Standard magnetic resonance-based measurements of the \( P_1 \rightarrow \text{ATP} \) rate do not index the rate of oxidative phosphorylation in cardiac and skeletal muscles

Arthur H. L. From\(^1,2 \) and Kamil Ugurbil\(^1,2,3 \)

\(^1\)Center for Magnetic Resonance Research and Departments of \(^2\) Medicine (Cardiovascular Division) and \(^3\) Radiology, University of Minnesota Medical School, Minneapolis, Minnesota

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From AH, Ugurbil K. Standard magnetic resonance-based measurements of the \( P_1 \rightarrow \text{ATP} \) rate do not index the rate of oxidative phosphorylation in cardiac and skeletal muscles. Am J Physiol Cell Physiol 301: C1–C11, 2011. First published March 2, 2011; doi:10.1152/ajpcell.00345.2010.—Magnetic resonance spectroscopy-based magnetization transfer techniques (MT) are commonly used to assess the rate of oxidative (i.e., mitochondrial) ATP synthesis in intact tissues. Physiologically appropriate interpretation of MT rate data depends on accurate appraisal of the biochemical events that contribute to a specific MT rate measurement. The relative contributions of the specific enzymatic reactions that can contribute to a MT \( P_1 \rightarrow \text{ATP} \) rate measurement are tissue dependent; nonrecognition of this fact can bias the interpretation of MT \( P_1 \rightarrow \text{ATP} \) rate data. The complexities of MT-based measurements of mitochondrial ATP synthesis rates made in striated muscle and other tissues are reviewed, following which, the adverse impacts of erroneous \( P_1 \rightarrow \text{ATP} \) rate data analyses on the physiological inferences presented in selected published studies of cardiac and skeletal muscle are considered.

magnetization transfer; oxidative ATP synthesis; striated muscle

MAGNETIC RESONANCE (MR) magnetization transfer techniques (MT) are commonly used for the in vitro and in vivo estimation of the rates of oxidative ATP synthesis [i.e., the net inorganic phosphate (\( P_1 \) \( \rightarrow \) ATP rate of mitochondrial ATP synthase] in heart, skeletal muscle, brain, liver, and other tissues. However, physiologically relevant interpretation of MT data depends on an accurate appraisal of the biochemical events that contribute to a specific MT rate measurement.\(^1 \)

This communication is focused on MT studies of the rate of oxidative ATP synthesis in cardiac and skeletal muscles because this technique has been employed in many studies of the physiology and pathophysiology of these tissues. The complexities of MT-based measurements of mitochondrial ATP synthesis rates made in striated muscle and other tissues are reviewed following which, the adverse impacts of erroneous \( P_1 \rightarrow \text{ATP} \) rate data analyses on the physiological inferences presented in selected published studies of cardiac and skeletal muscle are considered.

**MT As Applied to the Evaluation of Enzyme Rates: Methodological Considerations**

In MT studies of enzymatic rates, the nuclear spin of an atom in a reactant of an enzyme catalyzed process is selectively irradiated to disturb its magnetization away from the thermal equilibrium value. This perturbation subsequently migrates to the other reactants as the magnetically disturbed atom within the selectively irradiated reactant is incorporated into the other reactant(s) through chemical conversion. The selective irradiation can be applied so as to “saturate” an atomic moiety; i.e., the bulk magnetization of the saturated moiety becomes zero and this moiety is no longer detected in a MR spectrum. However, the same atom within the other reactants can be detected, albeit perturbed from its original thermal equilibrium signal intensity. Notably, the actual chemical processes (i.e., reactant distribution, chemical equilibrium, reaction rates, etc.) remain unaltered, but the “spin” equilibrium is perturbed. An alternative strategy is a selective irradiation pulse applied briefly to invert the spins of one atom in one reactant. Subsequently, these spins recover toward their thermal spin equilibrium and the recovery kinetics contain information about the intrinsic processes that induce spin relaxation as well as the chemical reaction itself.

In the ATP synthesis/hydrolysis reaction, the terminal phosphate of ATP (\( \text{ATP}_\gamma \)) becomes \( P_1 \) when the ATP molecule is cleaved to form ADP and \( P_1 \) (Fig. 1A). The phosphorus nuclear spin in the \( P_1 \) and \( \text{ATP}_\gamma \) moieties have very different resonance frequencies and, thus, can be detected as separate peaks in a \(^{31}\text{P} \) MR spectrum. However, when the thermal equilibrium magnetization of the \( \text{ATP}_\gamma \) resonance (which is directly proportional to the signal intensity in the MR spectrum) is perturbed by selective saturation, this perturbation is reflected in the signal intensity of the \( P_1 \) resonance (Fig. 1B) because when the perturbed \( \text{ATP}_\gamma \) is cleaved through ATP hydrolysis, \( P_1 \) is generated. Once within the \( P_1 \) moiety, the phosphorus nuclear spins have a different resonance frequency and are no longer subject to the direct influence of the selective saturation of \( \text{ATP}_\gamma \); therefore, they relax toward thermal equilibrium with a time constant equal to intrinsic \( T_1 \) (\( T_1; \) i.e., the \( T_1 \) that would be measured in the absence of any chemical exchange). But if

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\(^1 \) This article is the topic of an Editorial Focus by Balaban and Koretsky (1a).

Address for reprint requests and other correspondence: A. H. L. From, Center for Magnetic Resonance Research, University of Minnesota, 2021 6th Street SE, Minneapolis, MN 55455 (e-mail: fromx001@umn.edu).

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the rate of Pᵢ reincorporation into ATPγ is sufficiently fast compared with intrinsic T₁, the Pᵢ atoms reincorporated into ATPγ again experience the selective irradiation. Ultimately, a new spin equilibrium for Pᵢ is reached, reflected directly in the signal intensity of this peak in the MR spectrum (Fig. 1B). This new spin equilibrium is determined by the competition between the relaxation back to thermal equilibrium with the rate constant 1/T₁ and the rate of reconversion to ATPγ (i.e., Pᵢ + ADP → ATPγ). Thus, if the T₁ of the Pᵢ resonance is known, the unidirectional rate of the Pᵢ + ADP → ATP reaction (i.e., the unidirectional rate of ATP synthesis) can be calculated.

For simplicity, we will focus on the compounds directly detected in the magnetization transfer process and indicate this rate as the unidirectional Pᵢ → ATP rate. The necessary parameters to calculate this unidirectional rate can be measured in several different ways. For example, spectra acquired as a function of time after the onset of the selective irradiation to saturate the ATPγ peak can be used to extract both the rate of approach to the new spin equilibrium and the intensity of Pᵢ in this new spin equilibrium. Alternatively, the intensities of the new spin equilibrium can be measured in one spectrum after allowing sufficient time to establish a new steady state under saturation, and in addition, T₁ for the Pᵢ resonance in the presence of the selective saturation is separately measured. For the creatine kinase reaction, saturation of ATPγ leads to a perturbation of the phosphate moiety of phosphocreatine (PCr) (shown in Fig. 1B). This measurement then allows the determination of the unidirectional PCr → ATP rate.

An alternative method for determination of the Pᵢ → ATP rate involves selective inversion of one of the reactants (versus saturation), and, as mentioned above, has also been used. Depending on the intrinsic T₁ of the inverted or saturated resonance, one method can be more sensitive than the other. For simplicity we will focus on the MT method using saturation rather than inversion since this is the approach most commonly used in the in vivo studies.

Although the MT technique measures unidirectional rates (i.e., the number of molecules converted per unit time), one formulates the process mathematically using unidirectional rate constants (rate per unit molecule). Thus, for the unidirectional rates of A → B conversion, the apparent unidirectional rate constants are defined so that the unidirectional rates vᵢ = kᵢ[A] and vᵢ = kᵢ[A] are given by the equations vᵢ = kᵢ[A] and vᵢ = kᵢ[A][B], respectively. The designation “apparent” is used for the rate constant because, in an enzymatic reaction, the rate constant calculated in this way is a complex function of the reactants and products and does not have the straightforward meaning it would have in a nonenzymatic reaction.

Obviously, measurement of enzyme rates with MT is a powerful analytic technique. However, major interpretive complications are inherent in the method when applied in vivo. In aerobic tissues, the net rate of ATP synthesis by the mitochondrial ATP synthase (i.e., the rate of ATP synthesis through oxidative phosphorylation) is proportional to the oxygen consumption rate of that tissue by the P/O ratio. Under conditions where ATP concentrations are time independent (i.e., in a steady state), the net rates of ATP synthesis by the oxidative phosphorylation process and by other pathways (e.g., glycolysis) must be equal to the net rate of ATP utilization by all energy-consuming processes, such as muscle contraction and maintenance of ion gradients, etc. Ignoring for the time being possible contributions by all other pathways (like glycolysis) that can contribute to ATP synthesis, and assuming that all ATP synthesis is oxidative, we can consider the problem as composed of two unidirectional rates of the mitochondrial ATP synthase (in the Pᵢ → ATP and ATP → Pᵢ directions) and the rate of ATP utilization by energy-driven cellular processes, which operates unidirectionally in the ATP → Pᵢ direction. In this case, the difference between the two unidirectional rates of the mitochondrial ATP synthase (i.e., the net ATP synthesis rate) must be equal to the rate of ATP utilization under steady-state conditions. In other words,

\[ F_{\text{NET Cell ATPsynthesis}} = F_{\text{NET Mitochondria ATPsynthesis}} = v_{\text{mitochondria}}^{\text{mitochondria}} - v_{\text{mitochondria}}^{\text{mitochondria}} = v_{\text{ATP→Pᵢ}}^{\text{utilization}} \]

where \( F_{\text{NET Cell ATPsynthesis}} \) is the net intracellular ATP synthesis rate, and \( F_{\text{NET Mitochondria ATPsynthesis}} \) is defined as \( (v_{\text{Pᵢ→ATP}}^{\text{mitochondria}} - v_{\text{ATP→Pᵢ}}^{\text{mitochondria}}) \). It is a powerful analytic technique. However, major interpretive complications are inherent in the method when applied in vivo. In aerobic tissues, the net rate of ATP synthesis by the mitochondrial ATP synthase (i.e., the rate of ATP synthesis through oxidative phosphorylation) is proportional to the oxygen consumption rate of that tissue by the P/O ratio. Under conditions where ATP concentrations are time independent (i.e., in a steady state), the net rates of ATP synthesis by the oxidative phosphorylation process and by other pathways (e.g., glycolysis) must be equal to the net rate of ATP utilization by all energy-consuming processes, such as muscle contraction and maintenance of ion gradients, etc. Ignoring for the time being possible contributions by all other pathways (like glycolysis) that can contribute to ATP synthesis, and assuming that all ATP synthesis is oxidative, we can consider the problem as composed of two unidirectional rates of the mitochondrial ATP synthase (in the Pᵢ → ATP and ATP → Pᵢ directions) and the rate of ATP utilization by energy-driven cellular processes, which operates unidirectionally in the ATP → Pᵢ direction. In this case, the difference between the two unidirectional rates of the mitochondrial ATP synthase (i.e., the net ATP synthesis rate) must be equal to the rate of ATP utilization under steady-state conditions. In other words,

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Fig. 2. This schematic illustrates the point that the magnetization transfer technique (MT)-determined \( \frac{P_{i} \rightarrow ATP}{rate} \) is equal to the net rate of oxidative ATP synthesis if and only if the mitochondrial ATP synthase operates far out of equilibrium (i.e., \( v_{\text{ATP} \rightarrow P} \approx 0 \)). Otherwise, \( MT \text{exp} \gg NET \text{Mitochondria} \). Note that the rate calculated by the product of the oxygen consumption rate and the P/O ratio, which we can designate as \( F_{\text{Oxy Cons}} \), is always equal, by definition, to \( F_{\text{ATP Synthesis}} \). Consequently, \( F_{\text{Oxy Cons}} = MT \text{exp} \) if and only if the mitochondrial ATP synthase operates far out of equilibrium.

However, there is the potential confounding factor of additional contributions to the \( P_{i} \rightarrow ATP \) rate from enzymes and pathways other than ATP synthase. Thus, we must consider

\[
MT \text{exp} \Rightarrow NET \text{Cell ATP Synthesis} = NET \text{Other ATP Synthesis} + NET \text{Mitochondria} = v_{\text{utilization}} \frac{ATP}{P} - P_{i}^{\text{mitochondria}}
\]

or

\[
NET \text{Cell ATP Synthesis} = [(v_{\text{other}} P_{i} \rightarrow ATP) + (v_{\text{mitochondria}} P_{i} \rightarrow ATP)] - [(v_{\text{other}} P_{i} \rightarrow ATP) + (v_{\text{mitochondria}} P_{i} \rightarrow ATP)] = v_{\text{utilization}} \frac{ATP}{P} - P_{i}^{\text{mitochondria}}
\]

In this more general case, the experimentally measured rate by MT, \( MT \text{exp} \), is equal to \( [(v_{\text{other}} P_{i} \rightarrow ATP) + (v_{\text{mitochondria}} P_{i} \rightarrow ATP)] \). Notably, the net rate of ATP synthesis of all other pathways may be negligible compared with net rate of aerobic ATP synthesis, and, hence, insignificant with regard to the energy balance and ATP turnover of that tissue; yet, the unidirectional ATP synthesizing enzymes within that pathway may be very fast and may make a major contribution to the MT-measured unidirectional rate (e.g., Refs. 21 and 43 and references therein). Expressing this mathematically using the rate equations above, \( NET \text{other ATP Synthesis} = [(v_{\text{other}} P_{i} \rightarrow ATP) - (v_{\text{other}} P_{i} \rightarrow ATP)] \approx 0 \) can be the case in the tissue but \( v_{\text{other}} P_{i} \rightarrow ATP \) can be comparable or even larger than \( v_{\text{mitochondria}} P_{i} \rightarrow ATP \) so that the experimentally measured rate cannot be equated with \( v_{\text{mitochondria}} P_{i} \rightarrow ATP \) alone. Under these circumstances, although the apparent rate constant and unidirectional rate measurements may be accurate, they represent the sum of the unidirectional rates of all of the enzymes generating the MT effect (Fig. 2). As discussed in greater detail later, this is in fact the case in cardiac muscle. In the fortunate case that one of these component unidirectional reactions is much faster than all others, the measured rate can (reasonably) be assigned to that specific reaction. However, it is critically important to realize that this situation cannot, a priori, be assumed to be applicable. Thus, the rate calculated from the oxygen consumption rate multiplied by the P/O ratio is not necessarily equal to the experimentally measured rate by MT when selectively saturating ATPy.

The general case is that

\[
MT \text{exp} = [(v_{\text{other}} P_{i} \rightarrow ATP) + (v_{\text{mitochondria}} P_{i} \rightarrow ATP)] = [(v_{\text{other}} P_{i} \rightarrow ATP) + (F_{\text{Oxy Cons}} + v_{\text{mitochondria}} P_{i} \rightarrow ATP)]
\]

and thus \( MT \text{exp} > F_{\text{Oxy Cons}} \). To claim that \( MT \text{exp} = F_{\text{Oxy Cons}} \) requires that all other pathway contributions to the unidirec-
tion and catecholamine infusion. Furthermore, although the apparent P/O fell moderately as $\text{MV}_2\text{O}_2$ increased, it remained >4 at the highest cardiac work state achieved. One possible explanation was that, in the intact heart, mitochondrial ATP synthase had a significant rate in the ATP→$P_i$ direction. Hence, although the net rate of mitochondrial ATP synthesis must be proportional to $\text{MV}_2\text{O}_2$ by the P/O ratio, the two unidirectional $P_i$→ATP and ATP→$P_i$ rates can each be much larger than the net $P_i$→ATP rate. This speculation was theoretically possible because ATP synthase had long been known to be capable of generating a significant ATP→$P_i$ rate under some experimental conditions and this enzyme had also been considered to be “near equilibrium” in intact myocardium (12). If our early speculation had been correct, our data would have predicted that, as ATP demand increased, the net $P_i$→ATP rate increased solely as a consequence of a progressively decreasing ATP→$P_i$ rate through ATP synthase.

However, we soon realized that another potential contributor to the measured unidirectional $P_i$→ATP rate in myocardium was the glyceraldehyde dehydrogenase (GAPDH)/phosphoglycerate kinase (PGK) enzyme couple. In 1987, Brindle and Radda (5) reported data obtained from the study of isolated GAPDH/PGK enzymes (the assay milieu employed mimicked conditions thought to be present in the cytosol of cardiomyocytes) and we (21, 43) also reported new data obtained in the perfused rat heart bearing on this issue. The data in these reports strongly supported the view that the coupled GAPDK/PDK reaction pair operates near equilibrium (i.e., the forward and backward unidirectional rates of this enzyme couple are approximately equal) and both of these unidirectional rates are large and far exceeded the rates of net ATP production by and of carbon flux through the glycolytic pathway. These considerations led us to hypothesize that, in the myocardium, it might be impossible to evaluate the $P_i$→ATP rate through the mitochondrial ATP synthase in the presence of active glycolysis (i.e., when the GAPDH/PGK couple was active) even though the net ATP production rate by the glycolytic pathway is far overshadowed by the rate of oxidative ATP synthesis in this highly oxidative tissue. In short, we hypothesized that ATP synthase contributed only a fraction of the MT-measured $P_i$→ATP rate and that another large fraction was contributed by the GAPDH/PGK couple (Fig. 2).

To test this hypothesis, the perfused rat heart experiments were repeated while glycolysis was inhibited (by treatment with iodoacetate or by glycogen depletion induced by a preceding period of carbon substrate-free perfusion) (21, 43). In these studies, the perfusion medium was glucose free and contained pyruvate (in lieu of glucose) to support oxidative metabolism. Under these experimental conditions, the MT-measured unidirectional $P_i$→ATP rate increased in concert with increasing $\text{MV}_2\text{O}_2$ and the P/O ratio calculated from the MT data was ∼2.5 during the baseline work state and did not change as $\text{MV}_2\text{O}_2$ values increased (Fig. 3A, dashed-dotted line). These data indicated that the GAPDH/PGK couple did make a substantial contribution to the MT-measured $P_i$→ATP rate when glycolysis was active. Hence, when GAPDH/PGK activity was eliminated, the MT-determined $P_i$→ATP rate was proportional to $\text{MV}_2\text{O}_2$ by the P/O ratio. As discussed previously, this is possible if and only if no other pathways make a significant contribution to the MT-determined unidirectional $P_i$→ATP rate, and 2) if and only if the mitochondrial ATP synthase operates essentially unidirectionally (i.e., far out of equilibrium) in the $P_i$→ATP direction. That a glycolytic contribution to the MT-measured unidirectional $P_i$→ATP rates could occur was also shown in studies of Escherichia coli and yeast (6).

Figure 3B shows our concept of how the rates of each of the two subcomponents of the measured $P_i$→ATP flux change as $\text{MV}_2\text{O}_2$ increases in the glucose-perfused heart. The oxidative $P_i$→ATP rate due to mitochondrial ATP synthase at any given level of $\text{MV}_2\text{O}_2$ is expected to be essentially the same irrespective of whether glycolysis is active or inhibited. Hence, the observation that when glycolysis is active, the measured $P_i$→ATP rate is high at the lowest work state and does not increase further in conjunction with increasing $\text{MV}_2\text{O}_2$ (Fig. 3B, solid line) indicates on stoichiometric grounds that the GAPDK/PDK rate contribution to the measured $P_i$→ATP rate decreases moderately as $\text{MV}_2\text{O}_2$ increases (Fig. 3B, dashed-dotted line). In contrast, the mitochondrial $P_i$→ATP rate contribution increases (Fig. 3B, dashed-dotted line) just as shown in the studies in which glycolysis was blocked (Fig. 3A, dashed-dotted line). We cannot explain why, in the perfused heart, the absolute contribution of the GAPDH/PGK couple to the measured $P_i$→ATP flux decreases as $\text{MV}_2\text{O}_2$ increases; however, as will be seen later, this phenomenon does not appear to occur in autoperfused rat hindlimb muscles.

Using these methods for determining the “true” P/O in an intact tissue, we subsequently directly determined the extent of mitochondrial uncoupling induced by administration of high concentrations of fatty acids or dinitrophenol in perfused rat hearts (22). In another report, we also demonstrated that a period of nonlethal global ischemia sufficient to cause myocardial stunning did not necessarily induce mitochondrial uncoupling (37) as had been previously proposed. Importantly, these MT studies strongly supported the concept that, in intact myocardium operating under conditions of adequate oxygen and carbon substrate availability, there was not a significant reverse rate (i.e., in the ATP→$P_i$ direction) through mitochondrial ATP synthase.

When we extended these studies to in vivo canine hearts (with intact glycolysis), we found that the glycolytic contribution to the measured unidirectional $P_i$→ATP rate was also substantial as indicated by the presence of an unphysiologically high P/O (36). Portman (33) confirmed our finding that the GAPDH/PGK contribution to the $P_i$→ATP rate was quite high in in vivo canine hearts and did not increase further as the mitochondrial ATP synthase induced by administration of high concentrations of fatty acids or dinitrophenol in perfused rat hearts (22). In another report, we also demonstrated that a period of nonlethal global ischemia sufficient to cause myocardial stunning did not necessarily induce mitochondrial uncoupling (37) as had been previously proposed. Importantly, these MT studies strongly supported the concept that, in intact myocardium operating under conditions of adequate oxygen and carbon substrate availability, there was not a significant reverse rate (i.e., in the ATP→$P_i$ direction) through mitochondrial ATP synthase.

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Our Cardiac Pi→ATP Rate Data Are Consistent With Findings in Isolated Mitochondria

Using radioactive tracers, LaNoue et al. (24) showed that the ATP→Pi rate was very low in fully energized (isolated) cardiac mitochondria undergoing state 3 respiration. In contrast, under state 4 respiratory conditions, where ADP availability was limited but carbon substrate and oxygen availability were not limiting, ATP in the incubation medium was rapidly hydrolyzed to form ADP and Pi via ATP synthase. Because mitochondria in perfused working hearts appear to be working under “state 3-like” conditions, our data were consistent with the data obtained in isolated mitochondria. This concordance supports our conclusion that, in the perfused heart, the mitochondrial ATP→Pi rate was minimal and that the MT-measured unidirectional rate did reflect the net rate of mitochondrial ATP synthesis so long as glycolysis was inhibited.

How Do These Considerations Relate to the Possible Presence of Significant ATP→Pi Rates in Resting Skeletal Muscle?

Although rate data obtained in skeletal muscle will be discussed in considerable detail later, to our knowledge, no direct measurements of mitochondrial derived ATP→Pi rates have been made in resting skeletal muscles. As pointed out, LaNoue et al. (24) showed that there was a considerable ATP→Pi + ADP rate in isolated mitochondria operating under state 4 conditions and essentially none when state 3 conditions were present. In resting skeletal muscle, ATP synthetic rates are limited by ADP availability because the rate of ATP expenditure is low and carbon substrate and oxygen availability are not limiting (7). Hence, mitochondrial function in resting skeletal muscle may be more comparable to that present in isolated mitochondria. This concordance supports our conclusion that, in the perfused heart, the mitochondrial ATP→Pi rate was minimal and that the MT-measured unidirectional rate did reflect the net rate of mitochondrial ATP synthesis.

As previously discussed, the net rate of ATP synthesis must equal the sum of the forward and reverse reactions. Because the Pi→ATP synthase is unidirectional in the mitochondria, the ATP→Pi rate must exceed the net rate of mitochondrial ATP synthesis even during state 4 respiratory conditions, where ADP availability was limited. However, when experimental conditions are nonphysiological and these conditions are present in the oxygenated perfused heart or in resting human skeletal muscle.

Tissue Heterogeneity As a Confounding Factor

Our work and that of other investigators in the field have been (implicitly) premised on the concept that metabolic behavior described is homogeneous within the voxel or region sampled. However, as is well known, there is evidence of macroheterogeneity within large skeletal muscles (18) and microheterogeneity within the heart (10). This phenomenon can (to an unknown extent) complicate interpretation of data acquired using MR methods. However, a detailed discussion of this issue exceeds the scope of this review. Nevertheless, in the brain, the observation that MT-measured Pi→ATP rate in the brain does track the rate of oxidative phosphorylation may result from tissue heterogeneity (see discussion further on).

Problematic Interpretation of Pi→ATP Rate Data in a Perfused Heart Study

In a perfused guinea pig heart study examining the response of myocardial high-energy phosphate compound metabolism to nitro-l-arginine methyl ester (l-NAME) inhibition of nitric oxide synthase (NOS) (40), MT-measured Pi→ATP rates and measured MV\text{O}_2 values were used to evaluate the hypothesis that inhibition of NOS decreased energetic efficiency. In that report, efficiency was defined as the ratio of the MT-measured Pi→ATP rate to the measured MV\text{O}_2 value. The P/O ratio reflects mitochondrial efficiency and uncoupling reduces the efficiency of oxidative phosphorylation and thus the P/O ratio. However, as previously discussed, the ratio of the MT-measured unidirectional P_i→ATP rate to the measured MV\text{O}_2 value is equal to the P/O ratio when there are no other reactions, like GAPDH/PKG, contributing to the MT measurement and 2) if mitochondrial ATPase operates virtually unidirectionally in the P_i→ATP direction. The first condition is unlikely to have been met in this study because their
perfusionate carbon substrate was 11 mM glucose. Hence, the GAPDH/PGK couple must have been quite active even if it is assumed that cardiac metabolism was also being partially supported by utilization of endogenous lipids (23).

It was observed that the mean values of the MT-determined P¡→ATP rates were the same in the control and l-NAME groups although VO2 was significantly higher in the latter group. If the P¡→ATP rate measurements actually represented only the rate of oxidative ATP synthesis, then these data would imply the presence of some degree of mitochondrial uncoupling when NOS was inhibited. However, the VO2 values estimated from their Figure 4 [(40); ~25 and 29 µmol·g dry wt·min·1, respectively, for control and l-NAME treated hearts] can be used to calculate rates of oxidative ATP synthesis assuming a P/O of 2.5. Both groups of hearts were studied at heart rate-systolic pressure product (RPP) rates of ~22,000 mmHg/min and the ATP synthetic rates calculated from the VO2 data are ~125 and ~145 µmol·g dry wt·min·1 for the control and l-NAME-treated groups, respectively. Yet the means of the MT-measured P¡→ATP rates attributed to oxidative phosphorylation were ~3.5 and 3.8 mM/s in the control and treatment groups, respectively. When the latter rates are converted to the same units used for our VO2-based ATP synthase rate calculations [assuming cell water to be ~65% of tissue weight (16)], the measured unidirectional P¡→ATP rates for the two groups were ~1,050 and 1,140 µmol·g dry wt·min·1. These values are substantially higher than the rate of oxidative ATP synthesis estimated from the VO2-based calculations. As is evident, under the described experimental conditions, these unidirectional MT-based P¡→ATP rate data were inappropriately used to infer the presence of mitochondrial uncoupling because the ATP synthase-derived component of the measured P¡→ATP rates was substantially smaller than their GAPDH/PGK-associated P¡→ATP rate components, as we had previously reported (21, 43). Although acute inhibition of NOS could have caused some mitochondrial uncoupling and accounted for the small differences in VO2 between the groups, the P¡→ATP rate data reported cannot be used to support this hypothesis.

**MT Studies in Liver and Brain Further Illustrate the Presence of Tissue-Related Variations in the Size of the Glycolytic Contribution to the Measured P¡→ATP Rate**

**Liver.** A study performed in our laboratory by Thoma and Ugurbil (42) is instructive with regard to the tissue variability of the magnitudes of the glycolytic and mitochondrial contributions to the MT-determined unidirectional P¡→ATP rate. In the perfused liver (in which it is possible to accurately measure the rate of O2 consumption), it was found that MT-measured unidirectional P¡→ATP rate was almost entirely of glycolytic origin. In a similar (but noninvasive) study performed in human subjects, Schmid et al. (39) reported that the MT-measured P¡→ATP rate in the liver was ~29 mM/min. These authors pointed out, based on published estimates of in vivo human hepatic oxygen consumption and an assumed P/O ratio of 2, that glycolysis accounted for ~75% of the MT-measured unidirectional P¡→ATP rate.

**Brain.** Lei et al. (26) (also from our laboratory) have reported P¡→ATP rate measurements in the human visual cortex. In contrast to observations made in the liver and heart, estimates of the P¡→ATP rates calculated from published measurements of brain oxygen consumption rates were comparable to the MT-measured P¡→ATP rates. These findings were confirmed in a subsequent study in which MT-measured P¡→ATP rates and estimates of the oxidative ATP synthesis rate made from concomitant 31C measurements of the TCA cycle rate were also shown to be concordant in the human brain (11). Hence, the brain may be at the other extreme (as compared with liver) with regard to the degree that MT measurements of P¡→ATP rate reflect the activity of mitochondrial ATP synthase alone.

The actual reason(s) why MT-determined P¡→ATP rates in brain appear to be concordant with non-MT-based rate measurements is unknown. The authors speculated that the presence of two different primary cell types in the brain, i.e., neurons and astrocytes, might alter the results of the MT studies. It has been proposed that the main oxidative carbon substrate utilized by neurons might be lactate that is glycolytically generated by astrocytes (30). If so, then glycolytic activity mediating a P¡→ATP rate might be predominantly sequestered in the astrocytes (and other glial cells of the brain) while the MT-measured P¡→ATP flux measured in the brain might emanate primarily from neurons. The latter would be possible, for example, if the P¡ levels were higher in neurons (as compared with glial cells) and the P¡ content of glial cells were too low to be detectable in 31P MR spectra of the brain. In this construct, the P¡ detected in brain would arise mainly from neurons and the MT-measured P¡→ATP rate would be of neuronal origin while the major glycolysis associated GAPDK/PGK contribution in the P¡→ATP direction would occur in astrocytes (and other glial cells) and be “MR invisible.”

**Unidirectional P¡→ATP Rate Measurements in Skeletal Muscle**

**Animal studies.** One of the earliest (and most frequently cited) studies of MT-determined P¡→ATP rates in skeletal muscle was carried out by Brindle et al. (4). Because the data and analyses presented in this paper serve as the template on which virtually all subsequent MT-based P¡→ATP rate measurement studies of skeletal muscle are based, we analyzed it in some detail. This paper reported the effects of isometric contractions of increasing duration and frequency on MT-measured P¡→ATP rates in the (auto)perfused hindlimb muscles of anesthetized rats. The P¡→ATP rate was found to increase in concert with the tension-time index (an index of contractile energy expenditure; TTI). However, in resting muscle, a substantial (rather than a modest) unidirectional P¡→ATP rate was also present although the TTI was zero.

As already mentioned, Brindle and associates (5) had previously studied (in vitro) the capacity of GAPDH/PGK couple to generate a MT-detectable P¡→ATP rate. Thus, these authors analyzed their data with the possibility in mind that the GAPDH/PGK rate might contribute to their measurements of the P¡→ATP rate. On the basis of calculations using their TTI data and the oxygen consumption data obtained in rat skeletal muscle by Hood et al. (15), they concluded that the observed work-associated increase of measured P¡→ATP rate probably did not contain a major GAPDH/PGK component.

This conclusion is puzzling because the methods section of the Brindle et al. (5) report indicates that the study rats were
not fasted before the start of the experiments. Hence, blood fatty acid levels were likely not as high as they would have been in fasted animals and the blood insulin levels were probably high enough to foster significant glucose uptake in resting skeletal muscle. Hence, a significant GAPDH/PGK contribution to the unidirectional \( P_i \rightarrow ATP \) rate measurement would have been expected to be present in both the resting and exercising muscles. Furthermore, because resting rat hindlimb oxygen consumption rates were known to be very low (15), the rate of mitochondrial oxidative ATP synthesis must also have been commensurately low. However, Brindle et al. reported a resting muscle \( P_i \rightarrow ATP \) rate measurement of 0.8 \( \mu \text{mol·g dry wt}^{-1}·\text{s}^{-1} \). As will be shown, this value is much higher than ATP synthetic rates calculated from the data of Hood et al. (15), who directly measured oxygen consumption rates in glucose buffer-perfused resting rat hindlimbs at rest and during a progressive sciatic nerve stimulation protocol. The latter group’s resting muscle oxygen consumption measurements averaged ~0.37 \( \mu \text{mol·g dry wt}^{-1}·\text{min}^{-1} \). This can be converted into the same rate units used in the Brindle et al. paper by assuming a P/O of 3 and a tissue wet-to-dry ratio of 4.35. This calculation yields a value of 0.16 \( \mu \text{mol·g dry wt}^{-1}·\text{s}^{-1} \) of oxidative ATP synthesis as compared with the value of 0.8 \( \mu \text{mol·g dry wt}^{-1}·\text{s}^{-1} \) reported by Brindle et al. In other words, the net ATP synthesis rate calculated from published oxygen consumption data approximated ~20% of the MT-measured \( P_i \rightarrow ATP \) rate reported by Brindle et al. This implies that the rate measured in the resting muscle in the latter report likely did include a major contribution from the GAPDH/PGK couple and/or the mitochondrial ATPase had a significant unidirectional rate in the ATP→ \( P_i \) direction.

During the stimulation protocol, the TTI values reported in the Brindle et al. paper ranged from ~0.05 to ~0.35 N-s \(^{-1}\cdot\text{cm}^{-2} \); i.e., TTI increased sevenfold from the lowest to the highest stimulation induced work state. Over the same TTI range, the reported MT-measured \( P_i \rightarrow ATP \) rate increased by approximately ~2.8-fold. However, when the estimated resting muscle \( P_i \rightarrow ATP \) rate component that we presume was generated by glycolysis (0.64 \( \mu \text{mol·g dry wt}^{-1}·\text{s}^{-1} \); see calculations above) is subtracted from each of the data points obtained during muscle stimulation, the fold increase in \( P_i \rightarrow ATP \) rate over the TTI range increased to ~5.2-fold. This “corrected” estimate of the magnitude of the \( P_i \rightarrow ATP \) rate increase from the lowest to the highest stimulation rates approximates the expected increase of the rate of oxidative ATP synthesis if one assumes linearity between the TTI and oxygen consumption as do the authors. In contrast, the fold increase calculated from the “uncorrected” data in the report markedly underestimates the increase in the \( P_i \rightarrow ATP \) rate generated by ATP synthase during the stimulation protocol.

Our reanalysis of these data, taken together with the \( O_2 \) consumption data reported by Hood et al. (15), suggests that, in resting rat skeletal muscle, 1) the measured unidirectional \( P_i \rightarrow ATP \) rate contains a major GAPDH/PGK component and/or mitochondrial ATPase operates with a significant unidirectional rate in the ATP→ \( P_i \) direction, as reported for isolated mitochondria respiring under state 4 conditions; and 2) net rates of both glycolytic and oxidative ATP synthesis are low. During the muscle stimulation protocol, 1) the glycolytic contribution to the overall unidirectional \( P_i \rightarrow ATP \) rate measurement (unlike the oxidative contribution) and/or a possible unidirectional mitochondrial ATPase rate in the ATP→ \( P_i \) direction (24) may not have changed much, and 2) the component of the total unidirectional \( P_i \rightarrow ATP \) rate associated with mitochondrial ATPase increased appropriately in concert with the TTI.

In a different study of oxidative ATP synthesis in resting rat skeletal muscle, MT evaluation of the \( P_i \rightarrow ATP \) rate was done in concert with a \( ^{13} \text{C} \) MR-based measurement of the TCA cycle rate. It was reported that the transition from the fed to fasted state was not associated with mitochondrial uncoupling as evidenced by a lack of change of the ratio of the MT-measured \( P_i \rightarrow ATP \) rate to the TCA cycle rate (17). In another study by this group, the effects of triiodo-l-thyronine or dinitrophenol (the latter a classical mitochondrial uncoupling agent) were examined in resting rat skeletal muscle by using MT and \( ^{13} \text{C} \) methods (16). In that report, reductions of the ratio of the \( P_i \rightarrow ATP \) rate to the TCA cycle were observed with both interventions and this was claimed to be evidence of the presence of mitochondrial uncoupling. However, in both of the aforementioned reports, our calculations of oxidative ATP synthesis rates (from their measured TCA cycle turnover rates) yielded \( P_i \rightarrow ATP \) rate values that were far lower than those estimated from their MT data. Hence, it is likely that, in these experiments, a significant GAPDH/PGK contribution to the unidirectional \( P_i \rightarrow ATP \) rate and/or a significant unidirectional mitochondrial ATPase rate in the ATP→ \( P_i \) direction was present. This complication, in our view, invalidates their claimed demonstration of increased mitochondrial uncoupling in these muscles despite the fact that some degree of uncoupling was undoubtedly present following the cited pharmacological interventions.

Additional reports such as those of Yerby et al. (46) have also claimed that MT-based \( P_i \rightarrow ATP \) rates determined in resting rat skeletal muscle reflect the rate of oxidative ATP synthesis. However, their data analysis is also based on that presented by Brindle et al. (4). Notably, the measured \( P_i \rightarrow ATP \) rates in all groups studied in the Yerby et al. report were approximately three to six times higher than oxidative ATP synthesis rates calculated from the resting rat skeletal muscle oxygen consumption data reported by Hood et al. (15). The conclusions presented in the Yerby et al. report were challenged in a Letter to the Editor (19) whose authors pointed out the discrepancies between the MT-determined \( P_i \rightarrow ATP \) rate measurements and previous estimates of oxidative ATP synthesis rate made from either oxygen consumption or \( ^{13} \text{C} \) MRS TCA cycle turnover rate measurements.

**Human Skeletal Muscle**

**Relevant physiological characteristics of human skeletal muscle.** In resting human skeletal muscle, intracellular \( O_2 \) levels are high (34) and carbon substrate availability is non-limiting; furthermore, as pointed out above, regulation of oxidative ATP synthesis appears to be ADP (and \( P_i \)) limited in resting skeletal muscle (7).

**Oxygen consumption of resting human skeletal muscle.** Data reported by Richardson and Saltin (35) allowed us to calculate the oxygen consumption rate of resting human quadriceps muscle (i.e., ~0.39 \( \mu \text{mol·g wet wt}^{-1}·\text{min}^{-1} \)). In that study, thigh arteriovenous oxygen content differences, muscle blood flows, and muscle volume were measured and it was assu-
med that almost all blood flow and other data obtained were derived from the quadriceps. However, some of their reported blood flow and oxygen uptake values may have arisen from tissues in the leg other than the quadriceps. Hence, their estimate of quadriceps oxygen uptake may have been somewhat high. From these data (assuming an operational P/O ratio of 3), the oxidative ATP synthetic rate can be estimated to be ~2.4 μmol·g·wt⁻¹·min⁻¹ in the resting quadriceps. In another study in which human vastus lateralis muscle blood flow and oxygen uptakes were measured by means of positron emission tomography, the estimated resting muscle oxygen uptake ranged between ~0.04 and 0.08 μmol·g·wt⁻¹·min⁻¹ depending on whether the subject was untrained or trained and on which region of the muscle was sampled (18). The oxidative ATP synthetic rates calculated from these oxygen consumption measurements (again assuming a P/O of 3) ranged from ~0.24 and 0.48 μmol·g·wt⁻¹·min⁻¹. The true rate of human resting quadriceps muscle oxygen consumption (and the net rate of oxidative ATP synthesis estimated from this value) likely resides somewhere between the values stated in the aforementioned reports. For the purposes of the discussion of the human studies cited below, the resting quadriceps oxygen consumption values calculated from data reported by Richardson and Saltin (the largest literature estimate that we found) will be used for comparison with MT-derived Pᵢ→ATP rate measurements.

**MT-Based Pᵢ→ATP Rates in Resting Human Skeletal Muscle**

As noted above, the oxidative ATP synthetic rate calculated from the oxygen consumption rate present in resting quadriceps muscle was ~2.4 μmol·g·wt⁻¹·min⁻¹. These values are far below the range of the MT-derived ATP synthesis rates (~8–14 μmol·g·wt⁻¹·min⁻¹) reported by Szendroedi et al. (41) and Petersen et al. (31) in the quadriceps of awake normal human subjects and also in the nondiabetic offspring of type 2 diabetes mellitus (DM2) patients. Because the muscle oxygen consumption rate is the “gold standard” for indirect estimation of the rate of oxidative ATP synthesis (assuming normal mitochondrial coupling), it is clear that, in these reports, the MT Pᵢ→ATP rate values far exceeded the rates calculated from the aforementioned oxygen consumption data.

The view that the uncorrected MT-based Pᵢ→ATP rate measurements in resting skeletal muscle markedly overestimate the true rate of oxidative ATP synthesis is also supported by recent data reported by Befroy et al. (1). In that study, the turnover rates of the TCA cycle (using ¹³C MR methods) were measured in resting skeletal muscle of both normal subjects and in the nondiabetic offspring of DM2 patients. It was reported that the TCA cycle turnover rates were significantly lower in the offspring of the diabetics than in normal subjects (~60 vs. 96 μmol·g·wt⁻¹·min⁻¹, respectively), suggesting that the rate of oxidative ATP production was lower in the former. Oxidative ATP synthetic rates of 0.9 and 1.4 μmol·g·wt⁻¹·min⁻¹, respectively, can be calculated from the TCA cycle rate data cited by assuming a stoichiometry of 15 molecules of ATP synthesized from the reducing equivalents produced during each turn of the TCA cycle. This calculation is actually a modest underestimate of the rate of cytochrome oxidase catalyzed oxygen consumption because both the glucose and the fatty acid metabolic pathways themselves produce reducing equivalents that also contribute to the rates of mitochondrial ATP synthesis rates. However, in the normal subjects (despite the aforementioned simplification of our calculations), the reported TCA cycle based estimates of the rate of oxidative ATP synthesis are near the oxidative ATP synthetic rate calculated from the aforementioned oxygen consumption data (~2.4 μmol·g·wt⁻¹·min⁻¹) obtained in resting human skeletal muscle (35). In contrast, the estimates of the Pᵢ→ATP rates from the reported TCA cycle data (1) were markedly lower than the MT-acquired Pᵢ→ATP rate values previously reported in comparable groups of subjects (31, 41). We do not dispute the possibility that the true mitochondrial Pᵢ→ATP rate (i.e., the rate estimated from the TCA cycle data) may be lower in the muscle of insulin insensitive subjects. However, whether “mitochondrial inadequacy” (our term) is the basis of this difference is questionable as will be argued next.

**Can Mild to Moderate Reductions of the (Tissue) Mitochondrial Vₘₐₓ for Oxidative ATP Synthesis Affect the Oxidative Pᵢ→ATP Rate in Resting Skeletal Muscle?**

Decreased mitochondrial density in the skeletal muscle of DM2 subjects and their offspring has been reported (28). However, in a study that examined the functional consequences of this finding in more detail, it was found that although the Vₘₐₓ value for oxidative ATP synthesis was reduced in muscle homogenates obtained from patients with DM2, the Vₘₐₓ values for ATP synthesis per milligram of mitochondrial protein extracted from the homogenates were normal (3). These data indicate that, although the tissue Vₘₐₓ for ATP synthesis is modestly reduced in these subjects, the ATP synthetic capacity of individual mitochondria is not reduced (27).

Given the aforementioned data, the question arises whether a modest reduction of the tissue Vₘₐₓ for oxidative ATP synthesis (i.e., per mg of muscle) could limit the rate of oxidative ATP generation present in either resting or even during submaximally exercising skeletal muscle. On the basis of considerations of enzyme kinetic theory, the answer to this question is likely no. This theory posits that the Vₘₐₓ of a given quantity of an enzyme (E) is an indication of its maximum turnover rate if this measurement is obtained under conditions where substrate availability is in excess and product inhibition is minimal (i.e., during the initial phase of the reaction). However, in a cuvette, under conditions in which the actual turnover rate of E is constrained because the concentration of a substrate is significantly lower than the enzyme Kₘ for that substrate, then increasing concentration of that substrate will speed up the enzyme turnover rate. Hence, at the markedly submaximal level of ATP demand present in a resting muscle, a mildly reduced mitochondrial content would not limit the rate of ATP synthesis because of kinetic compensatory mechanisms such as 1) increased mitochondrial reducing equivalent (NADH or FADH₂) availability, 2) increased cytosolic ADP concentration ([ADP]), and 3) other mechanisms (see Ref. 13 for discussion of this issue). Furthermore, it is also well known that the turnover rates of the TCA cycle and ATP synthase are influenced by the availability of intracellular [Ca²⁺] and that mean cytosolic [Ca²⁺] in striated muscle increases with increasing intensity of muscle activity. Hence, in working muscle this could also compensate (at submaximal rates of muscle
ATP expenditure) for modest reductions in muscle mitochondrial density. The expected result of these compensatory phenomena would be the maintenance of the balance between ATP synthesis and ATP utilization rates. Hence, in resting skeletal muscle, the main observable “energetic” consequence of modestly reduced tissue mitochondrial density would be expected to be increased [ADP] and [Pi] and reduced [PCr] in the cytosol. As will be seen later, these changes do not appear to be present in human subjects with DM2.

We have reported related energetic compensatory phenomena in studies of perfused rat hearts in which we showed that the relationship between intracellular [ADP] and MV02 (at any observed MV02 value) was inconstant and that it was dependent on the composition of perfusate carbon substrate (13). In that report, it was presumed that the variable relationship between [ADP] and MV02 was due to the differences in the concentrations of mitochondrial reducing equivalents present under the different substrate conditions.

The relevance of these considerations to the specific case of skeletal muscle with decreased insulin sensitivity is that they indicate that a reduction of the (extremely submaximal) rate of oxidative ATP synthesis (see aforementioned TCA cycle rate data) present in resting skeletal muscle cannot be a consequence of a modest reduction of mitochondrial numbers and/or even a modest reduction of the Vmax of individual mitochondria. More likely is that the reduction of the TCA cycle rate observed in insulin-insensitive skeletal muscle indicates that the rate of muscle ATP utilization in these subjects is for some reason decreased.

In additional reports that employed MT measurements of P1→ATP rates to assess the rate of oxidative ATP synthesis resting in human skeletal muscle, data analysis also appears to be problematic. In one (25), the effects of hyperthyroidism (induced by exogenous thyroid hormone administration) on mitochondrial coupling were examined. In the mildly hyperthyroid experimental subjects, TCA cycle turnover rates in resting muscle were increased by ~40% as compared with those of control subjects while the MT-determined Pi→ATP rate was comparable in both groups. In consequence, the amount of mitochondrial activity in the Pi→ATP enzyme couple and/or arose because of the presence of significant amount of mitochondrial activity in the P1→ATP direction.

Similarly, in another study that employed the same analytic methods (2), mitochondrial uncoupling was reported to be increased in healthy, endurance trained (as compared with untrained) human resting skeletal muscle. However, comparison between the rates of oxidative ATP synthesis measured by MT and TCA cycle rates measured with 13C MR techniques yielded disparities similar to those present in the study of the mildly hyperthyroid subjects discussed above. Hence, these MT-derived data cannot be used to impute the presence of uncoupling in trained skeletal muscle. However, the increased TCA cycle turnover rates that were present in resting trained muscle (as compared with levels present in untrained muscle) are of interest. Although, this TCA cycle rate increase could have been due to mitochondrial uncoupling, it seems more likely that it was a consequence of increased mitochondrial density in the trained muscle. It is well known that mitochondrial content increases in the relevant skeletal muscles when untrained subjects undergo endurance training. Under these circumstances, even if the “resting” (i.e., obligatory near state 4 oxygen consumption rate) of individual mitochondria was not altered, the increased mitochondrial numbers present in each gram of trained muscle would be expected to increase the tissue TCA cycle rate because of the obligatory resting state metabolism of the additional mitochondria.

**Additional Evidence That Oxidative ATP Synthesis Is Not Significantly Limited in Skeletal Muscle With Reduced Insulin Sensitivity**

1) Nair et al. (29) have shown, in muscle biopsy samples obtained from Asian Indians with insulin insensitivity or frank DM2, that tissue mitochondrial DNA copy numbers were elevated as were the oxidative and ATP synthetic capacities of mitochondria isolated from biopsy specimens. Indeed, the mitochondrial oxidative capacities of these DM2 patients were “supranormal” when compared with similar data obtained from normal subjects of North American or European origin. Hence, in this population of DM2 patients, skeletal muscle mitochondrial ATP synthetic capacity was not reduced despite the presence of severe insulin resistance. These observations convincingly demonstrate that there is not an obligatory mechanistic link between the presence of severe skeletal muscle insulin resistance and a decreased mitochondrial capacity for oxidative phosphorylation.

2) Investigators from Maastricht University (9) studied a large group of patients with DM2 in whom in vivo resting skeletal muscle mitochondrial function was assessed with conventional 31P MR spectroscopy. Resting muscle values of [ATP], [PCr], and [ADP] and also the rates of [PCr] and [ADP] recoveries following a brief period of imposed ischemia were measured. It was reported that mitochondrial oxidative capacity was strongly correlated with a marker of overall fitness (i.e., the maximal whole body oxygen uptake rate). Moreover, it was also reported that the relationship between these variables in the DM2 group was quantitatively comparable to that found in a group of non-diabetic subjects. Hence, the presence of insulin insensitivity per se was not associated with variations in the kinetics of oxidative ATP synthesis (assessed by the rate of postischemic recovery). Last, these investigators showed that, in the DM2 group, resting skeletal muscle high-energy phosphate compound levels (including calculated [ADP]) were also comparable to those present in the non-diabetic group. The finding that [ADP] levels were not increased in diabetic skel-
et al. muscles further supports our view (see earlier discussion) that oxidative ATP synthetic capacity is not sufficiently limiting in these resting muscles to warrant invocation of kinetic compensatory mechanisms.

Last, this research group (using the same 31P MR spectroscopic techniques employed in their human study) also reported data from Zucker diabetic fatty rats (a genetic DM2 model). In that animal model, there was no evidence of abnormal mitochondrial oxidative function at any stage of the development of the disease (8).

3) As pointed out by Holloszy (14), in primary mitochondrial diseases in which skeletal muscle oxidative ATP synthetic capacity is known to be reduced, glucose uptake and insulin sensitivity are increased rather than being decreased. These data also contradict the assertion that, in skeletal muscle, limitation of oxidative ATP synthesis per se can cause insulin insensitivity.

Why Are the MT-Determined Pi→ATP Rates Abnormally Low in Insulin-Insensitive Skeletal Muscle?

In resting skeletal muscle, MT-measured Pi→ATP rates substantially exceed oxidative ATP synthesis rates calculated from either oxygen consumption measurements or TCA cycle turnover rate measurements. This is because, as discussed, the component of MT-based Pi→ATP rate measurements originating from the GAPDH/PGK couple and/or mitochondrial ATPase activity in the ATP→P i direction is substantial.

Thus, we ask what the lower Pi→ATP rates observed in insulin-insensitive muscle might mean in metabolic terms and why the MT-measured Pi→ATP rate increases substantially during a hyperinsulinemic, normoglycemic clamp, but not insulin-insensitive resting skeletal muscle? In normal resting (i.e., insulin-sensitive) muscle, it is not likely that augmentation of glucose uptake (such as occurs during an hyperinsulinemic, normoglycemic clamp) speeds up the rate of oxidative ATP synthesis to the extent that would be predicted by the increases of MT-determined Pi→ATP rates reported (31, 41). Additional data support the view that an increased rate of glucose uptake per se cannot cause insulin sensitivity.

Conclusions

We suggest that conventional MT-based measurements of Pi→ATP rate in skeletal and cardiac muscle, liver, and perhaps in other tissues as well (with the apparent exception of the brain) do not yield quantitative estimates of the rate of oxidative ATP synthesis unless the GAPDH/PGK contribution to the Pi→ATP rate is absent and/or the presence of significant mitochondrial activity in the ATP→Pi direction can be ruled out. This complication should be considered when interpreting MT Pi→ATP rate data obtained in any tissue. Our analysis based on considerations of the biochemical origins of measured Pi→ATP rates and basic enzyme kinetics is supported by non-MT-based studies of mitochondrial function in normal and insulin-insensitive skeletal muscle. We conclude that MT-based measurements of total skeletal muscle Pi→ATP rates recorded in insulin-insensitive and normal subjects are not indicative of the true net rate of oxidative (i.e., mitochondrial) ATP synthesis. Hence, these data cannot be used to infer a functionally significant limitation of mitochondrial capacity for oxidative ATP synthesis in insulin-sensitive resting skeletal muscle. The mechanism of the reduced Pi→ATP rates observed in insulin-insensitive resting skeletal muscle remains to be elucidated but may well be a consequence of the effects of decreased glucose uptake on the kinetics of the GAPDH/PGK enzyme couple.

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DISCLOSURES

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