Near infrared monitoring of human skeletal muscle oxygenation during forearm ischemia

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HAMPSON, NEIL B., AND CLAUDE A. PIANTADOSI. *Near infrared monitoring of human skeletal muscle oxygenation during forearm ischemia.* J. Appl. Physiol. 64(6): 2449–2457, 1988.—Changes in tissue oxygenation of forearm muscles were measured by near infrared (NIR) spectrophotometry in 10 healthy adults during tourniquet ischemia and venous outflow restriction. Muscle O_2 stores were depleted rapidly by forearm ischemia manifest by a progressive decrease in tissue oxyhemoglobin and oxymyoglobin over 4–5 min. Muscle ischemia significantly decreased the oxidation level of cytochrome aa_3 to below resting base line after only 1.5 min, and the enzyme became fully reduced after 6.5 min. After 8 min of ischemia, tourniquet release was accompanied by a transient increase in muscle blood volume due to influx of oxyhemoglobin. The cytochrome aa_3 oxidation level increased above resting base line within 1 min after tourniquet release. Transcutaneous Po_2 measurements recorded simultaneously from the same forearm correlated poorly with the kinetics of O_2 availability and cytochrome oxidation in the underlying muscle tissue; this was not unexpected because overlying skin did not contribute significantly to NIR muscle signals. Venous outflow restriction without inflow obstruction increased muscle deoxyhemoglobin and tissue blood volume but did not change muscle O_2 stores or cytochrome aa_3 oxidation level. The ability of the NIR technique to detect dynamic trends in tissue oxygenation reveals that muscle O_2 is rapidly consumed during tourniquet ischemia and rapidly restored by hyperemic responses after brief ischemia.

cytochrome oxidase; spectrophotometry; hemoglobin; myoglobin

SKELETAL MUSCLE is a major target for systemic vasoregulation in various pathophysiologic states (2). O_2 delivery to skeletal muscle is sacrificed early in many conditions where conservation of systemic O_2 delivery is necessary to preserve oxygenation of more hypoxia-sensitive tissues such as brain. Previous studies in animals and humans have utilized a variety of techniques to investigate both the response of skeletal muscle tissue to ischemia and the limits of muscle resistance to ischemic hypoxia (6, 7, 18, 20, 21, 24, 27, 29). These diverse approaches range from measurements of venous and tissue Po_2 to biopsies from ischemic muscle for ultrastructural and chemical analysis. The studies have yielded conflicting results regarding both temporal and quantitative responses of skeletal muscle to ischemia. None of the methods applied to date have proven to be useful for noninvasive sensitive monitoring of skeletal muscle oxygenation in humans.

Near infrared (NIR) multiple wavelength spectrophotometry provides a new technology capable of continuous noninvasive monitoring of changes in tissue O_2 stores and O_2 availability at the mitochondrial level (8, 17, 23). The technique is based on the relative ease with which NIR light passes through biological tissue, being significantly absorbed primarily by the oxy- and deoxy- fractions of -hemoglobin (Hb) and -myoglobin (Mb) and the oxidized copper moiety of cytochrome aa_3, the terminal member of the mitochondrial cytochrome chain. Because these molecular species have different absorption spectra in the NIR region of the spectrum (700–1,000 nm), it is possible to illuminate intact tissue with selected wavelengths of NIR light and calculate changes in the tissue concentration of each molecule based on changes in absorbance at the various wavelengths (17, 23). Changes in muscle O_2 stores are determined by changes in the relative amounts of oxygenated hemoglobin and/or myoglobin within the optical field. Intracellular O_2 availability is monitored by measuring changes in the reduction-oxidation (redox) level of cytochrome aa_3, a parameter dependent on mitochondrial availability of O_2, ADP concentration, and cellular substrate metabolism (14). O_2-dependent redox behavior by cytochrome aa_3 has been demonstrated in several tissues in vivo. Tissue microelectrode measurements of brain Po_2 have been shown to correlate with cytochrome aa_3 redox level (19). NIR parameters measured from intact hindlimb muscle in cats respond rapidly to progressive O_2 deprivation (9), readily detect changes in sympathetic vasomotor output (8), and correlate well with changes in blood flow and O_2 consumption of the hindlimb (9). The NIR optical technique thus noninvasively provides not only valuable trends in mitochondrial O_2 availability but is also sensitive to important changes in overall tissue O_2 stores.

In this study, forearm skeletal muscle of normal human volunteers was monitored with NIR multiwavelength spectrophotometry during pressure cuff-induced forearm ischemia and during cuff occlusion of venous outflow from the forearm. The experiments were designed to *1*) investigate changes in skeletal muscle oxygenation caused by ischemic hypoxia at rest in humans and *2*) evaluate the ability of the NIR technique to noninvasively distinguish changes in tissue oxygenation from changes in tissue blood (hemoglobin) volume caused by changes in venous pressure.

METHODS

NIR multiwavelength spectrophotometry. NIR spectrophotometry was performed using a laser-based instru-

ment constructed in our laboratory. A simplified schematic of the spectrophotometer is provided in Fig. 1. The instrument can monitor two sites simultaneously; therefore it contains two arrays of laser diodes, two sample photodetectors and two reference photodiodes all controlled by a single master timing circuit. The basic principles of NIR multiwavelength spectrophotometry have been described by Jöbsis-VanderVliet (16). The method relies on the ability of NIR light (700–1,000 nm) to pass readily through biological tissues including skin and bone. NIR light is diffusely scattered by the tissues and photons absorbed primarily by oxidized copper atoms of cytochrome aa_3 and the iron-porphyrin complexes of oxy- and deoxyhemoglobin and -myoglobin. Differences in the molecular absorption spectra of these iron and copper centers in the NIR region make it possible to illuminate the tissue with preselected wavelengths of monochromatic light and recover quantities of photons proportional to changes in the relative concentrations of oxidized cytochrome aa_3 in the tissue and deoxyhemoglobin (tHb) and oxyhemoglobin ($tHbO_2$) in the small blood vessels and capillaries. However, the absorption spectra of hemoglobin and myoglobin are too similar to separate the oxygenated and deoxygenated fractions of each in vivo. In other words, changes in O_2 saturation by either of the two pigments cannot be analyzed optically in terms of its relative contribution to a particular change in the optical signal. Therefore the two oxy- and two deoxy-species of each are summed respectively when monitoring skeletal muscle. The term "O_2 store" is used for convenience to describe changes in the $tHbO_2$ and oxymyoglobin (MbO_2) signal. If the concentration of myoglobin in the region of monitoring is constant over the time of the experiment, adding the signals (tHb + Mb) + ($tHbO_2$ + MbO_2) cancels changes in myoglobin saturation, allowing measurement of relative changes in tissue blood (hemoglobin) volume (tBV). Linearity with respect to changes in concentration results from expressing the data logarithmically in accordance with the Beer-Lambert relation.

Multiwavelength spectrophotometry of intact skeletal muscle was performed using the reflectance mode (13). Pulses of NIR light from a bank of four gallium aluminum arsenide laser diodes (M/A-Com, Laser Diode, New Brunswick, NJ) were presented to the tissue sites by means of a fiberoptic bundle coupled to the intact skin with optical gel (Math Associates, Westbury, NY). The laser diodes provided incident light of 1.5 nm spectral bandwidth at the following wavelengths: 775, 810, 870, and 904 nm. With the use of time-domain multiplexing, each laser diode was pulsed for 200 ns in sequence at a frequency of 1 kHz. NIR light from the tissue was collected by a second fiberoptic bundle located 4–5 cm away from the point of entry and measured by a side window photomultiplier (Hamamatsu R936). The photocurrents from the sample (S) and reference (R) (TRW-Optron photodiode, model OP 915) photodetectors were integrated, demultiplexed, and converted to log ratios using a log ratio amplifier. The four log S/R voltages from each side of the instrument were fed into a single microprocessor-based data acquisition system (Dianachart, Rockaway, NJ) where algorithms were applied to them to deconvolute the absorption spectra. The metabolic signals were then recorded on an Epson printer (model FX 100). The sensitivity of this NIR instrument varies as a function of the intensity of the NIR signal. With an attenuation factor of 8 optical density (OD) units, the noise level is ~0.15% for the hemoglobin signals and 1.0% for the cytochrome aa_3 signal. At 10 OD, the noise level increases to 0.4% for the hemoglobin signals and

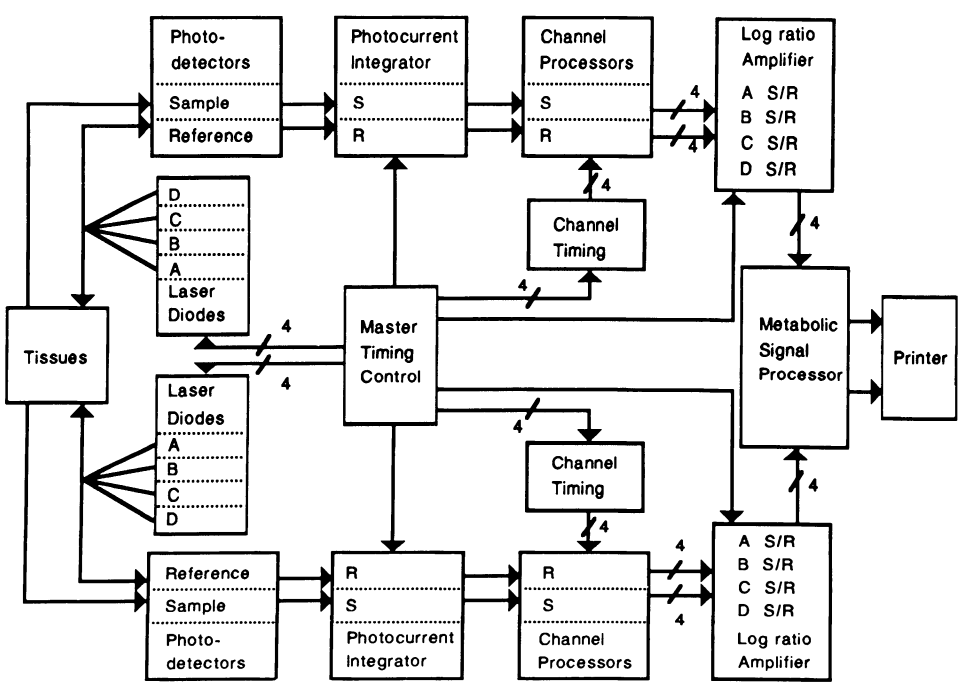

FIG. 1. Schematic diagram of 4 wavelength near infrared spectrophotometer used for optical monitoring in these studies.

2.5% for the cytochrome aa_3 signal. The instrument drift is <1%/h.

The three algorithms were of the form

$$\Delta[M] = a(\Delta OD_{775}) + b(\Delta OD_{810}) + c(\Delta OD_{870}) + d(\Delta OD_{904}),$$

where $\Delta[M]$ is the change in concentration of the molecule to be measured, *a–d* are weighting coefficients, and ΔOD is the total change in optical density at that wavelength. The weighting coefficients have been derived from animal data, where the shape and relative intensities of each of the three absorption spectra can be obtained independently with the same optical geometry and pathlength. A fourth wavelength (at 870 nm) is used to correct for a small shoulder of uncertain origin often found at a longer wavelength than the cytochrome copper peak at 830 nm (17). The use of a fourth wavelength also produces more even spacing of the measuring wavelengths and thus provides improved correction for wavelength-dependent light scattering. In the solution for each algorithm, the term variation in density (vd) has been substituted for OD because different weighting coefficients are used at each measuring wavelength. Variations in density are proportional to concentration, although absolute concentrations cannot be measured because of unquantified biophysical variables such as optical pathlength. However, changes in relative concentration caused by physiological responses can be compared between experiments in terms of vd with the caveat that minor differences in geometry between source and detector fiberbundles will contribute to the statistical variability of the data. One vd unit corresponds to a 10-fold change in a signal computed through its algorithm.

The NIR signals may also be analyzed by dividing the signal change produced with a specific metabolic event by the total NIR signal for that experiment. This approach normalizes all data to fractions or percentages of a total labile signal (TLS) and is useful to compare the optical responses from different experiments. In these experiments, TLS has been defined as the signal difference between the base-line cytochrome aa_3 oxidation level at normoxic control (100% oxidation) and maximum cytochrome aa_3 reduction during ischemia (0% oxidation). If the TLS cannot be determined, as may happen for the hemoglobin or tBV signals, the optical data are reported only in relative optical units (vd).

Experimental protocols. Ten healthy adult nonsmokers (8 male, 2 female) from 26 to 36 yr of age participated in the experiments. All measurements were made in awake subjects seated in a comfortable position after obtaining informed consent to a protocol approved by the Institutional Review Board of Duke University Medical Center. Arterial hemoglobin saturation was monitored with the probe of a Nellcor N-100 pulse oximeter attached to the tip of the right fourth finger. An adult probe from a transcutaneous O_2 monitor (Novametrix TCOM 818) was applied to the skin of the inner left forearm to measure transcutaneous O_2 tension (Ptc_{O_2}) after performing appropriate instrument calibration and skin site preparation. Resting arterial blood pressure was recorded from the left arm and the pressure cuff left loosely wrapped around the upper arm.

In the first protocol, designated forearm ischemia, one pair of NIR monitoring optrodes was applied to the skin 4–5 cm apart over the proximal left forearm musculature (primarily brachioradialis). Once stable optical base lines, resting arterial hemoglobin saturation, and Ptc_{O_2} were established, occlusion of the brachial artery was performed by inflating the pressure cuff to 50 mmHg above the systolic arterial pressure. This inflation pressure was maintained for 8 min and then released. These time and pressure parameters were chosen after preliminary experiments demonstrated that neither longer ischemia nor greater cuff pressure resulted in greater changes in TLS. Digital optical data, Ptc_{O_2} from the ischemic forearm, and arterial hemoglobin saturation monitored from the control forearm were recorded every 30 s during the 8 min of ischemia and a subsequent 7-min recovery period. Control NIR signals from muscle were obtained in 5 of the 10 subjects simultaneously by applying the second set of optrodes to a similar site on the contralateral forearm and recording the optical signals during the same 15-min period. Control NIR signals from skin were obtained from the remaining five subjects by positioning the second set of optrodes to transilluminate only a doublefold thickness of skin on the ischemic forearm.

A second protocol, designated venous outflow restriction, was used to measure the NIR responses of skeletal muscle during impaired venous return from the forearm in 5 of the 10 subjects. After establishing stable optical base lines, the pressure cuff was inflated to a pressure of 50 mmHg for 5 min and then released. This inflation pressure is well below that known to interfere with forearm arterial inflow (22). Digital optical data were recorded every 30 s during the 5 min of cuff inflation and a subsequent 5-min recovery period.

Data analysis. Summed data at each time point of the experimental protocols were expressed as means ± SE. Statistical evaluation of changes from a stable optical base line was done by performing one-way analysis of variance (ANOVA) on successive postocclusion optical values recorded at 30-s intervals. Paired *t* testing of pre- vs. postocclusion NIR values was used to determine that the optical signals had returned to the original values. A *P* value of <0.05 was accepted as significant.

RESULTS

For the 10 subjects, resting arterial blood pressure was 118 ± 5 mmHg systolic and 75 ± 3 mmHg diastolic, baseline arterial hemoglobin saturation by pulse oximetry was 97 ± 1%, and mean forearm Ptc_{O_2} was 79 ± 3 mmHg before ischemia. Figure 2 shows representative NIR optical tracings from the forearm of a single subject during the ischemia protocol. Within 1 min after beginning the ischemia, tHb + Mb began to increase and $tHbO_2$ + MbO_2 to decrease in a reciprocal fashion. Approximately 6 min after onset of ischemia, tissue O_2 stores were depleted and the two signals had stabilized at new steady states. The desaturation of muscle tissue hemoglobin and myoglobin was accompanied by a progressive decrease in the redox level of muscle cytochrome aa_3, con-

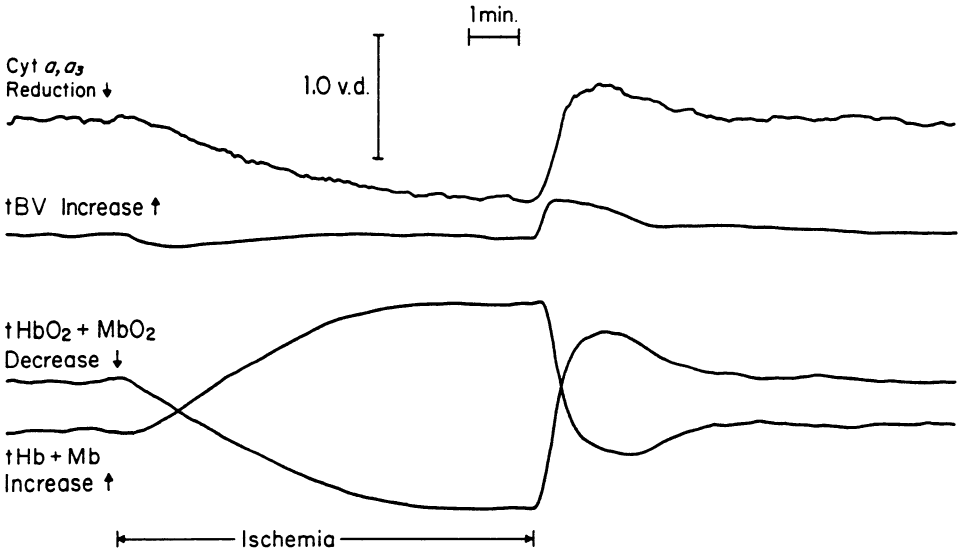

FIG. 2. Near infrared optical responses from forearm skeletal muscle of a representative subject during 8 min of tourniquet ischemia and subsequent recovery. Ischemia caused deoxygenation of tissue hemoglobin and myoglobin, associated with a decrease in muscle cytochrome aa_3 redox level. Arterial hyperemia followed tourniquet release as tissue blood volume transiently increased above base line, and both tissue oxyhemoglobin + oxymyoglobin and cytochrome aa_3 redox levels surpassed base-line values. Cyt aa_3, cytochrome aa_3; tBV, tissue blood volume; $tHbO_2$ + MbO_2, tissue oxyhemoglobin plus oxymyoglobin; tHb + Mb, tissue deoxyhemoglobin plus deoxymyoglobin; vd, variation in density.

sistent with intracellular O_2 depletion. Cytochrome aa_3 reached its maximum reduction level after ~6 min of ischemia in this subject. tBV was relatively constant during the ischemic period but increased above base line within 30 s after release of the pressure. The postischemic hyperemia was arterial in nature because the increase in $tHbO_2$ + MbO_2 exceeded the decrease in tHb + Mb during recovery from ischemia. This hyperemia was accompanied by an increase in the oxidation level of muscle cytochrome aa_3 above its previous resting state. All signals returned to or near the preischemia base line by the end of the 7-min recovery period.

Optical data from the 10 muscle ischemia protocols are summarized in Fig. 3. Muscle tHb + Mb and $tHbO_2$ + MbO_2 were significantly changed from base line within 1.0 min after onset of tourniquet ischemia ($P < 0.005$) (Fig. 3, *A* and *B*), but muscle tBV did not change significantly during the 8-min ischemic period (Fig. 3*C*). O_2 stores, represented by the changes in tissue hemoglobin and myoglobin oxygenation, were depleted during the initial 4–5 min of ischemia, after which these NIR signals became stable (Fig. 3, *A* and *B*). Cytochrome aa_3 oxidation level began to decrease within 1.5 min after onset of ischemia and became maximally reduced after 6.5 min (Fig. 3*D*). As the representative experiment in Fig. 2 shows, rapid signal recovery followed tourniquet release as both $tHbO_2$ + MbO_2 and cytochrome aa_3 surpassed base-line values within 1.0 min ($P < 0.05$). The maximum increase in muscle tBV occurred 1.0 min into recovery, and muscle cytochrome aa_3 was maximally reoxidized 1.5 min after tourniquet release, reaching a redox level 55 ± 12% above resting base line ($P < 0.01$). All of the optical signals subsequently returned toward base line, with no measurement significantly different from its resting value after 4.0 min of recovery from ischemia.

The control NIR parameters monitored simultaneously during contralateral forearm ischemia are displayed in Figs. 4 and 5. Figure 4 shows summarized optical data from the control forearm muscle of five subjects. No significant changes occurred in any of the NIR signals, and there was no change in arterial hemoglobin saturation measured by the pulse oximeter from the control limb of all subjects (not shown). Figure 5 summarizes optical data obtained from monitoring a doublefold thickness of skin on the ischemic forearm in the five remaining subjects. Again, no significant changes were seen in any optical signals from skin during either ischemia or recovery.

Figure 6 displays the mean Ptc_{O_2} as a function of time measured from the ischemic forearms of the 10 subjects. The Ptc_{O_2} significantly fell below its base line within 0.5 min after beginning ischemia ($P < 0.001$) and reached a nadir after only 2.0 min of ischemia. After relief of ischemia, Ptc_{O_2} recovered more slowly than the NIR signals, failed to show a hyperemic overshoot, and remained below base line throughout the recovery period ($P < 0.05$).

Figure 7 displays optical data tracings from a typical subject during venous outflow restriction of the forearm. The tHb + Mb and tBV signals progressively increased for ~3 min after inflating the pressure cuff to 50 mmHg. When the cuff was released after 5 min of venous occlusion, both signals rapidly returned to base-line levels. No appreciable change occurred in $tHbO_2$ + MbO_2 or cytochrome aa_3 signals during either the period of occlusion or subsequent recovery. Summarized data from venous restriction in the five subjects are shown in Fig. 8. Statistically significant increases in muscle tBV and tHb + Mb occurred within 1.5 min after increasing forearm venous pressure ($P < 0.05$). These two NIR signals reached new steady-state levels during the 5 min of venous occlusion and both returned to base line within 1.0 min after cuff deflation. No significant changes in either $tHbO_2$ + MbO_2 or cytochrome aa_3 redox level occurred during the 5 min of occlusion or the subsequent recovery period.

DISCUSSION

NIR multiwavelength spectrophotometry has been used previously to monitor changes in cerebral and skeletal muscle oxygenation in a variety of animal experiments (8, 9, 23). The method has proved to be a valid

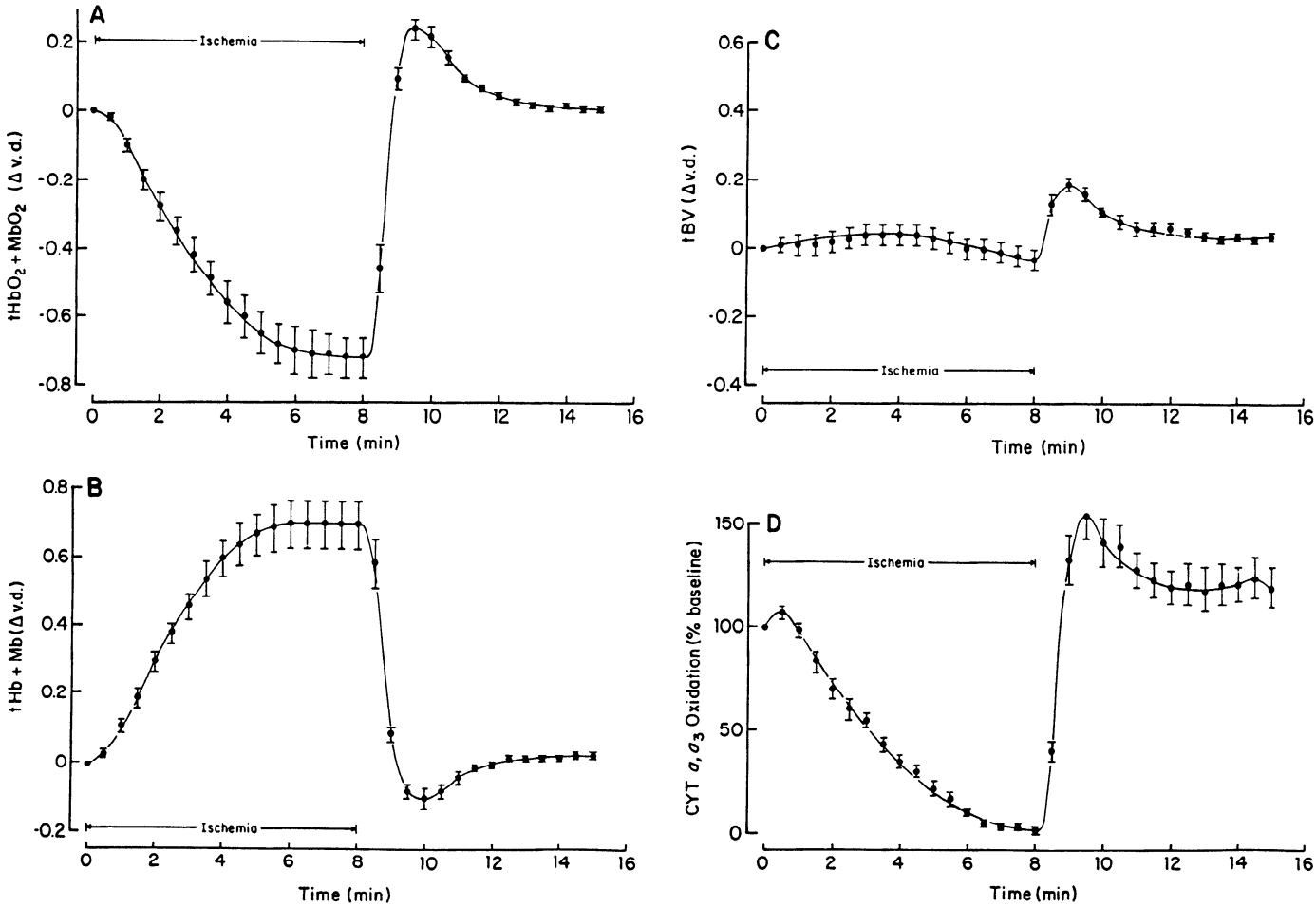

FIG. 3. Summarized muscle optical responses from 10 subjects during 8 min of tourniquet ischemia and subsequent recovery. Muscle O_2 stores are depleted after 6 min of ischemia, accompanied by a decrease in cytochrome aa_3 redox level. Transient arterial hyperemia follows tourniquet release and is associated with oxidation of muscle cytochrome aa_3 to 155% of its base-line value. Data are means ± SE. For definition of abbreviations, see Fig. 2 legend.

noninvasive trend monitor useful for investigating the physiology of O_2 transport to tissue. NIR spectrophotometry is sensitive to changes in tissue oxygenation both at the level of the small blood vessels and capillaries and at the intracellular sites of O_2 uptake. The former are evaluated by assessing tissue blood (hemoglobin) volume and oxygenation and the latter by changes in myoglobin saturation and changes in the redox level of mitochondrial cytochrome aa_3, estimated by Jöbsis (12) to be the site of 90% of O_2 consumption in the body.

The present study represents the first experimental application of NIR spectrophotometry to investigate changes in skeletal muscle oxygenation in healthy human adults. The new data indicate that sudden forearm ischemia is accompanied by very rapid depletion of muscle tissue O_2 stores that becomes maximal after only 4–6 min of arterial occlusion. On reperfusion of the extremity, forearm muscle hemoglobin, myoglobin, and cytochrome aa_3 recover within 1 min, surpassing base-line levels during a transient period of hyperemia. These changes are indeed attributable to muscle ischemia because no changes occurred in either simultaneously monitored control muscle or the skin overlying the ischemic arm. The latter observation is consistent with previous findings utilizing the NIR technique (8, 23) and is probably a result of both the small volume of skin between the optrodes relative to muscle and the low concentration of cytochrome aa_3 in skin (8, 16).

The rapid decrease in skeletal muscle cytochrome aa_3 redox level observed in these experiments has been interpreted to be due to a lack of available mitochondrial O_2 for electron transfer. It has long been known that the Michaelis-Menten constant (K_m) for O_2 of isolated mitochondria is <0.1 mmHg (4). Since tissue P_{O_2} in primate skeletal muscle is probably in the range of 20–40 mmHg (21) and the O_2 consumption of resting skeletal muscle is low (3), the rapid fall in cytochrome aa_3 redox level could be due to a decrease in mean mitochondrial P_{O_2} <0.1 mmHg only if substantial gradients of O_2 concentration were present throughout the resting muscle. Although Gayeski et al. (5) suggest that O_2 gradients in red muscles are not steep, there may be a substantial resistance to O_2 diffusion across the muscle capillary (10). In addition, the effective diffusion distance from capillary to mitochondria may be greatest at rest because the number of open capillaries in resting muscle is considerably less than that in exercising muscle (11). A greater reduction level of cytochrome aa_3 at physiological P_{O_2}

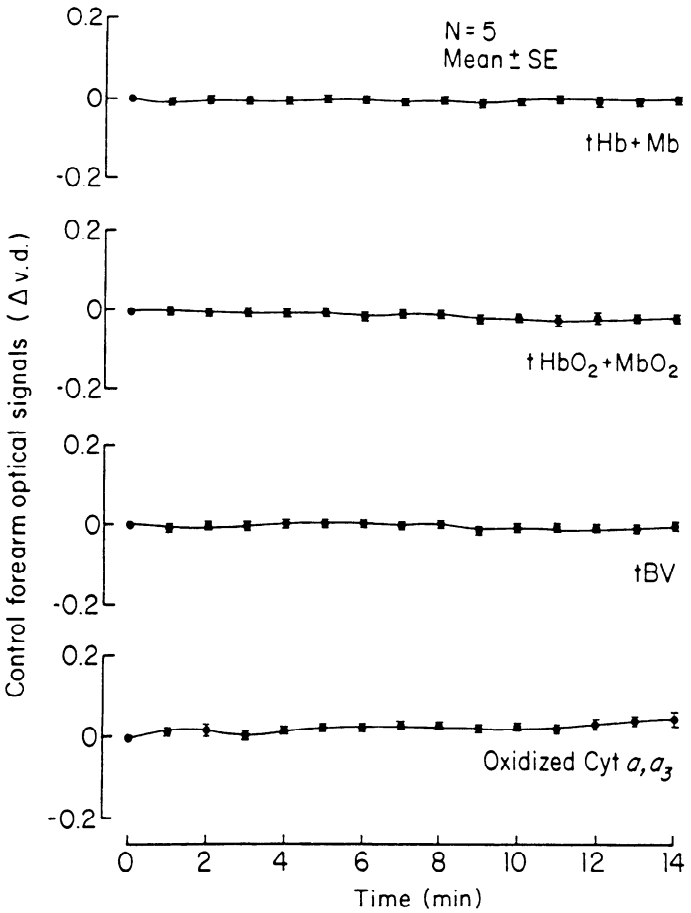

FIG. 4. Summarized optical data from control forearm muscle of 5 subjects, obtained during contralateral forearm ischemia. No significant changes occurred in any of NIR signals. Data are means ± SE. For definition of abbreviations, see Fig. 2 legend.

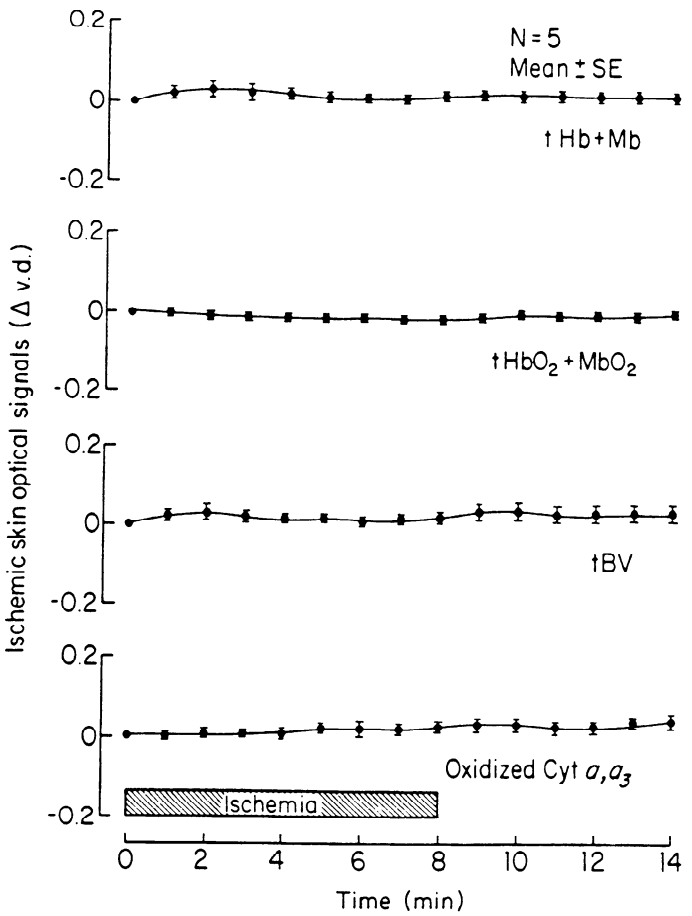

FIG. 5. Summarized optical data obtained from monitoring a doublefold thickness of skin on ischemic forearm in 5 subjects. No significant changes were seen in any optical signals during either ischemia or recovery. Data are means ± SE. For definition of abbreviations, see Fig. 2 legend.

could also be explained if the K_m for O_2 of cytochrome aa_3 in vivo is greater than that for isolated mitochondria in vitro (15). Either of the latter two concepts is supported by our observation that muscle cytochrome aa_3 redox level was able to increase above base line during the arterial hyperemia after pressure cuff release. If the K_m for O_2 of cytochrome aa_3 in vivo is as low as has been reported in vitro, the enzyme should be near totally oxidized in resting muscle. Since the mean cytochrome aa_3 redox level in these experiments increased >50% above base line during recovery from ischemia, the pool of cytochrome aa_3 in the monitored muscle is at least 33% reduced at rest. This most likely indicates that up to one-third of the muscle capillaries, and therefore many perfusion-metabolism units, are closed at rest. The postocclusion findings also may indicate that O_2 gradients are steep or that the K_m for O_2 of the resting oxidase is greater than reported from in vitro data. High resting reduction levels of cytochrome aa_3 in vivo have been reported previously in other intact tissues (15, 25).

Many experimental approaches have been devised to evaluate the effects of ischemia on skeletal muscle energy metabolism because histologic changes in muscle appear to develop quite slowly after the onset of tourniquet ischemia. Józsa et al. (18) performed electron microscopy on human muscle biopsies and found only depletion of some glycogen granules after up to 2 h of tourniquet

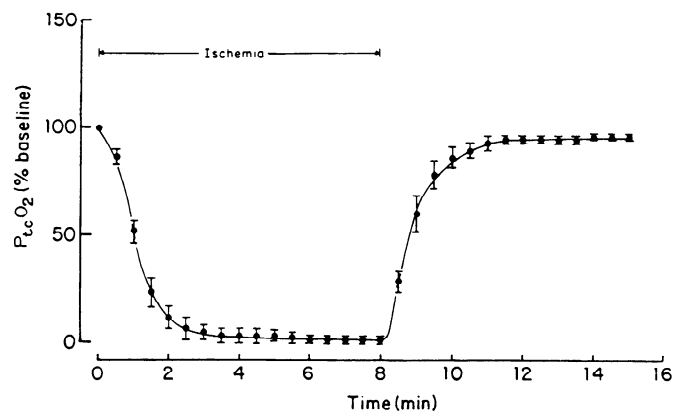

FIG. 6. Summarized transcutaneous P_{O_2} measurements from ischemic forearms of the 10 subjects. Transcutaneous P_{O_2} (Ptc_{O_2}) rapidly fell below its base line after beginning ischemia, reached a nadir after 2.0 min of ischemia, and recovered slowly after relief of ischemia. Data are means ± SE.

ischemia. Tountas and Bergman (27) also found no ultrastructural changes in human muscle with 2 h of ischemia and reported the appearance of mitochondrial swelling between the 3rd and 4th h of ischemia in the forearm flexor muscles of monkeys.

Additional techniques utilized to investigate skeletal muscle responses to ischemia in primates have included

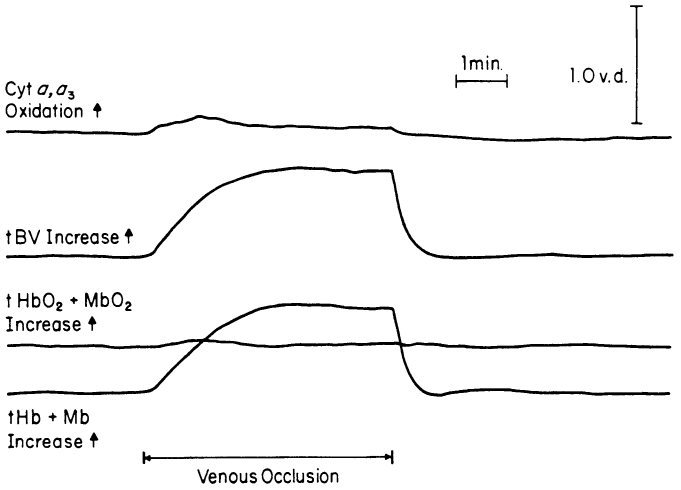

FIG. 7. Near infrared optical responses from skeletal muscle of a representative subject during 5 min of forearm venous outflow restriction and subsequent recovery. Venous occlusion caused an increase in muscle tissue blood volume that was comprised of deoxyhemoglobin. When cuff was released after 5 min of venous occlusion, signals rapidly returned to base-line levels. No appreciable change occurred in cytochrome aa_3 redox level during either period of occlusion or subsequent recovery. For definition of abbreviations, see Fig. 2 legend.

measurement of P_{O_2} in venous blood of ischemic limbs (21, 29), measurement of changes in muscle tissue P_{O_2} (21), and biochemical analysis of metabolic intermediates from biopsies of ischemic muscles (6, 7, 20, 24). Figure 9 compares changes in some of these parameters as a function of time with the changes in muscle cytochrome aa_3 redox level reported in our study. Several studies have examined changes in high energy intermediates in human skeletal muscle during ischemic hypoxia. Muscle ATP has been reported to remain stable for periods of tourniquet ischemia up to 2 h (6, 7, 20). Serial measurements of human skeletal muscle phosphocreatine (PCr) concentration during limb ischemia from the study of Häggmark et al. (6) are shown in Fig. 9. Muscle PCr measured in these 11 subjects did not decrease below base line until after 45 min of ischemia (*line a*), continuing then to decrease over an additional 75 min until tourniquet release (not shown). Haljamäe and Enger (7) reported 40% decreases in muscle PCr within 30–60 min of tourniquet ischemia, and Larsson and Hultman (20) reported significant decreases in PCr after 1.5–2.5 h of tourniquet ischemia. Simultaneous increases in muscle lactate and decreases in muscle glycogen suggest that glycolytic metabolism, a pH-mediated shift in the creatine kinase equilibrium favoring PCr dissociation, and muscle cooling maintain intracellular ATP concentration during ischemia of this duration (6, 7, 20). In all cases, decreased PCr levels returned to normal within 5–15 min after tourniquet release. Miller et al. (21) found that venous blood P_{O_2} in monkeys decreased 64% below base line after 60 min of limb ischemia (Fig. 9, *line b*), similar to the 56% decrease reported in humans by Wiglis (29). Skeletal muscle tissue P_{O_2} determined in monkeys by mass spectrometry changed more rapidly after tourniquet inflation (21), decreasing 42 and 87% below base line within 5 and 30 min, respectively (Fig. 9, *line c*).

Measurement of biochemical changes closer to the site of cellular O_2 utilization appear to assess the effects of ischemia on oxidative metabolism more sensitively than changes in either P_{O_2} or the pool of high energy compounds. Sahlin (24) measured the contents of NAD^+, NADH, lactate, and pyruvate in serial human skeletal muscle biopsies performed during tourniquet ischemia. NADH content increased by 100% within 5 min of circulatory occlusion and was a more sensitive indicator of muscle hypoxia than changes in lactate content. Changes in NAD^+/NADH during ischemia reported in his study are represented by *line d* of Fig. 9. The time course for changes in pyridine nucleotide concentration is very similar to that demonstrated for the reduction of cytochrome aa_3 in our study (Fig. 9, *line e*), with both parameters decreasing >75% below base line within 5 min. The close temporal relationship of these responses is not surprising, because both reflect the accumulation of electrons from substrate metabolism due to the lack of available O_2 for mitochondrial respiration.

The NIR optical responses to ischemia were in marked contrast to the NIR responses to impaired venous return from the forearm. Venous occlusion at 50-mmHg pressure resulted in increased muscle blood volume, entirely accounted for by an increase in tHb + Mb or deoxygenated hemoglobin. Since arterial inflow to the forearm was not affected significantly, no changes were seen in the oxygenated fractions of hemoglobin or myoglobin and no change occurred in the redox level of muscle cytochrome aa_3. In conjunction with the results of arterial occlusion, these data demonstrate differences in the behavior of the NIR signals in human muscle tissue consistent with our physiological expectations. The metabolic signals not only responded independently in the two experiments, but it was also shown that the cytochrome aa_3 signal was highly stable during large shifts in tissue blood volume caused by elevating venous pressure. Independence of the cytochrome aa_3 and $tHbO_2$ + MbO_2 signals has been demonstrated during other human and animal experiments in our laboratory, allowing assessment of functional shunt flow in tissue when it occurs.

NIR spectrophotometry was compared in these studies with a technique currently used clinically for noninvasive monitoring of oxygenation, measurement of transcutaneous P_{O_2}. The time course of Ptc_{O_2} change during ischemia correlated poorly with the change in mitochondrial O_2 availability in the underlying muscle tissue, as represented by the NIR optical responses. Transcutaneous O_2 monitoring measures O_2 diffusion from skin and has been shown to be highly dependent on blood flow (1, 28). Cutaneous blood flow and Ptc_{O_2} both reach zero levels in the forearm at plethysmographic pressures above 70–80 mmHg (1). In the present studies, inflation of the pressure cuff to ~170 mmHg would be expected to abruptly occlude skin flow, causing Ptc_{O_2} to fall rapidly to zero as we observed. During recovery from ischemia, Ptc_{O_2} returned more slowly to base line than did the NIR parameters, and the dramatic and early muscle hyperemia was not apparent from Ptc_{O_2} measurements. This comparison demonstrates that the NIR technique is better able to monitor changes in peripheral oxygenation during ischemia because it assesses O_2 sufficiency at the

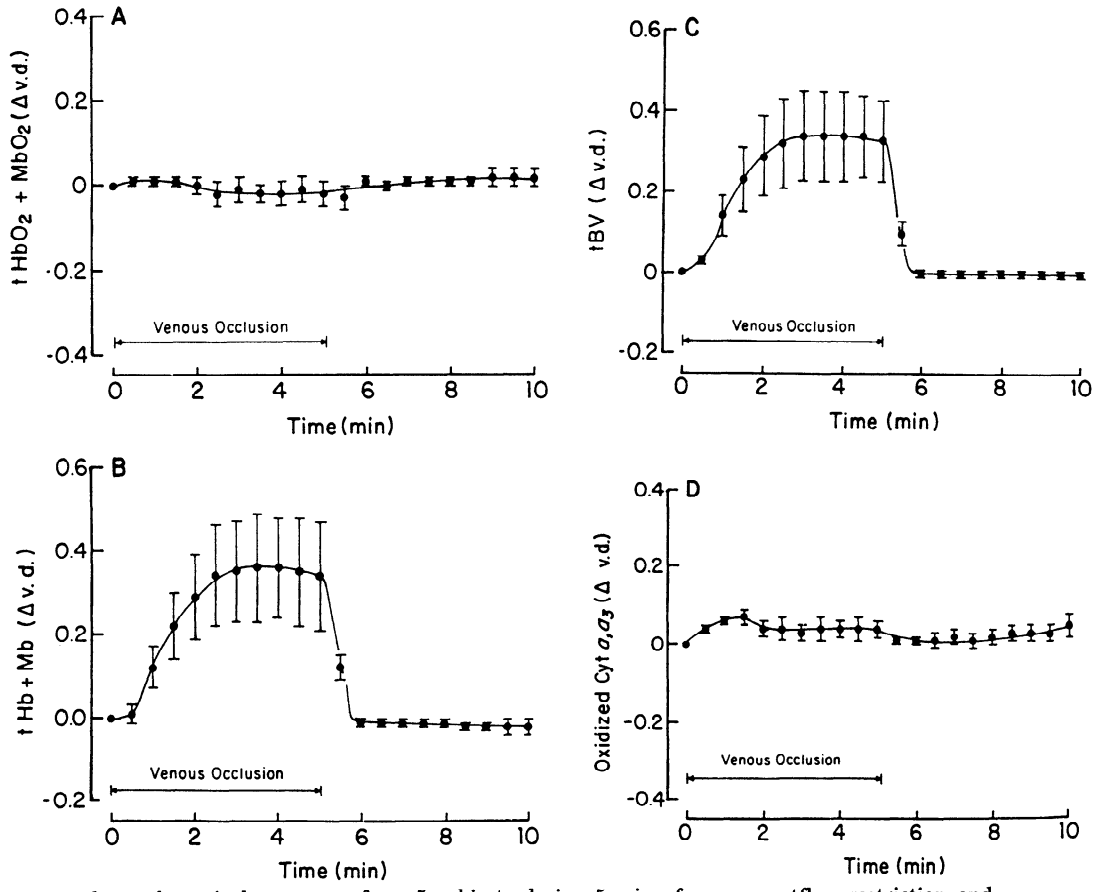

FIG. 8. Summarized muscle optical responses from 5 subjects during 5 min of venous outflow restriction and recovery. Increases in muscle blood volume and tissue deoxyhemoglobin + deoxymyoglobin occurred within 1 min after increasing forearm venous pressure and continued to increase for 3 min after cuff inflation. No significant changes in either tissue oxyhemoglobin + oxymyoglobin or cytochrome aa_3 redox level occurred during period of venous occlusion or subsequent recovery. Data are means ± SE. For definition of abbreviations, see Fig. 2 legend.

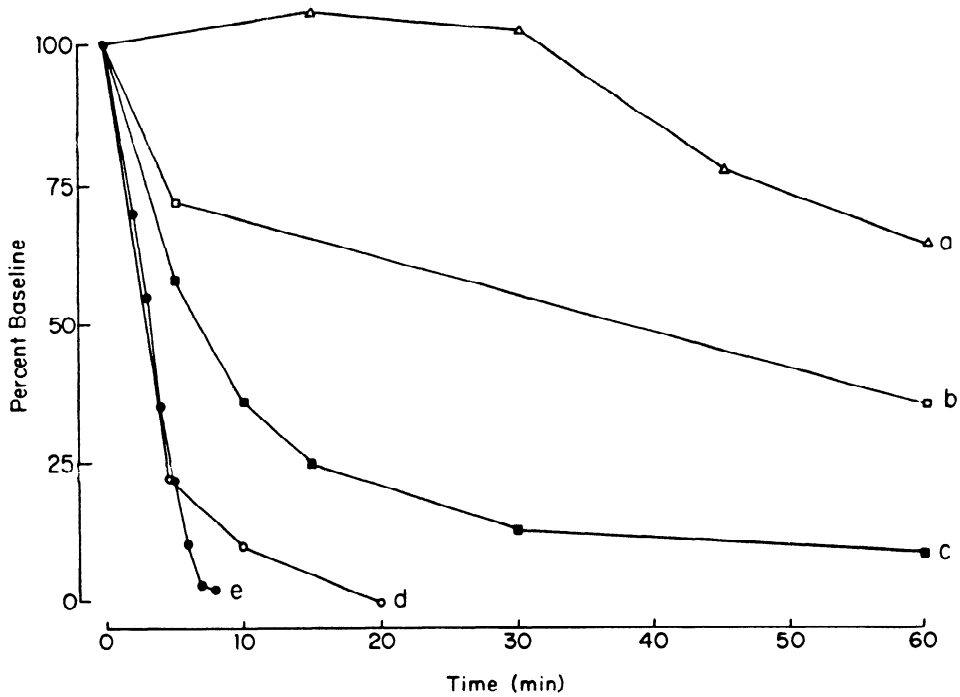

FIG. 9. Comparison of changes in various parameters of skeletal muscle during tourniquet ischemia. *Line a*, human muscle phosphocreatine levels from serial biopsy specimens (Häggmark et al., Ref. 6); *line b*, venous P_{O_2} from ischemic monkey limb (Miller et al., Ref. 21); *line c*, monkey muscle tissue P_{O_2} determined by mass spectrometry (Miller et al., Ref. 21); *line d*, muscle $NAD^+/NADH$ from serial human biopsies (Sahlin, Ref. 24); and *line e*, human muscle cytochrome aa_3 redox level responses reported in this study.

deep tissue level and does not depend on maintenance of blood flow for operation.

In conclusion, these studies have demonstrated that *1*) abrupt ischemia rapidly depletes the O_2 stores of normal human skeletal muscle, *2*) cytochrome aa_3 in human skeletal muscle at rest is not fully oxidized, and *3*) NIR multiwavelength spectrophotometry is sensitive to dynamic trends in muscle O_2 delivery and intracellular O_2 availability in humans.

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