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THE INCRETIN SYSTEM ABC'S IN HEALTH AND DISEASE - NOVEL APPROACHES TO OBESITY AND DIABETES TREATMENT

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Abbreviatons

BP	Blood pressure
T2DM	Type 2 diabetes mellitus
IR	Insulin resistance
GLP-1	Glucagon-like peptide 1
GIP	Glucose-dependent insulinotropic polypeptide
GI	Gastrointestinal
DPP-4	Dipeptidyl peptidase-4
GLP-1RA	GLP-1 receptor agonists
DPP-4i	DPP-4 inhibitors
BMI	Body mass index
GLP-1R	GLP-1 receptor
GIPR	GIP receptor
Gcg	Glucagon
ADA	Adenosine deaminase
sDPP-4	Soluble DPP-4
SDF-1α	Stromal-derived factor-1 alpha
NPY	Neuropeptide Y
PYY	Peptide YY
FFAs	Free fatty acids
NTS	Solitary tract nucleus
PVN	Paraventricular nucleus
ARC	Arcuate nucleus
POMC/CART	Proopiomelanocortin/cocaine and amphetamine-

	regulated transcript
NPY/AgRP	Neuropeptide Y/Agouti-related peptide
MSG	Monosodium glutamate
VTA	Ventral tegmental area
NAc	Nucleus accumbens
EX-4	Exendin-4
CNS	Central nervous system
BAT	Brown adipose tissue
BBB	Blood brain barrier
TGs	Triglycerides
VANs	Vagal afferent neurons
LepRs	Leptin receptors
Y2R	Y2 receptors
OXM	Oxyntomodulin
Gcg-R	Glucagon receptor
VAT	Visceral adipose tissue
IL-6	Interleukin 6
TNF-α	Tumor necrosis factor alpha
PAI-1	Plasminogen activator inhibitor-1
NAFLD	Non-alcoholic fatty liver disease
CRP	C reactive protein
HIF-1α	Hypoxia-inducible factor 1-alpha
MCP-1	Monocyte chemoattractant protein 1
T_h	T helper cell
T_{reg}	Regulatory T cell

HOMA-IR	Homeostasis model assessment of IR
LDL	Low-density lipoprotein
HDL	High-density lipoprotein
CTA	Conditioned taste aversion
DIO	Diet-induced obese
UCP-1	Uncoupling protein 1
RAAS	Renin-angiotensin-aldosterone system
AT_1R	Angiotensin II receptor type 1
ARAs	Angiotensin II receptor antagonists
ACE	Angiotensin-converting enzyme
ACEi	Angiotensin-converting enzyme inhibitors
EPCs	Endothelial progenitor cells
RYGB	Roux-en-Y gastric bypass
AGB	Adjustable gastric band
BAs	Bile acids

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Abstract

Incretins are gastrointestinal-derived hormones released in response to a meal that play a key role in the regulation of postprandial secretion of insulin (*incretin effect*) and glucagon by the pancreas. Both incretins, glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) have several other actions. GLP-1 regulates body weight by inhibiting appetite and delaying gastric emptying, actions that are dependent on central nervous system GLP-1 receptor activation. Several other hormones and gut peptides, including leptin and ghrelin, interact with GLP-1 to modulate appetite. GLP-1 is labile, being rapidly degraded by dipeptidyl peptidase-4 (DPP-4), a multifunction molecule whose role in obesity dynamics extends beyond incretin metabolism. DPP-4 is involved in adipose tissue inflammation, which is a pivotal event in insulin resistance and a key pathophysiological mechanism in the genesis of obesity-related complications. Furthermore, the incretin system appears to provide the basis for understanding the high weight loss efficacy of bariatric surgery, a current employed obesity treatment that also benefits diabetes. The present review brings together new insights into obesity pathogenesis, integrating GLP-1 and DPP-4 in the complex interplay between obesity epidemics and inflammation, namely in diabetic patients. This will in turn provide the basis for new perspectives regarding GLP-1 receptor agonists and DPP-4 inhibitors therapeutic potential.

Keywords: incretin-based therapies, glucagon-like peptide-1 (GLP-1), dipeptidyl peptidase-4 (DPP-4), obesity, type 2 diabetes mellitus (T2DM), bariatric surgery, inflammation.

1. Introduction

Obesity and its accompanying spectrum of complications is a major health care problem reaching epidemic proportions.(1) Together with elevated blood pressure (BP), elevated fasting plasma glucose and dyslipidemia, it is part of the so called metabolic syndrome, which represents a constellation of interactive risk factors for type 2 diabetes mellitus (T2DM) and cardiovascular disease.(2)

T2DM, estimated to affect around 415 million of people worldwide,(3) is characterized by glucose and lipid metabolism abnormalities leading to an impaired insulin secretion from pancreatic β-cells and a state of peripheral insulin resistance (IR).(4) In addition, incretin action is found to be impaired.(5) Incretins, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), are peptide hormones produced in the gastrointestinal (GI) tract following a postprandial nutrient supply. Incretin hormones have recently been the focus of extensive research because of their role in the physiological regulation of glucose homeostasis, mostly due to the enhancement of glucose-dependent insulin secretion.(6) In T2DM, the incretin effect is blunted,(5) in addition to the reduced bioavailability of these hormones resulting from their accelerated inactivation by dipeptidyl peptidase-4 (DPP-4).(6) Likewise, the incretin-based therapies, which comprise GLP-1 receptor agonists (GLP-1RA) and DPP-4 inhibitors (DPP-4i), have been gaining prominence in recent years as a tool for T2DM pharmacological management.(7) Moreover, a growing body of evidence suggests that the effects of incretins extend well beyond glycemic control, with incretin-based therapies exerting cytoprotective actions on both macro and micro-vascular complications of T2DM. The aforementioned pleiotropic effects mainly lie on the anti-inflammatory, antioxidant and anti-apoptotic properties of these agents.(7–9)

Incretin-based therapies have also been implicated as a promising new approach for obesity. In fact, incretin impairment seems to be a common key player of both T2DM and obesity.(10,11) In addition, incretins are thought to exert a physiological effect on food intake and satiety,(11) and some of these medications therefore have the additional benefit of promoting weight loss.(12) That is a key factor of the management of T2DM, once the vast majority of patients are overweight (Body mass index (BMI)= 25-29.9 kg/m^2) or obese ($BMI \geq 30 kg/m^2$).(13) Bariatric surgery, which remains the only effective way to lose weight in patients with both T2DM and obesity, seems to act through the incretin axis,(14) promoting a better glycemic control and a prolonged remission from diabetes.(15,16) Although the physiologic mechanisms underlying the outcomes achieved with bariatric surgery are complex and remain elusive, the incretin axis is believed to be a main player of the physiologic regulation of appetite and satiety, energy homeostasis and human metabolism.(17)

IR is another common denominator between T2DM and obesity.(18,19) Furthermore, obesity and IR are characterized by a low grade inflammatory response, illustrating a dysfunctional immunity.(20–22) Indeed, an abnormally high number of inflammatory cells is present in the adipose tissue of obese subjects and T2DM patients.(21) In this regard, anti-inflammatory actions that are very well recognized from incretin-based therapies (7–9) would be of potential benefit.

Research into incretin hormones is an exciting new field in Medicine and Biology. The present work will review the link between incretins and metabolic homeostasis, summarizing the actual placement of incretin-based therapies in T2DM management. Particular attention will be given to their potential positive effects on obesity, based on weight loss and inflammation, shedding light into the mechanisms underlying bariatric surgery efficacy, as well as new therapeutic opportunities.

2. Materials and Methods

Considering the non-systematic character of this review and the broad width of the topic, encompassing sometimes apparently non-related subjects, several successive literature researches were performed. The study was mainly supported by search of scientific articles in the PubMed database, and the last search took place on December 2015. The literature research used the following combination of terms:

- ("GLP-1" [Title]) AND ("type 2 diabetes" [Title]) – 155 articles
- ("GLP-1"[Title]) AND ("obesity"[Title])]) – 22 articles
- ("dipeptidyl peptidase-4"[Title]) AND ("inflammation"[Title] OR "obesity"[title])]) – 14 articles
- ("bariatric surgery"[Title/Abstract]) AND ("obesity"[Title/Abstract]) AND ("GLP-1"[Title/Abstract] OR "dipeptidyl peptidase-4"[Title/Abstract]) – 60 articles

Original studies, both clinical and experimental, as well as reviews were considered, and only articles in English language were selected. Preference was given to articles published between 2010 and 2015, which were sorted by relevance. In addition, the revision of the topic and references list of these articles was also considered.

3. Overview of incretins in health and in T2DM

3.1. Biology of incretins

Given the central role they play in glucose and energy homeostasis, incretins have been a matter of scientific interest for over a century. The *incretin effect* was described in 1964 as the physiological greater increase in insulin secretion after an oral glucose load when compared to a corresponding intravenous bolus.(23) This phenomenon can only be explained by an intrinsic GI sensing mechanism followed by an efferent β-cell response. Hence, incretins are peptide hormones secreted from the digestive tract into the bloodstream following nutrient ingestion that boost glucose-dependent insulin secretion.(24)

To date, only two substances satisfy the incretin criteria in humans – GLP-1 and GIP. Both are ligands that bind to specific G protein-coupled receptors present in plasma membrane, the GLP-1 receptor (GLP-1R) and the GIP receptor (GIPR). This will lead to activation of an intracellular signaling cascade mediated by an adenylate cyclase and subsequent increasing levels of intracellular cyclic adenosine monophospate and calcium in pancreatic beta cells, thereby stimulating exocytosis of insulin-containing granules.(25) This response accounts approximately for 70% of postprandial glucose-dependent insulin secretion.(24)

In the distal ileum and colon, L-cells secrete GLP-1, which is the post-transcriptional product of pro-glucagon. Once produced, GLP-1 will stimulate islet β-cells by directly binding to its receptor or through portal vein vagal activation. Furthermore, it exerts a trophic action on β-cell mass, stimulating proliferation and inhibiting apoptosis.(24) Besides the effect on insulin secretion and biosynthesis, GLP-1 also inhibits α-cell glucagon (Gcg) secretion, combined with the increased secretion of somatostatin by δ cells, which together with insulin further inhibit Gcg release.(24) Thus, the net effect is a decrease in plasma

glucose and the regulation of carbohydrate metabolism. Actually, as normoglycemia is reached, GLP-1 signals are removed from α islet cells, preventing further development of hypoglycemia.(6) GLP-1 extends its spectrum of action over the peripheral insulin sensing organs, such as muscle, liver and adipose tissue that express GLP-1R. In particular, it promotes muscle glucose uptake and storage and decreased hepatic glucose production, whereas insulinemic induced glucose uptake and lipogenesis are enhanced in adipose tissue (6), consequently preventing fatty acid release (Figure 1). Meanwhile, GIP is derived from the K-cells of the stomach, duodenum and proximal jejunum and also acts on β-cells receptors.(24)

Other extra-pancreatic incretin effects have been an enthusiastic research target, given the wide distribution of incretin receptors over the body, including GI tract, peripheral and central nervous system, bone, kidney and heart (Figure 1).(9,24) GLP-1 plays a role in food intake control, delaying gastric emptying and acting on hypothalamic regions responsibly for appetite regulation. This is of paramount importance in therapeutic terms, as early satiety leads to decreased caloric intake and thus weight reduction,(11) as will be further analyzed in detail. Concurrently, GIP has no considerable effects on gastric emptying, but it has been shown to influence several mechanisms responsible for fat accumulation in adipose tissue.(6) Furthermore, GLP-1 has been shown to positively influence BP, hydroelectrolytic balance and endothelial function, thus having a major contribution in cardiorenal metabolic regulation and vascular protection. Finally, neuroprotective properties have also been attributed to incretins.(9) Figure 1 schematically represents the described pancreatic and extra-pancreatic effects of GLP-1. The pathways involved in some of the considered repercussions of incretins on peripheral tissues have been extensively reviewed elsewhere (9,26,27) and we will not be discussed here in detail.

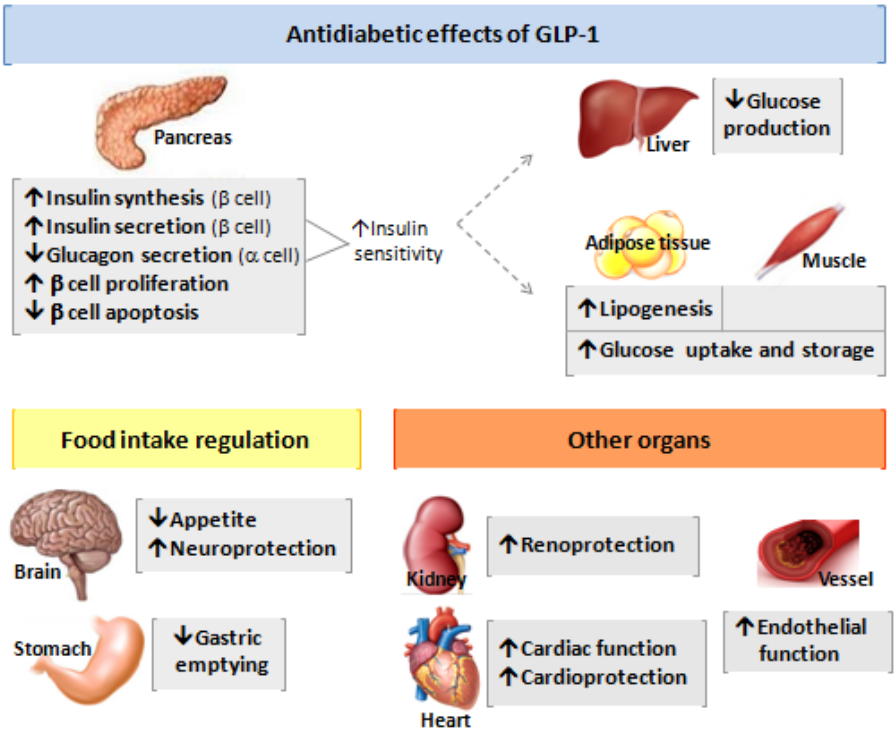

Figure 1 – Pleiotropic effects of GLP-1 on peripheral tissues. GLP-1 exerts an antidiabetic action by directly targeting the endocrine pancreas, producing subsequent insulin-dependent effects on liver, adipose tissue and muscle. Insulin-independent effects of GLP-1 comprise brain, stomach, kidney and cardiovascular GLP-1R activation, modulating food intake and multiple organs function.

Only a few minutes after being secreted into the circulation, GLP-1_{7-36} and GIP_{1-42} ($t_{1/2}$ ≈2 minutes) suffer rapid cleavage and inactivation by the enzyme DPP-4,(24) also known as adenosine deaminase (ADA) complexing protein 2 or CD26.(28) The truncated inactive metabolites that arise by the removal of their two N-terminal amino acids, GLP-1_{9-36} and GIP_{3-42}, undergo further renal elimination.(24)

DPP-4 is a multifunctional protein expressed in two molecular forms – a soluble form (sDPP-4) circulating in the plasma and a membrane-anchored form. The former either comes from a shedding process driven by proteinases,(29) namely from monocytic, endothelial and renal cells, as well as adipocytes,(30,31) or is released into circulation via vesicles in the form of exosomes, ectosomes and apoptotic bodies.(29) The latter usually serves as a surface

receptor and is highly expressed in the kidney, T lymphocytes and endothelial cells, but it is also found in several different cell types and tissues – pancreas, GI tract, liver, heart, brain and hematopoietic system.(32) Regarding its peptidase activity, which is exerted by both forms, DPP-4 cleaves a wide variety of other physiological substrates, accounting for incretin-independent effects. These substances include several chemokines, growth factors and regulatory peptides, such as stromal-derived factor (SDF)-1α, neuropeptide y (NPY), peptide YY (PYY) and substance P.(33) DPP4-mediated cleavage either inactivates or modifies the peptides thus exerting different biological properties.(28) Besides its peptidase activity, DPP-4 has a non-catalytic function, which is mediated by interaction with several ligands such as ADA, caveolin-1, CXCR4 and collagen, among others.(29) Adenosine, an ATP metabolite liberated from cells during inflammation, ischemia and infection,(34) markedly suppresses immune cells, comprising T cells. ADA degrades adenosine, thus allowing T cell activation and proliferation, such as in adipose tissue. On the other hand, interaction with matrix ligands, such as collagen and fibronectin, regulates cell adhesive and migratory behavior.(28)

3.2. The incretin defect in obese T2DM

IR, the cornerstone of T2DM pathophysiology, is defined as the inability of endogenous (or exogenous) insulin to promote proper glucose uptake and use.(19) The initial compensatory hyperinsulinemia tends to disappear with the progressive β-cell dysfunction, with ultimate exhaustion of its secretory capacity. As glucose levels rise, β-cell function declines further and apoptosis eventually occurs, reducing pancreatic islet cell mass; as so, impaired glucose tolerance and diabetes develop.(18)

Incretin dynamics is also known to be profoundly altered in T2DM.(5) Indeed, a major reduction in the incretin response was detected when comparing to healthy individuals,

presumably a consequence rather than a cause of the diabetic state, according to Knop *et al*.(35) This incretin defect has been attributed to impaired secretion of GLP-1 and defective GIP insulinotropic action.(5) This means that glucoregulatory effects of GLP-1 are still preserved in T2DM subjects despite its decreased levels, unraveling the conceivable applications of GLP-1 treatments.(36) In turn, GIP therapeutic application is vanished by the loss of effective GIP-stimulated insulin secretion observed in diabetes, although its levels are normal or even higher compared with healthy controls.(37) Several explanations for the impaired GIP responsiveness in T2DM have been proposed, namely GIPR mutations, downregulation and desensitization of GIPR, post-receptor signaling defects and reduced beta cell function and mass.(37)

Decline of β-cell function in T2DM is expressed by both first-phase and second-phase insulin secretion impairment. The former, corresponding to the early β-cell response through mobilization of stored insulin within 10 minutes after a postprandial peak in plasma glucose, appears to be particularly blunted.(38) As a compensation attempt, incretins contribution to first-phase insulin secretion is greater in diabetic subjects, thus playing a critical role in promoting a prompt insulin discharge in response to a glucose stimulus.(39) Nevertheless, the existent incretin defect favors a defective first-phase insulin secretion.(7)

DPP-4 can also play a significant role in T2DM. Despite contradictory studies,(40,41) DDP-4 protein levels and activity is known to be increased in diabetic animals and patients.(42,43) Specifically, Mannucci *et al.* has shown that increased enzyme activity correlated significantly with poor glycemic control in T2DM patients.(42) One of the possible explanations would be the hyperglycemia-induced cytotoxicity, leading to the shedding of DPP-4 from endothelial cell membranes into the circulation.(44) This further compromises glycemic control, as not only there is a lowered GLP-1 secretion, but also an enhanced degradation by DPP-4.

Since disturbed GLP-1 kinetics is involved in T2DM, i.e. decreased production and increased degradation, therapeutic strategies aiming at reversing these phenomena would improve glycemic control. In fact, two promising drug classes target these drawbacks: GLP-1 mimetics, which are agonists of the GLP-1R, and DPP-4i, also known as gliptins.(7) In addition, these agents are believed to confer direct β-cell cytoprotection.(45,46) Efficacy and safety of both medications have been proved in numerous clinical trials (reviewed in (47)). Moreover, T2DM is associated with long-term dysfunction and failure of several organs that culminate micro and macrovascular disorders, which are the major causes of the high morbidity and mortality rates found in diabetic patients.(3) The extrapancreatic effects of incretins would, therefore, be of valuable application in this context. Furthermore, DPP-4 activity also seems to be increased in these organs, probably as the result of endothelial dysfunction, known to be part of the pathophysiology of macro and microvascular complications.(43,44) Regarding this, the beneficial properties of gliptins on the kidney and retina have been previously reported on both type 1 and type 2 diabetes rodent models(7,43,48–50), and in humans.(8) Considering incretin mimetics, they have been implicated in the favorable modulation of cardiometabolic risk factors, such as dyslipidemia and elevated BP.(9,26) In sum, incretin mimetics and DPP-4i are a mainstay of diabetic therapy nowadays.

Moreover, a common dysmetabolic background can be identified between T2DM and obesity. In fact, obesity is one of the main players on the genesis of T2DM, as supported by the fact that the majority of T2DM subjects are overweight.(13) Actually, the positive energy balance translates into intra-abdominal fat deposition and increased plasmatic free fatty acids (FFAs). The resulting lipotoxicity combines with the arising glucotoxicity, leading to a perpetuous cycle that over the years culminates in overt diabetes.(2) In addition to the incipient IR, there is also an impaired regulation of the incretin effect in obesity.(10,11)

Thus, recent interest has been fostered in the potential profit of such treatments in obesity. This review will now focus on the principles behind this exciting hypothesis.

4. Incretins in obesity

4.1. Interplay between incretins and other hormones and peptides in obesity

Appetite control is a very important topic in obesity pathophysiology, since its (un)balance is responsible for weight gain. This regulation is under manipulation of several hormones and peptides,(51) that to some extent interact with GLP-1, as will be briefly described.

4.1.1. GLP-1

Concerning incretin dynamics, reduced GLP-1 secretion and impaired incretin effect are present in obese subjects with normal glucose tolerance, becoming more evident as glycemic control further deteriorates.(10) The mechanism behind incretin dysregulation is not fully understood, but it is postulated to result from the inadequate L-cell responsiveness to carbohydrates in an environment of elevated levels of circulating FFAs,(52) as commonly found in an obese state where IR and weight gain are hallmarks.(18) As mentioned in a previous section, GLP-1 modulates food intake and satiety. Although the ongoing dietary status was reported to alter the GLP1R expression in the brain,(53) these effects are nevertheless preserved in obese and T2DM subjects.(54) They result from a complex but harmonious interplay between the peripheral and central GLP-1 systems, as will be discussed below.

Along with its intestinal production, GLP-1 is synthesized in a similar fashion by a small population of neurons in the solitary tract nucleus (NTS) in the caudal brainstem, thereby also acting as a neurotransmitter. The projecting fibers access GLP-1R-rich regions known to be involved in energy homeostasis control, namely the hypothalamus and the hindbrain.(11) Concerning hypothalamic areas, paraventricular nucleus (PVN) and arcuate nucleus (ARC) have been implicated. Accumulating data point to the latter as the location of

GLP-1R-mediated activation of anorexigenic proopiomelanocortin/cocaine and amphetamine-regulated transcript (POMC/CART) neurons and simultaneous inhibition of orexigenic neuropeptide Y/Agouti-related peptide (NPY/AgRP) neurons, therefore promoting satiety (Figure 2).(11) In fact, monosodium glutamate (MSG), a substance used to produce an animal model of obesity,(55) was shown to annul the effects of GLP-1 by destroying the ARC after subcutaneous injection in rodents.(56) More recently, Boonnate *et al* showed that rats provided with MSG (2 mg/g body weight in drinking water, daily) suffered a reduction in pancreatic β-cell mass accompanied by hemorrhagic and fibrotic lesions, although glucose homeostasis remained unaffected, suggesting that in the presence of susceptibility to diabetes or sodium the pancreas might functionally manifest the dietary MSG negative effect.(57)

Additionally, GLP-1 also decreases food reward, further contributing to the reduced food intake (Figure 2). Mesolimbic GLP-1R plays a role in this extremely valuable effect, as known that motivational incentive of food is strongly responsible for overeating. Precisely, ventral tegmental area (VTA) and the nucleus accumbens (NAc) dopaminergic signaling have been implied, exerting communication with NTS, hindbrain and hypothalamus.(58,59) In pre-clinical studies, peripheral and central infusions of the GLP-1R agonist Exendin-4 (EX-4) reduced hunger-driven feeding, irrespective of macronutrient composition. Interestingly, mesolimbic behavioral response to GLP-1 is not limited to food-derived reward, but extends to alcohol and the psychostimulants cocaine and amphetamine reward.(59) Regarding energy expenditure, no consistent or significant effect seems to exist.(60) Central nervous system (CNS)-GLP-1R signaling was intended to activate brown adipose tissue (BAT) thermogenesis peripherally, but the exact contribution is unknown,(58) thus rendering food intake as the main party of the energy balance equation. Finally, Heppner *et al* questioned whether the demonstrated neuroprotective properties of GLP-1 such as in reduced neuronal death could directly contribute to a proper central homeostatic regulation. However, as inferred from

literature, it seems that this effect is most likely due to the decreased glucotoxicity and brain inflammation resulting from weight loss and enhanced glycemic control.(58) Figure 2 graphically summarizes the mechanisms behind GLP-1 mediated weight loss.

In addition to the locally released GLP-1, gut-produced GLP-1 also reaches the CNS to activate central GLP-1R. This may occur not only by crossing blood brain barrier (BBB)-free circumventricular organs such as the subfornical organ close to the hypothalamus and area postrema in the brainstem, as shown in rats, but also through vagal signaling, targeting GLP-1R of vagal afferent neurons with cell bodies on the nodose ganglion.(11) Furthermore, food intake elicits gastric distension, thus activating the gastro mechanoreceptors that contribute to vagal sensory input to the NTS as well.(11,61) It is worth mentioning that the degree of gastric distension is partially under influence of the rate of gastric emptying, which is in turn delayed by peripheral GLP-1, affecting postprandial glycemic excursions. Gut GLP-1 also contributes to gastric accommodation, changing the stomach volume when anticipating food intake, possibly affecting the perception of gastric distension and promoting satiety.(61) Finally, peripheral GLP-1 is considered an enterogastrone,(52,62) acting on enteric neurons and inhibiting GI postprandial motility in a negative feedback fashion in the face of a nutrient supply, maximizing absorption and digestion – the "ileal brake".(52) Efferent signals from CNS also contribute to the motility slowing action. Regarding the GLP-1 incretin effect, portal vein sensory neurons are activated and the elicited NTS descending responses stimulate pancreatic vagal motor neurons resulting in insulin secretion, which adds to the direct GLP-1R islet stimulation.(52)

4.1.2. Ghrelin

Ghrelin is a circulating polypeptide hormone mainly produced in the stomach, whose orexigenic characteristics led to the designation of “hunger hormone”,(7,63) and it has been shown to promote body weight gain and adiposity. Not only these effects result from increased appetite and food intake, but they are also achieved through reduced energy expenditure in the form of adipocyte storage of hepatic-synthesized fatty acids and triglycerides (TGs).(64) Whereas postprandially decreased, fasting plasmatic levels of ghrelin are high, thereby indicating its fast-acting role in meal initiation.(63) Interestingly, its peak concentration before feeding precedes the GLP-1 response to the ingested nutrients, suggesting that ghrelin acts as a secretagogue of the intestinal L-cells to induce GLP-1 release in response to the upcoming meal. Accordingly, both premeal ghrelin concentration and the nutrient-induced GLP-1 response are reduced in obese subjects.(65)

As exploited before in this review, vagus nerve assures the communication of peripheral GI satiety signals to higher brain centers. In this way, GLP-1 acts in a paracrine manner on vagal afferent neurons (VANs), but it is not alone in this process. Gut-derived hormones interact at this level to regulate energy balance, inasmuch as ghrelin.(66) As a matter of fact, VANs GLP-1R slightly differ from the other widespread receptors in the way that they experiment a “phenotypic switch” according to feeding and consequent modulation by GI hormones, including ghrelin. Indeed, in the fasted state ghrelin is able to block the anorexigenic GLP-1 signal by translocating the GLP-1Rs on VANs, while in a refed state characterized by low ghrelin levels GLP-1 binds to its receptor and induces satiation.(66)

In the CNS, gut-secreted ghrelin and to a lesser extent pituitary and hypothalamic produced ghrelin activate the orexigenic pathway through binding to its receptor on NPY/AgRP neurons of ARC, whereby inhibiting the anorexigenic POMC/CART subpopulation (Figure 2).(63,66) Thus, the stimulation of food intake is supposed to derive in

part from the ability to mitigate GLP-1 effects on meal ingestion. Peripherally, ghrelin attenuates the effects of GLP-1 on food intake.(11)

Somehow paradoxically, this stomach-derived hormone is able to both induce obesity when exogenously administrated and to prime the impaired incretin system in the (pre)diabetic obese population. This indicates how the whole-energy body metabolism is strictly regulated, in a coordinated balance in which ghrelin and GLP-1 seem to interact as the *yin* and the *yang*.

4.1.3. Leptin

Proper peripheral and central interaction of hormones is critical to the regulation of hunger and satiety signals. Another hormone seems to be part of this interactive regulation, being widely known for its appetite suppressing effects and representing the basis for a genetic form of both human phenotype and animal model of obesity (*ob/ob*).(67) Leptin is an adipokine, i.e., a hormone secreted by the white adipose tissue, and therefore its plasma levels correlate with body adiposity. After crossing the BBB, it targets neuronal ARC hypothalamic leptin receptors (LepRs), inhibiting the expression of orexigenic NPY/AgRP and stimulating anorexigenic POMC/CART (Figure 2).(63,66) These pathways are common to GLP-1R CNS signaling, leading to *ab initio* suspect of a link between leptin and GLP-1 in reducing food intake. The minor, albeit significant, gastric leptin production occurring shortly after food intake further supports this hypothesis, as it may correspond to the early phase of GLP-1 release that results from indirect stimulation of endocrine or neural mediators.(53) On the other hand, LepRs are present in intestinal L-cells, whose GLP-1 production was shown to be enhanced by leptin in both rodents and humans.(24) In fact, the charge of leptin from adipose tissue with increase of its postprandial plasmatic levels is conceivably responsible for the late phase of GLP-1 secretion, where L-cells directly respond to the luminal nutrient intake.(53)

Besides the feeding restraining behavior, leptin increases energy expenditure through fostered lipolysis and fatty acid oxidation and reduced lipogenesis.(19)

Nevertheless, leptin resistance represents the canonical state of diet-induced obesity, in which individuals are unable to respond to the established hyperleptinemia.(66) Leptin resistance has been postulated to cause the decreased meal-induced GLP-1 secretion observed in these subjects,(24) and recent evidence tends to further confirm the interaction between leptin and GLP-1 to induce satiety.(60,66) Similarly to ghrelin receptors, LepRs are expressed in VANs, and Ronveux *et* al demonstrated that in face of leptin resistance VANs experiment an obesogenic phenotype switch that precedes the development of leptin resistance in the ARC, highlighting the importance of peripheral signaling pathways in homeostatic regulation.(66) From this and what was previously mentioned in this review, one can infer that in a normal situation leptin opposes to ghrelin action on VANs.

The fact that plasma leptin concentrations obey a circadian rhythm pattern with nocturnal highest levels (66) gives rise a delightful question. Maybe it corresponds to an evolutional mechanism orchestrated in order to keep the body free from feeding impulses and decrease the metabolic rate, as seen in animal hibernation? Actually, this action seems to be interrupted by sleep deprivation, which has been associated not only with decreased circulating levels of leptin but also increased levels of the opposing hormone in appetite regulation, ghrelin, and epidemiologically linked to augmented BMI.(18) In addition, sleep deprivation has also been associated with increased risk for developing T2DM,(19,68) presumably due to the activation of the hypothalamo-pituitary-adrenal axis that the waken state hyperarousal triggers, resulting in altered diurnal cortisol secretion and consequent impaired glucose tolerance.(18) As a matter of fact, the typical cushingoid phenotype of primary or secondary hypercortisolemia comprehends truncal adiposity, dyslipidemia and T2DM.(69) Hypertension is another common feature,(69) and leptin has been linked to the

renal sympathetic hyperactivity and therefore BP characteristic of obese subjects, through hypothalamic-mediated effects.(2) Sleep disturbance may as well behave as a complication of obesity, as illustrated by obstructive sleep apnea,(2) possibly further aggravating insulin resistance through the mechanisms mentioned earlier. Current modern society work and sleep patterns certainly contribute to the alarming trend of metabolic and anxiety-related behavioral disorders so typical of our century and could in part explain the obesity epidemic.

4.1.4. Other peptides

PYY is mainly secreted by colon and rectum L-cells following a nutrient supply but, contrary to GLP-1, its degradation by DPP-4 yields a still biologically active peptide - PYY_{3-36}. This form inhibits appetite, contributing to the ileal brake (52) and inhibiting NPY/AgRP neurons through selective binding to Y2 receptors (Y2R).(11) As expected from a physiologic point of view, PYY levels are low during fasting and increase in postprandial period.(70) Fasting plasma PYY concentrations were shown to be lower in obese subjects and inversely correlated with BMI.(70) Nevertheless, the anorectic response to exogenous PYY infusion was preserved, pointing that a PYY deficiency rather than resistance may contribute to the pathogenesis of obesity. Furthermore, PYY administration reduced plasma ghrelin levels(70), which targets the same orexigenic neurons as PYY with an opposing effect, leading to a decreased appetite and food intake.(11) NPY belongs to same peptide family of PYY and also undergoes cleavage by DPP-4.(8) Other effects and subsequent implications of both these physiological substrates will be later revisited in this review.

Oxyntomodulin (OXM) is co-synthesized with GLP-1 by the processing of proglucagon in the L-cells and in the CNS, showing a reducing food intake effect.(24) This is thought to be at least in part achieved through the activation of both GLP-1R and Gcg receptor (Gcg-R).(33)

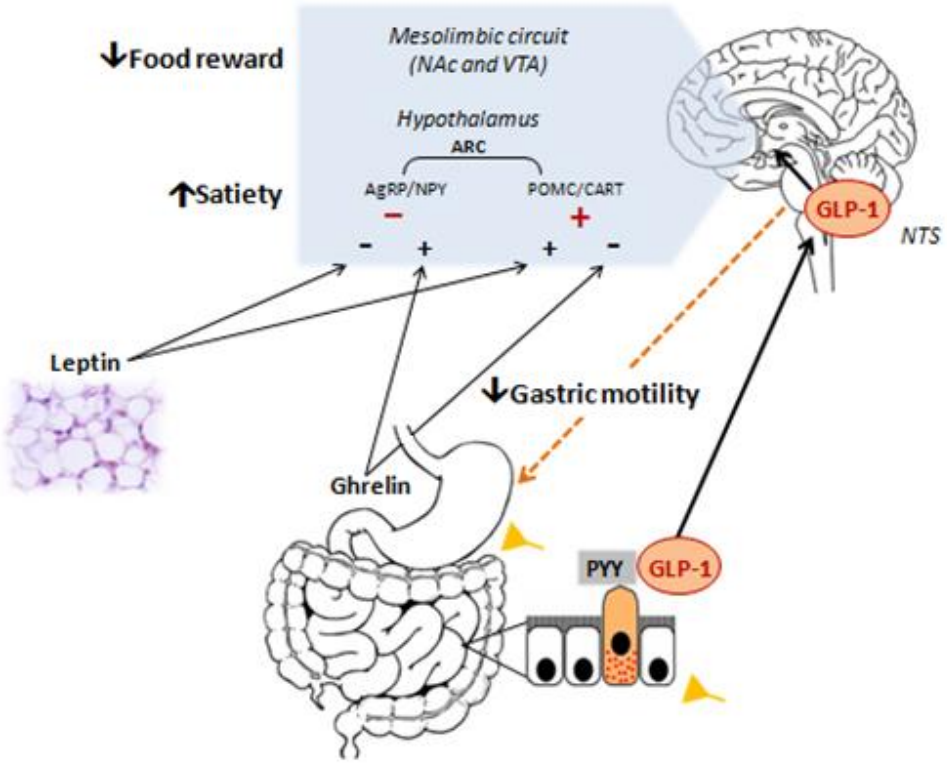

Figure 2 – Peripheral and central mechanisms behind GLP-1-induced weight reduction. Gut-produced GLP-1 reaches the CNS through vagal afferent signaling, and possibly by crossing the brain-blood-barrier (BBB). Meal-induced gastric distension also contributes to the afferent sensory input through activation of stomach mechanoreceptors. In the CNS, these signals reach solitary tract nucleus (NTS), where GLP-1 is also locally produced. Neuronal projections reach hypothalamic areas involved in food intake regulation, such as the arcuate nucleus (ARC) where GLP-1 inhibits the orexigenic neuropeptide Y/Agouti-related peptide (NPY/AgRP) neurons and activates the anorexogenic proopiomelanocortin/cocaine and amphetamine-regulated transcript (POMC/CART) neurons, therefore promoting satiety. Other hormones – PYY, co-secreted with GLP-1, and leptin released from adipose tissue also act as GLP-1 on ARC neurons, while ghrelin stimulates the orexigenic and inhibits the anorexigenic subpopulation. Additionally, GLP-1 decreases food reward further reducing food intake, by modulating ventral tegmental areas (VTA) and nucleus accumbens (NAc) signaling, components of the mesolimbic circuit. Efferent vagal signaling causes gastric motility to decrease, contributing to the satiety effect.

4.2. Incretins and inflammation in obesity

It is clear that obesity disrupts appetite signaling, and both GLP-1 and other gut hormones are implicated in dysregulation of food consumption. Furthermore, adipose tissue inflammation is another key player in obesity pathophysiology. Since GLP-1 has been associated with anti-inflammatory properties, establishing the connection between the incretin system and inflammation may actually offer new therapeutic challenges. The spotlight of this review will now turn into the underlying mechanisms.

Adipose tissue is more than a deposit of cells with energy-storage functions. In fact, the omentum, part of the visceral adipose tissue (VAT), is a key player in the body's response to intra abdominal infection. Its angiogenic and pro-regenerative properties have motivated its clinical use in surgery as an adjuvant to healing of gastrointestinal anastomosis or in reconstruction of soft tissue defects.(71) VAT represents an endocrine organ with a complex army of differentiated cells, where the generated microenvironment has repercussions in whole body homeostasis. Recalling the energy balance equation, chronic favoring of excessive energy intake ultimately leads to obesity, with excessive body fat accumulation. Adipocyte expansion, namely in VAT, is accompanied by infiltration of immune cells, especially macrophages, believed to be key players in pathophysiology of obesity-induced insulin resistance and its related comorbidities.(20,21) Indeed, Cancello *et al* pointed out that macrophage number in omental VAT correlated with insulin resistance and liver inflammation.(72) In fact, macrophagic cytokines elicit changes in adipokine secretion profile, propitiating the development of systemic inflammation that further echoes in specific organs (Figure 3). Indeed, obesogenic hypertrophic and hyperplasic adipocytes secrete, besides leptin, pro-inflammatory cytokines such as interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-α) and plasminogen activator inhibitor 1 (PAI-1).(2,18) Moreover, leptin itself

has also been described to prompt IL-6 and TNF-α production.(73) In turn, as an insulin sensitizer, adiponectin is decreased.(2) This inflammatory response has peculiar traits - devoid of typical acute cardinal signs, it exhibits an indolent low-grade character, nonetheless dangerous as long-lasting clinically silent. Meanwhile, it is responsible for the onset of IR (Figure 3).(20,21) On the other hand, the exaggerated calorie intake naturally stimulates insulin secretion and, thus, hyperinsulinemia and IR. As a result, contrarily to physiological conditions where lipogenesis would be promoted, there is fat breakdown with increased plasma levels of FFAs.(2,18) This promotes hepatic neoglucogenesis and decreases peripheral insulin sensitivity, primarily in muscle and liver, where they contribute to the development of non-alcoholic fatty liver disease (NAFLD). The liver turns as well into a source of inflammatory factors, synthesizing PAI-1, C reactive protein (CRP) and fibrinogen. Endothelium is another important target of insulin resistance and inflammation, whose dysfunction combines with dyslipidemia and increased sympathetic nervous system activation to boost atherosclerosis and essential hypertension onset.(2,18)

Regarding macrophage infiltration, several players are thought to be in its origin. Mechanical factors have been implicated, with enlargement of adipose tissue creating a hypoxic microenvironment.(2) In fact, hypoxia-inducible factor 1-alpha (HIF-1α) was found to be overexpressed in morbid obesity. Subsequent local necrosis and cytokines production, namely chemotactic cytokines such as monocyte chemoattractant protein 1 (MCP-1), stimulate macrophage infiltration.(74) Moreover, leptin also seems to enhance blood monocyte diapedesis through effects on endothelial cells.(75)

On the other hand, both $CD8^+$Tcells and different subsets of T helper (T_h)/$CD4^+$T cells have been implicated in the regulation of adipocyte enlargement and subsequent metabolic disease progression.(76,77) Indeed, $CD8^+$Tcells, also found to be increased in rodent and human VAT, are believed to promote macrophage accumulation.(20) $CD4^+$Th1 cells drive

classical macrophage/M1 differentiation, the phenotype implicated in IR through production of the pro-inflammatory cytokines mentioned above. On the contrary, alternative macrophage/M2 phenotype, provided with immunosuppressive and anti-inflammatory properties, is induced by $CD4^+$Th2 cells.(21) M2 macrophages will in turn promote induction of a special subset of $CD4^+$T cells that downregulate effector responses of other T cells - regulatory T cells (T_{reg}).(21) In the obese state, there is an increased ratio of M1/M2 with a subsequent diminution of T_{reg} in favor of pro-inflammatory T cells. These include Th17, a peculiar T cell subset that further contributes to diminished insulin sensitivity (Figure 3).(21)

In addition to the vast bulk of cytokines and hormones secreted by obese VAT, DPP-4 has also been suggested to fit in the concept of adipokine. In fact, both s-DPP-4 and visceral adipose tissue DPP-4 levels have been shown to be considerably elevated in obese humans when compared to lean controls.(29,31) Similarly, DPP-4 levels were also increased in the kidney of a T2DM rodent model.(43,78) Increased serum DPP-4 concentrations were shown to be positively correlated with BMI, adipocyte size, insulin and leptin levels, while presenting a negative correlation with adiponectin.(31) They are thought to derive from adipocyte-expressed DPP-4 release, with VAT displaying a higher release than subcutaneous adipose tissue. Soluble DPP-4 may in turn impair insulin sensitivity in peripheral organs, but also in adipocytes in an autocrine and paracrine manner.(31) Besides, DPP-4 mediates vascular cell proliferation, therefore playing a role in the genesis of obesity-associated vascular complications. These findings lead Lamers *et al* to consider DPP-4 for the detection of subjects at high risk of such complications, and suggest it as a novel biomarker of metabolic syndrome.(31) Furthermore, DPP-4 expression was found to be increased in VAT macrophages from obese humans and rodents, with higher levels compared to those of corresponding peripheral cells. This expression positively correlated with fasting insulin and IR, as evaluated using a homeostasis model assessment of IR (HOMA-IR).(77) As explored

in a previous section, DPP-4 acts as a ligand for ADA,(29) therefore promoting T cell proliferation. In this regard, macrophage-expressing DPP4 contributed to this effect.(77) In addition, ADA accounts for the autocrine and paracrine action of DPP-4 - adenosine presents antilipolytic effects, and binding of ADA presumably enhances hypertrophic adipocyte lipolytic activity. In addition, DPP4 inactivates NPY, reversing its antilipolytic properties.(31)

DPP4 relevance in the obesity context seems to be twofold – while its non-enzymatic function intervenes in ADA-mediated pathways and plays a role in T cell proliferation, known to contribute to IR and metabolic complications, peptidase activity is responsible for the activation of some cytokines, and, above all, for the degradation of incretins leading to chronic hyperglycemic effects. As so, VAT can be regarded as a powerful inflammatory source, that triggers and feeds the metabolic dysfunction seen in T2DM, which in turn helps sustaining the inflammation processes.(77) In this complex multivisceral interplay (illustrated in Figure 3), DPP4 establishes crucial connections.

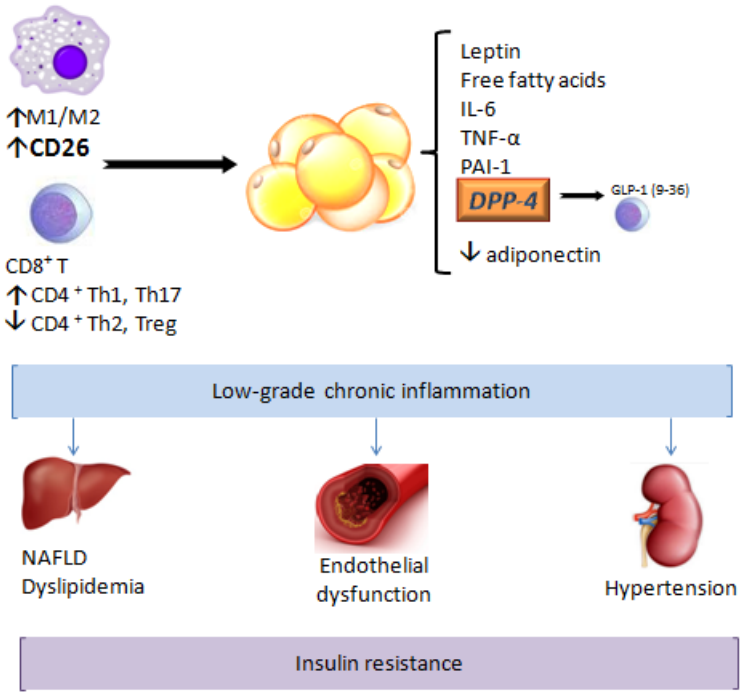

Figure 3 – DPP-4 plays a role in adipogenic-mediated inflammation in obesity. Adipocyte expansion following fat accumulation seen in obesity leads to immune cell infiltration, (macrophages and T cells). M1 macrophagic phenotype drives differentiation of pro-inflammatory T cell subsets ($CD4^+$ Th1 and Th17) and induces the adipose tissue to produce, besides leptin, pro-inflammatory cytokines, namely interleukin-6 (IL-6), tumor-necrosis factor α (TNF-α) and plasminogen activator inhibitor (PAI-1). Adiponectin production is in turn decreased. This triggers a state of on-going low-grade chronic systemic inflammation, responsible for the onset of insulin resistance. As a result, there is fat breakdown with increased circulating free fatty acids, contributing to the development non-alcoholic fatty liver disease (NAFLD) and dyslipidemia. Obesity-induced endothelial dysfunction boosts atherosclerosis and, together with increased sympathetic nervous system activation, is responsible for essential hypertension. DPP-4 is also secreted by obese adipose tissue, and expressed on macrophages. Its peptidase activity cleaves active GLP-1, while its non-enzymatic function mediates T cell proliferation. Both these effects worsen insulin resistance and metabolic complications.

5. Therapeutic approaches

Regarding obesity treatment, dietary control is often insufficient and the approved pharmacological treatments have an extremely low efficacy. The limited success of current therapies has been somehow related to the complex pathophysiological features involving many factors that control adipose tissue metabolism, which have been in part explained above and will now serve to discuss potential promising therapeutic strategies based on GLP-1 or under its influence.

5.1 Incretin-based therapies

5.1.1. GLP-1R agonists

The continuum between diabetes and obesity is of significant importance and this interdependency could be the basis for innovative approaches, based on the correction of the incretin defect. Moreover, the fact that pharmacological GLP-1R agonism consistently promotes weight loss has sparked major interest in the obesity context. As so, the application of GLP-1RA in the obese population seems to be of logic dual benefit, with reductions in BMI adding to the well-established improvement in glucose homeostasis, commonly impaired in these subjects.

As recently reviewed,(11,52,60,61) a meta-analysis of the GLP-1RAs exenatide and liraglutide on obese individuals with and without diabetes showed a greater weight loss when compared to controls. Weight reduction results mainly from the decrease in fat mass, particularly visceral and truncal adipose tissue. It is dose dependent and the greater the BMI the better the results. Waist circumference, which is actually a superior predictor of development of cardiovascular disease and T2DM than BMI, experiences a significant decrease too. In fact, liraglutide's contribution appears to include an improvement in hepatic

steatosis, as well as lean tissue reduction. Confronted with other strategies to combat obesity, the supraphysiological doses of GLP-1 have the advantage of significantly maintaining weight loss, known to be difficultly achieved with diet alone. Besides, dieting only leads to a mediocre or brief return to euglycemia, even when compliance is assured. The weight loss, even though moderate, is comparable to the approved drugs presently used to treat obesity, which in the case of the peripheral lipase inhibitor orlistat (Xenical®) cause inconvenient side effects, such as oily stools and fecal urgency.(11,52,60,61) Moreover, GLP-1R agonists may provide other beneficial effects on the obesity-related risk factors. Non-glycemic effects include improved fasting plasma lipid profile and decreased postprandial lipemia, recognized as a typical feature of obesity and IR dyslipidemia.(79) While the former consists of reduced fasting TGs, total cholesterol and low-density lipoprotein (LDL)-c, together with increased high-density lipoprotein (HDL)-c, the latter comprehends attenuated elevation of serum TG, apolipoprotein B-48, apolipoprotein C-III, and cholesterol and TG in remnant-like particles.(79) In addition, GLP-1 improves endothelial function through nitric-oxide-dependent and independent pathways, somehow antagonizing atherosclerosis.(9) On the other hand, anti-hypertensive properties were reinforced in a meta-analysis and meta-regression, with small but significant BP lowering occurring even independently of confounding factors.(80) In sum, this interesting drug class, via the reduction of cardiovascular risk and through weight-loss independent effects, likely satisfies the current urge for long-lasting pharmaceutical treatment in obesity.

However, as with every medication, GLP-1RA can present with side effects, namely nausea and vomiting, which represent the principal tolerance limiting factor of this drug class.(47) One could arguably question whether the elicited nausea could contribute to weight loss, but no direct correlation was observed, and weight loss persisted after nausea relief.(11,52,60,61) These reactions are attributed to the delayed gastric emptying, but also to

central mechanisms, namely through the BBB access to area postrema, which controls vomiting.(60,81) Albiglutide is a newer agent that does not easily diffuse into this region, due to its high molecular size, thus having a low prevalence of GI effects, though also promoting weaker weight loss.(82) We could speculate that albiglutide pharmacokinetics would perhaps be of benefit in a situation where nausea overtly interferes with daily life. Further, GLP-1 targets neurons within the central nucleus of the amygdala to cause the so-called conditioned taste aversion (CTA), a suitable indicator of visceral illness which is normally developed when a transient GI malaise follows the consumption of a novel taste.(83) CTA was pre-clinically demonstrated in rodent studies after EX-4 or GLP-1 administration. What is fascinating is that the neurophysiologic pathway behind the indicated effect seems to be anatomically separated from the food reward zones. As a result, the lack of association between nausea and GLP-1 in those locations leads to anticipate the conception of drugs that specifically target certain CNS zones, therefore dissociating the anorectic effects from illness-inducing events of GLP-1.(59) Subcutaneous administration, owing to the peptidic nature of GLP-1RA, can be pointed out as another obstacle, but it actually seems to be well accepted by most subjects seriously willing to lose weight.(52)

Nevertheless, innovative strategies have been proposed to overcome the modest 2-4% weight reduction potency of GLP-1RA and minimizing unwanted side effects. The so-called "GLP-1R co-therapies" combine different mechanisms of action, resulting in superior weight loss and improved glucose metabolism.(58) One of the modalities consists of single molecule multi-agonists. GLP-1/ Gcg co-agonism was demonstrated to enhance weight loss in obese mice, with Gcg increasing energy expenditure and BAT activation, and GLP-1 preventing its hyperglycemic action. These data still await further clinical testing.(58)

On the same basis, GLP-1R co-therapies could eventually be extended to the other above explored hormones recognized to be involved in appetite control. OXM, which seems

to activate both GLP-1R and Gcg-R,(33) could behave as a natural occurring GLP-1/Gcg co-agonist.

Considering ghrelin changes found in obesity, it could be regarded as a potential therapeutic weapon. Indeed, using GLP-1R wild-type and knockout ($Glp1r^{-/-}$) mice, Gagnon *et al* recently showed that exogenous ghrelin administration enhanced glucose-stimulated GLP-1 secretion and glucose homeostasis.(65) GLP-1 secretion was mediated through a ghrelin receptor and extracellular signal-related kinase ½-dependent pathway. The improvement of ghrelin effects on glucose metabolism was found to require GLP-1R. Overall, that study points to a key role of ghrelin in the enhancement of GLP-1 secretory response to ingested nutrients.(65)

5.1.2. DPP-4 inhibitors

DPP-4i have been thoroughly studied in recent years due to their distinct properties as T2DM therapeutic agents.(7) Regarding side effects, DPP-4i appear to be superior to GLP-1RA in the way they do not cause nausea, and this is postulated to result from differences in potency at the receptor level of external GLP-1 versus the endogenous peptide.(60) On the other hand, in detriment of their convenient tolerance profile, DPP-4i are neutral or modest respectively to weight loss (summarized in (84)), which has rendered them out of popularity in the attempt to pharmacologically interconnect T2DM and obesity. However, DPP-4i seem nevertheless to be implicated in energy homeostasis, namely in lipid metabolism. Using diet-induced obese (DIO) mice, Shimasaki *et al* showed that the DPP-4i sitagliptin attenuated body adiposity without affecting food intake, and increased BAT uncoupling protein 1 (UCP-1), known to enhance thermogenesis and energy expenditure.(85) Contrarily to what was previously accepted, BAT appears to remain important in adulthood,(86) and therefore its link with DPP4i would appear to deserve further investigation. Using a similar animal model,

Fukuda-Tsuru *et al* demonstrated that the DPP-4i teneligliptin was able to increase energy expenditure, as shown by a significant increase in oxygen consumption, together with suppression of adipocyte hypertrophy and hepatic steatosis.(87)

In addition, as disserted in this paper, DPP-4 roots up at the inflammatory ground that perpetuates obese metabolic dysfunction. Hereof, inhibition of DPP-4 is clearer than ever an interesting therapeutic approach for obesity. In fact, anti-inflammatory properties of such medications had already been demonstrated in the context of T2DM vascular complications, namely regarding the diminution of pro-inflammatory cytokines.(43,48,50)

Since DPP-4 is appreciably increased in obese animals and humans (21,31,88), its inhibition provides an accessible therapeutic strategy. Given the recognized expression of DPP-4 in immune cells, the modulatory effect of DPP4i has gained emerging importance. Indeed, DPP-4i treatment enhanced M2 polarization in obese or atherosclerotic mice, therefore reversing the M1/M2 shift. Accordingly, in another study DPP4 inhibition originated T_{reg} expansion, whereas renin-angiotensin-aldosterone system (RAAS) components induced the opposing effect.(21) Several organs are on the sights of obesity-induced inflammation. In this multiple biological crosstalk scenario, cardiovascular and renal IR are pivotal in the development of hypertension.(18,21) Angiotensinogen, whose expression is increased by hyperinsulinemia,(18) is also secreted by adipocytes, which therefore can potentially act as a small-scale RAAS.(2) Resistance to insulin-mediated nitric oxide formation causes endothelial dysfunction and subsequent peripheral arterial vasoconstriction. This, combined with kidney and heart sympathetic tone activation in the form of increased renal sodium reabsorption and increased cardiac output, results in hypertension.(2) Contrarily, DPP4-i have been show to lower BP through vasodilator effects and enhanced renal sodium excretion. The latter derives from decreased proximal tubular sodium uptake due to reduced sodium–hydrogen exchanger 3 activity, curiously the same transporter that is stimulated by

RAAS.(21) Moreover, angiotensin II receptor type 1 (AT_1R) antagonism, which is pharmacologically achieved with angiotensin II receptor antagonists (ARAs), has been shown to increase GLP-1R expression.(21) The crosstalk between DPP4-inhibition/GLP-1 signaling and AT_1R antagonism has led Aroor *et al* to propose such a therapeutic association in order to ameliorate IR and cardiorenal sequelae.(21) Actually, the rationale for this alliance can be pointed out, particularly when comparing to DPP-4i and angiotensin-converting enzyme inhibitors (ACEi) association. In fact, Marney *et al* reported an increased sympathetic tone following the DPP4i sitagliptin administration in hypertensive humans taking high-dose ACEi.(89) This was postulated to result from the existence of overlapping substrates of DPP4 and ACE, namely substance P, which increases sympathetic outflow. When ACE is inhibited, it is inactivated by DPP-4, except when this system is also pharmacologically suppressed. In addition, NPY represents another DPP-4-degraded substrate which acts as a peripheral vasoconstrictor through Y1-receptor activation, when not cleaved by DPP-4. Thus, decreased degradation of NPY with DPP-4i has also been implicated to justify the mentioned phenomenon.(89) However, ARAs are free from interactions with DPP-4 substrates, and could therefore help to counterbalance the increased sympathetic system activation.(90) Nevertheless, it is important to assess the real clinical relevance of the subsequent effects, such as fastened heart rate.

Among the wide range of DPP-4 substrates, SDF-1α should be mentioned, based on its important hematopoietic functions. This chemokine, expressed in the stroma of multiple organs,(91) is involved in the recruitment of adult progenitor cells from both bone marrow and circulating blood to the site of ischemic injury, where endothelial progenitor cells (EPCs) can contribute to vascular repair and compensatory angiogenesis.(88) In the face of increasing blood sugar and peripheral IR, lower SDF-1α plasma concentrations are found in mice.(92) When inhibiting DPP-4, SDF-1α inactivation is therefore prevented. In a study by Fadini *et*

al, patients taking DPP4i displayed a 2-fold increase of EPCs and a 50% increase of SDF-1α.(93) In this regard, several studies have addressed tissue healing properties of DPP4i in the setting of myocardial (94) or vascular ischemia in kidney (95) and lung.(96) Given the nature of obesity-related complications, exploiting intact SDF-1α through DPP4i would be an asset. Still, more recently, another function was attributed to this peptide – in obese mice, adipocyte-derived SDF-1α expression and secretion were increased, resulting in macrophage recruitment and accumulation in adipose tissue. This was reduced when blocking SDF-1α action, together with insulin sensitivity improvement.(91) These results raise some controversy in the light of what was previously explained, and perhaps point to a bimodal opposing biological role of SDF-1α that needs to be further elucidated. However, given that DPP-4 secretion is also enhanced in obesity, the extra SDF-1α would expectedly be degraded.

Recent obese rodent studies focusing on obesity-related organ dysfunction are summarized in Table 1, while completed and ongoing randomized controlled clinical trials with DPP-4i with cardiovascular endpoints are listed in a paper recently published by Zhong *et al.*(88) To sum up, even though DPP-4i do not have a visibly appreciable effect on weight loss, they modulate immune activation and inflammation that underlie obesity metabolic dysfunction and complications. As so, in the long-term they potentially exert precious effects, presumably beyond the pleiotropic effects of endogenous GLP-1, which should be subject of future clinical studies. Moreover, association with DPP4-i and GLP-1RA could also be of interest regarding synergistic effects.(97)

Table 1: Effects of dipeptidyl peptidase-4 inhibitors on obesity-related organ dysfunction in rodent models

Author /Year (Ref)	Type of study	Doses and comparators	Main outcomes
Liver			
Akaslan *et al.* 2013(114)	NAFLD-HFD induced rats	Sitagliptin (3mg/kg/day) or HFD continuation for 4 weeks	Treatment lowered serum glucose, plasma insulin, HOMA-IR index, serum triglycerides and cholesterol Less hepatic steatosis in treated group, with no effect on hepatomegaly nor liver transaminase levels
Ohyama *et al*. 2014(115)	ob/ob mice	MK0626 (3 mg/kg) or vehicle for either four or eight weeks	Treatment reduced glucose and insulin levels, and increased serum adiponectin levels, in a dose-dependent manner. It enhanced AMPK activity and inhibited hepatic lipogenic gene expression Treatment reduced serum ALT dose-dependently and attenuated hepatic steatosis
Heart			
Apaijai *et al.* 2014(116)	HFD rats with induced cardiac I/R injury	Vildagliptin (3mg/kg/day) Metformin (30mg/kg/day) Vildagliptin+Metformin or vehicle for 3 weeks	All treatments improved metabolic parameters, HRV, and LV function During I/R, all treatments improved LV function, reduced infarct size and pro-apoptotic proteins, and improved mitochondrial function Only combined treatment reduced cardiac arrhythmia score and mortality rate, and increased Cx43 phosphorylation
Bostick *et al.* 2014(117)	WD mice	MK0626 (10mg/kg /day) or vehicle for 16 weeks	Treatment improved insulin resistance, normalized diastolic relaxation, and ameliorated myocardial oxidant stress and fibrosis
Huisamen *et al*. 2010(118)	DIO rats I/R cardiac injury by ex vivo perfusion	PFK275-055 (10 mg/kg/day) or vehicle for 4 weeks	Treatment resulted in smaller infarct size after I/R, along with activation of cardioprotective kinases
Kidney			
Nistala *et al.* 2014(30)	WD mice	MK0626 (10 mg/kg/day) or vehicle for 16 weeks	Treatment decreased serum uric acid levels, proteinuria and oxidative stress, with concomitant improvement of glomerular and tubulointerstitial injury, oxidative stress, and increased levels of the anti-inflammatory IL-10. These effects occurred independently of changes in BP and insulin sensitivity
Nistala *et al.* 2014(119)	Zucker obese rats	Linagliptin (4mg/kg/day) or vehicle for 8 weeks	Treatment increased SDF-1 α in kidney and plasma, improved obesity-related glomerulopathy, filtration barrier injury and oxidative stress
Pancreas			
Omar *et al.* 2013(120)	Advanced-age HFD mice	Vildagliptin (3 μmol/day) or vehicle for 46 weeks	Treatment β cell function and improved insulin secretion, peri-insulitis and survival rates. β cell area remained unaffected

NAFLD – non-alcoholic fatty liver disease; HFD – high fat diet; HOMA-IR – homeostasis model assessment; ALT – alanine aminotransferase; AMPK – AMP-activated protein kinase; I/R – ischemia-reperfusion; LV – left ventricular; HRV – heart rate variability; Cx43 – connexin 43; WD – western diet; DIO – diet-induced obese; BP – blood pressure; IL-10 – interleukin 10; SDF-1 α - stromal derived factor 1 α .

5.2. Bariatric surgery and GLP-1 based mechanisms

Bariatric surgery represents the *gold-standard* of obesity treatment in terms of weight loss efficacy (approximately 40% reduction),(14) significantly improving the quality of life.(98) In addition, it has the opportune outcome of enhanced glucose metabolism, eliciting a sustained T2DM regression in both rodents and humans.(14–16) This has triggered the recent scientific popularity of bariatric surgery, aimed at dissecting the underlying molecular pathways, found to be incretin-based. Given the interdependency of obesity and T2DM, the same mechanisms that contribute to weight loss after surgery are probably also implicated in the metabolic improvement witnessed in diabetic patients. In fact, bariatric surgery, instead as being considered strictly for obesity, is more and more labeled as metabolic surgery.

Indeed, there are different types of procedures – malabsorptive and restrictive as bypass techniques with manipulation of GI tract, such as Roux-en-Y gastric bypass (RYGB) and bilio-pancreatic-diversion, and purely restrictive as stomach reduction without exclusion of the small intestine, such as vertical sleeve gastrectomy and adjustable gastric band (AGB).(61,99) The anatomical descriptive characteristics are self-explanatory in respect to the induced weight loss. Regarding the endocrine complementary justification, GLP-1 mediated levels rise three to five times after gastric bypass surgery.(14) Two mechanisms have been claimed to be behind these hormonal changes – the foregut hypothesis states that bypass exclusion of upper gut could change the compositional environment of bile and nutrients and their contact with intestinal mucosa, consequently reducing generation of anti-incretin signals; the hindgut theory argues that hastened gastric transit and accelerated nutrient exposure of ileal L-cells enhances GLP-1 secretion.(14,99) As co-secreted with GLP-1 by L-cells, PYY and OXM also experiment a postprandial increase,(14) potentially further contributing to weight loss. In addition, given that leptin levels and adipose tissue mass are correlated, when body mass diminishes leptin levels lower, improving leptin resistance, while

adiponectin increases.(14) Concerning ghrelin, postprandial levels have been reported to be decreased in rats,(100) but changes in humans are of more complex interpretation as there are discrepancies in postoperative fasting concentrations.(14,100) On the other hand, restrictive AGB also seems to mimic weight loss GLP-1ergic effects by interfering with NTS activation, despite it does not trigger an increase in GLP-1 secretion.(61)

For what is described, bariatric surgery embraces the effects of GLP-1R agonism, raising the possibility of combining surgical intervention with pharmacologic therapy. In particular, AGB has been suggested as an adequate element in this alliance, showing positive results in rats when combined with the GLP-1RA EX-4.(58) The nature of AGB explains its lower efficacy but also lower invasiveness when comparing to bypass maneuvers – as there is no intestinal rerouting, less complications arise.(58) Actually, post-operatory complications, together with the intervention cost and shortage of bariatric surgeons, prevent bariatric surgery from being a widespread population-based therapy.(11) AGB therefore seems a good option in this context, although there is concern for a high late failure rate of this technique.(101) Clinical use of GLP-1RA before bariatric surgery in diabetic obese patients in order to reduce the surgical risk has also been assessed, although with some study design limitations. In a prospective uncontrolled study, Iglesias *et al* reported that twice daily exenatide treatment during six months early reduced body weight and waist circumference, along with improvement of glycemic, lipidic and tensional profiles.(102) Glycemic improvement naturally results from the recovery of postprandial incretin effect and early phase insulin secretion, and to a minor extent from weight-loss related effects, as diminished lipotoxicity enhances insulin sensitivity.(14)

In addition, in the context of bariatric surgery, incretin physiology also involves its enzymatic degradation by DPP4. Given DPP-4's adipokine character, adipose tissue is on the origin of a great amount of its circulating levels, and one can therefore imagine that such

procedures will lead to their reduction. Indeed, Lamers *et al* demonstrated that surgery-induced weight loss restricted DPP-4 release from subcutaneous adipose tissue to levels similar to those observed in lean subjects.(31) Moreover, Cancello *et al* showed that fat reduction following RYGB lead to a significant reduction of macrophages found in subcutaneous adipose tissue of morbid obese patients, with the remaining macrophages found to positively stain for the anti-inflammatory interleukin-10. The expression of chemoattractant genes in adipose tissue, such as MCP-1, suffered a similar reduction after surgery, highlighting the role of the corresponding molecules in macrophage recruitment in obesity.(74) Taking into account what was previously stated in this review in respect to the link between DPP-4 and macrophages, DPP-4i could be, similarly to GLP-1RA, an interesting therapeutic tool to be combined with bariatric surgery. Furthermore, clinical studies following RYGB in morbid obese diabetic patients revealed a reduction in oxidative stress, systemic inflammation and insulin resistance,(103) effects that are also shared to some extent by these drugs.

5.2.1. Bile acids and GLP-1

Bile acids (BAs) also play a role on metabolic homeostasis after bariatric surgery. Classically recognized for promoting cholesterol metabolism, lipid digestion and lipid-soluble vitamins absorption, bile acids are as well engaged in incretin secretion.(99) Recognized changes of these biological molecules levels after bypass surgery justify the growing interest on BAs. Anatomical manipulation of the gut may augment enterohepatic BAs circulation, as bile is drained to the terminal ileum thus promoting BAs reabsorption and further hepatic delivery.(104–106) RYGB-submitted patients presented with plasmatic increment of serum BAs along with lightened postprandial glucose levels and increased GLP-1 concentration.(104) The underlying mechanism involves activation of TGR5, a membrane-

bound G protein coupled receptor mainly expressed in the colon, whose stimulation in enteroendocrine L-cells promotes GLP-1 secretion (Figure 4).(104,106) One can easily suppose the meaningful role BAs seem to play in glucose dynamics, and the fact that dampened gallbladder motility explains the reduced BAs intestinal flow in T2DM subjects (104) supports this connection. But taking a wider step and extending the scenario to obesity, BAs may herald an exciting supplementary explanation for the observed sustained post-operatory weight loss. Scholtz *et al* elegantly showed that feeding reward behavior decreases in a greater degree after RYGB than AGB.(107) As so, in the RYGB group functional MRI assessment demonstrated less degree of food reward-brain region activation, and high-calorie food was perceived as less palatable and tempting when compared to AGB or control group. Moreover, in RYGB subjects hormone profiling was characterized by increased GLP-1, PYY and plasma BAs.(107) Accordingly, other clinical studies have suggested that RYGB-submitted patients present reduced sucrose sensitivity and palatability.(108) GLP-1 and PYY are already known for centrally modulating nutrient ingestion, but some findings also support BAs intervention in this process, as TGR5 expression was demonstrated in brain neurons and astrocyte.(109) Perhaps investigating its presence in areas involved in satiety regulation, such as NTS, would be of interest given the metabolic regulation effects shown by this receptor. Moreover, TGR5 activation in skeletal muscle and BAT is thought to increase resting energy expenditure by mediating thyroid hormone function, namely through conversion of inactive T_4 to active T_3.(104) Nevertheless, the existing little amount of evidence supporting the increase of energy expenditure as an important player in surgery-induced weight loss should motivate future controlled studies in this regard.(108) In any case, BAs physiology raises the question whether its modulation could be a therapeutic target.

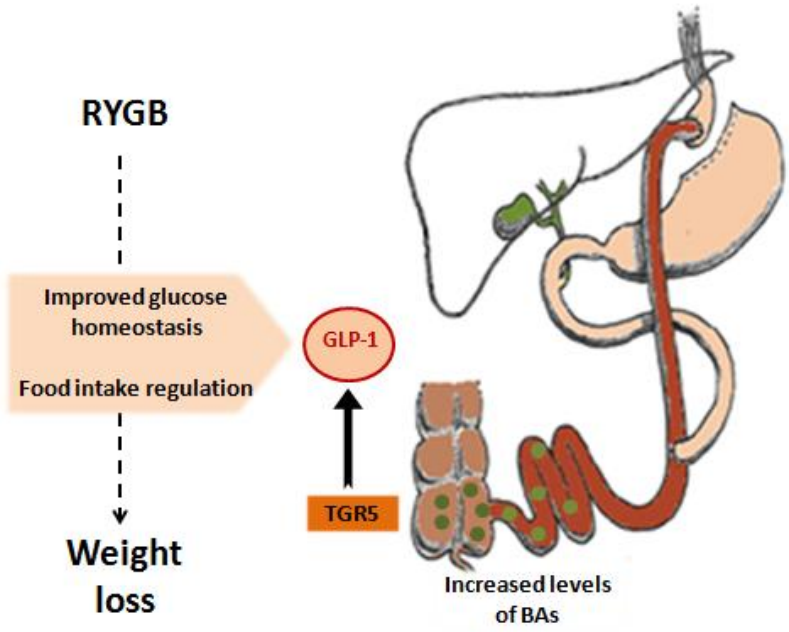

Figure 4 – Weight loss after gastric bypass surgery is partially mediated by an interplay between bile acids and TGR5/GLP-1. Roux-en-Y gastric bypass (RYGB) presumably results in increased exposure of ileal and colon mucosa to BAs. Here, the stimulation of TGR5 promotes GLP-1 secretion from L cells, potentiating the beneficial effects of surgery.

Current pharmacological modulation of BAs metabolism consists of BAs sequestrants, used as secondary oral therapy for hypercholesterolemia.(110) They are nonabsorbable resins that bind to BAs thus preventing ileal entrance in enterohepatic circulation, with consumption of cholesterol to form new BAs.(111,112) However, some BAs reach the colon, where they bind to cell-surface TGR5.(112) The existing studies focus on BAs sequestrants conceivable application in T2DM as they ameliorate chronic hyperglycemic and hyperinsulinemic profile in both animals and humans.(111) Regarding obesity, Potthoff *et al* concluded that administration of the sequestrant colesevelam to DIO mice ameliorated glycemic regulation profile, partially through increased glycogen storage mediated by TGR5/GLP-1.(112)

For what was explained, BAs seem to contribute to the well established impact of bariatric surgery in obesity. This raises the question whether associating BAs sequestrants to this surgical intervention could optimize surgery outcomes. After all, altered BAs

enterohepatic circulation is a common feature of both approaches, and therefore a synergic potentiating could be of benefit. Thinking of another association, adding the use of GLP-1RA to bariatric surgery may also turn out to provide valuable repercussions in highly selected situations. A third question inevitably arises – what is the value of the association of GLP-1RA with BAs sequestrants? Weight loss would be amplified, and impaired glucose homeostasis and dyslipidemia, so commonly present in obese patients,(2) would also be improved. As a matter of fact, one of the drawbacks of BAs sequestrants' mechanism of action is the increase in plasma TGs levels,(111) which justifies the association of this medication with other antidyslipidemics.(110) Given the pleiotropic effects of GLP-1RA on lipid profile, there might be an extra rationale for such a therapeutic alliance.

The general mechanistic principles behind bariatric surgery outcomes are progressively being unraveled. Weight loss is mainly conceived to result from reduced energy intake, which seems to have a multifactor origin, comprising early satiety and altered food taste perception. In addition, the latter may further contribute to the recognized improved executive control of feeding behavior. CTA has also been implicated since bariatric surgery elicits gastrointestinal symptoms (fullness, pain, nausea and vomiting),(108) and GLP-1 involvement in CTA has already been described in a previous section. From what is explored in this work, mediation of these processes by gut hormones and BAs seems to be of extreme importance, despite the forthcoming need of interventional rather than descriptive approaches as proposed in a recent review.(108) In fact, the mechanisms underlying weight loss after bariatric surgery are so complex and involve distinct parts of gut-adipose-brain axis that it is somewhat difficult to identify if weight loss is the cause or the consequence.

6. Conclusion

The serious proportions that obesity is reaching, presenting a high morbidity and mortality rate and accounting for the great public health expense,(1) impose the development of adequate treatments. Bariatric surgery is currently the only effective approach that provides a significant sustained weight loss in obese patients.(14) Unraveling the mechanisms behind bariatric surgery effects has brought us closer to understand the complex physiological interplay between GLP-1 and other regulators of food intake. Even though that GLP-1 effect on weight reduction is only moderate when translated to practice with GLP-1RA, it is undeniably valuable. First, its antidiabetic properties support their application in obese diabetics. Second, their pleiotropic effects may represent an advantage regarding the inflammatory background of obesity and its related complications. Third, when associated to other therapeutics the final weight loss outcome could be enhanced.

Getting down to brass tacks, IR is crucial to interconnect diabetes and obesity and the arising complications. Adipose tissue is in fact a dynamic organ whose inflammatory properties had already been recognized in different contexts. Contrarily, fat-induced inflammation is harmful in the obese diabetic situation, in which neoangiogenesis allows the expansion of fat tissue and the secreted pro-inflammatory factors sustain a self-fed vicious cycle. DPP-4 is actually part of this loop, an adipokine either aggravating the incretin defect or stimulating lymphocyte proliferation. These cells, together with macrophages, are part of the dysfunctional immune response that underlies IR.

What becomes clear from this review is that GLP-1 definitely modulates food intake, and this priceless feature should continue to be exploited in therapeutic terms. Incretin-based therapies indeed improve metabolic control with minimal adverse effects, and long-term risk remains to be proven.(113) Besides, putative cytoprotective effects have been demonstrated. Further investigation is warranted, not only to clarify basic molecular mechanisms, but also to

verify the concrete translation of these assumptions into tangible clinical outcomes, through randomized control trials with large study populations.

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