Nutrient-Sensing Mechanisms in the Gut as Therapeutic Targets for Diabetes

Danna M. Breen,[1,2] Brittany A. Rasmussen,[1,3] Clémence D. Côté,[1,3] V. Margaret Jackson,[4] and Tony K.T. Lam[1,2,3,5]

The small intestine is traditionally viewed as an organ that mediates nutrient digestion and absorption. This view has recently been revised owing to the ability of the duodenum to sense nutrient influx and trigger negative feedback loops to inhibit glucose production and food intake to maintain metabolic homeostasis. Further, duodenal nutrient-sensing defects are acquired in diabetes and obesity, leading to increased glucose production. In contrast, jejunal nutrient sensing inhibits glucose production and mediates the early antidiabetic effect of bariatric surgery, and gut microbiota composition may alter intestinal nutrient-sensing mechanisms to regain better control of glucose homeostasis in diabetes and obesity in the long term. This perspective highlights nutrient-sensing mechanisms in the gut that regulate glucose homeostasis and the potential of targeting gut nutrient-sensing mechanisms as a therapeutic strategy to lower blood glucose concentrations in diabetes. ***Diabetes*** **62:3005–3013, 2013**

Diabetes affects approximately 350 million people (1) and is characterized by a disruption in glucose homeostasis. The elevation of plasma glucose concentration is due secondarily to an elevation of hepatic glucose production and a decrease in glucose uptake. Since the discovery of insulin, laboratories have focused on characterizing signaling pathways in the liver that mediate the ability of insulin to inhibit glucose production and in the muscle and fat to increase glucose uptake (2). These studies have convincingly illustrated that reversing insulin resistance and/or enhancing insulin action directly in the liver, muscle, and fat helps restore glucose homeostasis in diabetes and obesity. However, is insulin resistance a dominant determinant of glucose disturbance in diabetes and obesity? Or more specifically, is reversing insulin resistance the most effective way to lower glucose production in diabetes and obesity?

Although the answer to these questions remains unclear, recent studies begin to suggest this may not be the case because administration of leptin is sufficient to lower glucose production and plasma glucose concentrations in insulin-deficient diabetic rodents (3–5). Further, duodenal-jejunal bypass (DJB) surgery lowers glucose production in insulin-deficient uncontrolled diabetic rodents independent of a rise in plasma insulin concentrations (6), whereas in insulin-resistant Zucker *fa/fa* rodents DJB lowers glucose production independent of reversing insulin resistance (7). The effect of leptin to lower glucose concentrations in insulin-deficient conditions has been attributed to a lowering of plasma glucagon levels (3), implicating glucagon action (instead of insulin resistance) as a potential major driver of hyperglycemia (8). However, the glucose-lowering effect induced by DJB (6) as well as central nervous center nutrient-sensing mechanisms (9) occur independent of changes in plasma glucagon levels, suggesting that other factor(s) responsible for the glucose-lowering effect may be present. In this article, we propose that activating nutrient-sensing mechanisms in the gut is an effective strategy to lower glucose production and plasma glucose concentrations in diabetes and obesity. Further, we postulate that insulin action is not required for the metabolic control of gut nutrient sensing such that glucose homeostasis could be rapidly restored in spite of insulin resistance in diabetes and obesity.

NUTRIENT-SENSING MECHANISMS IN THE DUODENUM

A rise in nutrients occurs in the small intestine after meal ingestion, and nutrients are released into the circulation after their absorption. While still in the preabsorptive state, nutrients activate sensing mechanisms in the duodenum to trigger a gut-brain–driven negative feedback system to inhibit exogenous nutrient intake and endogenous nutrient production by the liver (10–12). Consequently, metabolic homeostasis is maintained in response to nutrient intake. In diabetes and obesity, intestinal nutrient-sensing mechanisms fail to lower food intake and glucose production (13), leading to a disruption in metabolic homeostasis. With an aim to restore glucose and energy homeostasis, laboratories have focused on characterizing duodenal nutrient-sensing mechanisms that alter food intake and glucose production in diabetes and obesity.

This article will first highlight the role of duodenal nutrient-sensing mechanisms in the regulation of glucose homeostasis and its relevance in diabetes and obesity. After the absorption of nutrients such as lipids into duodenal enterocytes, long-chain fatty acids (LCFAs) from lipids are metabolized into LCFA-CoA via acyl-coA synthase (12). Short-term continuous intraduodenal intralipids infusion elevates duodenal LCFA-CoA levels and lowers glucose production in healthy rodents while plasma insulin concentrations are maintained at basal levels (14) (Fig. 1). Direct inhibition of duodenal acyl-coA synthase and accumulation of duodenal LCFA-CoA negates the gluco-regulatory effect of intralipids, and intraduodenal lipid

From the [1]Toronto General Research Institute, University Health Network, Toronto, Ontario, Canada; the [2]Department of Medicine, University of Toronto, Toronto, Ontario, Canada; the [3]Department of Physiology, University of Toronto, Toronto, Ontario, Canada; the [4]Department of Cardiovascular, Metabolic and Endocrine Diseases, Pfizer Global Research and Development, Cambridge, Massachusetts; and the [5]Banting and Best Diabetes Centre, University of Toronto, Toronto, Ontario, Canada.

Corresponding author: Tony K.T. Lam, tony.lam@uhnres.utoronto.ca.

Received 2 April 2013 and accepted 20 May 2013.

DOI: 10.2337/db13-0523

D.M.B. and B.A.R. contributed equally to the study.

D.M.B. is currently affiliated with Pfizer Global Research and Development, Cambridge, Massachusetts.

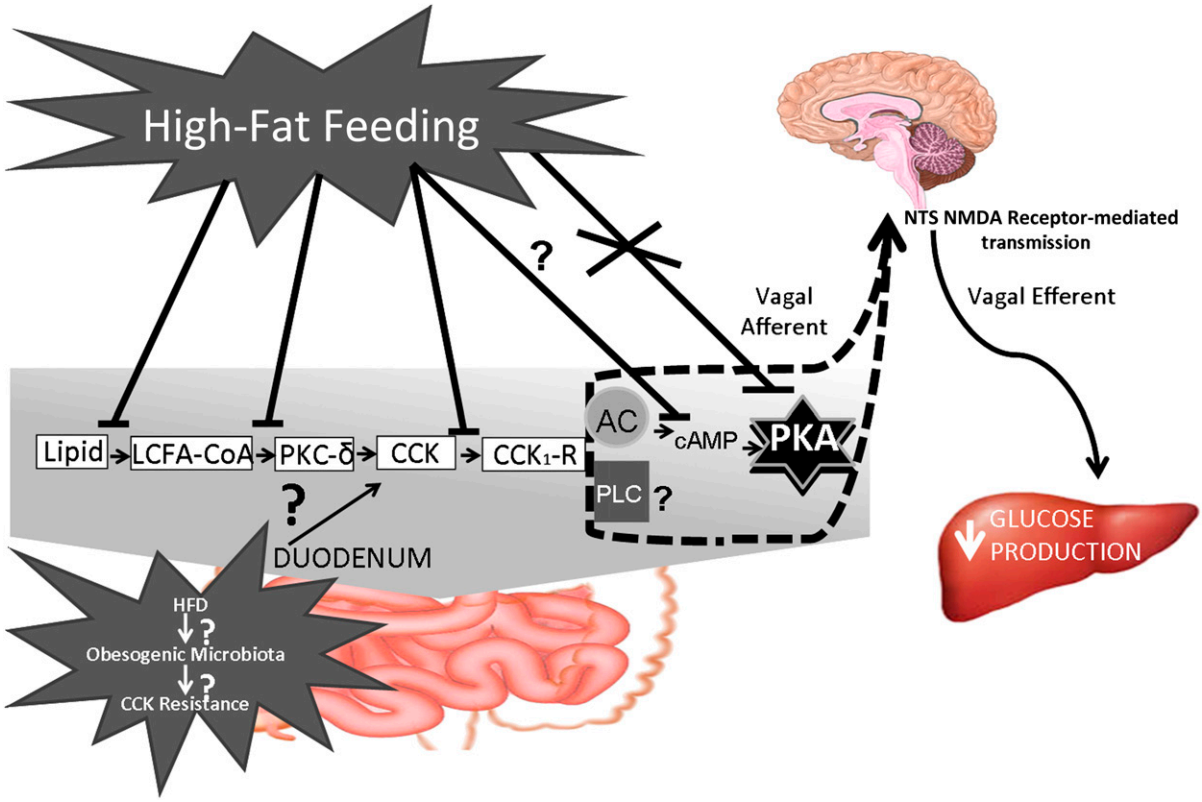

FIG. 1. Duodenal lipid-sensing mechanisms. Upon intake of nutrients, the duodenum activates a complex molecular and neuronal network to lower hepatic glucose production. High-fat feeding induces duodenal CCK resistance and direct activation of PKA bypasses the defect. High-fat feeding induces obesogenic microbiota and subequently alters CCK action. AC, adenylyl cyclase; CCK1-R, CCK receptor; HFD, high-fat diet.

infusion also fails to lower glucose production in high-fat diet–fed rodents (14). These findings illustrate the importance of elucidating the downstream duodenal lipid signaling events that regulate glucose production in normal and high-fat diet–fed rodents.

Intraduodenal lipid administration lowers food intake in rodents and humans through the release of the gut-derived peptide hormone cholecystokinin (CCK) from the duodenal I cells (11). The release of CCK induced by LCFA is confirmed in the STC-1 CCK-secreting cell line and is protein kinase C (PKC) dependent (15,16). Along with this, activation of duodenal mucosa PKC (particularly PKC-δ, expressed in both rodent and human duodenal mucosa) by PKC activator is sufficient to lower glucose production while molecular and chemical inhibition of duodenal PKC-δ negate the glucoregulatory effect of duodenal lipids (17). Further, given that direct inhibition of intestinal CCK-1 receptors negates the ability of intraduodenal lipids, PKC activator, or CCK-8 infusion to lower glucose production, the subsequent activation of duodenal CCK-1 receptors is required for the LCFA–PKC-δ–CCK signaling axis to lower glucose production in healthy rodents in vivo (17–19) (Fig. 1). It is believed that CCK binds to the CCK-1 receptors expressed on vagal afferents innervating the small intestine (20), and activation of CCK-1 receptors triggers a gut-brain-liver axis to lower glucose production in healthy rodents (19). Although PKC-δ is colocalized with CCK in the duodenal mucosa (18), suggesting that the LCFA-CoA ➔ PKC-δ ➔ CCK signaling event takes place in the duodenal I-cells, definitive in vivo experiments are lacking. Nonetheless, direct activation of the duodenal PKC-δ ➔ CCK signaling axis fails to lower glucose production in high-fat diet–fed rodents independent of changes in the duodenal expression of CCK-1 receptors (18,19,21).

Addressing the mechanism(s) underlying duodenal PKC-δ ➔ CCK resistance in lowering glucose in high-fat diet is essential for *1*) evaluating novel fatty acid G-protein receptors coupled to PKC-δ in duodenal I cells and *2*) in evaluating CCK-1 receptor agonists as antidiabetic therapies. In healthy volunteers, it has also been reported that CCK is released in response to intragastric fatty acids with chain lengths matching the ligand profiles of GPR40/FFAR1 and GPR120/O3FAR1, both of which have been shown to be present in rodent duodenal I cells and to modulate CCK release (22,23). GPR40 and GPR120 are both Gq-coupled receptors activating PKC, but is unknown if these receptors lower glucose production via a duodenual-brain-liver axis in rodents on a high-fat diet or if present in human I cells. Recent advances in isolation of human enteroendocrine cells and measuring peptide secretion will directly address the latter (24). Despite the earlier promising potential of CCK-1 receptor agonists in metabolism, selective activation of the receptor has failed to generate a new class of antiobesity or diabetic therapy primarily due to toleration and lack of efficacy. Systemic agonists must be minimized to avoid unwanted adverse effects via direct activation on the gall bladder and pancreas, whereas gut-selective approaches have also been dose limited as a result of gastrointestinal side effects such as vomiting (25). Understanding the endogenous CCK tone within the duodenum in addition to a deeper understanding of the CCK-1 receptor intracellular coupling mechanisms underlying glucose-lowering efficacy

may provide new insights into the doability of CCK-1 receptor agonists for diabetes.

CCK-1 receptors are G-protein coupled and are highly expressed in the gut (26). The signaling cascade of CCK-1 receptors in the acinar cells involves both protein kinase A (PKA) and phospholipase C (PLC) activation, which mediate CCK's ability to trigger pancreatic secretion (26–29). Although the CCK-1 receptor signaling cascade in the duodenum remains largely undefined, direct activation of duodenal PKA signaling has recently been demonstrated to be sufficient and necessary for CCK-1 receptor activation to stimulate vagal afferent firing in vivo (21). Consequently, a gut-brain-liver–driven neuronal network is ignited to lower glucose production in both normal and high-fat diet–fed rodents (21). However, duodenal PKA is not the sole mediator of this network as inhibition of PLC prevents CCK from lowering glucose production as well (21). Given that direct duodenal PKA activation bypasses duodenal CCK resistance in high-fed feeding to lower glucose production (21), high-fat diet–induced defects lie within the inability of duodenal CCK-1 receptor activation to stimulate PKA (Fig. 1). Future studies are warranted to clarify the duodenal signaling cascades of the CCK-1 receptor that regulate glucose production such that novel and effective antidiabetic therapeutic targets could be revealed.

Recently, it has been shown that glucose sensing in L-cells, enterochromaffin cells and activation of neural pathways is impaired in diabetes as determined using phosphorylated calcium calmodulin-dependent kinase II as a marker of cellular activation in vivo (30). No changes were detected in gastric inhibitory peptide (GIP) however. GIP secretion is primarily regulated by fats and was not tested (30,31). Could activation of duodenal PKA signaling mediate other gut-derived peptide hormones such as glucagon-like peptide-1 (GLP-1) and GIP to lower glucose levels in diabetes and obesity? GLP-1 and GIP receptors signal via PKA (32,33), and selective inhibition of intestinal dipeptidyl peptidase-IV (DPP-IV) activity, which enhances GLP-1 and GIP, can trigger vagal firing and improves glucose homeostasis in diet-induced obese rodents in a GLP-1 receptor–dependent manner (34). The role of GIP has not been directly tested. Although the specific site(s) of GLP-1 action in the gut remain to be clarified, a possibility remains that the local release of intestinal GLP-1 activates GLP-1 receptors expressed on the vagus (32) and triggers a gut-brain–driven system to lower blood glucose levels in diabetes and obesity. Of note, it is unknown how the GLP-1 receptor (34) but not CCK-1 receptor (19,21) signaling in the gut remains intact in diet-induced obesity to lower blood glucose levels. Nonetheless, we propose that PKA signaling (likely located on the vagal terminals that innervate the intestine) mediates multiple gut-derived hormones to trigger a gut-brain–driven system to lower blood glucose levels in diabetes (Fig. 1).

On the one hand, intestinal-specific signaling–targeted research may prove useful to unveil novel antidiabetic targets, but on the other hand, examining whether different types of fatty acids or nutrients other than lipids may trigger the gut-brain axis to lower glucose production may be equally important. CCK signaling in the gut is not only triggered by lipids (19), but also by refeeding (17,19) to regulate glucose production and homeostasis, respectively. Although the ability of the duodenal lipid-sensing pathway to regulate glucose production is assessed under the pancreatic euglycemic clamp conditions to ensure that any changes in glucose metabolism are due primarily to the treatment rather than changes in circulating glucoregulatory hormones, direct inhibition of the duodenal lipid-sensing pathway is also found to disrupt glucose homeostasis following a fasting–refeeding nonclamp protocol (17,19). These findings highlight the physiological relevance of a gut-brain–driven feedback system that is activated by duodenal nutrient sensing after a meal to maintain circulating nutrient homeostasis.

It is currently unknown whether other classes of nutrients, in addition to lipids, regulate glucose production in the proximal intestine. However, duodenal glucose sensing inhibits feeding (35,36) while enteric glucose sensing (37) (which includes intestinal, mesenteric, and hepatoportal sensing systems) or, specifically, jejunal glucose sensing (6) regulates peripheral glucose homeostasis via a gut-brain–driven system. Glucose is absorbed through the proximal intestine into the portal vein where selective portal glucose sensing triggers a gut-brain-liver axis to inhibit glucose production and mediate the glucose-lowering effect of bariatric surgery in high-fat diet–fed mice (38). Hence, the essential glucoregulatory role of nutrient-sensing mechanisms in the gut is highlighted and a possibility emerges that nutrient-sensing mechanisms may be conserved throughout the gut for glucose regulation.

NUTRIENT-SENSING MECHANISMS IN THE JEJUNUM

Nutrient absorption occurs predominantly in the duodenum, and it is believed that only under malabsorptive conditions undigested nutrients reach the distal gut (39). However, studies in animals (40–43) and humans (44,45) illustrate that ingested nutrients reach the distal gut during early phases of food ingestion, opening up the possibility that nutrient-sensing mechanisms in the jejunum or ileum are activated shortly after a meal to regulate peripheral metabolic homeostasis. In fact, direct jejunal lipid (46–50) or glucose (47) but not protein (47) administration lowers food intake. The underlying mechanisms of jejunal nutrient sensing remain unclear, but the roles of peptide YY (46,47), GLP-1 (46–48), CCK (46), and/or a gut-brain neuronal axis (46,51,52) have been implicated. Nonetheless, given that jejunal nutrient sensing regulates feeding (46–50) and that, as discussed above, duodenal lipid sensing regulates glucose production (12), a potential glucoregulatory role of jejunal nutrient sensing has been examined as well (6) (Fig. 2).

Direct administration of glucose or lipids (the main sources of food energy) into the jejunum lowers glucose production in the absence of a lowering of food intake (6). The effect of nutrient sensing is ignited within the jejunum since direct inhibition of glucose sensing mechanisms in the duodenum or ileum fails to negate the ability of a jejunal glucose infusion to lower glucose production (6). In addition, an equimolar infusion of glucose into the portal vein, as in the jejunum, fails to lower glucose production (6). These experiments do not exclude the glucoregulatory role of nutrient sensing in the duodenum, ileum, or portal vein but rather illustrate that direct infusion of glucose into the jejunum triggers signaling mechanisms within the jejunum to lower glucose production.

Nutrient sensing in the jejunum appears to share similar mechanisms with the duodenum, since both segments lower glucose production in response to lipids, require the formation of LCFA-CoA, and trigger a gut-brain-liver neuronal relay to lower glucose production (6,53). However,

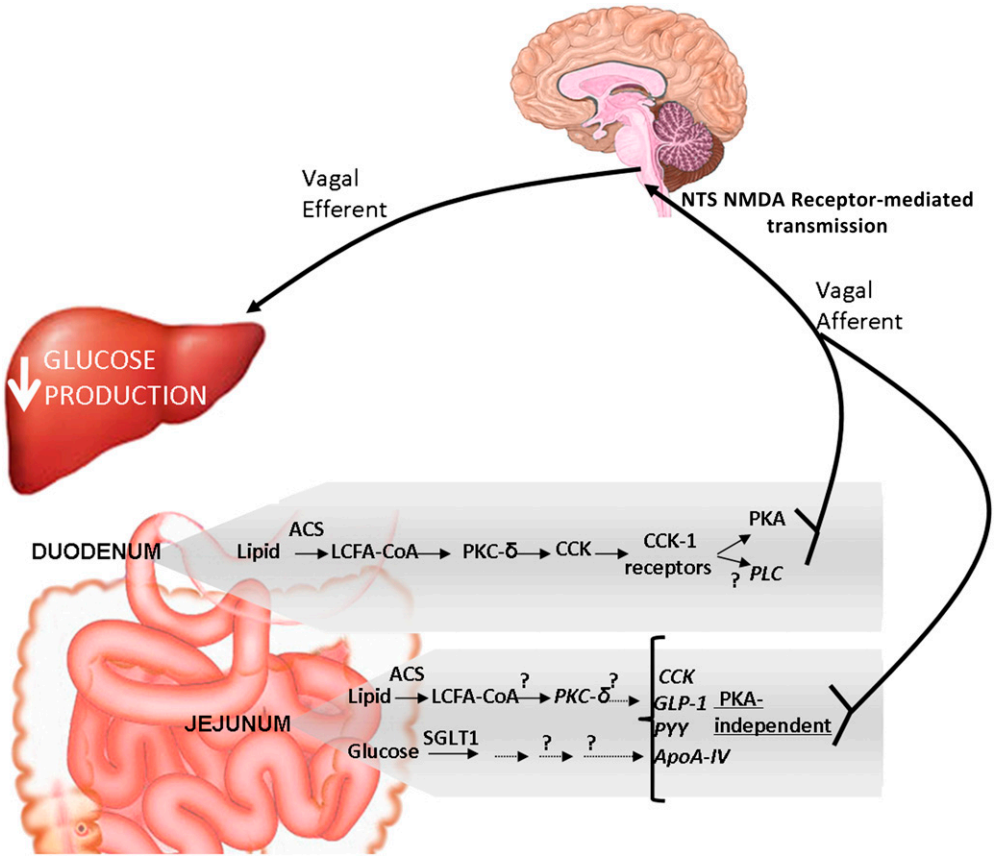

FIG. 2. Jejunal nutrient-sensing mechanisms. An influx of nutrients into the duodenum or jejunum activates a complex biochemical, molecular, neuronal, and physiological network to lower hepatic glucose production. Although there are some parallels regarding nutrient sensing in the duodenum and jejunum, it appears that unlike in the duodenum, jejunal nutrient sensing is PKA independent. ACS, acyl-CoA synthetase; NTS NMDA, nucleus of the solitary tract N-methyl-D-aspartate; PYY, peptide YY.

evidence suggests that important differences may exist between the two because direct administration of a PKA agonist (which activates downstream CCK-PKA signaling) into the duodenum, but not the jejunum, lowers glucose production (21), although CCK is also synthesized in the jejunum of animals and humans (54–56) but to a much lower extent (57). Given that CCK mediates duodenal lipid sensing mechanisms via PKA and/or PLC signaling pathways to lower glucose production (53) (Fig. 1), these findings suggest that either jejunal nutrient sensing is not CCK dependent or may potentially signal via the CCK-PLC signaling pathway. This working hypothesis remains to be tested.

Out of all the gut-derived satiety factors studied, apolipoprotein A-IV is principally synthesized and secreted from the jejunum, although its secretion is thought to be specific to lipids and not glucose (11). The potential role of apolipoprotein A-IV in jejunal regulation of glucose homeostasis remains unknown. Similarly, other major distal gut peptides that have documented anorectic effects mediated by the vagus are GLP-1 and peptide YY (58), either of which remain possible mediator(s) in maintaining glucose homeostasis triggered by jejunal nutrient sensing. Future investigation is required to dissect the downstream jejunal signaling pathway(s) of nutrient sensing that regulate peripheral metabolic homeostasis.

Inhibition of the sodium-glucose transporter proteins (SGLTs) in the jejunum negates the ability of intrajejunal glucose administration to lower glucose production (6), indicating that jejunal glucose uptake from the lumen is a necessary step for jejunal glucose sensing to regulate peripheral glucose homeostasis. Since galactose, like glucose, is also absorbed into the intestinal enterocyte from the lumen via the same glucose transporter (59), it would be important to assess whether galactose, at an equimolar concentration of glucose in the jejunum, also lowers glucose production. Another monosaccharide, fructose, is absorbed into the intestine via glucose transporter-5 (59), and the potential glucoregulatory role of fructose sensing in the jejunum deserves future attention. We propose that comparing the glucoregulatory impact of sensing mechanisms potentially triggered by any of these monosaccharides will be crucial in revealing novel nutrient-sensing mechanisms in the jejunum. In parallel, accumulation of jejunal LCFA-CoA is required for intrajejunal lipid administration to lower glucose production (6). Because the accumulation of LCFA-CoA in the brain and the liver are sufficient and necessary to mediate both lipid and glucose sensing mechanisms to regulate hepatic glucose metabolism (60), this raises the possibility that lipid and glucose sensing mechanisms within the jejunum may converge. However, this postulation remains to be confirmed.

In a physiological nonclamp (i.e., glucoregulatory hormones change at will) condition when nutrient-sensing mechanisms are activated by a fasting-refeeding protocol, direct disruption of duodenal nutrient-sensing mechanisms increases plasma glucose levels during refeeding and disrupts glucose homeostasis as compared with rodents

with intact duodenal nutrient sensing (17,19). In contrast, in the same experimental conditions, plasma glucose levels are not altered when jejunal nutrient-sensing mechanisms are disrupted (6). These data suggest that the key benefits of nutrient-sensing mechanisms in the jejunum to regulate glucose homeostasis are revealed under conditions in which normal nutrient flow is disrupted, for example, when sections of the upper intestine are removed in cases of intestinal cancer or inflammation (where short bowel resection is recommended) or after bariatric surgery in obese patients. Several key aspects of gut nutrient-sensing mechanisms and the regulation of glucose production have been uncovered, yet several questions remain. First, given the fact that direct nutrient administration into the duodenum or jejunum exerts similar effects on glucose production, would an additive or possibly synergistic effect on glucose control be observed if both duodenal and jejunal nutrient sensing were stimulated in parallel with a matched caloric load? Is there a humoral or neuronal crosstalk between the duodenum and jejunum? Given that the duodenum is exposed to the highest influx of nutrients from the stomach, are duodenal nutrient-sensing mechanisms dominant over jejunal nutrient sensing? If so, what is the functional relevance of jejunal nutrient sensing regarding glucose control?

The concept of a defect in duodenal nutrient sensing resulting in an increase in glucose production raises many fundamental questions when translating to a new antidiabetic therapy. Can duodenal nutrient-sensing defects simply be reversed by diet and exercise? Do the duodenal nutrient-sensing defects trigger the first step in the journey of diabetes or is this defect responsible for a select subset of patients with diabetes who need to be defined? How can defects in duodenal nutrient sensing in patients be identified early as part of an intervention strategy? Pharmacologically, any gut-targeted therapy to lower blood glucose will access both the duodenum and jejunum, so is there a predominant selective gut mechanism of action to sufficiently lower blood glucose in diabetes? Alternatively what combination approaches should be pursued to maximize efficacy while maintaining a well-tolerated safety profile?

GUT NUTRIENT-SENSING MECHANISMS IN BARIATRIC SURGERY

Bariatric surgeries exert a wide range of health benefits (61). Although the primary goal of bariatric procedures is weight loss, for which it is currently the most effective intervention, it also remains the only known treatment to induce remission of type 2 diabetes resulting in euglycemia. Bariatric surgery normalizes blood glucose concentrations in the majority of type 2 diabetic humans independent of weight loss (62–64). Furthermore, the effectiveness on glucose control induced by bariatric surgery is superior to conventional pharmacological therapy and lifestyle modifications (62–64). One additional study even indicates that bariatric surgery induces diabetes remission up to 6 years (65).

Although the effectiveness of bariatric surgery has set the standard for goals in glucose control for nonsurgical treatments, replicating its effect with nonsurgical tools has been relatively unsuccessful. Perhaps this is partly due to the complex interactions of numerous signaling pathways affected by the procedure in combination with the equally complex nature of the targeted diseases. Clearly, diabetes remission induced by bariatric surgery is associated with an enhancement of nutrient delivery to the distal small intestine. Could the enhancement of nutrient flux into the distal small intestine after bariatric surgery lead to an antidiabetic effect (Fig. 3)? Given that both the duodenum and jejunum are independently capable of sensing nutrients and generating a gut-derived signal mediated by vagal nerves to trigger a gut-brain-liver axis to lower glucose production independent of weight loss (as discussed above), a clear possibility remains. This hypothesis is also supported by medical devices that are being investigated as noninvasive alternatives to bariatric surgery. A trial using a novel device that electrically alters the vagal nerve firing as a treatment for morbid obesity reports a fast decrease in fasting plasma glucose within the first week in patients with type 2 diabetes (ENABLE trial; http://ir.enteromedics.com/releasedetail.cfm?ReleaseID=610191). Likewise a tube device that is endoscopically inserted in the gut extending from the duodenum to the proximal jejunum to prevent nutrients being absorbed within this region also reports rapid changes in glucose (http://www.gidynamics.com/endobarrier-overview.php).

DJB, a nonrestrictive experimental form of bariatric surgery (66), lowers glucose levels in nonobese rodents (66) and nonobese/mild-obese humans with type 2 diabetes (67,68) independent of weight loss. This surgical procedure redirects nutrient flow from the stomach to the distal jejunum and thus bypasses the duodenum. Can the enhancement of nutrient flux into the jejunum trigger a neurocircuit to lower glucose levels in diabetes with DJB? First, DJB is performed in two independent rat models in which diabetes is induced by destruction of the pancreatic β-cells by either a toxic injection of streptozotocin or a genetic autoimmune response (BioBreeding rats) (6). The use of these uncontrolled diabetic models enables the assessment of the effects of DJB in a nonobese setting with impaired insulin secretion. Within 2 days, DJB lowers blood glucose concentrations independent of lowering food intake/body weight or increasing insulin secretion in both uncontrolled diabetic models (6). Importantly, direct inhibition of jejunal nutrient-sensing mechanisms disrupts peripheral glucose control triggered by DJB during refeeding (6). Of note, the glucose response during refeeding in the DJB diabetic rodents with or without intact jejunal nutrient-sensing mechanisms closely resembles those observed in relatively mild-obese type 2 diabetic humans with or without DJB surgery, respectively (67). Together with the ability of jejunal nutrient sensing to trigger a gut-brain-liver neuronal relay to lower glucose production in normal rodents (see above), these findings collectively suggest that increased delivery of nutrients to the jejunum after DJB triggers a neuronal-mediated signaling mechanism to lower glucose production and hence blood glucose concentrations in diabetes.

The ability of DJB to rapidly lower blood glucose concentrations is potentially via a CNS-mediated reduction in hepatic glucose production (6). In fact, other reports have published similar results that DJB (7) or a variant of DJB (38) triggers the CNS to lower hepatic glucose production in obese type 2 diabetic rodents. Of note, DJB lowers blood glucose concentrations in insulin-deficient rodents (6) and lowers glucose production in obese type 2 diabetic rodents independent of an improvement on insulin action (7). Thus, we propose that insulin action is not required for the rapid glucose-lowering effect induced by DJB in diabetes. However, these findings do not exclude the possibility that DJB could also lower blood glucose concentrations

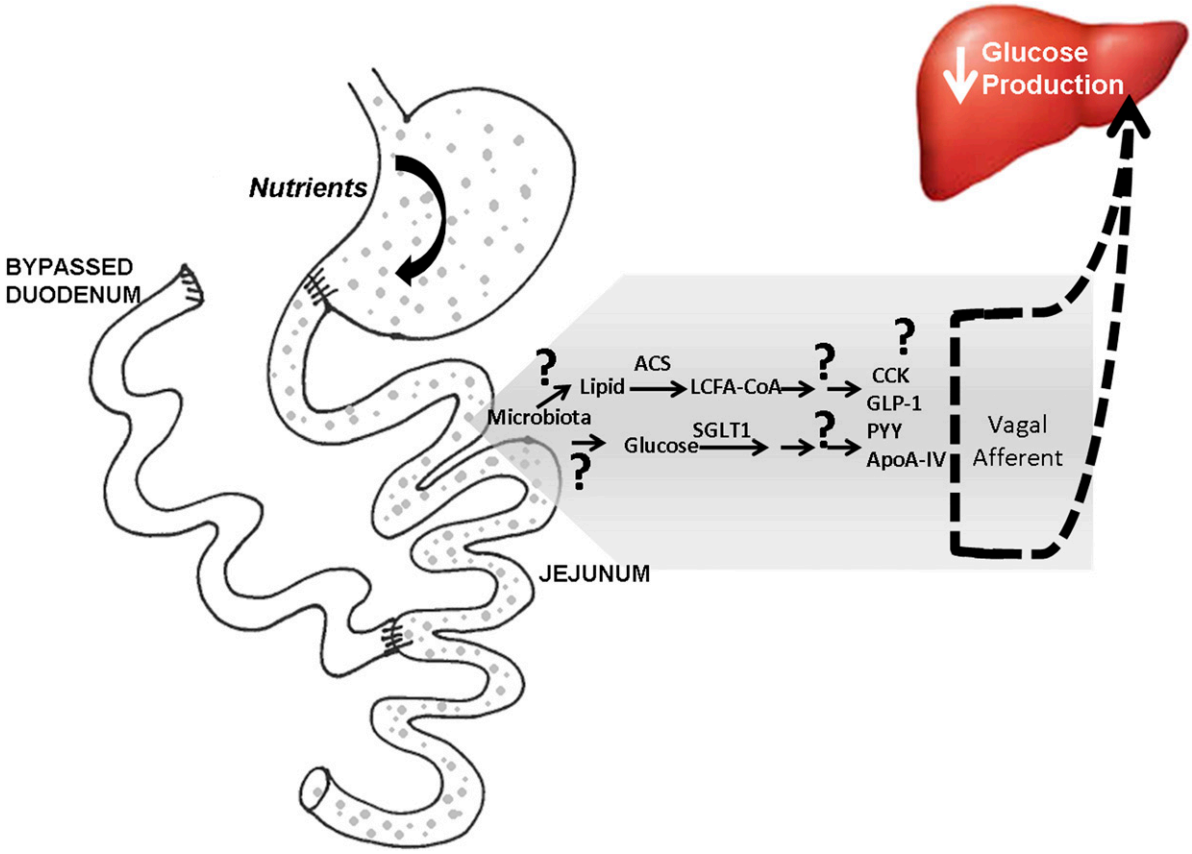

FIG. 3. Gut nutrient-sensing mechanisms and bariatric surgery. Bariatric surgery bypasses the duodenum, and nutrient flow is enhanced into the jejunum, resulting in activation of jejunal nutrient-sensing mechanisms involving a series of biochemical reactions, gut-derived hormones, and neuronal circuitry to lower glucose production and plasma glucose levels.

via increased insulin-dependent or independent glucose uptake, although studies have indicated that DJB does not improve insulin action in rodents with diabetes and/or obesity (7,69,70). In addition, improvement in β-cell function occurs after bariatric surgery in obese type 2 diabetic subjects (61). Thus, a potential increase in glucose uptake, improvement in insulin action and/or increase in insulin secretion could play important roles in the sustained glucose-lowering effect triggered by DJB.

Of note, changes in insulin secretion after bariatric procedures in obese type 2 diabetes are generally thought to coincide with increased GLP-1 secretion (61). However, changes in circulating GLP-1 are not consistent between models of diabetes that received DJB (6). Moreover, bariatric surgery still improves glucose tolerance in GLP-1 receptor–deficient high-fat diet–fed mice (71). In addition, clinical use of GLP-1 agonists and DPP-IV inhibitors does not mirror the full glycemic benefits observed after bariatric surgery, and although controversial, it is possible that other incretin hormones such as GIP may be involved in enhancing insulin secretion.

It is well known that bariatric surgery results in significant health benefits in obesity with type 2 diabetes, whereas only a few studies have demonstrated that bariatric surgery lowers glucose concentrations in mild/non-obese type 2 diabetic subjects (67,68) and in obese type 1 diabetic subjects (72). In clinical practice, there is much debate regarding the appropriate BMI level to determine eligibility for bariatric procedures. Therefore, until this debate is resolved, determining whether bariatric surgery could exert significant benefit in nonobese humans with type 1 diabetes will remain speculative. Despite this, the existence of a gut-brain-liver axis regulating glucose homeostasis and its relevance in the antidiabetic effect of bariatric surgery suggests that targeting the gut with drug therapy to influence the liver could be a valid therapeutic approach and would potentially mimic the antidiabetic effect of bariatric surgery. Given the accessibility of the gut to pharmacotherapy, we propose targeting nutrient sensing molecules in the gut will have therapeutic potential in lowering blood glucose concentrations in diabetes.

GUT MICROBIOTA AND NUTRIENT SENSING

The intestine has developed mechanisms to sense nutrients after a meal and trigger a negative feedback system to maintain glucose homeostasis. This triggering leads to the rapid glucose lowering effect of DJB in diabetes (Figs. 2 and 3). However, we must keep in mind that the intestinal environment is complex, given that an abundant collection of microorganisms (predominantly bacteria) termed microbiota resides within the gut. In the human intestine approximately 400 bacterial species are present and together resemble a multicellular organ that has evolved to provide metabolic functions (73). These bacteria belong to three main groups (phyla): *Firmicutes*, *Bacteriodetes*, and *Actinobacteria*, which represent >95% of the total bacteria in the intestine. Disruption in this symbiotic relationship can disturb host functions and in extreme cases can cause serious disease such as irritable

bowel disease (74), stressing the importance of these microorganisms in maintaining the intestinal environment and the roles they play in helping the host.

The gut microbiota is a dynamic organ that is constantly adapting to changes in its environment, and it is believed that the progression of obesity/diabetes can be attributed to the intestinal microbiota–host relationship. In mice switched from a regular chow to a high-fat diet, the gut microbiota composition is altered in as little as 24 h (75), resulting in increased levels of *Firmicutes* (76). This change is secondary to increased fat content rather than the obese state, rendering the microbiota to be more obesogenic and resulting in increased energy harvest from the diet in the distal gut. In addition, germ-free mice are protected from developing diet-induced obesity (77), further highlighting the relationship between the gut microbiome and obesity. Of note, it has recently been demonstrated that genetically identical mice fed the same high-fat diet differed in their metabolic phenotype due to differences in their microbiota composition (78). Thus, the relative contribution of diet versus the gut microbiota profile in causing metabolic diseases remains to be addressed.

Although a larger bacterial population resides in the more distal portion of the small intestine and in the colon, bacteria are also present in the upper intestine, including the duodenum and jejunum. In fact, direct administration of microbiota from lean donors into the duodenum of humans with metabolic syndrome significantly improves insulin sensitivity independent of weight loss (79), consistent with the view that the duodenum regulates peripheral glucose homeostasis (Fig. 1). Furthermore, gut microbiota alters intestinal permeability (80,81) and regulates both the synthesis and production of bile acids (82,83), which are important for fat emulsification and absorption in the intestine. Gut microbiota also regulates hormone release from enteroendocrine cells (84,85) and interestingly, ingestion of bacteria activates vagal afferent signaling (86). In addition, germ-free mice have altered CD36 expression (a proximal fatty acid transporter) (85) and SGLT-1 expression (84), indicating that gut bacteria affects nutrient absorption/uptake. Further, high-fat feeding promotes an obesogenic bacterial profile, which is linked to increased inflammation in the intestine and whole-body insulin resistance (80). Obesogenic mirobiota induces inflammation potentially via bacterial fragments (87). In light of these findings, we put forward a working hypothesis that the obesogenic microbiome disrupts nutrient-sensing CCK signaling mechanisms in the duodenum, leading to a dysregulation of glucose homeostasis (Fig. 1).

Changes in microbiota also occur after bariatric surgery in which the obese bacterial profile is altered to reflect a more lean profile (88). Whether these changes are associated with a decrease in food intake or are due to the physical rearrangement of the digestive tract still remains unknown. It has also been suggested that this change in microbiota after surgery may lead to changes in enteroendocrine cell function (85,89). However a direct correlation has not been shown. Thus, the link between bariatric surgery and changes in gut microbiota as well as its eventual impact on metabolic regulation warrants further investigation. Nevertheless, a possibility remains that the improvement of glucose homeostasis observed following bariatric surgery is mediated by a change in gut microbiota, leading to an enhancement of nutrient-sensing mechanisms in the jejunum and an improvement in glucose tolerance (Fig. 3).

SUMMARY

The gut possesses a unique role in regulating glucose and energy homeostasis whereby the presence of ingested nutrients into the small intestine activates sensing mechanisms that affect both glucose production and food intake. The nutrient-sensing mechanisms are conserved from the duodenum to the jejunum, and although it is evident that nutrient uptake and a neuronal network are required in both regions to lower glucose production, whether additional parallel mechanisms exist is not yet known. Duodenal nutrient sensing acts as a protective mechanism under normal physiological conditions to maintain glucose homeostasis during nutrient ingestion by ensuring that glucose production is decreased; however, this mechanism appears to be impaired after excess caloric intake. On the other hand, jejunal nutrient sensing is important in mediating the glucose lowering effect (via decreased glucose production) of bariatric surgery where nutrient influx to the jejunum is enhanced. Emerging evidence also suggests a relationship between gut microbiota and intestinal nutrient-sensing mechanisms; however, this concept has not been directly tested. Although many questions remain unanswered, recent advances in our understanding of the pathways regulating gut nutrient sensing provide compelling support for potential new therapeutic targets to restore glucose homeostasis in diabetes.

ACKNOWLEDGMENTS

The work discussed in this review from the Lam laboratory is supported by a research grant from the Canadian Institute of Health Research (CIHR-MOP-82701). D.M.B. was supported by a Canadian Diabetes Association postdoctoral fellowship. B.A.R. was supported by a CIHR Doctoral Vanier Canada scholarship.

No potential conflicts of interest relevant to this article were reported.

D.M.B., B.A.R., C.D.C., and T.K.T.L. developed the concept and wrote the manuscript. V.M.J. reviewed and edited the manuscript.

The authors apologize to colleagues whose work is not referenced as the result of space limitations.

REFERENCES

1. Scully T. Diabetes in numbers. Nature 2012;485:S2–S3
2. Saltiel AR, Kahn CR. Insulin signalling and the regulation of glucose and lipid metabolism. Nature 2001;414:799–806
3. Fujikawa T, Chuang JC, Sakata I, Ramadori G, Coppari R. Leptin therapy improves insulin-deficient type 1 diabetes by CNS-dependent mechanisms in mice. Proc Natl Acad Sci USA 2010;107:17391–17396
4. German JP, Thaler JP, Wisse BE, et al. Leptin activates a novel CNS mechanism for insulin-independent normalization of severe diabetic hyperglycemia. Endocrinology 2011;152:394–404
5. Wang MY, Chen L, Clark GO, et al. Leptin therapy in insulin-deficient type I diabetes. Proc Natl Acad Sci USA 2010;107:4813–4819
6. Breen DM, Rasmussen BA, Kokorovic A, Wang R, Cheung GW, Lam TK. Jejunal nutrient sensing is required for duodenal-jejunal bypass surgery to rapidly lower glucose concentrations in uncontrolled diabetes. Nat Med 2012;18:950–955
7. Jiao J, Bae EJ, Bandyopadhyay G, et al. Restoration of euglycemia after duodenal bypass surgery is reliant on central and peripheral inputs in Zucker fa/fa rats. Diabetes 2013;62:1074–1083
8. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. J Clin Invest 2012;122:4–12

9. Chari M, Lam CK, Wang PY, Lam TK. Activation of central lactate metabolism lowers glucose production in uncontrolled diabetes and diet-induced insulin resistance. Diabetes 2008;57:836–840
10. Badman MK, Flier JS. The gut and energy balance: visceral allies in the obesity wars. Science 2005;307:1909–1914
11. Cummings DE, Overduin J. Gastrointestinal regulation of food intake. J Clin Invest 2007;117:13–23
12. Lam TK. Neuronal regulation of homeostasis by nutrient sensing. Nat Med 2010;16:392–395
13. Breen DM, Yang CS, Lam TK. Gut-brain signalling: how lipids can trigger the gut. Diabetes Metab Res Rev 2011;27:113–119
14. Wang PY, Caspi L, Lam CK, et al. Upper intestinal lipids trigger a gut-brain-liver axis to regulate glucose production. Nature 2008;452:1012–1016
15. Chang CH, Chey WY, Chang TM. Cellular mechanism of sodium oleate-stimulated secretion of cholecystokinin and secretin. Am J Physiol Gastrointest Liver Physiol 2000;279:G295–G303
16. Takahashi A, Tanaka S, Miwa Y, et al. Involvement of calmodulin and protein kinase C in cholecystokinin release by bombesin from STC-1 cells. Pancreas 2000;21:231–239
17. Kokorovic A, Cheung GW, Breen DM, Chari M, Lam CK, Lam TK. Duodenal mucosal protein kinase C-δ regulates glucose production in rats. Gastroenterology 2011;141:1720–1727
18. Breen DM, Yue JT, Rasmussen BA, Kokorovic A, Cheung GW, Lam TK. Duodenal PKC-δ and cholecystokinin signaling axis regulates glucose production. Diabetes 2011;60:3148–3153
19. Cheung GW, Kokorovic A, Lam CK, Chari M, Lam TK. Intestinal cholecystokinin controls glucose production through a neuronal network. Cell Metab 2009;10:99–109
20. Broberger C, Holmberg K, Shi TJ, Dockray G, Hökfelt T. Expression and regulation of cholecystokinin and cholecystokinin receptors in rat nodose and dorsal root ganglia. Brain Res 2001;903:128–140
21. Rasmussen BA, Breen DM, Luo P, et al. Duodenal activation of cAMP-dependent protein kinase induces vagal afferent firing and lowers glucose production in rats. Gastroenterology 2012;142:834–843, e3
22. Sykaras AG, Demenis C, Case RM, McLaughlin JT, Smith CP. Duodenal enteroendocrine I-cells contain mRNA transcripts encoding key endocannabinoid and fatty acid receptors. PLoS ONE 2012;7:e42373
23. McLaughlin J, Grazia Lucà M, Jones MN, D'Amato M, Dockray GJ, Thompson DG. Fatty acid chain length determines cholecystokinin secretion and effect on human gastric motility. Gastroenterology 1999;116:46–53
24. Habib AM, Richards P, Rogers GJ, Reimann F, Gribble FM. Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells. Diabetologia 2013;56:1413–1416
25. Jordan J, Greenway FL, Leiter LA, et al. Stimulation of cholecystokinin-A receptors with GI181771X does not cause weight loss in overweight or obese patients. Clin Pharmacol Ther 2008;83:281–287
26. Williams JA, Blevins GT Jr. Cholecystokinin and regulation of pancreatic acinar cell function. Physiol Rev 1993;73:701–723
27. Berridge MJ. Inositol trisphosphate and diacylglycerol as second messengers. Biochem J 1984;220:345–360
28. Marino CR, Leach SD, Schaefer JF, Miller LJ, Gorelick FS. Characterization of cAMP-dependent protein kinase activation by CCK in rat pancreas. FEBS Lett 1993;316:48–52
29. Simonsson E, Karlsson S, Ahrén B. Involvement of phospholipase A2 and arachidonic acid in cholecystokinin-8-induced insulin secretion in rat islets. Regul Pept 1996;65:101–107
30. Lee J, Cummings BP, Martin E, et al. Glucose sensing by gut endocrine cells and activation of the vagal afferent pathway is impaired in a rodent model of type 2 diabetes mellitus. Am J Physiol Regul Integr Comp Physiol 2012;302:R657–R666
31. Lardinois CK, Starich GH, Mazzaferri EL. The postprandial response of gastric inhibitory polypeptide to various dietary fats in man. J Am Coll Nutr 1988;7:241–247
32. Drucker DJ. The biology of incretin hormones. Cell Metab 2006;3:153–165
33. Ding WG, Gromada J. Protein kinase A-dependent stimulation of exocytosis in mouse pancreatic beta-cells by glucose-dependent insulinotropic polypeptide. Diabetes 1997;46:615–621
34. Waget A, Cabou C, Masseboeuf M, et al. Physiological and pharmacological mechanisms through which the DPP-4 inhibitor sitagliptin regulates glycemia in mice. Endocrinology 2011;152:3018–3029
35. Chapman IM, Goble EA, Wittert GA, Horowitz M. Effects of small-intestinal fat and carbohydrate infusions on appetite and food intake in obese and nonobese men. Am J Clin Nutr 1999;69:6–12
36. Woltman T, Reidelberger R. Effects of duodenal and distal ileal infusions of glucose and oleic acid on meal patterns in rats. Am J Physiol 1995;269:R7–R14
37. Knauf C, Cani PD, Kim DH, et al. Role of central nervous system glucagon-like Peptide-1 receptors in enteric glucose sensing. Diabetes 2008;57:2603–2612
38. Troy S, Soty M, Ribeiro L, et al. Intestinal gluconeogenesis is a key factor for early metabolic changes after gastric bypass but not after gastric lap-band in mice. Cell Metab 2008;8:201–211
39. Spiller RC, Trotman IF, Higgins BE, et al. The ileal brake—inhibition of jejunal motility after ileal fat perfusion in man. Gut 1984;25:365–374
40. Lin HC, Doty JE, Reedy TJ, Meyer JH. Inhibition of gastric emptying by sodium oleate depends on length of intestine exposed to nutrient. Am J Physiol 1990;259:G1031–G1036
41. Lin HC, Zhao XT, Wang L. Fat absorption is not complete by midgut but is dependent on load of fat. Am J Physiol 1996;271:G62–G67
42. Meyer JH, Elashoff JD, Doty JE, Gu YG. Disproportionate ileal digestion on canine food consumption. A possible model for satiety in pancreatic insufficiency. Dig Dis Sci 1994;39:1014–1024
43. Rodriguez MD, Kalogeris TJ, Wang XL, Wolf R, Tso P. Rapid synthesis and secretion of intestinal apolipoprotein A-IV after gastric fat loading in rats. Am J Physiol 1997;272:R1170–R1177
44. Holgate AM, Read NW. Relationship between small bowel transit time and absorption of a solid meal. Influence of metoclopramide, magnesium sulfate, and lactulose. Dig Dis Sci 1983;28:812–819
45. Jian R, Pecking A, Najean Y, Bernier JJ. [Study of the progression of an ordinary meal in the human small bowel by a scintigraphic method (author's transl)]. Gastroenterol Clin Biol 1979;3:755–762 [in French]
46. Ogawa N, Ito M, Yamaguchi H, et al. Intestinal fatty acid infusion modulates food preference as well as calorie intake via the vagal nerve and midbrain-hypothalamic neural pathways in rats. Metabolism 2012;61:1312–1320
47. Dailey MJ, Tamashiro KL, Terrillion CE, Moran TH. Nutrient specific feeding and endocrine effects of jejunal infusions. Obesity (Silver Spring) 2010;18:904–910
48. Dailey MJ, Moghadam AA, Moran TH. Jejunal linoleic acid infusions require GLP-1 receptor signaling to inhibit food intake: implications for the effectiveness of Roux-en-Y gastric bypass. Am J Physiol Endocrinol Metab 2011;301:E1184–E1190
49. Drewe J, Gadient A, Rovati LC, Beglinger C. Role of circulating cholecystokinin in control of fat-induced inhibition of food intake in humans. Gastroenterology 1992;102:1654–1659
50. Ogawa N, Yamaguchi H, Shimbara T, et al. The vagal afferent pathway does not play a major role in the induction of satiety by intestinal fatty acid in rats. Neurosci Lett 2008;433:38–42
51. Randich A, Chandler PC, Mebane HC, et al. Jejunal administration of linoleic acid increases activity of neurons in the paraventricular nucleus of the hypothalamus. Am J Physiol Regul Integr Comp Physiol 2004;286:R166–R173
52. Lal S, Kirkup AJ, Brunsden AM, Thompson DG, Grundy D. Vagal afferent responses to fatty acids of different chain length in the rat. Am J Physiol Gastrointest Liver Physiol 2001;281:G907–G915
53. Rasmussen BA, Breen DM, Lam TK. Lipid sensing in the gut, brain and liver. Trends Endocrinol Metab 2012;23:49–55
54. Buffa R, Solcia E, Go VL. Immunohistochemical identification of the cholecystokinin cell in the intestinal mucosa. Gastroenterology 1976;70:528–532
55. Larsson LI, Rehfeld JF. Distribution of gastrin and CCK cells in the rat gastrointestinal tract. Evidence for the occurrence of three distinct cell types storing COOH-terminal gastrin immunoreactivity. Histochemistry 1978;58:23–31
56. Polak JM, Bloom SR, Rayford PL, Pearse AG, Buchan AM, Thompson JC. Identification of cholecystokinin-secreting cells. Lancet 1975;2:1016–1018
57. Sayegh AI, Ritter RC. CCK-A receptor activation induces fos expression in myenteric neurons of rat small intestine. Regul Pept 2000;88:75–81
58. Suzuki K, Jayasena CN, Bloom SR. Obesity and appetite control. Exp Diabetes Res 2012;2012:824305
59. Ferraris RP, Diamond J. Regulation of intestinal sugar transport. Physiol Rev 1997;77:257–302
60. Caspi L, Wang PY, Lam TK. A balance of lipid-sensing mechanisms in the brain and liver. Cell Metab 2007;6:99–104
61. Dixon JB, le Roux CW, Rubino F, Zimmet P. Bariatric surgery for type 2 diabetes. Lancet 2012;379:2300–2311
62. Schauer PR, Kashyap SR, Wolski K, et al. Bariatric surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med 2012;366:1567–1576
63. Mingrone G, Panunzi S, De Gaetano A, et al. Bariatric surgery versus conventional medical therapy for type 2 diabetes. N Engl J Med 2012;366:1577–1585

64. Dixon JB, O'Brien PE, Playfair J, et al. Adjustable gastric banding and conventional therapy for type 2 diabetes: a randomized controlled trial. JAMA 2008;299:316–323
65. Cohen RV, Pinheiro JC, Schiavon CA, Salles JE, Wajchenberg BL, Cummings DE. Effects of gastric bypass surgery in patients with type 2 diabetes and only mild obesity. Diabetes Care 2012;35:1420–1428
66. Rubino F, Marescaux J. Effect of duodenal-jejunal exclusion in a non-obese animal model of type 2 diabetes: a new perspective for an old disease. Ann Surg 2004;239:1–11
67. Lee HC, Kim MK, Kwon HS, Kim E, Song KH. Early changes in incretin secretion after laparoscopic duodenal-jejunal bypass surgery in type 2 diabetic patients. Obes Surg 2010;20:1530–1535
68. Cohen RV, Schiavon CA, Pinheiro JS, Correa JL, Rubino F. Duodenal-jejunal bypass for the treatment of type 2 diabetes in patients with body mass index of 22-34 kg/m2: a report of 2 cases. Surg Obes Relat Dis 2007;3:195–197
69. Gavin TP, Sloan RC 3rd, Lukosius EZ, et al. Duodenal-jejunal bypass surgery does not increase skeletal muscle insulin signal transduction or glucose disposal in Goto-Kakizaki type 2 diabetic rats. Obes Surg 2011;21:231–237
70. Kindel TL, Martins PJ, Yoder SM, et al. Bypassing the duodenum does not improve insulin resistance associated with diet-induced obesity in rodents. Obesity (Silver Spring) 2011;19:380–387
71. Wilson-Pérez HE, Chambers AP, Ryan KK, et al. Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like peptide-1 receptor deficiency. Diabetes 2013;62:2380–2385
72. Czupryniak L, Wiszniewski M, Szymański D, Pawłowski M, Loba J, Strzelczyk J. Long-term results of gastric bypass surgery in morbidly obese type 1 diabetes patients. Obes Surg 2010;20:506–508
73. Ley RE, Hamady M, Lozupone C, et al. Evolution of mammals and their gut microbes. Science 2008;320:1647–1651
74. Lepage P, Colombet J, Marteau P, Sime-Ngando T, Doré J, Leclerc M. Dysbiosis in inflammatory bowel disease: a role for bacteriophages? Gut 2008;57:424–425
75. Turnbaugh,PJ, Ridaura,VK, Faith,JJ, Rey,FE, Knight,R, Gordon,JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 2009;1:6ra14, 2009
76. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology 2009;137:1716–1724, e1–e2
77. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–1031
78. Serino M, Luche E, Gres S, et al. Metabolic adaptation to a high-fat diet is associated with a change in the gut microbiota. Gut 2012;61:543–553
79. Vrieze A, Van Nood E, Holleman F, et al. Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. Gastroenterology 2012;143:913–916, e7
80. Ding S, Chi MM, Scull BP, et al. High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. PLoS One 2010;5:e12191
81. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes 2008;57:1470–1481
82. Swann JR, Want EJ, Geier FM, et al. Systemic gut microbial modulation of bile acid metabolism in host tissue compartments. Proc Natl Acad Sci USA 2011;108(Suppl. 1):4523–4530
83. Claus SP, Tsang TM, Wang Y, et al. Systemic multicompartmental effects of the gut microbiome on mouse metabolic phenotypes. Mol Syst Biol 2008;4:219
84. Swartz TD, Duca FA, de Wouters T, Sakar Y, Covasa M. Up-regulation of intestinal type 1 taste receptor 3 and sodium glucose luminal transporter-1 expression and increased sucrose intake in mice lacking gut microbiota. Br J Nutr 2012;107:621–630
85. Duca FA, Swartz TD, Sakar Y, Covasa M. Increased oral detection, but decreased intestinal signaling for fats in mice lacking gut microbiota. PLoS One 2012;7:e39748
86. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci USA 2011;108:16050–16055
87. Burcelin R. Regulation of metabolism: a cross talk between gut microbiota and its human host. Physiology (Bethesda) 2012;27:300–307
88. Zhang H, DiBaise JK, Zuccolo A, et al. Human gut microbiota in obesity and after gastric bypass. Proc Natl Acad Sci USA 2009;106:2365–2370
89. Uribe A, Alam M, Johansson O, Midtvedt T, Theodorsson E. Microflora modulates endocrine cells in the gastrointestinal mucosa of the rat. Gastroenterology 1994;107:1259–1269