Estimation of β-Cell Mass by Metabolic Tests
Necessary, but How Sufficient?

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This Perspectives in Diabetes addresses the accuracy of metabolic testing as a measure of pancreatic islet β-cell mass in vivo in animals and in humans. The impetus for framing this question lies in the current intense interest in determining the fate of β-cell mass in transplanted islets, i.e., does it decrease, increase, or remain the same over time, as well as ascertaining whether drugs that enhance incretin levels, and consequently enhance glucose-induced insulin secretion, might also preserve β-cell mass. An important methodology recently making scientific strides in this arena is positron emission tomography (PET). The central question this Perspectives in Diabetes raises is whether it is likely that PET will provide significant advantages over the metabolic methods already in hand and routinely used to estimate β-cell mass. This article examines the fidelity with which published metabolic data correlate with independent measures of β-cell mass across multiple species. Correlation coefficients in the general range of $r = 0.80$ are routinely obtained and are robust for in vivo research. Whether PET can significantly improve on these correlations, given its inherent limitations in measurement sensitivity, remains to be seen. It is clear that investigators developing PET methodology to estimate β-cell mass should at the same time incorporate metabolic measures into their studies so that side-by-side comparisons of the accuracy of the two experimental approaches can be made. *Diabetes* 56:2420–2424, 2007

Every scientific epoch, regardless of length, has its own burning question, its own Holy Grail. This derives partly from a desire to extend knowledge beyond its current state and partly from dissatisfaction with existing technologies that seem to have been pushed to the point of diminishing returns. If we can cram 20,000 songs onto an iPod, what would it take to load 10 times as many? If we can diagnose 20 diseases using a routine panel of clinical tests, what might be accomplished if we had a microarray that identified a complete personal predilection to diseases? This restless quest raises a new set of questions. Is new always better? In a world of limited resources, is the cost-to-benefit ratio of new technologic development always favorable? Does the promise of technologic novelty divert our attention from more thoughtful and productive use of proven technologies already in hand? More succinctly put, are new technologic pastures always greener? Alphone Karr had a wry caveat about this in *Les Guepes* (1849), "Plus ca change, plus c'est la meme chose," or the more that changes, the more it's the same thing.

In the last several years, pathophysiologic and therapeutic questions have generated a keen desire to measure pancreatic β-cell mass in humans. One example is the question of the relative roles of β-cell apoptosis and neogenesis in the onset of type 2 diabetes (1). Another emanates from the advent of incretin-related drugs, such as exenatide and dipeptidyl peptidase-4 inhibitors, because data from animal experiments suggest that these drugs might not only enhance glucose-stimulated insulin release, but also increase β-cell mass (2). At the same time, impressive strides are being made with PET and its potential application to measurements of β-cell mass in humans (3,4).

This Perspective in Diabetes will consider the degree to which data from well established, clinically applicable metabolic tests correlate with β-cell mass in animals and humans. Included in this article will be an analysis of the accuracy afforded by, as well as limitations inherent in, metabolic methodologies and musings about the probability that current and future imaging techniques might provide significant improvements in the state of this art.

METABOLIC METHODOLOGIES AND CAVEATS

The most frequently used metabolic methods of assessing β-cell function in vivo in animals and in humans examine insulin secretion using the oral glucose tolerance test (OGTT), the intravenous glucose tolerance test (IVGTT), the intravenous arginine stimulation test (IVAST), and glucose potentiation of arginine-induced insulin secretion (GPAIS). Each of these has advantages and limitations. OGTT is the easiest to administer and takes into account the endogenous incretin effect that augments insulin secretion, but it does not allow the measurement of the acute insulin response, also known as first-phase insulin secretion. IVGTT is designed to directly examine the first-phase insulin secretion response, which is the β-cell response most sensitive to the adverse effects of hyperglycemia. Loss of first-phase insulin secretion is a major hallmark of type 2 diabetes. This response is diminished when fasting glucose levels are >100 mg/dl and absent when levels are >115 mg/dl (5,6). However, IVGTT does not assess incretin effects on insulin secretion. IVAST has the marked advantage of being able to assess first-phase insulin responses even when fasting glucose levels are >115 mg/dl, but it also does not assess incretin effects. GPAIS was devised to ascertain the maximal acute insulin response

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AIR, acute insulin response; AIRarg, AIR to intravenous arginine; AIRgluc, AIR to intravenous glucose; GPAIS, glucose potentiation of arginine-induced insulin secretion; IVAST, intravenous arginine stimulation test; IVGTT, intravenous glucose tolerance test; AIRargMax, glucose potentiation of arginine-induced AIR; OGTT, oral glucose tolerance test; PET, positron emission tomography.

TABLE 1
Examples of metabolic techniques used to correlate quantified β-cell mass with results of metabolic tests

Test	Species	Metabolic measurement	Insulin response	Ref.
IV injections of arginine and glucose	90% pancreatectomized rat	AIRgluc and AIRarg	Both significantly reduced, AIRgluc > AIRarg: $P <$ 0.005 vs. $P < 0.05$	Bonner-Weir et al. (8)
IV injections of arginine and glucose and GPAIS	64% pancreatectomized dog	AIRgluc, AIRarg, and AIRargMax	AIRarg but not AIRgluc significantly reduced; AIRargMax most significantly reduced: $P =$ 0.0002 vs. $P = 0.02$	Ward et al. (9)
IV injections of arginine and glucose and GPAIS	Streptozocin-administered baboon	AIRgluc, AIRarg, and AIRargMax	Correlation coefficients with β-cell mass: AIRgluc $r =$ 0.63, $P < 0.02$; AIRarg $r =$ 0.29, $P =$ NS; AIRargMax $r = 0.58$, $P < 0.02$	McCulloch et al. (10)
IV injections of arginine and glucose	Streptozocin-administered rat given various numbers of transplanted islets	AIRgluc, AIRarg, and AIRargMax	Correlation coefficients with islet transplant number: AIRgluc $r = 0.66$, $P <$ 0.0001; AIRarg $P =$ NS; AIRargMax: $P =$ NS	Tobin et al. (11)
IV injections of arginine and glucose and GPAIS	Streptozocin- and nicotinamide-administered minipig	AIRgluc, AIRarg, and AIRargMax	Correlation coefficients with β-cell mass: AIRgluc $r =$ 0.82, $P < 0.0001$; AIRarg $r = 0.76$, $P < 0.001$; AIRargMax $r = 0.88$, $P <$ 0.0001	Larsen et al. (12)
IV injections of arginine and glucose and GPAIS	Hemipancreatectomy in nondiabetic humans	AIRgluc, AIRarg, and AIRargMax	AIRgluc ($P < 0.05$) but not AIRarg significantly reduced, and AIRargMax most significantly reduced ($P < 0.001$).	Seaquist and Robertson (13)
IV injections of arginine and glucose and GPAIS	Pancreatectomized humans given auto-islet transplants	AIRgluc, AIRarg, and AIRargMax	Correlation coefficients with islet mass: AIRgluc $r =$ 0.84, $P < 0.01$; AIRarg $r =$ 0.65, $P < 0.05$; AIRargMax $r = 0.81$, $P < 0.01$	Teuscher et al. (14)
IV injections of arginine and glucose	Type 1 diabetic humans given alloislet transplants	AUCgluc and AIRarg	Correlation coefficients with islet mass: AUCgluc $r =$ 0.50, $P < 0.01$; AIRarg $r =$ 0.79, $P < 0.01$	Ryan et al. (15)

AUCgluc, area under the curve following intravenous glucose; IV, intravenous.

(AIR) to arginine. This technique assumes that a prestimulus exposure to hyperglycemia will enhance margination of insulin granules into the arginine-sensitive acutely releasable pool, priming the pump, so to speak. An important practical advantage of the IVGTT and the IVAST is that first-phase insulin response can be ascertained within 15 min, whereas the OGTT and GPAIS require 3 h and GPAIS requires an intravenous glucose infusion in addition to an arginine injection.

METHODS OF ALTERING B-CELL MASS AND CAVEATS

The focus of this article is the accuracy and limitations of metabolic testing, rather than imaging methodologies, that estimate β-cell mass. Understanding the value and limitations of experimental methods used to alter β-cell mass in vivo is essential to the intent of this Perspective on Diabetes. Methods of altering β-cell mass include partial or total pancreatectomy, use of drugs to destroy β-cell mass, and transplantation of islets. Major caveats about partial pancreatectomy are that it assumes an even distribution of islets throughout the pancreas, that no new islet generation occurs between the time the surgery is completed and the β-cell function tests are performed, and that islets not excised are not damaged by the surgery itself. The most common method to noninvasively decrease β-cell mass is the use of various doses of streptozotocin, a β-cell toxin. These experiments, while clearly the most convenient, carry with them the unlikely assumption that remaining β-cells counted by morphometry are not functionally harmed to any degree by the drug. Transplantation of islets has the distinct advantage of knowing quantitatively how many islets are provided to the recipient. However, this approach carries the assumption that all transplanted islets that survive isolation procedures contain only healthy β-cells. Moreover, this experimental approach is sometimes further confounded by the use of immunosuppressive drugs to protect allografted islets. These drugs, as well as transplant sites, can be hazardous for β-cells (7).

SCIENTIFIC LITERATURE: EXPERIMENTS PERFORMED AND CONCLUSIONS DRAWN

This analysis of published articles is limited to peer-reviewed scientific studies that, first, involved alterations

of β-cell mass by surgery, drugs, or transplantation and, second, correlated consequences with metabolic measurements of first-phase insulin secretory responses to glucose and/or arginine in vivo. Admittedly, this approach considerably limits the field of references (8–15), but it has the value of examining the most relevant and rigorously collected information about the central issue of the accuracy of estimating β-cell mass by metabolic testing in vivo.

Going in roughly historical sequence with the intent of examining animal studies first and human studies last, the initial citation to consider is that of Bonner-Weir et al. (8). In these experiments the question was: what functional β-cell abnormalities are caused by excessive glucose stimulation of surgically reduced β-cell mass? The approach was to compare the AIR to intravenous glucose (AIRgluc) and the AIR to intravenous arginine (AIRarg) in hyperglycemic 90% pancreatectomized versus sham-operated rats. The authors reported that AIRgluc was more dramatically affected than AIRarg (Table 1). Shortly thereafter, Ward et al. (9) asked: what test is more sensitive in detecting suboptimal β-cell mass? Before and after 64% pancreatectomy in dogs, they compared AIRgluc to AIRarg at fasting as well as glucose potentiation of arginine-induced AIR (AIRargMax) during increased plasma glucose levels caused by intravenous glucose infusion. They reported that AIRargMax was more sensitive than AIRgluc or AIRarg in reflecting the decrease in β-cell mass. McCulloch et al. (10) also examined which metabolic tests of β-cell function best correlate with reduced β-cell mass. They gave varying doses of streptozotocin to adolescent male baboons and determined pancreatic insulin content and β-cell mass 3 days later. These authors found that both AIRgluc and AIRargMax correlated with pancreatic insulin content and β-cell mass. In this study the correlation line intersected at zero when insulin content was compared with metabolic measures of β-cell function. However, the correlation line intersected higher on the *y*-axis when data comparisons were made using immunostaining methods of estimating β-cell mass. This raises the possibility that β-cells poorly granulated with insulin were included in the β-cell mass calculations and consequently that this method overestimated functional β-cell mass. Tobin et al. (11) examined whether insulin responses to arginine and glucose are proportional to transplanted islet cell mass. They transplanted a range (500–3,000) of Wistar-Furth rat islets into streptozotocin-induced diabetic rat recipients and performed metabolic tests 3 weeks later. Their results documented that increasing the numbers of islets transplanted gave increasingly better glycemic control and that AIRgluc correlated better than AIRarg with numbers of islets transplanted. Larsen et al. (12) studied this issue by including the important variables of obesity and insulin resistance. They gave obese and lean minipigs nicotinomide and streptozotocin, performed β-cell function studies, and then determined β-cell mass. They concluded that the β-cell response to combined glucose and arginine stimulation correlated most highly with β-cell mass.

Three studies of human subjects have been reported. The first was by Seaquist and Robertson (13) who examined whether tests of β-cell function are affected by removal of 50% islet mass. They examined AIRgluc, AIRarg, and AIRargMax in healthy human organ donors who underwent hemipancreatectomy. They observed that AIRgluc but not AIRarg was affected by removal of ~50% of pancreas in healthy humans. However, the measurement most significantly affected was AIRargMax. In another investigation involving nondiabetic humans, Teuscher et al. (14) examined tests of β-cell function as predictors of transplanted islet mass. These studies involved long-term follow-up (3–13 years) of nondiabetic patients with chronic pancreatitis who underwent pancreatectomy followed by infusion of their own islets (autografts) intrahepatically within 2 h of pancreas removal. In these studies both AIRgluc and AIRargMax were highly correlated with transplanted islet mass, as was AIRarg, albeit to a lesser degree (Fig. 1 and Table 1). Most recently, Ryan et al. (15) examined this issue in type 1 diabetic recipients of intrahepatic islet allografts receiving potentially β-cell toxic immunosuppressive drugs. They observed that AIRarg was highly correlated with transplanted islet mass (Fig. 2 and Table 1).

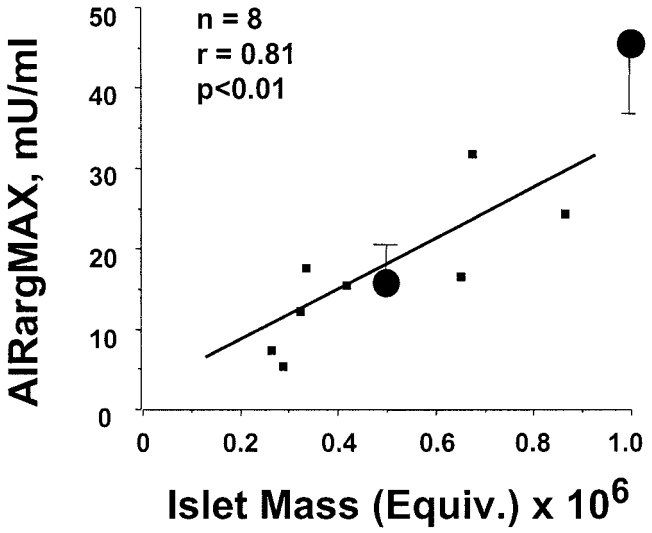

FIG. 1. Correlation of AIRglucMax with mass of intrahepatically transplanted autoislets in insulin-independent normoglycemic recipients of autoislet transplantation following total hemipancreatectomy for unrelentingly painful chronic pancreatitis. The correlation coefficient refers only to the data from the eight autoislet transplant recipients (■) who had fasting plasma glucose levels (mean ± SE) 99 ± 3 mg/dl and were 1–13 years posttransplant. For purposes of comparison, similar data from normal subjects (estimated as having 1 million islets) and healthy hemipancreatectomized pancreas organ donors (estimated as having 500,000 islets) are shown by large circles. Reproduced with permission from ref. 14.

WHAT CAN BE DEDUCED FROM INFORMATION RELATING B-CELL MASS TO METABOLIC TESTS?

Certainly, the weight of the evidence testifies that the metabolic tests used in the cited studies to one degree or another faithfully correlated with β-cell mass. Depending on which experimental model was used, each of the tests was shown to be affected by manipulations of β-cell mass, and where statistical correlations were examined, the coefficients describing the relationships between metabolic data and β-cell mass were statistically significant, with r values of ~0.80. This constancy is remarkable in view of the range of species examined (rat, dog, baboon, minipig, human) and the variety of methods used to alter β-cell mass (partial and total pancreatectomy, β-cell toxins, and islet transplantation in various sites with and without β-cell toxic immunosuppressive drugs). One critically important consideration, however, is that AIRgluc can be used reliably only when the animal or human being tested is not hyperglycemic. In the latter situation in humans, only AIRarg and AIRargMax should be used

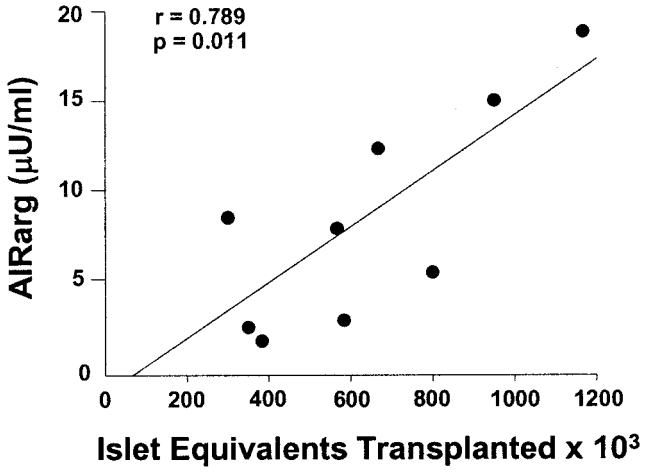

FIG. 2. Correlation of AIRarg with numbers of intrahepatically transplanted alloislets in immunosuppressed type 1 diabetic recipients. Reproduced with permission from ref. 15.

because AIRgluc is abnormal when fasting plasma glucose is >100 mg/dl and disappears altogether when it exceeds 115 mg/dl (5,6).

In the human experiments, AIRgluc, AIRarg, and AIRargMax reproducibly correlated well with estimates of β-cell mass, whether the experimental manipulation involved pancreas excision with or without replacement of pancreatic islets without or with posttransplant exposure to potentially β-cell toxic immunosuppressive drugs. Even in the long-term experiments involving autoislet transplantation with no use of immunosuppressive drugs in totally pancreatectomized nondiabetic recipients (14), correlation coefficients were at the 0.80 level. This, in a sense, is impressive because the times elapsed after transplantation when tests were performed were quite variable (1–13 years). Yet, a linear relationship was obtained between β-cell function and transplanted islet mass, and the slope of the regression line corresponded to similar data from normal subjects with and without hemipancreatectomy. One might have expected that the data from patients transplanted for the longest periods of time would have shown some deterioration in β-cell function and thus fall beneath the correlation line shown in Fig. 1. This suggests that ongoing apoptosis of islets over time was well compensated for by islet neogenesis.

THE CENTRAL QUESTION: HOW WELL DO METABOLIC RESULTS CORRELATE WITH ESTIMATES OF B-CELL MASS?

Data in Table 1 indicate that the best one can expect are correlation coefficient values in the range of 0.80–0.85, meaning that roughly 64–72% of the variability in the relationship can be accounted for by the two variables under examination. This leaves roughly 30% of the variation unaccounted for. Nonetheless, it seems remarkable that the correlation coefficients were so robust, given the many variables inherent in the experiments.

How do we deal with the residual unaccounted for variation? Should we conclude that available metabolic tests are fundamentally flawed and of minimal value? Do we conclude that more accurate measurements are required before we can attack the important questions of whether incretin-like pharmacologic agents favorably influence β-cell mass in diabetic patients? Are we unable to examine critically whether available therapeutic approaches might result in arrest of accelerated β-cell apoptosis or stimulate islet regeneration? My view is that the answer to these questions is a resounding "No." In studies of human physiology, the best result one usually obtains with correlation analysis involves r values of ~0.80. There are too many unidentified variables in the in vivo situation in humans (who certainly do not with total fidelity reflect the experimental advantages of genetically inbred rodents) to do much better. These other variables include innate variations in baseline native β-cell mass, levels of intrinsic insulin resistance, dietary factors, known or unknown illnesses that may be or have been present in the past, and drug exposure that might alter β-cell function.

A related but important issue is whether any method, such as the elegant imaging techniques that are undergoing increasing refinement in sensitivity, will be able to achieve better results than metabolic measures of β-cell mass in humans. The sticking point is that any technology, no matter how elegant, may not necessarily provide improved accuracy because of the inherent variables encountered in the in vivo situation. New techniques eventually have to be correlated with an independent measure of β-cell mass to answer this question. This puts all candidate technologies at the same disadvantages listed above for metabolic tests, ranging from unknowns such as intrinsic β-cell health to vagaries inherent in islet transplantation and immunosuppression. On a more positive note, the metabolic methods cited in this Perspective in Diabetes provide valuable tools for investigators interested in quantifying β-cells by imaging techniques in vivo. Investigators can compare the results of the correlation analysis of imaging data versus metabolic data, such as AIRgluc, AIRarg, and AIRargMax, as well as independent estimates of β-cell mass. This approach will provide a very valuable assessment of whether imaging methods are more accurate than metabolic testing or whether Karr's caveat applies. At a more practical level, currently available metabolic tests should be used with full confidence to prospectively examine in humans through paired data analysis whether incretin-like drugs augment glucose-induced insulin secretion only or whether they also preserve β-cell mass.

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