Mechanisms of current therapies for diabetes mellitus type 2

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Thulé PM. Mechanisms of current therapies for diabetes mellitus type 2. Adv Physiol Educ 36: 275–283, 2012; doi:10.1152/advan.00094.2012.—Recent investigations have clarified major aspects of type 2 diabetes mellitus (DM) pathophysiology. As demonstrated by Robert Rizza and colleagues, an oral glucose challenge increases blood sugars in subjects with mild type 2 DM more, and for a longer duration, than subjects without diabetes (56). Moreover, the expected rise in serum insulin occurs later and remains elevated longer (56). In addition to this β-cell dysfunction, glucagon concentrations remain unsuppressed (56). In subjects with more pronounced diabetes, glucose levels are greater throughout the challenge, β-cell dysfunction is more severe, and normal suppression of glucagon levels is absent (56).

Two additional aspects of pathophysiology among patients with type 2 DM can be observed when subjects are studied during an insulin clamp. In a stepped hyperinsulminemic-euglycemic clamp, circulating insulin is provided by an intravenous infusion and maintained at predetermined levels. Endogenous glucose production by the liver is then calculated by measuring the dilution of an administered radioactive glucose tracer. Finally, glucose disposal is calculated based on the rate of administered glucose needed to maintain blood sugar levels. When performed on patients with type 2 DM, endogenous glucose production is not appropriately suppressed, whereas glucose disposal is consistently subnormal (56).

Together, these data underscore the major abnormalities in diabetes: insufficient β-cell insulin secretion, unsuppressed postprandial glucagon secretion, hepatic insulin resistance producing excessive endogenous glucose production, and insulin resistance in peripheral tissues, predominantly fat and skeletal muscle. Recognizing the major pathology of DM, the modulators of blood glucose can be extrapolated and viewed schematically as 1) the quantity of calories, particularly carbohydrates, consumed; 2) the efficiency with which carbohydrates are absorbed; 3) the insulin and glucagon responses to blood glucose levels; 4) hepatic disposal and the production of glucose; and 5) the disposal of glucose into peripheral tissues, particularly fat and adipose tissue.

In this article, I will outline the major mechanisms underlying the efficacy of antihyperglycemic medications currently approved in the United States (U.S.), with the exception of insulin and insulin analogs. Antiabsorptives reduce the quantity of glucose entering the bloodstream from the intestinal tract. Insulin secretagogues, which include both sulfonylureas and meglitinides, stimulate the secretion of insulin from pancreatic β-cells. Incretin mimetics, glucagon-like peptide (GLP)-1, agonists and dipeptidyl peptidase (DPP)-4 inhibitors affect multiple axes, including β- and α-cell functions. The biguanide metformin inhibits hepatic glucose production. Peroxisome proliferator-activated receptor (PPAR)-γ agonists [thiazolidinediones (TZDs)] expand subcutaneous adipose tissue and secondarily reduce intramyocellular lipids (IMCLs) and intraheptocellular lipids and insulin resistance. Finally, the dopamine agonist bromocriptine (marketed as Cycloset) works in the central nervous system to increase nonoxidative glucose metabolism.

Antiabsorptive Agents

Carbohydrates commonly enter the body as starches or other compound sugars that must undergo stepwise enzymatic degradation to be absorbed. In the gut lumen, starches are first degraded by pancreatic amylase into smaller sugars, maltose, maltotriose, and dextrins, which are subsequently degraded into glucose by enterocyte membrane-bound α-glucosidases (33). Similarly, sucrose is degraded to fructose and glucose by sucrase. α-Glucosidase inhibitors reversibly limit the function of these enzymes and thus delay the absorption of ingested carbohydrates. While moderately effective at preventing postprandial elevations in blood sugar, the retention of ingested carbohydrates in the gut lumen leads to bacterial consumption and the major adverse reaction to these medications, flatulence (33). The three commercially available α-glucosidase inhibitors (acarbose, voglibose, and miglitol) are similarly effective in reducing blood sugars but exhibit some differences. Acarbose alone inhibits pancreatic amylase and has a greater affinity for sucrase than voglibose. Miglitol is the only compound absorbed to a significant extent and may also inhibit glucose transport across the enterocyte membrane (33).

Nonabsorbable bile acid sequestrants are one class of medication used to reduce serum cholesterol levels. Approved for this purpose in 2000, colesevelam, when used in patients with diabetes, was noted to also reduce blood sugars (17). When used as an add-on therapy in a 26-wk randomized, controlled trial, colesevelam reduced hemoglobin (Hb)A1c by ~0.5% compared with patients receiving sulfonylureas alone (9). However, the mechanism by which colesevelam lowers blood glucose remains undetermined.

In a double-blinded, randomized, controlled trial, Henry et al. (21) performed oral glucose tolerance tests and a two-step
hyperinsulinemic-euglycemic clamp both before and after 12 wk of colesevelam monotherapy in diabetic patients that had undergone an 18-wk washout period. The initial oral glucose tolerance test was performed immediately after acute colesevelam administration to assess effects on glucose absorption. Surprisingly, they discovered no difference in HbA1c or fasting blood glucose levels between treatment groups. Furthermore, they discovered no effects on endogenous glucose output or insulin suppression of the endogenous glucose output. Their findings did reveal a small reduction in acute glucose absorption, but this effect was not observed with chronic administration (21). Consequently, this carefully executed study failed to reveal a glucose-lowering mechanism for colesevelam.

Others have hypothesized that bile acids retained in the gut by colesevelam stimulate intestinal L-cells to secrete GLP-1. To investigate this possibility, Shang et al. (63) determined plasma GLP-1 levels in diet-induced obese rats after 8 wk of treatment with colesevelam and a high-fat diet and compared the results with treatment with a bile acid receptor blocker. After an oral glucose challenge, GLP-1 values in colesevelam-treated animals were elevated compared with untreated rats. This effect was not observed in rats treated with the bile acid receptor blocker, suggesting that although colesevelam treatment elevates serum GLP-1 concentrations, this effect is not mediated by bile acid stimulation via bile acid receptors (63). Another bile acid sequestrant has been shown to increase GLP-1 in human subjects. Suzuki et al. (67) observed significant declines in postprandial glucose values and GLP-1 elevations after a week of treatment with colestimide in 16 patients admitted to hospital for hyperglycemia (24). Both insulin and glucagon levels were unaffected. However, this small study was neither randomized nor placebo controlled. In summary, the currently available data remain inconclusive regarding the glucose-lowering mechanism of colesevelam.

**Insulin Secretagogues**

Insulin secretagogues, particularly sulfonylureas, remain a mainstay of therapy for DM due to their proven efficacy and low cost. Recently, enthusiasm for their use has been attenuated by their capacity to induce glucose-independent insulin release and subsequent hypoglycemia. In addition, their use is associated with weight gain, and some evidence in vitro suggests that they may accelerate β-cell failure. The meglitinides, also called glinides, possess a significantly shorter duration of action than the sulfonylureas and consequently produce less hypoglycemia. They tend, however, to be more costly.

Both sulfonylureas and meglitinides induce glucose-independent insulin release from β-cells by inhibiting K⁺ flux through ATP-dependent K⁺ (K̂_{ATP}) channels (61). This inhibition depolarizes the β-cell membrane, which opens voltage-dependent Ca²⁺ channels in the membrane (2). The entry of Ca²⁺ stimulates the recruitment of storage vesicles containing insulin to, and fusion with, the cell membrane, leading to insulin release (2).

K̂_{ATP} channels are heterooctamers consisting of four K⁺ pore units and four regulatory units, called sulfonylurea receptors (SURs) (62). The two isoforms of K⁺ pore units (Kir6.1 and Kir6.2) and the three isoforms of SURs (SUR1, SUR2A, and SUR2B) are widely distributed in cardiomyocytes, vascular smooth muscle, and skeletal muscle cells (61). However, the combination of Kir6.2 and SUR1 is dominant and operative in the pancreatic β-cell (62). Secretagogues bind to one or both of the two binding sites on SUR1 to inhibit the K̂_{ATP} channel and stimulate insulin secretion (61).

In addition to their binding to SUR1, sulfonylureas have been posited to signal via an additional alternate pathway to exert their full effect. Exchange protein directly activated by cAMP (Epac)2 is a guanine nucleotide exchange factor that interacts with the protein Rap1 to increase the rapidly releasable pool of insulin vesicles poised for membrane fusion in the β-cell (62). Epac2 usually responds to cAMP generated by alternative signaling pathways. However, recent evidence indicates that sulfonylureas bind not only to SUR1 but also to Epac2 (22). Insulin secretion was diminished in Epac2 knockout (KO) mice in vivo after a combined glucose and sulfonylurea challenge and from their isolated islets in vitro (72). Moreover, using isolated Epac2 synthesized with integrated fluorophores on either side of the cAMP responsive hinge region, Herbst et al. (22) were able to demonstrate that the addition of glyburide dose dependently reduced the fluorescence resonance energy transfer between fluorophores. This same reduction was reproduced by the addition of cAMP, an agent known to cause rotation of the molecular hinge, suggesting that sulfonylureas, like cAMP, bind to Epac2. However, other investigators (55) using an in vitro activity assay failed to confirm a direct interaction between Epac2 and sulfonylureas. Consequently, while the full effect of sulfonylureas appears to require both SUR1-mediated cell depolarization as well as an Epac2-mediated signal, the precise nature of the Epac2 signal remains controversial.

Like sulfonylureas, meglitinides also bind to SUR1 and induce β-cell membrane depolarization with a subsequent release of insulin (25). Their typically much shorter duration of action may be explained by their more rapid dissociation from SUR1 binding (25). This can be observed by comparing the K⁺ flux in rat β-cells after the addition of either nateglinide or glyburide to primary cultured rat β-cells stimulated with diazoxide, a K̂_{ATP} channel opener. Nateglinide reduces K⁺ flux for −50 min (25). In contrast, in the same assay, glyburide inhibits K⁺ flux for more than twice as long (25). Importantly, Seino et al. (62) reported that meglitinides do not activate Epac2, offering another explanation for the divergent mechanisms between sulfonylureas and glinides.

**Incretin Mimetics and DPP-4 Inhibitors**

It has been known for some time that oral glucose is a more robust stimulator of insulin secretion than intravenous glucose. Nauck et al. (48) demonstrated this by determining the quantity of intravenous glucose required to mimic the blood glucose curve after challenges with 25, 50, and 100 g of oral glucose. Only a fraction of the oral glucose load was required to mimic the blood sugar levels when administered intravenously. Moreover, the amount of intravenous glucose required remained stable, despite a quadrupling of the oral glucose. The observation that only a fraction of the oral glucose load was required to reproduce blood glucose levels when administered intravenously was explained by the insulin and C-peptide levels, which were elevated significantly more by the oral challenges than by the intravenous challenges (48). Indeed, the authors...
calculated that 20–80% of the insulin response after oral glucose was attributable to gut produced incretins that remained unstimulated after intravenous glucose loading (48).

The majority of the measurable incretin effect in humans is accounted for by the actions of glucose-dependent insulino-tropic polypeptide (GIP) and GLP-1 (3). GIP is produced by K-cells, which are most prevalent in the duodenum and ileum of the proximal gut, in response to fat and glucose (3). GLP-1 is produced by L-cells, which, in contrast, are more prevalent in the distal ileum and colon, in response to fats and carbohydrates (3).

Currently, only GLP-1 agonists are used for the treatment of DM. GLP-1 is derived from the proglucagon gene and message and prohormone (3). Selective cleavage by prohormone convertase (PC)2 in α-cells produces glicentin-related polypeptide, glucagon, intervening protein-1, and the major proglucagon fragment (MPGF) (3). Rather than PC2, L-cells express PC1, also called PC3, which cleaves proglucagon into glicentin, oxyntomodulin, GLP-1, intervening protein-2, and GLP-2 (3). No physiological actions have been determined for glicentin-related polypeptide, intervening protein-1, or MPGF (3). In contrast, glicentin is believed to exert trophic effects on the small bowel in rodents (3). Oxyntomodulin inhibits gastrointestinal secretion and motility, stimulates pancreatic enzyme secretion, affects the heart rate and intestinal glucose uptake, and promotes satiety (3). GLP-2 inhibits crypt apoptosis and stimulates proliferation, glucose transport, and barrier function and promotes neural proliferation and survival (3). GLP-1 circulates in multiple forms, undergoing both enzymatic amidi- nation and cleavage to produce its major forms, GLP-1(7–37) and (7–36)amine (3).

GLP-1 binds specifically to the GLP-1 receptor (GLP-1R), a class B G protein-coupled receptor that is widely expressed, including in the pancreatic islet cells, heart, lungs, kidney, stomach, vagus ganglion, brain stem, and hypothalamus (23). GLP-1 is degraded by DPP-4, a widely expressed serine protease with both membrane-bound and -soluble forms (23).

The action of DPP-4 limits the serum half-life of endogenous GLP-1 to under 2 min (23).

GLP-1 appears to exert anorexogenic effects on the central nervous system by both peripheral and central pathways. GLP-1 released from L-cells is posited to bind to GLP-1Rs located on vagus afferents in the intestinal wall, the hepatic portal system, and perhaps the liver, before it can be degraded by the robust activity of widely distributed DPP-4 (23). Afferent signals are transmitted to the vagus ganglion and, via a reflex arc, contribute to satiety signals, stimulate pancreatic islet function, and diminish gastrointestinal motility (23). The diminished anorexigenic effects of GLP-1 activation after nodose ganglion ablation supports a role for vagus afferents, whereas the inhibition of anorexia after the central administration of exendin(9–39), a GLP-1R antagonist, implicates a role for central GLP-1 action (23).

Central actions of GLP-1 may be responsible for the reductions in gut motility observed after the administration of GLP-1 mimetics. However, data have also suggested a direct, peripheral effect. Consistent with direct gut affects, Shirra et al. (59) were able to inhibit the suppression of antral and duodenal pressure waves induced by glucose instillation with the concomitant intravenous infusion of exendin(9–39), a selective GLP-1R antagonist.

An aspect of GLP-1 action that has been of particular clinical utility has been the propensity to lower glucose while infrequently inducing hypoglycemia, and potential mechanisms underlying this aspect are slowly being revealed. To demonstrate GLP-1 action in relationship to blood sugars, Vilsboll et al. (70) maintained blood sugars at three progressively higher levels by an intravenous glucose infusion in healthy volunteers and infused sufficient GIP or GLP-1 to mimic endogenous postprandial fluctuations. Simultaneous determination of serum insulin and glucagon allowed the authors to gauge GLP-1 and GIP effects relative to blood glucose (70). Strikingly, each elevation of either GIP or GLP-1 induced a peak in insulin secretion and a decline in glucagon secretion (70). Of note, however, was that the magnitude of the insulin stimulation tended to be proportional to the glucose level, whereas glucagon suppression was inversely proportional (70). Consequently, the stimulation of insulin secretion by GLP-1 was quite small when blood sugars were only 5 mM, but the suppression of glucagon was robust (70). Conversely, with each stepped increase in blood glucose, the magnitude of insulin stimulation increased, whereas the magnitude of glucagon suppression induced by the GLP-1 peaks diminished (70).

Recently developed knowledge about β-cell insulin secretion has shed light on the potential mechanisms underlying the glucose-dependent actions of GLP-1 on islet cells. As previously mentioned, ATP generated by the metabolism of glucose entering the β-cell closes K_{ATP} channels, depolarizing the β-cell membrane and permitting the influx of Ca^{2+} from the cell exterior and intracellular stores through voltage-regulated Ca^{2+} channels, which, in turn, induces the fusion of secretory vesicles to the cell membrane and insulin exocytosis (20). This process, called “triggering,” is necessary for insulin secretion but is powerfully modified by a collection of additional metabolic and neurohormonal processes, collectively called “amplifying” (20). Beyond the K^{+} channel closure that the ATP produced by glucose metabolism induces, additional metabolic products, potentially NADPH, GTP, and others, enhance the ultimate secretory response (20, 41). Importantly, amplifying mediators, while critical for insulin release, tend to only work in conjunction with triggering events and do not independently stimulate insulin secretion (20).

The presence and relative impact of an amplifying process can be observed by comparing cellular Ca^{2+} influx curves and insulin secretory responses from mouse islets exposed first to 1 mM glucose and then stimulated with either 15 mM glucose or 1 mM glucose combined with tolbutamide, a sulfonylurea (20). Exposure of islets to 1 mM glucose alone fails to induce either a significant increase in Ca^{2+} influx or insulin secretion. Increasing the glucose exposure to 15 mM stimulates both a robust Ca^{2+} influx and initiates a profound insulin secretory response, consistent with glucose stimulation of both triggering and amplifying pathways (20). Exposing islets to 1 mM glucose with tolbutamide produces a Ca^{2+} curve, or triggering event, similar to that produced by 15 mM glucose (20). However, the accompanying insulin secretory response is less than half that produced by 15 mM glucose, consistent with a diminished amplifying effect of tolbutamide compared with 15 mM glucose (20).

In β-cells, GLP-1 stimulation functions as an amplifying event. The binding of GLP-1 to GLP-1Rs increases cAMP concentrations, which, in turn, stimulate at least two, parallel, activation pathways (34). The first is mediated by cAMP-depen-
dent activation of PKA, which itself has multiple consequences. PKA activation leads to SUR1 phosphorylation, which inhibits $K_{\text{ATP}}$ activity (38), leading to the inhibition of voltage-dependent $K^+$ channels (42), thus delaying membrane repolarization. In addition, PKA activation may lead to the phosphorylation and activation of L-type Ca$^{2+}$ channels, which would directly enhance insulin secretion (11, 35). The second is mediated by the activation of Epac2, which has been reported to increase β-cell glucokinese activity and thus increase intracellular ATP production and also enhance $K_{\text{ATP}}$ channel closure (51). A variety of mechanisms coupling Epac activation to reduced $K_{\text{ATP}}$ channel activity has been proposed, including the activation of Rap1 and its target phospholipase C-ε (which would reduce local concentrations of the $K_{\text{ATP}}$ channel activator phosphoinositol 4,5-bisphosphate), activation of protein phosphatase 2B (protein phosphatase 2B/calcineurin, which would inhibit $K_{\text{ATP}}$ channels), and direct effects on $K_{\text{ATP}}$ channels (34). While the relative importance of these mechanisms remains to be clarified, data have supported the concept that GLP-1-induced Epac2 activation shifts the dose response of $K_{\text{ATP}}$ channels to ATP to the left, facilitating greater $K_{\text{ATP}}$ channel inhibition at lower ATP concentrations (29, 34).

Data have also indicated convergence of the PKA and Epac pathways at multiple molecular sites. Ca$^{2+}$ release from intracellular stores, leading to insulin exocytosis, is stimulated by PKA activation through inositol 1,4,5-trisphosphate receptors (28), but also via Epac2-mediated stimulation of ryanodine receptors (68). Similarly, the PKA actions of phosphorylation of Rab3A-interacting molecule (RIM) and facilitation of cell membrane tethering and priming of secretory vesicles, in cerebral neurons, at least (39), are very similar to findings of cAMP-activated Epac2 binding RIM to regulate docking and vesicular membrane fusion in both neurons and β-cells (30, 49).

In addition to the insulin secretory enhancement they exert on β-cells, GLP-1 agonists are suspected to inhibit β-cell apoptosis and thus prolong β-cell longevity (53). In an experiment in vitro supporting this contention, Farilla et al. (8) cultured human islets for 5 days. They quantified islet cell apoptosis and determined the expression of proapoptotic (activated caspase-3) and antiapoptotic (Bcl-2) proteins in islets that were cultured either with or without exendin-4, a GLP-1 agonist. Fluorescent micrographs of islets revealed that by culture day 5, islets treated with exendin-4 exhibited significantly less apoptosis, lesser amounts of activated caspase-3, and greater amounts of Bcl-2 than control islets (8). While human data in vivo to support the antiapoptotic effect of GLP-1 on islets are lacking, these results add to a growing body of evidence showing that GLP-1 may prolong islet and β-cell function in patients with type 2 DM.

In summary, GLP-1 produced by L-cells in the mid and distal intestine has diverse physiological effects. Aside from cardiovascular effects (not reviewed here), GLP-1 agonists appear to exert anorexogenic effects on feeding behavior through both central and vagus mediated pathways (3, 23). GLP-1 slows gastric and intestinal motility and exerts multiple effects on the pancreas (3, 23). In addition to inhibition of pancreatic exocrine excretion, GLP-1 stimulates β-cell insulin secretion and inhibits α-cell glucagon secretion, both in proportion to ambient blood glucose levels (3, 23). In the β-cell, GLP-1 functions to amplify the triggering signal produced by glucose, rather than functioning as a triggering stimulus itself, and thus promotes only glucose-dependent insulin secretion (3, 23). It remains plausible that this mechanism contributes to the low incidence of GLP-1 agonist-induced hypoglycemia. The available data suggest that while GLP-1 agonist treatment of patients with DM may improve insulin sensitivity in the liver, muscle, and fat, these effects are most likely secondary.

The propensity of GLP-1 agonists to be either weight neutral or even stimulate weight loss has contributed to the enthusiasm behind their clinical acceptance. The impact of GLP-1 agonist therapy on body weight has been shown in a study by Heine et al. (19), in which patients with type 2 DM, poorly controlled on combined sulfonylurea and metformin, were randomized to receive the addition of either exenatide or insulin glargine. Both add-on therapies successfully reduced HbA1c to the 7% target. However, whereas patients randomized to insulin glargine gained weight, patients that received exenatide lost a significant amount of weight (19). A seven-point glycemia evaluation during the final week of the study also revealed the expected reductions in postprandial glucose excursions with exenatide therapy, whereas postprandial values during insulin glargine treatment remained elevated (19).

The application of inhibitors to DPP-4, the enzyme responsible for degrading endogenous GIP and GLP-1, offers an alternative to the use of GLP-1 agonists. While currently approved GLP-1 agonists are injectable agents, DPP-4 inhibitors are administered orally and have been proven effective in combination with other oral agents (15). Similar to GLP-1 agonists, DPP-4 inhibitors raise serum incretin levels, although not to the levels obtained with injectable agonists, and may enhance glucose responsive insulin secretion and inhibit glucagon secretion (1, 47). In contrast to GLP-1 agonists, their use is not associated with nausea (14), but nor do DPP-4 inhibitors induce weight loss (6, 15). The reasons for the lack of body weight effects are unclear but may relate to the alternative actions of DPP-4. For example, non-GLP-1 substrates of DPP-4 include neuropeptide Y, a potent orexigen, which targets antilipolytic Y1 receptors (47). Reduced DPP-4-mediated neuropeptide Y degradation may artificially sustain Y1 receptor stimulation and prevent the stimulation of receptors targeted by degradation products (47). Moreover, DPP-4 inhibitors may affect enzymes other than DPP-4. Sangle et al. (57) recently reported that sitagliptin increased GLP-1 secretion from both murine and human L-cell lines, independent of DPP-4 inhibitory function.

**Pramlintide**

Technically, amylin is not an incretin hormone, as it is produced by β-cells rather than intestinal cells. However, it is included in the discussion of incretins because the actions of its pharmaceutical cognate, pramlintide, are similar to GLP-1 agonists. Amylin, or islet amyloid polypeptide, is cosecreted from pancreatic β-cell vesicles at a 1:100 ratio to insulin (26). Amylin is an additional member of a peptide family containing calcitonin, CGRP, and adenomedullin (54). Because it is produced by β-cells, it can be deficient in patients with type 2 DM and is deficient in all patients with type 1 DM (26).

There appears to be no distinct amylin receptor (18, 54). Instead, amylin binds to calcitonin receptors in the central nervous system that cooperate with receptor activity modifying proteins (RAMPs) (54). The existence of at least three isofoms...
of calcitonin receptor and their association with three widely expressed RAMP proteins creates at least six putative amylin receptors (54). Investigators have implicated the area postrema as an important amylin-binding area in rats (18, 26). In general, the cardinal effects of amylin action (enhanced satiety, diminished glucagon secretion, and delayed gastric emptying) appear to be centrally, rather than peripherally, mediated (26).

The use of unmodified amylin as a therapeutic agent is untenable because it is amyloidogenic, and the accumulation of islet amyloid has been implicated in the progressive β-cell loss that is associated with advanced type 2 DM (32). To address this issue, the pharmaceutical industry developed pramlintide, a synthetic peptide similar to amylin, with the exception that the amylin native amino acids 25, 28, and 29 have been replaced with proline (32). These changes produced a protein that was soluble, stable, nonamyloidogenic, and nonaggregating with amylin, but continued to bind to amylin receptors (32).

In a double-blind, crossover, randomized, controlled trial of patients with type 2 DM, administration of pramlintide successfully reduced total caloric intake (4). In another study (60), pramlintide reduced the postprandial glucagon area under the curve (AUC) in type 2 DM patients compared with placebo administration. The effects of diminished carbohydrate intake and hepatic glucose output secondary to diminished glucagon secretion combined to enable pramlintide therapy to reduce both HbA1c and body weight during a 52-wk dose response study (60).

Metformin

The biguanide derivative metformin remains a mainstay of therapy for type 2 DM. Used in Europe for decades before its approval in the U.S. in 1995, metformin is recommended as the first-line therapy by the American Diabetes Association and the European Association for the Study of Diabetes (71). In addition to demonstrated efficacy in reducing complications and mortality, metformin does not usually induce hypoglycemia (69, 71). Moreover, metformin appears to facilitate modest but significant average weight loss in overweight subjects (7a).

Metformin exerts the majority of its blood glucose-lowering effects in the liver. Sixteen weeks of metformin therapy reduced fasting blood glucose levels, whereas glucose disposal remained unchanged (66). Using isotopic magnetic resonance spectroscopy, Stumvoll et al. (66) demonstrated that blood glucose declines were accounted for by diminished hepatic glucose output. Moreover, they demonstrated that reductions in gluconeogenesis, rather than glycolysis, correlated most strongly with declines in fasting plasma glucose (66).

Recently, the presence of organic cation transporter (OCT)1 has been underscored as a factor restricting metformin’s effect in the liver. Shu et al. (64) were able to demonstrate that despite producing similar serum levels, metformin administration to transgenic mice harboring an OCT1 KO was unable to raise hepatic metformin levels to those of wild-type mice. Importantly, whereas metformin reduced fasting blood glucose in wild-type mice, it had no effect on OCT1−/− mice. The investigators subsequently demonstrated the clinical applicability of these findings to human subjects by demonstrating the absence of the metformin effect in subjects expressing OCT1 protein variants that are unable to transport metformin (64). Before they received metformin, nondiabetic subjects expressing either reference OCT1 or variant OCT1 exhibited similar glucose AUCs during an oral glucose tolerance test. However, after metformin therapy, the glucose AUC and 2-h insulin levels were greater in subjects expressing variant OCT1 (64). Interestingly, the authors’ previous data indicated that >20% of U.S. patients of European ancestry may express metformin transport-defective variant OCT1 (64).

Based on evidence showing that the provision of complex II substrate rescues metformin-induced inhibition of the mitochondrial electron transport chain, metformin is currently believed to inhibit complex I of the oxidative phosphorylation complex (71). Although the precise mechanism of complex inhibition remains unclear, the inhibition of hepatic oxidative phosphorylation is presumed to affect the AMP-to-ATP ratio and secondarily stimulate the activation of AMP-dependent kinase (AMPK), a molecular sensor of cellular energy status (71). These events were clearly demonstrated by providing metformin to either rat or human hepatocytes in primary culture and demonstrating an increase in AMP-to-ATP ratios, increased AMPK activity, and enhanced phosphorylation of acetyl-CoA carboxylase, an AMPK target gene (65).

By inducing the AMPK-mediated phosphorylation of target genes important for intermediary metabolism, the dominant metformin effects were presumed to be suppression of gene products critical for hepatic gluconeogenesis. As demonstrated by Foretz et al. (10), the addition of metformin to primary hepatocytes prestimulated with dibutyryl cAMP, a cAMP mimetic and activator of PKA that stimulates gluconeogenic gene expression, reduces the protein expression of both glucose-6-phosphatase and phosphoenol pyruvate carboxykinase (PEPCK). However, this same group recently challenged the necessity of AMPK in mediating metformin effects in the liver by demonstrating that metformin inhibits hepatic gluconeogenesis even in AMPK KO mice (10). Glucose production by hepatocytes from AMPK KO mice is reduced in a dose-dependent manner, similar to wild-type hepatocytes. Moreover, this same dose-dependent inhibition of glucose production by metformin is observed in wild-type hepatocytes expressing increased quantities of gluconeogenic genes, induced through the forced expression of PPAR-γ co-activator (PGC)-1α (10). Because glucose production was inhibited despite enhanced gluconeogenic gene expression, the authors concluded that the reduced ATP content of metformin-treated cells is in part responsible for the reduced gluconeogenesis (10).

Current assessments of mechanisms by which metformin lowers blood glucose indicate a role for both AMPK-dependent and AMPK-independent effects. Metformin clearly exerts effects predominantly in the liver to increase the AMP-to-ATP ratio, which, in turn, activates AMPK and subsequently reduces gluconeogenic gene expression (37, 71). However, the altered energy state of the hepatocyte appears to also be directly limiting to the energy-consumptive process of gluconeogenesis, irrespective of AMPK activation (46, 71).

TZDs

The TZDs are a class of compounds that bind and activate PPAR-γ (58). Introduced to the U.S. market in 1997, the initial commercially available TZD, troglitazone, was removed from clinical use after being associated with lethal, idiosyncratic
hepatic failure (31). The two currently used TZD compounds, rosiglitazone and pioglitazone, are insulin-sensitizing agents and, as such, do not typically produce hypoglycemia during monotherapy. However, treatment with TZDs has been associated with increased adiposity and weight gain as well as fluid retention and an increased risk of congestive heart failure (31, 50). In addition, data have revealed an association between prolonged TZD use and incident bladder cancer (36, 45).

PPAR-γ is an intranuclear hormone receptor that typically binds PPAR response elements as a heterodimer with alternate intranuclear receptors in the promoter region of target genes. PPAR-γ heterodimerizes with the retinoid X receptor, farnesoid X receptor, liver X receptor, mineralocorticoid receptor, and glucocorticoid receptor (50). With its described roles as a mediator of both ligand-dependent and -independent coactivation and corepression of target genes, PPAR-γ is anticipated to impact a broad scope of transcriptional events (58). Pharmacological stimulation of PPAR-γ induces adipogenesis as well as the expression of a number of genes important for lipogenesis in adipocytes, including fatty acid transport protein, CD36, acyl-CoA synthase, glucokinase, glucose transporter 4, and PEPCK (58). Indeed, patients treated with TZDs tend to increase total body adiposity and body weight. However, visceral adiposity tends to be diminished (31).

The capacity of TZDs to stimulate adipogenesis and adiposity has contributed to the lipid steal hypothesis of TZD action. The hypothesis posits that under conditions of modestly excessive caloric intake, excess calories are adequately stored as fat in adipocytes. When caloric intake exceeds the capacity of fat tissue to accept newly consumed or synthesized lipids, fat tends to accumulate in peripheral tissues, producing insulin resistance (58). By inducing the genesis of new, highly avid and insulin-sensitive fat cells, TZDs enhance the organism’s ability to store consumed calories, reducing their ectopic deposition in skeletal muscle and the liver (58).

According to the lipid steal hypothesis, everyone with increased skeletal muscle lipid content would be expected to exhibit insulin resistance. However, the actual relationship between IMCLs and insulin resistance is more complex. Three days of high-fat diet increases anterior tibialis IMCLs, which is associated with a decline in the glucose infusion rate (GIR), the quantity of glucose required to maintain a constant blood sugar, during an insulin clamp (43). A diminished GIR is a clear indication that the increased IMCLs are associated with increasing insulin resistance. However, highly fit individuals also tend to have elevated IMCLs while also exhibiting some of the greatest insulin sensitivities known. Consequently, whereas IMCLs correlate negatively with insulin sensitivity in unfit individuals, insulin sensitivity correlates positively with IMCLs in highly fit individuals (43).

These data point to additional TZD effects that may improve insulin sensitivity, including inhibiting the secretion of inflammatory cytokines and stimulating the secretion of advantageous adipokines from fat tissue. TZDs are among the few pharmaceutical agents known to elevate circulating adiponectin levels. Levels of adiponectin, an adipokine capable of activating AMPK kinase in both skeletal muscle and the liver, are strongly correlated with insulin sensitivity. In one study (44), 12-wk treatment of glucose-intolerant subjects with troglitazone dose-dependently increased adiponectin levels compared with controls. Additionally, TZDs are believed to reduce fat tissue expression of TNF-α (27).

The theory of β-cell lipotoxicity posits that deposition of circulating lipids in the β-cell leads to their premature apoptosis. Consequently, an additional supposition of the lipid steal hypothesis might be that TZD therapy should improve β-cell function. In confirmation of this theory, Gastaldelli et al. (12) treated drug naïve or sulfonylurea-treated patients for 4 mo with pioglitazone in a randomized, placebo-controlled trial. In a subsequent oral glucose tolerance test, drug-naïve patients exhibited smaller glucose AUCs and a decline in circulating free fatty acid levels after treatment with pioglitazone compared with before treatment (12). Patients that received sulfonylurea exhibited even greater responses compared with patients that received the placebo, who demonstrated an increase in glucose AUCs with no change in serum free fatty acid levels (12). Importantly, comparison of the results of two-step, euglycemic-hyperinsulinemic clamps performed before and after TZD treatment clearly demonstrated an increase in β-cell secretory capacity not observed in the placebo-treated groups (12).

Overall, TZDs appear to enhance peripheral and hepatic insulin resistance by expanding lipid storage capacity and affinity, thus reducing ectopic lipid deposition with its associated insulin resistance and creating a cytokine and adipokine milieu that favors insulin sensitivity. Additionally, TZD-induced declines in circulating free fatty acids are associated with improved β-cell responsiveness.

### Bromocriptine

Possibly as an adjustment to seasonal variability of food availability, Syrian hamsters predictably adjust insulin sensitivity in response to photoperiod. Some time ago, investigators working with these animals noted fluctuations of dopamine levels in the central nervous system that corresponded with metabolic status. In animals segregated according to their intrauterine glucose test responses, amounts of the dopamine metabolite homovanillic acid in the suprachiasmatic nucleus were greater in insulin-sensitive animals compared with glucose-resistant animals (40). Moreover, an intraperitoneal administration of a dopamine agonist, bromocriptine, reduced both glucose and insulin levels in insulin-resistant animals, indicating a profoundly positive effect on insulin sensitivity (5). Subsequently, an insulin clamp study (5) confirmed a reduction in hepatic glucose output, supporting an improvement specifically in hepatic insulin sensitivity.

A formulation of bromocriptine was subsequently investigated and ultimately approved for the treatment of hyperglycemia in humans. The marketed preparation is called Cycloset. One representative 16-wk, double-blind, randomized, placebo-controlled study (52) of this medication in obese patients with type 2 DM revealed a significant decline in both fasting plasma glucose and HbA1c levels. In these same patients, bromocriptine use was associated with diminished average glucose levels during an oral glucose tolerance test, and insulin clamp evaluations confirmed an increase in nonoxidative glucose disposal (52). Importantly, insulin levels remained unaffected (52). In combination with findings showing that bromocriptine had no effect on hepatic glucose production, free fatty acid levels, or body composition, these data suggest that bromocriptine’s effects are exerted primarily in peripheral tissues, skeletal
**Table 1. Listing of the drug classes currently approved to treat hyperglycemia, with the exception of insulin and insulin analogs, the United States followed by the drug names and relevant references from the text**

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Drug Name (Trade Name)</th>
<th>Mechanism(s)</th>
<th>Selected Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Glucosidase inhibitors</td>
<td>Acarbose (Precose); miglitol (Glyset); voglibose (Voglib)</td>
<td>Inhibits intestinal carbohydrate absorption</td>
<td>33</td>
</tr>
<tr>
<td>Bile acid sequestrant</td>
<td>Colesevelam (Welchol)</td>
<td>Unknown, possibly stimulates of incretin secretion</td>
<td>9, 17, 21, 24, 63</td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>Glyburide/glibenclamide (Diabeta, Glynase, Micronase); glipizide (Glucotrol); glimeperide (Amaryl)</td>
<td>Glucose-independent insulin secretion</td>
<td>22, 55, 61, 72</td>
</tr>
<tr>
<td>Meglitinides</td>
<td>Repaglinide (Prandin); nateglinide (Starlix)</td>
<td>Glucose-independent insulin secretion, enhances glucose-dependent insulin secretion, inhibits glucagon secretion, delays gastric emptying, stimulates satiety</td>
<td>25</td>
</tr>
<tr>
<td>Glucagon-like peptide-1 agonists</td>
<td>Exenatide (Byetta, Bydureon); liraglutide (Victoza)</td>
<td>GLP-1 receptor-β agonists</td>
<td>3, 8, 15, 20, 34, 53, 70</td>
</tr>
<tr>
<td>Dipeptidyl peptidase-4 inhibitors</td>
<td>Sitagliptin (Januvia); saxagliptin (Onglyza); linaclotide (Tradjenta)</td>
<td>Enhances glucose-dependent insulin secretion, inhibits of glucagon secretion</td>
<td>1, 6, 15, 47</td>
</tr>
<tr>
<td>Amylin analog</td>
<td>Pramlintide (Symlin)</td>
<td>Stimulates satiety, reduces glucagon secretion</td>
<td>4, 26, 32, 60</td>
</tr>
<tr>
<td>Biguanide</td>
<td>Metformin (Glucophage, Fortamet, Glumetza, Rio met)</td>
<td>Inhibits hepatic glucose production</td>
<td>7a, 64, 66, 71</td>
</tr>
<tr>
<td>Peroxisome proliferator-activated receptor-γ agonists</td>
<td>Pioglitazone (Actos); rosiglitazone (Avandia)</td>
<td>Reduces ectopic lipid accumulation, increases adiponectin, reduces deleterious cytokine secretion</td>
<td>12, 31, 50, 58</td>
</tr>
<tr>
<td>Dopamine agonist</td>
<td>Bromocriptine (Cycloset)</td>
<td>Unknown, probably reduces central adrenergic tone</td>
<td>5, 7, 13, 52</td>
</tr>
</tbody>
</table>

**Summary**

The above discussion should permit placement of the available antihyperglycemic agents in functional relation to our presented schematic indicating the modulators of blood glucose. Agents that increase satiety and consequently reduce the caloric and carbohydrate burden imposed on a stressed metabolic system include the GLP-1 agonists and pramlintide. α-Glucosidase inhibitors block the intraluminal and intestinal brush border degradation of complex carbohydrates, thus preventing their absorption into the bloodstream. Hepatic glucose output is either directly or indirectly diminished by treatment with metformin, GLP-1 agonists, and DPP-4 inhibitors, whereas insulin sensitivity in peripheral tissues is enhanced by treatment with TZDs. Postprandial glucagon secretion is reduced in patients with type 2 DM by treatment with GLP-1 agonists, DPP-4 inhibitors, and pramlintide, and GLP-1 agonists, DPP-4 inhibitors, and TZDs improve β-cell insulin secretory function. Finally, the precise mechanisms underlying the effects of both coleselam and bromocriptinewill require further investigation in humans. Table 1

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).

**AUTHOR CONTRIBUTIONS**

Author contributions: P.M.T. drafted manuscript; P.M.T. edited and revised manuscript; P.M.T. approved final version of manuscript.

**REFERENCES**


