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Review

Oxyntomodulin: Actions and role in diabetes

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ABSTRACT

Oxyntomodulin is a product of the glucagon precursor, proglucagon, produced and released from the endocrine L-cells of the gut after enzymatic processing by the precursor prohormone convertase 1/3. It corresponds to the proglucagon sequence 33–69 and thus contains the entire glucagon sequence plus a C-terminal octapeptide, comprising in total 37 amino acids. As might have been expected, it has glucagon-like bioactivity, but also and more surprisingly also activates the receptor for GLP-1. This has given the molecule an interesting status as a glucagon-GLP-1 co-agonist, which is currently attracting considerable interest for its potential in the treatment of diabetes and obesity. Here, we provide an update on oxyntomodulin with a focus on its potential role in metabolic diseases.

1. Introduction

Historically, it was realized already in the 1960's that endocrine cells in the gut contain material that reacts with antibodies against glucagon, hence the designation gut glucagon-like immunoreactivity (gut-GLI or "enteroglucagon") [1]. Biochemical purification of the immunoreactive material from gut extracts showed that two separate substances, a larger and a small molecule, were responsible for the immunoreactivity [2].

Further isolation and characterization showed that both substances contain the full glucagon sequence [3,4] and that the smaller one might constitute a fragment of the larger molecule. The larger molecule, glicentin ("gli-" for glucagon-like immunoreactivity, and "cent" because Sundby et al. [5] originally thought that it contained 100 amino acids) turned out to comprise 69 amino acids [6] and the smaller one 37 amino acids corresponding to the C-terminal 37 amino acids of the larger molecule [4,7] (Fig. 1). The glucagon sequence occupies position 33–61 of glicentin and it was therefore proposed that glicentin constitutes at least a part of the biosynthetic precursor, also for pancreatic glucagon, and this was supported by the identification of (and secretion of) both the 1–30 fragment of glicentin [8], as well as (small amounts of) the full glicentin molecule in the pancreas [9]. The subsequent cloning of the cDNA encoding the full proglucagon molecule confirmed this hypothesis [10].

The sequence of the smaller peptide was presented for the first time by two independent groups in Stockholm in 1982, and Dominique Bataille who had isolated the peptide together with Victor Mutt at the Karolinska Institute in Stockholm [7] had observed that the molecule influences acid secretion in certain stomach and parietal cell preparations [11], and proposed the catchy name: oxyntomodulin (with the name "oxynto-' derived from the "oxyntic glands' responsible for secretion of acid). Other groups were more interested in the possible glucagon-like effects of oxyntomodulin on pancreatic secretion of especially insulin (the incretin effect) and, indeed, oxyntomodulin preparations of varying purity appeared to be able to stimulate insulin secretion [12], a finding which was confirmed with synthetic oxyntomodulin [13].

The intestinal processing of proglucagon thus results in the formation of glicentin and oxyntomodulin + proglucagon 1–30 (glicentin-related pancreatic polypeptide, GRPP), but rarely glucagon [14]. (Glucagon is normally found in concentrations comprising < 1% of total (most often not detectable) but may be produced in significant amounts after surgical operations on the upper GI tract, notably Roux-en-Y gastric bypass and reconstruction after total pancreatetomy [15]). Proglucagon also gives rise to additional products with biological activity, namely glucagon like peptides 1 & 2. It remains unresolved whether oxyntomodulin is a specific product of intestinal proglucagon processing, or whether it mainly results from chemical instability of glicentin in solution. A specific enzyme catalyzing the cleavage has not been identified, but the prohormone convertases would be expected to be able to do so because of the presence of two basic amino acids between the GRPP and oxyntomodulin sequences. Since this cleavage occurs extensively in the pancreas but not in the gut, PC2 might be expected to be particularly effective (see below). Oxyntomodulin does

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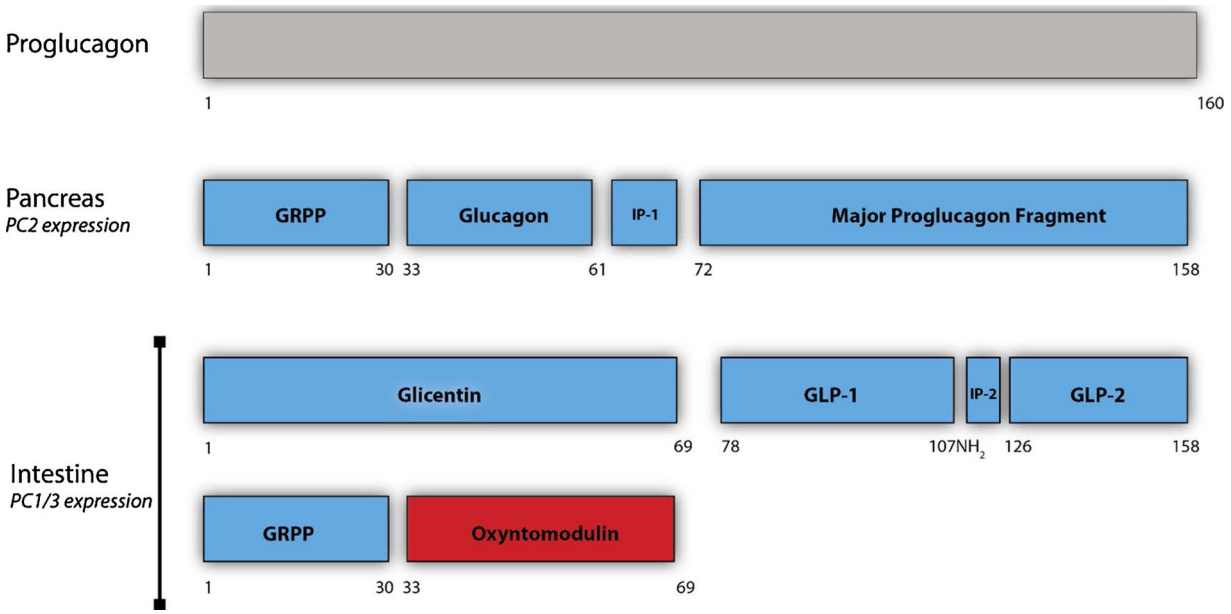

Fig. 1. Processing of Proglucagon to Oxyntomodulin.
In the pancreas, proglucagon is processed by prohormone convertase-2 to glicentin-related pancreatic polypeptide (GRPP), glucagon, a short intervening peptide (IP-1) and the so-called major proglucagon fragment. In the intestine, proglucagon is processed by prohormone convertase 1/3 activity to glicentin [1–69], which may be further cleaved into glucagon-reactive polypeptide (GRPP) and oxyntomodulin [33–69]. Due to these processing patterns, immune-based methods relying on a single antibody will not be specific for oxyntomodulin: antibodies raised against the N-terminal region will cross-react with glucagon, C-terminal antibodies with glicentin, and 'side-viewing' antibodies will bind to glucagon and glicentin. Full specificity requires a 'sandwich' approach, involving a combination of N- and C-terminally directed antibodies.

not seem to be formed from solutions containing glicentin, but may be formed in the circulation *in vivo* [16]. However, oxyntomodulin constitutes about 1/3 of the amount of extractable 'enteroglucagon' (gut GLI) [17], it is secreted from isolated gut segments in similar proportions, and also in the circulation it appears to make up about a third of total "enteroglucagon", in fasting plasma as well as in postprandial (stimulated) samples [18,19]. This would be consistent with a biosynthetic processing of proglucagon to 1/3 oxyntomodulin and 2/3 glicentin. A similar ratio is found in extracts of the brain stem where proglucagon is expressed in a group of neurons in and around the nucleus of the solitary tract [20], suggesting that it is not the intestinal milieu that is responsible for the formation. The processing at residues 31 and 32 (Lys-Arg) corresponds to the sites where PC-1 may cleave [21], and transfection experiments support that PC 1/3 may catalyze this cleavage [22,23] but apparently PC-2 may also result in cleavage *in vitro* [22] and other trypsin-like endopeptidases are capable of similar cleavages; thus, the processing mechanism cannot be said to be firmly established so far. The concentrations of enteroglucagon in the intestinal wall amount to around 100–200 pmol/g intestinal wet weight (a rather "high" concentration for a gut peptide), and as mentioned oxyntomodulin makes up about a third of this [17].

Oxyntomodulin is not produced in any appreciable amounts in the pancreas [24] under normal conditions, although increased PC1/3 activity has been reported leading to increased active GLP-1 in such cases [25,26]. Although traces of glicentin may be formed in the pancreas, oxyntomodulin has not been identified with certainty [9,24]. A number of studies have pointed to expression of PC1/3 in rodent alpha cells in the developing pancreas and during adaptation to pregnancy [27,28]. Similar alterations may occur during diabetes development in animals [29]. Most studies on altered processing in the alpha cells leading to formation of GLP-1 (and potentially oxyntomodulin) are based on immunohistochemical findings, but such studies are generally misleading because most GLP-1 antibodies will crossreact with proglucagon 72–107 (i.e. N-terminally extended GLP-1 which is inactive), which is a normal constituent of the pancreas [24]. Unequivocal demonstration of GLP-1 (as well as oxyntomodulin) in the pancreas therefore requires extraction and chemical identification.

2. Measurement

Oxyntomodulin has for long been difficult to measure in biological fluids because of its similarity to glicentin and glucagon. Thus, it is not possible to measure oxyntomodulin with a single antibody technique. A sandwich technique is theoretically possible but requires that both of the two antibodies involved are "terminal wrapping", meaning that they require the full, unmodified terminal of the peptide for reaction, allowing neither prolongations nor abbreviations of the termini they are directed against. Previously, measurements of oxyntomodulin relied upon assays for glucagon based on so-called "side-viewing" antibodies, which would pick up all proglucagon-derived moieties containing a mid-sequence of the glucagon molecule, followed by chromatography to separate oxyntomodulin from the other glucagon containing moieties by size [30]. As mentioned, such measurements identified oxyntomodulin to constitute about 1/3 of the entire "enteroglucagon" content in intestinal extracts and fasting and postprandial plasma samples. In plasma, this would correspond to concentrations around at the most 10–30 pmol/L. These findings were recently confirmed by mass spectrometry [31]. In addition, a sandwich ELISA has been established which, although not completely specific (10% cross-reaction to glicentin), appeared useful in validation experiments [31]. Measurements with the sandwich ELISA confirmed the previous chromatography data, including the estimates of circulating plasma concentrations. A couple of commercial assays for oxyntomodulin have been announced, but turned out, so far, to be totally misleading (giving 1000-fold readings in excess of those confirmed by mass spectrometry), emphasizing the need for all investigators to evaluate the reliability of commercial assays before use [32].

3. Secretion

As already mentioned, oxyntomodulin seems to rather constantly constitute about a third of circulating "enteroglucagon", and this means that data obtained with assays for "enteroglucagon" to a large extent also apply to oxyntomodulin (although the concentrations are lower). This means that any stimulus to the L-cell, whether determined from

changes in GLP-1, GLP-2 or enteroglucagon secretion will also apply to oxyntomodulin. Thus, luminal nutrients, including carbohydrates, lipids as well as protein will stimulate secretion, and the cellular mechanisms governing L-cell secretion will also apply. A neural regulation is unlikely to be significant [33]. Oxyntomodulin secretion in pathological conditions has very rarely been specifically investigated, but data obtained with the validated sandwich ELISA mentioned above suggested that the meal-induced responses were slightly lower in obese subjects and in subjects with type 2 diabetes concentrations [31]. In conditions with accelerated intestinal nutrient entry such as gastric bypass, responses were greatly exaggerated and concentrations measured specifically reached as high as 50–100 pmol/L which may be biologically relevant [31,34,35]. In humans, the metabolic clearance rate of plasma oxyntomodulin is around 5 ml/kgx min and its half-life about 12 min as determined after intravenous infusion of synthetic oxyntomodulin to steady state levels [36]. Oxyntomodulin may be subject to DPP-4 dependent degradation [34] which is why DPP-4 resistant oxyntomodulin analogues have been developed[37]. In theory, oxyntomodulin may also be subject to neutral endopeptidase degradation as shown for glucagon and recently for GLP-1 in mice [38].

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4. Actions

4.1. Effects on the pancreatic islets

A specific receptor for oxyntomodulin was extensively searched for [39], but was never identified. However, results from several groups using various experimental approaches clearly identified interaction with the glucagon receptor (consistent with the concept that activation of CGCR requires the N-terminal part of the glucagon molecule), but with an affinity of around 1/50–1/100 of that of glucagon [13]. This means that oxyntomodulin may affect insulin secretion via the GCGR expressed by beta cells as well as act on the hepatocytes, the classical site of GCGR expression, where it may lead to glucose production [40,41]. However, oxyntomodulin also interacts with the GLP-1 receptor, again with an affinity of about 1/100 compared to GLP-1 [42]. Interactions with this receptor have been probed experimentally with the use of the GLP-1 receptor antagonist, exendin-4 [9–39]. Oxyntomodulin, therefore, may also stimulate insulin secretion via the GLP-1 receptor. The overall actions of oxyntomodulin on glucose metabolism are less clear, since the actions on hepatic glucose production and insulin secretion would be expected to pull in different directions [43], but in mice, glucose tolerance was reported to be improved rather than inhibited by oxyntomodulin [44,45], mainly due to a stimulation of insulin secretion. Oxyntomodulin, has, surprisingly, been reported to increase glucagon secretion, both *in vitro* [13] and *in vivo* [46], and this would be expected to counteract the actions of the stimulated insulin secretion. This is clearly in contrast with oxyntomodulin's ability to activate the GLP-1R, which classically leads to lowered secretion rates of glucagon, and to the suggested autoregulation of glucagon secretion given that oxyntomodulin activates GCGR located on the pancreatic alpha cells.

4.2. Effects on gastric acid secretion

Regarding its actions on gastric acid secretion, there is more confusion. Originally, oxyntomodulin was found to inhibit acid secretion from isolated gastric glands more potently than glucagon (hence the name) [11,39] and it also inhibits acid secretion, when infused into man to reach slightly supraphysiological concentrations [36,47]. However, the question remains whether the gastric actions depend on interactions with GLP-1 or glucagon receptors or both, since both glucagon are GLP-1 powerful inhibitors of acid secretion [42]. The mechanism involved is unlikely to be direct since both GLP-1 and oxyntomodulin (via the GLP-1R) were reported to *stimulate* acid secretion (and cAMP) from isolated parietal cells [48,49]. An indirect action via stimulation of somatostatin cells in the gastric glands seems more likely (and was observed for GLP-1 in rats [50] but not in pigs [51]), and actions *in vivo* via inhibition of vagal efferent activity are also probable [52]. Oxyntomodulin also inhibits pancreatic exocrine secretion, but this effect may be secondary to its inhibitory action on gastric emptying (which may strongly influence postprandial glucose excursions as well) [47]. It is unclear whether physiological (postprandial) oxyntomodulin concentrations are sufficient influence gastric acid secretion *in vivo*.

4.3. Effects on food intake and body weight

The effect of oxyntomodulin on food intake and appetite has attracted considerable recent interest. Again both of the "parent" peptides, glucagon and GLP-1, have effects on appetite and food intake [53,54]. The anorexic actions of GLP-1 turned out to be of sufficient magnitude to allow development of long acting agonists that are now approved for obesity therapy [55], and a new GLP-1 analogue was recently reported to produce weight losses of up to 13.8% over a one year period (press release from Novo Nordisk, 2017 regarding semaglutide). As a weight losing agent, glucagon has received a lot less attention, undoubtedly because its hyperglycemic and diabetogenic potential would seem incompatible with a clinical application for obesity. Nevertheless, there is ample support in the literature for a pronounced effect of glucagon on both appetite and body weight (see the excellent review by N. Geary [56]). Several mechanisms might be involved. Geary et al. identified an acute central mechanism, mediated via vagal sensory afferents, whereby glucagon would influence appetite and food intake [56]. Patients with glucagon producing tumors [57,58] typically show major weight loss and loss of appetite [59,60], but in these patients, the weight loss is also associated with muscular wasting presumably due to accelerated amino acid turnover [61]. Rats with a transplantable glucagon-producing tumor showed profound anorexia and died from starvation as circulating glucagon levels increased after transplantation [62]. The tumor, on the other hand, also produced GLP-1 (which is of similar pharmacological interest) so the effects cannot be ascribed to glucagon alone [58,63]. However, the recent pharmaceutical development of long-acting glucagon agonists has provided striking evidence for a pronounced effect of glucagon alone [64]. The metabolism of glucagon, particularly in rodents is extremely rapid, but with long-acting (PEGylated or acylated) derivatives a more or less continuous exposure can be maintained and with such agonists, it is possible to induce massive weight loss (> 30% of body weight). This leaves little doubt that glucagon has the potential to inhibit food intake.

Oxyntomodulin itself also acts to inhibit food intake and thus leads to weight loss (37;65). In pair-feeding experiments, it was more efficient than paired restrictions in food intake, suggesting that oxyntomodulin (and glucagon) may also increase energy expenditure [66]. Indeed, this has been confirmed by direct experiments, even in humans, where oxyntomodulin injected 3 times daily s.c. caused a weight loss of 2.3 kg over 4 weeks [67]. In measurements of energy expenditure there was an overall increase in energy expenditure [68]. There was no effect on basal metabolic rate but there was a reported (small) effect on activity-related expenditure. There were no reported side effects, such as itching or restlessness. In rodents, its effects on food intake were abolished by exendin-4 [9–39] and absent in GLP-1R knockout mice [42]. The effect on energy expenditure, however, was not affected and may thus be due to activation of the glucagon receptor [69]. This may or may not be secondary to activation of the sympathetic nervous system [70–72].

5. Receptor pharmacology and physiology of oxyntomodulin

To evaluate this, it is important to consider the circulating levels of oxyntomodulin and the receptor interactions of oxyntomodulin. As discussed above, plasma levels are generally low (0–30 pmol/L), and

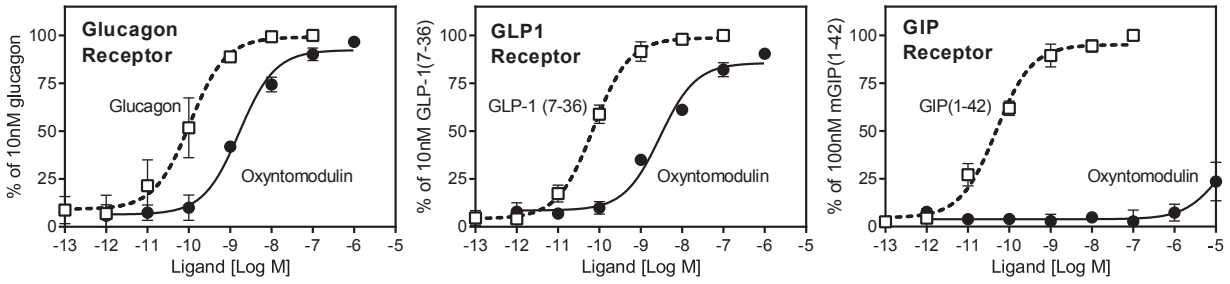

Fig. 2. Receptor activation profile of oxyntomodulin.
[86]COS-7 cells transiently expressing the glucagon, GLP-1 and GIP receptors following transfection using the calcium phosphate precipitation method with the addition of chloroquine. Receptor mediated cAMP accumulation was measured following the instructions of the HitHunter™ cAMP XS assay (DiscoveryRx, Herlev, Danmark) [87].The cells were washed twice in Hepes-buffered saline (HBS) buffer and incubated with 1 mM 3-isobutyl-1-methylxanthine (IBMX) for 30 min at 37 °C. Then either oxyntomodulin (black circles) or the cognate ligand for each receptor (white squares) were added and incubated for an additional 30 min at 37 °C and the intracellular cAMP accumulation was measured (studies carried out in the authors' laboratory).

given that its potency for both the GLP-1 and the glucagon receptor is lower than those of the cognate ligands, it seems unlikely that oxyntomodulin plays any significant metabolic/gastrointestinal role. In agreement with this, infusions of oxyntomodulin to physiological levels have little effect. However, although lower than the cognate ligands, the potency of oxyntomodulin is still in the nanomolar range for the glucagon- and the GLP-1 receptor. Fig. 2 illustrates the potencies in the murine system, where oxyntomodulin activates the glucagon- and the GLP-1 receptor with potencies (EC_{50} values) of 1,7 and 2,9 nM, respectively, compared to EC_{50} values of 107 and 72 pM for glucagon and GLP-1 on their respective receptors. In contrast, no activation is observed of the GIP receptor in concentrations up to 1 uM. These findings are consistent with previously described pharmacodynamics of oxyntomodulin [42,73]. As the oxyntomodulin concentration may increase considerably in conditions with accelerated gastric emptying/increased intestinal nutrient delivery, such as surgical operations of the gastrointestinal tract including gastric bypass surgery, a contribution to the changes of metabolism and body weight cannot be excluded in these cases, although this has not yet been investigated. However, given that the concentrations of for instance GLP-1 are also greatly increased in these patients and considering the ~50-fold higher potency of GLP-1 compared to oxyntomodulin on the GLP-1 receptor, it still seems that the relative role of oxyntomodulin must be limited. In human studies involving infusions to a concentration of ~300 pmol/L, there was no effect on energy expenditure, but both composite appetite scores and food intake were reduced. The effect was similar to that of physiological infusions of GLP-1 and glucagon together [74].

6. *In vivo* pharmacology and the perspectives of combination therapy versus co-agonism

Although the physiological role of oxyntomodulin may be limited, administration of pharmacological amounts may have considerable therapeutic potential, by simultaneously targeting both the glucagon and the GLP-1 receptors with resulting, perhaps additive, effects on appetite and food intake as discussed above. Given the structural requirements for ligand binding and subsequent receptor activation in class B receptors and the receptor expression profiles of the GLP-1 and the glucagon receptor, it is highly unlikely that a co-agonist would be able to activate two separate receptor molecules simultaneously. Indeed, co-administration of the two may under certain condition have additive effects, and this is likely to occur also in humans (although the effect was not miraculous as mentioned above [75]), where the hyperglycemic effect of glucagon may be neutralized by addition of GLP-1, due to the combined insulin-stimulating effects.

Undoubtedly because of the phylogenetic similarities between the two peptides and between the two responsible receptors, it is also possible to develop "co-agonists" and/or chimeric peptides with agonism for both receptors [64,65,76–78]. This is of particular interest for the pharmaceutical industry, since development of a co-agonist for clinical use requires a single development program, whereas a combination of two agents (glucagon and GLP-1) for regulatory reasons implies two (expensive) development programs. It has also been possible to modify the co-agonists to provide degradation resistance and long half-lives, and such co-agonists are currently in clinical development [79,80]. Because of the intrinsic dual agonism, the hyperglycemic risk of the glucagon part may apparently be abrogated by the GLP-1 part, whereas the weight loss potential is enhanced. In a study in diabetic rhesus monkeys, a co-agonist showed superior weight loss compared to a long acting GLP-1 agonist and improvements in glycemic control [81], and similar findings were made in another study in cynomolgus monkeys [78]. A co-agonist from Medimmune was demonstrated to improve steatohepatitis and liver regeneration in mice [78]. The clinical development of such co-agonists is difficult however, because the optimal ratio between the two agonist activities is difficult to extrapolate from findings in experimental animals due to differences between pharmacokinetics and receptor pharmacodynamics in animals and humans, whereas with the two-agonist combination approach, the optimal ratio can be determined clinically. An optimal ratio is critical to avoid diabetogenic reactions while obtaining maximum effects on appetite/food intake. Potential side effects of the co-agonists (and the two-agonist combination) should also be considered. Both agonists may cause gastrointestinal side effects including nausea and vomiting, and combinations might be additive. Both agonists increase heart rate, and although glucagon also is known to have inotropic effects and although long-term GLP-1 agonist therapy may be associated with improved cardiovascular risk [82,83], there is no guarantee that a co-agonist is without cardiovascular problems as for instance observed with certain DPP-4 inhibitors [84]. Glucagon physiology and co-agonist developments were recently reviewed [85].

7. Conclusions

Oxyntomodulin is a dual agonist interacting weakly with both the glucagon and the GLP-1 receptor, but physiologically oxyntomodulin seems to be a waste product of limited biological significance, and abnormalities of secretion do not seem to play a role in metabolic or surgical conditions including type 2 diabetes and post-gastric bypass surgery. However, the co-agonism is of interest because its glucagon and GLP-1 effects on food intake may be additive, whereas the diabetogenicity of the glucagon part may be offset by the antidiabetic potential of the GLP-1 part. Preclinical studies support the further development of therapies for obesity based on this combination.

Declaration of interest

We have no conflicts of interest to declare.

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