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REVIEW

Glucagon-like peptide 1 in the pathophysiology and pharmacotherapy of clinical obesity

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Abstract

Though the pathophysiology of clinical obesity is undoubtedly multifaceted, several lines of clinical evidence implicate an important functional role for glucagon-like peptide 1 (GLP-1) signalling. Clinical studies assessing GLP-1 responses in normal weight and obese subjects suggest that weight gain may induce functional deficits in GLP-1 signalling that facilitates maintenance of the obesity phenotype. In addition, genetic studies implicate a possible role for altered GLP-1 signalling as a risk factor towards the development of obesity. As reductions in functional GLP-1 signalling seem to play a role in clinical obesity, the pharmacological replenishment seems a promising target for the medical management of obesity in clinical practice. GLP-1 analogue liraglutide at a high dose (3 mg/d) has shown promising results in achieving and maintaining greater weight loss in obese individuals compared to placebo control, and currently licensed anti-obesity medications. Generally well tolerated, provided that longer-term data in clinical practice supports the currently available evidence of superior short- and long-term weight loss efficacy, GLP-1 analogues provide promise towards achieving the successful, sustainable medical management of obesity that remains as yet, an unmet clinical need.

Key words: Obesity pathophysiology; Glucagon-like peptide 1 analogues; Glucagon-like peptide 1; Clinical obesity

Core tip: Several lines of clinical evidence implicate an important functional role for glucagon-like peptide 1 (GLP-1) signalling in the pathophysiology of clinical obesity. Here we critically evaluate such findings in way that as yet has been unexplored; using the well established roles of GLP-1 as an incretin and meal to meal satiety signal to go some way toward explaining findings from interventional and observational clinical data that suggest functional deficits of GLP-1 to be a contributor to the obesity phenotype. We also explore

the promise shown by GLP-1 analogues in achieving and maintaining significant weight loss in obese individuals, and use findings to discuss to what extent they too may support a role for GLP-1 in obesity pathophysiology. We conclude by exploring what an association with functional GLP-1 deficit could mean for the clinical management of obesity; conducting cost and risk benefit analyses to evaluate the extent to which GLP-1 analogues may provide a successful and sustainable option for the medical management of obesity that remains as yet, an unmet clinical need.

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INTRODUCTION

Public health and economic impacts of obesity

Obesity is a global epidemic, perhaps the greatest challenge to global and public health of our time. With a doubling in prevalence from 1980 to 2008[1], 13% of the world's population at present are obese [body mass index (BMI) $\geqslant$ 30 kg/m^2] and 39% overweight (BMI $\geqslant$ 27 kg/m^2)[2]. If recent trends continue, by 2030 up to 57.8% of the world's adult population will be overweight or obese[3] (Figure 1). The World Health Organisation (WHO) has estimated that 44% of the global diabetes burden, and 23% and 7%-41% of the burdens for ischaemic heart disease and specific cancers respectively can be attributed to being overweight or obese[4]. Psychosocially, stigma and discrimination toward obese people can have consequences for psychological as well as physical health[5], with impaired quality-of life[6] and increased rates of depression[7] reported in this group. Even modest losses of 5%-10% of total body weight are associated with reduced risk of comorbidities in obese individuals[8-10]. Therefore, effectively managing rates of obesity is a major goal in public health policy.

In addition to its physical and psychological burdens, obesity and its comorbidities impose disproportionately high healthcare and economic demands at individual and societal levels[11]. Affecting the wider economy indirectly through increased rates of worker illness absenteeism and resultant losses in productivity, healthcare systems are burdened from direct healthcare related costs; obese individuals on average incurring healthcare related costs 30% greater that their healthy weight peers[12-16]. A global systematic review has estimated the direct costs of obesity related diseases to account for between 0.7% and 2.8% of a country's total healthcare expenditure[16]. In the United Kingdom alone, direct costs to the National Health Service (NHS) of treating overweight and obesity, and related co-morbidities were estimated at £5.1 billion in July 2006; representing around 5% of total NHS spending[17,18]. A computer based micro-simulation model predicting the direct healthcare related costs of overweight and obesity in the United Kingdom should 2001 prevalence remain constant, has forecasted the NHS spending £15.4 billion and £22.5 billion in 2015 and 2050 respectively[18,19] on the direct health costs of treating overweight and obesity and related co-morbidities in England alone. An upward trajectory prevented by significant weight loss in those currently obese (Figure 2), findings imply that whilst the prevention of obesity is the strategic imperative, the effective management of those already obese is an immediate priority.

Current management of obesity

Current medical management of obesity involves lifestyle, pharmacological and surgical interventions[20]. Lifestyle intervention, in the form of dietary, behavioural and exercise counselling, are currently the suggested first line treatment for obesity; however, whilst a recent meta-analysis reports such interventions to show small but significant benefits on weight loss maintenance, weight loss achieved and sustained with lifestyle intervention alone remains suboptimal[20-24]. In the face of such challenges, a number of pharmaceuticals have been marketed to assist weight management over the years[25,26] (Table 1). However, adverse effects of some and the transient weight losses associated with others[27] mean that the pharmacological management of obesity remains suboptimal. The only proven treatment to achieve and maintain weight loss in obesity is bariatric surgery[28-30]. However, surgical and anaesthetic risks associated with overweight and obese status sees these invasive procedures reserved to those patients classed morbidly obese (BMI $\geqslant$ 40 kg/m^2) or as a last resort in those failing more conservative management[20,31,32]. The minimally invasive and efficacious management of obesity therefore, remains an unmet clinical need.

Glucagon-like peptide 1 and the management of human obesity

The ideal management of any illness involves an understanding of its underlying pathophysiology; greater understanding facilitating the development of targeted pharmacotherapies to either replete physiological factors pathologically depleted, or antagonize pathological processes. The pathophysiology of obesity however, remains poorly understood. The WHO has defined the current obesity crisis epidemiologically, as the consequence of an increasing imbalance between energy intake and expenditure[33]. Physiologically, energy balance is a closely regulated system involving interactions between peripheral endocrine, nutritional and neural signals acting on regulatory central hypothalamic and hedonic brain regions[34-36]. Clinical obesity has been associated with deregulations in both homeostatic and hedonic controls of energy balance potentially facilitated by impaired glucagon-like peptide 1 (GLP-1) signalling[35-37] (a role for GLP-1 in the pathophysiology of clinical obesity). Pharmacologically targeting GLP-1

Table 1 Current and previously Food and Drug Administration licenced anti-obesity pharmacotherapeutics

Drug	Mechanism	Year	Clinical use and limitations	Suspension reason
Currently FDA licenced drugs				
Diethylpropion	NA releasing agent	1959	FDA approved for short term use (3 mo); not recommended with uncontrolled hypertension or heart disease	-
Phentermine		1959	FDA and EMA approved for long term use; treatment dependent weight loss	-
Orlistat (Xenical)	-	1999		-
Orlistat (Alli)	Pancreatic lipase inhibitor	2007		-
Phentermine-topamirate (Qysmia)	-	-	Approved for long term use; treatment dependent weight loss	-
Lorcaserin (Belviq)	5HT2c-R antagonist	2012	FDA approved for long term use, recommended in those with cardiovascular disease; treatment dependent weight loss	-
Liraglutide (Saxenda)	GLP-1 analogue	2014	FDA and EMA (2015) approved	-
Previously FDA licenced drugs				
Dinitrophenol	Unknown	1938	-	Dermatitis, neuropathy, agranulocytosis, visual impairment, death
Aminorex	Unknown	1968	-	Chronic pulmonary hypertension
Amphetamines	Monoamine reuptake inhibitor	1971	-	Addiction, hypertension, myocardial toxicity
Fenfluramine	Serotonin reuptake inhibitor	1997	-	Valvular heart disease
Phenylpropanolamine	NA-R and DA-R agonist	2000	-	Haemorrhagic stroke
Rimonabant	CB1R antagonist	2009	-	Psychiatric disorders, depression, suicidal ideation
Sibutramine	Serotonin-NA reuptake inhibitor	2010	-	Risk of major cardiovascular events

The pancreatic lipase inhibitor Orlistat and GLP-1 analogue liraglutide are the only currently UK licenced anti-obesity agents). 5HT2c: Serotonin receptor; NA: Noradrenaline; DA: Dopamine; CB1R: Cannabinoid receptor; R: Receptor; FDA: Food and Drug Administration; EMA: European Medical Association.

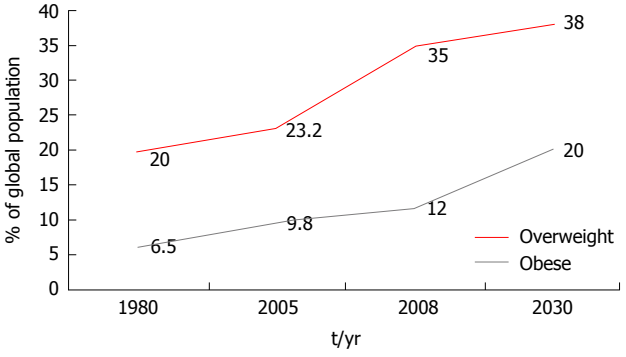

Figure 1 Global overweight and obesity trends and projections. If recent trends continue unabated, by 2030, 38% and 20% of the world's adult population are projected to be overweight or obese respectively[8].

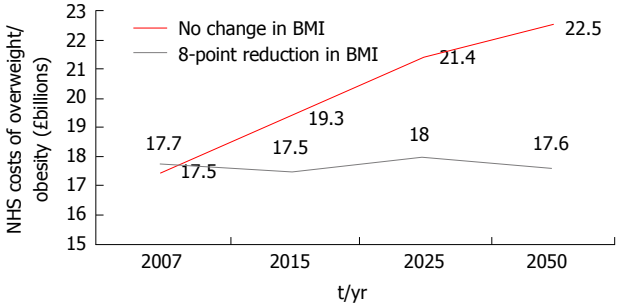

Figure 2 Projected trends in National Health Service costs from a micro-simulation model. A computer based micro-simulation model[18] predicted the direct healthcare related costs of overweight and obesity in the United Kingdom at five time points from 2001 (2001, 2007, 2015, 2025 and 2050) should prevalence of overweight and obesity remain constant (red) or if an average of an 8-point BMI reduction was achieved in all those obese in 2001 (grey). BMI: Body mass index; NHS: National Health Service.

therefore, may go some way towards achieving the successful and sustainable medical management of clinical obesity that as yet remains to be achieved.

GLP-1 is a 31 amino acid polypeptide primarily synthesized by the enteroendocrine L cells of the terminal ileum. Amongst its pleotropic central and peripheral effects, GLP-1 acts as a potent incretin first clinically used in the medical management of overweight or obese individuals with type 2 diabetes mellitus (T2DM)[38]. The repeatedly demonstrated ability of GLP-1 analogues to induce weight loss in this cohort[39,40] prompted phase Ⅲ trials studying the weight loss efficacy of the GLP-1 analogue liraglutide (3 mg; trade name Saxenda) *vs* placebo[41] and the pancreatic lipase inhibitor orlistat[42,43] (the only anti-obesity drug licensed in the United Kingdom) in non-diabetic overweight and obese adults. The greater weight loss efficacy achieved and maintained by GLP-1 analogues prompting the Food and Drug Administration (FDA) in 2014 to approve Saxenda as the first GLP-1 analogue for use as a weight loss aid in obese

adults and overweight adults with at least one weight related co-morbidity[44]. March 2015 saw the European Medical Association (EMA) grant marketing authorization for 3 mg liraglutide under the FDA approved criteria in all 28 European Union (EU) states[45]. However, launching in April 2015 in the United States at a cost of over $1000 per patient a month, cost-benefit is of greater issue in EU nations such as the United Kingdom where health care is primarily socially funded; undoubtedly contributing to the uncertainty of launch plans in the United Kingdom at present[46]. Clinical evidence however implicates a role for functional impairments in GLP-1 signalling in the pathophysiology of obesity, GLP-1 agonism therefore may be the first truly targeted therapeutic in the medical management of clinical obesity. Therefore, with its superior clinical efficacy to currently United Kingdom licensed therapies benefiting patients through greater achieved and maintained weight loss and the economy through the potential to reduce long-term financial burdens of obesity, the cost-benefit spectrum may therefore be swayed, favouring the use of GLP-1 analogues in the medical management of obesity in the United Kingdom[46].

THE HOMEOSTATIC AND HEDONIC CONTROL OF ENERGY BALANCE

Physiologically, energy balance is a closely regulated system involving interactions between peripheral endocrine, nutritional and neural signals acting on regulatory central hypothalamic[34] and hedonic[35,36] brain regions. Where previously the neurocircuits mediating the homeostatic and hedonistic control of energy balance were considered distinct entities, it has now emerged that considerable cross talk exists with implications for the pathophysiology of clinical obesity.

Peripheral afferents

Peripheral signals involved in energy homeostasis are often stratified as long or short acting. Long acting signals provide information about available energy stores, and in response, the brain makes corrective adjustments to food intake and energy expenditure to maintain body weight[47]. The white adipocyte hormone leptin[48] and pancreatic hormone insulin are the two major afferents governing long-term energy balance and act primarily as anorexigens. Food intake and energy expenditure in the short term are modulated by a wide variety of situational and meal-related factors, among the most important are short-term gut derived hormones such as GLP-1 that act to signal acute energy status. Originally thought to exert their effects on energy balance through modulating homeostatic hypothalamic circuits, both long and short term afferents may also modulate the hedonic drive toward food consumption, though these pathways remain less extensively studied[49] (Figure 3).

Central controllers

The homeostatic control of food intake: The hypothalamic arcuate nucleus (ARC) is believed to play a crucial role in the homeostatic control of energy balance. At a cellular level, the ARC contains two distinct neural populations exerting antagonistic effects on food intake; a medially located orexigenic (appetite stimulating) population consisting of neurons co-expressing Agouti related peptide (AgRP) and neuropeptide Y and a laterally located anorexigenic (appetite suppressing) population consisting of neurons co-expressing pro-opiomelanocortin (POMC) and cocaine and amphetamine related transcript (CART)[55-58]. Both neural subsets project to melanocortin 4 receptor (MC4R) positive neurons located in intra- and extra-hypothalamic sites. POMC is cleaved to produce α-MSH an agonist of MC4R whereas AgRP acts an inverse agonist[59-61]. The ARC may exert its effects on energy homeostasis by direct cortical projections or indirectly *via* second order neurons in adjacent hypothalmic nuceli of which the paraventricular nucleus (PVN) is believed to be play a crucial role[62,63]. GLP-1 receptors (GLP1-Rs) have been localized pre-clinically in the ARC and PVN[50,51] and stimulation of theses receptors reduce food intake to induce weight loss in rodents. Targeting the homeostatic controls of energy balance may therefore be the means by which GLP-1 agonism achieves its weight loss effects in the clinic, suggesting an underlying deregulation in GLP-1 signalling contributing to the multifactorial pathophysiology of human obesity.

The hedonic control of food intake: Despite a robust homeostatic system governing energy balance, feeding and meal termination are also influenced by hedonic, reward-related factors such as palatability and the perceived rewards associated with meal consumption. The drive to pursue such pleasurable experiences largely mediated by the mesolimbic rewards system originating from dopaminergic neurons in ventral tegmental area (VTA) that terminate on neurons in the nucleus accumbens. Though the relationship between peripheral afferents signalling acute and long term energy status and central hedonic control centres are less well defined, GLP-1Rs have been located in the dopaminergic neurons of the VTA[64] where activation inhibits neural firing, potentially reducing hedonic drives toward food consumption. Interestingly, where the homeostatic control of energy balance modulates food intake to regulate the amount of body fat an individual maintains[65], in obesity, despite an overall positive energy balance, hyperphagia is the norm. Where previously, the neurocircuits mediating the homeostatic and hedonistic control of energy balance were considered distinct entities, it has now emerged that considerable cross talk exists and central GLP-1 signalling has been implicated as a mediator of such interactions (detailed in a number of excellent reviews[36,37,54]). A skew toward hedonic and away from homeostatic controls of energy balance may explain the pathological hyperphagia seen in obesity; restoring the balance between homeostatic and hedonic drives towards food consumption may therefore be the means by which GLP-1 agonism achieves its sustained

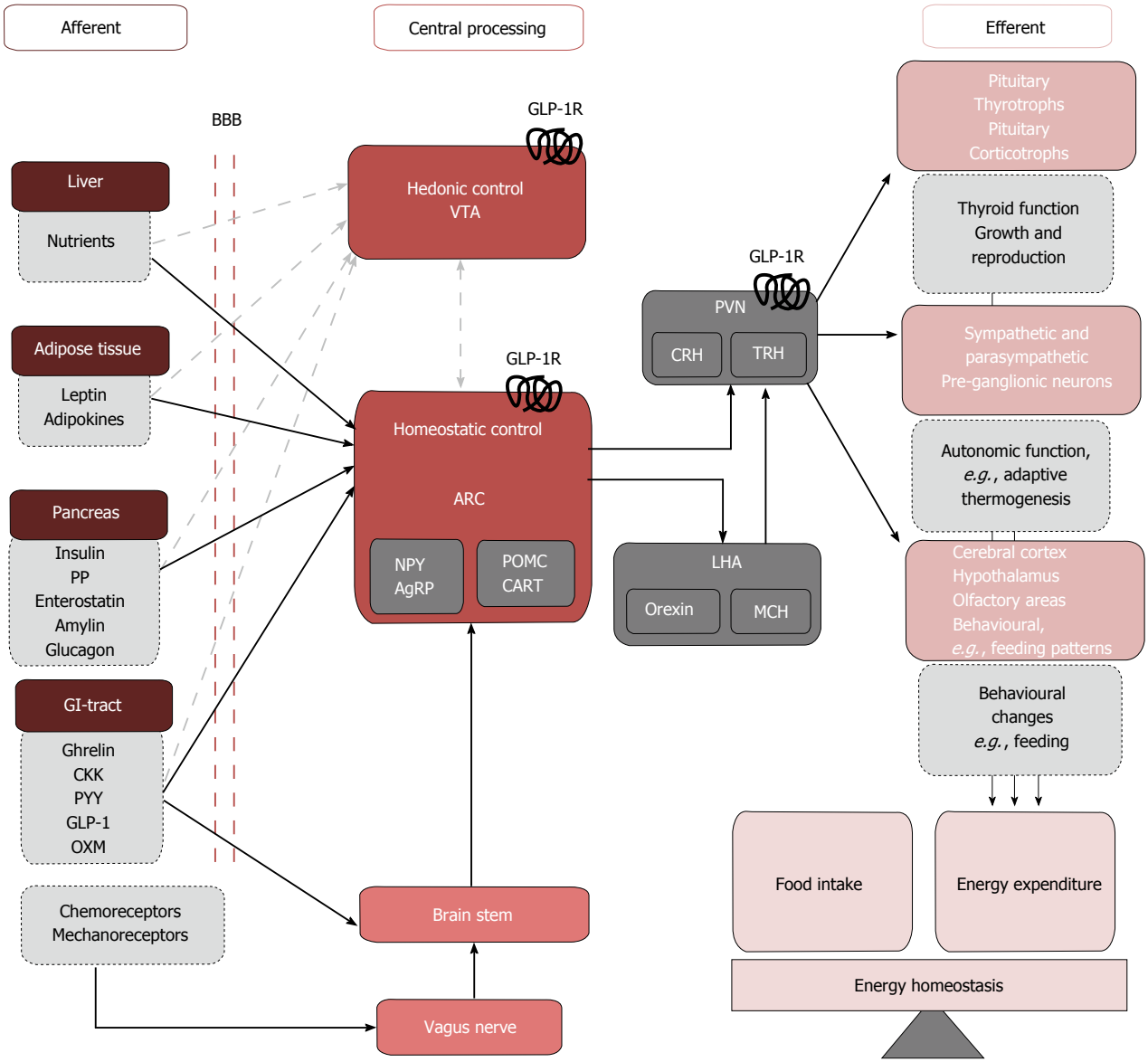

Figure 3 The hedonic and homeostatic controls of energy balance. Peripheral signals from the Liver, adipose tissue pancreas, GI-tract cross the BBB to directly signal to neurons of the ARC of the hypothalamus. GI-tract enteroendocrine hormones and chemo- and mechanoreceptor neural afferents can also indirectly activate the ARC *via* the vagus nerve and brainstem. The net output of the ARC neurons is relayed to second order intrahypothalmic neurons in the PVN, and LHA that express the MC4R. GLP-1Rs have been localized pre-clinically in the ARC and PVN[50,51], stimulation of theses receptors inducing reductions in food intake and weight loss potentially through efferent pathways that involve the activation of TRH and CRH expressing neurons and pre-ganglionic sympathetic and parasympathetic neurons. Feeding and meal termination are also influenced by hedonic, reward-related factors centrally processed in the VTA. Though the interactions between peripheral nutrient signals and rewards neurocircuitry are not extensively defined (grey dashed arrows) GLP-1Rs have been localized pre-clinically in the VTA[52]. Previously considered as separate entities, severe cross-interactions exist between central homeostatic and hedonic control centres[53]. This communication may be mediated by central GLP-1 signalling[36,37,54]. GI-tract: Gastrointestinal tract; BBB: Blood-brain barrier; ARC: Arcuate nucleus; PVN: Paraventricular nucleus; LHA: Lateral hypothalamic area; MC4R: Melanocortin 4 receptor; TRH: Thyrotrophin releasing hormone; CRH: Corticotrophin releasing hormone; VTA: Ventral tegmental area; PP: Pancreatic polypeptide; CKK: Cholecystokinin; PYY: Polypeptide-YY; OXM: Oxymodulin.

weight loss effects in the clinic, suggesting an underlying deregulation in GLP-1 signalling contributing to the multi-factorial pathophysiology of human obesity.

GLP-1

Synthesis, secretion and degradation

GLP-1 is a 31 amino acid polypeptide derived from post-translational processing of the native 160 amino acid peptide proglucagon by the enzyme prohormone convertase 1 (PC1/3). Peripheral proglucagon gene expression has been localized to the enteroendocrine L cells and pancreatic α-cells whilst centrally, proglucagon expressing neurons have been localized to brainstem regions such as the nucleus of the solitary tract (NTS)[66-68]. Tissue specific post-translational processing liberates different pro-glucagon derived peptides[69] depending on subtype of PC enzyme present. Figure 4 details the different post-translational products following PC1/3 and 2 cleavage.

GLP-1 is primarily synthesized by PC1/3 activity in the intestinal L cells[75]; open-type epithelial cells

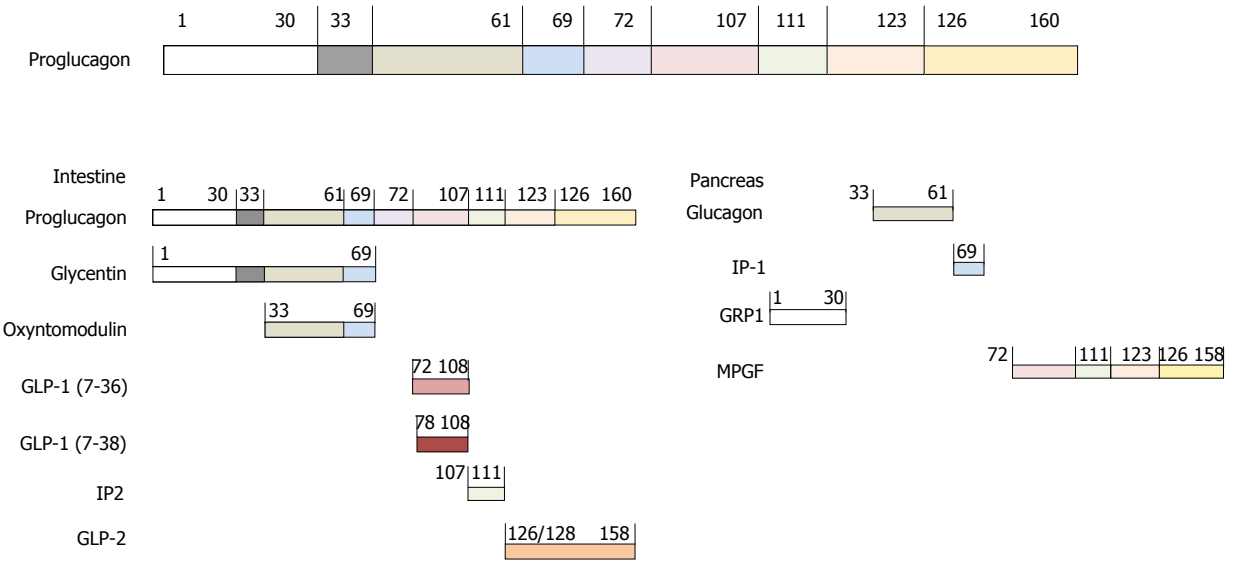

Figure 4 Post-translational tissue specific processing of proglucagon[70-72]. The 160 amino acid pro-glucagon gene (GCG) encoded on chromosome 2 undergoes tissue specific post-translational cleavage by prohormone convertase (PC) 1/3 and PC2 in central and peripheral sites. This figure shows the major cleavage products of the GCG with numbers indicating the amino acids between which the hormone product lies and at which the PC enzymes act. In the pancreas, PC2 dominates and liberates glucagon. In the intestine PC 1/3 activity dominates and produces GLP-1, of note, the other products of proglucagon cleavage by PC1/3 are produced in a 1:1 ratio with GLP-1[73,74]. The PC responsible for cleaving GCG in the central nervous system is not well established; both PC1/3 and PC2 may play a role. GLP: Glucagon-like peptide; GRP1: Gastrin-releasing peptide 1; IP2: Intervening peptide 1/2.

most densely located in the ileum and colon[76-78]. Long apical processes that extend toward the intestinal lumen[77] allow direct nutrient sensing by L cells, of which glucose has been implicated as the most potent GLP-1 secretagogue in both healthy and T2DM humans[79]. Being in close proximity to neurons of the enteric nervous system and the intestinal microvasculature[80,81], L cells also receive neural and hormonal signals that act as indirect nutrient sensors. Following synthesis, GLP-1 is secreted from the L-cells *via* secretory granules located in the basolateral membrane. GLP-1 secretion in response to nutrient sensing is biphasic; an initial rapid rise occurring within 10-15 min post-prandial, followed by a second longer phase peaking at 30-60 min[82]. The early phase of GLP-secretion has traditionally been attributed to signals from the parasympathetic vagal nerve and neurotransmitters such as gastrin-releasing peptide (GRP) and acetylcholine. However, more recently, GLP-1 secreting cells that show direct secretory responses to nutrient stimulation have been localised in significant numbers in the proximal small intestine implicating a role for this albeit sparser population of proximal GLP-1 releasing cells in the rapid postprandial rises of plasma GLP-1[83-85]. The second phase is mediated *via* direct nutrient contact with subsequent membrane depolarization or activation of second messenger systems mediating GLP-1 release. Figure 5 depicts the major nutrient, neural and hormonal secretagogues of GLP-1.

Secreted GLP-1 is rapidly degraded at its N-terminal residue by the ubiquitously expressed enzyme dipeptidyl peptidase Ⅳ (DPPV) to yield residues GLP-1 (9-36 amide) and GLP-1 9-37[88,89]. The majority of GLP-1 degradation is attributed to membrane-bound DPPV in the hepatic portal system resulting in an extremely short half-life (about 2 min)[81,90]. As such, only about 10%-15% of GLP1 secreted from intestinal L cells reaches peripheral downstream targets. The amount of GLP-1 reaching potential central targets involved in energy balance is unknown. As parenteral administration of GLP-1 avoids the physiological first-pass effect of hepatic DPPV, the supraphysiological plasma concentrations achieved by subcutaneous (SC) administration may explain the weight loss efficacy achieved by 3 mg liraglutide in obese and overweight patients in the clinic. Findings also go some way to suggest either a reduction in secretion of, or sensitivity to, physiological GLP-1 secretion as a contributor to the multifactorial pathophysiology of human obesity.

Central and peripheral effects of GLP-1

GLP-1 exerts its effects by intracellular signalling pathways activated after binding to the G-protein coupled receptor GLP-1R[91]. The extensive central and peripheral expression of the GLP-R reflects the pleotropic physiological roles of GLP-1 that are summarised in Figure 6 and extensively reviewed elsewhere[70,86]. From this point onward the review will focus on exploring the evidence surrounding a role for physiological GLP-1 signalling in the regulation of energy balance and deregulations of this signalling as one contributor to the multifactorial pathophysiology of clinical obesity.

GLP-1 AND THE REGULATION OF ENERGY BALANCE

Evidence: Effects of GLP-1 administration on food intake and energy expenditure in man

Numerous clinical studies have examined the relationship between acute physiological and supraphysiological

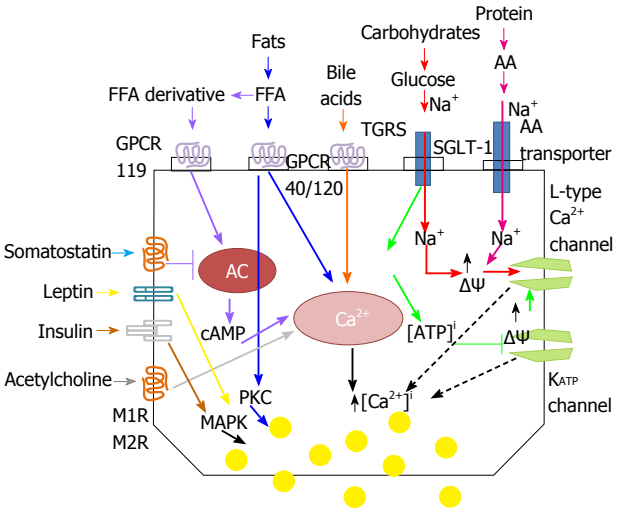

Figure 5 Mechanisms of glucagon-like peptide 1 release from entero-endocrine L cell. Glucagon-like peptide 1 (GLP-1) release from L-cells is regulated by direct nutrient sensing *via* receptors and channels on apical processes or indirectly *via* neuro-hormonal mechanisms[70,71,86,87]. A: Nutrient signals. Carbohydrates: Glucose derived from carbohydrate metabolism is the most potent stimuli for GLP-1 secretion. Glucose can trigger GLP-1 release by two mechanisms: (1) the sodium-glucose cotransporter-1 (SGLT-1) couples the transport of glucose with Na ions. Na = influx leads to membrane depolarization (ΔΨ) (red arrows); and (2) glucose metabolism generates adenosine triphosphate (ATP). Elevated intracellular ATP concentrations $[ATP]^i$ close KATP channels and leads to membrane depolarization (ΔΨ) (green arrows). Both routes to membrane depolarisation increase intracellular Ca levels ($[Ca^{2+}]^i$) by opening L-type Ca channels. Elevated $[Ca^{2+}]^i$ triggers the exocytosis of GLP-1 secretory granules located at the basolateral surface of the enteroendocrine L cell (dashed lines). Fats: Fats are potent stimuli for GLP-1 secretion. Free fatty acids (FFA) (blue arrows) interact with G-protein coupled receptors (GPCRs) that trigger Ca^{2+} release from internal stores and also activate protein kinase C (PKC). FFA derivates (purple arrows) interact with GPCRs that activate second messenger systems involving adenylate cyclase (AC) and cyclic AMP (cAMP) which increases $[Ca^{2+}]^i$. Bile acids (orange arrows) and short chained fatty acids (not shown) also increase $[Ca^{2+}]^i$ by GPCR interactions. Proteins: Protein is a weak stimulator of GLP-1 release when compared with sugars and lipids. Amino acids (AA) derived from protein breakdown are transported intracelluarly with Na^+ *via* Na^+ dependent AA transporters. Na^+ influx causes membrane depolarization and elevated $[Ca^{2+}]^i$ with resultant GLP-1 exocytosis (pink arrows); B: Hormonal signals. Somatostatin inhibits GLP-1 release by blocking AC activation (light blue arrows). The peripheral adiposity signals leptin (yellow arrows) and insulin (brown arrows) are thought to stimulate GLP-1 release via activation of mitogen-activated protein kinase (MAPK) signalling pathway; C: Neural signals. Acetylcholine binding to muscarinic receptors (M1R, M2R) elevates $[Ca^{2+}]^i$ stimulating GLP-1 release (grey arrows). GRP is though to stimulate GLP-1 release in association with the activation of mitogen activated protein kinase kinase (MAPKK) and subsequent phosphorylation and activation of MAPK (not shown).

doses of GLP-1 with measurements of food intake and feelings of hunger and satiety in healthy normal weight and obese adults with and without T2DM[92-99]. The main findings of these studies have been summarized in Figure 7. Though individual studies are conflicting, a meta-analysis reports that acute GLP-1 infusion induces a mean 11.7% decrease in food intake when compared with saline control in man[100]. Interestingly, whilst supra-physiological doses of GLP-1 reduces appetite and food intake in both lean and obese subjects, physiological GLP-1 doses reduces appetite and food intake in only lean subjects[93,94,97,99]. Findings go some way to suggest a role for resistance to physiological GLP-1 signalling as a factor contributing to obesity pathophysiology. Interestingly, whilst physiological GLP-1 infusions in obese subjects induce appetite reductions[92,95] similar to those observed in their lean peers, this is not translated into a reduction in food intake, suggesting pathological alterations of GLP-1 signalling in obesity that reinforce feeding despite a reduced physiological drive to food intake. One mechanism that this may be achieved is through a pathological skew toward hedonic and away from homeostatic controls of energy balance in obesity, potentially mediated by deregulated central GLP-1 signalling (a role for GLP-1 in the pathophysiology of clinical obesity).

Whilst evidence from clinical interventional studies suggests that physiological GLP-1 contributes to negative energy balance by decreasing food intake. The effects of GLP-1 on energy expenditure are less clear. Fasting plasma GLP-1 concentrations have been positively associated with increased rates of energy expenditure in man[101]. Clinical evidence regarding the effects of acute GLP-1 administration on energy expenditure however is conflicting. Physiological infusions of GLP-1 have been reported to reduce energy expenditure in lean and non-diabetic obese patients[95,102] associated with reduced carbohydrate metabolism. Others, however, have observed that supraphysiological infusions of GLP-1 increase energy expenditure in lean individuals in an insulin dependent manner[103].

Interpretations: Implications for the clinic

Evidence from clinical interventional studies suggests that acute post-meal rises in GLP-1 contribute to negative energy balance primarily through an anorexigenic effect. The long-acting GLP-1 analogue liraglutide (3 mg) has recently been approved as a once daily bolus SC injection for the medical management of obesity. The sustained anorectic effect of a long term agonist combined with supraphysiological dosing perhaps the mechanism of the clinical weight loss efficacy achieved by liraglutide 3 mg. Unfortunately, to date, clinical studies assessing the comparative efficacy of acute *vs* continuous GLP-1 administration on appetite reduction and weight loss remain scarce. Näslund *et al*[104] compared the effects of 4 doses of acute GLP-1 infused 30 min prior to meals [prandial subcutaneous infusion (PSI)] to an equivalent dose of continuous SC GLP-1 infusion (CSI) on food intake and weight loss in non diabetic obese patients. Though both acute and continuous GLP-1 infusion produced significant reductions in food intake when compared to placebo (P = 0.02 PSI and CSI), a statistically significant weight loss compared to placebo was only observed following PSI. With respect to the clinic, findings suggest that lowered dose; more frequent GLP-1 administration may prove more efficacious in inducing weight loss in obese patients. Nevertheless, in view of the negative impact of SC drug administration on patient adherence and the potential biases associated with the

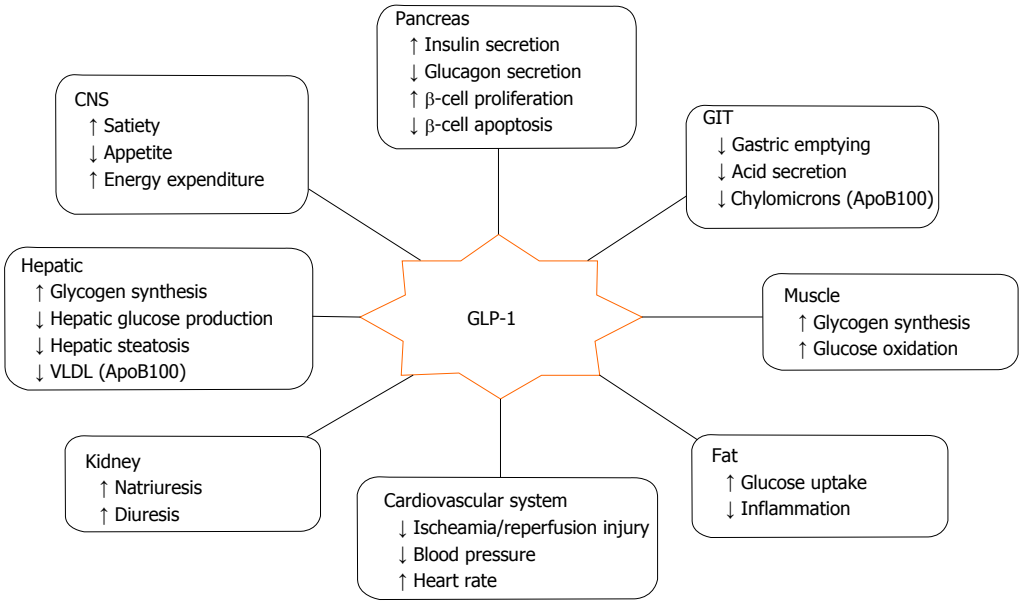

Figure 6 Central and peripheral effects of glucagon-like peptide 1. *Ex vivo* and *in vivo* studies in rodents, and observational and interventional studies in man have allowed the characterization of numerous central and peripheral effects of GLP-1. Peripheral effects of GLP-1 may be classed broadly as pancreatic or extra-pancreatic. Pancreatic effects of GLP-1 act to promote insulin secretion (incretin effect). Extra-pancreatic effects of GLP-1 include: (1) regulation of energy metabolism and nutrient storage (liver, muscle and fat); (2) efficient nutrient handling (stomach and GIT); and (3) others: Cardiovascular repair, blood pressure control, diuresis[86]. VLDL: Very low-density lipoproteins; GLP-1: Glucagon-like peptide 1; GIT: Gastrointestinal tract; CNS: Central nervous system.

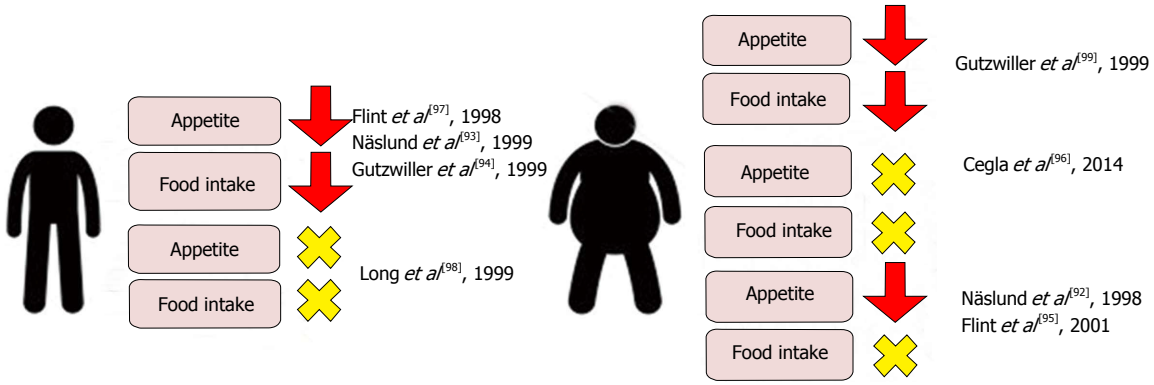

Figure 7 Effects on Visual Analogue Scale assessed appetite scores and ad libitum food intake in lean and obese subjects following physiological and supraphysiological[92-99] infusions of glucagon-like peptide 1. Though individual studies report conflicting data, a meta-analysis of clinical studies evaluating the acute effects of GLP-1 infusion on food intake reports a mean 11.7% decrease when compared with saline control[100]. GLP-1: Glucagon-like peptide 1.

significantly greater peak plasma GLP-1 concentrations achieved following PSI compared to CSI (269.4 pmol *vs* 88.7 pmol) once daily bolus administration at present, seems to be the most clinically efficacious means of therapeutic GLP-1 analogue delivery.

Interpretations: Potential effectors of GLP-1s negative energy balance effects

Clinical and pre-clinical evidence suggests that targeting peripherally and centrally located GLP-1Rs may exert the anorectic effects of physiological GLP-1 signalling.

Peripheral effectors: Histological studies in man have shown GLP-1Rs to be expressed in cells of the gastric mucosa and in pancreatic islet cells[105,106]. Pre-clinically, stimulation of gastric and pancreatic GLP1-Rs are associated with reductions in food intake that occurs alongside activation of hedonic and homeostatic brain regions[47,63,86]. Findings suggest physiological GLP-1 signalling may induce its anorectic effects in man by indirectly activating central controllers of appetite through gastric and pancreatic receptors.

Gastric mechanoreceptors are activated by gastric distension following acute nutrient intake, and gastric mechanoreceptor signalling plays an important role as a meal-to-meal satiety signal, activating the NTS which in turn modulates neural activity in both the ARC the VTA[107] (the homeostatic and hedonic control of energy balance). By relaying to the NTS, mechanoreceptor induced anorectic effects may therefore be exerted through modulation of both homeostatic and hedonic appetite control. The amount of gastric distension in response to a given meal is negatively associated with the rate of gastric emptying; delayed gastric emptying

positively associated with increased satiety and fullness in both healthy and obese patients[108-112]. GLP-1 has been found to delay gastric emptying in healthy lean, obese and T2DM subjects, and histological studies in man have shown that GLP-1Rs are expressed in gastric mucosa[92,105,113-116]. Post-prandial GLP-1 secretion may therefore exert its anorectic effect through activating GLP-1Rs in gastric mucosa, which in turn increase mechanoreceptor firing and signalling to the NTS. Though the neurotransmitters involved in relaying signals from the NTS to homeostatic and hedonic appetite controls remain to be defined, physiological gastric distension in rodents has been shown to up-regulate *GLP-1* gene expression in the NTS associated with central proglucagon processing[117], implicating a role for centrally synthesised GLP-1.

In the fasted state, the stomach is empty and so gastric motility is reduced to basal levels. That reductions in appetite after GLP-1 administration have been observed in fasting human subjects[99], suggests that mechanisms other delaying gastric motility contribute to the physiological anorectic effect of GLP-1. The glucoregulatory hormone insulin, traditionally viewed as an anorectic signal involved in the regulation of long-term energy balance[47,63], displays both basal and acute meal-related secretion[118]. With acute insulin administration associated with reduced *ad libitum* food intake in healthy lean individuals[119], findings implicate a role for insulin as an anorexigen involved in the regulation of short term energy balance. Insulin receptors are widely expressed in the ARC and VTA[120-122], thus modulation of both homeostatic and hedonic appetite control may be the means by which insulin exerts its anorectic effects on short-term energy balance.

The most extensively studied of GLP-1's physiological roles is as a positive modulator of insulin secretion from pancreatic β-cells[123] (evidence: Effects of GLP-1 administration on food intake and energy expenditure in man). Whilst GLP-1 has been shown to increase energy expenditure in healthy lean individuals in an insulin dependent manner[103], no clinical evidence to date exists exploring the role of insulin as a mediator of GLP-1 anorexigenic signalling. Studies assessing the effects of GLP-1 interactions with the oriexigen ghrelin however, suggest that this may indeed be the case. Ghrelin receptors have been localised preclinically in Agrp/NpY neurons of the ARC and dopaminergic neurons of the VTA[124,125], with activation of neurons in either brain region producing orexigenic effects. GLP-1 infusion in healthy lean humans is associated with significant suppression of postprandial rises in ghrelin[126]; the decline in orexigenic signalling a potential indirect mediator of GLP-1s anorexigenic effect. Interestingly, the reductions in ghrelin concentration observed with GLP-1 infusion inversely correlate with coinciding rises in insulin concentration and elsewhere, insulin infusion has been shown to display a reciprocal relationship with ghrelin secretion in man[127]. Together, findings suggest that GLP-1's anorectic effects may be mediated secondary to its incretin effect that in turn that suppresses ghrelin release, thus orexigenic signalling.

Central controllers: Histological and *in vivo* studies in rodents have shown that GLP-1Rs are expressed in anorexigenic POMC/CART neurons of the ARC and in dopaminergic neurons of the VTA[59,61,18,128] where they stimulate and inhibit neural firing respectively. Preclinical studies have shown that the stimulation of the POMC/ARC neurons of the hypothalamus and inhibition of the dopaminergic neurons of the VTA reduce food intake. Findings suggest that GLP-1 may exert its negative energy balance effects in man through direct activation of central GLP-1 receptors in the ARC and VTA; activating the anorexigenic homeostatic and inhibiting the hedonic hyperphagic drives to food intake. With the development of neuroimaging techniques, *in vivo* clinical studies substantiate the effects of GLP-1 on brain regions involved in the homeostatic and hedonic controls of energy balance. Whether these effects are mediated by direct central GLP-1R activation or indirectly *via* peripherally located GLP-1Rs however, remain to be defined.

Using fluorodeoxyglucose positron emission tomography Alvarez *et al*[129] demonstrate that GLP-1 infusion in lean individuals reduces glucose metabolism in the hypothalamus and brainstem. With patients fasted during the study and with no changes in peripheral hormone profiles observed, the effects of gastric mechanoreceptor activation or other hormonal influences respectively on observed effects are negated. Elsewhere, correlations between PET assessed increases in hypothalamic blood flow and physiological post-prandial rises in serum GLP-1 have been observed[130]. Both findings may represent altered neural activity in brain regions associated with homeostatic energy balance secondary to direct or indirect GLP-1/GLP-1R signalling. The effects of this alteration in central neural activity on food intake and appetite however, have not been explored. Using functional magnetic resonance imaging (fMRI), De Silva *et al*[131] demonstrate that GLP-1 infusion in lean individuals attenuates neuronal activity in 6 brain regions involved in rewards processing and hedonic feeding accompanied with reductions in food intake. Though neither parameter reached statistical significance *vs* placebo, results support the idea that central GLP-1 signalling may at least in part exert its negative energy balance effects through modulations in hedonic appetite control centres, potentially by reducing the hedonic value associated with food and food-driven motivation.

Clinical evidence exists to suggest that the SNS modulates energy expenditure through increased thermogenesis assessed *in vivo* as muscle sympathetic nerve activity (MSNA)[132,133]; increased MSNA positively associated with increased short and longer term energy expenditure in otherwise healthy human subjects[134,135]. Peripheral GLP-1 infusion has been shown to significantly

Table 2 Obesity and endocrine phenotypes in probands with *PCSK1* gene deletion monogenic obesity

Ref.	Jackson *et al*[144], 1997	Jackson *et al*[143], 2003	Farooqi *et al*[148], 2007	Frank *et al*[145], 2013	Parker *et al*[147], 2013	Bandsma *et al*[146], 2013
Obesity phenotype						
Hyperphagic, early-onset	Yes	Yes	Yes	Yes	Yes	Yes
Endocrine phenotype						
Abnormal glucose metabolism	Yes	Yes		Yes	Yes	Yes
Hypogonadotrophic hypogonadism	Yes			Yes	Yes	
Hypocortisolaemia	Yes	Yes		Yes	Yes	
Hypothyroidism			Yes	Yes	Yes	
Central diabetes insipidus					Yes	
Others						
Early onset malabsorptive diarrhoea	Yes	Yes	Yes	Yes	Yes	Yes

Proband details: Jackson *et al*[144] (1997) - a 40-year-old Caucasian woman; Jackson *et al*[143] (2003) - female Caucasian infant, non consanguineous; Farooqi *et al*[148] (2007) - 6 North African boy, consanguineous; Frank *et al*[145] (2013) - male infant; Parker *et al*[147] (2013) - 13 children aged 3 to 17; Bandsma *et al*[146] (2013) - 2 children age 2 and 7 Arab, consanguineous, 2 children aged 1 and 10, African, non-consanguineous.

increase MSNA in healthy human controls[136] and suggest that GLP-1 signalling may produce its negative energy balance effects not only through anorexigenic signalling, but also by increasing energy output.

A ROLE FOR GLP-1 IN THE PATHOPHYSIOLOGY OF CLINICAL OBESITY

Genetics

Genetic analyses in man suggest clinical obesity is associated with a lack of functional GLP-1 signalling that may contribute to the development of the obesity phenotype.

Monogenic human obesity: Monogenic human obesity is a rare form of clinical obesity that shows Mendelian patterns of inheritance; the obesity phenotype attributed to the loss or gain of function in a single gene[137]. Two broad classes of Mendelian human obesity exist; syndromic obesity encompasses about 30 Mendelian disorders wherein obesity co-presents alongside characteristic physical and developmental anomalies. Though causative genes have been identified, the mechanisms through which the genetic mutations induce obesity are not completely understood in all cases[138]. Non-syndromic obesity is characterized by a severe, early onset hyperphagic obesity attributed to loss of function mutations in 1 of 11 genes[139-141]. Interestingly, 8 of these genes have physiological roles in the central control of energy balance[142]. One such gene is PCSK1 encoding the enzyme PC1/3 involved in the proteolytic processing of proglucagon, to yield, amongst other peptides, GLP-1 (GLP-1). Six studies to date document the relationship between autosomal recessive, compound heterozygous or homozygous[143-148] mutations in PCSK1 in 21 probands associated with reduced or absent function of PC1/3. Table 2 details the phenotypes of probands, all of whom presented with an early onset hyperphagic obesity and malabsorptive diarrhoea with varying, though extensively overlapping endocrine phenotypes.

Though the cause of the obesity and endocrine phenotypes associated with PCSK1 mutation are unknown, they may well be attributed to the loss of PC1/3 prohormone processing function. Signs of impaired intestinal[146] pro-glucagon processing have been described in probands with PC1/3 deficiency and may contribute to the development of the obesity phenotype secondary to reduced GLP-1 synthesis. Disappointingly, only 2 of 6 studies detailing the phenotypes PCSK1 mutant probands assess post-prandial GLP-1 secretory responses and report conflicting results; whilst an oral glucose load (OGTT) yields significantly reduced GLP-1 response in three child probands compared to age matched controls, post-prandial responses in a 40-year-old proband match those of healthy age-matched controls. One interpretation of such findings may be that whilst other PCs may compensate for lacking PC1/3 to allow for GLP-1 synthesis in response to mixed nutrient secretagogues, PC1/3 is necessary and essential for GLP-1 synthesis in response to its most potent secretagogue, glucose. An alternative interpretation comes from observations that GLP-1 secretion following OGTT in the 3 child probands studied by Bandsma *et al*[146] seem to show an age dependent impairment improving with increasing age. Following follows reports by Parker *et al*[147] who observed that the pattern of endocrinopathy in probands with PSCK1 mutant monogenic obesity change with age, perhaps GLP-1 secretion too may show an age-dependent alteration, potentially compensated for over time. One way to test this hypothesis would be to histologically examine the enteroendocrine expression of GLP-1 in adult PCSK1-mutant probands; enteroendocrine expression of GLP-1 is significantly reduced compared to control in children with PCSK1 monogenic obesity[146], if indeed the normal post-prandial GLP-1 responses seen in adulthood are a reflection of the activation of redundant PC activity in intestinal cells up regulation of enteroendocrine GLP-1 expression would be observed. Though the cause of the hyperphagic obesity in PCSK1 mutant human monogenic obesity remains ill defined,

monogenic obesity implicates a role for deregulated GLP-1 signalling in the development of the obesity phenotype.

Polygenic obesity and Genome Wide Association Studies: Monogenic obesity is a rare form of clinical obesity, accounting for less than 1% of total cases of obesity worldwide. The obesity epidemic of the past 10-50 years has been largely attributed to environmental and societal changes facilitating a positive energy balance; "the obesogenic environment"[149,150]. However evidence from adoption, twin and family studies suggest the genetic contribution to BMI ranges between 60% and 84%[151]. As such, the current obesity epidemic may be defined as the interaction between a genetic predisposition and the "obesogenic environment"[149,150,152-154]. Genome wide association studies have identified 119 independent gene loci implicated as risk factors toward "common" obesity[155,156], as such today's obesity epidemic may be referred to as a polygenic obesity. One such susceptibility gene is PCSK1 encoding the enzyme PC1/3 involved in the proteolytic processing of proglucagon, to yield, amongst other peptides, GLP-1.

Single-nucleotide polymorphisms (SNPs) at three independent *PCSK1* loci have been consistently linked to an increased risk of obesity[157-161]. Though it is unclear how these minor alleles predispose to obesity, *in vitro* studies suggest that the encoded PC1/3 variants may not be as enzymatically active or physiologically available as the common form, potentially resulting in a partial PC1/3 deficiency. Decreased GLP-1 synthesis secondary to reduced proglucagon processing by PC1/3 in enteroendocrine L cells may therefore be the mechanism by which identified PCSK1 SNPs confer an increased risk toward the obesity phenotype.

Intestinal neuroendocrine gene expression: Neuroendocrine signals from the gut play an important role in the physiological control of energy balance. Findings from a recent study by Ritze *et al*[162] studying the gene expression of several proteins in the intestinal neuroendocrine network go some way to suggest intestinal GLP-1 expression and/or function may be altered in obesity. Though GLP-1 was not directly tested in the study, the anorectic neuropeptide PYY shown to co-localise and be co-secreted with GLP-1 in enteroendocrine cells[163] was tested. Taking PYY levels as proxy measures of GLP-1, Ritze *et al*[162] report significant correlations between GLP-1 with the GLP-1R in non-obese subjects (suggesting physiological ligand-receptor signalling), a correlation lost in obese subjects and replaced by correlations with the orexigen ghrelin ($P < 0.01$). Ritze *et al*[162] also observed correlations between the long-term satiety signal leptin and GLP-1R in obese subjects not seen in their lean counterparts.

A recent *in vitro* study on human L cells has shown that ghrelin is a positive modulator of GLP-1 release[164]. Ghrelin levels have also been reported to be reduced in humans with obesity[165,166]. The correlations between intestinal PYY (GLP-1) and ghrelin reported in obese subjects suggests that ghrelin decreases in obesity coincide with decreased GLP-1 levels, the latter potentially antagonising the anorexigenic effects of the former and may explain the difficulty to attain and maintain weight loss observed by many obese patients. Intestinal GLP-1 signalling has been suggested to promote small-intestinal motility in humans[167] and preclinically, central administration of leptin has been shown to increase the satiating effect of GLP-1, possibly through enhancing GLP-1R signalling. Correlations between the leptin and GLP-1R in obese subjects may therefore reflect a leptin-mediated enhancement of intestinal GLP-1/GLP-1R increasing intestinal motility to promote increased gastric emptying and reduced gastric mechanoreceptor activation in response to a given meal; the resultant decrease in anorexigenic signalling potentially explaining the persistent hyperphagia seen in obesity despite an overall positive energy balance.

Clinical studies in polygenic obesity

Interventional and observational clinical evidence suggests that malfunctioning of GLP-1 contributes to the development and/or maintenance of the obesity phenotype, rationalizing the use of GLP-1 analogues as novel therapeutic agents in the medical management of obesity.

Post meal and oral glucose GLP-1 secretory responses: A number of clinical studies have assessed the effect of physiological GLP-1 secretion responses in obese and lean subjects following an oral 75 g glucose load (OGTT) or post-prandial following a balanced meal. Where an OGTT consistently demonstrates a reduced GLP-1 secretion in obese subjects compared to their lean control post-prandial GLP-1 responses are conflicting; some observing significant reductions and others no change[168-178] in obese subjects when compared to their lean counterparts (Figure 8). That oral glucose, the most powerful GLP-1 secretagogue consistently demonstrates reduced GLP-1 responses in obese subjects may suggest that the impaired GLP-1 response observed are secondary to a reduced L-cell glucose sensing capacity in obesity. Support for such a postulate comes from findings by Ranganath *et al*[170] who demonstrate that whilst GLP-1 secretion to an oral fat load remains intact, GLP-1 secretion in response to an oral carbohydrate load is decreased in obesity. However, reports by Adam *et al*[168] that demonstrate reductions in GLP-1 response in obesity to a balance meal with retained responses to an oral carbohydrate load challenge such an interpretation. Interestingly however, in their observational study, Adam *et al*[168] also demonstrate that whilst carbohydrates stimulate post-prandial GLP-1 release similarly in obese and non-obese subjects, these rises are positively correlated with increased satiety only in lean subjects and put forward an alternate hypothesis that rather than impaired GLP-1 secretion, downstream receptor resistance may be the root of GLP-1 dyshomeostasis in

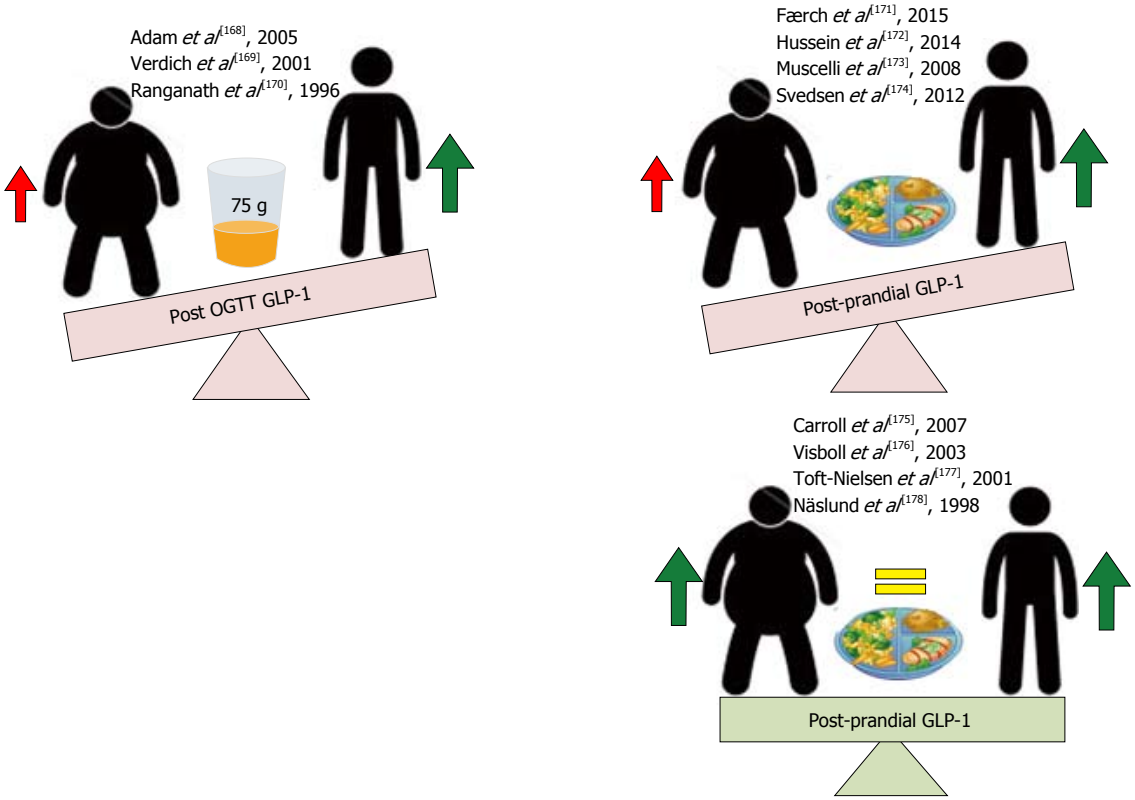

Figure 8 Effects of obesity on glucagon-like peptide 1 responses post oral glucose load and post-prandial. Obese subjects consistently demonstrate reduced glucagon-like peptide 1 (GLP-1) secretory responses following a 75-g oral glucose load compared to lean controls. Post-prandial GLP-1 secretory responses in obese subjects are conflicting, with some studies observing significant reductions and others observing no change[168-178] when compared to lean controls.

obesity. That deranged GLP-1 signalling is observed only in obese subjects suggests that obesity induces changes in functional GLP-1 signalling that through resultant reductions of signalling at central and peripherally located receptors (GLP-1) may facilitate the maintenance of the obesity phenotype. Pharmacologically targeting GLP-1 to restore physiological signalling may therefore be an efficacious method to prevent the propagation of, and potentially reverse weight gain in obesity.

Evidence from clinical studies suggests that weight gain induces alterations in functional GLP-1 signalling that facilitates and propagates the obesity phenotype. Though the mechanisms of reduced functional post-prandial GLP-1 signalling in obesity remain to be defined, clinical evidence implicates a role for interactions between GLP-1 and the long-term satiety signals insulin and leptin, and short-term orexigen ghrelin. The most extensively studied of post-prandial GLP-1's physiological roles is as a positive modulator of pancreatic β-cell insulin secretion[123]. Hyperinsulinaemia is positively associated increased BMI in individuals with normal glucose tolerance and increased BMI and increasing glucose intolerance have been shown to independently and additively impair GLP-1 secretion[171-173,175,179]. The chronic hyperinsulinaemia positively associated with increasing levels of obesity therefore, may acting as a negative feedback signal to inhibit physiological post-prandial GLP-1 release observed in obese subjects when compared with healthy lean control[171-173]. The long term adiposity signal leptin acts as a satiety signal governing long term energy balance and clinically, increased BMI has been shown to be positively correlated with fasted leptin, however obese subjects are thought to be resistant to leptin's effects[180,181]. *In vitro* studies of human intestinal L-cells have shown that leptin acts a GLP-1 secretogogue and go some way to suggest that the leptin resistance associated with obesity may account for the decreased post-prandial GLP-1 secretion observed in obese humans[168-170,182]. Ghrelin is the only orexigenic gut derived hormone[183]; released pre-prandial, ghrelin promotes meal initiation and increases food intake, and complex reciprocal interactions exist between GLP-1 and ghrelin that have implications for obesity pathophysiology. Preclinically, physiological ghrelin signalling has been shown to enhance post-prandial GLP-1 release[164], clinical obesity has however been associated with reductions in fasting ghrelin levels that may contribute to the reduced post-prandial GLP-1 release observed[168-170]. Conversely, clinical data exists to suggest that suppression of late post-prandial rises in ghrelin is one mechanism by which GLP-1 exerts its anorexigenic effect[126]; reduced post-prandial GLP-1 secretion in obesity potentially explaining the attenuated decreases of post-prandial serum ghrelin observed in this cohort[168-170,184,185] (Figure 9).

Together, evidence exists to suggest that the hyper-insulinaemia, leptin resistance and impaired ghrelin secretion occurring secondary to obesity cause functional deficits in GLP-1 signalling; the resultant reductions in

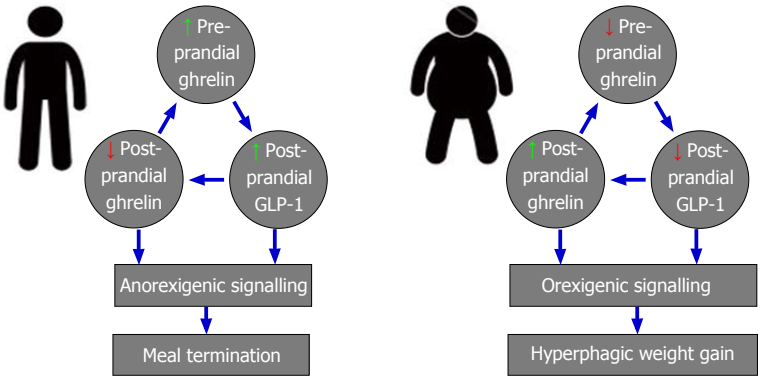

Figure 9 Physiological and pathophysiological interactions between orexigenic ghrelin and anorexigenic glucagon-like peptide 1 signalling in lean and obese individuals. GLP-1: Glucagon-like peptide 1.

GLP-1 mediated anorexigenic signalling facilitating post-meal hyperphagia, weight gain and thus perhaps the obesity phenotype. Pharmacologically targeting GLP-1 to restore homeostatic signalling may therefore be an efficacious method to prevent the propagation of, and potentially reverse the weight gain in obesity.

GLP-1 secretion post RYGB: Weight losses following bariatric surgery, pharmacotherapy or diet and lifestyle modification are all associated with decreases in circulating leptin and improved insulin sensitivity. The resultant reductions in anorexigenic signalling potentially facilitating weight gain and may explain the difficulty obese subjects have in attaining and maintaining weight loss. Bariatric surgery remains the most effective treatment modality for morbid obesity, with a meta-analysis reporting the Roux-en-y gastric bypass (RYGB) to produce a greater and more sustained weight loss than currently available pharmacotherapeutics, diet and lifestyle interventions or other bariatric options. Prospective studies assessing the effects of RYGB on post-prandial GLP-1 responses in non-diabetic obese patients consistently report statistically significant increases in post-prandial GLP-1 when compared to the pre-operative state, following equivalent weight losses with GB[186-193] and when compared with healthy lean control[188] (Figure 10). This post-operative supraphysiological GLP-1 secretory response therefore may explain the greater short- and long-term weight loss efficacy achieved with this treatment modality.

Evidence from clinical studies implicate the supra-physiological[188] post-prandial GLP-1 responses achieved following RYGB in the superior weight loss efficacy of this treatment modality. Though the mechanisms by which RYGB may induce increases in GLP-1 secretion remain poorly understood, clinical studies implicate a role for altered gut mechanics and L cell resensitisation.

That increases in post-prandial GLP-1 responses following RYGB are observed as early as 3 d post-operatively[190] suggest physical changes associated with RYGB, rather than gene-mediated up-regulations GLP-1 synthesis play a role in the increased GLP-1 secretory responses observed. Where both RYGB and GB induce weight loss through volume restriction, the former also redirects nutrient flow from the upper stomach directly into the distal jejunum. The exaggerated GLP-1 response following RYGB likely secondary to the increased glucose load delivered to the distal small intestine where L-cells are more densely populated. Such a concept is supported by the observed reductions in foregut and increases in hindgut hormones following RYGB and the hyperplasia of GLP-1 containing ileal cells in biopsy samples of obese humans after bypass[194-196].

RYGB induces a weight loss greater time-for-time and reaches a plateau more successfully maintained when compared with weight loss following GB[186-189]. Post-prandial GLP-1 responses following RYGB are also significantly greater than those following GB (that show no change from pre-operative levels[186,190,191]) however this response does not plateau but instead shows a tendency to increase with time past surgery. A relationship between the exponentially increasing post-prandial GLP-1 response and greater weight loss maintenance achieved post-RYGB may be explained by findings observed Kellum *et al*[189] who report that at 1 year post RYGB, alongside significantly greater achieved and maintained weight loss when compared to GB, GLP-1 responses were significantly increased in response to a carbohydrate meal in subjects post RYGB; a response positively associated with amount of weight lost. With derangements in L-cell carbohydrate sensing implicated in the pathophysiology of human obesity (see 5.1.1B, 5.2.1B), and with no alterations in response to a protein-fat meal observed following weight loss with RYGB and no altered response to either meal following weight loss with GB, findings suggest that weight loss following RYGB may be associated with a restoration of L cell sensitivity to the most potent GLP-1 secretagogue; a resensitisation that may occur proportionately to the amount of weight lost, the feed forward effect of weight loss on increased GLP-1 secretion resulting in supraphysiological GLP-1 signalling with the potential to antagonize the increased orexigenic drives of decreased leptin and insulin signalling associated with weight loss.

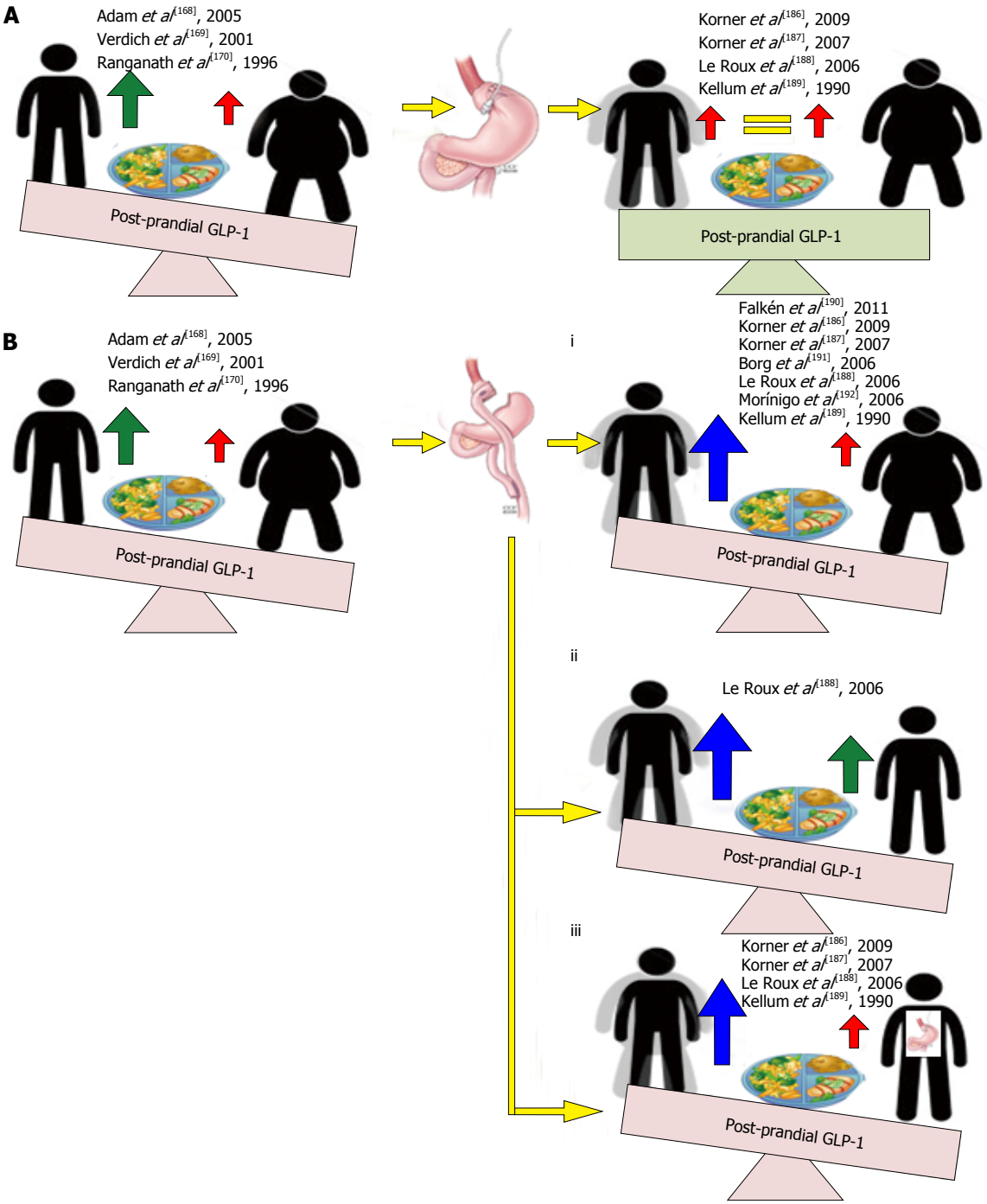

Figure 10 Effects of weight loss induced by gastric banding and Roux-en-Y gastric bypass on post-prandial glucagon-like peptide 1 secretion. Weight loss following gastric banding induces no changes in post-prandial glucagon-like peptide 1 (GLP-1) levels from pre-operative levels (A). All 7 studies assessing the effects of post-prandial GLP-1 secretion following weight loss with RYGB show significantly increased responses compared with pre-surgery responses and healthy obese controls (Bi), healthy lean controls (Bii) and following weight losses following gastric banding (Biii).

Such a concept may explain the long-term weight loss efficacy associated with RYGB.

Together, evidence exists to suggest that the supraphysiological[188] upregulation in GLP-1 signalling seen following RYGB contributes to the superior short and long term weight loss efficacy observed with this treatment modality. Not only do findings go some way to suggest a role for impaired GLP-1 signalling in the pathophysiology of human obesity, findings support the potential for pharmacological mimicry of this supraphysiological GLP-1 secretion as a minimally invasive, thus risk reducing and cost-effective alterative of achieving and maintaining similarly significant weight loss in obese subjects in the clinic.

Functional neuroimaging and self-assessments of appetite: fMRI studies have provided evidence *in vivo* to suggest that central nervous system responses in brain regions involved in rewards processing are altered in obese individuals; reduced brain activity in response to the

consumption of, and increased activity in response to the anticipation of palatable food consistently observed when compared with healthy lean controls[197-199]. Interestingly, GLP-1 agonism reverses these functional brain changes to match those of lean control with associated reductions in *ad libitum* food intake[199], an effect prevented by pre-treatment with a GLP-1 antagonist[200] (Figure 11). Together, findings suggests that obesity induced decreases in functional GLP-1 signalling contribute to altered rewards processing in obesity to facilitate hyperphagic weight gain despite an overall positive energy balance.

Further support for a role for GLP-1 and altered rewards processing in obesity pathophysiology comes from self-assessments of appetite. Subjectively assessed emotional eating scores have been defined as hedonic markers of appetite that display strong positive associations with the degree of human obesity, and, in obese subjects, relate to the extent to which GLP-1 receptor activation in brain regions involved in rewards processing are reduced[201-203]. Together, findings suggests that obesity induced decreases in functional GLP-1 signalling creates a feed-forward loop of hyperphagic weight gain despite an overall positive energy balance, an effect perhaps secondary to a GLP-1 deficit mediated skew toward hedonic and away from homeostatic controls of food intake. Together, findings from fMRI and subjective appetite assessment scores implicate obesity-associated reductions in functional GLP-1/GLP-1R signalling in the pathophysiology of hyperphagic weight gain in obesity. As such, findings support the role of the GLP-1R as a novel therapeutic target in the medical management of obesity, providing rationale for the use of liraglutide 3 mg in the pharmacotherapy of obesity in the clinic.

A ROLE FOR GLP-1 IN THE PHARMACOTHERAPY OF CLINICAL OBESITY

The balance between drug efficacy and cost determines the selection of a pharmacological agent for use in the medical management of any disease; greater understanding of underlying disease pathophysiology facilitating the development of targeted therapeutics with the potential for greater efficacy. Several lines of clinical evidence implicate a role for altered GLP-1 function in the pathophysiology of human obesity and a number of recent clinical trials have validated the clinical efficacy of long-term once daily SC 3 mg liraglutide (Saxenda) as an adjunct to calorie-restriction and exercise counselling in obese and overweight individuals with at least one weight related comorbidity. Significant improvements in clinical outcome measures such as body weight, anthropometric and cardiometabolic parameters, and indices of glucose tolerance have been observed and recently reviewed elsewhere[204]. Though March 2015 saw the EMA grant marketing authorization for 3 mg liraglutide as a weight-management agent in all 28 EU states[45], cost-benefit of funding treatment on the NHS undoubtedly contributes to the uncertainty of launch plans in the United Kingdom at present[46].

Weight loss efficacy of GLP-1 analogues

Evidence: One phase Ⅱ (NCT00422058)[43], and a number of phase Ⅲ multi-national double-blinded randomized control trials conducted in non-diabetic obese adults (NCT00480909)[42], overweight adults with at least one weight related co-morbidity (SCALE Obesity and Pre-diabetes, and SCALE Maintenance[205,206]), non-diabetic obese adults with obstructive sleep apnoea (OSA) (SCALE OSA[41]) and obese adults with T2DM (SCALE diabetes[207]) have established the efficacy of once daily 3 mg SC liraglutide as an adjunct to an energy-deficient low-calorie diet and physical activity counselling for weight management in this cohort. Results from the first study; a 20-wk phase Ⅱ trial in non-diabetic obese subjects showed that weight loss with liraglutide is dose-dependent up to 3.0 mg once daily[42,206]. Significantly more liraglutide 3 mg/d recipients than placebo or orlistat recipients achieved a 5% or 10% reduction of body weight at 20 wk. In a 2-year phase Ⅲ extension of the same study[42], double-blind treatment (liraglutide 1.2-3 mg/d) was continued until week 52, after which all liraglutide (< 2.4 mg/d) and placebo recipients were switched to liraglutide 2.4 mg, then 3.0 mg (week 70-96) based on 20-wk and 1-year results respectively (Figure 12) that indicated this was the optimal dosage. At 2 years, mean bodyweight reductions in those randomized to liraglutide were significantly greater than pancreatic lipase inhibitor orlistat, the only alternative licenced weight loss agent in the United Kingdom. Results from the SCALE maintenance and SCALE obesity and prediabetes[205,206] trials report similarly significant reductions in bodyweight in subjects randomized to 3 mg liraglutide when compared with placebo at 56 wk (P < 0.0001) alongside increased 5% and 10% responder rates. Findings are supported by results of the 32 wk SCALE Sleep Apnoea trial[41] in obese non-diabetic subjects with moderate to severe OSA and in the 56-68 wk SCALE Diabetes[207] trial in obese subjects with T2DM (Figure 13). With even modest losses of 5%-10% of total body weight associated with reduced risk of comorbidities in obese individuals[8-10], findings provide rationale for the licensing and funding of 3 mg liraglutide as an adjunct to lifestyle alteration as the first line anti-obesity pharmaceutical agent for weight management in obese and comorbid overweight adults in the United Kingdom.

Interpretations - obesity pathophysiology: Excessive consumption of palatable food can trigger neuroadaptive responses in brain reward circuits similar to that of alcohol and drugs of abuse[208] and clinical studies provide evidence to suggest that human obestiy is associated with altered rewards processing mediated in part by altered GLP-1 function that may render the hyperphagia of obesity the manifestation of a "food addiction". Whilst 3 mg liraglutide has been shown to induce weight loss in man by reductions appetite pre-

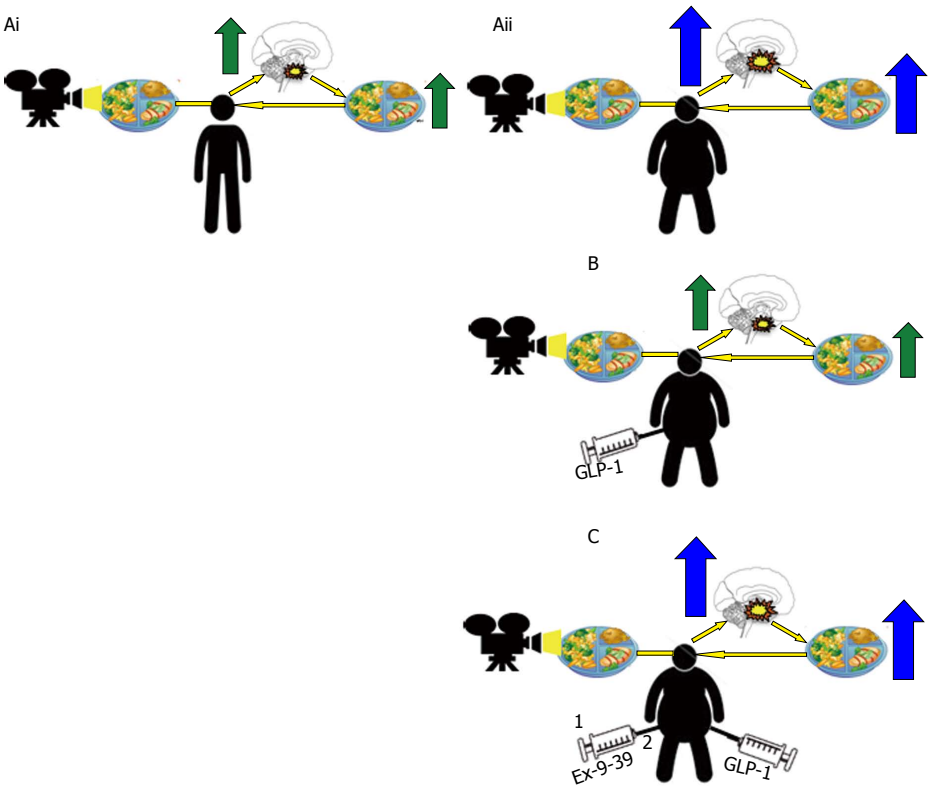

Figure 11 Altered central nervous system responses in brain regions involved in rewards in obese subjects is reversed upon glucagon-like peptide 1 receptor activation. Central nervous system activity in regions involved in rewards processing show increased responses in obese subjects when exposed to food related images (Aii) associated with increased ad libitum food consumption when compared to healthy lean control (Ai). A response reversed upon administration of a GLP-1 analogue with concomitant reductions in ad libitum food intake (B), this effect antagonized by pre-treatment with GLP-1 antagonist Extendin 9-39 (EX9-39) (C). GLP-1: Glucagon-like peptide 1.

clinically, liraglutide attenuates the reinforcing properties of alcohol *in vivo*[209,210]. As such, perhaps GLP-1 agonism may attenuate produce its weight loss effects in part, by attenuating the negative reinforcement of hyperphagia in obesity. Interestingly, though all aforementioned trials[41-43,205-207] advise participants to restrict food consumption throughout the treatment period, adherence rates are not reported. If indeed GLP-1 agonism induces its weight loss effects by modulating food related rewards that potentially reverses the negative reinforcement of hyperphagia in obesity, an increased adherence to caloric restriction would be expected. It would be interesting to see if this were the case.

Interpretations - cost-benefit of 3 mg liraglutide as an anti-obesity agent on the NHS: Follow-up period (FUP) assessments in the SCALE Maintenance, and SCALE Diabetes[205-207] trials suggest that weight loss with 3 mg liraglutide is treatment dependent; weight gains in excess to those seen in placebo control and subjects re-randomized to treatment observed in liraglutide treated participants upon treatment cessation (Figure 12).

Though reductions in bodyweight have been shown to significantly improve health outcomes and thereby reduce healthcare costs long term[211], in 2013, 24.9% of the United Kingdom adult population were classed obese and 90% of the £2.7 million T2DM adults in the United Kingdom were overweight. Following FDA and EMA approval criteria, all these individuals are potential candidates for treatment with liraglutide 3 mg. Costing in excess of $1000 per patient a month, and with prevalence of obesity and overweight predicted to rise (introduction), prolonged treatment seems unsustainable. A potential solution comes from longitudinal observations from Astrup *et al*[43] who observed that subjects randomized to liraglutide 3 mg achieve maximal rates of weight loss in the initial 0-20 wk treatment period with a tendency toward weight gain beyond 36 wk[42,43]. Findings suggest that whilst initially treatment with a GLP-1 analogue may compensate for functional deficits in obesity, treatment beyond 20 wk may be associated with the development of treatment resistance, most apparent 36 wk from initiation (Figure 14). Based on this, perhaps treatment with liraglutide 3 mg should be prescribed for 20 to a maximum of 36 wk alongside behavioural therapies promoting lifestyle changes and developing strategies to combat the addiction driven hyperphagia implicated in obesity pathophysiology (GLP-1 secretion post RYGB); combing behavioural therapies in the initial 20 wk of drug induction where weight loss is most pronounced may act as a positive reinforcer of sustained behavioural change facilitating continuation of these behaviours. This approach, integrating the psychosocial empowerment associated with patient self-management of chronic illness, alongside cost benefits associated with limited in-treatment period seems an

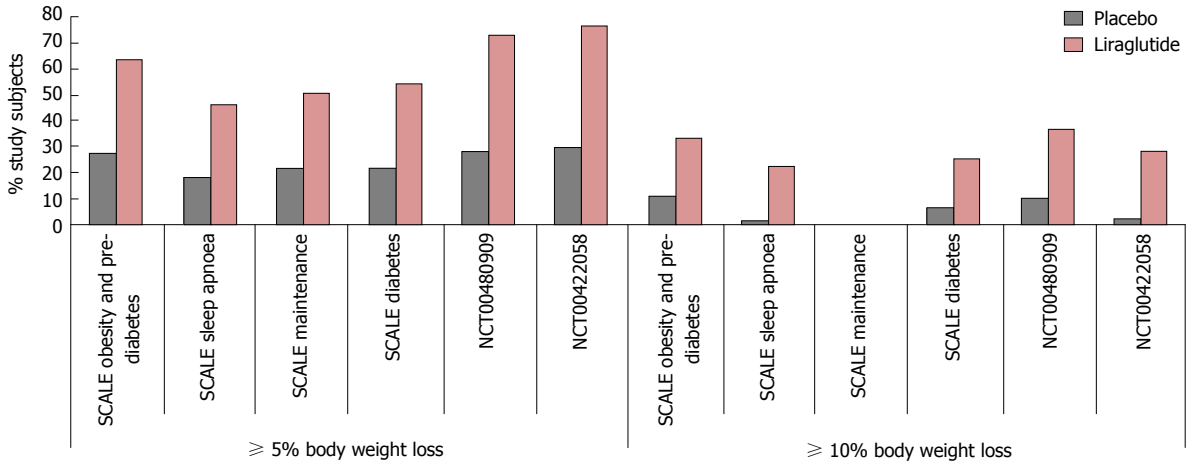

Figure 12 Significantly greater 5% and 10% weight loss achieved following 3 mg subcutaneous liraglutide compared to placebo and orlistat. Five percent and 10% responder rates in NCT00480909 reported at 1 year (see text).

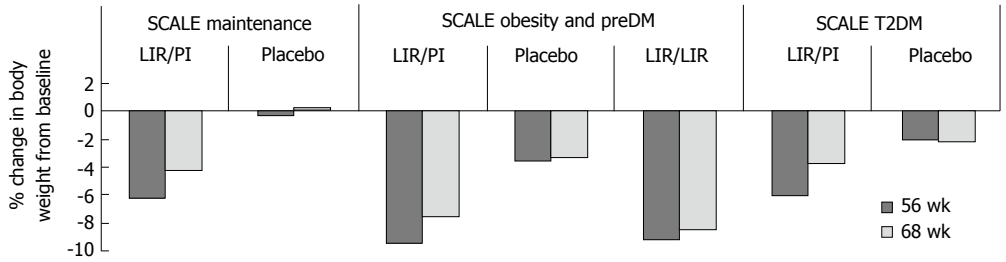

Figure 13 Body weight changes with liraglutide are treatment dependent. Following the 56-wk treatment period a 12-wk follow-up (FUP) was conducted in SCALE maintenance, SCALE obesity and pre-diabeties and SCALE diabetes trials. Twelve weeks FUP was an off-treatment period in SCALE Maintenance and SCALE diabetes. In SCALE Obesity and pre-diabetes FUP period involved a re-randomisation to either 3 mg liraglutide (LIR/LIR) or placebo (LIR/PI) and though weight gain occurred in all three groups weight gain was significantly higher following cessation of liraglutide. T2DM: Type 2 diabetes mellitus.

attractive one, especially if sustainable long-term weight losses with resultant reductions in the socioeconomic impacts of weight-related comorbidities can be achieved.

Glucose tolerance

Evidence: Clinical studies in obese subjects with pre-diabetes consistently demonstrate a greater reversion to normal glycaemic control following treatment with liraglutide 3 mg coinciding reduced T2DM incidence[42,206,212].

Interpretations - obesity pathophysiology: Being overweight or obese is the main modifiable risk factor for T2DM and increasing BMI is positively associated with hyperinsulinaemia even in those with normal glycaemic control[175,179,213] suggesting a common pathophysiology to both conditions. That increased BMI and impaired glucose tolerance have been shown to independently and additively impair GLP-1 secretory responses following an OGTT[171-173] suggests that this common pathophysiology may lie in a functional deficit of GLP-1. Support for the existence of a common pathophysiology between obesity and T2DM comes from observations that treatment with the insulin sensitizer metformin (currently the first line pharmacotherapeutic agent in the management of T2DM) upregulates GLP-1 secretory response following an OGTT, the restoration of physiological anorexigenic and incretin effects perhaps explaining the weight loss, and insulin sensitising properties of the drug seen in the clinic respectively[214,215].

Interpretations: Cost-benefit of 3 mg liraglutide as an anti-obesity agent on the NHS: Being overweight or obese is the main modifiable risk factor for T2DM[213] and T2DM is one of the major indirect financial burdens of obesity and overweight. Treating T2DM and its complications alone current costs the NHS £8.8 billion a year with indirect costs estimated at £13 billion[216]. With the incidence of obesity projected to rise (introduction), so too can be expected the incidence of T2DM, the management of which therefore, may become unsustainable on the NHS. Though costly, treatment with liraglutide 3 mg is associated with reductions in the rate of development of T2DM in overweight and obese subjects[42,206,212] and goes some way to suggest that treatment may reduce both direct burdens of obesity and overweight and the large indirect burden posed by new incidences of T2DM. However, weight loss in itself is associated with an improvement in glycaemic control. It may be argued therefore, that true cost-benefit of funding liraglutide 3 mg on the rationale of T2DM prevention in overweight and obese

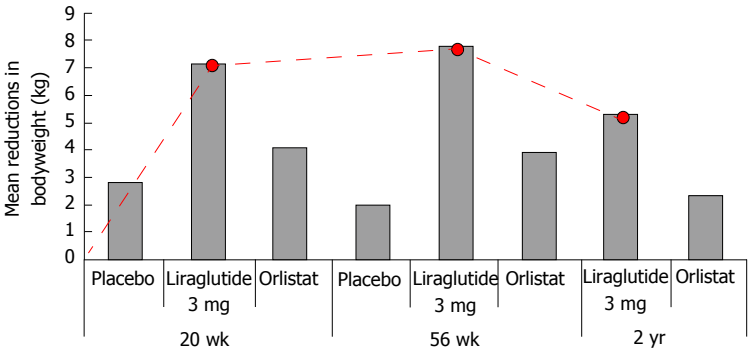

Figure 14 Liraglutide 3 mg shows greater, dose dependent, reductions in body weight compared to placebo and orlistat with maximal effect between 0-20 wk of treatment. Red line dotted trace shows patters of weight loss from baseline in those subjects treated with 3 mg liraglutide. Greatest rate of weight loss was observed between 0-20 wk, reducing from 20-36 with a tendency toward weight gain from 36 wk and beyond.

subjects exists only if the improved glucose tolerance achieved following a given weight loss with liraglutide 3 mg exceeds those attained following other, arguably cheaper treatment modalities. Calculating correlation coefficients between percentage weight lost and percentage changes in glucose tolerance in non-diabetic obese subjects randomized to liraglutide 3 mg compared to those administered orlistat or placebo[41-43,205,206] in aforementioned phase Ⅲ trials may be one way in which this could be assessed. Figure 15 interprets the possible findings of such a test.

If indeed pharmacological GLP-1 agonism improves glucose tolerance independent of weight lost, the potential curb in prevalence, thus socio-economic burden of T2DM achieved with treatment provides a second rationale for the licensing and funding of liraglutide 3 mg as an adjunct to lifestyle alteration as the first line anti-obesity agent for weight management in obese and co-morbid overweight individuals in the United Kingdom.

Adverse drug events

Evidence - in-treatment tolerability: Whilst evidence exists to suggest GLP-1 agonism may be a targeted agent with long term cost-benefit for use in the medical management of obesity, tolerability and safety are important considerations in determining the choice of any pharmaceutical, especially in the management of chronic disease. The safety and efficacy of liraglutide 3 mg has been evaluated in 5 phase Ⅲ double-blinded placebo controlled trials comprising 3384 overweight or obese subjects receiving liraglutide 3 mg and 1941 placebo controls for a treatment periods of 32, 52 and 56 wk[42,42,205-207]. In a pooled analysis of the 5 aforementioned trials, liraglutide 3 mg in obese and overweight subjects was generally well tolerated, with most adverse drug events gastrointestinal in nature, transient and of mild to moderate intensity[42,43,205-207]. However, 9.8% of liraglutide and 4.3% of placebo recipients discontinued treatment because of an adverse event[217]. Figure 16 details adverse reactions occurring with a higher incidence to placebo with an incidence of ⩾ 10% in liraglutide 3 mg recipients, stratified by system.

Of interest, 0.6% of subjects receiving liraglutide 3 mg experienced increases in mean heart rate (an average baseline increase of 2.5 beats/min) compared to 0.1% of placebo recipients[218]. Potentially a manifestation of GLP-1 induced increases in SNS activity (potential effectors of GLP-1s negative energy balance effects: Central controllers) contributing to GLP-1 induced weight loss *via* increases energy expenditure, elsewhere tachycardia associated with 3 mg liraglutide treatment in non-diabetic obese subjects yields no associated increases in 24 h energy expenditure[209]. Thus, whilst the clinical significance of this finding remains to be determined, observations may warrant more intense monitoring in patients with pre-existing cardiovascular disease.

Interpretations - long term risk-benefit of liraglutide 3 mg as an anti-obesity agent on the NHS: Though generally well tolerated in the acute setting, safety concerns have been raised regarding the potential risk of pancreatitis and pancreatic and thyroid cancer with long-term use of GLP-1 analogues[219,220]. Confirmed cases of acute pancreatitis and papillary thyroid carcinoma were reported in 0.3% of liraglutide 3 mg treated compared to 0.2% of placebo treated participants, however the relative rarity of events means the relationship between treatment with disease incidence and severity remains to be defined. On-going clinical experience and thorough post-marketing surveillance should help clarify any such associations and also identify other potential adverse drug events. To this end, episodes of acute renal failure and medullary thyroid carcinoma (not observed during in-treatment and FUP period assessments[42,43,205-207]) have been reported in the post-marketing period, though again, insufficient data exists to establish or exclude a causal relationship. Figure 17 details other potentially serious medical conditions observed in during in-treatment and FUP assessments[42,43,205-207].

Though potentially associated with serious long-term adverse effects, the rarity of incidence and lack of causal relationship mean that current knowledge supports benefit over risk, supporting the licensing and funding of liraglutide 3 mg as the first line anti-obesity pharmaceutical agent for weight management in obese and overweight adults with at-least one weight related

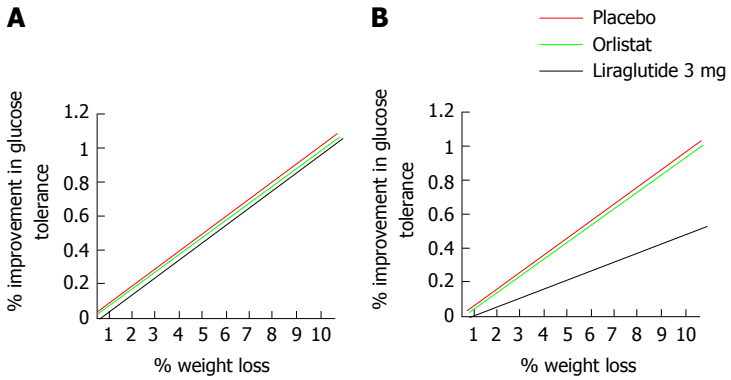

Figure 15 Theoretic model to assess glucose tolerance as a factor of weight. Liraglutide 3 mg produces increased weight loss and increased improvements in glucose control compared to both placebo and control in non-diabetic obese and overweight subjects[41-43,205,206]. If the greater glucose sensitivity seen following treatment with liraglutide 3 mg is secondary to the greater weight loss achieved, the relationship between a given weight reduction and the percentage improvement in glucose tolerance should be the same in all treatment groups (A). If however, the relationship between a given weight loss and change in glucose tolerance is less strong (B) following treatment with liraglutide, findings would suggest that mechanisms beyond weight loss contribute to the greater improvements of glucose tolerance seen following GLP-1 agonism. One mechanism may be *via* activation of GLP-1R in the pancreas, where endogenous GLP-1 signalling has a well established incretin effect. GLP-1: Glucagon-like peptide 1.

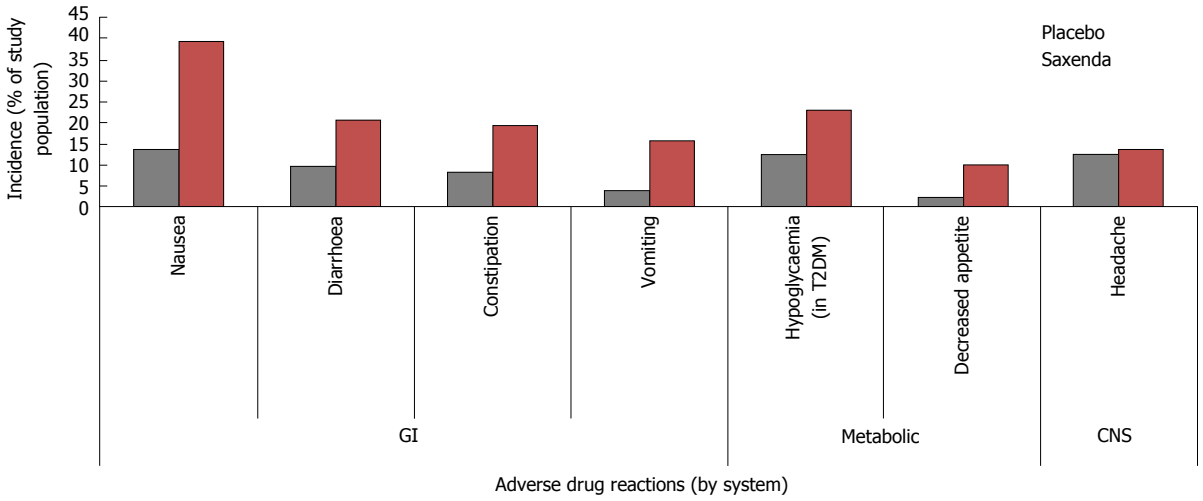

Figure 16 Adverse drug reactions reported in ≥ 10% of liraglutide 3 mg recipients with a higher incidence than placebo stratified by system. GI: Dyspepsia, abdominal pain, dry mouth, gastritis, gastroesophageal reflux disease, flatulence, eructation and abdominal distension were also more prevalent in liraglutide treated participants but occurred with incidence ≤ 10%, not shown; 6.2% with Saxenda *vs* 0.8% with placebo discontinued treatment as a result of gastrointestinal adverse reactions; CNS: Dizziness, malaise and fatigue occurred were more also prevalent in liraglutide treated participants but occurred with incidence ≤ 10%, not shown; Metabolic: Liraglutide reduces blood glucose and thus, there is a potential for hypoglycaemia to occur. In the SCALE diabetes trial severe hypoglycemia occurred in 3 (0.7%) of 422 Saxenda-treated patients and in none of the 212 placebo-treated patients. In clinical trials involving patients without T2DM[42,43,205,206] no systematic reporting of hypoglycemia occurred but spontaneously reported symptomatic episodes potentially hypoglycemic in cause were reported by 1.6% (46/2962) of liraglutide 3 mg and 1.1% of (19/1729) placebo treated non diabetic patients. T2DM: Type 2 diabetes mellitus; GI: Gastrointestinal; CNS: Central nervous system.

co-morbidity in the United Kingdom.

CONCLUSION

Obesity is a global epidemic, perhaps the greatest challenge to global and public health of our time. Whilst public health initiatives should continue to focus on curbing the projected upward trends in the incidence of obesity and overweight, effective management of those individuals already obese remains an important and as yet unmet clinical need. The current medical management of obesity in the United Kingdom is suboptimal, with the only treatment modality with proven long-term efficacy being bariatric surgery. Both risky and costly, this treatment option is not viable for the widespread management of obesity, and remains reserved for extreme cases. The ideal medical management of any illness utilizes a targeted pharmacotherapy that either repletes physiological factors pathologically depleted, or antagonizes pathological processes, the development of such an agent requiring an understanding of the pathophysiology underpinning a disease. Though the pathophysiology of clinical obesity is undoubtedly multifaceted, several lines of clinical evidence implicate a role for functional impairments in GLP-1 signalling. Whilst genetic studies implicate a role for primary altered GLP-1

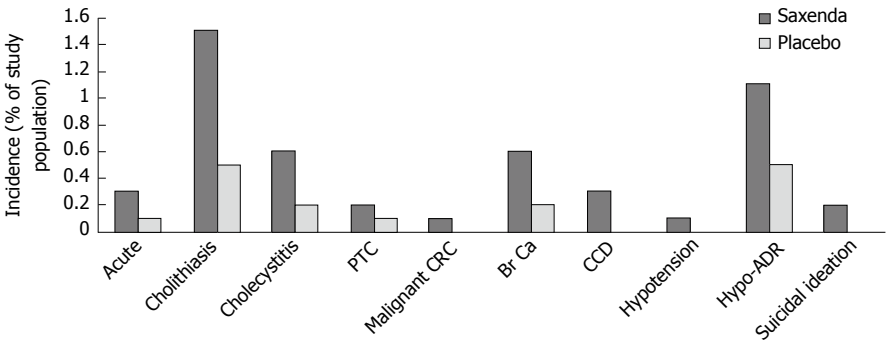

Figure 17 Reported incidences of serious medical conditions from pooled analyses of 5 double-blinded placebo controlled trials studying the safety and efficacy of 3 mg liraglutide (Saxenda) in 3384 overweight or obese subjects receiving liraglutide and 1941 placebo controls. Hypotension associated adverse reactions (Hypo-ADR) refer to hypotension, orthostatic hypotension, circulatory collapse, and decreased blood pressure). One of the six (0.2%) liraglutide treated subjects reporting suicidal ideations attempted suicide. PTC: Papillary thyroid carcinoma; CRC: Colorectal carcinoma; Br Ca: Breast cancer; CCD: Cardiac conduction disorders.

signalling as a risk factor towards development of the obesity phenotype, clinical studies assessing physiological GLP-1 responses in normal weight and obese subjects suggest weight gain may induce functional deficits in GLP-1 that facilitates maintenance of the obesity phenotype. Whatever the relationship, cause or effect, reductions in functional GLP-1 signalling seems to play a role in clinical obesity, as such, the pharmacological replenishment of this functional deficit seems a promising target for the medical management of obesity in the clinic. Indeed, the GLP-1 analogue liraglutide 3 mg has shown promising results in achieving and maintaining greater weight loss in obese individuals when compared to control or currently licensed anti-obesity medication. Though results from extended phase Ⅲ and phase Ⅳ studies report the development of potentially fatal adverse drug events in those randomized to or prescribed liraglutide 3 mg respectively, the scarcity of incidence and lack of causal relationship sees such potential risks overshadowed by the proven superior weight loss efficacy of treatment. Cost-benefit, however, may pose a barrier toward viable NHS funding, though this may be overcome by strategic treatment delivery; combining short-term liraglutide 3 mg treatment (≤ 36 wk) with behavioural therapies targeted toward promoting healthy lifestyle changes. With drug induced weight loss potentially reinforcing adherence to long-term lifestyle changes, if successful, shortened in-treatment period alongside decreases in direct and indirect socioeconomic burdens of obesity and overweight secondary to achievement and maintenance of significant weight loss associates a long-term cost-benefit to funding treatment. Such a concept supports the use of liraglutide 3 mg as the first line anti-obesity agent on the NHS when conservative lifestyle management alone has failed in achieving clinically significant weight loss in comorbid overweight or obese adults.

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MINIREVIEWS

Place of technosphere inhaled insulin in treatment of diabetes

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Abstract

Technosphere insulin (TI), Afrezza, is a powder form of short-acting regular insulin taken by oral inhalation with meals. Action of TI peaks after approximately 40-60 min and lasts for 2-3 h. TI is slightly less effective than subcutaneous insulin aspart, with mean hemoglobin A1c (HbA1c) reduction of 0.21% and 0.4%, respectively. When compared with technosphere inhaled placebo, the decrease in HbA1c levels was 0.8% and 0.4% with TI and placebo, respectively. Compared with insulin aspart, TI is associated with lower risk of late post-prandial hypoglycemia and weight gain. Apart from hypoglycemia, cough is the most common adverse effect of TI reported by 24%-33% of patients *vs* 2% with insulin aspart. TI is contraindicated in patients with asthma and chronic obstructive pulmonary disease. While TI is an attractive option of prandial insulin, its use is limited by frequent occurrence of cough, need for periodic monitoring of pulmonary function, and lack of long-term safety data. Candidates for use of TI are patients having frequent hypoglycemia while using short-acting subcutaneous insulin, particularly late post-prandial hypoglycemia, patients with needle phobia, and those who cannot tolerate subcutaneous insulin due to skin reactions.

Key words: Afrezza; Efficacy; Safety; Technosphere insulin; Cough

Core tip: Technosphere insulin is the only approved form of inhaled insulin. It is a short-acting insulin that can be taken with meals in patients with type 1 or type 2 diabetes. In this minireview, the author provides an appraisal of this new formulation of insulin to help determine its place in the management of diabetes.

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INTRODUCTION

In June 2014, the Food and Drug Administration (FDA) approved technosphere insulin (TI) under the trade name Afrezza (MannKind Corp., Valencia, CA) for use in

type 1 and type 2 diabetes[1]. Afrezza is a dry powder of recombinant human insulin adsorbed onto an inert excipient of fumaryl diketopiperazine (FDKP) particles[2]. In this powder, insulin and FDKP are present in a 1:9 ratio by dry weight[3]. The median aerodynamic diameter of technosphere microparticles is 2-2.5 μm, suited for deposition in distal lungs[3]. In initial studies, Afrezza was delivered by a device called MedTone. In 2010, the manufacturer produced a smaller and more efficient device called Gen2[4]. The main purpose of this article is to identify the best candidates for the use TI based on its pharmacodynamics, efficacy and safety. More emphasis will be placed on data derived from clinical trials using the current Gen2 device of TI.

SEARCH METHODOLOGY

PubMed search was conducted until July 2016 to identify all humans studies related to efficacy and safety of TI published in the English, Spanish and French literature. The search included pertinent animal and *in-vitro* studies. Review articles, and prescribing information of afrezza are also reviewed. Search terms included "inhaled insulin", "diabetes mellitus", "technosphere", "pulmonary safety", "cough", "lung cancer".

ABSORPTION AND METABOLISM

Upon inhalation, a mean of approximately 60% of the emitted dose of TI reaches the lungs. The remainder 40% is swallowed and enters the gastrointestinal tract[3]. The technosphere particles dissolve rapidly at the physiological pH of the lungs. This dissolution allows both insulin and the excipient FDPK to be independently absorbed across the alveolar wall to the systemic circulation with a time to maximum concentration (Tmax) of 15 and 10 min, respectively[1,3]. The inhaled insulin and its carrier FDKP are rapidly cleared from the lungs. Thus, clearance half-life of insulin and FDKP from the bronchoalveolar lavage is approximately 1 h. By 12 h post-inhalation, their concentrations in the bronchoalveolar lavage are below or near the limit of quantification[3]. After systemic absorption, the carrier FDKP is excreted unchanged in urine without any evidence of pharmacological activity[5].

PHARMACOKINETICS AND PHARMACODYNAMICS

Bioavailability of TI (calculated based on actual insulin content of the TI cartridge) is approximately 20%-27% relative to subcutaneous regular insulin, and 33% relative to lispro[6]. TI has rapid onset of action characterized by a sharp rise in serum concentrations reaching peak levels after 12-15 min[7]. The median time to maximum effect in 12 patients with type 1 DM was shown to be 53 min (SD 74 min) compared to approximately 90-120 min with insulin lispro[7]. In another study of 11 healthy volunteers, mean time for maximal effect of TI was 42-58 min for doses 25-100 units compared to 171 min with subcutaneous regular insulin given in a single dose of 10 units[8]. Duration of action of TI is short and fades away by approximately 160 min or 2-3 h[7,9]. Median terminal half-life of TI is 28-39 min (for doses 4-32 units) *vs* 145 min for subcutaneous regular insulin (15 units)[7]. Based on the above pharmacodynamics, TI is considered an ultra-rapid insulin given at the start of a meal or within 20 min after starting a meal[10].

EFFICACY OF TI

Effect on blood glucose concentrations

Reduction in post-prandial hyperglycemia is the main target action of TI. In one placebo-controlled trial of patients with type 2 diabetes, patients randomized to TI had 43% more reduction in maximal postprandial glucose levels compared with inhaled placebo[11]. The study of Rosenstock *et al*[12] shed the light on timing of action of TI. Thus, compared with biaspart insulin injected 15 min before meals, the inhalation of TI within 90 s of meal ingestion was associated with significant decrease in self-monitored blood glucose (SMBG) at 1 h after meals (171 mg/dL *vs* 209 mg/dL with biaspart). Yet, 2 h after meals, blood glucose concentrations were similar between biaspart and TI, and after 2 h, postprandial glucose levels became higher in patients randomized to TI than biaspart[12]. The previous finding is in agreement with pharmacodynamics studies described above showing that peak action of TI occurs at approximately 1 h and fades away after 2 h[6,7].

Effect on hemoglobin A1c levels

Overall, available data suggest that TI is slightly less effective than subcutaneous insulin. In one meta-analysis of 8 clinical trials of type 1 and type 2 diabetes, mean hemoglobin A1c (HbA1c) reduction with subcutaneous insulin was slightly greater than TI, with net statistically significant difference of 0.16% (95%CI: 0.06%-0.25%)[13]. The same meta-analysis showed that mean HbA1c reduction compared to baseline was 0.55% (95%CI: 0.34%-0.78%) based on data compiled from 12 clinical trials using the older MedTone device and the current Gen-2 device[13]. The efficacy of the TI Gen2 device was published in 2 trials summarized in Table 1[9,10]. In one trial, the mean reduction in HbA1c levels among patients randomized to TI and aspart was 0.21% and 0.4%, respectively with a significant difference of 0.19% (95%CI: 0.02-0.36)[10]. In the second study, TI was superior to placebo, as expected, with HbA1c reduction of 0.8% *vs* 0.4%, respectively[9]. Although the MedTone device is no longer used, 2 randomized trials using this older device are presented in Table 2 because of their large size, and relatively long-duration (1-2 years)[12,14]. Studies that directly compare the efficacy of the 2 devices are not available. However, their short-term safety was compared in single head to head trial discussed in the safety section below[10].

SAFETY PROFILE OF TI

Hypoglycemia

In clinical trials of TI-Gen 2, non-severe hypoglycemia

Table 1 Clinical trials of technosphere insulin using Gen2 device

Ref.	Rosenstock *et al*[9]	Bode *et al*[10]
Design	Randomized, double-blind, placebo-controlled, 24 wk-duration	Randomized, open-label, 24 wk-duration
Type of diabetes	Type 2	Type 1
Intervention	TI (n = 177) *vs* placebo (n = 177), both groups were on oral agents	TI (n = 174) *vs* prandial aspart (n = 170). Both groups received basal insulin (NPH or detemir, or glargine)
Mean HbA1c levels at baseline	8.26%	7.93%
Reduction in HbA1c *vs* baseline	-0.8% with TI and -0.4% with placebo	-0.21% with TI *vs* -0.4% with aspart
Reduction in mean HbA1c with TI *vs* comparator	-0.4% *vs* placebo (95%CI: -0.57 to -0.23)	0.19% *vs* aspart (95%CI: 0.02 to 0.36)
Proportions of patients reaching HbA1c ⩽ 7%	38% with TI *vs* 19% with placebo (P = 0.0005)	18% with TI *vs* 31% with aspart (P = 0.01)
Proportions reporting adverse effects	61% TI *vs* 51.1% placebo	58% TI *vs* 43% aspart
Proportions of patients reporting hypoglycemia	67.8% TI *vs* 30.7% placebo (P < 0.0001)	96% TI *vs* 99.4% aspart (P = 0.06)
Proportions of patients reporting cough	23.7% TI *vs* 19.9% placebo (difference not statistically significant)	31.6% TI *vs* 2.3% aspart P < 0.05
Withdrawal due to cough	1.1% with TI *vs* 3.4% with placebo	5.7% with TI *vs* 0% with aspart
Change in mean weight	+ 0.5 kg TI *vs* -1.1 kg placebo (P < 0.0001)	-0.4 kg with TI *vs* +0.9 kg aspart (P = 0.01)
Change in mean FEV1 (L)	- 013 L with TI *vs* -0.04 L with placebo	-0.07 L with TI *vs* -0.04 L with aspart
Withdrawal due to adverse effects	4% with TI *vs* with 5.1% placebo	9.2% with TI *vs* with 0% aspart

FEV1: Forced expiratory volume in 1 s; TI: Technosphere insulin; HbA1c: Hemoglobin A1c.

Table 2 Clinical trials of technosphere insulin using the Med-Tone device

Ref.	Raskin *et al*[14]	Rosensensk *et al*[12]
Design	Randomized, open label, 2 yr-duration, pulmonary safety trial	Randomized, open-label, 52-wk duration
Type of diabetes	Types 1 and 2	Type 2
Groups of subjects and intervention	TI (n = 938), usual diabetes care (n = 951), control subjects without diabetes (n = 164)	Glargine qhs + prandial TI (n = 334) *vs* [1]biaspart insulin bid (n = 343)
Proportions of patients with adverse effects	79% TI *vs* 71% usual care	84% TI *vs* 89% biaspart
Mean HbA1c at baseline	8.7%	8.7%
Reduction in HbA1c *vs* baseline	-0.59% with TI and -0.50% with usual care	-0.68% with TI/glargine *vs* -0.76% with biaspart
Reduction in HbA1c with TI *vs* comparator	0.09% (not significant)	0.07% (not significant)
Proportions of patients reporting hypoglycemia	39.5% TI *vs* 39.1% usual care	48% glargine/TI *vs* 69% biaspart. OR 0.4 (95%CI: 0.3-0.5)
Proportions of patients reporting cough	27.8% TI *vs* 4.4% usual care	33% glargine/TI *vs* 6% biaspart
Withdrawal due to cough	4.7% TI *vs* 0% usual care	2% glargine/TI *vs* 0% biaspart
Change in mean weight	Not reported	+ 0.9 kg glargine/TI *vs* +2.5 kg biaspart. Mean difference 1.6 kg (95%CI: -2.4 to -0.7)
Decline in mean FEV1 (liters)	More decline in TI group *vs* usual care. Mean difference 0.037 (95%CI: 0.017-0.06)	-0.13 glargine/TI *vs* -0.09 biaspart (difference not significant)
Withdrawal due to adverse effects	11% TI *vs* 0.6% usual care	9% glargine/TI *vs* 4% biaspart

[1]Biaspart insulin is pre-mixed insulin composed of 70% insulin protamine suspension + 30% insulin aspart. FEV1: Forced expiratory volume in 1 s; TI: Technosphere insulin; HbA1c: Hemoglobin A1c.

was defined as SMBG < 70 mg/dL and/or presence of symptoms of hypoglycemia, whereas severe hypoglycemia was an event that required assistance of another person[9,10]. Compared with insulin aspart, incidence of all hypoglycemia was numerically lower in patients randomized to TI-Gen2, 96.0% and 99.4%, respectively (P = 0.062), and incidence of severe hypoglycemia was significantly lower with TI Gen2-treatment than with aspart, 18.4% and 29.2%, respectively[10]. Importantly, the timing of hypoglycemic events reported in patients treated with TI was consistent with its short duration of action. Hence, hypoglycemic event rates (events/patient-months) within 2 h after meals were similar in patients randomized to TI and insulin aspart. Meanwhile, 2-5 h after meals, those rates were 2-3 times less with the use of TI compared with insulin aspart[10]. On the other hand, when compared with inhaled placebo, the incidence of all hypoglycemia was higher with TI-Gen 2 therapy (67.8%) compared with placebo (30.7%), (P < 0.0001), and incidence of severe hypoglycemia was 5.1% with TI *vs* 1.7% with placebo (P = 0.09)[9].

Cough and throat symptoms

Cough is the most common non-hypoglycemic adverse effect of TI reported by 24%-33% of patients randomized to TI compared to 2%-6% of patients randomized to subcutaneous insulin or usual diabetes care[9,10,13,14]. Cough induced by TI is characterized by several features. First, it is generally mild, described as severe in approximately 1% of patients[10]; second, it occurs within 10 min of inhalation[9]; third, the percentage of patients reporting cough is highest in the first week after treatment, then decreases gradually with time[10]; fourth, cough is reversible, and resolves within 1-2 d after drug discon-

tinuation[9]; fifth, the occurrence of cough did not seem to be related to changes in pulmonary function as discussed below[14]; sixth, proportions of patients who reported cough was slightly higher in patients taking TI *vs* technosphere inhaled placebo powder: 23.7% (42 of 177) *vs* 19.9% (35 of 176), respectively[9]. The latter observation suggests that cough is mainly due to the inhaled excipient powder (FDKP), and that the insulin component contributes to a lesser extent to the development of cough. The exact mechanism of cough is unclear, and is probably due to stimulation of cough reflex by dry powder inhalation[9]. Unfortunately, frequency of cough associated with the use of the current Gen2 device is markedly greater than the older MedTone device, 31.6% and 22.5%, respectively[9]. This finding was attributed to the high amount of powder being inhaled in a single inhalation with Gen2, whereas with MedTone, the amount of powder inhaled per dose was distributed over 2 inhalations[9].

Throat pain or irritation occurred in 4.4% of patients with type 2 DM (n = 1991) compared with 0.9% of patients using comparator (non-inhaled) therapy (n = 1363)[7].

Effect of TI on pulmonary function tests

The effect of TI delivered by MedTone device on pulmonary function was studied in a large randomized trial composed of 3 groups of subjects followed for 2 years: Patients with type 1 or type 2 diabetes receiving TI (n = 730), patients with type 1 or type 2 diabetes receiving usual care n = 824), and a smaller group of subjects without diabetes not taking any medications (n = 145) (Table 2)[14]. After 3 mo, the authors recorded an initial decline among the 3 patient groups in all parameters of pulmonary functions studied including the forced expiratory volume in 1 second (FEV1) with the largest decline occurring in the TI-treated group. The difference in decline in FEV1 from baseline to 24 mo between the TI-treated group and usual care group was small but statistically significant: 0.037 liters (95%CI: 0.014 to 0.060)[14]. However, after 3 mo, the rate of change in respiratory parameters was not statistically different between patient groups. This suggests that worsening of pulmonary function in patients treated with TI occurred early in the first 3 month, and do not progress further up to 2 years of follow-up. The manufacturer recommends that pulmonary function tests (*e.g.,* spirometry) should be assessed before treatment initiation, after 6 mo of therapy and annually thereafter[7]. If there is reduction of 20% or more in FEV1 compared to pre-treatment values, consideration should be given for drug-discontinuation[7]. Although Raskin *et al*[14] did not found a relationship between the changes in pulmonary function and the occurrence of cough, the manufacturer recommends more frequent monitoring of pulmonary function in patients with any pulmonary symptoms such as persistent cough, wheezing and breathing difficulties, and to discontinue the drug if symptoms persist[7]. Available data are insufficient regarding reversibility of pulmonary function abnormalities after discontinuation of long-term use of TI[7]. However, in a 24-wk trial, the authors documented reversibility of FEV1 4 wk after discontinuation of TI[10]. The exact mechanisms of decline in pulmonary function after TI inhalation are unclear. Animal studies showed that the previous form of inhaled insulin (Exubera) forms amyloid aggregates in lungs of mice and may induce mitochondrial dysfunction leading to a significant reduction in pulmonary air flow[15].

Effect on weight

The effect of TI on weight gain was less pronounced compared with subcutaneous insulin formulations. Thus, when TI was compared with twice daily premixed biaspart, mean weight gain after 52 wk was 0.9 kg and 2.5 kg, respectively[12]. Moreover, the use of TI was associated with mean weight loss of 0.4 kg as opposed to a mean weight gain of 0.9 kg among patient randomized to prandial insulin aspart[10]. One meta-analysis has shown that TI was associated with less weight gain than subcutaneous insulin with a net difference of -1.1 kg (95%CI: -2.1 to -1.6 kg)[14]. Meanwhile, an average weight gain of 0.5 kg was recorded in patients randomized to TI *vs* a weight loss of 1.1 kg in patients randomized to placebo[9]. The reasons for low propensity of TI to cause weight gain are not entirely clear. Possible causes include its somewhat inferior efficacy and lower risk of causing late post-prandial hypoglycemia compared to subcutaneous insulin. The latter advantage might lead some patients to avoid "overeating" in an attempt to prevent hypoglycemia.

Diabetic ketoacidosis

In clinical trials of TI, no reports of ketoacidosis were reported[9-12,14]. However, a meta-analysis that examined regulatory documents reported a nearly 5 times higher incidence of DKA among patients treated with TI compared with prandial short-acting insulin[13]. Likewise, the manufacturer reports higher frequency of DKA in trials of type 1 diabetes among patients using TI *vs* subjects receiving comparators: 0.43% (n = 13) and 0.14% (n = 3), respectively[7]. The reasons for this increase in DKA with TI are not understood, but could be partly attributed to its ultra-short duration of action of TI creating times of day with relative insulin deficiency.

Lung cancer

In patients exposed to TI in clinical trials, the manufacturer reported 2 cases of lung cancer (2 cases in 2750 patient-years of exposure) both having prior history of heavy tobacco abuse[7]. Two other cases (both squamous cell carcinoma) occurred in non-smokers after clinical trial completion. Thus, 4 cases of lung cancer were reported in patients exposed to TI *vs* none in control group[7]. Although the number of affected patients is too small to draw a valid conclusion, lung cancer is certainly a major concern of inhaled insulin, particularly that lung cancer rates were found to be increased in association with the previous inhaled insulin Exubera[16]. The long-term local effects of TI and its carrier on pulmonary cell are unknown. *In vitro* studies of lung cell line (Calu-3) showed that TI did not affect insulin transport, cell viability, and plasma membrane integrity[17]. Meanwhile,

Table 3 Candidate patients for technosphere insulin

Patients with type 1 diabetes who are taking basal insulin once daily, but prefers to take their prandial insulin in the inhaled formulation
Patients with type 2 diabetes uncontrolled on oral agents, and are reluctant to start subcutaneous insulin due to needle phobia or other reasons
Patients already on subcutaneous prandial insulin who develop frequent late post-prandial hypoglycemia (4-5 h after meals)
Any patient who develops skin reactions to insulin subcutaneous injections such as lipoatrophy or lipohypertrophy
In combination of automated artificial pancreas to provide rapid insulin delivery right after meals[20]

Table 4 Advantages and limitations of technosphere insulin

Advantages
Relatively easy and non-painful administration
Flexible timing of administration either inhaled directly before meals or within 20 min after finishing a meal[10]
Hypoglycemia is less frequent than subcutaneous insulin, particularly late postprandial hypoglycemia
Weight gain is slightly less pronounced than subcutaneous insulin
Limitations
Frequent cough (24%-33% of patients)
Available only as prandial short-acting insulin. Hence, long-acting basal subcutaneous insulin should be added in patients with type 1 diabetes
Slightly less effective than subcutaneous insulin
Need for baseline and then serial pulmonary function testing
Safer to switch to subcutaneous insulin in case of upper or lower respiratory infections to avoid exacerbation of the disease and possible unreliable pulmonary absorption
No data available for pediatric and pregnant populations
Limited strength options and difficult fine titration of doses
Lack of long-term safety data
High cost, *e.g.*, average price of ninety 4-unit cartridges and 2 inhalers is $271[21]

insulin is a growth factor that binds to insulin receptors and if present in high concentrations, can bind to IGF-I receptors in lungs. This binding could potentially induce new-onset pulmonary cancer or accelerate growth of pre-existing malignant cells. Indeed, isolated human bronchial carcinoma cells (H292) were shown to express insulin receptors 4-5 times higher than normal bronchial epithelial cells[18]. In fact, the FDA requested the manufacturer to conduct a clinical trial with sufficient power to examine this issue.

Patients with pulmonary diseases and smokers

TI is contraindicated in any chronic pulmonary disease such as asthma or chronic obstructive pulmonary disease (COPD)[7]. Indeed, acute bronchospasm and wheezing were observed in 29% (5 of 17) of patients with asthma following inhalation of TI compared with none of 13 individuals without asthma[7]. Moreover, in asthmatic patients, a substantial mean reduction in FEV1 of 400 mL was recorded 15 min after a single dose of TI[7]. Similarly, in a small group of patients with COPD (n = 8), a mean decline in FEV1 of 200 mL was observed 18 min after TI inhalation[7]. These acute and severe reactions to TI among patients with asthma and COPD could be the result of airway irritation upon contact with the inhaled insulin and/or the excipient. Interestingly, no significant differences in pharmacokinetics (time to maximum concentration, peak plasma insulin concentrations, and plasma insulin exposure) were found between patients with COPD and healthy subjects after a single dose of TI[19]. In case of common cold or flu, some workers recommend switching to subcutaneous insulin until the disease resolves[4]. It is not recommended that patients who smoke use TI[7].

PLACE OF INHALED INSULIN TI IN DIABETES THERAPY

Based on available data, the use of TI is most appropriate in the following selected groups of patients (Table 3). First, patients with type 1 diabetes who are taking basal insulin once daily, but prefers to take their prandial insulin in the inhaled formulation; second, patients with type 2 diabetes uncontrolled on oral agents, and are reluctant to start subcutaneous insulin due to needle phobia or other reasons; third, patients already on subcutaneous prandial insulin who develop frequent late post-prandial hypoglycemia (4-5 h after meals); fourth, patients who develop skin reactions to insulin subcutaneous injections such as lipoatrophy or lipohypertrophy. Another potential place of TI that is under investigations includes its use in combination of automated artificial pancreas to provide rapid insulin delivery right after meals[20].

CONCLUSION

Despite its limitations, TI represents a useful addition to the treatment of diabetes. Its easy non-invasive way of administration is a major advantage to patients who do not like injections. Although TI is slightly less effective than the subcutaneous insulin analog aspart, this is balanced by its lower risk of causing late postprandial hypoglycemia and weight gain. Cough remains a major limiting factor of TI occurring mainly in early treatment. Long-term clinical trials of adequate power along with post-marketing (phase IV) studies are needed to clarify the long-term safety of TI and its relationship to lung cancer. Advantages and limitations of TI are summarized in Table 4.

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ORIGINAL ARTICLE

Basic Study

Evaluation of extraction protocols for anti-diabetic phytochemical substances from medicinal plants

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Author contributions: Okoduwa SIR and Umar IA got the concept and design of the study; Okoduwa SIR and Habila JD perform the study and data analysis; James DB, Inuwa HM and Habila JD participated in the interpretation of data; Okoduwa SIR wrote the first version of the manuscript; Umar IA, James DB, Inuwa HM and Habila JD critically revised the manuscript for important intellectual content; all the authors read and gave a final approval of the revised version of the manuscript to be published.

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Institutional animal care and use committee statement: The experimental protocol was review and approved by the Research Ethics Committee of the Institute. All experimental protocol was in conformity with the Institutional guidelines that are in compliance with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research.

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Abstract

AIM

To examine the efficacy of three extraction techniques: Soxhlet-extraction (SE), cold-maceration (CM) and micro-wave-assisted-extraction (MAE) using 80% methanol as solvent.

METHODS

The study was performed on each of 50 g of *Vernonia amygdalina* (VA) and *Occimum gratissimum* (OG) leaves respectively. The percentage yield, duration of extraction, volume of solvent used, qualitative and quantitative phytoconstituents present was compared. The biological activities (hypoglycemic effect) were investigated using albino wistar rat model of diabetes mellitus (n = 36) with a combined dose (1:1) of the two plants leaf extracts (250 mg/kg b.w.) from the three methods. The

extracts were administered orally, once daily for 21 d.

RESULTS

In this report, the percentage VA extract yield from MAE was highest (20.9% ± 1.05%) within 39 min using 250 mL of solvent, when compared to the CM (14.35% ± 0.28%) within 4320 min using 900 mL of solvent and SE (15.75% ± 0.71%) within 265 min using 500 mL of solvent. The percentage differences in OG extract yield between: MAE *vs* SE was 41.05%; MAE *vs* CM was 46.81% and SE *vs* CM was 9.77%. The qualitative chemical analysis of the two plants showed no difference in the various phytoconstituents tested, but differs quantitatively in the amount of the individual phytoconstituents, as MAE had significantly high yield (P > 0.05) on phenolics, saponins and tannins. SE technique gave significantly high yield (P > 0.05) on alkaloid, while CM gave significant high yield on flavonoids. The extracts from CM exhibited a significantly (P > 0.05) better hypoglycemic activity within the first 14-d of treatment (43.3% ± 3.62%) when compared to MAE (36.5% ± 0.08%) and SE methods (33.3% ± 1.60%). However, the percentage hypoglycemic activity, 21 d post-treatment with 250 mg/kg b.w. extract from MAE was 72.6% ± 1.03% and it was more comparable to 10 mg/kg b.w. glibenclamide treated group (75.0% ± 0.73%), unlike the SE (69.5% ± 0.71%) and CM (69.1% ± 1.03%).

CONCLUSION

CM technique produces extract with better hypoglycemic activity, whereas; MAE is a better option for high yield of phytoconstituents using less solvent within a short time.

Key words: Extraction techniques; Microwave-assisted-extraction; Maceration; Phytoconstituents; Medicinal plants; Soxhlet; Anti-diabetes

Core tip: Extraction of active phytoconstituents from medicinal plants rely mostly on the use of appropriate extraction method. Different extraction techniques affect the yield and biological activity of phytocomponents. In this study, we observed that microwave assisted extraction produces significantly higher overall extract yield as well as in phenolic, saponin and tannin content. Cold maceration and soxhlet extraction produced higher flavonoid and alkaloid yield respectively. Maceration extracts exhibited significantly better hypoglycemic activities in diabetic rats compared to extracts from soxhlet and microwave assisted extraction. This study reveals that the choice of extraction protocol should depend primarily on the purpose of interest.

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases globally, affecting the quality of human life of all ages across the world[1,2]. The disease has become a global public health problem affecting the socio-economic status of the individual[3]. It is an age long, serious heterogeneous metabolic disorder characterized by hyperglycemia and glucose intolerance, due to endogenous insulin deficiency, impaired effectiveness of insulin action, or both[4]. With DM the body cannot regulate the amount of sugar in the blood. This leads to increased glucose in the body that causes deregulation of the metabolism, often accompanied by glycosuria, polydipsia, and polyuria[4].

According to International Diabetic Foundation report, every 6 s, a person dies from diabetes[5]. In 2013, 5.0 million deaths were recorded across the globe with a prevalence of 8.3%[5]. A total of 415 million people are affected with diabetes, worldwide as at December, 2015. The figure is estimated to rise above 642 million by 2040[5]. In Nigeria, 3921500 cases have been reported as at 2013 with a prevalence rate of 4.99%. This alarming rate calls for urgency to find better treatment and novel prevention strategies for the disease.

Several hypoglycemic drugs are available for managing diabetes since it is incurable but they suffer from generally inadequate efficacy and number of serious adverse effects[6,7]. Hence, the shift to the use of plant source, a new hopeful approach that has long been authenticated by World Health Organization in its general assembly[8].

Plants are the major source of potential therapeutic agents worldwide. The uses of Plants for therapeutic purposes have been recorded to be as long as history. Plants which contain substances that could be used for medicinal purposes or which are precursors for the synthesis of useful drugs are considered as therapeutic plants[9]. According to the report of Farnsworth and Soejarto[10], there are between 35000 and 70000 plant species that have been used for medicinal purposes in the world[10]. The bioactive components present in plants can only be utilized in disease treatment/management after being extracted with suitable solvent and prepared into substances such as ointment, cream, gel, moisturizer, pills and so on[11,12].

Extraction is the partitioning of therapeutically dynamic segments of plant utilizing suitably selective solvents through standard methods[12,13]. The basis of all extraction in medicinal plant research is to isolate the dissolvable plant metabolites, excluding the insoluble cell marc. The preliminary unrefined extracts obtained utilizing these strategies contain complex blend of numerous plant metabolites, for example, alkaloids,

glycosides, phenolics, terpenoids and flavonoids[12,14,15]. Some bioactive components present in plants are either heat sensitive or solvent specific hence the quality and composition of the extracts as well as their biological activities are affected by the type of extraction procedure. To get the most astounding biological efficacy and yield of plant extract, it is important to consider these limitations and utilize a standardized method for a specific bioactive molecule[14,16].

The needs of standardized extraction methodology for unrefined medications are to accomplish the remedially craved part and to dispose of the inactive portion by treatment with a specific solvent called menstruum. The extract consequently obtained might be prepared for use as a therapeutic agent in the form of tinctures and fluid extracts, it may be further processed to be fused in any dosage form such as tablets or capsules, or it might be fractionated to seclude singular synthetic substances, for example, vincristine, hyoscine and ajmalicine which are modem drugs. For that reason, standardization of extraction methodologies contributes fundamentally to the final nature of the medicinal drug[13,15].

However, with the expanding interest for natural therapeutic products and nutraceuticals for healthcare everywhere throughout the world, producers of medicinal plant extracts are in continuous search for the most suitable extraction strategy keeping in mind the end goal to produce extracts of characterized quality with minimal variability from batch to batch. Conventional extraction is generally carried-out using reflux, maceration, soxhlet and distillation techniques. These techniques which have been utilized for a long time are extremely tedious and require generally a lot of solvents. Extraction utilizing non-routine techniques, for instance, microwave techniques can produce high yield, within a shorter time utilizing a smaller amount of solvents[17,18]. Among the different customary and routine extraction systems, Soxhlet extraction has been the most generally utilized. Unfortunately, there is paucity of literature on the best extraction method for a specific bioactive molecule. The evaluation on some of these methods by most previous investigators focuses on either the yield or duration of extraction, without considering the effects of the protocol on the various bioactive entities that works in a synergic manner.

Soxhlet extraction serves not just as a method for extraction of phyto-constituents but additionally as a reference to look at more current extraction procedures. Previously, it has been suggested that the microwave-assisted-extraction (MAE), a present day extraction method is a superior method for extricating phyto-components from plants[19]. Several reports on the usefulness of the MAE as it concerns medicinal plants have been published[20-24].

Vernonia amygdalina (VA) commonly known as bitter leaf and *Occimum gratissimum* (OG) generally refers to as scent leaf, have been reported to have anti-diabetic properties[25]. The efficacy of the combined use of both plants with respect to diabetes has been documented[26]. Also, their hypoglycemic activities have been attributed to the presence of flavonoids, alkaloids and saponins among others[27-29]. It is therefore imperative to examine the effect of different extraction methods on their biological activities.

From our insight, the extraction of phytoconstituents from VA and OG using MAE strategy has not yet been accounted for. These two plants were chosen as a reference point for other therapeutic plants owing to the fact that their anti-diabetic potentials have been established in recent publications[26-31]. Therefore, this study evaluated three extraction technologies, *viz*: MAE, Soxhlet extraction and cold maceration with an aim to present a comparison among the distinctive strategies utilized for extraction of hypoglycemic bioactive constituents from medicinal plants. The primary goal of the research is to give a successful and effective, straightforward, safe and less time consuming with maximal yield strategy for extricating specific bioactive parts from therapeutic plants.

MATERIALS AND METHODS

Plant preparation

Fresh specimen of VA and OG leaves were harvested in the month of May, 2015 from a local farm in Samaru, Zaria, Kaduna State, Nigeria. The plant samples were identified and authenticated by the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen number 1166 and 1285 were deposited for VA and OG respectively. The leaves of the plants sample were dried under shade at room temperature to constant weights for seven days. The dried samples were then pulverized into powder using a laboratory milling machine (Thomas-Wiley Laboratory mill Model 4, United States). The powders were preserved in clean plastic containers, kept away from light, heat and moisture until use.

Reagents and chemicals

All the chemicals and reagents used were of analytical reagent grade.

Experimental duration

The research was conducted between May and December, 2015.

Apparatus

A conventional microwave oven (2450 MHz, Toshiba, and Tokyo, Japan) with variable power up to 1000 watts, a time controller, beam reflector and a stirring device was used.

Procedure

Two conventional extraction techniques namely, Soxhlet and cold maceration were used in comparison with a new modern technology, the MAE technique.

Soxhlet extraction method

Exhaustive Soxhlet extraction was performed using classical apparatus with accurately weighed 50 g of the powdered leaf samples of VA and OG respectively. Extraction was performed with 80% methanol as the extraction solvent. After extraction, the methanol solvent was evaporated by concentrating under vacuum with rotary evaporator (Senco Rotary Evaporator, Model RE 801) at 40 ℃ under reduced pressure. The solvent free methanol extract was thereafter evaluated.

Cold maceration method

Maceration was carried out in a closed conical flask for 72 h. In both case 50 g powdered VA and OG leaf sample and 80% methanol as the extraction solvent were used. The suspension after maceration was centrifuged and the supernatant evaporated under reduce pressure. The solvent free methanol extracts obtained were similarly evaluated.

Microwave assisted extraction method

Accurately weighed 50 g of the homogeneous powder leaf samples was mixed with 60 mL, 80% methanol. After allowing a preleaching time of 5 min the suspension was irradiated with microwave at optimized conditions[32-34]. The samples were treated under microwave irradiation in an intermittent way, *i.e.*, Irradiation: cooling: irradiation. The microwave irradiation time was set at three minutes and cooling time of five minutes was allowed. After 5 repeats, the samples were centrifuged at 4000 rpm and the supernatant evaporated under pressure. The dried residue was evaluated accordingly.

Percentage recovery yield of extraction

The percentage extraction yield (w/w) by the three extraction methods was calculated using the formula: Percentage extraction yield for plant extract = [mass of extract (g)/mass of plant sample (g)] × 100

Aliquots of the extracts were stored in screwed cap vials at 4 ℃-8 ℃ until further use. The extracts were re-dissolved in distilled water when required and given orally through gastric intubations.

Phytochemical analysis

Standard protocols were used in detecting the phytochemical constituents present in the two plants samples[35,36]. Tannins according the method describe by Markkar *et al*[37], Saponins as described by Bruneton[38], Alkaloids as described by Harbone[39], Flavonoids as described by Bohm *et al*[40].

Biological assay

To test biological activities of the plant extract from the different extraction techniques, thirty six albino wistar rats (150-200 g of either sex) fed with rat pellet diet (Grand Cereals Ltd, Nigeria) and water *ad libitum* were used. Animals were first acclimatized for two weeks before used. The study was conducted at the Research and Development Laboratory of Nigerian Institute of Leather and Science Technology (NILEST), Zaria Nigeria. The anti-hyperglycemic effect of the extracts obtained from the three extraction methods were assessed using rat model of DM. The experimental protocol was approved by the Institutional Animal Ethic Committee. All experimental protocol was in conformity with the institutional guidelines that are in compliance with National and International Laws and Guidelines for Care and Use of Laboratory Animals in Biomedical Research. The rules and regulations in accordance to the Ethical Committee directive were strictly followed.

Induction of diabetes

The rat model of diabetes used for this study was developed as followed. First, the rats were fasted overnight after which they were given a single intra-peritoneal injection (*ip*) of 55 mg/kg b.w. of streptozotocin (STZ) (Adooq Bioscience, LLC, United States) dissolved in 0.1 mL fresh cold citrate buffer pH 4.5.

Confirmation of diabetes was done 72 h after STZ induction, using a One Touch Glucometer (Lifescan Inc 1995 Milpas, California, United States). Blood samples were obtained from the tail puncture of the rats. Animals with fasting blood glucose ⩾ 200 mg/dL, after 10 d of STZ induction were considered diabetic and included in the study as diabetic animals[30].

Experimental design

Thirty six rats were divided into 6 groups of 6 rats per group. The treatments were as follows: (1) diabetic rats treated with extracts from cold maceration 250 mg/kg b.w.; (2) diabetic rats treated with extracts from soxhlet extraction (SEE) 250 mg/kg b.w.; (3) diabetic rats treated with extracts from MAE 250 mg/kg; (4) diabetic rats as positive control treated with standard drug (10 mg/kg b.w, glibenclamide); (5) diabetic rats as negative control. No treatment was given; and (6) non-diabetic rats as standard control (no induction, no treatment).

Route of administration

The extracts were administered orally, once daily for 21 d using combined dose (1:1) of the two plants leaf extracts (250 mg/kg b.w.)

Blood sample collection

Blood sample was withdrawn from the tail vein and tested using glucose test strips and glucometer (On-Call Plus, Acon Laboratories Ins, United States) after an overnight fast.

Statistical analysis

The results obtained were expressed as mean ± SD where applicable. The data were analyzed using analysis of variance and significant differences among means were determined by Duncan's multiple range test at $P < 0.05$ using Statistical Package for Social Sciences software version 20 for windows.

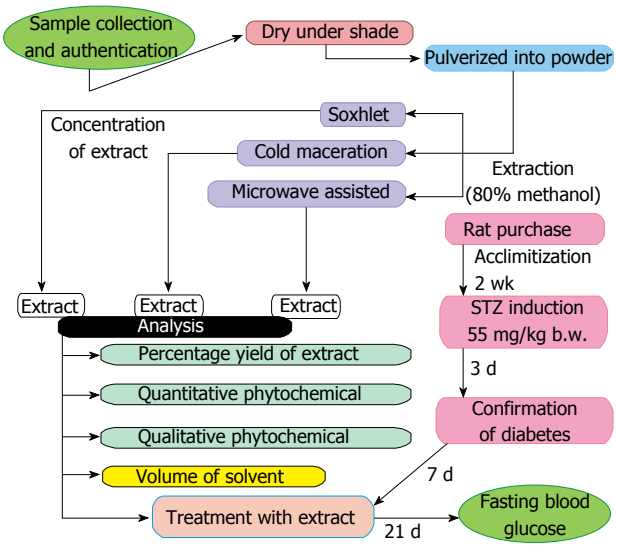

Figure 1 Experimental design for the evaluation of three extraction methods. STZ: Streptozotocin.

RESULTS

The efficacy of three extraction methods were compared by evaluating the anti-hyperglycemic effects of the two medicinal plants (VA and OG) leaf extracts. A flow chart illustrating the experimental design in detail is presented in Figure 1. The experiments were performed using the same quantity of plant samples (50 g each) and the biological activities were analyzed using rat model of diabetes with the same dose of extract (250 mg/kg body weight). A comparison of the extraction of the medicinal plants (VA and OG) using conventional microwaves, soxhlet extractor and cold maceration are shown in Table 1.

The yield of MAE extracts from VA for 39 min were higher (20.90% ± 1.05%) than that of soxhlet extract (15.75% ± 0.71%) for 265 min and that of maceration extract (14.35% + 0.28%) for 4320 min. Similarly, the yield of microwave extracts from OG (19.10% ± 1.67%) for 39 min were higher than that of soxhlet extract (11.25% ± 0.42%) for 255 min and that of maceration extract (10.15% ± 0.65%) for 4320 min. Regarding the extraction time, our result show that MAE appeared to be the fastest method since the extraction could be achieved within minutes. The MAE also consumed the least amount of solvent 250 mL when compared to the other methods, 500 mL and 900 mL for soxhlet and cold maceration respectively (Table 1).

The percentage differences in the recovery yield between two specific extraction methods are shown in the Table 2. It was observed that for VA, the difference in percentage recovery yield between MAE *vs* soxhlet extraction method was 24.59%; but with OG it was 41.05%. The difference in percentage yield between MAE *vs* cold maceration method was 31.29% for VA and 46.81% for OG. Although there were no significant differences between the soxhlet and cold maceration method for both plants, the percentage difference was 8.88% and 9.77 for VA and OG respectively (Table 2).

The results from the present study showed that there was no difference between the qualitative phytoconstituents obtained by the various extraction technologies under investigation (Table 3).

However, the quantitative chemical analysis (Table 4) revealed a statistically higher yield in alkaloid from the soxhlet extraction when compared to the MAE and cold maceration for VA. But there was no significant difference in the alkaloid yield between the soxhlet and MAE method for OG. The cold maceration technology recorded the highest yield in flavonoid in both plant when compared to the MAE and soxhlet extraction method. The difference was statistically significant ($P < 0.05$). The MAE had the highest yield in phenolics, saponins and tannins from the two plants studied when compared to the conventional extraction techniques (soxhlet and cold maceration). However, there was no significant difference between MAE and soxhlet in the yield of tannin from VA.

Soxhlet, cold maceration and MAE extracts of VA and OG were tested and compared for anti-diabetic activities. All the extracts exhibited comparable anti-diabetic activities with that of standard drug (glibenclamide) under the same dose rate of 250 mg/kg body weight tested according to the method of Abdulazeez *et al*[26]. The cold maceration extract exhibited a better hypoglycemic effect within the first 7 (17.9%) and 14 (43.2%) d of treatment when compared to others. But the hypoglycemic activity of extract obtained from MAE was more comparable to the standard drug glibenclamide in reducing the blood glucose of the animals after 28 d post induction (21 d treatment).

The extract from MAE exhibited the least hypoglycemic effect within the first 7 day of treatment. There was no significant difference between the hypoglycemic effects of extracts obtained from soxhlet and the cold maceration method at the end of the 21 d (Tables 5 and 6).

DISCUSSION

Developing nations are rich in therapeutic plants at the same time, because of trouble in getting reliably effective extraction equipments; esteem expansion to this rich bioresources are difficult. Usually and prevalently in extremely poor nations, the advancements utilized are improper and not efficient. The crucial setback is identified with the nature of the products. Primitive extraction techniques don't promise a steady and top notch reliable quality and, sometimes, unseemly innovations and techniques result in creating defiled products which has low market value[41]. The present study, evaluated three different technologies for extraction of hypoglycemic compounds from medicinal plants, with a view to ascertain the best option for the isolation of specific bioactive entity.

In the study it was noticed that Soxhlet extraction and cold maceration spent longer time for complete extraction of same quantity of the plant sample studied. Also, the amount of the solvent utilized in MAE was

Table 1 Comparison of percentage recovery yield, extraction time and volume of solvent used for the different extraction methods for the two plants

Parameter	*Vernonia amygdalina*			*Ocimum gratissimum*		
	MAE	Soxhlet	Maceration	MAE	Soxhlet	Maceration
Sample (g)	50	50	50	50	50	50
Solvent volume (mL)	250	500	900	250	500	900
Extraction time (min)	39	265	4320	39	255	4320
Recovery yield (g)	10.45 ± 0.53[b]	7.88 ± 0.35[a]	7.18 ± 0.14[a]	9.55 ± 0.84[b]	5.63 ± 0.21[a]	5.08 ± 0.32[a]
Percentage recovered (%)	20.90 ± 1.05[b]	15.75 ± 0.71[a]	14.35 ± 0.28[a]	19.10 ± 1.67[b]	11.25 ± 0.24[a]	10.15 ± 0.65[a]

Values are mean ± SD of 3 replicate determinations; same superscript across the column under the same plant indicate no significant difference ($P > 0.05$). MAE: Microwave-assisted-extraction.

Table 2 Calculated percentage differences in recovery yield of extracts between the extraction methods

Vernonia amygdalina	*Ocimum gratissimum*		
	MAE	Soxhlet	Maceration
MAE		41.05%	46.81%
Soxhlet	24.59%		9.77%
Maceration	31.29%	8.88%	

Percentage difference between two extraction methods = [(values from method with higher recovery yield - values from method with lower recovery yield)/values from method with higher recovery yield] × 100.

Table 3 Comparison of some qualitative phytochemicals obtained with the three extraction methods for the two plants

S/N	Constituents	Maceration		Soxhlet		Microwave	
		VA	OG	VA	OG	VA	OG
1	Carbohydrates	+	+	+	+	+	+
2	Anthraquinones	-	-	-	-	-	-
3	Glycosides	+	+	+	+	+	+
4	Cardiac glycosides	+	+	+	+	+	+
5	Saponins	+	+	+	+	+	+
6	Steroids	+	+	+	+	+	+
7	Triterpenes	+	+	+	+	+	+
8	Tannins	+	+	+	+	+	+
9	Flavonoids	+	+	+	+	+	+
10	Alkaloid	+	+	+	+	+	+

The symbol (+): Indicate detected; (-): Not detected; VA: *Vernonia amygdalina*; OG: *Ocimum gratisimum*.

less, proving that, MAE is indeed truly economical. Comparable results were reported by Vongsangnak *et al*[42], when contrasting routine extraction procedures with MAE throughout the isolation of saponins from cell culture of *Panax notoginseng* and analgesic substances from the roots of *Ximenia americana*[20]. Besides, microwave illumination technique has been reported to be extremely quick, dependable for making of Schiff bases[21]. Possible explanation observed in the various methods of extraction could be due to differences in rate of chemical reaction. For instance, in soxhlet extraction approach, the applied heat supplies the activation energy needed to rupture the plant tissues in order to release its phytoconstituents. Whereas in MAE, the activation energy is achieved due to oscillatory wave generated by the system and by the ionic conduction and dipole rotation of the molecules of the plant sample. This brings about increase in the cell pressure, thereby rupturing to release its contents on attaining its elastic limit. The heat in MAE is internally generated by the molecules unlike in soxhlet whereby the heat is externally generated. In cold maceration, the release of phytoconstituents from plant matrix is due to differences in ionic solvent concentration gradients, hence more solvent is require to create a positively dynamic concentration gradients. This account for the higher volume of solvent utilized by the cold maceration as observed in the present study. In a nutshell, with MAE, the plant cell tissues are effectively broken and separated to release phytoconstituents with ease, hence the greater yield as recorded.

The result of the quantitative phytochemical screening within the limit of the analyzed components in the present study suggests that the phytoconstituents of the studied plants were not destroyed since the results obtained showed that the same phytoconstituents tested in all the extracts from the different procedures were the same. This finding is in agreement with earlier reports of Mandal *et al*[19], that, the plant components obtained from MAE are neither decomposed nor oxidized under optimized conditions. Several phytochemicals have been found to give an increase in their extractive yields when compared to their yields on subjection to conventional extraction techniques[21]. Chan *et al*[22] also recorded a higher yield in the extraction of anti-diabetic ingredient from herbal plant. In their experiment using 5 g sample with 150 mL solvent recorded a yield of 1.63 mg/g sample in 5 min using MAE as against 0.47 mg/g in 3 h obtained with soxhlet extraction technology. In a related study for extraction of caffeine and polyphenols from leaves of green tea, MAE achieved higher extraction yield within 4 min than any extraction methods at room temperature for 20 h[43]. Ginsenosides extraction yield from ginseng root was obtained in 15 min using focused MAE technique which was also better than other conventional solvent extraction technologies for 10 h[24,44]. The higher yield obtained from MAE and soxhlet extraction when compared to the cold maceration may be attributed to the heat exchange and mass transfer.

In soxhlet extraction, the heat exchange and the mass transfer are restricting variables contrast with MAE, where heat exchange happens from the focal point of

Table 4 Comparison of the quantitative phytochemicals obtained with the three extraction methods for the two plants

	Vernonia amygdalina (mg/100 g)			*Ocimum gratissimum* (mg/100 g)		
	MAE	Soxhlet	Maceration	MAE	Soxhlet	Maceration
Alkaloid	4.0 ± 0.65^{a}	7.0 ± 0.57^{b}	5.0 ± 0.46^{a}	6.5 ± 0.75^{b}	7.0 ± 0.15^{b}	5.0 ± 0.90^{a}
Flavonoid	13.0 ± 0.35^{b}	9.0 ± 0.50^{a}	15.0 ± 0.21^{c}	10.0 ± 0.35^{b}	8.0 ± 0.18^{a}	12.0 ± 0.25^{c}
Phenolic	17.5 ± 0.25^{c}	15.0 ± 0.21^{b}	12.0 ± 0.70^{a}	15.0 ± 0.22^{c}	14.0 ± 0.21^{b}	11.0 ± 0.65^{a}
Saponin	4.5 ± 0.45^{b}	3.0 ± 0.30^{a}	2.0 ± 0.75^{a}	6.1 ± 0.75^{b}	4.0 ± 0.92^{a}	5.0 ± 0.20^{a}
Tannin	14.0 ± 0.55^{b}	13.0 ± 0.50^{b}	10.0 ± 0.25^{a}	12.0 ± 0.18^{b}	9.0 ± 0.24^{a}	9.1 ± 0.25^{a}

Values are mean ± SD of 3 replicate readings. Same superscript across the column under the same plant signifies no significant difference at $P > 0.05$. MAE: Microwave-assisted-extraction.

Table 5 Effect of the extract from the different extraction methods on blood glucose in streptozotocin-induced diabetic rats

Group	Before induction	7 d after induction	Days after treatment		
			7th	14th	21st
CME	79.4 ± 4.5	344.6 ± 10.3	282.8 ± 17.6	195.8 ± 18.3	106.5 ± 6.7
SEE	85.3 ± 3.2	396.8 ± 15.7	342.5 ± 14.7	264.9 ± 16.8	121.1 ± 10.2
MAE	82.7 ± 5.1	373.3 ± 13.6	331.3 ± 11.4	237.1 ± 8.9	102.4 ± 7.6
PC	91.6 ± 4.5	389.4 ± 11.9	359.0 ± 12.6	266.3 ± 13.2	97.3 ± 5.8
NC	80.6 ± 3.8	365.5 ± 9.8	395.1 ± 16.3	419.6 ± 10.2	448.4 ± 17.4
HC	83.2 ± 6.2	84.8 ± 4.7	83.9 ± 7.8	84.7 ± 6.5	84.2 ± 7.1

Values are mean ± SD (mg/dL) of readings from 6 rats per group. CME: Cold maceration extract; SEE: Soxhlet extraction extract; MAE: Microwave-assisted extraction extract; PC: Positive control; NC: Negative control; HC: Healthy control.

Table 6 Calculated percentage change in blood glucose after treatment with the extracts in streptozotocin-induced diabetic rats

Group	Initial blood glucose on day 0 (mg/dL)	Percentage change after treatment (%)		
		Day 7	Day 14	Day 21
CME	344.6 ± 10.3	18.0 ± 2.66^{e}	43.3 ± 3.62^{e}	69.1 ± 1.03^{c}
SEE	396.8 ± 15.7	13.7 ± 0.29^{d}	33.3 ± 1.60^{c,d}	69.5 ± 0.71^{c}
MAE	373.3 ± 13.6	11.2 ± 0.18^{c,d}	36.5 ± 0.08^{d}	72.6 ± 1.03^{d}
PC	389.4 ± 11.9	7.8 ± 0.42^{c}	31.6 ± 1.30^{c}	75.0 ± 0.73^{d}
NC	365.5 ± 9.8	-8.1 ± 1.57^{a}	-14.8 ± 0.29^{a}	-22.7 ± 1.47^{a}
HC	84.8 ± 4.7	1.4 ± 3.96^{b}	0.1 ± 2.12^{b}	0.7 ± 2.88^{b}

Same superscript down the column signifies no significant difference at $P > 0.05$. Percentage change in blood glucose = [(blood glucose before treatment - blood glucose after treatment)/blood glucose before treatment] × 100. CME: Cold maceration extract; SEE: Soxhlet extraction extract; MAE: Microwave-Assisted Extraction extract; PC: Positive control; NC: Negative control; HC: Healthy control.

the specimens to the external colder environment, and volumetric warming impact prompts a speedier ascend in temperature. Additionally, the interior warming of *in situ* water inside of the plant material expands the plant cells and prompts the burst of the plant tissues[45]. This may be the explanation behind the higher extraction of phytochemicals from MAE when compared to soxhlet and cold maceration extracts observed in the present study. It is very obvious from these results, that microwave extraction represents a promising substitute for extracting hypoglycemic compounds from natural substrate.

The hypoglycemic activity of the 250 mg/kg b.w. cold maceration extract was significantly better than others within the first 14 day of treatment. Surprisingly after 21 d treatment, the blood glucose lowering capacity of the MAE extract was almost the same with that of 10 mg/kg b.w. glibenclamide. The percentage change in blood glucose by MAE extract after the experimental duration of 21 d treatment was 72.6% whereas that obtained from the standard drug glibenclamide was 75.0%. The higher yield of alkaloid by soxhlet extraction may account for its better efficacy when compared to the cold maceration in the present study. Saponin, alkaloid and flavonoid are known to play a significant role in anti-diabetic action[27-29].

MAE had the highest yield in three different phyto-constituents *viz* phenolics, saponins and tannins. This suggests that microwave methanol extract could be used even at 250 mg/kg b.w. to complement currently available oral hypoglycemic drugs. The results suggest that microwave technology is a viable means for extracting valuable anti-diabetic components from medicinal plants. The reason for the observed differences in hypoglycemic activities by the various extract within the first 14 d and after the 21 d of experimental treatment could be due to the concentrations of the phyto-chemicals which vary in the extracts. It could also be possible that the different phytochemical exhibiting hypoglycemic effect have different rate of reaction in reducing the blood glucose level.

The main advantages of MAE over the conventional extraction techniques is that it reduces solvent consumption, it has a shorter operational time, modestly high recoveries, decent reproducibility and negligible

specimen control for extraction process[46]. Several biologically active compounds have been extracted by application of MAE, such as extraction of azadiractine related limonoids from *Azadirachta indica* seed kernels[47], extraction of artemisinin from *Artemisia annua*[48] and ginsenosides extraction from roots of *Panax ginseng*[44], quercetin from herbal plant[22]. According to Pan *et al*[49], antioxidant activity of phenolic substances extricated from the peel of *Dimocarpus Longan* utilizing MAE was better than that of Soxhlet extraction. Besides, MAE of curcumin from *Curcuma longa* showed a better results and a higher extraction yield with noteworthy diminishing in the extraction time when compared to that of Soxhlet extraction, maceration and stirring extraction[50]. In the present study, there was higher yield in phenolics, saponins and tannins from both plants-VA and OG. This implies that MAE is a better technology for the extraction of these phytoconstituents. The soxhlet extraction technology showed a higher yield in alkaloid whereas maceration technology was best for the extraction of flavonoids.

We find the use of MAE leads to very fast extraction rate with high value of phytoconstituents compared to soxhlet and cold maceration technique. The findings obtained from the present research showed that the choice of extraction technology should be based primarily on the phytochemical entity of interest. For instance, with respect to the result from the present research, the use of soxhlet extraction technology would be recommended when alkaloid is the main phytoconstituent of interest, whereas, the cold maceration would be preferred for extraction of flavonoids. However, since there was no apparent destruction of any bioactive components by the extraction technologies studied, the MAE is recommended as the most suitable technology for routine extraction processes because it is faster, utilizes relatively less amount of solvent and saves more time. Nevertheless, since extraction efficiency differs from efficacy, no single method can be rated as best for extracting all forms of phyto-components. So, a further study on the isolation of the precise bioactive component(s) and its structural elucidation is recommended to ascertain the best technology for obtaining pure bioactive hypoglycemic compound(s) from medicinal plants.

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COMMENTS

Background

Although several methods are available for extraction of active phytoconstituents from medicinal plants. The technique that produces higher yield of specific phytoconstituents has not been reported. The biological activities of these active phytoconstituents are known to be affected by the extraction protocol employed. To address these issues, this study examines three different procedures of extracting anti-diabetic substances from medicinal plants and evaluated their hypoglycemic activities.

Research frontiers

There is active research in the field of investigation of a more effective extraction technique of active ingredient from medicinal plant. Hence, it is imperative to ascertain which extraction protocol produces a significant amount of specific phytoconstituents.

Innovations and breakthroughs

The hypoglycemic activity of anti-diabetic plant is revealed in this study to be subject to the extraction method employed. For the first time, extraction method producing significantly high yield of specific phytoconstituents is presented.

Applications

Since the biological efficacy of phytoconstituents is subject to the extraction protocol employed, the use of an appropriate extraction technique with respect to a specific active ingredient would enhance the process of drug development.

Terminology

Phytoconstituents are chemical compounds that occur naturally in plants. Some of which are responsible for the medicinal effect of the plant.

Peer-review

The authors provided the complete review of this issue. This manuscript provides the updated evidence to the readers.

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ORIGINAL ARTICLE

Case Control Study

Brain-derived neurotrophic factor plasma levels and premature cognitive impairment/dementia in type 2 diabetes

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Abstract

AIM

To assess the relationship of brain-derived neurotrophic factor (BDNF) with cognitive impairment in patients with type 2 diabetes.

METHODS

The study included 40 patients with diabetes mellitus type 2 (DM2), 37 patients with chronic kidney disease in hem dialysis hemodialysis therapy (HD) and 40 healthy subjects. BDNF in serum was quantified by ELISA. The Folstein Mini-Mental State Examination was used to evaluate cognitive impairment.

RESULTS

The patients with DM2 and the patients in HD were categorized into two groups, with cognitive impairment and without cognitive impairment. The levels of BDNF showed significant differences between patients with DM2 (43.78 ± 9.05 *vs* 31.55 ± 10.24, $P = 0.005$). There were no differences between patients in HD (11.39 ± 8.87 *vs* 11.11 ± 10.64 $P = 0.77$); interestingly, ferritin levels were higher in patients with cognitive impairment (1564 ± 1335 *vs* 664 ± 484 $P = 0.001$). The comparison

of BDNF values, using a Kruskal Wallis test, between patients with DM2, in HD and healthy controls showed statistical differences ($P < 0.001$).

CONCLUSION

Low levels of BDNF are associated with cognitive impairment in patients with DM2. The decrease of BDNF occurs early and progressively in patients in HD.

Key words: Diabetes mellitus type 2; Hemodialysis; Brain-derived neurotrophic factor; Folstein mini-mental; Premature cognitive impairment

Core tip: The objective was to compare serum levels of brain-derived neurotrophic factor (BDNF) between patients with and without cognitive impairment, patients with diabetes mellitus type 2 (DM2) and chronic kidney disease patients on hemodialysis, in order to increase our knowledge on the possible role of BDNF in early cognitive impairment in DM2. We found differences in serum BDNF levels; they were lowest in patients with DM2 with cognitive impairment. In patients on hemodialysis, serum BDNF levels were lower than in patients with DM2 and healthy controls and ferritin levels were higher in patients with cognitive impairment.

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INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is a growth factor that belongs to the neurotrophin family; its mature isoform binds specifically to the tropomyosin receptor kinase B, a tyrosine kinase receptor, whereas the precursor pro-BDNF binds to the pan-neurotrophin receptor p75NTR; each mediate different neurotrophic signals[1,2]. BDNF is also important for learning and memory processes, as it induces long-term potentiation in hippocampus and structural changes in synapses.

A positive correlation between brain BDNF concentration and cognitive performance has been described, while decreased BDNF production has been proposed as one possible pathogenetic factor for Alzheimer's disease and major depression[3,4]. Interestingly, plasma BDNF levels are decreased in patients with diabetes mellitus type 2 (DM2) and have been inversely correlated with plasma glucose and insulin resistance as assessed by homeostatic model assessment. Moreover, the output of plasma BDNF from the human brain is abrogated by hyperglycemia, but it is not regulated by hyperinsulinemia[5].

Zhen *et al*[6] found both lower serum BDNF levels and impaired cognitive functions in diabetic patients compared to controls; furthermore, a positive relationship between serum BDNF and delayed memory was observed in diabetic patients, suggesting a role for BDNF in cognitive deficit associated with DM2.

A longer duration of DM2 has been associated with a major risk of chronic kidney disease (CKD), and has been considered a possible new determinant of cognitive decline and dementia[7]. Most recent prospective studies have found an association between CKD and cognitive decline[8-11]. The Health, Aging, and Body Composition Study demonstrated that more advanced stages of CKD are associated with an increased risk for cognitive impairment[12]. BDNF plays a critical role in the functioning of the brain[13-20]. It has been observed that the concentration of serum BDNF reflects the changes in brain BDNF levels[21-23]; therefore, measuring the concentration of serum BDNF can be used to monitor its changes in the brain[24]. It was recently demonstrated that BDNF stimulates the production of prostacyclin in cerebral arteries[25]; it plays an important role in endothelium-dependent relaxation and has also antiplatelet, vasculoprotective, cardioprotective and anti-atherogenic properties[26-28]. Zoladz *et al*[29] demonstrated that the decrease in serum BDNF levels after hemodialysis is accompanied by elevated levels of F-isoprostanes and decreased plasma total antioxidant capacity, which might be caused by the increase in oxidative stress induced by hemodialysis.

The aim of the present study was to compare serum levels of BDNF and the results of the mini mental state examination between patients with DM2 and patients with CKD on hemodialysis, in order to obtain more information on the possible role of BDNF in premature cognitive impairment/dementia in type 2 diabetes. We also investigated whether BDNF predicted premature cognitive impairment, and if it was associated with any clinical parameters in a group of patients with chronic kidney disease.

MATERIALS AND METHODS

A cross-sectional study was carried out in three groups of patients from the Unidad Médica de Alta Especialidad (UMAE) No. 1 Bajío, Instituto Mexicano del Seguro Social (IMSS), León, Guanajuato, México; the patients were matched by age.

Patients with DM2

We selected 37 diabetic male patients, aged 39-59 year (mean age 50.57 ± 5.9 year) with a history of DM2 with a duration of 14.3 ± 6.22 year.

Patients with chronic kidney disease on hemodialysis

We investigated 40 men, aged 18-67 year (mean ± SD, mean age 42.30 ± 12.8 years), with chronic kidney disease, who had started hemodialysis therapy (HD).

Table 1 Clinical characteristics of hemodialysis patients

Baseline characteristics	HD patients with cognitive impairment (n = 17)	HD patients without cognitive impairment (n = 23)	P
Age (yr)	51.88 ± 12.81	42.30 ± 12.87	0.02
Duration of hemodialysis (mon)	41.29 ± 42.01	32.08 ± 36-76	0.13
BDNF (ng/mL)	11.39 ± 8.87	11.11 ± 10.64	0.77
Creatinine (mg/dL)	8.90 ± 1.90	9.21 ± 2.61	0.68
Urea (mg/dL)	128.68 ± 54.23	127.74 ± 51.54	0.77
Hemoglobin (g/dL)	12.13 ± 1.38	11.53 ± 1.92	0.51
Ferritin (ng/mL)	1564 ± 1335.05	664.22 ± 484.99	0.001
Mini-mental state examination	19.58 ± 3.24	26.08 ± 1.50	0.0001

BDNF: Brain-derived neurotrophic factor; HD: Hemodialysis therapy.

We excluded patients older than 69 years of age and those with acute infectious diseases, psychiatric diseases or severe liver dysfunction. Baseline demographic and clinical data such as age, primary cause of renal disease and current medications were collected from the patients' records.

Healthy control subjects

The control group was formed by forty healthy male volunteers from the same demographic group as the patients; they were aged 39-60 years (mean age 42 ± 2.2 year) and received annual health examinations.

Fasting blood samples were collected from patients and healthy controls at 8 am. Serum BDNF concentrations were determined by ELISA using the Human BDNF Quantikine Kit. The concentrations of ferritin in serum and other biochemical parameters were measured at the Central Clinical Laboratory. A neurological assessment was performed before each hemodialysis session (Mini Mental).

The study was performed in accordance with the Declaration of Helsinki of the World Medical Association and was approved by the local Bioethics Committee of the UMAE No. 1 Bajío, IMSS, León, Guanajuato, México. All patients signed an informed consent form for this investigation.

Statiscal analysis

The statistical analysis was performed using Microsoft Excel and Statistica software. The statistical significance of the differences observed between patients and controls was assessed using two-tailed t-test, χ^2 and Kruskal-Wallis ($P < 0.05$).

RESULTS

The patients with DM2 and on hemodialysis were categorized according to the score obtained in the Folstein Mini-Mental State Examination into a group with cognitive impairment and a group without cognitive impairment. The group of patients with type 2 diabetes and cognitive impairment had 19 patients and the group without cognitive impairment had 18 patients. The average age was 50.57 ± 5.9 years with a history of type 2 diabetes mellitus with a duration of 14.3 ± 6.22 years. We were able to analyze the differences between patients with and without cognitive impairment (Table 1). We observed significant differences in the levels of glycated Hb, which were higher in patients with cognitive impairment (8.36 ± 1.52 *vs* 7.33 ± 1.42 P = 0.02). There were also differences in the duration of diabetes; patients with cognitive impairment had more years of DM2 (14.31 ± 6.22 *vs* 9.05 ± 4.64, P = 0.007). The values of serum BDNF also showed significant differences between patients with and without cognitive impairment (31.55 ± 10.24 *vs* 43.78 ± 9.05, P = 0.005).

The group of patients on HD with cognitive impairment had 17 patients; the group without cognitive impairment had 23 patients. The average age of the patients was 42.30 ± 12.8 years. The most common cause of renal failure was diabetes mellitus (45%), followed by glomerulonephritis (20%), renal hypoplasia (15%), hypertension (10%) and other causes (10%). Sixty-five point seven percent of the patients had been subjected to a vascular access procedure using a catheter, while only 31.2% had an arteriovenous fistula. There were also significant differences in patients with chronic renal disease on replacement therapy with hemodialysis between those with and without cognitive impairment (Table 2). Ferritin levels were higher in patients with cognitive impairment (1564 ± 1335 *vs* 664 ± 484, P = 0.001), in contrast to serum levels of BDNF (11.39 ± 8.87 *vs* 11.11 ± 10.64, P = 0.77); however, both groups of patients on hemodialysis had lower levels than healthy controls.

The serum BDNF levels of healthy control subjects were 39.36 ± 8.9 ng/mL. The comparison of BDNF levels, using a Kruskal Wallis test, between patients with DM2, HD and healthy controls showed statistically significant differences ($P < 0.001$) (Figure 1).

DISCUSSION

Most recent prospective studies associate chronic kidney disease with cognitive impairment. There has been a significant increase in the prevalence of chronic degenerative diseases worldwide; thus, there is a particular interest in learning how to modify the conditions that cause cognitive decline and dementia. DM2 has been strongly associated with an increased loss of cognitive functions. A recent cohort study showed that high glucose levels may be a risk factor for dementia and

Table 2 Clinical characteristics of diabetes mellitus type 2 patients

Baseline characteristics	DM2 patients with cognitive impairment (n = 19)	DM2 patients without cognitive impairment (n = 18)	P
Age (yr)	50.57 ± 5.90	54.05 ± 3.63	0.06
Duration of DM2 (yr)	14.31 ± 6.22	9.05 ± 4.64	0.007
BDNF (ng/mL)	31.55 ± 10.24	43.78 ± 9.05	0.005
Glucose (mg/dL)	177 ± 64.91	138 ± 43.90	0.07
Glycated hemoglobin (HBA1c) (%)	8.36 ± 1.52	7.33 ± 1.42	0.02
Minimental state examination	20.26 ± 2.15	25.44 ± 1.50	0.0001

BDNF: Brain-derived neurotrophic factor; DM2: Diabetes mellitus type 2.

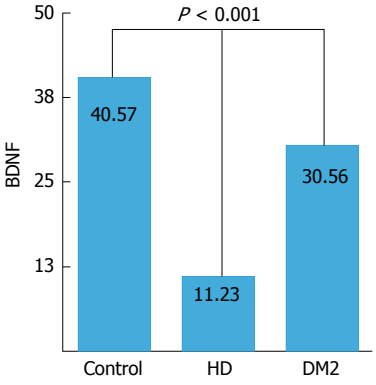

Figure 1 Difference between serum brain-derived neurotrophic factor levels between control subjects and patients in hemodialysis therapy patients with diabetes mellitus type 2. BDNF: Brain-derived neurotrophic factor; HD: Hemodialysis therapy; DM2: Diabetes mellitus type 2.

that the combination of DM2 and hypertension greatly increase the risk of cognitive impairment. Beside vascular factors, other risk factors include the formation of advanced glycosylation products, oxidative inflammation and stress, alterations in the hypothalamic-pituitary-adrenal axis and cortisol levels[30], and abnormalities in insulin secretion and signaling that promote cerebral amyloidosis.

The analysis of the relationship between serum ferritin and Mini-Mental scores in HD patients showed a significant difference between serum ferritin levels and the presence of cognitive impairment according to the Folstein test. Therefore, we can say that a higher iron overload corresponds to greater cognitive impairment.

Becerril-Ortega *et al*[31] analyzed the relationship between iron and neurodegenerative diseases (especially Alzheimer's disease) that affect cognitive impairment in a transgenic mouse model; they observed that iron interferes with the processing of the amyloid precursor protein (APP), neuronal signaling and cognitive behavior. The proposed mechanism is that iron overload increases the production of amyloidogenic KPI-APP and amyloid beta; this is mediated by N-methyl-D-aspartate receptors (NMDAR), mainly GluN2B, which is overexpressed. These data suggest that the damage induced by iron overload through APP accelerates cognitive impairment due to excessive extrasynaptic NMDAR activity 30, causing a significant memory and learning deficit, and inhibiting synaptic plasticity, mitochondrial dysfunction and neuronal apoptosis, which can lead to neurodegeneration.

This is also supported by a study that showed evidence of iron overload in brain structures such as the putamen, dentate nucleus, substantia nigra and red nucleus of patients with beta-thalassemia[32]. Another group of patients with thalassemia also showed iron overload and increased oxidative damage[33]. Blasco *et al*[34] found a significant positive association between obesity, insulin resistance and iron overload in the caudate nucleus, hypothalamus and hippocampus, and poor cognitive performance. Furthermore, it has been shown that iron overload causes oxidative stress *in vitro*[35] and can affect the hematopoiesis of bone marrow in mice by increasing oxidative stress[36].

Although there are multiple factors that influence cognitive impairment, several studies have shown an association with circulating levels of BDNF[37,38], and have suggested a synergistic effect between the presence of dementia and BDNF levels in in DM2[39]. Our study found this association and also that patients on HD had increased oxidative stress, probably induced by iron overload, which was evidenced by elevated levels of ferritin. This was significantly associated with greater cognitive impairment and with serum BDNF levels well below the levels found in patients with type 2 diabetes mellitus and healthy controls.

One of the most common advanced complications of DM2 is CKD. The progressive loss of renal function could make it necessary for the patient to receive renal replacement therapy such as hemodialysis, and the progressive loss of circulating levels of BDNF should be prevented in patients with DM2 to avoid premature cognitive decline. There have been several experimental studies with curcumin[40] and resveratrol, both of which increase serum BDNF levels. Curcumin has an antidepressant effect, mediated by its antioxidant activity and up-regulation of phosphor Akt and mTOR levels in the hippocampus and prefrontal cortex[41]. Resveratrol has antidepressant-like effects, mediated in part by the normalization of serum corticosterone levels and the up-regulation of Perk, pCREB and BDNF levels in the hippocampus and amygdala[42]. This is an alternative that should be investigated further in future randomized clinical trials.

COMMENTS

Background

Diabetes mellitus type 2 (DM2) has been strongly associated with an increased

loss of cognitive functions, while decreased brain-derived neurotrophic factor (BDNF) production has been proposed as one possible pathogenetic factor for premature cognitive impairment/dementia. A longer duration of DM2 has been associated with a major risk of chronic kidney disease, and has been considered a possible new determinant of cognitive decline and dementia.

Research frontiers

The analysis of the relationship between serum ferritin and Mini-Mental scores in hemodialysis therapy (HD) patients showed a significant difference between serum ferritin levels and the presence of cognitive impairment according to the Folstein test. Therefore, the authors can say that a higher iron overload corresponds to greater cognitive impairment, and it is of interest which factors modify the decrement of BDNF production. Measuring the concentration of serum BDNF can be used to monitor its changes in the brain, in order to influence the course of the disease.

Innovations and breakthroughs

The authors confirm the serum BDNF levels between patients with DM2, HD and healthy controls showed statistically significant differences. Ferritin levels were higher in patients in HD with cognitive impairment, is a breakthrough in the understanding of the factors contributing to the loss of BDNF and cognitive impairment.

Applications

Monitoring levels of BDNF can prevent cognitive decline implementing new measures such as the use of antioxidants proposed recently and currently under research.

Peer-review

The study is original and evaluates the cognitive impairment in diabetes mellitus in relationship with the brain-derived neurotrophic factor plasma levels and ferritin. The article has interest and likes suitable for the publication in the Journal.

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Retrospective Study

Double diabetes in Saudi Arabia: A new entity or an underestimated condition

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Abstract

AIM

To determine the clinical and biological characteristics of double diabetes (DD) among young people in Saudi Arabia.

METHODS

This was a retrospective descriptive chart review study including 312 young newly diagnosed diabetic patients (aged 12-20 years), whom were admitted over a five year period (January 2009 to December 2013). Family history of diabetes mellitus (DM) (first degree), physical body mass index (BMI), acanthosis nigricans, history of auto-immune disease and laboratory information for glycosylated hemoglobin, basal C peptide level and diabetes autoantibody response (anti-GAD, anti-IA2 and anti-ICA) were collected from medical report. A mean follow-up of 3 years for these patients was performed.

RESULTS

Patients were categorized into 4 groups, based on the autoantibody response (Ab+ or Ab-) and C-peptide secretion (β+ for fasting level 0.4-2.1 ng/mL and β- if < 0.4 ng/mL). Group1 (type 1a): Ab+ β- (21%), group 2 (type 1b): Ab- β- (9%), group 3 (DD): Ab+ β+ (31%)

and group 4 (classic type 2 DM): Ab- β+ (39%). The mean age of the DD patients in our study was 15.1 ± 6.4 years. A total of 41% of the study population presented with diabetic ketoacidosis and 61% of the study population presented with positive family history of DM. The mean BMI was 26.8 kg/m^2 with 64% of overweight or obese patients. Ninety two percent of the patients were started on insulin at the time of diagnosis. During a mean follow-up of 3 years, only 32% of the patients with DD required insulin and 78% were on metformin alone or with insulin.

CONCLUSION

Our findings enable us to arrive at the conclusion that almost one-third of the young Saudi diabetic patients reveal atypical forms of DM (double diabetes) expressing features resulting from both T1D and T2D.

Key words: Double diabetes; Therapeutic approaches; Hybrid diabetes; Autoantibody response; Saudi Arabia

Core tip: Almost one-third of the young Saudi diabetic patients reveal atypical forms of double diabetes (DD) expressing features resulting from both type 1 diabetes and type 2 diabetes. Therefore, identification of DD patients becomes important as this will give direction for the selection of the most apt diagnostic and therapeutic lines of treatment.

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INTRODUCTION

Almost one-third of the young Saudi diabetic patients reveal atypical forms of double diabetes (DD) expressing features resulting from both type 1 diabetes and type 2 diabetes. Therefore, identification of DD patients becomes important as this will give direction for the selection of the most apt diagnostic and therapeutic lines of treatment.

Diabetes mellitus (DM) is a chronic disease, recognized as a high-ranking and daunting health problem of the 21st century[1]. The rising incidence and prevalence of DM are becoming alarmingly evident irrespective of age groups or gender and occur in the developed and developing countries[1]. The International Diabetes Federation alert indicates that if lifestyle and health habits are not drastically and quickly changed, one-half of the Saudis will be diabetic by 2030. Also, as nearly 3 million (18%) Saudi children are overweight or obese, they will be most vulnerable to acquiring DM[2].

Type 1 diabetes is the presence of antibodies which attack the insulin-producing pancreatic beta cells an indication that type 1 diabetes is an autoimmune disorder. Whereas type 2 diabetes is characterized by insulin resistance and relative insulin deficiency, either or both of which may be present at the time diabetes is diagnosed. Traditionally, anyone exhibiting polyuria, polydipsia, and polyphagia, the classic symptoms of DM, and who also possess a family history of type 1 DM (T1D), obesity, acanthosis nigricans and the absence of both ketosis and diabetes-associated autoantibodies is recognized as a type 2 diabetic (T2D)[3]. However, T1D patients are most often thin and may have ketosis and diabetes-linked autoantibodies[4]. The DD patients exhibit characteristics of both T1D and T2D, which can be evident either during diagnosis or develop subsequently over time[5-7].

It was in 1991 that the nomenclature "double diabetes" was first given to T1D patients with a family history of T2D, as they were found to more likely be overweight and rarely had sufficient glycemic control, even on high insulin dosages[8]. The present classification makes it difficult to describe the type of heterogeneous DM affecting such young patients, whether to categorize them as T2D because of their obesity and insulin resistance, or as T1D due to the presence of auto-antibodies to the β cells[6]. Further, DD is quite hard to control, including the micro- and mostly macro-vascular typically T2D-associated complications[1,6].

Although the prevalence and incidence of DD is yet to be clearly defined, however nearly 25% of the T1D children showed obesity or were overweight[9]. Also, roughly 35% of children and adolescents with T2D possessed at least one diabetes-related antibody[9-11]. The rapid increase in the prevalence of T1D and T2D in the Kingdom caught the interest of the medical world soon after the rapid industrialization resulted in a dramatic spurt in the standard of living and incorporation of "Westernized" habits, including the selection of unhealthy dietary patterns, and reduction in physical activity[12]. In Saudi Arabia the prevalence of DM is at an alarming juncture and rising[1]. However, no study, to our knowledge, is currently available on the prevalence of DD in Saudi Arabia. Therefore, our objective is to ascertain the prevalence, clinical and biological features of DD among the young Saudi populace.

MATERIALS AND METHODS

Study design, setting and sampling

This is a retrospective descriptive study was conducted among 312 young newly diagnosed diabetic patients (aged 12-20 years) admitted over a 4-year period (January 2009 to December 2013) at Prince Sultan Military Medical City (PSMMC). PSMMC is a 1200-bed, tertiary medical center in Riyadh, Saudi Arabia, with round 40000 annual admissions (950000 active patients files) and 118000 emergency room visits per year from different region of the country. Patients selection of this

Table 1 Characteristics of the study population (n = 312)

Patients characteristics	Yes % (n)	No % (n)
Family history of diabetes	57 (178)	43 (134)
Patients with overweight or obese	64 (199)	36 (113)
Diabetic ketoacidosis at presentation	39 (122)	61 (190)
Autoantibodies positivity	52 (162)	48 (150)
Acanthosis nigricans	34 (106)	66 (206)
Family history of auto-immune disease	23 (72)	77 (240)
History of auto-immune disease	18 (56)	82 (256)

study was conducted using eligibility screening.

Data collection

Family history of DM (first degree), physical body mass index (BMI), acanthosis nigricans, history of auto-immune disease, first degree family history of auto-immune disease (celiac disease, systemic lupus erythematosus, Sjögren's syndrome, thyroid dysfunction (primary hypothyroidism, graves' disease), psoriasis, Crohn's disease, Addison's disease, multiple sclerosis, and myasthenia gravis) and laboratory data for glycosylated hemoglobin (HbA1c), basal C-peptide level and diabetes autoantibody response (anti-GAD, anti-IA2 and anti-ICA) were collected.

BMI

BMI was calculated by dividing the weight (kg) by the square of height in meters (BMI; kg/m^2) and BMI z score (adjusted for child age and gender). The *z* score (standard deviation scores), was figured as per the formula (Xi-Mx)/SD, where Xi is the actual measurement, Mx is the mean value for that age and gender, and SD is the standard deviation corresponding to that age and gender[13].

Diabetic ketoacidosis

Diabetic ketoacidosis (DKA) is defined as all three of the following must be present: (1) blood glucose level higher than > 250 mg/dL; (2) presence of urine ketones ++ or more; and (3) venous pH level lower than 7.30 and/or serum bicarbonate lower than 15 mEq/L[14].

HbA1c

HbA1c $\geqslant$ 6.5% makes the diagnosis of DM. The HbA1c test was performed in our laboratory using a standard method (National Glycohemoglobin Standardization Program certified) and standardized to the diabetes control and complications trial assay. In the absence of unequivocal hyperglycemia, results were confirmed by repeat testing[15].

Statistical analysis

Data analysis was carried out using Microsoft Excel 2010, Microsoft Corporation, Seattle, WA, United States and the Statistical Package for Social Sciences version 20, SPSS Inc., Chicago, IL, United States. In addition to descriptive statistics t test and χ^2 analyses were carried out to compare between DD and others groups. A P-value of < 0.05 was considered to be statistically significant.

RESULTS

Table 1 lists the characteristics of the population studied. The mean age of patients presenting with DD was 15.1 ± 6.4 years with BMI 26.8 kg/m^2, and 1:2 sex ratio (male *vs* female). The results indicated that 57% of the population studied had a family history, 64% showed obesity, 39% had DKA, 52% were positive for autoantibodies, 34% had acanthosis nigricans, 23% possessed a family history of autoimmune disease and 18 had a history of autoimmune disease.

The patients were divided on the basis of the auto-antibody response (Ab+ or Ab-) and C-peptide secretion (β+ for fasting level 0.4-2.1 ng/mL and β- if < 0.4 ng/mL) as listed in Figure 1. Depending on the autoantibody response (Ab+ or Ab-) and C-peptide secretion, the patients were segregated into four groups, *viz.*, group1 (type 1a): Ab+ β- (21%); group 2 (type 1b): Ab- β-(9%), group 3 (DD): Ab+ β+ (31%) and group 4 (classic T2D): Ab- β+ (39%).

The characteristics of the patients are shown based on the presence or absence of auto-antibodies and the C-peptide secretion are shown in Table 2. More than 25% of the DD population had a family history of diabetes and the mean BMI of the DD population was 26.8 (kg/m^2). Among the DD population, 28 (38.8%) had family history of auto immune disease, 17 (30.4%) had history of auto immune disease, ninety (31.4%) required insulin at diagnosis and thirty one (21%) required multiple dose of insulin injection during follow-up.

DISCUSSION

Globally, as the DD population is steadily increasing in number it becomes harder to diagnose and treat because these individuals experience symptoms of both T1D and T2D with the hybrid diabetes[5,7,16]. Identifying DD in children and adolescents is crucial as it affects the diagnostic method and choice of treatment. Within the scope of our knowledge, no other study regarding the prevalence of DD in Saudi Arabia is currently available. Therefore, this study was performed to ascertain the prevalence, clinical and biological characteristics of DD among the young Saudi population at PSMMC, a tertiary medical center in Saudi Arabia.

The current study, using the autoantibody response and C-peptide secretion, showed 31% of the population studied with DD and 26.8 mean BMI. However, at present research is limited regarding the incidence and prevalence of DD[9]. The results from another study indicated that almost 25% of T1D children are either overweight or obese and have DD[11]. This condition usually develops insidiously and initially manifests as a rising requirement for more insulin to control the glucose levels. T2D patients too can be diagnosed with blood tests to identify the specific pancreas-attacking proteins. Some studies also recorded that nearly 35% of children and adolescents with T2D possessed at least one diabetes-related

Table 2 Characteristics of the patients are shown based on the presence or absence of auto-antibodies and the C-peptide secretion

Auto-antibodies	Ab+		Ab-	
C-peptide secretion	β- (G 1)	β+ (double diabetes)	β- (G2)	β+
Age of diagnosis	13.16[a]	15.3	16.6	17.02[e]
Family history of diabetes	24 (13.5%)	45 (25.3%)	14 (7.9%)	95 (53.4%)[e]
Family history of auto immune disease	32 (44.4%)[a]	28 (38.8%)	5 (6.9%)	7 (9.7%)[e]
History of auto immune disease	29 (51.7%)[a]	17 (30.4%)	2 (3.6%)	8 (14.3%)[e]
DKA at presentation	57 (46.7%)[a]	33 (27%)	5 (4%)	27 (22.1%)[e]
BMI (kg/m^2)	21[a]	26.8	24.2	29.6[e]
HbA1c (%)	10.2[a]	8.9	10.8	11.7[e]
Patients requiring insulin at diagnosis	65 (22.6%)[a]	90 (31.4%)	28 (9.8%)	104 (36.2%)
Patients requiring insulin multiple dose injection (follow-up)	65 (44.2%)[a]	31 (21%)	28 (19%)[c]	23 (15.6%)[e]
Patients on metformin only during follow-up	0[a]	48 (38.1%)	0[c]	78 (61.9%)[e]
Patients on metformin with insulin during follow-up	4 (6.1%)[a]	27 (41.5%)	2 (3.1%)[c]	32 (49.2%)

Groups compared by Students' *t* test and χ^2 test: [a]$P < 0.05$, Ab+ β+ *vs* Ab+ β-; [c]$P < 0.05$, Ab+ β+ *vs* Ab- β-; [e]$P < 0.05$, Ab+ β+ *vs* Ab- β+. DKA: Diabetic ketoacidosis; BMI: Body mass index.

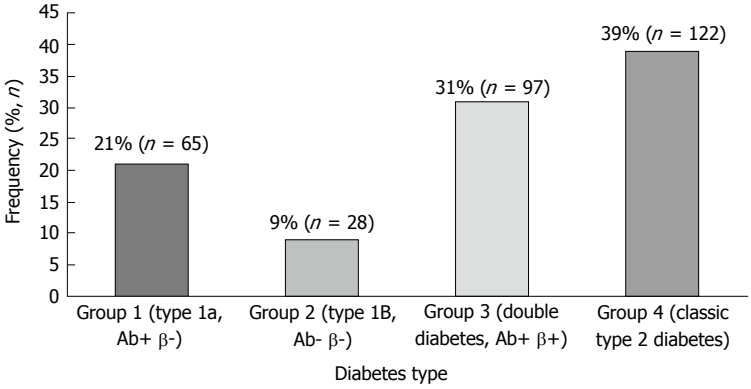

Figure 1 Patients categorized based on the autoantibody response (Ab+ or Ab-) and C-peptide secretion (β+ for fasting level 0.4-2.1 ng/mL and β- if < 0.4 ng/mL).

antibody[10]. Other studies reported that from among the patients newly diagnosed with diabetes, roughly one out of three children and adolescents suffer from DD[7]. DD can be produced by different factors, based on whether the individual initially has T1D or T2D[7]. If the patient commences with T1D and begins to gain excess or surplus weight, the individual may begin to be insulin resistant, implying that besides the body being ineffective in secreting insulin due to T1D, the typical insulin injections will no longer be effective as the patient becomes insulin-resistant, which causes the T2D. Such patients then develop DD necessitating medications plus insulin injections for blood sugar level control[9].

Several studies support the fact that DD is often seen in patients with a family history of T2D[8,17], a finding confirmed by the present study where 45% of the study population had a family history of DM. It is noteworthy that nearly 65% of the DD group required insulin, a discovery concurring with earlier reports in the literature[8,17]. During follow-up, nearly half the DD patients (46%) were managed solely on metformin (without necessitating insulin therapy).

Significantly, almost 64% of the population in this study was overweight or obese. This higher BMI percentage may possibly be a result of the dramatic rise in the standard of living and the "Westernized" lifestyle habits adopted in Saudi Arabia. Unhealthy and unwise dietary choices coupled with reduced physical activity have produced this situation[12,18-20]. The growing "obesogenic" state that induced insulin resistance could account for the development of islet cell autoimmunity *via* different mechanisms. Therefore, the trend of increasing obesity seems to play a prominent part in the rising incidence and changing phenotype of T1D among adolescents and children[7]. Some lifestyle changes and precautions can be incorporated to deter the development of DD for those with and without DM[12,18,19].

Diagnostic evaluation

Universally applicable clinical diagnostic criteria as well as methods enabling the identification of DD, either at the time of onset of hyperglycemia or during the course of the disease process, must be established. In 2009, Pozzilli *et al*[9] introduced the idea of "metabolic load" to define the T2D characteristics and "autoimmune load" to define the T1D features. They revealed that in obese children with hyperglycemia, the presence of a high "metabolic load" and a low "autoimmune load" are indicators of DD[9]. Therefore, they presented some biochemical and clinical guidelines to identify DD, as listed: (1) evidence of the clinical characteristics of T2D, dyslipidemia, hypertension and higher BMI with increased

cardiovascular risk, compared with children having classical T1D. Family history for T2D and T1D could be present; (2) a drop in the number of the clinical features of T1D, including polyuria and polydipsia, weight loss, formation of ketoacidosis; in this case insulin therapy is not the first line of treatment, unlike for patients with classical T1D; and (3) the number of autoantibodies to islet cells, although lesser in number and titer when compared with T1D, and sometimes a lower degree of risk linked with the MHC locus compared with T1D patients. Similar to T1D, where insulin resistance and obesity are not the usual characteristics, DD is always distinguished by an obese phenotype, besides the coexistence of β cell autoimmunity[9].

Limitations

This study includes a few limitations such as the limited number of risk, social, and demographic factors studied and demonstrated in a single center. Further research, preferably conducted on a greater scale, is required to overcome these limitations. However, this study offers pertinent information regarding DD among the young Saudi population.

In conclusion, our findings enable us to arrive at the conclusion that almost one-third of the young Saudi diabetic patients reveal atypical forms of DM (double diabetes) expressing features resulting from both T1D and T2D. Therefore, identification of DD patients becomes crucial as this will give direction for the selection of the most apt diagnostic and therapeutic lines of treatment.

COMMENTS

Background

The double diabetes (DD) population is steadily increasing worldwide in number it becomes harder to diagnose and treat because these persons experience symptoms of both type 1 diabetes mellitus (DM) (T1D) and type 2 DM (T2D) with the hybrid diabetes. Classifying DD in children and adolescents is crucial as it affects the diagnostic method and choice of treatment.

Research frontiers

The present classification makes it difficult to describe the type of heterogeneous DM affecting such young patients, whether to categorize them as T2D because of their obesity and insulin resistance, or as T1D due to the presence of autoantibodies to the β cells. Although the prevalence and incidence of DD is yet to be clearly defined, however nearly 25% of the T1D children showed obesity or were overweight. Also, roughly 35% of children and adolescents with T2D possessed at least one diabetes-related antibody. The rapid increase in the prevalence of T1D and T2D in the Saudi Arabia caught the interest of the medical world soon after the rapid industrialization resulted in a dramatic spurt in the standard of living and incorporation of "Westernized" habits, including the selection of unhealthy dietary patterns, and reduction in physical activity. In Saudi Arabia the prevalence of DM is at an alarming juncture and rising.

Innovations and breakthroughs

No study, to our knowledge, is currently available on the prevalence of DD in Saudi Arabia. Therefore, the authors ascertained the prevalence, clinical and biological features of DD among the young Saudi populace.

Applications

The authors' findings enable people to arrive at the conclusion that almost one-third of the young Saudi diabetic patients reveal atypical forms of DM (double diabetes) expressing features resulting from both T1D and T2D. Therefore, identification of DD patients becomes crucial as this will give direction for the selection of the most apt diagnostic and therapeutic lines of treatment.

Peer-review

This study provides the prevalence, clinical and biological features of DD in Saudi Arabia. Therefore, the manuscript is good for the readership.

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SYSTEMATIC REVIEWS

Intermittent energy restriction in type 2 diabetes: A short discussion of medication management

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Abstract

AIM

To discuss type 2 diabetes mellitus (T2DM) medication changes required during the popular 5:2 intermittent energy restriction (IER) diet.

METHODS

A search was conducted in MEDLINE, EMBASE, AMED, CINAHL and Cochrane library for original research articles investigating the use of very low calorie diets (VLCD) in people with T2DM. The search terms used included "VLCD" or "very low energy diet" or "very low energy restriction" or "IER" or "intermittent fasting" or "calorie restriction" or "diabetes mellitus type 2" and "type 2 diabetes". Reference lists of selected articles were also screened for relevant publications. Only research articles written in English, which also included an explanation of medication changes were included. A recent pilot trial using the 5:2 IER method, conducted by our research group, will also be summarized.

RESULTS

A total of 8 studies were found that investigated the use of VLCD in T2DM and discussed medication management. Overall these studies indicate that the use of a VLCD for people with T2DM usually require the cessation of medication to prevent hypoglycemia. Therefore, the 5:2 IER method will also require medication changes, but as seen in our pilot trial, may not require total cessation of medication, rather a cessation on the 2 IER days only.

CONCLUSION

Guidelines outlined here can be used in the initial stages of a 2-d IER diet, but extensive blood glucose monitoring is still required to make the necessary individual reductions to medications in response to weight loss.

Key words: Diabetes mellitus/therapy; Fasting; Caloric restriction; Diabetes complication; Intermittent energy restriction; Obesity; Very low calorie diet; Medication management; Type 2 diabetes mellitus

Core tip: Use of the popular 5:2 intermittent energy restriction diet in people with type 2 diabetes requires careful manipulation of oral hypoglycemic agents and insulin to prevent poor blood glucose control. This short review fills a very important gap in the literature, reviewing necessary medication changes required in severe energy restriction and outlining how these changes may apply during the 5:2 diet by sharing our experiences from our recent 5:2 pilot trial.

Carter S, Clifton PM, Keogh JB. Intermittent energy restriction in type 2 diabetes: A short discussion of medication management. *World J Diabetes* 2016; 7(20): 627-630 Available from: URL: http://www.wjgnet.com/1948-9358/full/v7/i20/627.htm DOI: http://dx.doi.org/10.4239/wjd.v7.i20.627

INTRODUCTION

Approximately 80% of people with type 2 diabetes mellitus (T2DM) are overweight or obese[1]. Weight loss is known to reduce glycemia and increase insulin sensitivity[1] and large amounts of weight loss can lead to remission of T2DM[2]. However, weight loss for this population group is often difficult[3], with poor adherence to weight loss programs, suggesting people find continuous energy restriction (CER) difficult to maintain. Recently attention has been given to a new method of weight loss, known as intermittent energy restriction (IER), which in the overweight and obese populations, without diabetes, has shown to be comparable to CER in achieving weight loss[4,5]. IER uses short periods (usually 2 d) of severe energy restriction, 400-800 kcal/d, followed by longer periods of habitual diet. There are however, very few studies comparing the effects of IER to daily CER in T2DM. Therefore, we have limited information on how to manage diabetes medications to prevent hypoglycemia, which is likely to occur during the short periods of severe energy restriction. We evaluated continuous very low calorie diet (VLCD) trials to provide a starting point for medication management and to provide guidance to future IER weight loss trials for people with T2DM.

MATERIALS AND METHODS

A search was conducted in MEDLINE, EMBASE, AMED, CINAHL and Cochrane library for original research articles investigating the use of VLCD in people with T2DM. The search terms used included "VLCD" or "very low energy diet" or "very low energy restriction" or "IER" or "intermittent fasting" or "calorie restriction" or "diabetes mellitus type 2" and "type 2 diabetes". Reference lists of selected articles were also screened for relevant publications. Only research articles written in English, which also included an explanation of medication changes were included.

VLCDs are defined as diets with an energy intake of < 800 kcal (< 3344 kJ) per day with at least 50 g of high-quality protein, essential fatty acids, daily requirements of vitamins and minerals as well as the addition of approximately 2 cups of non-starchy vegetables to prevent constipation. VLCDs can be given as a complete liquid formula or if food-based diets are used they often include a multivitamin supplement[6].

RESULTS

Seven trials using continuous VLCD in participants with T2DM were found, and one controlled trial was found using intermittent VLCD (Table 1). In six trials, including the intermittent VLCD trial, all oral hypoglycemic agents (OHA) were discontinued before the start of the trial[2,7-11] regardless of the degree of glycemic control. In two trials, medications were reinitiated if blood glucose levels (BGL) were above a pre-determined level[7,9]. In one trial, medications, including insulin, were restarted if the mean of two weekly fasting BGL averaged > 13.3 mmol/L for two weeks, dosages were increased thereafter on a case-by-case basis[7]. In the second trial, medications were reinitiated at half the original dose if fasting BGLs increased > 13.9 mmol/L[9]. In the other four trials, there was no mention of reinitiating medications[2,8,10,11].

In the two remaining trials, diabetic medications and insulin were reduced by 50% at either the commencement of the VLCD treatment[12] or in accordance with self-monitored BGLs[13]. In one trial, participants measured fasting and postprandial BGLs daily for two days before the start of the VLCD and if the mean result was < 8 and < 10 mmol/L, respectively, diabetic medications were halved[13]. Conversely, if levels were > 9 and > 11 mmol/L, respectively, medications were increased[13]. Medication changes occurred in the following order; insulin was decreased first followed by sulfonylureas and lastly metformin, and when increasing, medications were increased in reverse order[13]. In the second trial, medications were halved at the initiation of the VLCD and reduced further if the fasting weekly average was < 8.4 mmol/L or if participants experienced hypoglycemia (< 3.4 mmol/L) and increased if fasting weekly BGLs averaged > 8.4 mmol/L[12]. All changes to dosages occurred on a case-by-case basis in both trials[12,13].

One trial used a VLCD in an overweight population with T2DM on an intermittent basis. The severe energy restriction was used at a frequency of either 1 d or 5 d per week over 20 wk. Oral glycemic agents were discontinued 2 wk before the start of the trial and people with fasting glucose > 16.7 mmol/L were excluded. People using insulin were also excluded from this trial. Medication was reinstated, at half the original dose, if fasting BGLs increased to > 13.9 mmol/L; participants were only required to measure their fasting BGLs levels twice per week.

We recently conducted a 3-mo pilot trial testing the effects of a 2-d IER compared to a CER diet in people with T2DM[14]. Our pilot trial demonstrated that 2 d of

Table 1 Summary of trials

Ref.	Design	Duration	Subjects	Aim	Diet groups	Medication protocol
Wing *et al*[7]	Randomized parallel study	50 wk 1-yr follow-up	*n* = 93 Male/female: 33/60 Mean age: 51.8 ± 9.7 Mean diagnosis (yr): 6.8 ± 6.1	Effects of a weight control program, with and without 2 × 12-wk VLCD restriction	VLCD = 400-500 kcal *via* liquid or food-based diet from 1-12 wk and from 24-36 wk. LCD was followed at all other times LCD = 1000-1200 kcal	All medications (inc. insulin) were discontinued at the start of the trial. Insulin was discontinued and monitored for 3 d. Dosages of oral medications or insulin were reinstated if the mean of two fasting blood glucose levels averaged > 13.3 mmol/L over a fortnight. Dosages increased on a case-by-case basis
Kelley *et al*[8]	Single arm study	24 wk	*n* = 7 Male/female: 2/5 Mean age: 59 Mean diagnosis (yr): N/A	Evaluating the efficacy of VLCD treatment in obese T2DM participants	VLCD = 400-800 kcal *via* liquid and food-based diet	Discontinued all oral glycemic medication 3 wk before commencement on the VLCD
Williams *et al*[9]	Randomized parallel study	20 wk	*n* = 54 Male/female: 23/31 Mean age: 51.9 ± 7.8 Mean diagnosis (yr): N/A	Evaluating the efficacy of intermittent VLCD restriction on weight loss and glycemic control compared to moderate calorie restriction	VLCD = 400-600 kcal *via* food-based diet. LDC (1500-1800 kcals) at all other times 2 groups: 1-d: 1 d/wk plus 5 consecutive days in week 2 5-d: 5 d/wk for 15 wk	Discontinued all oral glycemic medication 2 wk before the trial and people with fasting glucose > 16.7 mmol/L were excluded. People using insulin were also excluded Medications were only reinstated if fasting BGLs (measured twice weekly) increased > 13.9 mmol/L. Restarted medication occurred at half of the original dose
Uusitupa *et al*[10]	Single arm study	12 wk	*n* = 10 Male/female: 6/4 Mean age: 51 ± 2.2 Diagnosis (yr): Ranged 4-16	Evaluating the effects of weight loss using a VLCD on metabolic control and cardiovascular risk factors in obese participants with T2DM	VLCD = 500-800 kcals *via* liquid and food-based diet	Discontinued all oral glycemic medications before the start of the trial

VLCD: Very low calorie diet; LCD: Low calorie diet; T2DM: Type 2 diabetes mellitus; BGL: Blood glucose levels.

IER compared to CER achieves similar reductions in HbA1c (-0.7% ± 0.9%; $P < 0.001$) and weight loss (-5.8 ± 3.9 kg; $P < 0.001$)[14]. In the pilot trial, our protocol was to discontinue OHA likely to cause hypoglycemia (*e.g.*, sulfonylureas) at baseline if HbA1c was < 8%. Medications such as metformin, gliptins, and SGLT2 inhibitors remained unchanged. Participants using insulin were also asked to reduce their dose by 10 units/d if randomized to the CER group or halved on the IER days. If HbA1c was > 8% at baseline OHA remained the same and insulin dose was decreased by 5-10 units on IER days. However, due to low BGLs in some participants we changed the medication protocol in preparation for our 12-mo intervention trial, which is currently ongoing. The new protocol requires discontinuation of sulfonylureas as well as insulin if baseline HbA1c is < 7% for both groups. If HbA1c is > 7% but < 10% then medications are discontinued only on IER days and if HbA1c is > 10% medications remain unchanged. Following this change, there has been a reduction in hypoglycemic events for participants taking insulin on IER days and a reduction in hyperglycemic events on non-IER days and in the CER group. It is important to note that in addition to changes made based on baseline values, it is also essential to monitor daily BGLs. Each participant requires individual medication changes, especially to insulin units, in response to weight loss.

DISCUSSION

IER is an alternative method to achieve weight loss, which can be used for the management of T2DM. Due to the severe energy restriction required for IER diets to be effective, management of OHA, as well as insulin, requires constant supervision as well as ongoing blood glucose monitoring by the participant to prevent unwanted hypo- or hyperglycemic events. Medication changes will differ depending on the number of days the intermittent restriction is followed and is likely to only require intervention on these days unless glycemic control is excellent. The treatment method promoted by popular media suggests 2 d of restriction. We tested this

method and we suggest baseline medication changes based on HbA1c, as outlined in the second protocol above, as well as individual changes in response to weight loss. Participants, therefore, need to be willing to monitor their BGLs at least twice daily and report any episodes of hypo- or hyperglycemia, which would indicate the need to further adjust medications.

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COMMENTS

Background

Recently attention has been given to a new method of weight loss, known as intermittent energy restriction (IER), which has demonstrated positive results for weight loss in overweight and obese populations.

Research frontiers

For this new diet method to be used safely in the type 2 diabetes mellitus (T2DM) population, medication management protocol must be established.

Innovations and breakthroughs

Very low calorie diets used in the treatment of T2DM provide insight, but as seen from the research, medication changes may only be required on the IER treatment days and after weight loss.

Applications

IER is a successful treatment method for weight loss and glycemic control in T2DM, and with regular blood glucose levels monitoring, medications can be safely adjusted to limit unwanted episodes of hypo-or hyperglycemia.

Peer-review

The review though very short is written well. The authors state that intermittent energy restriction which requires severe energy restriction needs to be discussed as it is a developing concept.

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