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Editorial Focus. A needle in a haystack: focus on “Proteomic alterations of distinct mitochondrial subpopulations in the type 1 diabetic heart”

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Diabetes mellitus is increasingly common throughout the world, due to both an increase in incidence and to decreased mortality of those with diabetes. Unfortunately, diabetes more than doubles the risk of cardiovascular disease in adults, and it even causes its own peculiar form of “diabetic” cardiomyopathy. These facts are conspiring, in turn, to increase the prevalence of heart failure (reviewed in Ref. 2). In fact, cardiovascular disease is now the leading cause of morbidity and mortality among individuals with diabetes, which raises the importance of understanding the mechanism of diabetic cardiomyopathy.

Proteomics is a very appealing tool for the study of complicated disease processes, such as diabetes, since it allows one to compare the protein expression profile of tissues, cells, or even organelles harvested from normal and diseased hearts. With luck, this unbiased approach might reveal the proverbial “needle in a haystack”—a specifically altered protein or pathway offering a potential therapeutic angle. However, as the results reported in the accompanying article by Baseler et al. (1) demonstrate, it is important to be certain that one is searching in the right “haystack.” Then, once the “needle” is found, there is also the not-insignificant task of working out what it means.

Whereas the more common type II diabetes is due to loss of insulin sensitivity (“insulin resistance”), up to 10% of cases are due to insufficient insulin production and are categorized as type I. In both types, there is a progressive increase in blood glucose concentration which defines the disease. Paradoxically, despite the plentiful glucose, glucose oxidation rates actually decrease in the diabetic heart, and fatty acid oxidation increases (2). This method of ATP production is less efficient, and contributes to the development of heart failure. Diabetes can, therefore, be considered a metabolic disease, which naturally raises the possible involvement of the mitochondria in diabetic cardiomyopathy.

Previous proteomic investigations of the ventricular myocardium of type I diabetic mouse models have detected an increase in mitochondrial proteins involved in fatty acid oxidation (2, 8, 19). Interpretation is complicated, though, by a potentially confounding increase in mitochondrial volume in the diabetic hearts (19). A subsequent proteomic analysis of mitochondria purified from type I diabetic rat hearts apparently confirmed an increase in proteins involved in fatty acid β-oxidation, with little change in tricarboxylic acid cycle proteins and a modest decrease in electron transport proteins (21). However, in all studies of cardiac mitochondria, a critical factor is the method used for their separation and purification, since there are at least two distinct populations of mitochondria that require different techniques for their isolation. The mitochondria purified most easily, by simple cellular lysis and centrifugation, are the subsarcolemmal mitochondria (SSM), positioned just beneath the sarcolemma (Fig. 1A). These are believed to be specialized for their role in producing ATP for ion pumps, and hence provide energy for the maintenance of the plasma membrane potential and generation of the cardiac action potential (10% of all cellular energy expenditure) and maintenance of internal stores of calcium (15% energy expenditure) (10). In the above study by Turko and Murad (21), the mitochondria are likely to have been mainly of subsarcolemmal origin, raising the possibility that differences might be detected in other mitochondrial subpopulations (other “haystacks”). The ideal arrangement on a farm, presumably, is to locate the haystacks close to the livestock, i.e., the source of energy. One finds a similar arrangement in the cardiomyocyte, wherein the majority of mitochondria are positioned immediately adjacent to the myofibrillar contractile units, providing the energy for contractile force production, which, in itself, accounts for 75% of energy expenditure (10). The close physical association with these interfibrillar mitochondria (IFM) is important for efficient cardiac energy utilization and function (22).

When an additional, mild proteolysis step is used to release the IFM from the myofibrils (15), they are found to have an innately higher respiratory rate than the SSM (15). Importantly, these differences are not an artefact of the isolation procedure, since differences persist even if SSM are exposed to protease (15). On the other hand, a recent study suggesting that mitochondrial respiratory defects only become apparent in aged skeletal muscle after mitochondrial isolation and are not present in the original myofibrils, may be cause for concern about the isolation procedure (16).

The mitochondrial subpopulations also exhibit differences in internal structure, with the cristae of the IFM being relatively more tubular than the lamelliform cristae of the SSM (Fig. 1B), and these differences are reasonably well preserved during isolation (17). Though the IFM were believed to be relatively “fixed” in place due to the physical constraint of the sarcosomes, recent evidence suggests that they are able fuse into elongated, “thread”-like mitochondria under certain conditions (14). Conversely, in pathological situations, such as ischemia, hypertrophy, and diabetes, they may fragment into smaller individual units, though whether this reflects an injury or a
Ca²⁺ accumulation in mitochondria, which was ascribed to microdomains of elevated Ca²⁺ regulation in other cell types, and this may well vary between SSM and IFM.

The authors of the accompanying article by Baseler et al. (1) previously confirmed that the streptozotocin type I diabetic model resulted in the expected cardiac contractile abnormalities in their hands (5). Furthermore, they found that the IFM were more susceptible than SSM, decreasing in size and complexity, with respiration more severely decreased (5) and with a concomitant decrease in membrane potential (23). The IFM produced more superoxide, resulting in oxidative damage of proteins and lipids in IFM and were more susceptible to opening of the mitochondrial permeability transition pore under certain conditions (23). Here, Baseler et al. (1) report the results of the first proteomic investigation using this model, which separates the IFM and SSM populations of myocardial mitochondria. Comparing these two “haystacks,” they found a number of “needles,” i.e., changes occurring preferentially in the IFM. These include a decrease in the content of proteins involved in fatty acid oxidation, electron transport chain activity, cristae morphology (mitofilin), and, interestingly, mitochondrial HSP70 (involved in mitochondrial protein import). Following this thread of investigation, they went on to examine the efficiency of mitochondrial protein import in the subpopulations and identified a selective deficiency in IFM mitochondria. This, in turn, may be due to a decrease in IFM membrane potential (which drives mitochondrial protein uptake). Thus, a defect in the respiratory capability to generate a membrane potential may be self-reinforcing, as it diminishes the ability of the mitochondria to import replacement proteins. At the risk of overextending the barnyard analogies, it then becomes a matter of “the chicken and the egg,” in terms of which is the initial damage responsible for the mitochondrial changes.

The question remains as to why the IFM are more susceptible than SSM to injury in type I diabetes. One possibility is that their higher respiratory rates generate more damaging oxygen free radicals. Some of the observed posttranslational protein modifications were suggestive of oxidative damage (1), and a more extensive proteomic examination might confirm specific oxidative damage in components of fatty acid oxidation and respiration. Knowing the primary defect raises therapeutic possibilities. For example, overexpression of mitochondrial antioxidants may prevent diabetic cardiomyopathy (2). A therapeutic approach might make use of the emerging class of mitochondrial HSP70 (involved in mitochondrial protein import). These include a decrease in the content of proteins involved in fatty acid oxidation, electron transport chain activity, cristae morphology (mitofilin), and, interestingly, mitochondrial HSP70 (involved in mitochondrial protein import). Following this thread of investigation, they went on to examine the efficiency of mitochondrial protein import in the subpopulations and identified a selective deficiency in IFM mitochondria. This, in turn, may be due to a decrease in IFM membrane potential (which drives mitochondrial protein uptake). Thus, a defect in the respiratory capability to generate a membrane potential may be self-reinforcing, as it diminishes the ability of the mitochondria to import replacement proteins. At the risk of overextending the barnyard analogies, it then becomes a matter of “the chicken and the egg,” in terms of which is the initial damage responsible for the mitochondrial changes.

The observed difference may depend upon the diabetic model. The same group has previously performed a similar study in db/db mice as a model for type II diabetes (4). In these mice, SSM had decreased size and internal complexity, while IFM had increased internal complexity. Though IFM were relatively unchanged, SSM had decreased respiratory rates, lower membrane potential, and increased oxidative damage, with corresponding decreases in mitochondrial protein import machinery and IMM proteins in SSM (4). This suggests insulin resistance may affect SSM specifically, although an early study...
also observed impaired fatty acid oxidation in IFM of type II diabetic mice (2, 11).

There are, of course, limitations to the proteomic approach. There are increasing numbers of reports of signaling proteins being localized to mitochondria, and even proteins such as connexin-43, proposed as mediating cardioprotection selectively via the SSM (18). Unfortunately, the standard proteomic approach is unlikely to detect these changes, as it is limited to proteins of high abundance, although an interesting extension to the current study may be to examine posttranslational modifications related to activity. Other “haystacks” may merit investigation. For example, there is an unexplored, third population of mitochondria in a sarcomere-free region near the nuclei (Fig. 1A) (12), which presumably provide ATP for transcription and translation. Furthermore, mitochondria in other cell types, particularly the endothelium may be important in diabetic cardiomyopathy (6). In conclusion, the authors may have succeeded in finding a needle in a haystack, but diabetic cardiomyopathy is not yet sewn up.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author.

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