ESSAYS ON APS CLASSIC PAPERS

Muscle, liver, and pancreas: Three Musketeers fighting to control glycemia

Amira Klip1,2 and Mladen Vranic2
1The Hospital for Sick Children, Toronto; 2Department of Physiology, University of Toronto, Toronto, Ontario, Canada

This essay examines the historical significance of two APS classic papers that are freely available online:


The work described in these two articles made it possible for the first time to measure, accurately and outside of steady-state conditions, 1) the rate of hepatic glucose production and glucose utilization (Wall et al., Ref. 15) using a radio-tracer infusion method, and 2) insulin sensitivity and the effect of insulin by the glucose clamp method (DeFronzo et al., Ref. 6). This pair of papers is ideally matched because, individually and in aggregate, they made strides in our ability to assess the dynamic behavior of glucose in humans. That this was achieved as early as 1957 is remarkable. These papers have the distinction of measuring glucose dynamics in vivo, in a way that allows us now to determine how effective insulin is and, therefore, to establish whether an individual is insulin-resistant or insulin-insufficient (i.e., suffers from glucose toxicity). Put in context of other discoveries, in the 1950s the use of radioactivity for biomedical research was incipient (a fallout of the war efforts), the translocation of glucose transporters to the membrane of fat cells in response to insulin was first sketched in the late 1970s, and the tyrosine kinase activity of the insulin receptor was discovered in 1985, the same year that GLUT4 was cloned. The crux of these studies is to understand the triple receptor was discovered in 1985, the same year that GLUT4 metabolism was the invention of a new tracer method involving primed tracer infusion of [1-14C]glucose at a constant rate (12, 13, 15). In their paper, “Effect of insulin on utilization and production of circulating glucose,” Wall et al. (15) postulate that the entire body glucose pool can be represented as a plasma compartment and two interstitial compartments. One of the interstitial fluid compartments exchanges glucose very rapidly with the plasma so that an intravenous dose of [14C]glucose mixes completely in less than 5 min. A key assumption of these studies is that the glucose in the plasma plus that in the fast compartment amount to half the glucose in the body pool, with the other half being located in a compartment that slowly exchanges glucose with the plasma. When [14C]glucose is infused continuously, the specific activity of glucose ([14C]/[12C] glucose) will decline as glucose production increases. The opposite will happen if glucose production decreases. Glucose utilization does not influence the glucose specific activity, because the rate of [12C]glucose released from the liver remains in balance with the constant [14C]glucose infusion. Wall et al. concluded the following. 1) The insulin-induced increase in glucose utilization is of greater magnitude than the decrease in glucose production. 2) When [1-14C]glucose in infused, an error arises resulting from the recycling of the radioactive label through hepatic metabolism. 3) Eventually, following the insulin injection, plasma glucose rebounds because of increased glucose production and not because of a decrease in tissue uptake of glucose.

The fight for tracer validation. In 1957, methods were not available for an in vivo validation of each of the three conclusions, although the assumptions seemed reasonable. The team used an electronic analog of the dog body glucose pool that was prophetic. In this circuit, glucose flow is represented by current, each compartment of the body glucose pool is represented by fixed capacitors, and the restriction of flow in and out of the compartments is represented by resistors (13). Because of this indirect approach, there was some reluctance of other laboratories to use the tracer method. Nonetheless, the analysis allowed the team to make key observations on the effects of glucose load (12), insulin, growth hormone, glucocorticoste-
roids, epinephrine, glucagon, prolactin, and thyroid hormones on glucose and free fatty acid turnover in normal dogs (1).

What about the second conclusion? Wrenshall and Hetenyi (16) used successive tracer injections to determine glucose turnover whereby each injection was followed by an exponential fall in specific activity with a minimal standard error. This afforded precise calculations at steady state but was not accurate for changes between steady states. There was animated discussion between the two groups with respect to advantages and disadvantages of the two approaches. Validation during changes in steady state was achieved when Katz et al. (8) and others used diverse glucose tracers (2-3H, 3-3H, and 6-3H) to assess not only recycling but also futile cycles (related to glucose entering and leaving the liver). A few years later, Cowan and Hetenyi (5) used an integral method to measure the average glucose production and calculated the pool fraction to be 0.65 of the total glucose pool. Finally, Radziuk et al. (10) compared known infusion rates of 12C-glucose to the tracer infusion method using a variety of tracers and the formula of Wall et al., thereby ascertaining on a continuing basis the appropriate pool fraction outside of steady state. It is remarkable how close the assumption of Wall et al. was to this value! Following this confirmation (10), the tracer method was widely used for both clinical and animal research.

Finally, Wall et al. had indicated that the recovery from hyperglycemia depends exclusively on increased glucose production, without a change in glucose utilization. Years later we found that muscle GLUT4 glucose transporters and tracer-determined glucose clearance decrease with hyperglycemia and increase with hypoglycemia (9), and hence constant glucose utilization occurs because changes in glucose clearance compensate for changes in glucose mass. Thus tracer and GLUT4 measurements explained the early observation of Wall et al.

**Taking control through clamps.** The second paper, “Glucose clamp technique: a method for quantifying insulin secretion and resistance” (6), is groundbreaking because it enabled researchers for the first time to measure the reciprocal effects of glucose on insulin secretion and of insulin on glucose uptake, without the complications of both variables changing simultaneously. Previous workhorse tests were the oral glucose tolerance test and the intravenous insulin tolerance test. The parameters garnered from these tests (plasma and insulin and glucose levels) were used to calculate insulin secretion (from the insulin/glucose ratio) and insulin tissue sensitivity (from the glucose/insulin ratio). Because glucose and insulin vary in response to each other (glucose induces insulin secretion and insulin induces glucose clearance from the blood), those calculations were inaccurate. The new “trick” consisted of artificially holding the glucose levels at a desired level through gauged, arterial infusions of the sugar since hyperglycemia curbs glucose production, the amount of sugar required to maintain a certain level of plasma glucose is hence equivalent to the amount being removed from the blood by the tissues. This in turn is pegged to the amount of insulin that is either secreted from the pancreas (when hyperglycemia is being held constant), or to the response to a maximal arterial infusion of insulin (when euglycemia and hyperinsulinemia are being held constant). Such “glucose clamp” was designed to keep blood glucose levels fixed, at either high values (hyperglycemic clamp) or normal values (euglycemic clamp) through the arterial infusion of glucose. In the latter case, insulin is concomitantly infused (euglycemic/hyperinsulinemic clamp). Thus the measurements operate on a negative feedback principle. The term “clamp” was suggested by J. J. Blum of the Department of Physiology at Duke University, by analogy to the voltage-clamp strategy used to measure current (see Ref. 6). The strategy, which began with the senior author of the paper, Reubin Andres (2), was beautifully developed into digestible elements in this paper by first author Ralph DeFronzo (6). Through a series of elegant studies, R. Sherwin et al. (11) and others buttressed the concept. The two modalities of the clamp, termed the hyperglycemic clamp and the euglycemic-hyperinsulinemic clamp, respectively, still stand today at the core of our approach to measure glucose sensitivity of insulin secretion and insulin sensitivity of glucose uptake.

In the DeFronzo study (6), in addition to the accurate description of the empirical and absolute values used and of the technical approach, the authors found that, during a hyperglycemic clamp, the plasma insulin response is biphasic, with an early burst in insulin release of about 45 μU/ml for about 6 min and a subsequent progressive, more sustained increase to 65 μU/ml. Secondly, they reasoned that the amount of glucose infused to keep glucose at “clamped” levels is a measure of glucose clearance from the blood, most typically via uptake into muscle (and to some extent fat cells). Because the liver is very insulin-sensitive, the high insulin levels infused are expected to largely block glucose output from the liver (the insulin-induced curb in hepatic glucose output), thereby virtually removing the liver from the equation. It follows that the euglycemic/hyperinsulinemic clamp afforded a measurement of glucose uptake into muscle (and to a lesser degree fat).

**The legacy of the clamps.** How pervasive has the use of the clamps been in assessing insulin secretion and action? Suffice it to say that a PubMed search (http://www.ncbi.nlm.nih.gov/entrez/) for the use of the two modalities of the clamp renders almost 4,000 papers! Strikingly, the clamp began with human studies and is now amply used on rats and mice, especially as new transgenic and gene knockout animal models are generated that require thorough assessment of glucose dynamics. While the vast majority of those studies examined metabolic defects underlying the metabolic syndrome, obesity and diabetes, there is currently one study that is recruiting subjects to study the effect of dark chocolate on insulin sensitivity in people with high blood pressure!

Glucose clamps allowed assessment of the effect of metabolic hormones independently of changes in glucose. In addition, the combination of glucose clamps and tracer method allows assessment of the effect on glucose turnover of endogenous insulin and of infused insulin independently of glycemia. The development of insulin clamps made it possible to segregate hormonal and metabolic defects from endogenous insulin release (14). This is important because glucose, by itself, can inhibit glucose production in the liver and increase peripheral glucose uptake. Unexpectedly, however, negative glucose production rates were frequently observed during glucose clamps. This was a blow to the method, because glucose production can only be positive. The issue was resolved by Finegood et al. (7), using the hot GINF (glucose infusion) and a regression method calculation. By this protocol, in addition to constant infusion of tracer, tracer was also added to the exogenous glucose used for clamping. The novelty consisted in maintaining constant the glucose specific activity during the non-steady state and
thereby avoiding mistakes due to rapid changes of specific activity. Finally, building on the innovations of the two clamps, Bergman et al. (4) developed an ingenious “minimal model” calculation that differentiates between insulin secretion and sensitivity with a single glucose injection. This method has made it possible to assess insulin sensitivity and secretion and their relationship to physiology and disease in large epidemiological studies, including scans of the human genome.

In closing, as the field of insulin action moves toward understanding the complex insulin signaling cascades that are activated in time and space within insulin-sensitive tissues and the pliable nature of islet tissue renewal, the tracer method and glucose clamps continue to be mainstays in establishing whole-body glucose homeostasis. This highlights once again that, in the race for knowledge, the horse-vs.-cart relationship between method and idea is almost as interdependent as the glucose-insulin cycle so beautifully examined in these classic papers (6, 15). Together, these two papers put numbers to the interrelationship among muscle, liver, and pancreas that are simultaneously “one for all, and all for one.”

REFERENCES

16. Wrenshall GA and Hetenyi G Jr. Successive measured injections of tracer as a method for determining characteristics of accumulation and turnover in higher animals with access limited to blood: tests in hydrodynamic systems and initial observations on insulin action on dogs. Metabolism 8: 531–543, 1959.