Proglucagon-Derived Peptides, GIP and Dipeptidyl Peptidase-4-Mechanisms of Action in Adipose Tissue

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Abstract
Proglucagon-derived peptides (PGDPS) and related gut hormones exemplified by glucose-dependent insulintropic polypeptide (GIP) regulate energy disposal and storage, through actions on metabolically sensitive organs, including adipose tissue. The actions of glucagon, GLP-1, GLP-2, GIP, and their rate limiting enzyme dipeptidyl peptidase-4, include direct and indirect regulation of islet hormone secretion, food intake, body weight, all contributing to control of white and brown adipose tissue activity. Moreover agents mimicking actions of these peptides are in use for the therapy of metabolic disorders with disordered energy homeostasis, such as diabetes, obesity and intestinal failure. Here we highlight current concepts and mechanisms for direct and indirect actions of these peptides on adipose tissue depots. The available data highlights the importance of indirect peptide actions for control of adipose tissue biology, consistent with the very low level of endogenous peptide receptor expression within white and brown adipose tissue depots. Finally, we discuss limitations and challenges for the interpretation of available experimental observations, coupled to identification of enduring concepts supported by more robust evidence.

Key words: Diabetes, Obesity, Inflammation, Fat, G protein coupled receptors, Peptides
Introduction

Proglucagon-derived peptides (PGDPS) and structurally-related gut hormones such as glucose-dependent insulino-tropic polypeptide (GIP) exert multiple actions on energy intake and disposal, impacting the activity of adipose tissue through direct and indirect mechanisms (1, 2). These hormones act through structurally related G protein coupled receptors (GPCR), several of which are expressed in adipose tissue. Moreover, they are rapidly inactivated by dipeptidyl peptidase-4 (DPP4), itself expressed and active within multiple adipose tissue cell types (3). Beyond their basic biological importance, this family of peptides has assumed considerable translational relevance. Notably, glucagon is approved for the treatment of hypoglycemia, and GLP-1R agonists are used for the therapy of type 2 diabetes (T2D) and obesity (4). A GLP-2R agonist teduglutide is approved for the chronic therapy of short bowel syndrome (5), whereas DPP4 inhibitors are widely utilized for treatment of T2D. Moreover, multi-agonists containing two or three peptide epitopes, including glucagon GLP-1 and GIP, are under investigation for the therapy of metabolic disorders (6, 7). Although gain and loss of function studies reveal adipose tissue as an important target for these peptides, the precise mechanisms through which glucagon, GLP-1, GIP and GLP-2 modulate adipose tissue activity, is poorly understood.

GLP-1, GLP-2 and GIP are rapidly secreted from the gut following nutrient ingestion, yet exert opposing actions on lipid assimilation. GLP-1 inhibits gastric emptying, and indirectly reduces postprandial secretion of triglyceride-rich chylomicron particles through poorly understood mechanisms (8-10). In contrast, GLP-2 promotes lipid absorption, and increases plasma triglycerides within minutes of meal ingestion. (11, 12). GIP infusion diminished the postprandial increment in triglyceride levels in rats (13) however acute infusion of GIP did not modify plasma triglyceride levels but increased accumulation of triglyceride levels in saturated fat of obese human subjects with T2D (14). In contrast, acute GIPR blockade using the antagonist GIP(3-30) reduced the uptake of triacylglycerides into adipose tissue of healthy lean human subjects (15). Interpretation of these collective findings is challenging as GIP, GLP-1, and GLP-2 simultaneously regulate islet hormone secretion and even glucagon has been shown to modulate insulin secretion and peripheral insulin action (16, 17). Here we update current concepts of how this family of peptides directly impacts the biology of white and brown adipose tissue (Figures 1,2), highlighting areas of uncertainty and limitations of the existing data.
Glucagon

The glucagon (GCG) peptide is encoded by the proglucagon gene and is comprised of 29 amino acids with a molecular weight of 3,485 (2, 4). Proglucagon is processed to yield glucagon in the pancreas, and PGDPs, including glicentin, oxyntomodulin, GLP-1 and GLP-2 in the intestine (18). GCG mediates its actions through a single Class B G protein coupled receptor (GCGR), structurally and functionally related to the GLP1R and GLP2R (19). Physiologically, the main action of GCG is to counteract hypoglycemia through increased hepatic glucose production. Here we summarize direct and indirect actions of GCG in white and brown adipose tissue.

The Glucagon Receptor

The GCGR is expressed at high levels in liver (predominantly hepatocytes), and to a lesser extent in peripheral tissues such as the kidney, heart, adrenal glands, spleen, pancreatic islets, ovary, thymus, stomach, duodenum, and brain (20-22). Gcgr mRNA transcripts have been identified in whole adipose tissue and in isolated adipocytes from mesenteric, inguinal, epididymal, retroperitoneal and BAT depots (21-24). However, relative levels of murine Gcgr mRNA transcripts are at least 100-fold lower in white adipose tissue (WAT) depots, relative to levels in liver. High affinity binding sites for glucagon, albeit at a lower binding capacity than those detected for GLP-1, were identified in solubilized membranes from human abdominal wall subcutaneous adipose tissue (25).

Glucagon action in WAT

Analysis of direct glucagon action on adipose tissue has yielded conflicting results. In static or perfused cultures of isolated rat epididymal adipocytes, glucagon increased rates of lipolysis and enhanced oxygen consumption at concentrations from $10^{-6}$ M to $10^{-11}$ M (26, 27). Glucagon exerted its effects on lipolysis independent from sympathetic nervous system (SNS) activation, as glucagon-induced glycerol release was not perturbed by denervation of the lumbar fat depot in rats (28). Glucagon also exhibited direct lipolytic actions in studies with human adipocytes prepared from subcutaneous adipose tissue, at concentrations ranging from $10^{-6}$ M to $10^{-8}$ M (29). Similarly, glucagon stimulated adenylate cyclase activity and cAMP accumulation in plasma membranes from human subcutaneous adipose tissue isolated from the mid-sternal thoracic wall (30).

Injection of glucagon (0.075 mg) into the brachial artery of healthy human subjects increased plasma levels of free fatty acids (FFAs) (31). Nevertheless, these findings could not be independently replicated, as infusion of glucagon, at doses (525 pg/kg/min) that substantially elevated circulating glucagon levels (mean values of 1.216 pg./ml), had no effect on plasma
levels of glycerol or amino acids in human subjects studied after overnight fasting (32).
Furthermore, infusion of glucagon into humans at a rate of 1.5ng/kg/min failed to alter local adipose tissue concentrations of interstitial glycerol, despite several fold elevations of plasma glucagon (110-130 ng/ml) (33). Similarly, glucagon infusion (1.2-3ng/kg/min) elevated circulating glucagon levels in normal human subjects or individuals with type 1 diabetes, yet had no effect on levels of palmitate or free fatty acids over a 2h period (34). Collectively, these studies question the importance of glucagon as a direct adipose tissue lipolytic hormone in humans.

Loss of glucagon action - impact on WAT

Interpretation of the importance of endogenous glucagon action within adipose tissue depots is challenged by the lack of suitable mouse models, and the non-tissue-selectivity of glucagon antagonists. Gcg/− mice exhibit lower rectal body temperatures, increased oxygen consumption lower Ucp1 mRNA expression in BAT, and reduced BAT mass following cold exposure (35). Supplementation with glucagon partially restored a subset of thermogenesis-associated defects in Gcg/− mice and increased Ucp1 expression in BAT. Nevertheless, the simultaneous germline loss of multiple PGDPs challenges the interpretation of phenotypes arising in Gcg/− mice.

Gcgr/− mice exhibit profound metabolic phenotypes characterized by resistance to diet-induced obesity, improved glucose tolerance, and elevated circulating levels of bile acids, GLP-1, GLP-2, and amino acids, associated with hyperglucagonemia and α-cell hyperplasia (36). Gcgr/− mice exhibit a reduction in adipose tissue mass, and attenuated induction of a thermogenic gene expression program in WAT after cold exposure, yet maintain their body temperature when exposed to 4C (37). Interpretation of the tissues and mechanisms contributing to these defects is challenging due to the pleiotropic and widespread adaptations arising secondary to loss of GCGR signaling in multiple tissues.

Glucagon action in BAT

BAT is uniquely different from WAT in that it does not store lipids but metabolizes them to generate heat. Multiple studies demonstrated that acute glucagon administration increases energy expenditure, measured as oxygen consumption, in rodents (24, 38) and humans (39-41). Daily administration of glucagon to rats increased BAT weight, protein content, DNA content and mitochondrial mass, findings partially attenuated by surgical denervation of BAT tissue (42). Interpretation of these studies is challenging due to the potential for direct and indirect actions of glucagon on thermogenesis. Notably, mice lacking FGF21 exhibit an
attenuated thermogenic response to acute glucagon administration (24, 43). Moreover, glucagon also acutely increases energy expenditure in mice in part through hepatic FXR activity (44). Further complicating interpretation of mechanisms, acute glucagon-stimulated increases in energy expenditure in humans were detected in the absence of increased interscapular BAT activity assessed by PET scanning using 2-deoxyglucose (41).

**Direct glucagon action in BAT**

Historical studies dating back to the 1960’s demonstrated that glucagon (10⁻⁷ to 10⁻⁹ M) stimulated oxygen consumption and release of free fatty acids in BAT slices from male Holtzman rats (45). Similarly, Kuroshima and colleagues demonstrated that glucagon (1 µg/ml) directly increased heat production in isolated cultures of rat brown adipocytes obtained from rats housed at 5C (46). Species-specific differences in the direct thermogenic response to glucagon have been observed, as glucagon (1-10 µm) stimulated oxygen consumption in isolated BAT cells from rats and mice but not hamsters (47, 48). Moreover, glucagon stimulated lipolysis in a dose-and GCGR-dependent manner and enhanced the expression of genes important for thermogenesis in an immortalized BAT cell line (24).

**Loss of glucagon action in BAT**

Genetic disruption of the Gcgr in mice results in lower amounts of adipose tissue mass, and lower BAT weights in mice studied on a low-fat or a high-fat diet controls (36, 49). The endogenous physiological importance of the GCGR in BAT was studied in mice (Gcgr⁵⁺⁻) with inactivation of the Gcgr within the Myf5 expression domain. Gcgr⁵⁺⁻ mice exhibited no basal perturbation in control of body weight, food intake, energy expenditure, fat or lean mass, or BAT mass (24). Furthermore, the thermogenic response to glucagon administration, BAT oxygen consumption ex vivo, levels of circulating TGs or NEFAs, and glucose, insulin or lipid tolerance were not dysregulated in Gcgr⁵⁺⁻ mice (24). Hence, the endogenous importance of the GCGR in BAT, expressed at very low levels, is uncertain (Figure 2).

**GLP-1 receptor expression in adipose tissue**

The GLP-1 receptor (GLP-1R) belongs to the class B family of the G protein-coupled receptors (19). The GLP-1R is expressed in islets and peripheral tissues such as lung, brain, kidney, stomach and heart express GLP1R mRNA transcripts (50, 51); however, the majority of studies do not report GLP-1R expression within adipose tissue. A few studies describe GLP-1R expression within differentiated mouse 3T3-L1 pre-adipocytes (52) and in epicardial, and visceral WAT (53, 54). Nevertheless, the relative magnitude of GLP-1R
expression in adipose tissue, compared to levels in islets or brain, has not been carefully quantified. Although immunoreactive GLP-1R protein has been reported in subcutaneous and visceral adipose tissue, as well as in preadipocytes and differentiated adipocytes ex vivo, detection of the GLP-1R with commercially available antisera has been problematic (55, 56), and the sensitivity and specificity of the antibodies used to detect immunoreactive GLP-1R protein within adipose tissue is not described (54, 57).

Several reports describe direct actions of GLP-1 in 3T3-L1 cells or in adipocyte-like cells differentiated from stromal vascular progenitor cells ex vivo. The GLP-1R agonist exendin-4 promoted the proliferation and survival of human omental adipose-derived stromal cells, actions sensitive to inhibition by exendin(9-39) (58). Similarly, the GLP-1R agonist (liraglutide 10-100nM) directly promoted proliferation and preadipocyte differentiation, from primary cultures of adipocyte progenitors, or 3T3-L1 cells, ex vivo (57). Related studies have shown that GLP-1 regulates expression of genes important for adipogenesis, lipogenesis, or lipolysis in human adipocytes differentiated ex vivo. Notably, exendin(9-39), an antagonist of the canonical GLP-1R, did not attenuate the actions of GLP-1 on human adipocytes (59). Mechanistically, knockdown of SIRT1 reduced the direct lipolytic and oxidative actions of exendin-4 in 3T3-L1-derived adipocytes (60).

Treatment of animals and humans with GLP-1 receptors agonist frequently reduces body weight and adipose tissue mass, and may be associated with acute changes in plasma levels of free fatty acids, insulin, glucagon, and adipokines (Figure 1). Conversely, loss of GLP-1 action, achieved using antagonists or genetics, is generally associated with hyperphagia, weight gain, and increased adipose tissue mass (8). Nevertheless, it seems likely that many of these alterations are indirect, postulated to reflect actions of GLP-1 on adipose tissue blood flow, insulin secretion, and the CNS control of food intake, and body weight. Indeed, infusion of GLP-1 into the abdominal subcutaneous adipose tissue via microdialysis failed to demonstrate any effect of native GLP-1 on adipose tissue blood flow or lipolysis as assessed by changes in glycerol levels (61). The available literature does not support the expression of a functional canonical GLP-1 receptor in white adipose tissue (Figure 1).

**GLP-1 action in brown adipose tissue**

The GLP-1R is not expressed in BAT (62). GLP-1R agonists increase energy expenditure in preclinical studies, through indirect central nervous system pathways linking central GLP-1R
activation (Figure 2) to increased sympathetic nervous system activity and enhanced BAT activity (63-65). Indeed, transient neonatal administration of exendin-4 for 6 days protected older female mice from diet-induced obesity through enhanced browning of perigonadal WAT, findings dependent on the presence of hypothalamic GLP-1Rs within the Sim1-Cre expression domain (66). Follow-up studies examined mechanisms of GLP-1 action in the brain that stimulate BAT thermogenesis and adipocyte browning independent of food intake. Central liraglutide administration increased i) browning of WAT and ii) BAT temperature, associated with induction of a thermogenic gene expression profile within interscapular BAT (63). Intracerebroventricular liraglutide administration reduced levels of pAMPK in the hypothalamus, whereas adenoviral AMPK activation in the hypothalamic ventromedial nucleus attenuated the liraglutide-mediated BAT activation (63).

The importance of endogenous GLP-1R signaling for BAT activity in mice is not clear. Whole body germline inactivation of the Glp1r−/− mice exhibited a normal induction of BAT activity in response to cold exposure (64), resistance to diet-induced obesity, reduced fat mass, and increased energy expenditure, confounded by increased physical activity in some (67), but not all (68) studies. Lentiviral-mediated knockdown of the nodose ganglion Glp1r in high fat diet-fed rats resulted in increased energy expenditure and BAT temperature, consistent with the importance of indirect GLP-1R-dependent autonomic neural inputs for control of basal BAT activity (62). Nevertheless, Glp1rΔPhox2b−/− mice with marked reduction of Glp1r expression within the nodose ganglion do not exhibit disturbances of body weight on a regular chow diet (69). In humans, careful controlled metabolic studies have shown that weight loss associated with acute or sustained GLP-1R agonism is not associated with increases in energy expenditure (70-73).

**Glucagon-like peptide-2**

Although GLP-2 secretion is stimulated by fat, and in turns augments intestinal lipoprotein secretion (5), a direct role for GLP-2 in adipose tissue has not been demonstrated. GLP2R expression has been reported in human epicardial fat (74) and at low levels in mouse adipose tissue (75). Whether GLP-2R expression within adipose tissue reflects contributions from nerves, blood vessels, or adipocytes has not been determined. Although the data is limited, GLP-2 had no direct effect on fatty acid synthesis in rat omental adipose tissue explants (76).
Glucose-dependent insulinotropic polypeptide

The GIP receptor is expressed within white and brown adipose tissue (Figures 1,2); however, the precise cell types within WAT that express the GIPR remain poorly understood. Expression of a reporter gene under the control of endogenous mouse Gipr transcriptional sequences was detected within some but not all adipocytes within interscapular BAT and inguinal WAT (77). Gipr mRNA has been detected in rat adipocytes (78), yet the majority of studies examining the functional biology of the adipose GIPR utilize adipocytes derived from progenitor cells ex vivo. GIPR mRNA transcripts were detected by qPCR in human subcutaneous and visceral adipose tissue from lean and obese subjects; however, the precise level of expression, relative to a known positive control, was not reported (79). Although GIPR protein expression was detected in adipose tissue, in both adipocyte and stromal vascular fractions, by Western blotting and immunohistochemistry, the sensitivity and specificity of the antibody used in these analyses was not validated. Critically, the specific antisera used in these studies, sc-98795, is no longer commercially available (79).

Differentiation of human preadipocytes to adipocytes was associated with marked upregulation of GIPR expression, and GIP acutely upregulated CALC1 and CGRP1 mRNA transcripts in human adipocytes via H89-sensitive mechanisms ex vivo (80). GIPR expression assessed by qPCR in biopsies from postmenopausal women was relatively higher in visceral compared to subcutaneous fat depots, was reduced in women with central obesity, but did not change after weight loss (81). Nevertheless, the relative expression of GIPR in fat tissue was not quantitated nor compared to classical sites of GIPR expression, for example, within the pancreas. Ablation of the GIP gene in mice led to upregulation of Gipr mRNA transcripts in visceral adipose tissue, findings of uncertain physiological significance (82).

Interpreting the actions of GIP on adipose tissue is complex, as GIP infusions may increase insulin levels, reduce glucose, and modify adipose tissue blood flow. Moreover, the extent to which differentiated adipose cell lines, such as 3T3-L1 cells recapitulate the physiology of GIP action in normal adipose tissue, remains unclear. Further complicating interpretation of the data, several analyses of GIPR expression in adipose cell lines such as 3T3-L1 cells have employed GIPR antisera subsequently invalidated for the detection of immunoreactive GIPR protein (83, 84). Moreover, the majority of studies demonstrating actions of GIP on adipose tissue cell lines or explants are carried out in the presence of insulin, as GIP alone often exhibits a negligible effect on lipogenesis. Nevertheless, in some studies, GIP alone dose-
dependently increased lipogenesis in the absence of insulin and stimulated osteopontin secretion in primary cultures of rat adipocytes in the presence of exogenous insulin (85).

The use of mouse genetics to manipulate Gipr expression in adipose tissue has not yet yielded useful results. Re-expression of the GIPR cDNA under the control of the Fabp4 (ap2) promoter in Gipr<sup>−/−</sup> mice resulted in greater weight gain, reflected predominantly by an increase in lean, but not fat mass (86). Hence the functional importance of the GIPR within murine fat depots was not determined. Conversely, mice expressing Cre under the control of the Fabp4 promoter were used to generate an “adipocyte-specific’ knockout of the Gipr. Gipr<sup>adipo<sup>−/−</sup> mice exhibited resistance to diet-induced obesity and improved insulin sensitivity, yet without any changes in adipose tissue mass or adipocyte size; however, Il6 mRNA and IL-6 protein were selectively reduced in visceral fat from high fat diet-fed Gipr<sup>adipo<sup>−/−</sup> mice (87). Interpretation of these studies using Fabp4-Cre for selective manipulation of adipocyte Gipr expression is complicated by demonstration that the Fabp4 promoter can direct expression to multiple non-adipose cell types, including endothelial cells and neurons within the central and peripheral nervous system (88-90).

**GIP and brown adipose tissue**

Acute infusion of GIP (4 hrs, 2-4 pmol/kg/min) in lean healthy humans or obese subjects with T2D had no consistent effect on resting energy expenditure (91, 92). Gipr<sup>−/−</sup> mice exhibit resistance to diet-induced obesity following prolonged high fat feeding (93), associated with increased expression of Ucp1 in BAT (67). Moreover, genetic reduction of GIP secretion achieved through K cell ablation or genetic activation of the Gip gene in K cells was also associated with increased energy expenditure and attenuation of weight gain in high fat diet-fed mice (82, 94). Notably, Gipr<sup>−/−</sup> mice resisted a drop in body temperature after cold exposure, and exhibited greater thermogenic responses to i) cold, or ii) the adrenergic agonist CL316,243 (95). A full length Gipr mRNA transcript was detected in BAT, albeit at levels ~10-fold lower than in murine islets. GIP directly activated Il6 mRNA and had minimal impact or reduced the expression of a subset of genes important for thermogenesis in a BAT cell line, whereas knockdown of the Gipr increased inflammatory gene expression and enhanced levels of mRNA transcripts important for thermogenesis (95). Selective elimination of the BAT Gipr using Myf5-Cre had little impact on body weight or energy expenditure in regular chow or high fat diet-fed mice studied at room temperature or 30C. Nevertheless, Gipr<sup>BAT<sup>−/−</sup></sup> mice exhibited resistance to diet-induced obesity and increased basal BAT oxygen consumption when studied at 4C. Hence, these findings indicate that while the BAT GIPR is
functional (Figure 2), it seems unlikely to make a major contribution to whole animal energy homeostasis under physiological conditions.

**GIP and adipose tissue inflammation**

Several studies demonstrated that GIP may activate a subset of proinflammatory genes within adipose tissue. Acute GIP infusion for 4 hrs increased the mRNA transcripts for MCP1, MCP2, IL6, and CD68 in subcutaneous adipose tissue from health male obese subject (96). Notably, GIPR mRNA transcripts were detected in human peripheral blood mononuclear cells, monocytes and monocyte-derived macrophages and GIP stimulated cAMP accumulation and mitogen activated kinase phosphorylation, but not MCP1 expression in human monocytes and macrophage cell lines (96). Although GIPR protein expression was reported in extracts from monocyte and macrophage cell lines, the precise identity, sensitivity and specificity of the GIPR antisera used in these studies was not reported. An indirect role for the hematopoietic GIPR in the control of adipose tissue inflammation was suggested by analysis of wildtype irradiated mice reconstituted with Gipr⁻/⁻ bone marrow. These mice exhibited increased fat mass, reduced adipose expression of Ucp1 and Ppargc1a, increased myelopoiesis, enhanced expression of proinflammatory S100A8, and infiltration of mononuclear cells into adipose tissue depots (97). These findings were largely phenocopied in mice with Lysozyme-Cre-mediated deletion of the Gipr from macrophages. Hence GIPR expression within adipocytes and immune cells may normally restrain inflammation, and depletion of GIPR within these cells may contribute to adipose tissue phenotypes in GIPR loss of function studies.

**Dipeptidyl Peptidase-4 in Adipose Tissue**

DPP4 is widely expressed within adipose tissue and has been localized to both the stromal vascular and adipocyte fractions (98), consistent with its known distribution of expression within endothelial cells, immune cells and adipocytes (3). Treatment of overweight men with the DPP4 inhibitor sitagliptin for 12 weeks increased uptake of [¹⁸F] fluorodeoxyglucose by 53% in subcutaneous WAT but not BAT (99). DPP4 inhibitors produce complex metabolic actions, and alter the metabolism of dozens of regulatory peptides and chemokines, complicating attribution of a subset of their activities directly to DPP4 within adipose tissue (3). Experimental and clinical obesity is commonly accompanied by an increase in circulating DPP4 activity together with increased adipose tissue expression of DPP4 (100, 101). Although DPP4 is secreted from multiple adipose tissue depots (100, 102), its relative
contribution to the circulating pool of soluble DPP4 (sDPP4) and DPP4 activity, as revealed through mouse genetics, is modest (98). Moreover, genetic inactivation of the Dpp4 gene within murine adipocytes has little impact on glucose homeostasis or insulin sensitivity in regular chow fed or high fat diet-fed mice (98). Intriguingly, reduction of DPP4 expression within mouse hepatocytes attenuates adipose tissue inflammation, whereas attenuation of adipocyte Dpp4 expression in high fat diet-fed mice reduces liver inflammation, through incompletely understood mechanisms (98, 103). Recent studies suggest that DPP4 expression within the reticular interstitium of WAT marks a proliferative progenitor mesenchymal progenitor population that gives rise to preadipocytes within adipose tissue (104). Hence, adipose tissue DPP4 exerts complex local actions, and also serves as an adipokine to modulate tissue inflammation and systemic metabolism.

Summary, Limitations and Future Directions
Understanding the direct and indirect actions of glucagon, GLP-1, GLP-2 and GIP on adipose tissue is challenging due to the wide-ranging metabolic actions of these peptides, and the lack of robust functional adipose tissue expression of their cognate receptors. Pharmacological use of GCG, GIP and GLP-1R agonists and mult agonists modifies food intake, body weight and adipose tissue mass and function, the latter predominantly through indirect mechanisms. Glucagon and GLP-1 communicate with white and brown adipose tissue in mice and rats indirectly through the central and autonomic nervous systems (Figures 1,2). The importance of these neural pathways for control of adipose tissue metabolism in humans has not been established. The available evidence supports expression and activity of the GCGR and GIPR within WAT and BAT; however, the endogenous physiological significance of low level GCGR and GIPR expression within adipose tissues remains unclear. Furthermore, the precise cellular localization of GCGR and GIPR within WAT and BAT has not been determined, and localization efforts in part are hampered by the lack of sensitive and specific validated antisera for detection of receptor expression.

Compelling loss of function data has not yet been reported to support important roles of the murine Gcgr or Gipr in adipose tissue function. Moreover, genetic deletion of these receptors in BAT reveals the lack of physiological importance of these receptors for control of BAT metabolism and energy homeostasis. DPP4 is expressed within multiple adipose tissue cell types, and may exert roles in control of cell differentiation, and as an adipokine regulating inflammation. Collectively, although the PGDPS, GIP and DPP4 display fundamental actions
critical for acute and long term energy storage, the available data does not support fundamental direct actions of these peptides and their endogenous receptors in the control of adipocyte biology.
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Figure Legends

**Figure 1**
Direct and indirect adipose tissue actions of glucagon, GLP-1, GLP-2 and GIP. Glucagon and GLP-1 communicate with white adipose tissue through modulation of islet hormones and sympathetic nervous systems pathways via the central nervous system. Receptors for glucagon (GCGR) and GIP (GIPR) are also detected within WAT depots in adipocytes and macrophages (GIPR); however, the importance of these receptors for the direct actions of these peptides has not been established.

**Figure 2**
Glucagon and GLP-1 activate BAT through indirect pathways. BAT expresses glucagon and GIP receptors (GCGR and GIPR, respectively) at low levels, however the physiological importance of these receptors for direct control of BAT activity remains uncertain.