

# Type 2 Diabetes and the Evolving Paradigm in Glucose Regulation

Muhammad A. Abdul-Ghani, MD, PhD

**T**ype 2 diabetes mellitus (T2DM) is a disease characterized by the dysregulation of glucose homeostasis resulting in hyperglycemia.<sup>1</sup> The etiology of T2DM is complex and comprises a variety of different dysfunctions involving multiple organs and tissue types. Whereas previous understanding of the pathophysiology of T2DM largely focused on beta cell dysfunction and insulin resistance in skeletal muscle and liver, that understanding has expanded in recent years to include defects in the adipose tissue, pancreatic alpha cells, gastrointestinal tract, brain, and kidney.<sup>2</sup> Indeed, it is now apparent that T2DM is a multisystem disease with multiple metabolic abnormalities that contribute in varying degrees to the development and maintenance of hyperglycemia (Figure 1). Chronic hyperglycemia is a primary factor in the pathophysiology of T2DM because of its contribution to the development of insulin resistance and beta cell dysfunction, both of which, in turn, exacerbate hyperglycemia.<sup>2</sup> To successfully manage T2DM, it is important that clinicians be apprised of the current and expanding state of knowledge regarding the complex interaction of organs and tissues in T2DM-related hyperglycemia. This article summarizes what is currently known about this multifaceted condition, including a description of the central role of the kidneys in glucose homeostasis.

## Natural History of T2DM: Insulin Resistance Versus Beta Cell Dysfunction

The natural history of T2DM has been well studied in multiple ethnic and demographic groups.<sup>3,4</sup> Insulin resistance is the earliest metabolic abnormality which can be detected in patients who will ultimately develop T2DM. For example, lean healthy individuals with normal glucose tolerance (NGT) who are the offspring of 2 T2DM parents manifest severe insulin resistance while still maintaining NGT.<sup>5</sup> This observation indicates that insulin resistance alone is not sufficient for the development of hyperglycemia. Moreover, the majority of obese individuals are insulin resistant.<sup>6</sup> The maintenance of normoglycemia is possible because in response to insulin resistance, the beta cells augment their secretion of insulin to offset the defect in insulin action and maintain NGT. Thus, as long as the beta cells are able to augment their secretion of insulin sufficiently to offset the insulin

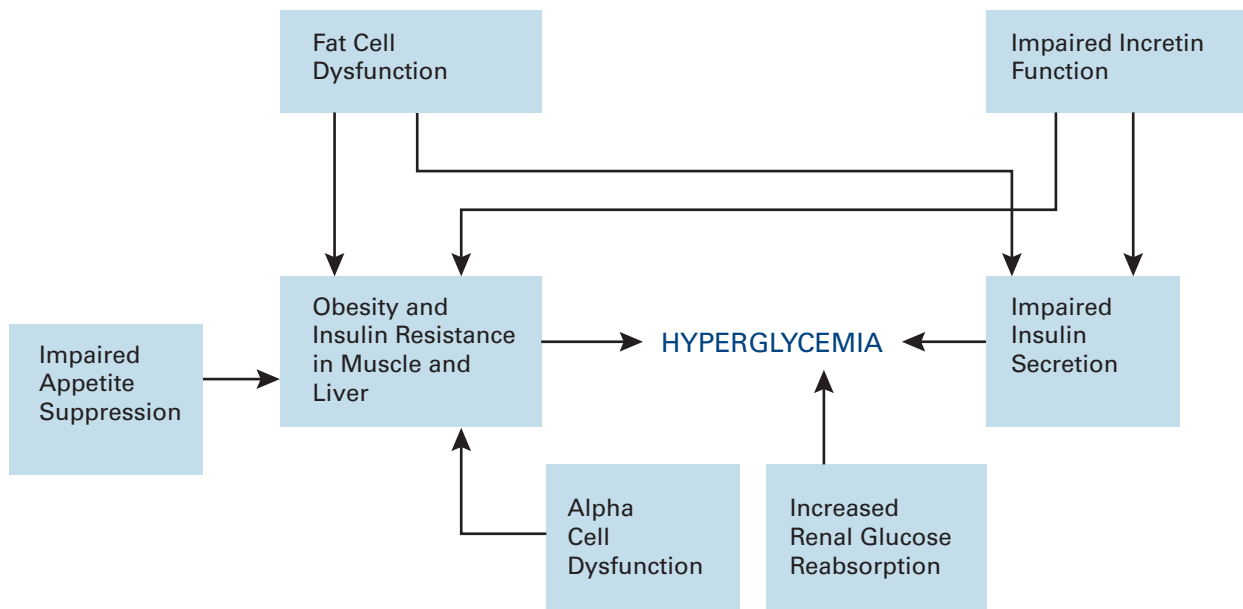
### Abstract

Type 2 diabetes mellitus (T2DM) is a multi-system disease comprising numerous metabolic defects that contribute to the development of hyperglycemia. Although insulin resistance in the skeletal muscle and liver together with progressive beta cell failure are traditionally thought of as the core defects responsible for the development and progression of hyperglycemia, research over the past 2 decades has revealed a far more complex interaction of organs and tissues, with consequences for the fundamental understanding of the mechanisms of glucose disequilibrium and the nature of T2DM itself. Dysfunctions in the gastrointestinal tract, adipose tissue, pancreatic alpha cells, brain, and kidneys have all been described, and together with insights into the involvement of liver, muscle, and beta cells produce a more robust picture of T2DM. The function of the kidneys in abnormal glucose homeostasis is a striking example of this evolution in T2DM knowledge, as the role of glucose transporters in regulating plasma glucose levels and producing hyperglycemia has enhanced current understanding of T2DM. As pathophysiologic mechanisms and defects continue to be discovered, they offer an expansion of potential targets for treatment of T2DM.

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For author information and disclosures, see end of text.

■ **Figure 1.** Metabolic Abnormalities That Contribute to Hyperglycemia



resistance, glucose tolerance remains normal.<sup>3</sup> However, in patients who develop T2DM, the beta cells begin to fail over time, leading to deterioration in glucose homeostasis. This initially manifests as impaired glucose tolerance (IGT) and later as overt diabetes.<sup>2,3</sup> The resultant hyperglycemia and poor metabolic control may cause a further decline in insulin sensitivity and other metabolic derangement (ie, glucotoxicity); however, it is progressive beta cell failure that ultimately determines the rate of disease progression.<sup>7</sup>

#### *Relationship of Insulin Resistance in Tissues to Glucose Disequilibrium*

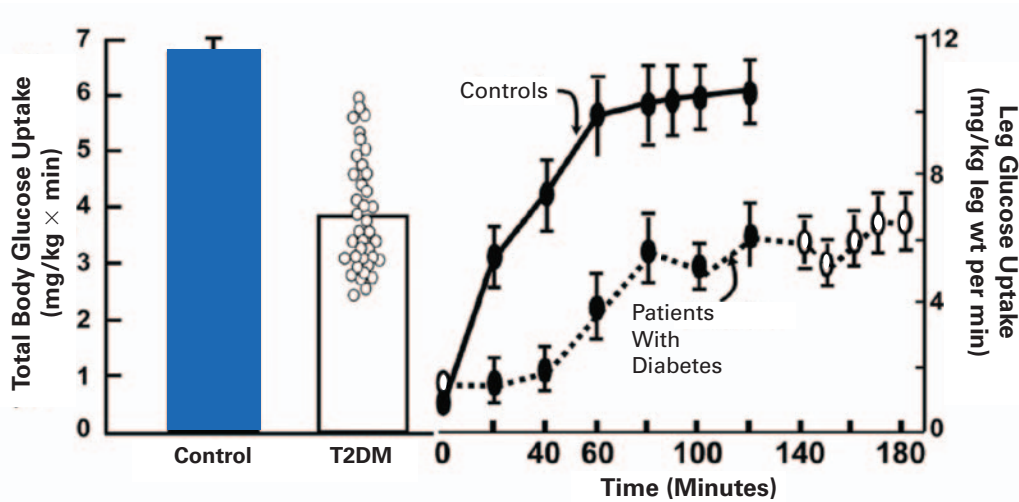
The term insulin resistance refers to impairment in insulin action in insulin-target tissues, such as skeletal muscle, liver, and adipocytes (fat cells). Insulin is the major anabolic hormone in the body and it is the major regulator of glucose metabolism. It stimulates glucose uptake and metabolism in skeletal muscle, suppresses hepatic glucose production (HGP), and restrains lipolysis in adipocytes.<sup>8,9</sup> In the presence of insulin resistance, all of these insulin actions are markedly impaired, leading to impaired insulin-mediated muscle glucose uptake and increased rates of HGP and lipolysis.

#### *Skeletal Muscle*

The primary action of insulin in skeletal muscle is to stimulate glucose uptake and metabolism. In lean healthy individuals, insulin stimulates glucose uptake into skeletal muscle in a dose-dependent manner. In insulin-resistant states, insulin-

stimulated glucose uptake is markedly reduced in skeletal muscle (Figure 2).<sup>2</sup> Both genetic and environmental factors contribute to the development of insulin resistance in skeletal muscle.<sup>1,10</sup> The important role of genetic background in the pathogenesis of insulin resistance can be demonstrated by the fact that lean healthy NGT offspring of 2 parents with T2DM manifest severe insulin resistance in skeletal muscle.<sup>5</sup> Also, obese patients with NGT as well as patients with IGT and T2DM demonstrate severe insulin resistance in skeletal muscle.<sup>11</sup> The euglycemic-hyperinsulinemic clamp represents the gold standard method for quantitation of insulin resistance, and studies with the euglycemic-hyperinsulinemic clamp in combination with femoral artery and vein catheterization have demonstrated multiple defects in insulin action in skeletal muscle.<sup>11,12</sup> The dose response curve relating insulin-stimulated glucose uptake and the plasma insulin concentration shifts to the right with an increase in half maximal effective concentration (EC50) to approximately 120 to 140  $\mu\text{U}/\text{mL}$  in patients with T2DM. In addition, the onset of insulin action in skeletal muscle in patients with T2DM is markedly delayed (Figure 2).<sup>2,11</sup> The molecular basis of insulin resistance in skeletal muscle has been extensively studied.<sup>11</sup> The ability of insulin to activate the insulin-signaling cascade in skeletal muscle is severely impaired in insulin-resistant individuals and this defect plays a paramount role in the pathogenesis of insulin resistance. In addition to the defect in insulin signaling, multiple intramyocellular (IMCL) defects in insulin action are present in insulin-resistant individuals, including impaired

■ **Figure 2.** Insulin-Stimulated Total Body Glucose Uptake and Insulin-Stimulated Leg Glucose Uptake in Patients With T2DM and Controls<sup>2</sup>



T2DM indicates type 2 diabetes mellitus.

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glucose transport and phosphorylation, reduced glycogen synthesis, and decreased glucose oxidation.<sup>11</sup> However, more proximal defects in the insulin signal transduction system play an important role in muscle insulin resistance.

#### Liver

In the fasting state, the body utilizes about 2 mg glucose/kg/min.<sup>13</sup> This glucose demand is perfectly matched by glucose production by the liver,<sup>8</sup> and glucoregulatory hormones maintain circulating glucose concentrations within a narrow range.<sup>14</sup> HGP is regulated by the direct and indirect effects of insulin, and a small increase in portal insulin concentration directly inhibits HGP.<sup>15</sup> The ED<sub>50</sub> for HGP inhibition by insulin is approximately 30 to 40  $\mu$ U/mL.<sup>3</sup> Because of the development of hepatic insulin resistance in T2DM, the rate of basal HGP is increased despite fasting hyperinsulinemia, averaging about 2.5 mg/kg/min.<sup>2</sup> In an average 80-kg person, this amounts to the addition of an extra 25 g to 30 g of glucose released to the systemic circulation every night.<sup>2</sup> Thus, the fasting plasma glucose concentration in individuals with T2DM strongly correlates with the rate of HGP (Figure 3).<sup>2</sup> In addition to hepatic insulin resistance, multiple other factors contribute to accelerated rate of HGP, including: 1) increased circulating glucagon levels and enhanced hepatic sensitivity to glucagon; 2) increased circulation of gluconeogenic precursors such as lactate, alanine, and glycerol; and 3) increased free fatty acid (FFA) oxidation.<sup>3</sup>

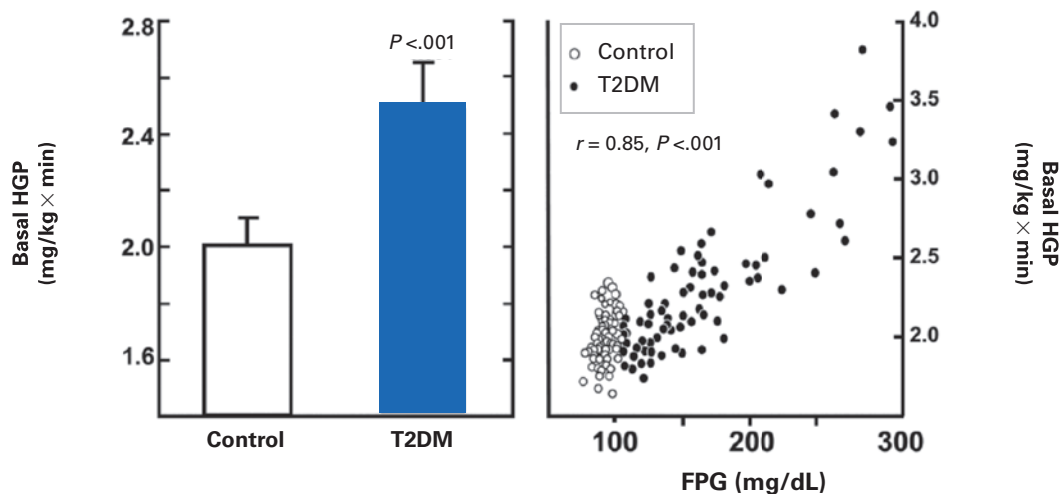
#### Adipocytes

Although the genetic background of the individual plays a pivotal role in the development of insulin resistance, the diabetes epidemic we are witnessing is driven primarily by the epidemic of obesity.<sup>16</sup> Obese patients display marked insulin resistance in extrahepatic tissues compared with lean matched individuals, and the extent of insulin resistance is related to the increase in body mass index (BMI).<sup>17-19</sup> Conversely, weight loss in obese, insulin-resistant, and diabetic patients improves insulin sensitivity.<sup>20</sup> Collectively, these studies demonstrate a causal role of obesity in the development of skeletal muscle insulin resistance.<sup>11</sup>

The mechanism via which obesity causes insulin resistance in skeletal muscle is related to the accumulation of fat in the myocytes.<sup>11,21</sup> Muscle biopsy studies have demonstrated an inverse relationship between muscle insulin sensitivity and intramuscular triglyceride content.<sup>22</sup> More recent studies using magnetic resonance spectroscopy have demonstrated that, although IMCL fat content contributes only a small fraction, a mean of 2.21%, to the total muscle fat content,<sup>23</sup> it plays a key role in the development of insulin resistance in skeletal muscle compared with extramyocellular fat content. Muscle insulin resistance strongly correlates with IMCL, independent of BMI, fasting blood glucose, and age.<sup>23</sup>

Considerable evidence links abnormal adipocyte metabolism to the pathogenesis of T2DM. The clinical sequelae of

■ **Figure 3.** Basal Hepatic Glucose Production in Patients With T2DM and Controls<sup>2</sup>



FPG indicates fasting plasma glucose; HGP, hepatic glucose production; T2DM, type 2 diabetes mellitus. Reprinted with permission from DeFronzo RA. *Diabetes*. 2009;58(4):773-795.

obesity are consequent to the pathological hyperplasia and hypertrophy of fat cells that increase FFA secretion, impair hepatic insulin clearance, and alter peripheral metabolism.<sup>19</sup> Chronic elevation of plasma FFA concentration has been shown to cause severe insulin resistance in skeletal muscle and liver and may impair insulin secretion in patients with a positive family history of T2DM.<sup>24,25</sup> Moreover, enlarged adipocytes have diminished capacity to store fat, and when the maximal fat storage capacity of the adipocytes is exceeded, excess lipid spills over to lean tissue (eg, skeletal muscle, liver, and beta cells), causing insulin resistance and impaired insulin secretion.<sup>26</sup> Consistent with this fat overflow hypothesis, acipimox, a drug which inhibits lipolysis and reduces plasma FFA concentration, markedly improves insulin sensitivity and insulin secretion in both obese nondiabetic individuals and in patients with T2DM.<sup>27-29</sup> Lastly, dysfunctional fat cells produce excessive amounts of insulin resistance-inducing inflammatory adipocytokines (eg, TNF alpha and interleukins), and fail to produce insulin-sensitizing adipokines such as adiponectin.<sup>30</sup>

#### Brain

As noted, the current epidemic of diabetes is driven by excessive body weight and obesity, and increased food intake is likely the major factor contributing to high rates of obesity.<sup>16</sup> Insulin resistance and compensatory hyperinsulinemia are characteristic among the obese, both diabetic and nondiabetic individuals.<sup>2</sup> Studies in experimental animals have demonstrated that insulin suppresses appetite and decreases food intake.<sup>31</sup> Despite hyperinsulinemia, obese patients dem-

onstrate increased food intake. Furthermore, peripheral tissue insulin resistance may also include the brain.<sup>2</sup> Studies using functional magnetic resonance imaging (MRI) have demonstrated that following a meal, there is suppression of the activity of 2 hypothalamic areas which are key centers for appetite regulation, and the magnitude of inhibition of these 2 areas is markedly attenuated in obese individuals. Moreover, obese individuals manifest a delay in the time taken to reach the maximum inhibitory response compared with lean individuals, even though the plasma insulin response was markedly increased.<sup>32</sup> Whether the impaired functional MRI response in obese patients contributes to or is a consequence of the insulin resistance and weight gain, or is due to impaired secretion and/or action of other peptides secreted from the gut in response to a meal and that regulate appetite (eg, GLP-1), remains to be seen. Nonetheless, these observations demonstrate the presence of a defect in the brain-gut axis which is responsible for the regulation of appetite and food intake. One also can speculate that it is likely that this defect plays an important role in the development of obesity and T2DM.

#### Relationship of Beta Cell Failure to Glucose Disequilibrium

Progressive beta cell failure is the primary defect responsible for the development and progression of hyperglycemia in individuals with T2DM.<sup>33</sup> The exact mechanism of progressive beta cell failure in T2DM remains poorly understood. However, it is now apparent that the impairment in beta cell function begins long before overt T2DM is evident.<sup>2</sup> Both genetic and other factors contribute to this process. Several

lines of evidence indicate a pivotal role of the genetic makeup of the individuals in the development of beta cell failure. Thus, beta cell failure clusters in families and NGT offspring of parents with T2DM manifest impaired first phase insulin secretion.<sup>34</sup> Due to the inheritability of defects in insulin secretion and action among patients with T2DM, there are substantial implications for future genetic studies.<sup>34</sup>

Other factors that contribute to beta cell failure include lipotoxicity, glucotoxicity, and incretin deficiency, as described below.

#### *Lipotoxicity Due to Elevated Plasma FFA Levels*

Physiological elevation of the plasma FFA concentration for as little as 48 hours markedly impairs insulin secretion in genetically predisposed individuals.<sup>25</sup> Conversely, a sustained reduction in plasma FFA concentration with acipimox in nondiabetic patients improved insulin sensitivity.<sup>29</sup> Moreover, peroxisome proliferator-activated receptor (PPAR) agonists (ie, rosiglitazone and pioglitazone), which deplete beta cell fat content, have been shown to improve beta cell function.<sup>35,36</sup>

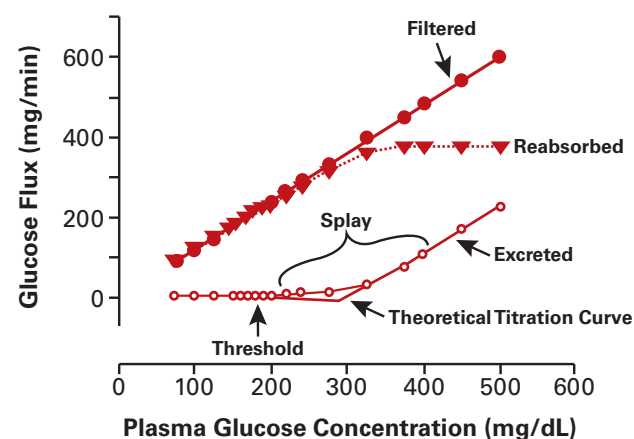
#### *Glucotoxicity*

Persistent elevation in plasma glucose concentration impairs beta cell function, and this has been referred to as glucotoxicity. Studies by Rossetti et al in partially pancreatectomized rats provided definitive proof of this concept. Following treatment with phlorizin, an inhibitor of renal glucose transport, the plasma glucose profile was normalized without changes in any other circulating metabolites. Normalization of the plasma glucose profile was associated with restoration of both the first and second phases of insulin secretion.<sup>37</sup> In vitro studies with isolated rat islets have also demonstrated that chronic exposure to elevated plasma glucose levels impairs insulin secretion.<sup>35</sup> In rats, Leahy et al showed that even a small elevation of the mean day-long plasma glucose concentration in vivo leads to a marked inhibition of glucose-stimulated insulin secretion in the isolated perfused pancreas.<sup>38</sup> Thus, strict glycemic control is essential not only to prevent the microvascular complications of diabetes but also to reverse the glucotoxic effect of chronic hyperglycemia on the beta cells, as well as on hepatic and muscle insulin resistance.

#### *Incretin Deficiency*

Patients with T2DM manifest multiple abnormalities in the incretin axis.<sup>2</sup> The ratio between insulin secretion during the oral glucose tolerance test and insulin secretion during

■ **Figure 4.** Glucose Reabsorption and Excretion by the Kidney<sup>49</sup>



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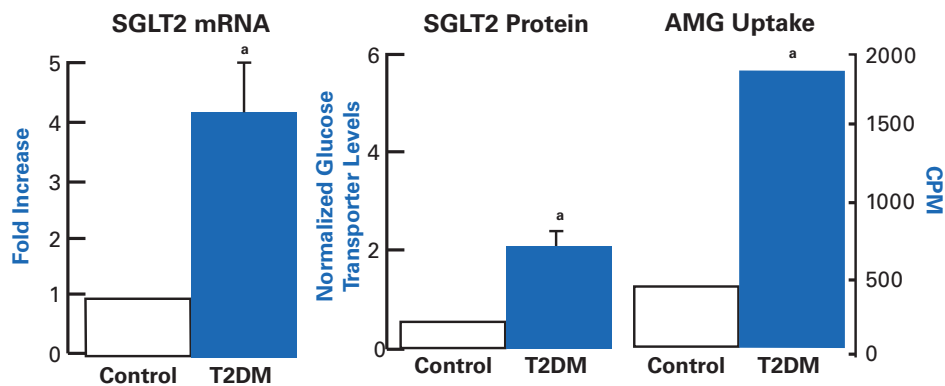
similar glucose stimulus produced with intravenous glucose administration represents the incretin effect and it is markedly reduced in patients with T2DM.<sup>39</sup> Two incretins, GLP-1 and GIP, account for the incretin effect.<sup>40</sup> The decrease in incretin effect in patients with T2DM can be explained primarily by resistance to the action of GIP,<sup>41</sup> and to a lesser extent by a resistance to GLP-1 action.<sup>42</sup> In addition, there is a deficiency of GLP-1 in T2DM.<sup>43</sup> The deficiency of GLP-1 can be observed in individuals with IGT and worsens progressively with progression to T2DM.<sup>41</sup> In contrast to GLP-1, plasma levels of GIP are elevated in T2DM, yet circulating plasma insulin levels are reduced.<sup>44</sup> This emphasizes the severity of beta cell resistance to the stimulatory effect of GIP on insulin secretion. Recent studies have shown that tight glycemic control can restore the beta cells' insulin secretory response to GIP.<sup>45</sup> Thus, beta cell resistance to GIP is another manifestation of glucotoxicity. Although the resistance to GIP and GLP-1 action reflects a defect in the beta cell, the impaired GLP-1 secretion in IGT and T2DM in the presence of hyperglycemia represents a defect in the L-cell in the gut and demonstrates the contribution of the gastrointestinal tract abnormality to the pathogenesis of T2DM.<sup>2,41</sup>

### **Additional Mechanisms of Glucose Disequilibrium in T2DM**

#### *Alpha Cells*

Glucagon is secreted from the alpha cell in the pancreatic islet in response to hypoglycemia; glucagon then acts on the liver to stimulate HGP and restore normoglycemia. Despite

■ **Figure 5.** SGLT2 Transporter mRNA, Protein, and Glucose Transport in Cultured Renal Proximal Tubular Epithelial Cells of Patients With T2DM and Controls<sup>54</sup>



<sup>a</sup> $P < .05$ -.01.

AMG indicates methyl- $\alpha$ -D-[U14C]-glucopyranoside; CPM, counts per minute; mRNA, messenger ribonucleic acid; SGLT2, sodium-glucose cotransporter 2; T2DM, type 2 diabetes mellitus.

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fasting hyperglycemia, the basal plasma glucagon concentration is elevated in individuals with T2DM. Moreover, the suppression of glucagon level after a meal is markedly impaired in individuals with T2DM.<sup>1,46</sup> Clinical study data have demonstrated that the hyperglucagonemia in diabetic individuals significantly contributes to the increased basal rate of HGP which is characteristic of T2DM.<sup>47</sup> Since an increased rate of basal HGP is the principal factor responsible for fasting hyperglycemia in T2DM individuals, these observations demonstrate the pivotal role of hyperglucagonemia in the pathogenesis of fasting hyperglycemia in T2DM and establish glucagon as a therapeutic target for lowering the fasting plasma glucose concentration. Indeed, several glucagon antagonists are in development and have been shown to be effective in lowering fasting hyperglycemia in individuals with T2DM.<sup>48</sup>

### Kidneys

The kidneys filter approximately 180 liters of plasma each day. In normal healthy individuals with a plasma glucose concentration of approximately 90 to 100 mg/dL, this filtrate contains around 162 grams of glucose.<sup>49</sup> In patients with NGT virtually all of this glucose is reabsorbed in the proximal tubule and no glucose is excreted in the urine.<sup>50</sup> The maximum glucose transport capacity ( $T_m$ ) of the proximal tubule has a value of about 375 mg/min, on average (Figure 4).<sup>49</sup> Renal glucose reabsorption takes place in the proximal tubule primarily in the S1 and S3 segments, and is mediated by sodium-glucose co-transporters (SGLTs) which couple glucose reabsorption to sodium reabsorption. The sodium electrochemical gradient generated by active sodium trans-

port provides the energy required for glucose transport.<sup>49</sup> To date, 6 human SGLTs have been identified.<sup>51</sup> However, only 2 transporters are responsible for renal glucose reabsorption. SGLT2 is exclusively expressed in the kidney and has low affinity and high capacity for glucose transport; it is primarily located in the S1 segment of the proximal tubule and absorbs 80% to 90% of filtered glucose.<sup>49,51,52</sup> SGLT1 is expressed in the kidney and gut and transports glucose and galactose, and is responsible for the majority of glucose and galactose uptake in the gut.<sup>50,53,54</sup> SGLT1 is located in the S3 segment of the proximal tubule in the kidney and is responsible for the reabsorption of the remaining 10% of filtered glucose.<sup>49,51</sup> In normal individuals, the filtered glucose load is less than the maximal glucose transport capacity ( $T_m$ , 375 mg/min). Thus, all of the filtered glucose is reabsorbed and returned to the circulation. However, if the filtered glucose load exceeds 375 mg/min, as often occurs in individuals with poorly controlled diabetes, the  $T_m$  is exceeded, and all of the glucose in excess of the  $T_m$  is excreted in the urine. The plasma glucose concentration at which the filtered glucose load reaches the  $T_m$  (375 mg/min) is called the threshold and in healthy individuals is approximately 180 mg/dL; above this threshold, the glucose excretion rate increases linearly and parallels the filtered load (Figure 4).<sup>49</sup> However, the reabsorption and excretion curves display a non-linear transition as the  $T_m$  for glucose is approached. This “rounding” of the curves is termed splay, and has been explained by heterogeneity in the  $T_m$  of individual nephrons and/or glomerulotubular imbalance.<sup>54</sup>

Patients with NGT have a  $T_m$  for glucose that is well above the filtered glucose load. This has major survival

benefits, since it allows the kidneys to conserve this critical energy source for the brain, which (with the exception of prolonged fasting) can utilize only glucose to generate energy for neuronal function.<sup>49</sup> Studies in human cellular models have demonstrated that the increase in plasma glucose concentration is associated with upregulation of SGLT2 expression in the kidney (Figure 5).<sup>54,55</sup> Consistent with this observation, individuals with T2DM have increased renal T<sub>m</sub> for glucose. Thus, one can postulate that, because hyperglycemia results in glucose filtration load in excess of the T<sub>m</sub>, the increase in T<sub>m</sub> during hyperglycemia represents an adaptive mechanism to prevent glucosuria and preserve energy during conditions when food was sparse. Thus, the increase in renal T<sub>m</sub> in diabetic individuals contributes to the maintenance of hyperglycemia. Today, when food is abundant and diabetes has reached epidemic proportions, this adaptive mechanism has become maladaptive, because the increase in renal T<sub>m</sub> in diabetic individuals contributes to the maintenance of hyperglycemia. In patients with diabetes it would be desirable for the kidney to excrete the excess filtered glucose load, to restore normoglycemia. Instead, the increased T<sub>m</sub> for glucose minimizes glucosuria and exacerbates the hyperglycemia.<sup>49</sup>

Since both SGLT1 and SGLT2 utilize active cotransport to couple glucose transport to the sodium gradient, increased glucose uptake in the proximal tubule in patients with diabetes is expected to be accompanied by increased sodium reabsorption by SGLT2.<sup>54</sup> One can speculate that the increased sodium reabsorption may lead to extracellular volume expansion and an increase in blood pressure. Although this hypothesis never has been tested in humans, a recent micropuncture study in experimental animals has demonstrated that acute inhibition of SGLT2 causes a 2- to 3-fold increase in single nephron sodium excretion. However, the increased sodium excretion waned after 2 weeks, suggesting an important role of tubuloglomerular feedback in long-term fluid and salt balance.<sup>56</sup>

### Summary

T2DM is a disease characterized by the dysregulation of glucose homeostasis, resulting in hyperglycemia. The pathophysiology of T2DM involves a complex relationship of dysfunction occurring in various organs and tissues. New insights into the pathophysiology of T2DM, and the role of various organs and tissues such as the kidneys in regulating blood sugar, may potentially reveal pathophysiological mechanisms and defects that could be targeted for the insulin-independent pharmaceutical treatment of T2DM.

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**Address correspondence to:** Muhammad A. Abdul-Ghani, MD, PhD, Diabetes Division, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Dr, San Antonio, TX 78229. E-mail: [abdulghani@uthscsa.edu](mailto:abdulghani@uthscsa.edu).

## REFERENCES

1. Powers AC. Diabetes mellitus. In: Longo DL, Fauci AS, Kasper DL, Hauser SL, Jameson JL, Loscalzo J, eds. *Harrison's Principles of Internal Medicine*. 18th ed. New York, NY: McGraw-Hill; 2012. <http://www.accessmedicine.com/content.aspx?aID=9141196>. Accessed March 6, 2012.
2. DeFronzo RA. Banting lecture: from the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes*. 2009;58(4):773-795.
3. DeFronzo RA. Pathogenesis of type 2 diabetes mellitus. *Med Clin North Am*. 2004;88:787-835.
4. Saad MF, Knowler WC, Pettitt DJ, Nelson RG, Mott DM, Bennett PH. The natural history of impaired glucose tolerance in the Pima Indians. *N Engl J Med*. 1988;319:1500-1505.
5. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*. 2004;350:664-671.
6. Neeland IJ, Turer AT, Ayers CR, et al. Dysfunctional adiposity and the risk of prediabetes and type 2 diabetes in obese adults. *JAMA*. 2012;308(11):1150-1159.
7. Rossetti L, Giaccari A, DeFronzo RA. Glucose toxicity. *Diabetes Care*. 1990;13:610-630.
8. DeFronzo RA, Ferrannini E, Simonson DC. Fasting hyperglycemia in non-insulin dependent diabetes mellitus: contributions of excessive hepatic glucose production and impaired tissue glucose uptake. *Metabolism*. 1989;38:387-395.
9. Groop LC, Bonadonna RC, DelPrato S, et al. Glucose and free fatty acid metabolism in non-insulin-dependent diabetes mellitus: evidence for multiple sites of insulin resistance. *J Clin Invest*. 1989;84(1):205-213.
10. Watanabe RM, Valle T, Hauser ER, et al. Familiarity of quantitative metabolic traits in Finnish families with non-insulin dependent diabetes mellitus: Finland-United States Investigation of NIDDM Genes (FUSION) Study Investigators. *Hum Hered*. 1999;39:159-168.
11. Abdul-Ghani MA, DeFronzo RA. Pathogenesis of insulin resistance in skeletal muscle. *J Biomed Biotechnol*. 2010;2010:476279.
12. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in non-insulin-dependent (type II) diabetes mellitus. *J Clin Invest*. 1985;76:149-155.
13. DeFronzo RA, Ferrannini E. Regulation of intermediary metabolism during fasting and refeeding. In: DeGroot LJ, Jameson JL, eds. *Endocrinology*. Philadelphia, PA: Elsevier; 2006:1015-1043.
14. Aronoff SL, Berkowitz K, Shreiner B, et al. Glucose metabolism and regulation: beyond insulin and glucagon. *Diabetes Spectrum*. 2004;17(3):183-190.
15. Girard J. The inhibitory effects of insulin on hepatic glucose production are both direct and indirect. *Diabetes*. 2006;55(suppl 2):S65-S69.

16. Hu FB. Globalization of diabetes: the role of diet, lifestyle, and genes. *Diabetes Care*. 2011;34:1249-1257.
17. DeFronzo RA, Soman V, Sherwin RS, et al. Insulin binding to monocytes and insulin action in human obesity, starvation, and refeeding. *J Clin Invest*. 1978;204-213.
18. Garcia-Estevez DA, Araujo-Vilar D, Saavedra-Gonzalez A, et al. Analysis of the relationship between body mass index, insulin resistance, and beta-cell function: a cross-sectional study using the minimal model. *Metabolism*. 2004;53(11):1462-1466.
19. Bray GA. Medical consequences of obesity. *J Clin Endocrinol Metab*. 2004;89(6):2583-2589.
20. Corpeleijn E, Saris WHM, Blaak EE. Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle. *Obes Rev*. 2009;10:178-193.
21. Greco AV, Mingrone G, Giancaterini A, et al. Insulin resistance in morbid obesity: reversal with intramyocellular fat depletion. *Diabetes*. 2002;51:144-151.
22. Pan DA, Lillioja S, Kriketos AD, et al. Skeletal muscle triglyceride levels are inversely related to insulin action. *Diabetes*. 1997;46(6):983-988.
23. Krssak M, Petersen KF, Dresner A, et al. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a <sup>1</sup>H NMR spectroscopy study. *Diabetologia*. 1999;42:113-116.
24. Roden M, Price TB, Perseghin G, et al. Mechanism of free fatty acid-induced insulin resistance in humans. *J Clin Invest*. 1996;97:2859-2865.
25. Kashyap S, Belfort R, Gastaldelli A, et al. A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. *Diabetes*. 2003;52:2461-2474.
26. DeFronzo RA. Dysfunctional fat cells, lipotoxicity, and type 2 diabetes. *Int J Clin Pract Suppl*. 2004;143:9-21.
27. Bajaj M, Suraamornkul S, Romanelli A, et al. Effect of sustained reduction in plasma free fatty acid concentration on intramuscular long chain-fatty acyl-CoAs and insulin action in patients with type 2 diabetes. *Diabetes*. 2005;54:3148-3153.
28. Paulisso G, Tagliamonte MR, Rizzo MR, et al. Lowering fatty acids potentiates acute insulin response in first degree relatives of people with type II diabetes. *Diabetologia*. 1998;41:1127-1132.
29. Santomauro ATMG, Boden G, Silva MER, et al. Overnight lowering of free fatty acids with acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. *Diabetes*. 1999;48:1836-1841.
30. Bays H, Mandarino L, DeFronzo RA. Role of the adipocyte, free fatty acids, and ectopic fat in pathogenesis of type 2 diabetes mellitus: peroxisomal proliferator-activated receptor agonists provide a rational therapeutic approach. *J Clin Endocrinol Metab*. 2004;89(2):463-478.
31. Porte D. Central regulation of energy homeostasis. *Diabetes*. 2006;55(suppl 2):S155-S160.
32. Matsuda M, Liu Y, Mahankali S, et al. Altered hypothalamic function in response to glucose ingestion in obese humans. *Diabetes*. 1999;48:1801-1806.
33. van Raalte DH, Diamant M. Glucolipotoxicity and beta cells in type 2 diabetes mellitus: target for durable therapy? *Diabetes Res Clin Pract*. 2011;93S:S37-S46.
34. Vauhkonen I, Niskanen L, Vanninen E, Kainulainen S, Uusitupa M, Laakso M. Defects in insulin secretion and insulin action in non-insulin-dependent diabetes mellitus are inherited: metabolic studies on offspring of diabetic probands. *J Clin Invest*. 1998;101(1):86-96.
35. Patane G, Anello M, Piro S, Vigneri R, Purrello F, Rabuazzo AM. Role of ATP production and uncoupling protein-2 in the insulin secretory defect induced by chronic exposure to high glucose or free fatty acids and effects of peroxisome proliferator-activated receptor- $\gamma$  inhibition. *Diabetes*. 2002;51:2749-2756.
36. Gastaldelli A, Ferrannini E, Miyazaki Y, et al. Thiazolidinediones improve  $\beta$ -cell function in type 2 diabetic patients. *Am J Physiol Endocrinol Metab*. 2007;292:E871-E883.
37. Rossetti L, Shulman GI, Zawulich W, DeFronzo RA. Effect of chronic hyperglycemia on in vivo insulin secretion in partially pancreatectomized rats. *J Clin Invest*. 1987;80:1037-1044.
38. Leahy JL, Cooper HE, Deal DA, et al. Chronic hyperglycemia is associated with impaired glucose influence on insulin secretion. *J Clin Invest*. 1986;77:908-915.
39. Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. *Diabetologia*. 1986;29:46-52.
40. Kim W, Egan JM. The role of incretins in glucose homeostasis and diabetes treatment. *Pharmacol Rev*. 2008;60:470-512.
41. Meier JJ, Nauck MA. Incretins and the development of type 2 diabetes. *Curren Diab Reports*. 2006;6:194-201.
42. Holst JJ, Gromada J. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *Am J Physiol Endocrinol Metab*. 2004;287:E199-E206.
43. Vilsbøll T, Krarup T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes*. 2001;50(3):609-613.
44. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest*. 1993;91:301-307.
45. Hojberg PV, Vilsbøll T, Rabøl R, et al. Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. *Diabetologia*. 2009;52:199-207.
46. Matsuda M, DeFronzo RA, Glass L, et al. Glucagon dose response curve for hepatic glucose production and glucose disposal in type 2 diabetic patients and normal individuals. *Metabolism*. 2002;51:1111-1119.
47. Baron AD, Schaeffer L, Shragg P, Kolterman OG. Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics. *Diabetes*. 1987;36:274-283.
48. Petersen KF, Sullivan JT. Effects of a novel glucagon receptor antagonist (Bay 27-9955) on glucagon-stimulated glucose production in humans. *Diabetologia*. 2001;44:2018-2024.
49. Abdul-Ghani MA, DeFronzo RA. Inhibition of renal glucose reabsorption: a novel strategy for achieving glucose control in type 2 diabetes mellitus. *Endocr Pract*. 2008;14:782-790.
50. DeFronzo RA, Davidson JA, Del Prato S. The role of the kidneys in glucose homeostasis: a new path towards normalizing glycaemia. *Diabetes Obes Metab*. 2012;14(1):5-14.
51. Wright EM, Loo DD, Hirayama BA. Biology of human sodium glucose transporters. *Physiol Rev*. 2011;91:733-794.
52. Vallon V, Platt KA, Cunard R, et al. SGLT2 mediates glucose reabsorption in the early proximal tubule. *J Am Soc Nephrol*. 2011;22:104-112.
53. Wright EM, Hirayama BA, Loo DF. Active sugar transport in health and disease. *J Intern Med*. 2007;261:32-43.
54. Abdul-Ghani MA, Norton L, DeFronzo RA. Role of sodium-glucose cotransporter 2 (SGLT2) inhibitors in the treatment of type 2 diabetes. *Endocr Rev*. 2011;32:515-531.
55. Rahmoune H, Thompson PW, Ward JM, et al. Glucose transporters in human renal proximal tubular cells isolated from the urine of patients with non-insulin-dependent diabetes. *Diabetes*. 2005;54:3427-3434.
56. Thomson SC, Rieg T, Miracle C, et al. Acute and chronic effects of SGLT2 blockade on glomerular and tubular function in the early diabetic rat. *Am J Physiol Regul Integr Comp Physiol*. 2012;302:R75-R83.