MAYO CLINIC

REVIEW

The Role of Glucagon in the Pathophysiology and Treatment of Type 2 Diabetes

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Abstract

Type 2 diabetes is a disease involving both inadequate insulin levels and increased glucagon levels. While glucagon and insulin work together to achieve optimal plasma glucose concentrations in healthy individuals, the usual regulatory balance between these 2 critical pancreatic hormones is awry in patients with diabetes. Although clinical discussion often focuses on the role of insulin, glucagon is equally important in understanding type 2 diabetes. Furthermore, an awareness of the role of glucagon is essential to appreciate differences in the mechanisms of action of various classes of glucose-lowering therapies. Newer drug classes such as dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 receptor agonists improve glycemic control, in part, by affecting glucagon levels. This review provides an overview of the effect of glucose-lowering therapies on glucagon on the basis of an extensive PubMed literature search to identify clinical studies of glucose-lowering therapies in type 2 diabetes that included assessment of glucagon. Clinical practice currently benefits from available therapies that impact the glucagon regulatory pathway. As clinicians look to the future, improved treatment strategies are likely to emerge that will either use currently available therapies whose mechanisms of action complement each other or take advantage of new therapies based on an improved understanding of glucagon pathophysiology.

 ■ Mayo Clin Proc. 2018;93(2):217-239

The opposing actions of insulin and glucagon were demonstrated nearly a century ago.[1,2] Today, the role of glucagon is recognized as important in glucose homeostasis and diabetes pathophysiology.[3-6] Glucagon, a 29-amino acid peptide hormone, is counterregulatory to insulin, stimulating hepatic glucose production, thereby increasing plasma glucose levels.[7] Glucagon also stimulates ketogenesis, thus working in tandem with insulin to maintain a normal "fuel balance."[8,9] Simply put, insulin acts as a glucose-depositing and anabolic hormone, whereas glucagon is glucose mobilizing and catabolic.[8,10]

During the past decade, the use of medications that modulate glucagon levels has gradually increased in the treatment of patients with type 2 diabetes (T2D). Dipeptidyl peptidase-4 inhibitors (DPP-4is) and glucagon-like peptide-1 (GLP-1) receptor agonists (GLP-1RAs), which both suppress glucagon secretion, have received increasing attention as add-on therapies for patients with T2D.[11,12] Other glucose-lowering drug classes also affect glucagon secretion (either positively or negatively), including sulfonylureas, exogenous insulin, amylin mimetics, and sodium-glucose cotransporter 2 inhibitors (SGLT2is).

Although plasma glucagon levels are not used in a clinical stratification of diabetic treatment, health care providers may gain clinical insight from understanding how glucagon levels can be pharmacologically controlled in patients with T2D. Of particular interest are the abilities of glucose-lowering drugs to preserve a normal counterregulatory glucagon response in hypoglycemic conditions and, thus, avoid hypoglycemic adverse events. Moreover, as will be discussed, potential benefits can be gained from emerging glucagon-modulating therapeutic strategies.

To explore the relationship between glucagon and T2D, we performed a literature review of glucagon and diabetes, including glucagon in normal physiology and the pathophysiology of T2D. We also made an extensive effort to identify studies that assessed the effect

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ARTICLE HIGHLIGHTS

- Under normal physiological conditions, glucagon, which is secreted by pancreatic alpha cells, works alongside insulin to regulate plasma glucose levels, including an increase in hepatic glucose production and release of glucose into circulation during hypoglycemia.
- The pathophysiology of type 2 diabetes includes aberrant secretion of glucagon, resulting in elevated glucagon concentrations in both the fasting state and after a meal.
- Some glucose-lowering drug classes, including dipeptidyl peptidase-4 inhibitors and glucagon-like peptide-1 receptor agonists, decrease glucagon secretion, resulting in amelioration of the inappropriately high glucagon concentrations characteristic of type 2 diabetes and improvement of the insulin:glucagon ratio.
- As clinicians consider treatment strategies for patients with type 2 diabetes, knowledge of the effect of glucose-lowering therapies on glucagon can inform treatment choices.

on glucagon of glucose-lowering drugs within the most commonly used drug classes in current clinical practice. We performed a 2-stage literature search for sulfonylureas, pioglitazone, pramlintide, DPP4-is, GLP-1RAs, and SGLT2is: (1) search of the PubMed database for names of the glucose-lowering drugs and the terms "diabetes" and "glucagon" in the title or abstract (restricted to English-language articles and clinical trials in humans with no date restriction); and (2) assessment of each large, phase 3 clinical trial identified in articles describing each drug's clinical development program to identify studies in which glucagon levels were measured. For metformin and insulin, a less extensive literature search was conducted that relied primarily on mention of glucagon results in other publications. Although this is neither a systemic nor comprehensive review of all clinical studies with results related to glucagon, the literature search did identify an extensive number of relevant studies.

DISCOVERY OF GLUCAGON AND ITS IMPORTANCE

Glucagon, a peptide hormone,[13] was identified and named in 1923 when 2 chemists experimenting with "aqueous extracts of pancreas" found a substance that had a hyperglycemic effect in dogs whose pancreas was removed and in normal rabbits.[2] Despite the initial confusion about whether glucagon was merely a contaminant during purification of insulin,[1,5] researchers recognized by the 1950s that glucagon was secreted from pancreatic alpha cells,[14] thus establishing the existence of 2 distinct pancreatic hormones,[15,16] both responsive to plasma glucose levels.[16] In 1975, Unger and Orci[9] proposed a "bihormonal hypothesis" of diabetes on the basis of evidence that the metabolic effects of T2D result from absolute or relative hyperglucagonemia, as well as absolute or relative insulin deficiency.[9] The authors further suggested that controlling glucagon secretion could potentially improve the treatment of diabetes.[9]

A substantial body of evidence suggests that hyperglucagonemia contributes to the hyperglycemic state of patients with T2D.[17-20] Although the mechanisms behind diabetic hyperglucagonemia and to what extent it affects the T2D state remain to be elucidated, studies with transgenic mice provide insight into the role of glucagon and its receptor. Transgenic mice lacking the glucagon receptor do not develop diabetes, even after nearly-complete beta-cell destruction.[21,22] However, this finding has not been confirmed by other groups.[23] Furthermore, transgenic mice with defective leptin receptors develop the phenotype of severe T2D, whereas transgenic mice defective in both glucagon and leptin receptors do not.[24] Adenoviral reintroduction of the glucagon receptor in these leptin receptor– and glucagon receptor–deficient mice resulted in severe hyperinsulinemia and hyperglycemia.[24]

ROLE OF GLUCAGON IN HEALTHY INDIVIDUALS

Normal glucose homeostasis depends largely on balanced secretion of glucagon and insulin from pancreatic alpha and beta cells, respectively, in a tightly regulated, multiloop feedback system (Figure).[7,25] Following a meal, high plasma glucose levels (hyperglycemia) stimulate the pancreas to release insulin. Insulin promotes glucose uptake and use by insulin-dependent tissues, stimulates formation of glycogen from glucose (glycogenesis) in the liver and muscle, and suppresses glucagon secretion.[7,25] When plasma glucose levels fall too low

Mayo Clin Proc. ■ February 2018;93(2):217-239 ■ https://doi.org/10.1016/j.mayocp.2017.12.003
www.mayoclinicproceedings.org

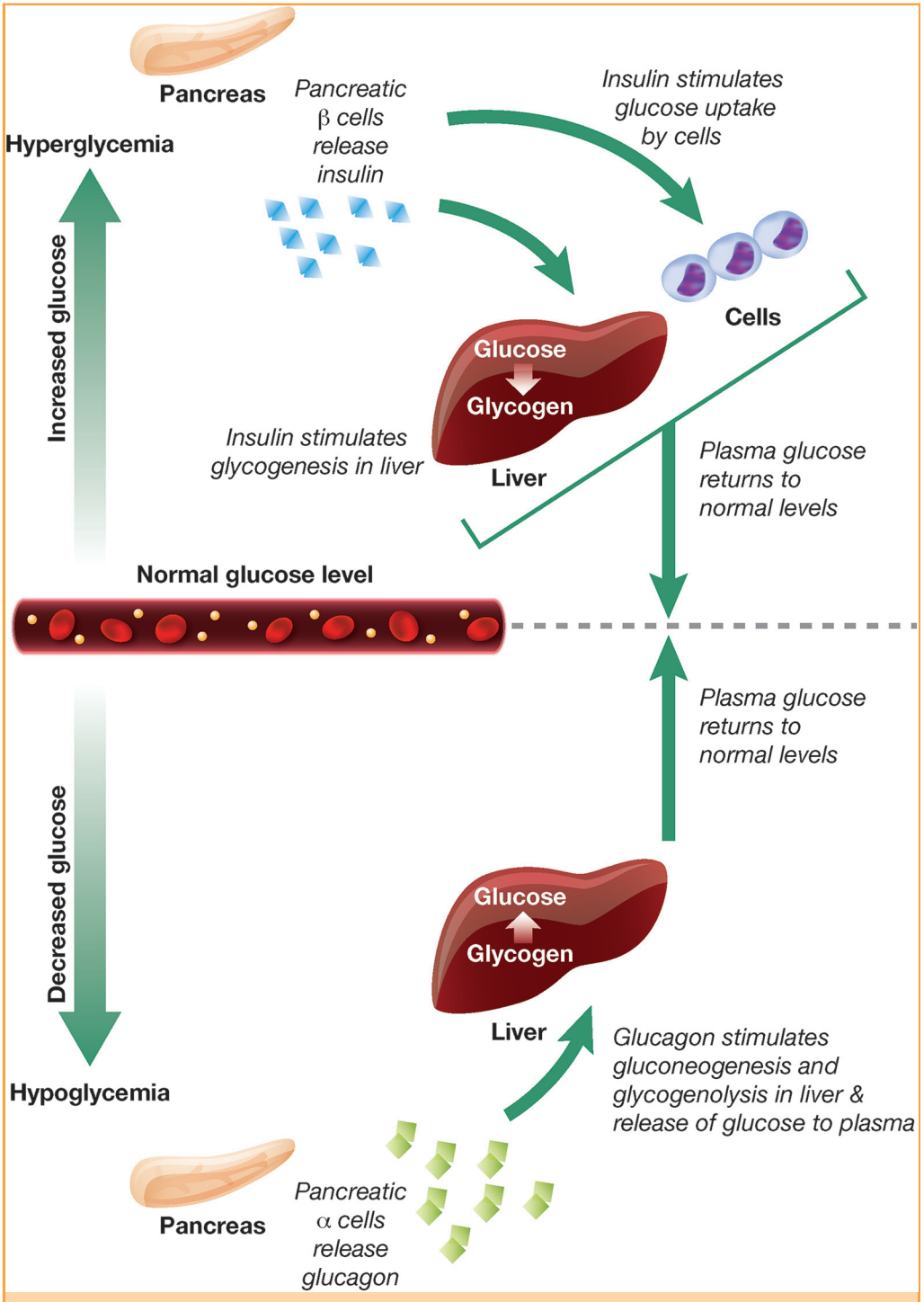

FIGURE. Regulation of blood glucose levels by glucagon and insulin in healthy individuals. In healthy individuals, glucagon and insulin work together to maintain normal plasma glucose levels. During hyperglycemia, pancreatic beta (β) cells release insulin, which stimulates glucose uptake by energy-consuming cells and the formation of glycogen in the liver. During hypoglycemia, pancreatic alpha (α) cells release glucagon, which stimulates gluconeogenesis and glycogenolysis in the liver and the release of glucose to the plasma.

TABLE 1. Factors Regulating Glucagon Secretion[43]

Stimulatory factors	Inhibitory factors
Hypoglycemia	Carbohydrate meal
Stress	Glucose
Protein meal	Isoleucine[10]
Most amino acids	Nonesterified fatty acids
Some fatty acids[10]	Ketones
Parasympathetic nerves	Gamma-aminobutyric acid[10]
Sympathetic nerves	Adenosine triphosphate
Pituitary adenylate cyclase–activating polypeptide	Zinc[3,10]
Cold exposure[44]	
Stimulatory hormones	**Inhibitory hormones**
Adrenaline (epinephrine)	Amylin[10]
Cholecystokinin	Glucagon-like peptide-1
Gastrin-releasing peptide	Insulin
Ghrelin[45]	Leptin[46,47]
Glucose-dependent insulinotropic polypeptide[48]	Secretin
Oxytocin	Somatostatin
Vasoactive intestinal peptide	
Vasopressin	

Adapted from *Diabetologia*,[43] with permission of Springer.

(hypoglycemia), the pancreas releases glucagon. Upon reaching the liver, glucagon promotes breakdown of glycogen to glucose (glycogenolysis), promotes glucose synthesis (gluconeogenesis), inhibits glycogen formation (glycogenesis), and thus mobilizes export of glucose into the circulation. Thus, glucagon provides a critical response to hypoglycemia. Interestingly, our group recently demonstrated that glucagon is also secreted in patients who have undergone total pancreatectomy, demonstrating extrapancreatic secretion of glucagon in humans.[26] The potential implications of this finding for the treatment of T2D are discussed below, though open questions remain: Is extrapancreatic secretion primarily mediated by enteroendocrine cells? Does it occur in both healthy individuals and patients with T2D? Is its metabolic effect physiologically relevant?

Glucagon's physiological role is broader than as a direct counterregulatory hormone to insulin. Glucagon also plays a role in lipid metabolism,[27-31] influences food intake, affects body weight, promotes autophagy, and has pleiotropic effects on the cardiovascular system.[3] Notably, many of these glucagon functions are associated with physiological processes awry in T2D. Increasingly, glucagon is recognized for its role in amino acid metabolism[32] through its regulation of hepatic ureagenesis,[33] alteration of transcription of key enzymes in the urea cycle,[28,34,35] and a probable indirect influence on alpha-cell mass.[36,37]

In humans, pancreatic islets are composed of approximately 40% alpha and 60% beta cells, whereas the relative proportions in mouse islets are approximately 20% and 80%, respectively, hinting at the relative importance of glucagon secretion.[10,38] An altered alpha- to beta-cell ratio may be a contributing factor in T2D pathogenesis.[39,40] Interestingly, glucagon receptor antagonism is associated with alpha-cell proliferation,[36] and there is much current interest in transdifferentiation of beta cells from alpha cells.[39,41,42] In the human pancreas, alpha and beta cells are spatially intermixed, which has important physiological implications because glucagon's effects on insulin-producing beta cells may occur, at least partially, in a paracrine manner.[38] The relative hyperglucagonemia of diabetes resulting from an altered alpha- to beta-cell ratio might be a consequence of beta-cell dedifferentiation to glucagon-producing islet cells, greater beta-cell susceptibility to cell apoptosis, and alpha-cell–resistant mechanisms to cellular stress.[42]

Various factors and hormones modulate glucagon secretion from the pancreatic alpha cell (Table 1).[3,10,43-48] They may be either stimulatory or inhibitory and involve multiple physiologic pathways. The GLP-1 inhibition of glucagon secretion is particularly noteworthy, because the GLP-1RA class of glucose-lowering drugs mimics the effects of GLP-1.[49,50] Together, these factors regulate glucagon secretion to prevent the potentially lethal state of hypoglycemia.[43]

The insulin-glucagon interaction is important in energy homeostasis in the entire body: (1) a high insulin:glucagon ratio promotes biosynthesis of proteins, inhibits glucose production, and reduces release of free fatty acids, whereas (2) a low insulin:glucagon ratio helps the body access stored nutrients, increases hepatic glucose production from glycogen and amino acids, and promotes the breakdown of adipose tissue into free fatty acids and glycerol.[8] Kalra and Gupta[51] called the insulin:glucagon ratio a "physiological fulcrum," balancing the 2 opposite ends of the metabolic spectrum to conserve energy if possible, but provide it when needed.

Mayo Clin Proc. ■ February 2018;93(2):217-239 ■ https://doi.org/10.1016/j.mayocp.2017.12.003
www.mayoclinicproceedings.org

The insulin:glucagon ratio determined by measurement of plasma glucagon levels may vary from the portal insulin:glucagon ratio, and conclusions on peripheral measures should always be approached with caution because of higher insulin and glucagon concentrations in portal circulation compared with peripheral.[3] Modest changes in insulin and/or glucagon secretion lead to amplified responses on glucose metabolism, in part because of the portal insulin:glucagon ratio. Thus, this provides a strong physiological argument for the rationale of targeting glucagon secretion.

Although studies have not established the utility of measuring glucagon routinely in clinical practice, the insulin:glucagon ratio is a comprehensive metabolic index that has been used for many years in research. Understanding how different drug classes interact with glucagon and how they influence the insulin:glucagon ratio can help to explain the efficacy and safety profiles of such drugs and inform the clinician's choice of treatment agent or potential combinations of treatments.[51]

ROLE OF GLUCAGON IN THE PATHOPHYSIOLOGY OF T2D

In patients with T2D, regulation of glucagon secretion is flawed, with elevated plasma glucagon concentrations in the fasting state and defective postprandial glucagon suppression that results in undesirably high plasma glucagon concentrations in the context of hyperglycemia.[4,6,52,53] Thus, hyperglucagonemia contributes to the increased hepatic glucose output characterizing patients with T2D.[4,43,54] Furthermore, in patients with T2D, plasma glucagon concentrations may even increase in response to a meal.[55] Although the mechanisms responsible for elevated glucagon levels in patients with T2D under hyperglycemic conditions are not fully understood, one theory is that pancreatic alpha cells are resistant to the glucagon-suppressive effects of glucose and insulin.[4,10]

Mechanisms underlying hyperglucagonemia in T2D remain to be fully elucidated. Interestingly, hyperglucagonemia in T2D is aggravated by oral glucose intake but not by intravenous glucose administration, which results in significant glucagon suppression.[56,57] This suggests that factors originating from nutrient stimulation of the gastrointestinal tract may play an important role. The recent confirmation that glucagon can be secreted from extrapancreatic tissues constitutes an interesting explanation of the postprandial hyperglucagonemia observed in T2D, namely, that postprandial glucagon production may be gut-derived, not originating from the pancreas.[26]

An interesting aspect of glucagon pathophysiology in T2D with therapeutic implications is the dynamic nature of the glucagon response to and regulation of both hypoglycemia and hyperglycemia. As will be discussed further below, therapeutic agents shown to have a short-term effect on postprandial glucagon levels (~180 minutes) may not be equally effective over longer treatment periods.

RELEVANCE OF GLUCAGON TO TREATMENT OF T2D AND EFFECTS OF GLUCOSE-LOWERING THERAPIES ON GLUCAGON

Because glucagon has a central role in glucose homeostasis[7] and patients with T2D have glucagon-mediated elevated hepatic glucose production,[43,54] addressing the fasting and postprandial hyperglucagonemia of patients with T2D has emerged as an interesting strategy for existing and future glucose-lowering therapies.[17,19] The introduction of new drug classes that modulate glucagon has underscored the limited attention previously shown to older drug classes in this regard. Table 2[54,58-168] summarizes the effects of the major classes of glucose-lowering drugs on fasting and postprandial glucagon levels and reports results of less common glucagon assessments, such as those under experimentally induced hypoglycemia.

Older Agents

In general, older therapies have been studied less extensively than newer drug classes regarding effects on glucagon secretion; however, the effect of glucose-lowering drugs on glucagon secretion is becoming more apparent as research in this area expands. In studies with older therapies, results regarding glucagon are often inconsistent, which may be a consequence of variability in study design. Furthermore, the difficulties associated with measuring glucagon should be recognized. Glucagon assays have evolved over time with inconsistencies in both sensitivity and specificity, complicating cross-study comparisons.[169] Cross-reaction with other glucagon-like peptides has

TABLE 2. Summary of Studies Identified That Assess the Effect of Glucose-Lowering Therapies on Glucagon in Patients With Type 2 Diabetes[a]

Drug class	Drug	No. of studies[b]	Effect on fasting glucagon	Effect on postprandial glucagon	Additional glucagon measures	Mention in label (PI or SPC)
Older agents						
Biguanides	Metformin[c]	2 Large studies[58,59] 2 Small studies[60,61]	Significant decrease vs BL[58] Decrease vs BL at 52 wk; no effect at 26 wk[59] Increase vs PBO[60]	No effect[58]	Decrease in glucagon vs BL after administration of oral or IV glucose[60] Increase in 12-h plasma glucagon vs glimepiride[61]	None
Insulin	Exogenous insulin[c]	4 Small studies[62-65]	NR	NR	No effect on glucagon response to arginine stimulation[65] Decrease in glucagon after arginine stimulation vs pretreatment[64] Decrease in plasma glucagon after IV administration of insulin + glucose to fasting patients[63] Significant decrease in 48-h plasma glucagon in insulin-optimized period compared with "uncontrolled" period[62]	None
SU	Glyburide/glibenclamide	5 Small studies[66-71]	NR	No effect after an OGTT[70] Nonsignificant increase after meal[71] No effect vs PBO[68]	No effect on plasma glucagon (*2 studies*)[68,69] No effect on 24-h plasma glucagon[67] Suppressed glucagon secretion during hypoglycemia[66]	Exerts an inhibitory effect on glucagon-producing alpha cells[72]
SU	Glipizide	1 Large study[58] 4 Small studies[68,73-75]	No effect[58] Significant increase vs BL[73]	No effect[58] No effect vs PBO[68] Significant increase after liquid meal challenge[73]	No difference in plasma glucagon during hyperglycemic clamp[75] No effect on plasma glucagon[68] Elevated plasma glucagon[74]	None

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TABLE 2. Continued

Drug class	Drug	No. of studies[b]	Effect on fasting glucagon	Effect on postprandial glucagon	Additional glucagon measures	Mention in label (PI or SPC)
Older agents, continued						
SU	Gliclazide[c]	7 Small studies[70,76-81]	NR	No effect after an OGTT[70]	No effect on plasma glucagon[78,80] No effect during IV glucose tolerance test or arginine test[76] No effect on plasma glucagon or in OGTT[77] Significant decrease in plasma glucagon during hyperinsulinemic pulse[79] No effect on plasma glucagon during glucose infusion[81]	None
SU	Glimepiride	4 Large studies[54,82-84] 4 Small studies[61,85-88]	No effect (*4 studies*)[83,85,86,88] Increase from BL[82]	Increase from BL (*2 studies*)[54,86] No effect[87]	Decreased 12-h plasma glucagon vs metformin[61] No effect on plasma glucagon[84] Significant increase in glucagon vs BL after arginine stimulation test[88]	None
TZD	Pioglitazone (Actos)	1 Large study[89] 1 Small study[90]	No effect[90]	No effect (*2 studies*)[89,90]	None reported	None
Amylin mimetics						
Amylin mimetic	Pramlintide (Symlin)	1 Small study[91]	NR	Decrease[91]	None reported	Suppresses glucagon secretion; reduces postprandial rise in glucagon[92]

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TABLE 2. Continued

Drug class	Drug	No. of studies[b]	Effect on fasting glucagon	Effect on postprandial glucagon	Additional glucagon measures	Mention in label (PI or SPC)
DPP-4is						
DPP-4i	Sitagliptin (Januvia)	3 Large studies[89,93,94] 4 Medium studies[95-98] 6 Small studies[60,88,99-102]	Decrease relative to BL[94] No effect (*2 studies*)[60,88]	Significant decrease vs PBO (*4 studies*)[89,93,96,98] Significant decrease vs BL (*2 studies*)[97,100] Decrease, but not vs PBO[60] Decrease, but less than with exenatide twice a day[95] No change when added to liraglutide[102] No effect[99]	No significant change after hyperglycemic clamp[101] Nonsignificant decrease during isoglycemic clamp[98]	Lowers glucagon secretion from pancreatic alpha cells Decreases glucagon levels in circulation in a glucose-dependent manner After a meal, results in decreased glucagon concentrations[103]
DPP-4i	Vildagliptin[d] (Galvus/Zomelis)	5 Large studies[54,84,104-106] 10 Small studies[87,107-115]	No difference vs PBO (*4 studies*)[105,109,111,113] Decrease[104]	Significant decrease vs PBO (*8 studies*)[105-107,109-111,113,114] Significant decrease vs BL[108] Significant decrease vs glimepiride[54] Decrease vs BL Increase, but less than PBO[112]	Increased glucagon during hypoglycemic clamp[107] No difference vs PBO during hypoglycemic clamp[112] Decreased plasma glucagon[84] No difference in AUC_{0-24} between AM and PM dosing; results similar to PBO[115]	Makes pancreas produce less glucagon; enhanced insulin:glucagon ratio during hyperglycemia; enhances the sensitivity of alpha cells to glucose, resulting in more glucose-appropriate glucagon secretion[116]
DPP-4i	Saxagliptin (Onglyza)	6 Large studies[117-122] 1 Small study[123]	NR	Significant decrease vs PBO (*2 studies*)[119,122] Decrease vs PBO[121] Decrease vs glyburide[118] No effect[117,120]	Significant decrease vs PBO during IV oral hyperglycemic clamp; decrease during IV hyperglycemic clamp but not significant vs PBO[123]	Decreases glucagon after a meal; lowers glucagon secretion from pancreatic alpha cells[124]
DPP-4i	Linagliptin (Tradjenta/Trajenta)	1 Large study[125] 2 Small studies[86,126]	No effect[86]	Decrease (both peak postprandial excursion and area under curve)[125] Decrease vs BL[86]	Increase during hypoglycemia, but not vs PBO[126]	Lowers glucagon secretion Decreases level of glucagon in circulation[127]

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TABLE 2. Continued

Drug class	Drug	No. of studies[b]	Effect on fasting glucagon	Effect on postprandial glucagon	Additional glucagon measures	Mention in label (PI or SPC)
DPP-4is, continued						
DPP-4i	Alogliptin (Nesina)	2 Large studies[128,129] 2 Medium studies[130,131] 1 Small study[132]	No effect (*2 studies*)[128,132]	Significant decrease vs PBO (*3 studies*)[128,130,131] Decrease vs BL, but not significant vs PBO[129]	None reported	Decreases postprandial glucagon[133]
GLP-1RAs						
GLP-1RA (short acting)	Exenatide twice a day (Byetta)	4 Large studies[134-137] 2 Medium studies[95,97] 6 Small studies[88,138-142]	Decrease vs BL (*2 studies*)[136,137] No effect (*2 studies*)[88,140] No difference vs insulin (*2 studies*)[135,141]	Significant decrease vs PBO (*3 studies*)[138,139,142] Significant decrease vs BL (*2 studies*)[95,97] Significant decrease vs sitagliptin (*2 studies*)[95,97] Significant reduction vs insulin glargine postbreakfast, significant increase postlunch[141] No effect[140]	Decreased plasma glucagon[134]	Suppresses inappropriately elevated glucagon secretion Lowers serum glucagon concentrations during periods of hyperglycemia Does not impair normal glucagon response to hypoglycemia[143]
GLP-1RA (short acting)	Lixisenatide (Adlyxin/ Lyxumia)	2 Large studies[144,145] 2 Small studies[146,147]	Decrease vs BL[145]	Significant decrease vs PBO[145] Significant decrease vs liraglutide[144]	No effect on glucagon suppression after IV glucose challenge vs PBO[145] Significant decrease vs PBO at 3.5 mmol/L glucose; no difference vs PBO at 2.8 mmol/L glucose[146]	Decreases glucagon secretion Decreases postprandial glucagon[148]
GLP-1RA (long acting)	Exenatide QW (Bydureon)	1 Large study[136]	Decrease vs BL[136] Significant decrease vs exenatide twice a day	NR	None reported	Suppresses inappropriately elevated glucagon secretion Moderates glucagon secretion and lowers serum glucagon concentrations during periods of hyperglycemia Does not impair normal glucagon response to hypoglycemia[149]

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TABLE 2. Continued

Drug class	Drug	No. of studies[b]	Effect on fasting glucagon	Effect on postprandial glucagon	Additional glucagon measures	Mention in label (PI or SPC)
GLP-1RAs, continued						
GLP-1RA (long acting)	Liraglutide (Victoza)	7 Large studies[82,85,137,144,150-152] 1 Medium study[153] 5 Small studies[126,154-157]	No effect (*2 studies*)[85,150] Significant decrease vs BL[82] Decrease vs BL[137] Decrease vs BL, but not significant vs PBO[152] Significant decrease vs PBO[151] Significant decrease vs PBO at wk 12, but not significant at subsequent visits[153]	Significant decrease vs PBO (*2 studies*)[155,156] Decrease vs BL[144] Significant reduction in incremental ratio of postprandial plasma glucagon to BL plasma glucagon, but no effect on postprandial plasma glucagon[157]	Increase during hypoglycemic clamp[126] Increase after OGTT at 12 wk but not sustained at wk 24, 36, or 48; delayed time to peak glucagon response[153] Significant decrease vs PBO in 24-h glucagon[154]	Decreases glucagon in a glucose-dependent manner. A single dose of liraglutide did not impair glucagon response to low glucose conditions[158]
GLP-1RA (long acting)	Albiglutide (Tanzeum/Eperzan)	1 Large study[159] 1 Small study[160]	No effect[159]	Decrease, but not significant vs PBO[159]	Significant increase vs PBO during hypoglycemia (glucose clamp 3.3 mmol/L [59.4 mg/dL]); no effect vs PBO during glucose clamp 9.0 mmol/L (162 mg/dL)[160]	Single dose did not impair glucagon response to low glucose concentrations[161]
GLP-1RA (long acting)	Dulaglutide (Trulicity)	2 Large studies[59,162]	Numeric decrease vs BL; no difference vs insulin glargine[162] Significant decrease vs metformin at 26 wk; decrease vs BL, but no difference vs metformin at 52 wk[59]	NR	None reported	Decreases glucagon secretion Decreases fasting glucagon[163]
GLP-1RA (long acting)	Semaglutide (FDA marketing application filed Dec 2016)	1 Large study[164]	Significant decrease vs PBO[164]	NR	None reported	Not available

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TABLE 2. Continued

Drug class	Drug	No. of studies[b]	Effect on fasting glucagon	Effect on postprandial glucagon	Additional glucagon measures	Mention in label (PI or SPC)
SGLT2is						
SGLT2i	Canagliflozin (Invokana)	No studies identified				None
SGLT2i	Dapagliflozin (Farxiga/ Forxiga)	1 Large study[117] 3 Small studies[165-167]	Significant increase vs BL (*2 studies*)[166,167]	Increase vs BL (*2 studies*)[117,165]	Significant increase in fasting glucagon vs PBO during euglycemic hyperinsulinemic clamp (*2 studies*)[166,167] Significant increase in fasting glucagon:insulin ratio vs BL or PBO (*2 studies*)[166,167]	None
SGLT2i	Empagliflozin (Jardiance)	1 Medium study[168]	Increase vs BL after single dose[168] Nonsignificant increase vs BL after 4-wk treatment[168]	Significant increase vs BL after single dose and after 4- wk treatment[168]	Decrease in insulin:glucagon ratio after single dose and after 4-wk treatment vs BL[168]	None

[a]$AUC_{0\text{-}24}$ = area under curve from time 0 to 24 h; BL = baseline; DPP-4i = dipeptidyl peptidase-4 inhibitor; FDA = Food and Drug Administration; GLP-1RA = glucagon-like peptide-1 receptor agonist; IV = intravenous; NR = not reported; OGTT = oral glucose tolerance test; PBO = placebo; PI = prescribing information; QW = once weekly; SGLT2i = sodium-glucose cotransporter 2 inhibitor; SPC = summary of product characteristics; SU = sulfonylurea; TZD = thiazolidinedione.

[b]Literature search for metformin and insulin was less extensive than for other drugs.

[c]Large studies have N≥100, medium studies N=50 to N<100, and small studies N<50.

[d]Not marketed in the United States.

been especially challenging regarding specificity, and many assays have not been sensitive enough to detect glucagon levels of less than 10 pmol/L.[169] The study design, lack of studies with older agents and glucagon as a primary end point, and the recognition of unreliable older glucagon assays make it difficult to determine the contribution of the effect of glucagon to the overall pharmacologic profile of the drug.

Metformin has been in use for decades, yet its mechanism of action is not fully understood. As summarized in Table 2, the effect of metformin on glucagon levels varies widely across clinical studies.[58-61] The primary action of metformin is to decrease hepatic glucose production; this contrasts with the physiologic action of glucagon to stimulate gluconeogenesis in the liver.[170,171] The overall glucose-lowering effect of metformin is, at least in part, the result of its inhibition of glucagon-induced stimulation of gluconeogenesis rather than its effect on the levels of glucagon or action on insulin.[172] An important murine study demonstrated that metformin acts to antagonize glucagon by affecting the cyclic adenosine monophosphate pathway in hepatocytes,[173] a finding that has generated much interest in revisiting the mechanism of metformin.

Exogenous insulin is widely used to treat T2D; however, its effects on glucagon levels have not been studied extensively in humans. Although endogenous insulin suppresses glucagon secretion under normal physiologic conditions, the molecular mechanisms of its action are not fully understood.[174] In small mechanistic studies from the 1970s, exogenous insulin was shown to decrease plasma glucagon levels in patients with T2D.[62-64]

The primary mechanism of action of sulfonylureas is to increase insulin secretion through effects on beta cells.[171] Based on their mechanism of action, a direct effect of sulfonylureas on pancreatic glucagon secretion would not be expected; the bulk of studies examining glucagon secretion are consistent with this view (Table 2).[58,67-70,75-78,80,81,83-85] However, sulfonylureas did increase fasting and/or postprandial glucagon levels in some studies,[54,71,73,74,82,86] while decreasing it in 3 others.[61,66,79] However, in most of the sulfonylurea studies listed in Table 2, measurement of glucagon was not the primary aim of the study, sulfonylurea was often the comparator to another therapy that was the focus of the study, and any sulfonylurea effects on glucagon that did occur were not interpreted as pharmacologically significant.

Finally, among older agents, pioglitazone, the only thiazolidinedione widely marketed globally, did not have an effect on either fasting or postprandial glucagon concentrations in 2 studies that measured glucagon.[89,90]

Amylin Mimetics

Pramlintide, the only amylin mimetic marketed in the United States, decreases postprandial glucagon responses.[91,92,175] This is not unexpected because the hormone amylin inhibits glucagon secretion.[10,175]

DPP-4is

The DPP-4i and GLP-1RA (discussed later) drug classes have been studied to a greater extent regarding their effects on glucagon than the older drug classes because their effects in this regard are widely recognized.[12,51,171]

The DPP-4is are "incretin enhancers" that prevent the degradation of endogenously produced glucose-dependent insulinotropic polypeptide and GLP-1, thus extending their action. The GLP-1 is a gut incretin hormone that stimulates pancreatic beta cells to secrete insulin and also suppresses glucagon secretion from the pancreatic alpha cells.[176] Preclinical studies showed that GLP-1 inhibits glucagon release enough to influence physiological glucose regulation.[43] Supraphysiological doses of native GLP-1 can restore alpha-cell glucose sensitivity in patients with T2D, facilitating a strict glucose-dependent inhibition of glucagon secretion.[177] Thus, GLP-1 suppresses glucagon secretion during hyperglycemia, with no major role during hypoglycemia. Interestingly, the other incretin hormone, glucose-dependent insulinotropic polypeptide, increases glucagon secretion during hypoglycemic and euglycemic conditions but not during hyperglycemia.[178-180] Consequently, DPP-4is would suppress glucagon secretion during hyperglycemia but not impair glucagon response to hypoglycemia.[126]

Both DPP-4is and GLP-1RAs exert inhibitory actions on glucagon-producing alpha cells that may be, at least partially, responsible for their role in ameliorating T2D. The DPP-4 is expressed in pancreatic islets, almost exclusively in alpha cells in the human pancreas.[181,182] The DPP-4 localization to alpha cells suggests a direct

Mayo Clin Proc. ■ February 2018;93(2):217-239 ■ https://doi.org/10.1016/j.mayocp.2017.12.003
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effect on alpha cells, implying a paracrine effect on insulin-producing beta cells. Human islets treated with the DPP-4i vildagliptin secreted higher levels of GLP-1 and insulin; thus, the inhibition of islet DPP-4 activity may contribute to the insulinotropic and glucose-lowering action of DPP-4is.[182] Likewise, a local GLP-1 system may exist in human pancreatic islets, with alpha cells containing GLP-1 and the enzyme prohormone convertase 1/3, which has a role in both glucagon and insulin biosynthesis.[183]

In clinical studies, DPP-4is, including sitagliptin, vildagliptin, saxagliptin, linagliptin, and alogliptin, consistently decrease postprandial glucagon secretion compared with the usual glucagon response to a meal, with either no effect or a slightly decreased effect on fasting glucagon (Table 2). Unsurprisingly, given the glucose-dependent effect of the incretin hormones, DPP-4is are glucose dependent in both their action on insulin and glucagon secretion.[11]

As alluded to earlier, DPP-4is do not affect the normal counterregulatory responses to hypoglycemia.[11] In patients with T2D, the normal counterregulatory response to hypoglycemia is impaired because of the hyperglucagonemic characteristic of T2D.[4] Thus, a therapeutic challenge is to reduce glucagon levels when necessary but, in doing so, not to disrupt the normal, appropriate increase in glucagon in response to hypoglycemia. The effects of DPP-4is on these counterregulatory pathways have been explored in clinical studies that used a "hypoglycemic clamp" to hold plasma glucose at a low target concentration (often by the addition of intravenous insulin).[107,126] A hypoglycemic clamp study with linagliptin demonstrated no disruption of normal counterregulatory responses to hypoglycemia in patients with T2D.[126] In contrast, a study of vildagliptin suggests that alpha-cell response to hypoglycemia may even be enhanced by treatment with a DPP-4i.[107] Protection of the counterregulatory response to hypoglycemia may, in part, provide a mechanistic explanation for the low risk of hypoglycemia that is characteristic of both the DPP-4i and GLP-1RA drug classes.[107,184]

GLP-1RAs

The GLP-1RAs are peptides that act as "incretin mimetics" to increase GLP-1 receptor signaling. They increase postprandial insulin secretion (even before plasma glucose levels start to rise), inhibit glucagon secretion, slow gastric emptying, and induce satiety. Like DPP-4is, GLP-1RAs are glucose dependent in their action on insulin secretion and their glucagonostatic effect.[4,11,12]

Clinical studies have generally demonstrated that, similar to DPP-4is, GLP-1RAs substantially lower glucagon concentrations in both the fasting state and after a meal (Table 2), thus reducing the hyperglucagonemia of T2D. Early studies with exenatide twice daily (the first drug marketed in this class) and liraglutide demonstrated reduced postprandial glucagon concentrations in patients with T2D.[138,139,154] These results have since been confirmed in other mechanistic studies and large clinical trials across the GLP-1RA class. Exenatide twice daily, lixisenatide, exenatide once weekly, liraglutide, and dulaglutide all decrease glucagon secretion.[59,82,136-139,142-146,148,149,151-158,162,163,171] In addition, semaglutide, a GLP-1RA still in clinical development, decreased fasting glucagon levels in a study.[164]

Although GLP-1RAs generally decrease glucagon levels, results are not uniform across the drug class. This may be a consequence of GLP-1RA class members differing in both structure and pharmacokinetic profile. Albiglutide, a large (>600 amino acid) peptide, does not appear to significantly decrease postprandial glucagon, compared with placebo, or to affect the magnitude of the glucagon response in hypoglycemia,[159,160] likely because it is too large to enter the central nervous system. However, albiglutide affected the timing of the glucagon response to hypoglycemia in a study.[160]

Interestingly, long-term treatment (48 weeks) with liraglutide in the Liraglutide and Beta-cell Repair (LIBRA) trial resulted in an increase in glucagon after an oral glucose tolerance test.[153] Although glucagon enzyme-linked immunosorbent assay cross-reaction with other glucagon-like peptides might possibly contribute to this, the mechanism for this unexpected observation remains unclear and warrants additional study.

In patients with T2D, the glucagon-suppressive effect of GLP-1RAs likely contributes approximately one-third of the overall glucose-lowering effect.[50,185] Decreased glucagon levels with GLP-1RA treatment result in an increased insulin:glucagon ratio.[139,153,154] Interestingly, in a

study comparing the short-term effects of the GLP-1RA exenatide twice daily and the DPP-4i sitagliptin, patients treated with exenatide twice daily had significantly lower postprandial glucagon levels than those treated with sitagliptin.[95] Overall, most studies on the glycemic contribution of the glucagon-lowering ability of GLP-1RAs are based on short-term effects, whereas data on chronic effects of GLP-1RAs on glucagon suppression are scarce and need to be investigated further.

The effects of GLP-1RA on the physiological counterregulatory response during hypoglycemia have been explored in clinical studies using a hypoglycemic clamp, similar to the studies described for DPP-4is. These studies demonstrated no disruption of normal counterregulatory responses to hypoglycemia in patients with T2D by GLP-1RAs, including lixisenatide,[186] albiglutide,[160] and liraglutide.[126] In addition, exenatide did not disrupt these responses in healthy volunteers.[184] In summary, GLP-1RAs decrease inappropriately elevated glucagon levels but do not impair the counterregulatory response of glucagon to hypoglycemia.

SGLT2is

The SGLT2i class of glucose-lowering drugs prevents reabsorption of glucose in the kidney, which facilitates the excretion of glucose in urine and, in turn, reduces plasma glucose levels.[171,187] Because these actions are insulin independent, there is minimal potential for hypoglycemia.[187] Results from clinical studies examining the effects of treatment with dapagliflozin[117,165-167] and empagliflozin[168] on glucagon secretion demonstrated increased fasting glucagon and postprandial glucagon levels and decreased insulin:glucagon ratios. To our knowledge, no clinical studies have reported the effects of canagliflozin on glucagon (Table 2).

Although there is limited information in humans regarding whole-body metabolic adaptation to treatment with SGLT2is,[188] 2 independent research teams uncovered an apparent SGLT2i class effect. Although SGLT2is lower fasting glucose in patients with T2D, they elicit a paradoxical rise in endogenous glucose production.[167,168] Merovci et al[167] found that dapagliflozin induced glucosuria and improved peripheral insulin sensitivity; however, endogenous glucose production increased substantially and was accompanied by an increase in fasting plasma glucagon concentration. In the other study, Ferrannini et al[168] reported that patients exhibited glycosuria after empagliflozin treatment, but empagliflozin was also associated with increased endogenous glucose production, beta-cell glucose sensitivity, and postprandial glucagon response.[168] Notably, studies measuring glucagon are often of short duration and may not provide insight into longer-term glucagon effects. In the Ferrannini et al study, the magnitude of the glucagon increase after 4 weeks of treatment was decreased compared with the increase after a single dose.[168]

Thus, it appears that SGLT2is may have a direct effect on glucagon in T2D. Merovci et al[167] speculate on several potential mechanisms for the relative increase in glucagon after SGLT2i treatment: acute decline in insulin-induced plasma glucose concentration from one hyperglycemic level to a lower hyperglycemic level; activation of a neural reflex that connects the kidney directly with pancreatic alpha cells; or activation of neuronal centers in the central nervous system that communicate with the alpha cells.[167] Two mechanistic studies provide insight into the increase in glucagon secretion with SGLT2i treatment via a direct effect on SGLT2 expression found in pancreatic alpha cells.[189,190] The SGLT2is have been associated with an increased risk of diabetic ketoacidosis.[191] The SGLT2i-induced increase in glucagon may provide a partial explanation for this observation given that increased levels of glucagon are linked with diabetic ketoacidosis.[192]

FUTURE OF DIABETES TREATMENT RELATED TO GLUCAGON

On the basis of findings described above, Merovci et al,[167] among others, have suggested that the combination of an SGLT2i and an incretin-based therapy could provide a potential synergism for the treatment of patients with T2D.[51,167,193,194] The rationale is that the incretin-based drug would block the increased production of endogenous glucose and elevated glucagon levels associated with SGLT2is and enhance the glucose-lowering effect of the SGLT2i.[167,193] A number of studies have reported a reduction in glucose (glycated hemoglobin [A1C]) levels after treatment with the

Mayo Clin Proc. ■ February 2018;93(2):217-239 ■ https://doi.org/10.1016/j.mayocp.2017.12.003
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TABLE 3. Randomized Phase 3 Clinical Studies That Combine Either a DPP-4i or a GLP-1RA With an SGLT2i in the Treatment of Type 2 Diabetes[a]

Reference, year	Duration to end point	Background therapy	Treatment arms	n	Change from baseline in A1C (%) [mmol/mol][b]
DPP-4i + SGLT2i[c]					
Lewin et al,[195] 2015	24 wk	None	Linagliptin 5 mg + empagliflozin 10 mg	135	−1.24 ± 0.06 [−13.6 ± 0.7]
			Linagliptin 5 mg + empagliflozin 25 mg	134	−1.08 ± 0.06 [−11.8 ± 0.7]
			Linagliptin 5 mg	133	−0.67 ± 0.06 [−7.3 ± 0.7]
			Empagliflozin 10 mg	132	−0.83 ± 0.6 [−9.1 ± 0.7]
			Empagliflozin 25 mg	133	−0.95 ± 0.06 [−10.4 ± 0.7]
DeFronzo et al,[196] 2015	24 wk	Metformin	Linagliptin 5 mg + empagliflozin 10 mg	135	−1.08 ± 0.06 [−11.8 ± 0.7]
			Linagliptin 5 mg + empagliflozin 25 mg	134	−1.19 ± 0.06 [−13.1 ± 0.7]
			Linagliptin 5 mg	128	−0.70 ± 0.06 [−7.6 ± 0.7]
			Empagliflozin 10 mg	137	−0.66 ± 0.06 [−7.2 ± 0.7]
			Empagliflozin 25 mg	140	−0.62 ± 0.06 [−6.8 ± 0.7]
Rosenstock et al,[197] 2015	24 wk	Metformin	Saxagliptin 5 mg + dapagliflozin 10 mg	179	−1.47 ± 0.08[d] [−16.1 ± 0.9]
			Saxagliptin 5 mg	176	−0.88 ± 0.08 [−9.6 ± 0.9]
			Dapagliflozin 10 mg	179	−1.20 ± 0.08 [−13.1 ± 0.9]
Mathieu et al,[198] 2015	24 wk	Metformin	Dapagliflozin 10 mg + saxagliptin 5 mg	146	−0.82[d] ± 0.07 [−9.0[d] ± 0.8]
			Placebo + saxagliptin	129	−0.10 ± 0.07 [−1.1 ± 0.8]
Matthaei et al,[199] 2015	24 wk	Metformin	Saxagliptin + dapagliflozin 10 mg	139	−0.51[d] (−0.63 to −0.39) [−5.6[d] (−6.9 to −4.3)]
			Placebo + dapagliflozin 10 mg	149	−0.16[d] (−0.28 to −0.04) [−1.7[d] (−3.1 to −0.04)]
Mathieu et al,[200] 2016	52 wk	Metformin	Dapagliflozin 10 mg + saxagliptin 5 mg	160	−0.74[d] (−0.90 to −0.57) [−8.1[d] (−9.8 to −6.2)]
			Placebo + saxagliptin 5 mg	160	+0.07[d] (−0.13 to +0.27) [−0.8[d] (−1.4 to −3.0)]
Jabbour et al,[201] 2014	24 wk	± Metformin	Dapagliflozin 10 mg + sitagliptin 100 mg	223	−0.5 (−0.6 to −0.4)[e] [−4.9 (−6.0 to −3.8)]
			Placebo + sitagliptin 100 mg	224	0.0 (−0.1 to +0.1) [+0.4 (−0.7 to +1.5)]
GLP-1RA + SGLT2i					
Frías et al,[202] 2016 (DURATION-8)	28 wk	Metformin	Exenatide 2 mg QW + dapagliflozin 10 mg every day	228	−2.0 (−2.1 to −1.8) [−21.9 (−23.0 to −19.7)]
			Exenatide 2 mg QW	227	−1.6 (−1.8 to −1.4) [−17.5 (−19.7 to −15.3)]
			Dapagliflozin 10 mg every day	230	−1.4 (−1.6 to −1.2) [−15.3 (−17.5 to −13.1)]

[a]A1C = glycated hemoglobin; DPP-4i = dipeptidyl peptidase-4 inhibitor; DURATION-8 = Diabetes Therapy Utilization: Researching Changes in A1C, Weight and Other Factors Through Intervention with Exenatide Once Weekly-8; GLP-1RA = glucagon-like peptide-1 receptor agonist; QW = once weekly; SGLT2i = sodium-glucose cotransporter 2 inhibitor.

[b]Data are presented as mean ± standard error or mean (95% CI).

[c]Dosing of DPP-4i and SGLT2i was once daily in all DPP-4i + SGLT2i studies.

[d]Adjusted mean.

[e]Last observation carried forward analysis.

combination of an SGLT2i and either a DPP-4i or a GLP-1RA (Table 3).[195-202]

Combination of DPP-4i and SGLT2i

To our knowledge, only 1 post hoc analysis has examined the effect of 1 of these combination treatments on glucagon in patients with T2D.[117] Glucagon levels increased approximately 10% after a liquid meal tolerance test with dapaglifozin treatment but did not increase with saxagliptin or the combination of saxagliptin and dapagliflozin. Interestingly, the change in glucagon levels did not correlate with changes in A1C levels.

Several large clinical studies have demonstrated that the combination of a DPP-4i and an SGLT2i improves glycemic control in patients with T2D (Table 3).[117,195-201] In each study, the DPP-4i/SGLT2i combination resulted in a greater A1C decrease than each drug alone: this was true for the combinations of linagliptin and empagliflozin,[195,196] saxagliptin and dapagliflozin,[197-200] and sitagliptin and dapagliflozin.[200] Notably, although the combination of linagliptin and empagliflozin[195,196] and the combination of saxagliptin and dapagliflozin[197] resulted in greater A1C decreases than the individual drugs and a significant increase in the proportion of patients attaining a target A1C of less than 7.0%, in each case, the reductions in A1C were less than additive.[195-197]

Combination of GLP-1RA and SGLT2i

Similar to the results from studies of DPP-4i/SGLT2i combinations, GLP-1RA/SGLT2i combination treatment was more effective than either drug alone in reducing glucose (Table 3); however, none of these studies reported glucagon results.

The combination of a GLP-1RA and an SGLT2i has, to our knowledge, been studied in only 1 phase 3 trial. In the DURATION (Diabetes Therapy Utilization: Researching Changes in A1C, Weight and Other Factors Through Intervention with Exenatide Once Weekly)-8 study, the combination of exenatide once weekly and dapagliflozin significantly reduced A1C levels from baseline compared with either drug alone, although the effects were not additive.[202]

Results from the DURATION-8 study are consistent with findings from previous small studies and post hoc analyses of GLP-1RA/SGLT2i combinations in T2D.[203-205] In each of these studies, the addition of an SGLT2i to a GLP-1RA augmented A1C reductions compared with the GLP-1RA alone. Similarly, the combination of daily dapagliflozin and exenatide once weekly was significantly more effective than placebo in improving body weight and reducing the risk of prediabetes than either drug alone.[206] Ongoing large clinical studies are examining the effects of adding liraglutide to canagliflozin (NCT02324842)[207] or dulaglutide to SGLT2is (AWARD-10 [A Study of Dulaglutide in Participants with Type 2 Diabetes Mellitus]; NCT02597049).[208]

Antagonism of the Glucagon Receptor

Antagonism of the glucagon receptor is being investigated in another approach for treating T2D.[209] Inhibiting glucagon-induced hepatic glucose production should, in theory, effectively decrease both fasting and postprandial hyperglycemia.[4] In a murine model of T2D, treatment with a glucagon receptor antagonist normalized plasma glucose and A1C levels to within nondiabetic ranges.[210,211] Phase 2 clinical studies have demonstrated reduced glycemia in patients with T2D treated with 2 glucagon receptor antagonists, LY2409021[212] and PF-06291874.[213] Despite good effect on glycemic end points, no drug in this class has reached the market for the treatment of T2D. This may be a consequence, in part, of observations that glucagon receptor antagonism results in elevation of liver transaminases,[4,212-215] liver fat,[216,217] body weight,[4,214] systolic blood pressure,[215] low-density lipoprotein cholesterol levels,[214,215] and alpha-cell hyperplasia.[209] The mechanisms for these side effects have been investigated, and though yet unknown, the mechanism for the increase in transaminase levels might be a result of increases in hepatic fat content (triglycerides).[213] Glucagon receptor antagonists have been associated with increased cholesterol absorption from the gut.[218]

Glucagon Receptor/GLP-1 Receptor Coagonists

Another emerging therapeutic strategy for the treatment of T2D and associated obesity that capitalizes on the underlying pathophysiology of glucagon is coagonism of the glucagon and GLP-1 receptors by a single molecule, of which there are now several examples with

Mayo Clin Proc. ■ February 2018;93(2):217-239 ■ https://doi.org/10.1016/j.mayocp.2017.12.003
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experimental evidence in rodent models.[219-222] Readers are directed to the excellent review by Müller et al[3] and references therein for a fuller discussion than space allows here.

CONCLUSION

To normalize metabolic control of glucose in the treatment of T2D, support has increased for targeting not only abnormalities in insulin secretion but also dysfunctional glucagon secretion. Glucagon is a key regulator of normal fuel metabolism, and both fasting and postprandial hyperglucagonemia make substantial contributions to the fasting hyperglycemia and postprandial glucose excursions that characterize T2D. Because patients with T2D have defects in glucagon control, improved restoration of metabolic control by therapies that also suppress glucagon, including DPP-4is and GLP-1RAs, would be beneficial. Future studies should focus on how novel strategies such as glucagon antagonism, glucagon/GLP-1 receptor coagonism, or combining DPP-4is or GLP-1RAs with SGLT2is can best control both insulin and glucagon in patients with T2D.

ACKNOWLEDGMENTS

Elizabeth Strickland, PhD, of inScience Communications, Springer Healthcare (Philadelphia, PA), and Faith Reidenbach, on behalf of inScience Communications, provided medical writing support, which was funded by AstraZeneca.

Abbreviations and Acronyms: **A1C** = glycated hemoglobin; **DPP-4i** = dipeptidyl peptidase-4 inhibitor; **DURATION-8** = Diabetes Therapy Utilization: Researching Changes in A1C, Weight and Other Factors Through Intervention with Exenatide Once Weekly-8; **GLP-1** = glucagon-like peptide-1; **GLP-1RA** = glucagon-like peptide-1 receptor agonist; **SGLT2i** = sodium-glucose cotransporter 2 inhibitor; **T2D** = type 2 diabetes

Grant Support: Medical writing support was funded by AstraZeneca.

Potential Competing Interests: Dr Hædersdal has no conflicts of interest to disclose. Within the past 36 months, Dr Lund has served as a consultant for Novo Nordisk. Within the past 36 months, Dr Knop has served on scientific advisory panels and/or speakers bureaus for, served as a consultant to, and/or received research support from Amgen, AstraZeneca, Boehringer Ingelheim, Eli Lilly and Company, Fractyl, Gubra, Merck Sharp & Dohme, Novo Nordisk, Sanofi, and Zealand Pharma. Within the past 36 months, Dr Vilsbøll has served on scientific advisory panels and/or speakers bureaus for, served as a consultant to, and/or received research support from Amgen, AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly and Company, Merck Sharp & Dohme, Novo Nordisk, Sanofi, and Takeda.

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