dot{Q}$ in exercise is suggested by the finding that human and experimental defects in muscle oxidative phosphorylation result in exaggerated increases in $\dot{Q}$ relative to increased $\dot{V}O_2$ and subnormal oxygen extraction from blood (low a-v O_2 diff) (6, 50, 51). Impaired oxidative phosphorylation causes an abnormally steep decline in the muscle phosphorylation potential, [ATP]/[ADP][Pi], or [ATP]/[ADP] relative to muscle work and metabolic rate. A close inverse relationship between exaggerated oxygen transport and the fall in [ATP]/[ADP][Pi] has been documented in experimental and inferred in human oxidative defects (6, 52, 53), supporting the hypothesis that metabolic factors related to the fall in the muscle phosphorylation poten-

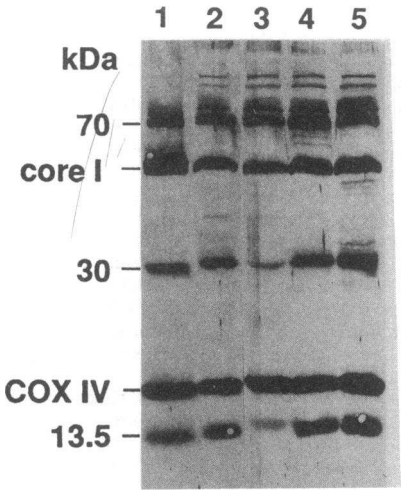

Figure 7. Immunoblot analysis of complex II. (Lane *1*), Beef heart mitochondria (40 μg of protein); (lanes *2* and *4*) mitochondria from frozen muscle of two controls (100 μg of protein); (lane *3*) mitochondria from frozen muscle of patient (100 μg of protein); (lane *5*) mitochondria from fresh muscle of control (100 μg of protein). The blot was incubated first with an antibody to holo complex II and, subsequently, with an antibody to subunit IV of cytochrome oxidase. Numbers on left represent M_r of subunits of complex II; Cox IV indicates subunit IV of cytochrome c oxidase.

tial or to increases in hydrolysis products of ATP participate in regulating oxygen delivery to working muscle (52). Patients with intrinsic respiratory chain defects have $\dot{Q}$ responses that are approximately threefold normal relative to $\dot{V}O_2$ (i.e., $\Delta\dot{Q}/\Delta\dot{V}O_2 \approx 15$) (6, 50). The mean slope of increase in cardiac output in our patient was four to sixfold normal ($\Delta\dot{Q}/\Delta\dot{V}O_2 \approx 20$–30), similar to that found in the patients studied by Larsson and Linderholm (Table I, Fig. 2) suggesting that muscle SDH/aconitase deficiency is exceptional among oxidative defects in disrupting oxygen delivery relative to utilization resulting in remarkably high $\dot{Q}$ with trivial exercise.

Compared to the profoundly exaggerated increase in cardiac output relative to metabolic rate, pulmonary ventilation during exercise in our patient was less strikingly increased relative to $\dot{V}O_2$ and the relationship between $\dot{V}E$ and $\dot{V}CO_2$ was virtually normal. Also, maximal ventilation in exercise in the patient was only ⅓ to ½ that of normal subjects whereas maximal cardiac output was high. Ventilation as measured during high intensity submaximal exercise in the patients of Larsson, Linderholm et al. (Table I) suggest a similar attenuation of maximal ventilation. Though available data is limited, this pattern of ventilation in exercise apparently differs from patients with intrinsic respiratory chain defects in whom exaggerated increases in $\dot{V}E$ relative to $\dot{V}O_2$ and $\dot{V}CO_2$ have been reported (6) and in whom pulmonary ventilation in maximal exercise is comparable to normal subjects (6, 44, 54). The presence of symptoms of ventilatory effort or dyspnea at relatively low levels of ventilation may relate to fatigue of the muscles of ventilation. The possibility that the metabolic defect involves the respiratory muscles is supported by the finding of Larsson et al. (1) that muscles exhibiting fiber necrosis included the diaphragm in a patient who developed fatal myoglobinuria.

Our results define a novel mitochondrial myopathy characterized by a deficiency of skeletal muscle SDH and mitochondrial aconitase and associated with a distinctive clinical syndrome and pattern of exercise pathophysiology. The findings suggest that the abnormal metabolic, circulatory, and ventilatory responses to exercise in this disorder are attributable to impaired function of the TCA cycle in skeletal muscle. These data contribute to the growing body of evidence that severe disorders of skeletal muscle oxidative metabolism dramatically alter physiologic responses to exercise and imply a crucial role for oxidative phosphorylation in active muscle in mediating the normal coupling between oxygen utilization and delivery in exercise.

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