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Metabolic effects of glucagon in humans

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ABSTRACT

Diabetes is a common metabolic disorder that involves glucose, amino acids, and fatty acids. Either insulin deficiency or insulin resistance may cause diabetes. Insulin deficiency causes type 1 diabetes and diabetes associated with total pancreatectomy. Glucagon produces insulin resistance. Glucagon-induced insulin resistance promotes type 2 diabetes and diabetes associated with glucagonoma. Further, glucagon-induced insulin resistance aggravates the metabolic consequences of the insulin-deficient state. A major metabolic effect of insulin is the accumulation of glucose as glycogen in the liver. Glucagon opposes hepatic insulin action and enhances the rate of gluconeogenesis, increasing hepatic glucose output. In order to support gluconeogenesis, glucagon promotes skeletal muscle wasting to supply amino acids as gluconeogenic precursors. Glucagon promotes hepatic fatty acid oxidation to supply energy required to sustain gluconeogenesis. Hepatic fatty acid oxidation generates β-hydroxybutyrate and acetoacetate (ketogenesis). Prospective studies reveal that elevated glucagon secretion at baseline occurs in healthy subjects who develop impaired glucose tolerance at follow-up compared with subjects who maintain normal glucose tolerance, suggesting a relationship between elevated glucagon secretion and development of impaired glucose tolerance. Prospective studies have identified animal protein consumption as an independent risk factor for type 2 diabetes and cardiovascular disease. Animal protein intake activates glucagon secretion inducing sustained elevations in plasma glucagon. Glucagon is a major hormone that causes insulin resistance. Insulin resistance is an established cardiovascular risk factor additionally to its pathogenic role in diabetes. Glucagon may be a potential link between animal protein intake and the risk of developing type 2 diabetes and cardiovascular disease.

Introduction

Diabetes mellitus is a wide metabolic disorder caused by insulin deficiency or insulin resistance (inability of insulin to accomplish its action). Glucagon is a major human hormone that produces insulin resistance mainly in the liver. Glucagon-induced insulin resistance may cause diabetes by itself in patients with glucagonoma, a rare tumor that secretes glucagon. Despite no insulin deficiency, these patients experience insulin-dependent diabetes that disappears after the surgical resection of the tumor [1]. Glucagon-induced insulin resistance aggravates the metabolic consequences of the insulin-deficient condition that characterizes type 1 diabetes (T1D). Patients with either total pancreatectomy or diabetic ketoacidosis highlight the crucial role of glucagon modulating the insulin deficient status. Patients with total pancreatectomy lack pancreatic glucagon secretion. As a result, they exhibit outstanding insulin sensitivity, being predisposed to life-threatening hypoglycemic episodes. In contrast, patients with diabetic ketoacidosis show strikingly high plasma glucagon level and they experience profound insulin resistance that requires high amounts of insulin for metabolic control [2–5].

Glucose administration inhibits glucagon secretion in healthy humans and this response is attenuated in subjects with impaired glucose tolerance or diabetes. By contrast, intake of animal protein activates glucagon secretion in healthy subjects and this response is exaggerated in patients with glucose intolerance or diabetes. Therefore, patients with impaired glucose tolerance and diabetes experience excessive glucagon secretion in response to normal stimuli. Glucagon secretion is exaggerated in response to animal protein intake while is not inhibited by glucose compared to normal humans (Table 1) [2,6].

Prospective studies show that excessive glucagon responses to glucose and arginine predict the development of impaired glucose tolerance, being present for several years prior to the diagnosis of the disorder. Excessive glucagon secretion occurs at baseline in subjects who develop impaired glucose tolerance at follow-up, compared to subjects who

Table 1
Glucagon secretion in response to dietary components.

	Healthy subjects	Impaired glucose tolerance and diabetes
Carbohydrate meals and glucose ingestion	Glucagon suppression	No or less suppression
Animal protein meals and arginine infusion	Glucagon stimulation	Amplified glucagon stimulation
Fatty acids	Glucagon suppression	Glucagon suppression
Mixed meal	Slight glucagon stimulation	Amplified glucagon stimulation

who remain glucose-tolerant, suggesting an association between glucagon excess and future impaired glucose tolerance [7,8].

Animal protein intake activates glucagon secretion, which in turn causes insulin resistance. Insulin resistance has a pathogenic role in T2D. In addition, insulin resistance by itself is a solid cardiovascular risk. Multiple prospective investigations demonstrate that the intake of animal protein is associated with increased risk of developing T2D compared to vegetable protein. Replacing meat for vegetable protein in the diet has demonstrated definite advantages regarding insulin sensitivity [9–11]. Numerous prospective studies also show that animal protein intake is associated with increased cardiovascular risk [12–16]. Animal protein consumption stimulates glucagon secretion and plasma glucagon remains elevated during extended periods, inducing a prolonged insulin-resistant state. Glucagon-induced insulin resistance might be the link between animal protein intake and the increased risk of developing T2D and cardiovascular disease.

Metabolic relevance of glucagon in healthy subjects

About a century ago, the existence of glucagon was inferred in pancreatic extracts expected to contain only insulin because such extracts caused transient hyperglycemia. This effect was thought to be produced by a "contaminant" that was eventually separated from insulin and named glucagon ("glucose agonist") or hyperglycemic factor of the pancreas.

In human pancreatic islets of Langerhans, α-cells are arranged at the periphery while insulin-secreting β-cells are located at the core. Blood vessels penetrate and branch inside the islets, so that endocrine cells are usually adjacent to them [17,18]. The α-cells secrete glucagon. In addition, secretion of extrapancreatic glucagon by enteroendocrine cells has been postulated to occur, although the physiological role of extrapancreatic glucagon remains unclear [19]. Human α-cells express *SLC5A2*, the gene that encodes sodium-glucose co-transporter-2 (SGLT2) protein and hepatocyte nuclear factor 4α regulates the expression of this gene in α-cells. Silencing of the *SLC5A2* gene (via siRNA) triggers glucagon secretion through ATP-sensitive potassium channel (K_{ATP}) activation. Consistently, inhibition of the transporter protein SGLT2 in isolated α-cells with dapagliflozin leads to glucagon secretion in vitro [20,21].

Hyperglucagonemia occurs in clinical conditions involving adrenergic stimulation and metabolic stress, such as exercise, infections, acute hypoglycemia and starvation, to assure availability of glucose to peripheral tissues, such as the brain and the skeletal muscle. Glucagon opposes insulin action in the liver preventing glucose accumulation as hepatic glycogen. As a consequence, glucose remains in the blood stream, being accessible to be used by peripheral tissues [22,23].

Glucagon secretion in response to glucose, amino acids, fatty acids, and mixed meals in healthy subjects

Either oral ingestion or intravenous infusion of glucose inhibits glucagon secretion in healthy humans, leading to a fall in plasma glucagon level [2,6,24,25].

In contrast, either animal protein intake or amino acid infusion activates glucagon secretion. The infusion of arginine is associated with a transient rise in plasma glucagon that occurs within the first 5 min of the infusion and disappears within the next 30 min. [2,26] L-alanine infusion also increases plasma glucagon [27]. Animal protein intake stimulates prolonged glucagon secretion. The increase in plasma glucagon elicited by animal protein is long-lasting, being maintained for at least four hours after the ingestion [6].

In a randomized controlled crossover pilot trial, the effect of meal sequence on post-prandial glucose was investigated. Glucagon levels were more elevated when fish was ingested before rice compared with the ingestion of rice before fish [28]. Hyperglycemia attenuates the increase in plasma glucagon in response to animal protein meals. When healthy individuals receive a glucose infusion during a beef meal to render them hyperglycemic, protein-induced glucagon secretion is reduced [2,29].

Like glucose, elevation of plasma free fatty acids within the physiologic range decreases plasma glucagon levels by approximately 50%. In addition, the increase in glucagon secretion that follows arginine administration is attenuated after elevation of plasma free fatty acids [24,26].

The effect of individual dietary components on glucagon secretion is diverse. Animal protein intake stimulates whereas glucose and fatty acids ingestion inhibits glucagon secretion. The intake of a standard meal that contains glucose, amino acids and fatty acids induces a slight but long-lasting increase in plasma glucagon level. Plasma glucagon remains elevated more than 6 h after the meal, declining gradually to basal values. It has been estimated that plasma glucagon is 62% above basal values at 360 min (6 h) after the ingestion of a liquid mixed meal [30,31].

Metabolic effects of glucagon in healthy humans

Glucagon has profound effects on glucose, amino acid and fatty acid metabolism that enable survival in conditions such as starvation and metabolic stress (Fig. 1). Metabolic actions of glucagon take place mostly in the liver rather than the peripheral tissues [32–35].

Effects of glucagon on glucose metabolism in healthy humans

A major metabolic effect of insulin is the accumulation of glucose in the liver as glycogen thus reducing hepatic glucose output. Glucagon opposes insulin action preventing glucose accretion as hepatic glycogen. Glucagon action maintains glucose available to peripheral tissues (such as the brain) in conditions of low exogenous glucose supply (starvation) and in conditions of metabolic stress such as infections [36–40].

A number of investigations demonstrate glucagon action producing hepatic insulin resistance in normal humans.

In healthy subjects receiving a glucose infusion, arterial insulin concentration rises and hepatic glucose output falls by 80–100%. Under these conditions, a glucagon infusion that produces a physiologic increment in plasma glucagon results in a rapid reversal in the insulin-mediated inhibition of hepatic glucose output. The increase of plasma glucagon opposes the effect of insulin suppressing hepatic glucose output [41].

When insulin is infused to normal subjects while maintaining euglycemia with glucose infusions, net hepatic glucose production is suppressed. A 15 ng/kg/min glucagon infusion under these conditions results in stimulation of net hepatic glucose production, indicating that
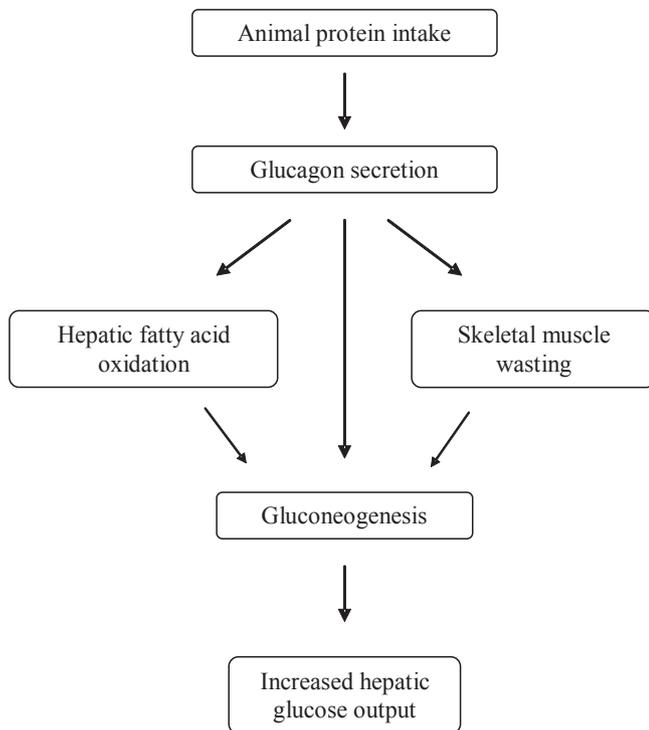
Fig. 1. Metabolic effects of glucagon.

glucagon opposes the inhibitory effect of insulin over hepatic glucose output [42].

The effect of prolonged (48 h) hyperglucagonemia on insulin-mediated hepatic glucose production in normal subjects was examined using the euglycemic insulin clamp technique. Insulin suppresses hepatic glucose production and that this action is opposed by glucagon administration. In addition, the effect of glucagon is entirely due to diminished non-oxidative glucose utilization (glycogen synthesis) [34].

In healthy individuals who receive an amino acid infusion to assess the effects of glucagon under hyperinsulinemic conditions, the amount of glucose required to maintain euglycemia is approximately 40% lower with high-dose glucagon infusion compared with low-dose glucagon infusion. When low plasma glucagon is present, insulin is allowed to store glucose as hepatic glycogen and the amount of glucose required to maintain euglycemia is greater. By contrast, when high plasma glucagon is present, glucose is not stored as glycogen in the liver and remains available in the blood stream. Therefore, the amount of glucose required to maintain euglycemia is lower [43].

Consistently with the action of glucagon inducing hepatic insulin resistance, selective glucagon deficiency achieved by infusing somatostatin + insulin while maintaining euglycemia with a glucose infusion results in a marked and long-lasting suppression of net hepatic glucose production, as the absence of glucagon allows optimal glucose storage in the liver [44].

The amount of glucose required to maintain hyperglycemia (220–240 mg/dl) in glucagon-deficient subjects is approximately three-fold the quantity of glucose required to maintain the same level of hyperglycemia in the glucagon-replaced individuals. Since the plasma levels of insulin and glucose are equal in the two groups, the amount of glucose infused to maintain the same level of glycemia is a measure of glucose tolerance. The striking difference in glucose tolerance is due to the presence or absence of glucagon. When glucagon is deficient, the liver is able to dispose of three times more net glucose than when glucagon is replaced. During glucagon deficit, glucose is efficiently stored by insulin whereas during glucagon excess, glucose is not stored by insulin as liver glycogen. Selective glucagon deficiency in normal subjects rendered hyperglycemic by glucose infusion induces hepatic

glucose uptake [45].

Similar results are obtained when hepatic glucose output is assessed isotopically with 3-[3H]glucose in the basal state and during infusion of somatostatin alone and in conjunction with replacement infusions of glucose and insulin. During isolated glucagon deficiency attained with somatostatin + insulin with serum glucose maintained at basal level, hepatic glucose output is suppressed by 71% in healthy subjects. During infusion of somatostatin alone (that suppresses glucagon and insulin), hepatic glucose output is suppressed by 25% of the basal value [46].

In healthy subjects under conditions of hyperglycemia, inhibition of glucagon secretion results in increased hepatic glycogen accumulation compared with conditions of basal glucagonemia. In addition, the relative contribution of gluconeogenesis (indirect pathway) to glycogen synthesis is lower during hypoglucagonemia than during basal glucagonemia [47].

In healthy subjects with normal glucose tolerance, glucagon suppression in response to oral glucose appears related to insulin sensitivity. Increased fasting glucagon levels develop in parallel with insulin resistance [48].

Effects of glucagon on amino acid metabolism in healthy subjects

In healthy subjects during the postabsorptive state, there is a net release of alanine from the skeletal muscle and a net hepatic uptake of this amino acid, which is a major precursor to glucose synthesis in the liver. An amino acid infusion induces a marked increase in hepatic amino acid uptake whereas leg balance reverses from a net release to a net amino acid uptake. Hepatic uptake accounts for approximately 70% of the total amino acid disposal and is greatest for the glucogenic amino acids, such as alanine. In contrast, leg amino acid uptake is dominated by the branched-chain amino acids (BCAAs). Body skeletal muscle is estimated to remove approximately 25–30% of the total infused amino acid load and approximately 65–70% of the infused BCAAs [49,50].

Glucagon infusion to healthy subjects reduces the plasma level of most amino acids [22,23,35,51–54]. Threonine, serine, and glycine experience the largest decrease [52,54]. Consistently, glucagon deficiency increases plasma level of most amino acids. Glycine, lysine, alanine, and arginine experience the largest increase [54].

Glucagon-induced hypoaminoacidemia is predominantly due to increased hepatic utilization of amino acids to generate glucose, since no changes in the uptake of amino acids by peripheral tissues has been documented [35,51].

The effects of glucagon on the disposal of an amino acid load have been assessed in healthy subjects receiving an amino acid infusion under euglycemic hyperinsulinemic conditions.

Physiological increments of plasma glucagon enhance the conversion of amino acids into glucose in the liver. During hyperglucagonemia, between 70 and 100% of the amino acids are converted to glucose while at basal glucagon concentration, hepatic glucose output does not rise during amino acid infusions, indicating that essentially none of the metabolized amino acids is converted to glucose [43].

A glucagon infusion resulting in supraphysiologic arterial levels of glucagon (ranging from 1700 to 5000 pg/ml) increases the conversion of alanine to glucose in the liver, but has no effect on the hepatic extraction of alanine [55].

Unlike most amino acids, glucagon has no effect on BCAAs and the plasma concentration of leucine, valine, and isoleucine remains unaltered by glucagon status. By contrast, the BCAAs are very responsive to insulin. Insulin decreases plasma concentration of BCAAs, insulin deficiency being associated with elevated plasma BCAAs level [35,53,54].

A physiological elevation of plasma glucagon concentration achieved by glucagon infusion raises urinary nitrogen excretion with urea accounting for the increased excretion. Enhanced gluconeogenesis induced by glucagon explains the increased urea formation in the liver. The carbon skeletons of amino acids are used to produce glucose whereas the amino groups generate urea. Excretion of 3-methyl-

histidine is unchanged, suggesting that muscle proteolysis is not the source of the nitrogen loss [22,43].

The effect of glucagon on amino acid metabolism is highlighted in two clinical conditions, glucagonoma (glucagon excess) and total pancreatectomy (lack of pancreatic glucagon).

Patients with glucagon-secreting glucagonoma typically experience severely depressed blood amino acid levels and advanced muscle wasting due to enhanced gluconeogenesis that requires skeletal muscle amino acid utilization. By contrast, patients with total pancreatectomy show elevated plasma concentration of most amino acids due to reduced gluconeogenesis associated with glucagon deficiency [4,43,54,56].

Patients with critical illness typically show excessive glucagon secretion. In critically ill patients, amino acid infusion intensifies glucagon secretion while glucose infusion does not suppress it, unlike normal subjects. Elevated plasma glucagon level is a major factor responsible for muscle wasting and hypoaminoacidemia associated to critical illness [23].

Effects of glucagon on fatty acids metabolism in healthy subjects

In healthy humans, increased lipolysis in the adipose tissue elevates plasma level of free fatty acids and induces fatty acid oxidation in the liver with formation of β-hydroxybutyrate and acetoacetate (so-called ketone bodies). Insulin has a major antilipolytic action and prevents free fatty acids from leaving the adipose tissue. Glucagon stimulates ketogenesis by enhancing fatty acid oxidation in the liver. Therefore, insulin deficiency (by increasing adipose lipolysis) and glucagon excess (by increasing hepatic fatty acid oxidation) promote β-hydroxybutyrate and acetoacetate formation [57–59].

In vitro studies using cultured human hepatocytes isolated from healthy liver biopsy specimens indicate that glucagon increases β-hydroxybutyrate and acetoacetate production owing to enhanced fatty acid oxidation. After 24 h exposure to glucagon, oleate oxidation increases and the rate of β-hydroxybutyrate and acetoacetate production rises by 150% compared with basal conditions. The addition of insulin to the culture medium abolishes the effect of glucagon [60].

Investigations conducted in one patient with glucagonoma before and after surgical removal of the tumor suggest that chronic hyperglucagonemia stimulates hepatic ketogenesis. Before surgery, the plasma concentrations of glucagon, β-hydroxybutyrate, and acetoacetate were elevated and approximately 48% of free fatty acid taken up by the liver was utilized for β-hydroxybutyrate and acetoacetate production. Postoperatively, plasma glucagon decreased to normal. Arterial free fatty acid level and net hepatic free fatty acid uptake remained unchanged. However, only 19% of the net hepatic free fatty acid uptake was utilized for β-hydroxybutyrate and acetoacetate formation. Ketogenesis was reduced by 63% despite that the liver extracted the same amount of fatty acids as it did before surgery. Postoperatively, arterial level of β-hydroxybutyrate and acetoacetate decreased to about 50% of the preoperative values [61].

The stimulatory effect of glucagon on hepatic ketogenesis occurs predominantly during insulin deficiency, a situation that allows lipolysis in the adipose tissue and increase free fatty acid availability to the liver [62].

Importance of glucagon during starvation in normal humans

Starvation requires adaptive processes that affect glucose, fatty acid, and amino acid metabolism in order to supply endogenous fuel to cells. The rate of hepatic gluconeogenesis increases to provide endogenous glucose to peripheral tissues, such as the brain. The skeletal muscle releases amino acids (mainly alanine) that are used to generate glucose in the liver. The adipose tissue releases free fatty acids that are oxidized in the liver to provide the energy required for enhanced gluconeogenesis. Hepatic oxidation of free fatty acids also produces β-hydroxybutyrate and acetoacetate that are used as fuel by the skeletal muscle. Glucagon secretion is a major driving force to the metabolic adaptation

to starvation. Plasma glucagon level increases after 24–48 h of fasting, inducing hepatic insulin resistance that prevents glucose from being stored. Glucagon also promotes gluconeogenesis and ketogenesis [49,63–65].

The increase in plasma glucagon level associated with starvation persists for several hours after refeeding. Glucose tolerance is markedly impaired in healthy subjects after 3 days of starvation. The blood glucose concentration averages 210 mg/dl one hour after ingestion of 100 g of glucose [52].

Starvation intensifies the increase in glucagon secretion elicited by amino acids. Arginine infusion to healthy volunteers at the end of 3 days of starvation produces a marked rise in glucagon secretion that is greater than the glucagon response of fed controls [66]. Like arginine, glucagon response to L-alanine infusion is enhanced by starvation [27].

Relevance of glucagon in patients with diabetes

Glucagon induces insulin resistance that worsens the insulin-deficient condition of T1D and has a pathogenic role in T2D. Pancreatic islets of patients with T2D contain a higher proportion of glucagon-producing α-cells and a reduced proportion of insulin-producing β-cells compared to normal controls. In addition, α-cells are distributed throughout the islet instead of in their usual location in the periphery of the islet [17]. Further, the expression of the glucagon gene (*GCG*) in α-cells is higher in islets from patients with T2D compared to nondiabetic individuals [20].

Monoclonal antibodies that block glucagon receptor are beneficial to control diabetes in patients with T1D and T2D [67,68]. However, a theoretical concern that might be associated to glucagon receptor blockade is development of pancreatic neoplasia, as patients with biallelic inactivating mutations in the glucagon receptor gene (*GCGR*) develop α-cell hyperplasia that may progress to neoplasia (Mahvash syndrome) [69].

Unlike healthy subjects, patients with T1D and T2D fail to suppress glucagon secretion in response to glucose intake and show exaggerated glucagon secretion in response to animal protein ingestion and mixed meals. As a result, plasma glucagon concentration is elevated compared to healthy subjects. The highest level of plasma glucagon is observed in patients with poorly controlled diabetes and diabetic ketoacidosis [2,3,31,48,70]. Patients with T2D showed higher day-long plasma glucagon levels compared to normal subjects in a study that measured plasma glucagon at several points in time during the day [71].

Glucagon secretion in response to glucose, amino acids, fatty acids, and mixed meals in patients with diabetes

The normal decline in plasma glucagon associated to carbohydrate meals or oral glucose ingestion does not occur in patients with T1D and T2D, despite the greater rise in plasma glucose [6,25,39,72]. Insulin therapy corrects partially the lack of suppression of glucagon in response to hyperglycemia in patients with diabetes, but glucagon response remains altered [25]. Patients with T1D and insulin-deficient T2D show no increase in glucagon secretion in response to hypoglycemia, unlike healthy individuals. This defect appears early in the course of the disease in patients with T1D [25,70,73,74].

Arginine infusion to healthy subjects stimulates glucagon secretion and this response is attenuated by hyperglycemia. Glucagon response to arginine infusion in patients with T1D and T2D is amplified compared to normal individuals, despite the presence of hyperglycemia. In T1D, the rise in plasma glucagon induced by arginine indicates that α-cells continue to function many years after β-cell function has been lost [2,73]. Insulin and sulfonylureas attenuate the excessive glucagon response to arginine in patients with T1D and T2D, unlike the abnormal glucagon response to glucose that is not readily corrected by insulin [25]. Hyperglycemia attenuates the increase in glucagon secretion associated to animal protein intake in healthy subjects. However, patients

with T1D and T2D maintain an elevated glucagon secretion after animal protein ingestion despite hyperglycemia being present [6,39].

Glucagon secretion in response to changes in plasma free fatty acid level has been scarcely investigated. In patients with T1D, suppression of glucagon levels by elevation of plasma free fatty acids is similar to that found in normal subjects [24].

The normal long-lasting rise in plasma glucagon after ingestion of a balanced mixed meal is intensified in patients with diabetes so that the increase in plasma glucagon is greater in these patients compared to healthy subjects [31]. The exaggerated response of glucagon to mixed meals in patients with T1D compared to normal subjects is an early feature of the disease, having being observed within the first 2 years of diagnosis [75].

Metabolic effects of glucagon in patients with diabetes

In patients with T1D and T2D, the amount of insulin required for glycemic control is higher in patients with more elevated plasma glucagon level, reflecting that glucagon induces insulin resistance. Patients with diabetic ketoacidosis require high amount of insulin for control during the early hours of therapy due to markedly elevated glucagon secretion that causes severe insulin resistance [3].

Effects of glucagon on glucose metabolism in patients with diabetes

Patients with T2D show elevated rate of hepatic glucose production compared to healthy individuals despite higher plasma insulin level in the diabetic group, reflecting hepatic insulin resistance. Elevated hepatic glucose production (due to enhanced gluconeogenesis) is the primary factor responsible for both fasting and postprandial hyperglycemia in patients with diabetes [39].

Glucagon contributes to fasting and post-prandial hyperglycemia in patients with T1D and T2D by increasing gluconeogenesis and hepatic glucose output. The normal suppression of hepatic glucose output following meals or glucose ingestion is impaired in patients with T2D and the magnitude of the defect is correlated with the increase in plasma glucagon concentration [39].

In patients with T1D, isolated glucagon deficiency achieved by infusion of somatostatin + insulin normalizes plasma glucose concentration while restoration of hyperglucagonemia by infusion of glucagon + somatostatin + insulin raises plasma glucose level [76].

In patients with T1D, hepatic glucose production increases simultaneously with elevated plasma concentration of glucagon after insulin removal, suggesting that glucagon is a major factor responsible for the rise in hepatic glucose release [77].

The contribution of pancreatic glucagon to insulin sensitivity has been evaluated in patients with T1D. Plasma glucagon level increased after arginine infusion. During a euglycemic hyperinsulinemic clamp, plasma glucagon was negatively correlated with the glucose infusion rate, suggesting that glucagon secretion in response to arginine deteriorates insulin sensitivity in patients with T1D [78].

Effects of glucagon on amino acid metabolism in patients with diabetes

In patients with diabetes, glucagon excess promotes gluconeogenesis that requires utilization of gluconeogenic precursors, including amino acids, particularly alanine. Hepatic uptake of alanine is greater in patients with diabetes compared to healthy subjects. Consistently, plasma alanine levels are higher during glucagon suppression by somatostatin in patients with T1D, owing to gluconeogenesis inhibition [79]. Augmented amino acid utilization to produce glucose in the liver due to enhanced gluconeogenesis induces protein loss in skeletal muscle of patients with diabetes [80].

Effects of glucagon on fatty acid metabolism in patients with diabetes

Similarly to healthy subjects, glucagon supports hepatic ketogenesis in patients with diabetes, particularly when excess free fatty acids are available due to insulin deficiency. This action of glucagon contributes to the elevated plasma level of β-hydroxybutyrate and acetoacetate [57]. In patients with T1D, an increase in plasma glucagon level is accompanied by a rise in blood ketone levels [81]. Accordingly, during glucagon deficiency attained by infusion of somatostatin + insulin, the urinary excretion of β-hydroxybutyrate and acetoacetate declines [82]. Glucagon suppression by somatostatin delays the development of ketoacidosis in patients with T1D. Isolated insulin absence does not lead to immediate diabetic ketoacidosis, glucagon being a prerequisite to the development of this condition [79].

Role of the lack of pancreatic glucagon in diabetes secondary to total pancreatectomy

Like patients with T1D, subjects with total pancreatectomy experience diabetes due to insulin deficiency. However, the lack of pancreatic glucagon after surgical removal of the pancreas induces striking sensitivity to exogenous insulin that modulates the metabolic disorder. Diabetes secondary to total pancreatectomy shows distinctive features that highlight the relevance of glucagon on intermediate metabolism. Glucagon deficiency suppresses hepatic gluconeogenesis and ketogenesis and allows unrestrained action of exogenous insulin that predisposes to life-threatening hypoglycemia [4,5,83,84].

Glucose metabolism in patients with total pancreatectomy

Patients with total pancreatectomy are strikingly sensitive to insulin due to the lack of pancreatic glucagon. They require lower amount of insulin for glycemic control compared to patients with T1D and they experience remarkable tendency to severe hypoglycemia due to unrestrained insulin action. In patients diagnosed with diabetes before the surgical intervention, the quantity of insulin required for metabolic control decreases after total pancreatectomy [5,85].

Glucagon enhances gluconeogenesis in healthy subjects and patients with diabetes thus increasing hepatic glucose output. In patients with total pancreatectomy, glucagon deficiency is associated with reduced rate of gluconeogenesis and subsequent plasma accumulation of gluconeogenic precursors. Plasma level of alanine and lactate are elevated in patients with total pancreatectomy compared to patients with T1D, even when exogenous insulin is available to achieve glycemic control [5,84,86].

Accordingly, glucagon replacement increases hepatic glucose production and blood glucose levels in patients with total pancreatectomy to values similar to those in patients with T1D. Glucagon infusion also reduces the plasma level of gluconeogenic precursors such as alanine and lactate in patients with total pancreatectomy [86].

Amino acid metabolism in patients with total pancreatectomy

The reduced rate of hepatic gluconeogenesis due to glucagon deficiency after removal of the pancreas accounts for the accumulation of amino acids in plasma observed in these patients. Fasting plasma level of most amino acids, including alanine, serine, glycine, threonine, arginine, citrulline, α-aminobutyrate, aspartate, proline, phenylalanine, and tyrosine is elevated compared to values obtained from normal subjects. Alanine, threonine, serine and glycine are the amino acids most responsive to changes in glucagon level. Restoring physiological glucagon concentration by glucagon infusion in patients with total pancreatectomy diminishes plasma level of the previously elevated amino acids. Plasma concentration of BCAAs is within the normal range in patients with total pancreatectomy, as these amino acids are unaffected by glucagon status [4].

Plasma level of alanine is particularly elevated in patients with total pancreatectomy, reflecting strikingly reduced gluconeogenesis. The high concentration of alanine does not return to normal when

exogenous insulin is available to attain glycemic control, suggesting that the gluconeogenesis rate remains low due to glucagon deficiency in spite of insulin being present [5,86]. Before the surgical intervention, plasma alanine level is similar to that of healthy individuals in patients undergoing total pancreatectomy. However, blood concentration of alanine increases quickly after removal of the pancreas [87].

Fatty acid metabolism in patients with total pancreatectomy

In healthy subjects and patients with diabetes, glucagon promotes hepatic fatty acid oxidation and subsequent β-hydroxybutyrate and acetoacetate production (ketogenesis), particularly when free fatty acid availability is increased due to insulin deficiency that allows adipose lipolysis. Patients with total pancreatectomy are highly resistant to ketosis because the lack of pancreatic glucagon reduces the ability to generate β-hydroxybutyrate and acetoacetate in the liver. Insulin removal induces similar increase in plasma free fatty acid level in patients with total pancreatectomy and T1D. However, the elevation in plasma ketone bodies is attenuated in patients with total pancreatectomy compared to T1D [5,81].

Excessive glucagon secretion is present in subjects with impaired glucose tolerance

Fasting plasma glucagon is elevated and abnormal patterns of glucagon secretion in response to dietary components (similar to diabetes) are already present in glucose-intolerant patients before the diagnosis of T2D.

Fasting plasma glucagon level is higher in obese patients compared to normal controls [88–90]. In addition, there is a nonlinear relationship between fasting glucagon concentration and insulin resistance, such that more severe insulin resistance is associated with higher fasting glucagon levels [48]. Furthermore, fasting plasma glucagon decreases in obese subjects after weight loss. Both bariatric surgery and dietary intervention result in comparable reduction in fasting plasma glucagon level [91].

The normal suppression of glucagon following a glucose load is reduced in subjects with impaired glucose tolerance, so that plasma glucagon concentration is higher in the obese subjects compared to the lean subjects after oral glucose challenge [48,88–90,92–94].

In obese subjects, the hepatic uptake of glucose precursors is increased despite basal hyperinsulinemia and the relative contribution to total glucose release attributable to gluconeogenesis is 70% higher compared to lean subjects. In addition, the suppression of hepatic glucose output following either oral glucose ingestion or intravenous glucose infusion is impaired in patients with impaired glucose tolerance compared with subjects with normal glucose tolerance. The magnitude of the defect is correlated with the increase in plasma glucagon concentration, indicating that impaired glucose tolerance is associated with hepatic insulin resistance due to glucagon excess [39,92].

In healthy subjects, intravenous infusion of arginine increases glucagon secretion and this response is exaggerated in patients with T1D and T2D. Like diabetic patients, subjects with impaired glucose tolerance show an exaggerated glucagon response to arginine infusion compared to subjects with normal glucose tolerance [93,95–97]. Weight loss normalizes the exaggerated glucagon response to arginine infusion in subjects with impaired glucose tolerance to the range of the lean individuals [95]. The infusion of high doses of insulin also normalizes the exaggerated glucagon response to arginine in subjects with impaired glucose tolerance [96]. The exaggerated response of glucagon to arginine infusion in subjects with impaired glucose tolerance precedes the altered glucagon response to glucose in these individuals [95]. In healthy subjects, hyperglycemia reduces the increase in glucagon secretion secondary to arginine infusion. The inhibitory effect of hyperglycemia is attenuated in glucose-intolerant subjects compared to subjects with normal glucose tolerance [90,97].

Excessive glucagon secretion predicts the development of impaired glucose tolerance

Prospective studies show that altered glucagon responses are present at baseline in subjects with normal glucose tolerance who develop impaired glucose tolerance at follow-up, compared to subjects who remain glucose-tolerant. In a prospective study that enrolled post-menopausal women with normal glucose tolerance, excessive glucagon response to arginine at baseline predicted future glucose intolerance. Exaggerated glucagon response to arginine occurs at baseline in subjects that develop impaired glucose tolerance at 3 years follow-up compared to those subjects that remain glucose-tolerant [7]. Similarly, excessive glucagon response to glucose at baseline predicted the development of impaired glucose tolerance at 12 years of follow-up in a prospective study that recruited post-menopausal women with normal glucose tolerance. Subjects that developed impaired glucose tolerance at follow-up failed to suppress glucagon secretion in response to glucose at baseline [8]. Therefore, altered glucagon responses to arginine and glucose at baseline independently predicted impaired glucose tolerance. Subjects who show excessive glucagon secretion in response to arginine or subjects who do not suppress glucagon secretion in response to glucose develop future glucose intolerance. Excessive glucagon secretion and exaggerated patterns of glucagon response to dietary components are present before the diagnosis of impaired glucose tolerance, suggesting a causative relationship between excessive glucagon response and insulin resistance.

Animal protein intake induces insulin resistance via glucagon secretion in healthy subjects and patients with T1D and T2D

In healthy subjects, animal protein intake stimulates sustained glucagon secretion, as elevated plasma glucagon level persists several hours after the protein meal. The increase in glucagon secretion in response to animal protein intake is intensified in patients with impaired glucose tolerance and diabetes who endure day-long increased plasma glucagon level. Glucagon is a major hormone that opposes the action of insulin.

In healthy subjects, a high animal protein diet induces hepatic insulin resistance due to glucagon excess compared to a normal protein diet. Nondiabetic subjects on a high protein diet show increased gluconeogenesis and hepatic glucose output compared to subjects on a normal protein diet. Further, the normal suppression of hepatic glucose output by insulin is impaired in the subjects on a high protein diet compared with those consuming a normal protein diet. Glucagon secretion correlates with the magnitude of the impairment, indicating that impaired inhibition of hepatic glucose production by insulin is mediated by enhanced glucagon secretion [80,98].

In patients with T1D and T2D, the increase in plasma glucagon associated with animal protein intake is accompanied by an increase in plasma glucose despite a constant infusion of insulin, suggesting that animal protein intake contributes to the post-prandial hyperglycemia through an increase in glucagon secretion [29]. Consistently, dietary animal protein increases insulin requirements in patients with T1D. The post-prandial rise in plasma glucose and therefore the insulin requirement for glycemic control is greater after the ingestion of a standard meal with added animal protein than following a standard meal with added fat. The addition of animal protein but not fat energy to a meal intensifies insulin resistance and therefore increases the amount of insulin required for metabolic control. The added protein to the standard meal activates glucagon secretion which in turn amplifies insulin resistance [99]. Similarly to healthy humans, the rate of gluconeogenesis and hepatic glucose output is increased in patients with T1D on a high protein diet compared to a normal protein. Further, the ability of insulin to suppress hepatic glucose output in the high protein group is reduced compared to the normal protein group. A marked parallelism between hepatic glucose production and glucagon secretion is

observed, indicating that impaired inhibition of hepatic glucose output by insulin is mediated in part by enhanced glucagon secretion in the patients with high animal protein intake [80].

Insulin resistance has a pathogenic role in T2D. Numerous prospective studies demonstrate that animal protein consumption increases the risk of developing impaired glucose tolerance and T2D whereas ingestion of vegetable protein has a protective effect on the risk of T2D [9–11,100]. In addition, insulin resistance is a well-established cardiovascular risk factor in healthy subjects and patients with diabetes. Multiple investigations show that animal protein consumption increases the risk of cardiovascular mortality whereas ingestion of vegetable protein has a protective effect on the risk of cardiovascular events [13–16,101,102]. Glucagon secretion induced by animal protein intake might be the link between animal protein and insulin resistance.

Conclusion

Conclusive evidence from prospective studies shows that animal protein consumption increases the risk of developing type 2 diabetes and cardiovascular disease. Animal protein intake activates glucagon secretion, inducing a sustained elevation in plasma glucagon level. Glucagon is a primary hormone that opposes insulin action. Glucagon secretion associated with animal protein intake might be the connection between animal protein and the elevated risk of developing cardiovascular disease and T2D.

Acknowledgement

Conflict of interest

The authors declare that they have no conflict of interest.

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