

## Regulation of lactic acid production during exercise

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KATZ, A., AND K. SAHLIN. *Regulation of lactic acid production during exercise.* J. Appl. Physiol. 65(2): 509-518, 1988.—Lactic acid accumulates in contracting muscle and blood beginning at ~50–70% of the maximal O<sub>2</sub> uptake, well before the aerobic capacity is fully utilized. The classical explanation has been that part of the muscle is O<sub>2</sub> deficient and therefore lactate production is increased to provide supplementary anaerobically derived energy. Currently, however, the predominant view is that lactate production during submaximal dynamic exercise is not O<sub>2</sub> dependent. In the present review, data and arguments in support of and against the hypothesis of O<sub>2</sub> dependency have been scrutinized. Data underlying the conclusion that lactate production during exercise is not O<sub>2</sub> dependent were found to be 1) questionable, or 2) interpretable in an alternative manner. Experiments in human and animal muscles under various conditions demonstrated that the redox state of the muscle is reduced (i.e., NADH is increased) either before or in parallel with increases in muscle lactate. Based on experimental data and theoretical considerations, it is concluded that lactate production during submaximal exercise is O<sub>2</sub> dependent. The amount of energy provided through the anaerobic processes during steady-state submaximal exercise is, however, low, and the role of lactate formation as an energy source is of minor importance. It is proposed that the achievement of increased aerobic energy formation under conditions of limiting O<sub>2</sub> availability requires increases of ADP, P<sub>i</sub>, and NADH and that the increases in ADP (and therefore AMP via the adenylate kinase equilibrium) and P<sub>i</sub> will stimulate glycolysis, and the resulting increase in cytosolic NADH will shift the lactate dehydrogenase equilibrium toward increased lactate production.

skeletal muscle; heart; reduced nicotinamide adenine dinucleotide; oxygen; hypoxia; high-energy phosphates

THE REGULATION of lactic acid production has been of interest to exercise physiologists and biochemists for many years. Part of this interest undoubtedly stems from the close association between lactate accumulation and muscle fatigue under various, but not all, conditions (76). Lactate accumulation as an index of accelerated glycolysis has long been appreciated, although the lack of increase in lactate does not necessarily imply that glycolysis is not accelerated. For example, exercise at 40% of maximal O<sub>2</sub> uptake ( $\dot{V}O_{2\text{ max}}$ ) results in glycogenolysis (80), whereas there is neither an increase in content of lactate in the contracting muscle (79) nor in lactate release from the muscle (2, 47). Thus an increased glycolytic rate can occur in the absence of an increase in lactate and is thus balanced by an equal rate of pyruvate

oxidation.

An increase in lactate production occurs already at ~50–70%  $\dot{V}O_{2\text{ max}}$  (47, 48, 55, 65, 69, 79, 81) well before the aerobic capacity is fully utilized. However, the mechanism for the increase in lactate production is not fully understood. The classical explanation has been that the contracting muscle is O<sub>2</sub> deficient and therefore part of the energy requirement must be supplemented through increased lactate production (33).

During isometric contraction, when the muscle is anoxic (32), lactate production accounts for 60% of the ATP turnover (56) and is thus an important source of energy. On the other hand, during steady-state submaximal bicycle exercise (~70%  $\dot{V}O_{2\text{ max}}$ ) with intact circulation, it can be calculated that lactate production ac-

counts for only 2% of the total ATP turnover (79). Lactate production is thus of minor importance in terms of ATP production during submaximal dynamic exercise.

The  $O_2$  dependency of lactate formation is supported by numerous studies where exercise under conditions of impaired  $O_2$  transport results in elevated lactate concentrations in blood (36, 38, 55, 62, 64, 67, 73, 84, 86, 94) and muscle (36, 55, 62) and where increasing the fraction of inspired  $O_2$  results in decreased lactate concentrations in blood (38, 62, 64, 88) and muscle (62). Subsequent findings by Wasserman and co-workers (87) were consistent with the  $O_2$  dependency hypothesis.

Recently, however, the idea that lactate formation during submaximal exercise is dependent on the availability of  $O_2$  has been questioned (8, 17-19). The main reasons for this are that 1) the NAD(P)H fluorescence from the surface of the muscle is decreased under conditions of increased lactate production (44) and 2) measurements of cellular  $O_2$  tension, estimated from the myoglobin saturation, show that relatively high levels of  $O_2$  are present in lactate-producing muscle (17-19).

It is the intent of this brief review to summarize a series of recent studies on muscle NADH content during exercise and to critically examine the data (and the interpretation of these data) that serve as bases for hypotheses explaining the regulation of lactate production during exercise.

#### *Methods for Measuring Tissue Lactate Production*

In the absence of blood flow, the muscle can be regarded as a closed system and lactate production can be obtained simply from measurements of lactate accumulation. This condition exists during isometric contraction where blood flow is restricted by the high intramuscular pressure (25) and during circulatory occlusion.

In nonischemic tissue, part of the formed lactate is released into the blood (or perfusate) and lactate production is therefore the sum of accumulation and release. From measurements of the arteriovenous lactate difference, blood flow, and muscle lactate accumulation, it was estimated that 17% of the produced lactate was released from the muscle during 5 min of bicycle exercise at 100%  $\dot{V}O_{2\text{ max}}$  (53). The proportion between release and accumulation is, however, likely to vary with both the intensity and duration of the exercise. Although data on muscle lactate accumulation alone cannot provide quantitative information, it is clear that they will provide a good qualitative measure of lactate production during short-term exercise.

The concentration of lactate in blood is dependent not only on the release of lactate from the contracting muscle but also on the rate of lactate removal from the blood and is therefore influenced by many factors. Increases in blood lactate, however, have been found to correlate with lactate in the contracting muscle in many situations (48, 81) and could therefore provide an adequate index of lactate production during short-term exercise.

Skeletal muscle is composed of different fiber types with different metabolic capacities. It has been suggested that a substantial part of the lactate metabolism occurs by a lactate shuttle where lactate is produced in fast-

twitch glycolytic fibers and oxidized in fibers with a high oxidative capacity (8). Measurements of the release and accumulation of lactate would not include the oxidized lactate, resulting in an underestimation of the total lactate production. It has been argued that the use of carbon-labeled lactate tracers would enable measurement of the total lactate production (8). However, it was recently demonstrated that an apparent uptake of [ $^{14}\text{C}$ ]-lactate may occur in the absence of net lactate utilization and thus  $^{14}\text{CO}_2$  production does not provide a measure of true lactate oxidation (57). This is probably due to an exchange mechanism where [ $^{14}\text{C}$ ]lactate will exchange with intracellular lactate and pyruvate (77). A virtually complete isotopic equilibration between lactate and pyruvate has recently been demonstrated (93). Consequently the tracer techniques using labeled lactate cannot distinguish between pyruvate and lactate metabolism (77). It is likely that the major part of the "lactate production" and "lactate oxidation" observed with the tracer method at rest and during low-intensity exercise is due to formation and oxidation of pyruvate (which is derived from glucose or glycogen). Although there is little doubt that simultaneous production and oxidation of lactate can occur within adjacent fibers in a muscle, it is clear that the magnitude of this so-called lactate shuttle has been overestimated.

#### *Criticisms Against the Hypothesis of $O_2$ Dependency*

The main criticisms that have been used to refute the idea that lactate formation during submaximal exercise is  $O_2$  dependent are the following. 1) The  $P_{O_2}$  in a contracting, lactate-producing muscle is higher than the value where respiration in isolated mitochondria is impaired. 2) The redox state of the muscle, measured by surface fluorometry, is not reduced (i.e., mitochondrial NADH is not increased). 3) Pulmonary  $O_2$  uptake ( $\dot{V}O_2$ ) at a given submaximal exercise intensity is not altered by training or hyperoxia, although lactate production is diminished. These criticisms are discussed below.

*High  $P_{O_2}$  in contracting muscle.* The  $O_2$  dependence of isolated mitochondria has been extensively studied by Chance and co-workers (9) and from these results it has been concluded that the respiration is  $O_2$  independent when the  $P_{O_2}$  exceeds 0.01–0.1 kPa. These observations on isolated mitochondria have been extrapolated to contracting muscle tissue and have been used as evidence that the metabolism is independent of  $O_2$  above these values (8, 18). It has, however, been pointed out that intact tissues (or cells) differ from isolated mitochondria in terms of  $O_2$  transport,  $O_2$  gradients, and energetic and redox states (3, 39, 45, 46). The  $P_{O_2}$  where maximal respiration is affected could therefore be higher in contracting muscle tissue than in isolated mitochondria.

In many previous papers and reviews it has been explicitly stated (8, 18) or implied (37) that if cellular respiration is not impaired, then cellular metabolism (including lactate formation) would not be dependent on  $P_{O_2}$ . In a series of recent studies, Connett et al. (17-19) have estimated the cytosolic  $P_{O_2}$  from the degree of myoglobin saturation in contracting dog gracilis muscle. They found that lactate production occurred, although

the  $\text{PO}_2$  ( $>0.3$  kPa) was well above the value where respiration in isolated mitochondria is affected. From these data, Connell et al. (17-19), as well as others (8, 83), concluded that the lactate production was not  $\text{O}_2$  dependent.

However, it has been suggested that a decrease in  $\text{PO}_2$  may have important metabolic consequences at values above those required to lower the respiratory rate (91). Thus during higher rates of respiration, aerobic metabolism may be  $\text{O}_2$  dependent at a relatively high  $\text{PO}_2$ , but due to adaptive changes in the mitochondrial redox state and the cytosolic phosphorylation potential (or ADP), cellular respiration during steady state is maintained. Furthermore it is likely that these cellular adaptations to a decreased  $\text{PO}_2$ , or an increased energy demand, result in the enhancement of the breakdown of phosphocreatine (PCr) and the activation of glycolysis (see below). According to this view, the increased lactate production during submaximal exercise should be regarded more as a regulatory phenomenon than a necessary supplementation of the energy production.

*Measurement of redox state by surface fluorometry.* A study that is frequently cited to provide evidence that lactate production during exercise is not  $\text{O}_2$  dependent is that of Jöbsis and Stainsby (44), who used surface fluorometry to monitor the redox state in dog skeletal muscle at rest and during contraction. The basis for this method is that light in the 366-nm region, when focused on the surface of an exposed muscle, elicits fluorescence from what is considered to be mitochondrial NADH and NADPH. It was shown that the fluorescence decreased (i.e., NAD(P)H decreased) when lactate production was reported to occur (44). Even the breathing of  $\text{N}_2$  during electrical stimulation of the gastrocnemius muscle resulted in a fluorescence that was lower than the baseline value. From their results, Jöbsis and Stainsby (44) concluded that the  $\text{O}_2$  supply was not limiting the activity of the respiratory chain and that lactate formation was not due to hypoxia. This technique, however, has recently been criticized. Some of these criticisms include the inability to differentiate between NADH and NADPH (1, 32, 85); the inability to differentiate between reduced pyridine nucleotides in the mitochondria and other compartments (61, 70), especially at high temperatures (11), movement artifacts during contraction (89); and possible damage to the microcirculation of the muscle during exposure of the muscle surface to the fluorometer. Additionally, only the muscle surface is monitored, and consequently changes occurring deep within the muscle cannot be detected (1). Furthermore, Chance et al. (11, 13) showed that when fluorescence in the perfused rat heart was compared with chemical assay of tissue NADH, the relative increases in the analytically determined NADH were severalfold higher than the relative increase in surface fluorescence. Thus it is apparent that surface fluorometry does not provide quantitative estimates of muscle NADH and under some conditions may not even provide correct qualitative results.

*Unchanged pulmonary  $\dot{\text{V}}\text{O}_2$ .* It is well documented that the measured  $\dot{\text{V}}\text{O}_2$  at a given exercise intensity before and after training (29, 49), or between normoxic and

respiratory hypoxic (or hyperoxic) conditions (55, 62), is similar, although lactate production is altered. The constant  $\dot{\text{V}}\text{O}_2$  in a lactate-producing muscle has been used as an argument that lactate production is not  $\text{O}_2$  dependent (e.g., Ref. 37). It should be emphasized, however, that the amount of  $\text{O}_2$  corresponding to the lactate production (in terms of ATP production) is small (<2%) (50) and less than the methodological error in determining  $\dot{\text{V}}\text{O}_2$  during submaximal exercise (coefficient of variation is ~4%) (4). Furthermore this line of reasoning does not consider the possibility that, to achieve a constant  $\dot{\text{V}}\text{O}_2$  (after training or with different fractions of inspired  $\text{O}_2$ ) at a given energy demand, adaptive changes occur within the muscle (20) and that these changes result in the observed differences in lactate production. This is more fully discussed below.

It is well documented that lactate production during submaximal exercise at a given work load increases with the inspiration of a gas with a decreased fraction of  $\text{O}_2$  and that lactate production decreases with the inspiration of a gas with an increased fraction of  $\text{O}_2$  (38, 55, 62, 73). Despite the fact that the increase in blood lactate during respiratory hypoxia is associated with increases in the lactate content of contracting muscle (55, 62) and the release of lactate from contracting muscle (36), Brooks (8) suggested that the elevated blood lactate content was due to a decreased lactate clearance. Available experimental data demonstrate, however, that respiratory hypoxia in humans during submaximal exercise results in a splanchnic removal of lactate that is, on the average, more than fivefold greater than the hepatic lactate removal during normoxia (73). The elevated blood and muscle lactate concentrations during hypoxia are thus due to an increased production, and it is therefore evident that lactate production by contracting muscle during submaximal exercise can be  $\text{O}_2$  dependent.

From the above discussion it is apparent that a number of criticisms against the  $\text{O}_2$  dependency hypothesis are based on 1) questionable data and/or 2) data that can be interpreted in an alternative manner. It is thus necessary to adopt other approaches to determine whether muscle lacks sufficient  $\text{O}_2$  during submaximal ( $<\dot{\text{V}}\text{O}_{2 \text{ max}}$ ) exercise.

#### *Determination of the NAD Redox State in Skeletal Muscle*

The most straightforward way to determine whether the cell is  $\text{O}_2$  deficient is to examine the organelle that utilizes  $\text{O}_2$ . For all practical purposes,  $\text{O}_2$  is utilized solely in the mitochondria. Thus one might consider measuring the  $\text{O}_2$  content of the mitochondria in the contracting muscle. Even if this was possible, the results would be difficult to interpret because we do not know which intramitochondrial  $\text{PO}_2$  must be reached before the mitochondria can be considered to be relatively  $\text{O}_2$  deficient. As discussed above, it is not appropriate to apply findings (or constants) from isolated mitochondria to *in vivo* conditions. The most sensitive index of mitochondrial  $\text{O}_2$  availability has been suggested to be the reduction state of the NAD redox couple, where an increase in mitochondrial NADH denotes a state of  $\text{O}_2$  deficiency

(9–11). There are a number of methods to determine the mitochondrial redox state, each with its inherent advantages and limitations. These methods include the metabolite indicator technique, tissue fractionation, and determination of total tissue NAD<sup>+</sup> and NADH.

**Metabolite indicator technique.** The principle of this method can be illustrated by determining the cytosolic redox state using the lactate dehydrogenase (LDH) reaction: lactate + NAD<sup>+</sup> ↔ pyruvate + NADH + H<sup>+</sup>. The redox state can be calculated as follows: [NAD<sup>+</sup>]/[NADH] = 1/K<sub>app</sub> × [pyruvate]/[lactate], where K<sub>app</sub> is the apparent equilibrium constant for the LDH reaction (1, 90). The total tissue concentrations of lactate, pyruvate, and H<sup>+</sup> can then be used to estimate the cytosolic redox state if all of the following requirements are satisfied (see Ref. 1). 1) The physicochemical conditions (e.g., ionic strength and pH) in the compartment under study are known to be able to use an appropriate K<sub>app</sub> in the calculation. 2) The dehydrogenase used for the calculation is located exclusively in that compartment. 3) The measurement of indicator metabolites like pyruvate and lactate should not be affected by spatial and/or binding compartmentation. 4) For each application of the method, it must be established that the indicator reaction is near equilibrium. In consideration of these requirements, Akerboom et al. (1) suggested that the LDH system should be applicable as a reliable indicator of the cytosolic redox state, and it has been used for different tissues, including liver, heart, skeletal muscle, and erythrocytes (see Ref. 1 for references). For practical purposes, [lactate]/[pyruvate] will be proportional to cytosolic [NADH]/[NAD<sup>+</sup>] (79).

The same principles apply in the use of the glutamate dehydrogenase and 3-hydroxybutyrate dehydrogenase reactions to estimate the mitochondrial redox state. These reactions have been used in rat liver, where the activity of these enzymes is relatively high (90). The glutamate dehydrogenase reaction was also recently used in dog skeletal muscle (92). However, the activity of glutamate dehydrogenase (and 3-hydroxybutyrate dehydrogenase) is extremely low in mammalian skeletal muscle (90), and it is therefore likely that it does not catalyze a reaction close to equilibrium (1, 51). Because of this and the inability to satisfy some of the other requirements, use of these reactions is not appropriate for estimating the mitochondrial redox state in mammalian skeletal muscle (1, 51).

**Tissue fractionation.** The principle of this technique is that the cytosolic and mitochondrial (or other organelles) compartments of the intact cell are fractionated and the metabolites are measured in each fraction. Two requirements must be met to obtain reliable results: 1) metabolism must not occur during fractionation, since this could alter the concentrations of the metabolites under study, and 2) adequate fractionation must be achieved. Essentially three fractionation methods have been described (see Ref. 1): 1) fractionation of freeze-clamped, freeze-dried tissue in organic solvents, 2) fractionation of hepatocytes by digitonin treatment, and 3) fractionation of hepatocytes by mechanical shearing forces.

Methods 2 and 3 are questionable because changes in

metabolites (e.g., NADH) could occur during the isolation of cells before fractionation and possibly during the fractionation (41, 90). The organic solvent method appears, from a theoretical viewpoint, to be potentially useful. We are, however, not aware that any of these techniques have been used for determination of NADH in skeletal muscle, a tissue that is relatively poor in mitochondria.

**Total tissue NADH.** The analytically determined content of tissue NADH could serve as a marker of mitochondrial O<sub>2</sub> availability. Unfortunately the NADH content will include that of all cellular compartments (whether free or bound, e.g., to proteins). The potential problem of not being able to differentiate between cytosolic and mitochondrial NADH can, to an extent, be overcome by use of the LDH system to estimate the cytosolic redox state. From the lactate, pyruvate, NAD<sup>+</sup>, and H<sup>+</sup> values in the tissue, the free content of NADH in the cytosol can be estimated (1, 78, 90). Such calculations from data in humans indicate that at rest, after 20 min of circulatory occlusion, and after maximal dynamic exercise to fatigue, cytosolic NADH accounts for 3.0, 1.2, and 5.5% of total muscle NADH, respectively (75). Occlusion and maximal exercise result in a >100% increase in total muscle NADH (74, 75, 79). In rat skeletal muscle (soleus and extensor digitorum logus) both at rest and after cyanide incubation (which resulted in 65–180% increase in NADH), the cytosolic NADH could account for only 0.5–1.5% of total muscle NADH (78). In the perfused rabbit heart, the NADH content during control conditions (74 μmol/kg dry wt) increased to almost 1,800 μmol/kg dry wt during anoxia, where the NADH content was similar to the total mitochondrial NAD<sup>+</sup> + NADH content (54; also see Ref. 58). Similar calculations for cytosolic NADH result in an increase from 2 μmol/kg dry wt during control to 19 μmol/kg dry wt during anoxia. Thus the increase in cytosolic NADH could account for only 1% of the increase in total NADH in anoxic hearts. Moreover, submaximal exercise (40% V<sub>O<sub>2</sub> max</sub>) results in ~40% decrease in human muscle NADH with no change in [lactate]/[pyruvate] (i.e., no change in cytosolic NADH) (79). Similarly, in the perfused rabbit heart, hyperkalemia (which arrests contractile activity) results in ~100% increase in NADH, whereas [lactate]/[pyruvate] does not change (54). Consistent with these observations is the finding of almost a 10-fold increase in [lactate]/[pyruvate] 1 min after isometric contraction (vs. fatigue), whereas NADH decreased by 65% (32). Additional arguments to support the idea that changes in tissue NADH mainly reflect changes in the mitochondria are given elsewhere (32, 50, 78).

It might be argued that use of the LDH system will provide data only on the free form of NADH (which is the relevant form regarding participation in enzymatic reactions) and that part of the NADH will be protein bound (both in the cytosol and mitochondria). Whether any of the measured NADH or NAD<sup>+</sup> is bound, or the extent of possible binding in mammalian skeletal muscle, is, to our knowledge, not known. It has, however, been suggested that in a given cellular compartment a constant relationship exists between the free and bound

[NADH]/[NAD<sup>+</sup>] (59), and evidence for this has been provided in isolated rat liver mitochondria (34). It is therefore likely that the relative changes in total NADH reflect a similar relative change in the free form of NADH.

Based on the above discussion it seems reasonable to conclude that 1) most of the analytically determined NADH is confined to the mitochondrial compartment and, more importantly for the purpose of this review, 2) changes in tissue NADH primarily reflect those within the mitochondria and can serve as indexes of mitochondrial O<sub>2</sub> availability.

### *Muscle NADH and Lactate Content*

In a series of recent studies the muscle contents of NADH and lactate have been determined under a variety of conditions. In the initial study, it was shown that circulatory occlusion of the thigh results in a rapid increase in muscle NADH (within 5 min), which leveled off after 10–20 min, whereas the lactate content did not increase significantly (74). During ischemia significant increases in lactate occur after ~20–30 min (15, 52). The muscle O<sub>2</sub> store is apparently depleted in ~3 min during ischemia (52), and this depletion thus coincides with the increase in muscle NADH. Similar data were obtained from rat muscle, where soleus and extensor digitorum longus (EDL) muscles were either freeze-clamped *in situ* or dissected out and incubated *in vitro* in a medium containing cyanide, which inhibits cytochrome oxidase, and thereafter frozen. Cyanide incubation resulted in a two- to threefold increase in NADH without any increase in lactate in the soleus and only a minor increase in lactate in the EDL muscle (78). In another study samples were taken from the quadriceps femoris muscle before and after isometric contraction at two-thirds of the maximal voluntary contraction force (32). Contraction at this intensity will increase the intramuscular pressure to a level where the local blood flow is occluded (25), and it can be calculated that the local O<sub>2</sub> store will be depleted after 2–3 s of contraction (32). Muscle NADH increased twofold already after 5 s of contraction and remained at about this level when the contraction was continued to fatigue (52 s). Muscle lactate did not change significantly after 5 s but was increased 12-fold at fatigue.

The conclusion from these studies is that an impairment of mitochondrial respiration by ischemia, cyanide, or depletion of O<sub>2</sub> by isometric contraction results in a large increase in muscle NADH, which precedes the increase in lactate production. From these data it appears that changes in muscle NADH are indicative of changes in the mitochondrial O<sub>2</sub> availability.

To further investigate the relationship between O<sub>2</sub> availability, muscle NADH, and lactate, subjects cycled at submaximal and maximal work loads (75, 79). Muscle biopsies were obtained at rest and after exercise. At low exercise intensities (40%  $\dot{V}O_{2\text{ max}}$ ) muscle NADH decreased, whereas at higher intensities (75 and 100%  $\dot{V}O_{2\text{ max}}$ ) NADH increased above the value at rest, which suggests that the O<sub>2</sub> availability was limiting for the respiratory chain. The increase in NADH coincided with lactate accumulation in both blood and muscle. The

decrease in NADH during submaximal exercise was not accompanied by changes in either blood or muscle lactate and is consistent with data from isolated mitochondria where an increase of ADP in the presence of sufficient substrate and O<sub>2</sub> results in an oxidation of mitochondrial NADH (12).

Human muscle is composed of different fiber types with different metabolic characteristics. Therefore the possibility of fiber-type heterogeneity in the pattern of NADH changes during exercise was investigated by analysis of NADH in single fibers (71). During low-intensity exercise (40%  $\dot{V}O_{2\text{ max}}$ ) NADH decreased only in type I fibers, whereas at higher intensities (75 and 100%  $\dot{V}O_{2\text{ max}}$ ) NADH increased above the preexercise value in both fiber types, suggesting that the availability of O<sub>2</sub> was restricted in both fiber types.

The effect of decreased O<sub>2</sub> availability on the energy metabolism at a constant energy requirement was investigated in subjects exercising at a submaximal work load (50% of  $\dot{V}O_{2\text{ max}}$ ; 120 W) while breathing air (control) or a gas mixture poor in O<sub>2</sub> [11% O<sub>2</sub>; respiratory hypoxia (55)]. The  $\dot{V}O_2$  during exercise was similar during both conditions, but the muscle lactate content was increased fourfold during respiratory hypoxia vs. control. Muscle NADH was significantly higher after exercise during respiratory hypoxia than during control. These results suggest that a decrease in the fraction of inspired O<sub>2</sub> during submaximal exercise results in a reduction of the mitochondrial redox state and an increase in lactate production, although  $\dot{V}O_2$  is maintained constant.

The O<sub>2</sub> dependence of metabolism was further investigated in isolated rabbit heart, which offers the advantage of being homogeneous in its fiber composition (54) (i.e., changes in NADH content and lactate production must be occurring in the same fibers). The isolated heart was perfused according to Langendorff, and the perfusate (providing glucose as substrate) was saturated with gas mixtures containing 95% O<sub>2</sub>-5% CO<sub>2</sub> (control) or 50% O<sub>2</sub>-5% CO<sub>2</sub>-45%-N<sub>2</sub> (hypoxia). In additional experiments cardiac arrest was induced by increasing the K<sup>+</sup> concentration to 15 mmol/l in the control perfusate.  $\dot{V}O_2$  was similar during control and hypoxia, but lactate production was fourfold higher during hypoxia. Muscle NADH increased about sevenfold during hypoxia compared with control (normoxia), again demonstrating the relationship between decreased O<sub>2</sub> availability and increases in NADH content and lactate production. Muscle NADH was lower in the contracting muscle (control) than in noncontracting muscle (K<sup>+</sup> arrest) whereas lactate production was not altered, again demonstrating that activation of respiration in the presence of sufficient substrate and O<sub>2</sub> results in an oxidation of NADH without affecting lactate production.

### *Why is Lactate Formation O<sub>2</sub> Dependent During Submaximal Exercise?*

A theoretical formula has been derived by Kushmerick (61) to describe the dependence of cellular respiration ( $\dot{V}O_2$ ) on P<sub>O<sub>2</sub></sub>, the mitochondrial redox state, and the phosphorylation potential. The formula predicts that an increased  $\dot{V}O_2$  can be achieved by increases in NADH,

ADP, or  $P_i$ . It was observed by Wilson et al. (91) that the metabolism is  $O_2$  dependent at rather high  $O_2$  tensions but that respiration is maintained constant during conditions of decreased  $O_2$  availability by increases in NADH, ADP, and  $P_i$ . There are also experimental data to support the concept that mitochondrial respiration depends on intramitochondrial NADH and extramitochondrial phosphates over a wide range of respiratory rates (60). The data presented above on increases in muscle NADH during various conditions where respiratory demand is increased are consistent with this idea. In addition to the increase in NADH, a decrease in PCr (reflecting an increase in free ADP) and an increase in  $P_i$  were observed during conditions of low  $O_2$  availability (55), which are also consistent with the findings of Wilson et al. (91). ADP, AMP (which will increase together with ADP due to the near equilibrium of the adenylate kinase reaction), and  $P_i$  are important activators of glycolysis (68), and AMP and  $P_i$  are also activators of glycogenolysis (16, 68). At low exercise intensities the increased rate of pyruvate formation is balanced by a similar increase in the rate of pyruvate oxidation, which is apparent from the absence of lactate accumulation in muscle and blood. From the existing data it is clear that the imbalance between the glycolytic and oxidative processes with increasing exercise intensities coincides with an increase in NADH above the value at rest in both fiber types. The parallel increase in muscle lactate and NADH under a variety of conditions could be explained by an increased stress on aerobic metabolism. An increased stimulation of mitochondrial respiration (by NADH, ADP, and  $P_i$ ) would be required both when the energy turnover increases at a constant tissue  $O_2$  tension and when the  $O_2$  availability decreases (at a given or increased energy turnover). Simultaneously the glycolytic rate would be accelerated.

Training is known to result in an increase in mitochondrial content and the aerobic capacity of the muscle (37). According to the hypothesis of respiratory control exerted by NADH, ADP, and  $P_i$  one would expect an attenuation of the increase in these regulators in a trained muscle when contracting at a given intensity. Data from contracting rat skeletal muscle show that  $P_i$ , ADP, and lactate increase less after training (20) and are consistent with this hypothesis. Similar data are also available for human muscle before and after training (31, 49). Data on the effects of aerobic training on NADH contents in contracting skeletal muscle are currently not available.

During exercise the increased glycolytic rate is associated with an increased formation of cytosolic NADH. The mitochondrial membrane is, however, impermeable to NADH, and reducing equivalents are therefore transported from the cytosol to the mitochondria by shuttle systems (see Ref. 23), thereby reoxidizing cytosolic NADH back to  $NAD^+$ . The malate-aspartate shuttle appears to be most important, whereas the glycerophosphate shuttle is likely to be of less importance in this respect in human skeletal muscle (82).

The exchange of glutamate-aspartate across the mitochondrial membrane (which is essential for the func-

tion of the malate-aspartate shuttle) is electrogenic, and thus the difference in NADH concentration between the mitochondria and the cytosol is influenced by the mitochondrial membrane potential (22). An increase in mitochondrial NADH, or alternatively a decreased mitochondrial membrane potential [which could result from either a decrease in  $[ATP]/[ADP]$  (22) or a decrease in the  $O_2$  availability (10)] is thus expected to lead to an increase in cytosolic NADH (79). The decrease in  $[ATP]/[ADP][P_i]$  at the higher respiratory rates, as well as the increase in NADH, can be regarded as compensatory changes necessary to achieve a given respiratory rate in the face of falling  $PO_2$  levels in the mitochondria. Thus an increased cytosolic NADH concentration relative to mitochondrial NADH is expected to occur at high glycolytic rates to create the driving force necessary to increase the transport of reducing equivalents from the cytosol to the mitochondria. An increased cytosolic  $[NADH]/[NAD^+]$  as reflected by an increased  $[lactate]/[pyruvate]$  has been demonstrated both during incremental exercise (79) and during conditions of decreased  $O_2$  availability (55). Formation of lactate will therefore increase by the law of mass action due to both an increased pyruvate concentration and an increased cytosolic  $[NADH]/[NAD^+]$ .

The relationship between muscle pyruvate and  $[lactate]/[pyruvate]$  vs. muscle lactate is presented (Fig. 1). It can be seen that during low exercise intensities (up to

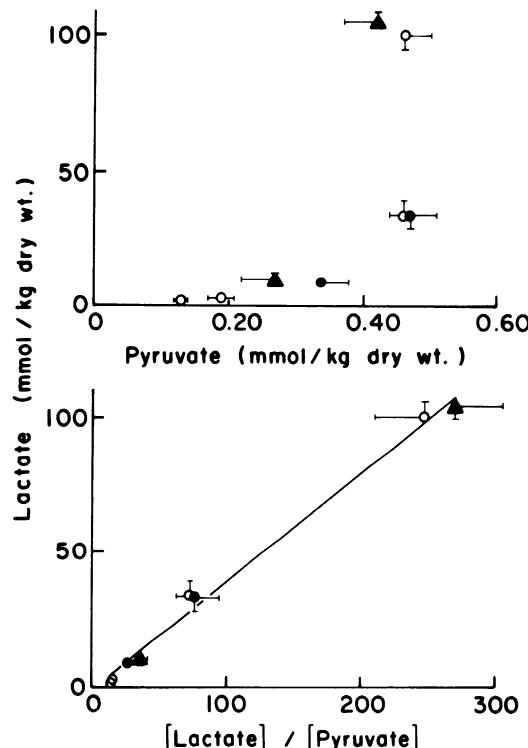


FIG. 1. Relationship between lactate and pyruvate (top) and  $[lactate]/[pyruvate]$  (bottom) in human skeletal muscle at rest and after dynamic exercise. Values are means  $\pm$  SE from 7–10 subjects. SE bars not shown lie within symbol. Exercise intensities are  $\sim 40$ ,  $50$ ,  $65$ ,  $75$ , and  $100\% V_{O_2 \text{ max}}$  with durations of  $\sim 5$  or  $10$  min. See Katz et al. (53) ( $\blacktriangle$ ), Katz and Sahlin (55) ( $\bullet$ ), and Sahlin et al. (79) ( $\circ$ ) for further details. Linear regression analysis showed lactate =  $0.41 [lactate]/[pyruvate] - 1.73$ ;  $r = 0.997$ ;  $n = 8$ .

50%  $\dot{V}O_{2\text{max}}$ , where lactate is ~9 mmol/kg dry wt), relatively large increases occur in pyruvate, which are accompanied by only small increases in lactate. In fact, the lactate values observed when pyruvate increased more than threefold are similar (~9 mmol/kg dry wt) to the values at rest reported from various laboratories (e.g., Ref. 7, 21, 26, 48). At higher exercise intensities, pyruvate remained fairly constant, whereas large increases were observed in lactate. On the other hand, [lactate]/[pyruvate] was linearly related to lactate.

These data indicate that although increases in muscle pyruvate can induce small increases in lactate, the major stimulus for the conversion of pyruvate to lactate during dynamic exercise is an elevated cytosolic redox state. By and large the data support the hypothesis that lactate production during submaximal (and maximal) exercise is, to a large extent, dependent on the  $O_2$  availability in the contracting muscle.

#### *Other Explanations for Increases in NADH and Lactate Production*

**NADH.** The observed increases in NADH suggest that the mitochondria in the muscle are  $O_2$  deficient (9). An alternative explanation for the increase in NADH during dynamic exercise could be that the mitochondrial NADH formation from the tricarboxylic acid (TCA) cycle, together with the reducing equivalents transported from the cytosol into the mitochondria, exceeds the enzymatic capacity of the respiratory chain. However, when the  $O_2$  delivery to the working muscle is increased (experimentally obtained by one-leg exercise), the maximal  $O_2$  uptake by the leg muscles can be increased to levels considerably higher than during two-leg exercise (31). Hence the maximal capacity of the respiratory chain is not utilized during two-leg exercise and thus is probably not limiting the oxidation of NADH.

Yet another explanation for the increase in NADH during steady-state dynamic exercise could be that at the onset of exercise there is a relatively large production of substrate (i.e., pyruvate) for oxidation in the TCA cycle, which decreases thereafter. The rates of electron transport and  $O_2$  utilization are under acceptor (ADP availability of  $[ATP]/[ADP]$  [ $P_i$ ]) control and are therefore determined by the rate of ATP hydrolysis. Thus as the result of the initial increase in glycolysis there could be a large influx of pyruvate (and possibly malate) into the mitochondria, in excess of what is required for oxidation, resulting in an increased formation of reducing equivalents (i.e., reduction of mitochondrial  $NAD^+$  to NADH) in well-oxygenated muscle. If this interpretation were correct, one would expect the recovery of NADH to resting levels to be very slow during the initial stages of recovery from exercise, since respiration decreases rapidly and pyruvate (coming from the accumulated lactate) actually increases (32, 53). One might also expect NADH to be elevated as long as substrate for the TCA cycle was elevated (i.e., pyruvate and lactate). The recovery of NADH is, however, rapid after isometric contraction (half-time < 1 min; Ref. 32). Moreover, 10 min after maximal dynamic exercise both pyruvate and lactate are ~11 times the values at rest (53), whereas NADH has

reverted to the value at rest (75). Thus available data do not support this explanation.

Similarly it might be argued that the increase in mitochondrial NADH during exercise may be due to  $Ca^{2+}$ -dependent activation of mitochondrial dehydrogenases, such as pyruvate dehydrogenase (PDH, via activation of PDH phosphate phosphatase),  $NAD^+$ -isocitric dehydrogenase, and 2-oxoglutarate dehydrogenase (30, 66). Such a mechanism would be consistent with the observed rapid increase in NADH during contraction and the rapid decrease in NADH after contraction. If, however,  $Ca^{2+}$  was the major regulator of mitochondrial NADH, then one might expect to see an increase in NADH whenever contractile activity is increased. During low-intensity exercise (40%  $\dot{V}O_{2\text{max}}$ ), however, NADH decreases. Furthermore, during respiratory hypoxia, where the  $\dot{V}O_2$  and metabolic demand (power output) are the same as during exercise during normoxia (55), suggesting similar concentrations of cytosolic  $Ca^{2+}$ , NADH is higher during hypoxia. These observations do not support the concept that  $Ca^{2+}$  is the major regulator of the mitochondrial redox state during exercise.

**Lactate.** The exponential increase in muscle lactate production with increasing work loads (>50%  $\dot{V}O_{2\text{max}}$ ) is associated with a similar profile in plasma catecholamines (28). Furthermore the decreased lactate production during respiratory hyperoxia is also associated with a decreased catecholamine response, whereas the converse occurs during respiratory hypoxia (see Ref. 27 for references). It has been argued that the increased lactate production during exercise is a consequence of the rise in plasma epinephrine (83). The scenario suggested by Stainsby et al. (83) is that the epinephrine-mediated increase in adenosine 3',5'-cyclic monophosphate will activate glycogen phosphorylase in excess of what is required for oxidative function and that an imbalance between glycolysis and pyruvate oxidation occurs, resulting in increased lactate production. Indeed there are data that may appear to be consistent with this hypothesis. Thus, Jansson et al. (43) infused epinephrine into one femoral artery (resulting in a high femoral venous epinephrine concentration) during two-leg bicycle exercise at 50%  $\dot{V}O_{2\text{max}}$ . From arteriovenous differences of both legs it was found that on the average the epinephrine-infused leg released twice as much lactate as the control leg did (assuming similar blood flows), although the muscle lactate contents after exercise were similar in the two legs.

On the other hand, it is well recognized that lactate production decreases as exercise duration increases (e.g., Refs. 6, 31); in contrast, epinephrine increases continuously (see Ref. 27). Moreover, bilaterally adrenalectomized subjects, whose plasma epinephrine concentration during exercise at 60%  $\dot{V}O_{2\text{max}}$  is <50% of the plasma epinephrine concentration in normal subjects at rest, achieve the same blood lactate concentration (if not slightly higher) during exercise as normal subjects (35). These findings indicate that epinephrine, at physiological levels per se, plays a minor role in stimulating lactate production.

It has been suggested that lactate production during

exercise may be due to a limitation in the transport of reducing equivalents from the cytosol into the mitochondria by the malate-aspartate shuttle (82). The maximal activity of the shuttle enzymes is, however, considerably higher than necessary during two-leg exercise at 100%  $\dot{V}O_{2 \text{ max}}$  (82). Nevertheless this does not rule out the possibility that *in vivo* the activities of these shuttle enzymes may be limiting for the transport of reducing equivalents, although compelling evidence for this is, to our knowledge, not available. It should be noted that in our studies we have never observed an increase in the cytosolic redox state before observing an increase in total tissue (i.e., mitochondrial) NADH. The increase in cytosolic NADH is apparently due to a relative decrease in the activity of the malate-aspartate shuttle, but this decrease may be due to limited  $O_2$  availability in the muscle, resulting in an increase in mitochondrial NADH and an accelerated cytosolic NADH production. The resulting increase in cytosolic NADH may be viewed as being necessary to create the driving force for increasing the flux of reducing equivalents from the cytosol into the mitochondria.

It is not likely that the availability of  $O_2$  is the sole determinant or a prerequisite for lactate production during exercise or any other condition. It is apparent that many factors, such as diet (40, 42), epinephrine (43), glycogen levels (72), temperature (24), enzymatic profile of the muscle, i.e., fiber type (14), pattern of fiber recruitment (5), pedal rate (63), affect lactate levels in body fluids. These factors indicate the diversity of the various modulators, or "fine tuners," of lactate metabolism. The rate of lactate production is determined by the relative kinetics of glycolysis, LDH, and mitochondrial respiration. Many factors could influence these processes *in vivo*, but from the data presented in this review it is clear that  $O_2$  availability is of major importance for mitochondrial respiratory function and thereby also for the regulation of the rate of lactate production.

### Conclusions

Within the last decade numerous reviews on lactate metabolism have been published. In many of these reviews it has been argued that lactate production during submaximal exercise is not due to hypoxia. In our view the existing experimental findings can be interpreted in an alternative manner where the  $O_2$  dependence of the metabolism during submaximal exercise is indeed due to a restricted availability of  $O_2$  in the mitochondria. Part of the apparent controversy exists because hypoxia at the cellular level can be defined in at least two ways: 1) cellular respiration is affected by  $PO_2$ , and 2) cellular metabolism is affected by  $PO_2$ . Many investigators have explicitly stated or implied that if cellular respiration is not impaired then cellular metabolism (including lactate formation) would not be dependent on  $O_2$ .

In the present review we have summarized a series of recent papers where the NADH and lactate contents in human and animal muscle have been determined under a variety of conditions. In contrast to the study by Jöbsis and Stainsby (44), where redox changes were monitored by surface fluorescence, we have shown that decreased

$O_2$  availability and submaximal exercise ( $>50\% \dot{V}O_{2 \text{ max}}$ ) result in increases in NADH that occur in parallel with increased lactate production. Thus there are both experimental data and theoretical reasons to support the idea that cellular metabolism is affected by the  $O_2$  availability long before respiration is compromised. It is proposed that under conditions of relatively limited  $O_2$  availability mitochondrial respiration is stimulated by increases in ADP,  $P_i$ , and NADH. These changes in the adenine nucleotides will favor stimulation of glycolysis and thereby increase cytosolic NADH, which will shift the LDH equilibrium toward increased lactate production.

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