NADH Shuttle and Insulin Secretion
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In their report (*1*), Eto *et al*. hypothesize that nicotinamide adenine dinucleotide (NADH) is the signal for the coupling of glycolytic and mitochondrial glucose metabolism that triggers insulin secretion. Their conclusions, based on their innovative and meticulous experiments, do not consider the possibility that the glycolytic end-product in the cytosol is not pyruvate, but lactate. Thus, when lactate enters the mitochondrial tricarboxylic acid cycle, it must first be converted to pyruvate by the lactate dehydrogenase (LDH) reaction that also produces NADH. The LDH reaction is an important source of NADH that allows cellular utilization of lactate as the sole oxidative energy substrate (*2*). Whether or not the LDH-produced NADH plays a role in triggering insulin secretion could be determined by use of the well-designed system described in the report (*1*). Incubation of *mGPDH*$^{-/-}$ islets with 22.2 mM glucose + aminooxyacetate (AOA) + (20 to 40 mM) lactate should provide ample amounts of LDH-produced NADH. Alternatively, incubation of wild-type (WT) islets with 22.2 mM glucose + iodoacetic acid (IAA) + AOA + (20 to 40 mM) lactate should work as well. Instead of IAA, the use of a pharmacological dose of the glucose analog 2-deoxy-D-glucose might work. Performing these experiments, one should be able to determine if the LDH reaction produces an NADH signal and, if so, whether or not such a signal is sufficient to trigger insulin secretion.

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It has been known since the early 1980s that the glycerol phosphate shuttle and the malate- aspartate shuttle are important for glucose-induced insulin secretion (*1*). These shuttles enable mitochondrial metabolism to reoxidize NADH formed in the reaction catalyzed by the cytosolic enzyme glyceraldehyde phosphate dehydrogenase (GAPDH). Eto *et al*. (*2*) are to be congratulated for obtaining a mouse deficient in mitochondrial glycerol phosphate dehydrogenase (mGPD), the key enzyme of the glycerol phosphate shuttle, because there are no known potent specific inhibitors of mGPD, which would be useful for studying its role in insulin secretion. The major conclusion of Eto *et al*. (*2*) is the observation that insulin release and glucose metabolism were normal in pancreatic islets from mice lacking mGPD and thus the glycerol phosphate shuttle, but when AOA, an inhibitor of transaminases, was applied to these islets to inhibit the malate-aspartate shuttle, insulin secretion was inhibited (*2*). In their wild-type (WT) islets AOA inhibited aspartate aminotransferase, but only minimally inhibited insulin release (*2*). However, Eto *et al*. (*2*) did not mention that others have seen inhibition of glucose-induced insulin release in WT rat islets and mouse islets treated with AOA (*3*). In regard to the site of action of IAA, the authors cannot unambiguously conclude that inhibition of [U-^{14}C]glucose metabolism by IAA in WT islets is caused in inhibition of only GAPDH (an idea crucial to their argument), because numerous enzymes are attacked by IAA (*4*). Eto *et al*. (*2*) also observed that in islets from the mGPD-deficient AOA-treated mouse $^{14}CO_2$ metabolism from [6-^{14}C]glucose was inhibited, but metabolism of [U-^{14}C]glucose to $^{14}CO_2$ was not inhibited. This is biochemically impossible [see (*5*)].

Eto *et al*. conclude (*2*, p. 983), "We thus hypothesized that abolition of insulin secretion observed in *mGPDH*$^{-/-}$ islets treated with AOA was caused not by an insufficient supply of pyruvate to mitochondria but by a halt of NADH supply to mitochondria." This conclusion is untenable because, without reoxidation of cytosolic NADH, glycolysis would be inhibited at the triose phosphates, and formation of all subsequent glycolytic intermediates, including pyruvate, would be decreased. A minimal conclusion one could make from the data in the report might be that there are only two NADH shuttles in the insulin cell, and that either shuttle alone can support glucose-induced insulin secretion. However, if both shuttles are inhibited, NADH reoxidation would be decreased, which would inhibit glycolysis and pyruvate formation and insulin release. Inhibition of cytosolic NADH reoxidation, glycolysis, and pyruvate formation and metabolism are essentially inseparable from one another (*5*).

Finally, some new information suggests that the conventional thinking about the glycerol phosphate shuttle as only using NADH may be oversimplified. At the normal pH of 7.1, the islet cytosolic glycerol phosphate dehydrogenase (cGPD) prefers NADPH as a cofactor over NADH, and there is evidence that enzyme-enzyme interaction influences the preference of cGPD for NADPH over NADH (*6*). Furthermore, islets possess at least one shuttle capable of exporting NADPH equivalents from the mitochondria (*7*). Thus, in the β cell, a major role of cGPD might be to supply cytosolic NADP.

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Response: Schurr and Payne note that lactate can be an alternative substrate that provides the cytosol with NADH and pyruvate through a reaction catalyzed by LDH. However, lactate at 40 mM could not restore the 22.2 mM glucose-induced insulin secretion in *mGPDH*$^{-/-}$ islets treated with 5 mM AOA, where activities of both the NADH shuttles were blocked. Two possibilities might explain this result. One is that LDH activity in the islets was too low to generate ample NADH and pyruvate from lactate in the cytosol. Indeed, LDH activity reportedly must be quite low to ensure aerobic glucose metabolism in β cells (*1*). The other is that although LDH activity was sufficiently high to utilize lactate, the products, NADH and pyruvate—which in combination may stimulate insulin secretion—were unable to do so because these conditions block the NADH shuttle system.

Glucose-induced insulin secretion is known to be abolished in the presence of IAA, which inhibits glycolysis at a step catalyzed by glyceraldehyde-3 phosphate dehydrogenase, before generation of glucose-derived NADH (*2*). In wild-type islets treated with 1 mM IAA, lactate at 40 mM was also unable to restore the secretion. In this case, the inability to restore secretion can again be explained by low LDH activity in β cells, but not by the blocking of the NADH shuttle system when LDH activity is sufficiently

high. If the ample supply of NADH and pyruvate is not sufficient to cause insulin secretion, then a third, hitherto unknown glycolysis-derived signal, possibly originating from between 1,3-bisphosphoglycerate and phosphoenolpyruvate, may be required for reconstitution of glucose-induced insulin secretion, a possibility that might be investigated by overexpressing LDH in β cells.

Turning to the comments of MacDonald and Fahien, we note that our report (*3*) demonstrated that the NADH shuttle system is essential for glucose-induced insulin secretion by coupling glycolysis with activation of mitochondrial metabolism, thereby promoting efficient adenosine triphosphate generation in mitochondria. MacDonald (*4, 5*) suggested the potential role of the glycerol phosphate shuttle or the malate-aspartate shuttle in glucose-induced insulin secretion, noting that mGPDH is expressed in islets 40 to 70 times as much as in liver, heart, or skeletal muscle and that AOA, an inhibitor of the malate-aspartate shuttle, partially inhibits glucose-induced insulin secretion. The absence of an inhibitor of the glycerol phosphate shuttle, however, has made it difficult to determine the role of the NADH shuttle system, composed of the two distinct shuttles. We have directly demonstrated the role of the system in glucose-induced insulin secretion by treating *mGPDH*$^{-/-}$ islets with AOA.

AOA at 5 mM suppressed glucose-induced insulin secretion from wild-type mice islets by ~15% in static incubation for 60 min (*3*). In perifusion, the small decrease was mainly explained by impairment in the early part of the second-phase secretion. When we applied 5 mM AOA to *mGPDH*$^{-/-}$ mice islets, glucose-induced insulin secretion was almost completely abolished. The inhibition by AOA was dose-dependent, and the half-maximal inhibitory concentration was ~1 mM at 22.2 mM glucose (*6*). The apparent difference in the ~15% inhibitory effect of 5 mM AOA on insulin secretion in wild-type islets observed by us (*3*) and the ~60% inhibition observed by other workers (*5, 7, 8*) may have its roots in the relative activity of the glycerol phosphate shuttle compared with that of the AOA-sensitive malate-aspartate shuttle. The activity of the glycerol phosphate shuttle may have been decreased during their incubation of rat islets (detailed procedures of islet culture after isolation were not described). We used the mouse islets within a few hours after isolation for insulin secretion experiments. It is notable that 15-mM glucose-stimulated insulin secretion is inhibited by more than 85% in clonal INS-1 β cells treated with as low as 0.25 mM AOA (*9*).

Oxidation of [6-^{14}C]glucose is thought to reflect the TCA (trichloroacetic acid) cycle activity more precisely than that of [U-^{14}C]glucose, mainly because the isotope-labeled carbons of [3-^{14}C]glucose and [4-^{14}C]glucose are lost at a step catalyzed by pyruvate dehydrogenase before entering the TCA cycle, although some portion of them enters the cycle as [1-^{14}C]oxaloacetate for oxidation (*10*). This is not the case with glucose labeled in position 1, 2, 5, or 6. In addition, oxidation of [1-^{14}C]glucose produces $^{14}CO_2$ during the oxidative part of the pentose phosphate pathway.

In contrast to the prevailing hypothesis, our report clearly demonstrates that glycolysis can proceed when the NADH shuttle system is halted, a proposition that might be explored through measurement of NAD^+/NADH ratio in the cytosol. If the ratio is comparable to wild-type islets, reoxidation of NADH to NAD^+ may be compensated for by other dehydrogenases present in the cytosol, one candidate being the reaction catalyzed by LDH. However, if that reaction predominates, [U-^{14}C]glucose oxidation in mitochondria should be decreased, which we did not observe (*3*). Thus, dehydrogenases other than LDH are more probable candidates. If the ratio is low, it may follow that glyceraldehyde-3 phosphate dehydrogenase functions well enough to maintain the normal glycolytic flux under the reduced cytosolic conditions.

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