Contraction duration affects metabolic energy cost and fatigue in skeletal muscle

MICHAEL C. HOGAN, ERICA INGHAM, AND S. SADI KURDAK
Division of Physiology, Department of Medicine, University of California, San Diego, La Jolla, California 92093-0623

Hogan, Michael C., Erica Ingham, and S. Sadi KurdaK. Contraction duration affects metabolic energy cost and fatigue in skeletal muscle. Am. J. Physiol. 274 (Endocrinol. Metab. 37): E397–E402, 1998.—It has been suggested that during a skeletal muscle contraction the metabolic energy cost at the onset may be greater than the energy cost related to holding steady-state force. The purpose of the present study was to investigate the effect of contraction duration on the metabolic energy cost and fatigue process in fully perfused contracting muscle in situ. Canine gastrocnemius muscle (n = 6) was isolated, and two contractile periods (3 min of isometric, tetanic contractions with 45-min rest between) were conducted by each muscle in a balanced order design. The two contractile periods had stimulation patterns that resulted in a 1:3 contraction-to-rest ratio, with the difference in the two contractile periods being in the duration of each contraction: short duration 0.25-s stimulation/0.75-s rest vs. long duration 1-s stimulation/3-s rest. These stimulation patterns resulted in the same total time of stimulation, number of stimulation pulses, and total time in contraction for each 3-min period. Muscle O2 uptake, the fall in developed force (fatigue), the O2 cost of developed force, and the estimated total energy cost (ATP utilization) of developed force were significantly greater (P < 0.05) with contractions of short duration. Lactate efflux from the working muscle and muscle lactate concentration were significantly greater with contractions of short duration, such that the calculated energy derived from glycolysis was three times greater in this condition. These results demonstrate that contraction duration can significantly affect both the aerobic and anaerobic metabolic energy cost and fatigue in contracting muscle. In addition, it is likely that the greater rate of fatigue with more rapid contractions was a result of elevated glycolytic production of lactic acid.

oxygen uptake; exercise; acid-base balance; adenosine 5’-triphosphate; lactate; lactic acid; mitochondrial respiration; phosphocreatine; glycolysis

IN MOST CONDITIONS of muscle contractile activity, O2 uptake (VO2) and the total metabolic energy cost of the working muscle are tightly coupled with the developed force of the muscle (for example, see Ref. 17). However, it has been suggested that anywhere from 20 to 50% (2, 3, 8, 19, 20, 27) of the ATP used during a contraction may be used for ion transport related to activation and relaxation, independent of the ATP hydrolysis by the actomyosin adenosinetriphosphatase (ATPase) necessary for force generation. In addition, there is evidence (7, 13) that total ATPase activity in a single contraction is greater at the onset of contraction than the ATPase activity needed to subsequently maintain the steady-state tetanic force that is developed. Although these processes have been studied by thermodynamic methods (see Ref. 19), isolated fibers and muscles in solution (3, 8, 10, 13, 23), there have been few studies investigating these energetics using intact muscle in vivo or in situ. It would follow then that repetitive contractions of short duration may be more energy consuming than contractions of long duration, if the total time of contraction remains constant. Fales et al. (11) and Stainsby and Fales (26) demonstrated in working skeletal muscle in situ that VO2 is lower per stimulus the longer an isometric contraction is maintained. However, only single contractions of different durations were compared, and anaerobic metabolism was not measured. Chastiotis et al. (4) and Bergstrom and Hultman (2) demonstrated in electrically stimulated, totally ischemic contracting human quadriceps a significantly greater energy cost for a series of contractions of short duration compared with long when total contraction times were kept equal. In examining the energetics of terrestrial locomotion, Heglund et al. (14) demonstrated that the metabolic cost of constant speed locomotion increases as animals turn on and off their muscles at a faster rate. These researchers (2, 4, 14) have suggested that short-duration contractions may require higher amounts of total energy due to the high energy cost of ion pumping in each activation-contraction cycle. This extra energy cost may become important to working muscle because a greater energy cost may affect the ability of working muscle to maintain a steady state of force development, as previously shown in ischemic contracting muscle (2, 4).

Although these various studies (2, 4, 11, 14, 26) suggest that contraction duration may influence muscle energetics, there has been no information gathered from normally perfused contracting whole muscle concerning the total metabolic energy requirements of the activation-contraction cycle and the subsequent effects on steady-state muscle function. It was the purpose of the present study to use fully perfused contracting muscle in situ (in which both anaerobic and aerobic energy supply pathways could be assessed) to investigate the effect of two different contraction durations, while keeping the total contraction time equal, on metabolic energy cost and fatigue.

METHODS

Six adult mongrel dogs of either sex with a weight range of 10–18 kg were anesthetized with 30 mg/kg pentobarbital sodium. Maintenance doses were given as required. The dogs were intubated with cuffed endotracheal tubes, and ventilation was maintained with a Harvard 613 ventilator at a rate that achieved normal values of PO2 and PCO2. Esophageal temperature was maintained near 37°C by the use of heating pads. The animals were given heparin at a dosage of 1,500 U/kg after the surgery.
Surgical preparation. The left gastrocnemius-flexor digitorum superficialis muscle complex (for convenience referred to as gastrocnemius) was isolated as described previously (18). Briefly, the muscle was isolated from nearby muscle groups, and all vessels draining into the popliteal vein except for those from the gastrocnemius were ligated to isolate the venous outflow from the gastrocnemius. The arterial circulation to the gastrocnemius was isolated by ligating all vessels from the femoral and popliteal artery that did not enter the gastrocnemius. The left popliteal vein was cannulated, and the venous outflow from the isolated muscle was returned to the animal via a jugular catheter.

The right femoral artery was catheterized for arterial blood sampling. This catheter was connected to the left femoral artery so that the isolated muscle was perfused by blood from this contralateral artery. Perfusion was accomplished either directly from the contralateral (systemic pressure, self-perfused) or via a Sigma motor pump to control flow. A pressure transducer in this line at the head of the muscle constantly monitored perfusion pressure. A carotid artery was also catheterized to monitor systemic blood pressure. The left sciatic nerve, which innervates the gastrocnemius, was doubly ligated and cut between ties. To prevent cooling and drying, all exposed tissues were covered with saline-soaked gauze and with a sheet of Saran. After the muscle was surgically isolated, the Achilles tendon was attached to an isometric myograph to measure force development.

The hindlimb was fixed at the knee and ankle and attached to the myograph with struts to minimize movement. Weights were used at the end of each experiment to calibrate the force myograph. The isometric force developed by each muscle was normalized to the weight of that muscle. Before each contraction period, the resting muscle was passively stretched until a tension setting of 10 g force per gram muscle mass (estimated before the experiment) was recorded. This ensured that the initial tension development was not affected by slippage in the system that might have occurred during the prior contraction period. This resting muscle length was slightly less than the length at which the contractile response was greatest.

Experimental protocol. Isometric muscle contractions (tetani) were elicited by stimulation of the sciatic nerve with square-wave impulses (4–6 V) of 0.2-ms duration at a rate of 40 impulses/s. Each muscle (n = 6) was stimulated for two contraction periods (45-min rest between each period) in a balanced order design. Each of the two contraction periods had stimulation patterns that resulted in a 1:3 contraction-to-relaxation ratio, with the difference in the two contractile periods being in the contraction duration (short duration 0.25-s stimulation/0.75-s rest vs. long duration 1-s stimulation/3-s rest). The total time of stimulation, number of stimulation pulses, and total contraction time were the same for each 3-min contraction period.

Before the first contraction period the blood supply to the isolated muscle was switched from self perfusion to pump perfusion, and enough time was allowed for conditions to stabilize at a blood flow similar to the self-perfused level. All blood flows were measured from the venous outflow using a graduated cylinder to collect the blood for a 20-s time period. Muscle blood flow was set at a level that achieved a perfusion pressure of 120 mmHg during the first contraction period. The same blood flow was used in the subsequent contraction period so that muscle blood flow was matched for both conditions.

Measurements. Arterial blood samples from the arterial line entering the muscle and venous samples from the left popliteal vein as close to the gastrocnemius as possible were drawn anaerobically at the end of each rest period and during the last few seconds of each of the two stimulation periods. These samples were kept on ice for the brief time before measurement. Venous blood flow measurements were made at the same time the blood samples were drawn by timed blood collections into a graduated cylinder. Barbee et al. (1), using muscle contractions similar to those used in this investigation, determined that a near steady-state flow and VO\textsubscript{2} had been achieved by the end of 2 min.

Muscle biopsies were obtained immediately after the blood measurements were taken. These biopsies were obtained using an Alko rapid freezing biopsy drill model 950B, which was able to freeze the sample in liquid N\textsubscript{2} in <1 s. In all, four biopsies were obtained from each muscle (one at rest and one at the end of the stimulation period, for each of the two stimulation periods). The dog gastrocnemius is composed of only high-oxidative fibers (22), with about 50% being slow-twitch, so there was little variability in fiber type distribution throughout the more superficial areas of the muscle where biopsy sampling occurred. The muscle was removed and weighed at the end of each experiment.

Fatigue was measured as the percent decline in developed force from the maximum developed force during each 3-min stimulation period. Blood lactate concentrations [lactate] were determined from the arterial and venous samples using a YSI 23L blood lactate analyzer. Blood PO\textsubscript{2}, PCO\textsubscript{2}, and pH were measured within 5–8 min with a blood gas analyzer (IL model 813) at 37°C, whereas hemoglobin concentration, percent O\textsubscript{2} saturation, percent CO\textsubscript{2} saturation, and O\textsubscript{2} content were measured with an IL 282 CO\textsubscript{2} Oximeter. These instruments were calibrated before each experiment and often throughout each experiment.

Tissue samples (50–70 mg) were extracted once in 0.5 M perchloric acid. Samples were analyzed with a fluorometer for ATP (10), phosphocreatine (PCr) (15), lactate (23), and creatine (28). These values are reported as millimole per kilogram wet weight after being normalized to the total creatine concentration ([creatine]) and creatine plus PCr in that sample. Total [creatine] has been shown to be an excellent normalization parameter for skeletal muscle because of its constancy at different work intensities (5, 7). The mean value of total [creatine] was measured in these experiments to be 20 ± 2 mmol/kg wet wt. This value for total [creatine] is similar to that measured by Piiper et al. (25) of 22 mM in the same muscle group and similar to the value of 21 mM measured by Connett et al. (6) in dog gracilis. Muscle H\textsuperscript{+} concentration ([H\textsuperscript{+}]) was estimated from the measured muscle [lactate], using the relationship developed in previous research in this in situ muscle model when both muscle [lactate] and [H\textsuperscript{+}] were measured (18). Our values of PCr concentration ([PCr]) and ATP concentration ([ATP]) at rest are similar to those measured by Connett and Honig (7) in dog gracilis muscle at rest and reflect values found in highly oxidative muscle (21).

The Fick principle was used to calculate muscle VO\textsubscript{2} and lactate efflux. ATP utilization (energy cost) was calculated from the aerobic changes in VO\textsubscript{2} (assuming a P/O of 3:1) and the anaerobic changes in muscle [ATP], [PCr], [lactate], and lactate efflux (assuming a P/lactate of 1.5:1).

Statistics. Repeated measures analysis of variance was used for the statistical analysis. Values are expressed as means ± SE. In all statistical analyses, the 0.05 level of significance was used.
RESULTS

Mean weight of the gastrocnemius muscles (n = 6) removed after the end of the experiment was 82 ± 3 (SE) g. The principal variables of O₂ transport, gas exchange, and acid-base balance in the blood perfusing the muscles during the two stimulation conditions are presented in Table 1. The arterial Po₂ (and arterial O₂ content) and muscle blood flow were not significantly different between the two conditions, so that O₂ delivery (16.1 ± 0.8 ml·100 g⁻¹·min⁻¹ during short-duration contractions vs. 16.8 ± 0.7 during long) to the working muscle was also no different. Muscle perfusion pressure was also no different between conditions (133 ± 6 mmHg during short-duration contractions vs. 138 ± 7 during long). However, there was a significantly higher O₂ uptake and O₂ extraction ratio (V̇O₂/O₂ delivery) during the short-contraction duration period (see Table 1).

Table 2 lists the biochemical, force, and bioenergetic parameters for the two stimulation paradigms. There was no difference in the initial developed force between the two contraction periods (indicating complete recovery between treatments); however, force development during contractions of short duration fell to a lower level over the 3-min time period, indicating significantly greater fatigue, as shown in Fig. 1. Developed force fell to 70% of initial developed force during the short-duration contraction period vs. 91% during the long-duration contraction period. In addition to the measured changes in developed force, estimates of the tension-time integral were made from the chart recordings. The tension-time integral for a single contraction (averaged from 5 contractions) fell from the initial maximal value of 15.5 ± 0.5 g force·s to 10.9 ± 0.8 during the short-duration contraction period compared with the respective values of 55.0 ± 2.5 and 51.4 ± 1.9 during the long-duration contraction period. These values followed the same patterns as developed force, and the tension-time estimates indicated that the time for activation and relaxation for a single contraction was not different between the two stimulation patterns and that there were no significant changes in the time to achieve maximal force or relaxation rates as the fatigue process occurred in the two stimulation conditions. The measured values of developed force were therefore representative of a comparative index of isometric energy demand.

As indicated in Table 2, intracellular levels of ATP did not change significantly from rest to work and were not different between the two conditions. Muscle [PCr] fell significantly during the short-contraction duration condition but was not significantly different from the values during the other contraction period. Therefore, muscle Pᵢ concentration ([Pᵢ]) (which increases proportionally with decreasing PCr) was also no different at the end of the two contractile periods.

Table 2. Developed force parameters, muscle contents of selected metabolites, and calculated rates of ATP utilization during 2 different stimulation paradigms

<table>
<thead>
<tr>
<th>Contraction Duration for 1 Contraction, s</th>
<th>0.25</th>
<th>1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial developed force, g force/g mass</td>
<td>287 ± 3</td>
<td>290 ± 32</td>
</tr>
<tr>
<td>Final developed force, g force/g mass</td>
<td>201 ± 40*</td>
<td>264 ± 25</td>
</tr>
<tr>
<td>Fatigue index, %</td>
<td>70 ± 2*</td>
<td>91 ± 2</td>
</tr>
<tr>
<td>Muscle [ATP], mmol/kg wet wt</td>
<td>4.0 ± 0.6</td>
<td>4.2 ± 0.4</td>
</tr>
<tr>
<td>Rest Contractions</td>
<td>3.3 ± 0.8</td>
<td>4.4 ± 0.6</td>
</tr>
<tr>
<td>Muscle [PCr], mmol/kg wet wt</td>
<td>12.4 ± 1.2</td>
<td>10.2 ± 1.3</td>
</tr>
<tr>
<td>Rest Contractions</td>
<td>7.9 ± 0.9</td>
<td>8.2 ± 1.2</td>
</tr>
<tr>
<td>Lactate efflux, µmol·100 g⁻¹·min⁻¹</td>
<td>66 ± 13*</td>
<td>26 ± 11</td>
</tr>
<tr>
<td>Muscle [lactate], mmol/kg wet wt</td>
<td>3.4 ± 0.8</td>
<td>2.9 ± 0.7</td>
</tr>
<tr>
<td>Rest Contractions</td>
<td>13.9 ± 1.5*</td>
<td>6.1 ± 1.0</td>
</tr>
<tr>
<td>Muscle [H⁺], nM</td>
<td>101 ± 3</td>
<td>99 ± 3</td>
</tr>
<tr>
<td>Rest Contractions</td>
<td>142 ± 5*</td>
<td>112 ± 4</td>
</tr>
<tr>
<td>V̇O₂/final developed force, µl·g force⁻¹·min⁻¹</td>
<td>0.53 ± 0.09*</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>ATP utilization rate, mmol ATP·100 g wet wt⁻¹·min⁻¹</td>
<td>34.5 ± 5.2*</td>
<td>26.1 ± 4.3</td>
</tr>
<tr>
<td>ATP utilization/final developed force, µmol ATP·g force⁻¹·min⁻¹</td>
<td>0.17 ± 0.03*</td>
<td>0.10 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6. Fatigue index, ratio of final force development/initial force development; lactate efflux, muscle lactate output (flow × arteriovenous lactate difference). Brackets denote concentration. *Significantly different (P < 0.05) from other condition.
Muscle [lactate] increased significantly from rest to work during both contractile conditions and was significantly higher at the end of the short-duration contraction period compared with the long, as was lactate efflux. Calculated intracellular [H$^+$] was also significantly higher during short- duration contractions compared with long.

The O$_2$ cost for the developed force was significantly greater (39%), as shown in Table 2, during the contractions of short duration. The calculated ATP utilization rate was 32% greater (P < 0.05), and the ratio of ATP utilization to developed force was 70% (P < 0.05) greater during the short-duration contractions compared with long.

**DISCUSSION**

This study demonstrated for the first time in fully aerobic, contracting muscle that contractions of short duration resulted in significantly higher V$_{\text{O2}}$, V$_{\text{O2}}$/developed force, calculated rate of ATP utilization, calculated energy cost (ATP utilization/developed force), and fatigue compared with contractions of long duration but with the same total contraction time. The estimated energy cost, from calculations of both aerobic and anaerobic metabolic energy-generating pathways, to produce an equal amount of force per time was 70% greater (P < 0.01) in the contractions of short duration compared with long duration. In addition, contractions of short duration resulted in significantly higher intracellular [lactate], muscle lactate efflux, and a threefold increase in glycolysis that likely influenced the faster rate of fatigue.

Energy cost of contractions. The total energy cost of a muscle contraction reflects the summed ATPase activity of the muscle, which consists of actomyosin, Ca$^{2+}$, and membrane ion transport ATPases. Although it has been well demonstrated that the energy cost of continuous muscular work is generally proportional to the amount of work being done, there have been relatively few studies examining the energy requirements of the nonactomyosin ATPases. Thermodynamic studies (measurements of activation and maintenance heat) have suggested that this energy cost may be 20–50% of the total energy cost (see Ref. 19). Because of the relatively small movement of membrane ions, it is thought that the vast majority of nonactomyosin ATPase activity is from the resequestering of Ca$^{2+}$ by the sarcoplasmic reticulum Ca$^{2+}$ pumps (19). In addition to the energy cost of ion pumping, there is evidence that the actomyosin ATPase rate may be higher at the onset of a single contraction (10, 13), with this ATPase rate falling as the tetanic contractile force is maintained. However, this higher rate at the onset of a contraction (lable heat production) becomes significantly reduced as single contractions follow each other in a continuous pattern (9).

With this in mind, it has been postulated that the frequency of the activation-contraction cycle can strongly influence the total energy requirement of the work being conducted. In comparisons of single contractions of different durations, Fales et al. (11) and Stainsby and Fales (26) demonstrated that O$_2$ consumption is lower per stimulus in long isometric tetanic contractions vs. short. Chastiotis et al. (4) and Bergstrom and Hultman (2) demonstrated in electrically stimulated human quadriceps, contracting with complete blood flow restriction (ischemia), a greater energy cost for contractions of short duration vs. long when the total contraction time was the same. They (2, 4) suggested that this may have been due to the energy cost of activation and relaxation (the main component being Ca$^{2+}$ pumping) being a significant percentage of the total energy cost.

Because normal exercising muscle does not work in an anaerobic environment, the intent of the present study was to examine these questions concerning muscle energetics by using an in situ muscle preparation in which both the anaerobic and aerobic contribution to energy expenditure could be calculated. The fourfold difference between the time the muscle was in the contracted state in the present study (0.25 vs. 1 s) was the same as the fourfold difference used by Bergstrom and Hultman (2). We found that the energy cost (ATP utilization) for the contractions of short duration was significantly greater than that calculated for contractions of long duration, consistent with the findings of Bergstrom and Hultman (2). In addition, our present study shows a significantly greater contribution to ATP production from both aerobic (V$_{\text{O2}}$) and anaerobic (changes in levels of ATP, PCr, and lactate) energy sources in the period of short-duration contractions. The O$_2$ cost (aerobic component of energy cost) for a given amount of force development during the short-duration contractions was 39% greater (Table 2) than during the long-duration contractions, whereas the glycolytic supply of ATP was increased threefold. Our estimates of total energy cost/developed force (aerobic and anaerobic) show that the shortest contraction duration had a 70% greater total energy cost than contractions having a duration four times greater, which is similar to the 62% difference found by Bergstrom and Hultman (2). This suggests that the value calculated (from comparisons with a single continuous contraction) by Bergstrom and Hultman (2) for the energy cost for activation and relaxation (mostly Ca$^{2+}$ cycling) of a 1-s tetanic contraction in human muscle, 37% of the total energy consumption, is similar in aerobically contracting dog muscle. This ~40% energy demand by the sarcoplasmic reticulum Ca$^{2+}$-ATPases is similar to that estimated from thermodynamic studies (3, 20) and is similar to the value recently calculated in skinned slow-twitch muscle fibers of Xenopus laevis by Steinen et al. (27).

Although it is known that the energy cost of the Ca$^{2+}$ cycling represents a significant portion of the total energy cost of muscle contractions and may explain our present results, it is also possible that the higher energy cost related to the time for the cross-bridge cycling to achieve full tetanic force (10, 13) was a contributing factor. During continuous contractions, this higher actomyosin ATPase demand at the onset of a single contraction diminishes (9), but the magnitude of this fall with repeated contractions is unknown.
Although the muscles used in the present study had been stretched to their optimal length for isometric contractions and there was virtually no work of shortening, we cannot exclude that part of the excess energy requirement during contractions of short duration vs. long may have been due to a greater actomyosin ATPase energy demand at the onset of each single contraction.

Fatigue. One important finding from the present study was that the higher energy cost of the short-duration contractile period resulted in a significantly greater fall in developed force (to 70% of initial force development over the 3-min contraction period, Fig. 1) compared with the long-duration contractions (to 91% of initial). This occurred even though V\textsubscript{\text{O}2} was significantly greater in the short-duration contractile period. The factors that reduce force development over time (fatigue) when stimulation patterns remain constant in contracting muscle are complex (30, 32). The relative contribution of these factors to the fatigue process is highly dependent on the conditions of the work (intensity, duration, etc.). Typically, fatigue during relatively high-intensity work results from an imbalance between the processes of ATP demand and ATP production. In the present study, the higher energy cost of the short-duration contractions resulted in both higher rates of oxidative phosphorylation and glycolysis (a threefold increase as estimated by the higher intracellular [lactate] and lactate efflux) in an attempt to accommodate the higher ATP demand. The modulator of the reduced force generation under the conditions of short-duration contractions was likely related to the altered intracellular environment resulting from the higher energy demand. Increased levels of H\textsuperscript{+} and P\textsubscript{i} have been implicated (31, 33) in the etiology of high-intensity contractile fatigue (by interfering with Ca\textsuperscript{2+} release, Ca\textsuperscript{2+} binding, or actomyosin binding; see Refs. 11 and 32). In addition, increases in muscle [lactate] may decrease contractility independent of changes in pH (16). Because muscle [P\textsubscript{i}] was not different during the two contractile conditions of this study, it is likely that the elevated rate of glycolysis and the subsequent increases in muscle [lactate] and [H\textsuperscript{+}] during the short-duration contraction period contributed to the significantly greater reduction in force development.

An interesting possibility to explain the accelerated rate of glycolysis and lactate production in the short-duration contraction period, which occurred even though O\textsubscript{2} consumption was also increased, may have been due to preferential usage of glycolytically produced ATP by the Ca\textsuperscript{2+} pumps. It has been suggested (24, 29, 34) that the ATP hydrolyzed by the sarcoplasmic reticulum ATPase is preferentially rephosphorylated by cytosolic glycolytic enzymes, whereas ATP generated by oxidative phosphorylation may preferentially support actomyosin ATPases. If this compartmentalization of energy supply was a factor in the current study, then ATP produced by glycolysis would be activated (due to extra demand of ion pumping) to a greater extent than oxidative phosphorylation in the short-duration contraction condition. Whereas our data cannot address this issue directly, the threefold increase in glycolytic rate in the short-duration contraction period would support this hypothesis.

In conclusion, this study demonstrated that contraction duration can significantly affect the metabolic energy requirements of contracting muscle and the subsequent maintenance of developed force. Calculations of both aerobic and anaerobic energy costs in fully perfused contracting muscle indicate that when total contraction time was kept the same, contractions of short duration resulted in significantly higher calculated rate of ATP utilization and calculated energy cost (ATP utilization/developed force) compared with contractions of long duration. In addition, contractions of short duration resulted in a threefold increase in glycolysis and greater fatigue that may have been related to the increased intracellular [lactate] and [H\textsuperscript{+}].

This research was supported by the National Institutes of Health Grants AR-40155 and HL-17731. S. S. Kurdak was supported by Çukurova University, Faculty of Medicine, Adana, Turkey.

Address for reprint requests: M. C. Hogan, Dept. of Medicine, 0623-A, Univ. of California, San Diego, 9500 Gilman Dr., La Jolla, CA 92039-0623.

Received 8 September 1997; accepted in final form 11 November 1997.

REFERENCES


