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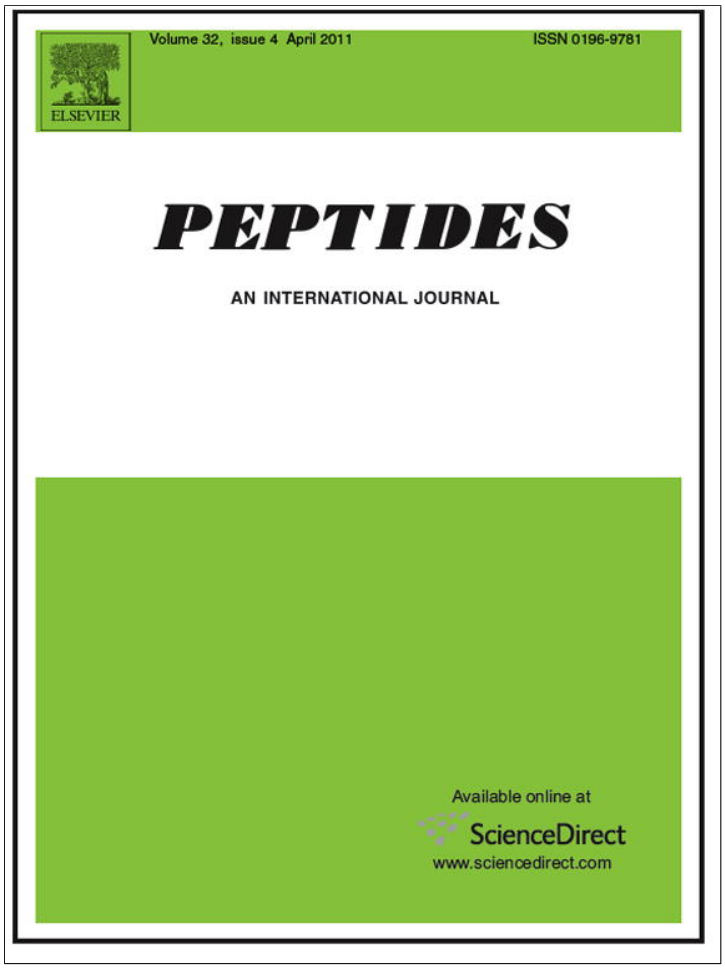

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Chronic treatment with a stable obestatin analog significantly alters plasma triglyceride levels but fails to influence food intake; fluid intake; body weight; or body composition in rats

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ABSTRACT

Obestatin (OB(1-23) is a 23 amino acid peptide encoded on the preproghrelin gene, originally reported to have metabolic actions related to food intake, gastric emptying and body weight. The biological instability of OB(1-23) has recently been highlighted by studies demonstrating its rapid enzymatic cleavage in a number of biological matrices. We assessed the stability of both OB(1-23) and an N-terminally PEGylated analog (PEG-OB(1-23)) before conducting chronic *in vivo* studies. Peptides were incubated in rat liver homogenate and degradation monitored by LC-MS. PEG-OB(1-23) was approximately 3-times more stable than OB(1-23). Following a 14 day infusion of Sprague–Dawley rats with 50 nmol/kg/day of OB(1-23) or a N-terminally PEGylated analog (PEG-OB(1-23)), we found no changes in food/fluid intake, body weight and plasma glucose or cholesterol between groups. Furthermore, morphometric liver, muscle and white adipose tissue (WAT) weights and tissue triglyceride concentrations remained unaltered between groups. However, with stabilized PEG-OB(1-23) we observed a 40% reduction in plasma triglycerides. These findings indicate that PEG-OB(1-23) is an OB(1-23) analog with significantly enhanced stability and suggest that obestatin could play a role in modulating physiological lipid metabolism, although it does not appear to be involved in regulation of food/fluid intake, body weight or fat deposition.

1. Introduction

Obestatin (OB(1-23))is a 23 amino acid ghrelin-associated peptide produced in the gastric mucosa from post-translational cleavage of residues 76–98 of the preproghrelin peptide [33]. Rodent and human OB(1-23) sequences are relatively conserved differing by only 3 amino acids. Furthermore, NMR and molecular modeling studies indicate that rodent [30] and human [32] isoforms adopt similar alpha-helical conformations, underlining their homogeneity. OB(1-23) appears to be enzymatically degraded in a number of tissues (e.g. plasma, liver, kidney and stomach), although the physiological significance of this observation has yet to be determined [37].

There is substantive evidence supporting a close correlation between plasma OB(1-23) concentrations and total body weight. A study by Nakahara et al. found that obese human subjects have significantly lower plasma levels of OB(1-23) compared to control subjects, whereas anorexic patients have significantly higher levels [27]. Elevated circulating OB(1-23) levels in anorexic patients have also been reported independently by other groups [10,24]. Similarly, several other studies in obese individuals are supportive of these findings. Circulating OB(1-23) concentrations were found to be are significantly lower in overweight and obese individuals compared with those of normal controls [2,8] and OB(1-23) levels are reported to be reduced in obese compared with normal weight or anorectic women [39]. Furthermore, plasma OB(1-23) concentrations are decreased in overweight and obese patients, and biopsy studies have revealed a reduced number of obestatin-positive cells in the gastric body mucosa of these individuals [9].

Interestingly, OB(1-23) levels appear to change following periods of weight reduction [9,43]. A study involving 88 obese and 25 normal weight children found significantly lower OB(1-23) levels in the obese group, and OB(1-23) levels were elevated in children subsequent to weight loss due to 'summer camp' intervention [43]. Similarly, adults undergoing significant weight loss after gastric banding experienced increases in OB(1-23) levels [18]. However, one contrasting report noted that plasma OB(1-23) levels in females

Abbreviations: OB(1-23), obestatin (1-23); PEG-OB(1-23), N-terminally PEGylated obestatin (1-23); WAT, white adipose tissue; PEG, polyethyleneglycol.

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suffering from severe obesity remained stable for up to 2 years after Roux-en-Y gastric bypass surgery which was accompanied by massive weight loss [29].

Many studies have investigated the effects of OB(1-23) administration on body weight and food intake, although these reports have been frequently conflicting [1,3,5,11,15,21,26,28,31,35,36,41]. It is important to note that these studies employed a range of doses and different animal models, but the reasons for such discrepancies remain unclear. A rather understudied area relates to potential effects of OB(1-23) on lipid metabolism. Some researchers have found that injecting mice for a period of 8 days with OB(1-23) reduced epididymal and perirenal fat stores and lowered plasma lipid concentrations, although this investigation was rather limited [25,26].The aim of the present study was to further investigate these initial reports of beneficial metabolic actions of obestatin by continuously infusing Sprague–Dawley rats with native OB(1-23) or a novel and stable OB(1-23) analog over an extended period. This was accompanied by a detailed serial assessment of food/fluid intake and body weight changes and terminal analysis of morphometric tissue weights, plasma lipids and glucose, and fat distribution between various depots including liver, gastrocnemius/soleous muscle, epididymal/subcutaneous fat pads.

2. Materials and methods

2.1. Peptides

Obestatin (1-23) (referred to as OB(1-23)) and N-terminally PEGylated obestatin(1-23) (referred to as PEG-OB(1-23)) were custom made and purified (≥95%) by GL Biochem (Shanghai, China). Peptides were C-terminally amidated rodent obestatin containing the amino acid sequence: FNAPFDVGIKLSGAQYQQHGRAL-NH_2. The molecular weights of OB(1-23)and PEG-OB(1-23) were 2516.8 and 2661.9 Da, respectively.

2.2. Experimental animals

Male Sprague–Dawley rats (Harlan, UK) were used for all studies. They were housed individually at 21 °C with a 12:12-h light–dark cycle and were given free access to drinking water and Teklad global rodent diet consisting of 16.4% protein, 4.0% fat and 3.3% carbohydrate (energy content 3.0 kcal/g; Harlan UK). All experiments were carried out in accordance with the UK Animals Scientific Procedures Act, 1986.

2.3. Assessing biological stability of obestatin peptides

The biological stability of OB(1-23) and PEG-OB(1-23) was assessed by monitoring enzymatic degradation of peptides in liver homogenate obtained from male Sprague–Dawley rats (8–10 weeks of age). Briefly, 1.5 g of rat liver was homogenised in (28 ml) Krebs Henseleit Buffer (KHB; composition in mM: 118.5 NaCl, 3.8 KCl, 1.18 KH_2PO_4, 25 $NaHCO_3$, 1.19 $MgSO_4$, 10 glucose, and 1.25 $CaCl_2$), the homogenate the centrifuged (100 × *g* for 5 min at 5 °C) and the supernatant stored at −80 °C. OB(1-23) or PEG-OB(1-23) (20 μg) were dissolved in 50 μl of KHB buffer and incubated (37 °C; 0, 10, 20, and 30 min) with 50 μl of rat liver homogenate. Reactions were terminated by the addition of 20 μl formic acid (10%, v/v) solution. The formation of peptide fragments from OB(1-23) or PEG-OB(1-23) was analyzed on a WatersAquityUltra Performance Liquid Chromatography (UPLC) system coupled to a photodiode array detector (for quantifying peptide degradation), and a mass spectrometer (for confirming the identity of peptide fragments). Samples were injected (10 μl) onto a reversed-phase C-18 UPLC column (1.7 μm; 2.1 mm × 50 mm; Aquity, Waters, Milford, MA, USA). The column was equilibrated with TFA/H_2O (0.05%, v/v) at a flow rate of 0.4 ml/min. Using 0.05% TFA in 95% acetonitrile/H_2O, the concentration of the eluting solvent was raised from 0% to 100% over 10 min. Chromatogram peaks were integrated at an absorbance of 214 nm, percentage intact peptide calculated, and half-lives determined by linear-regression analysis.

2.4. Chronic treatment studies

Rats (6–8 weeks) were randomly divided into 3 groups ($n = 6$) and surgically implanted with subcutaneous osmotic mini-pumps (model 2002, ALZET; Cupertino, CA) for 14 day continuous infusion of either saline, OB(1-23) (50 nmol/kg/day) or PEG-OB(1-23) (50 nmol/kg/day). Food and fluid intake and body weight were recorded at 3–4 day intervals. At the end of the treatment period rats were fasted overnight (16 h), sacrificed by sodium pentobarbitone overdose (200 mg/kg i.p.) and blood samples taken by cardiac puncture using a heparinized syringe. Blood was centrifuged for 30 s at 13,000 rpm (IEC Micromax RF) and plasma stored at −80 °C for subsequent analysis of glucose, cholesterol and triglyceride concentrations. Liver, epididymal and subcutaneous white adipose tissue and skeletal muscle (gastrocnemius and soleus) were excised, weighed, snap frozen in liquid N_2 and stored at −80 °C for measurement of tissue triglyceride content.

2.5. Extraction of tissue triglycerides

Extraction of triglycerides was performed by incubating tissues in a solution containing 66% chloroform, 33.5% methanol and 0.5% concentrated sulphuric acid. Tissue samples were weighed, placed in 1.5 ml Eppendorf tubes containing extraction solution and homogenized (Stuart SS10; Davidson and Hardy Ltd) with a pellet pestle attachment (Sigma, Dorset, UK). Tubes were incubated at 4 °C for 24 h, centrifuged (1000 rpm for 5 min), and the supernatant collected and dried to completeness using a Dri-Block Sample Concentrator (Techne, Cambridge, England). After drying, appropriate amounts of 2-propanol were added to solubilise lipids. Triglyceride concentrations were analyzed using an Analox GM7 Micro-Stat Analyser as described below.

2.6. Analysis of glucose, cholesterol and triglycerides

Plasma and tissue samples were analyzed using enzymatic assay kits (Analox Ltd) for glucose (GMRD-002A using glucose oxidase), cholesterol (GMRD-084 using cholesterol esterase) and triglyceride (GMRD-195 using lipase) detected on a GM7 Micro-Stat Analyser (Analox Instruments Ltd; London, UK). Prior to analysis reagents were warmed to 37 °C in a water bath and plasma/tissue samples thawed at room temperature.

2.7. Data analysis

Plasma analytes were expressed as mmol/l, tissue triglycerides as mmol/g of tissue and tissue weights as a percentage of total body weight. All results were expressed as mean ± standard error of the mean (SEM). Data were compared using a one-way ANOVA followed by Bonferoni post hoc testing. *P* values < 0.05 were considered to be statistically significant.

Table 1A
Degradation of obestatin peptides in rat liver homogenate. Percentage of peptide intact and the calculated half-lives.

Peptide	% of peptide intact				$t_{1/2}$ (min)
	0 min	10 min	20 min	30 min	
OB(1-23)	100	67.9	58.7	30.6	21.7
PEG-OB(1-23)	100	97.0	96.9	68.5	67.5

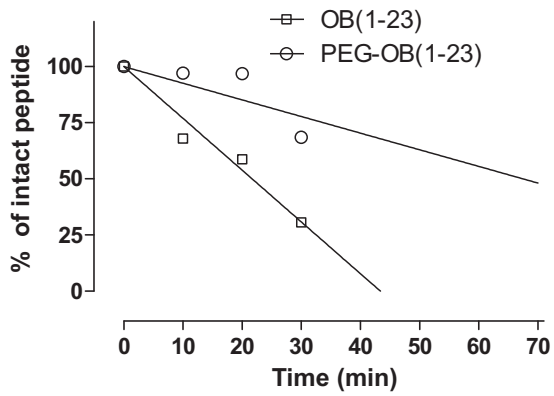

Fig. 1. Stability of OB(1-23) and PEG-OB(1-23) in rat liver homogenate. Figure shows the % of intact peptide detected for 0, 10, 20 and 30 min incubations in rat liver homogenate. Both OB(1-23) (open circles) and PEG-OB(1-23) (open squares) were progressively degraded and after 30 min, 30.6% and 68.5% remained intact, respectively. Following linear regression analysis the observed half-lives of OB(1-23) and PEG-OB(1-23) were 21.7 and 67.5 min, respectively.

3. Results

3.1. Stability of obestatin peptides in rat liver homogenate

Rat liver homogenate progressively degraded both OB(1-23) and PEG-OB(1-23) and after 30 min, 30.6% and 68.5% of the peptides remained intact, respectively (Table 1A and Fig. 1). Following linear regression analysis the observed half-lives of OB(1-23) and PEG-OB(1-23) were 21.7 and 67.5 min, respectively. A number of peptide fragments were detected by LC–MS analysis and these are consistent with enzymatic cleavage at F^1–N^2, P^4–F^5 G^{13}–A^{14}, L^{11}–S^{12}, G^{20}–R^{21}, and G^8–I^9 (Table 1B).

3.2. Effects of chronic OB(1-23) or PEG-OB(1-23) infusion on food intake, body weight, body weight gain and fluid intake in rats

Over the course of the 14-day treatment period food intake (Fig. 2A), body weight (Fig. 2B), body weight gain (Fig. 2C) and fluid intake (Fig. 2D) were not significantly altered by chronic treatment with either OB(1-23) or PEG-OB(1-23).

Effects of chronic OB(1-23) or PEG-OB(1-23) infusion on fasting plasma glucose, cholesterol and triglyceride concentrations in rats.

Table 1B
Degradation of obestatin peptides in rat liver homogenate. The cleavage site(s), detected peptide fragments with theoretical and experimental molecular weights.

Cleavage site(s)	Fragments detected in rat liver homogenate	Theoretical mass	Experimental mass
None	**OB(1-23)** FNAPFDVGIKLSGAQYQQHGRAL-NH_2	2516.8	2515.1
F1–N2	**OB(2-23)** NAPFDVGIKLSGAQYQQHGRAL-NH_2	2369.7	2368.6
P^4–F^5	**OB(5-23)** FDVGIKLSGAQYQQHGRAL-NH_2	2087.4	2086.1
F^1–N^2 G^{13}–A^{14}	**OB(2-13)** NAPFDVGIKLSG-COOH	1217.4	1216.4
F^1–N^2 L^{11}–S^{12}	**OB(2-11)** NAPFDVGIKL-COOH	1073.3	1072.0
G^{20}–R^{21}	**OB(1-20)** FNAPFDVGIKLSGAQYQQHG-COOH	2177.4	2176.0
G^8–I^9 G^{13}–A^{14}	**OB(9-13)** IKLSG-COOH	516.6	516.0
F^1–N^2 G^8–I^9	**OB(2-8)** NAPFDVG-COOH	718.8	717.9

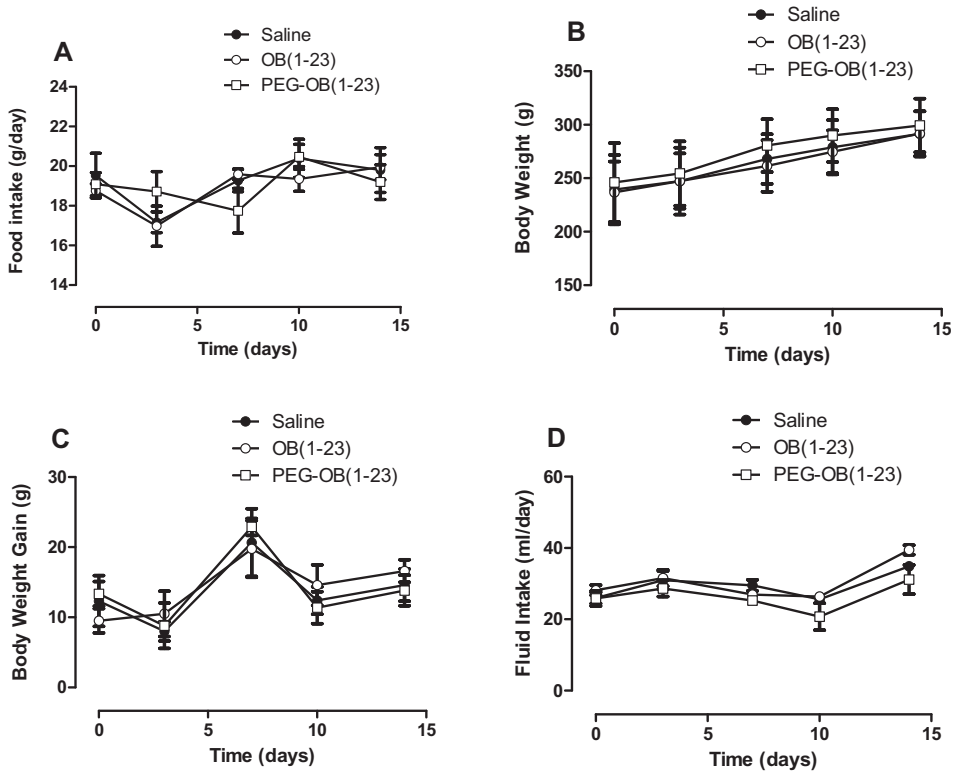

Fig. 2. Effects of chronic OB(1-23) or PEG-OB(1-23) treatment on (A) food intake, (B) body weight, (C) body weight gain and (D) fluid intake in rats. Sprague–Dawley rats (5 week old) were implanted with osmotic mini-pumps for a period of 14 days which contained either saline (closed circles), OB(1-23) (50 nmol/kg/day; open circles) or PEG-OB(1-23) (50 nmol/kg/day; open squares). Food intake, fluid intake and body weight were measured at −3, 0, 3, 7, 10 and 14 days. All values are mean ± SEM ($n = 6$).

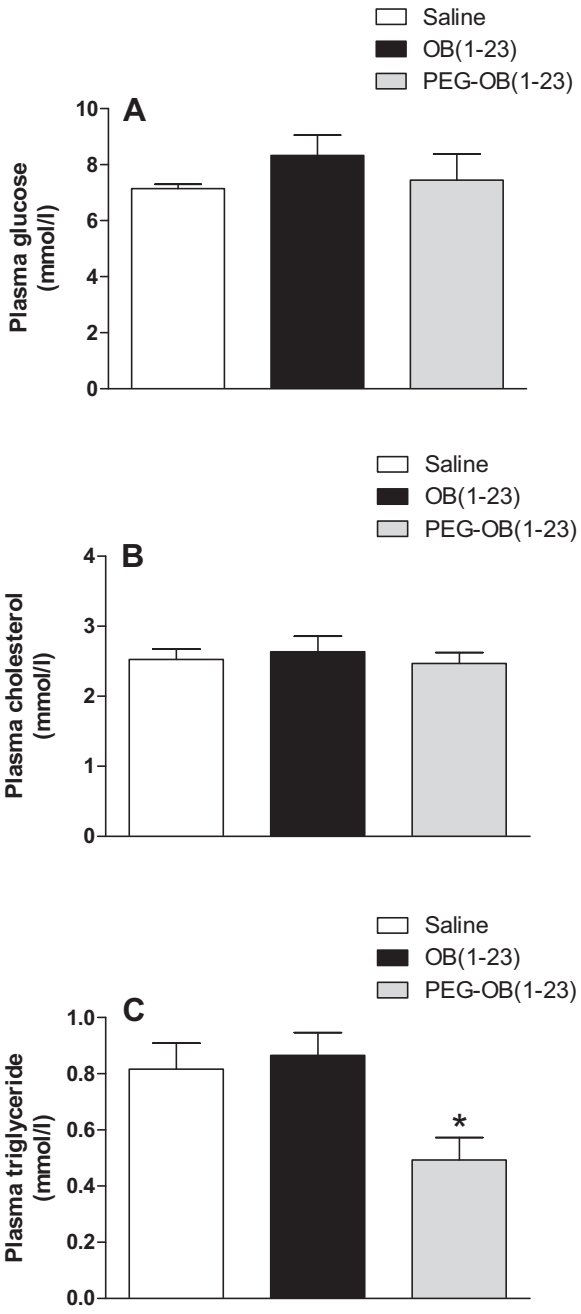

Fig. 3. Effects of chronic OB(1-23) or PEG-OB(1-23) treatment on fasting plasma (A) glucose, (B) cholesterol and (C) triglyceride concentrations. Sprague–Dawley rats (5 week old) were implanted with osmotic mini-pumps for a period of 14 days which contained either saline (white bar), OB(1-23) (50 nmol/kg/day; black bar) or PEG-OB(1-23) (50 nmol/kg/day; gray bar). On the final day of treatment rats were fasted for 16 h, anaesthetised and plasma obtained by cardiac puncture using a heparinised syringe. Analytes were measured using an AnaloxGM7 Micro-Stat Analyser and appropriate enzymatic assay kits. All values are mean ± SEM ($n = 6$). $^{*}P < 0.05$ compared with saline-treated rats.

Fasting plasma concentrations of glucose and cholesterol in rats infused with OB(1-23) or PEG-OB(1-23) were not significantly different from those of saline-infused rats (Fig. 3). However, in rats chronically infused with PEG-OB(1-23) fasting triglyceride levels were significantly decreased compared with rats infused with saline alone (0.82 ± 0.09 mmol/l versus 0.49 ± 0.08 mmol/l; $P < 0.05$).

3.3. Effects of chronic OB(1-23) or PEG-OB(1-23) infusion on liver, adipose and muscle tissue weights

The liver/body weight ratios of rats were unaffected by chronic OB(1-23) or PEG-OB(1-23) infusion (Fig. 4A). Fig. 4B and C indicated possible increases in the ratio of epididymal and subcutaneous WAT to body weight after OB(1-23) or PEG-OB(1-23) infusion but these did not reach statistical significance. No significant changes were detected in combined WAT/body weight ratios (Fig. 4D). Finally, in OB(1-23) or PEG-OB(1-23) treatment groups muscle morphometrical measurements for gastrocnemius (Fig. 4E); soleus (Fig. 4F); or combined (Fig. 4G) were not significantly different compared with saline controls.

3.4. Effects of chronic OB(1-23) or PEG-OB(1-23) infusion on the triglyceride content of liver, white adipose and muscle tissues

The triglyceride content of all measured tissues was unaffected by chronic infusion with either OB(1-23) or PEG-OB(1-23). No appreciable changes were detected in liver (Fig. 5A), subcutaneous WAT (Fig. 5C), gastocnemius muscle (Fig. 5D) and soleus muscle (Fig. 5D). Slight increases in triglycerides in epididymal WAT were observed in OB(1-23) and PEG-OB(1-23) infused rats but these did not reach statistical significance (Fig. 5B).

4. Discussion

Like many physiological peptides OB(1-23) is biologically degraded. Extensive degradation studies have reported rapid enzymatic cleavage of OB(1-23) at several sites in a number of biological matrices [37]. For example, Vergote et al. found that OB(1-23) was most rapidly degraded in liver homogenate with a recorded half-life of around 12 min [37]. These researchers concluded that strategies are required to improve the biological stability of OB(1-23) and suggested that N-terminal modification such as PEGylation could be used to prevent aminopeptidase activity [37].

We compared the stability of OB(1-23) and PEG-OB(1-23) in rat liver homogenate and observed peptide fragments consistent with cleavage of peptides at F^1–N^2, P^4–F^5 G^{13}–A^{14}, L^{11}–S^{12}, G^{20}–R^{21}, and G^8–I^9. The majority of these cleavage sites have previously been reported in studies where human or rodent OB(1-23) was incubated with either mouse plasma or kidney/liver homogenate [37]. However cleavage of OB(1-23) at G^8–I^9 has not been previously observed, and the calculated half-life of 21.7 min in rat liver homogenate was longer than that reported for those from mice (12.6 min [37]). Such disparities may relate to differing enzyme expression and activity in rat and mouse species, although there appears to be some interspecies homogeneity.

Although both peptides in the present study underwent some degradation, PEG-OB(1-23) was much less susceptible to breakdown compared with OB(1-23). Calculated half-lives indicated that PEG-OB(1-23) was approximately 3 times more resistant to enzymatic degradation than OB(1-23). We hypothesized that degradation would lead to inactivation of OB(1-23) and as a consequence PEG-OB(1-23) would have prolonged bioactivity. In order to investigate biological activity peptides were chronically infused into rats and a wide range of metabolic and morphological parameters were measured.

There is some consensus of opinion to support a relationship between physiological OB(1-23) concentrations and body weight, but the notion that OB(1-23) exerts actions which alter food intake or body weight is becoming more and more disputed. Several early studies reported reductions in food and fluid intake and body weight gain following both acute and chronic administration of OB(1-23) to rodents [3,5,22,26,41]. However, an increasing num-

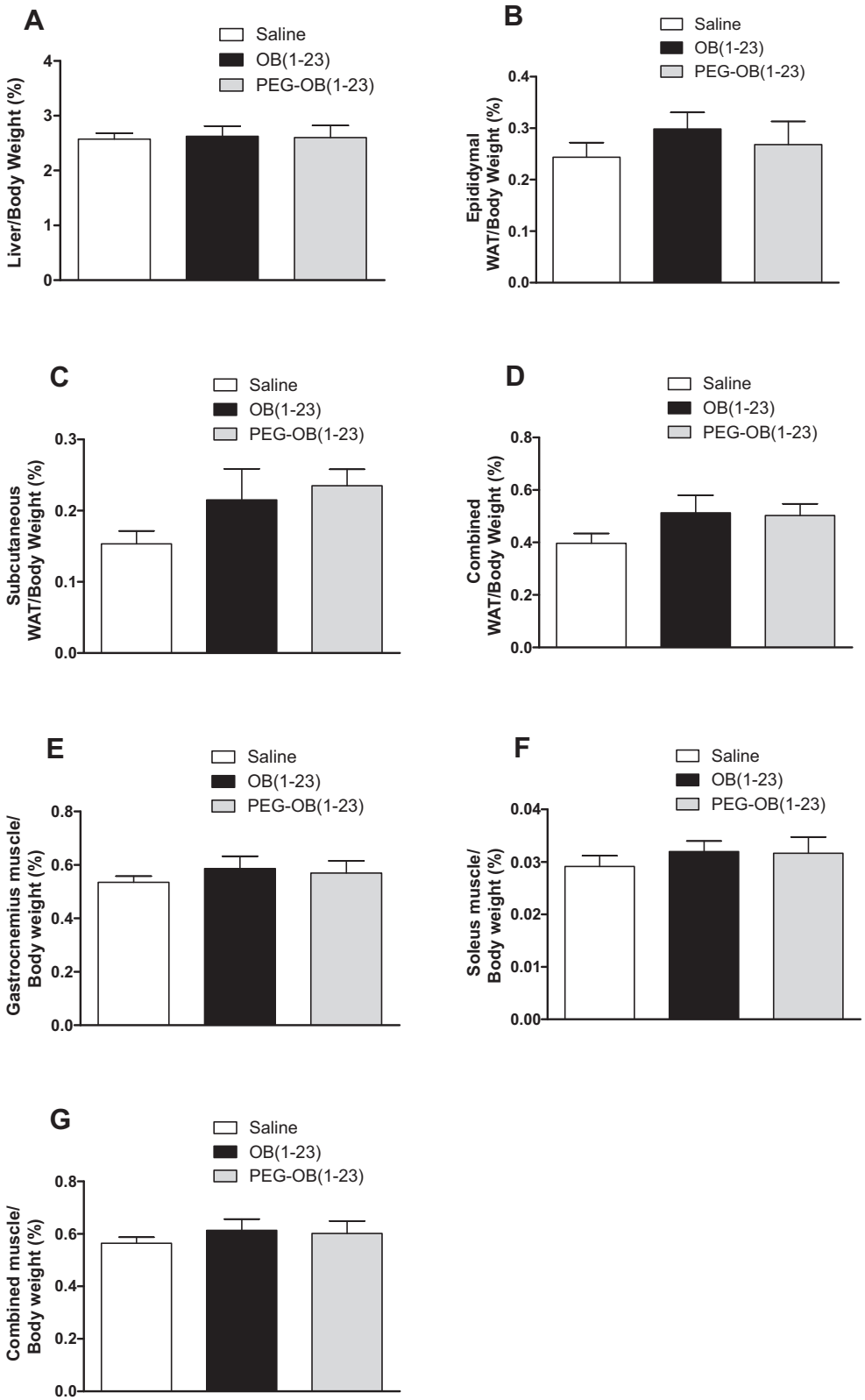

Fig. 4. Effects of chronic OB(1-23) or PEG-OB(1-23) on liver, muscle and white adipose tissues (WAT). Sprague–Dawley rats (5 week old) were implanted with osmotic mini-pumps for a period of 14 days which contained either saline (white bar), OB(1-23) (50 nmol/kg/day; black bar) or PEG-OB(1-23) (50 nmol/kg/day; gray bar). After the treatment period rats were sacrificed and tissues (A) liver, (B) epididymal WAT, (C) subcutaneous WAT (D) combined WAT (E) gastrocnemius muscle, (F) soleus muscle and (G) combined muscle were dissected, weighed and expressed as a percentage of body weight. All values are mean ± SEM ($n = 6$).

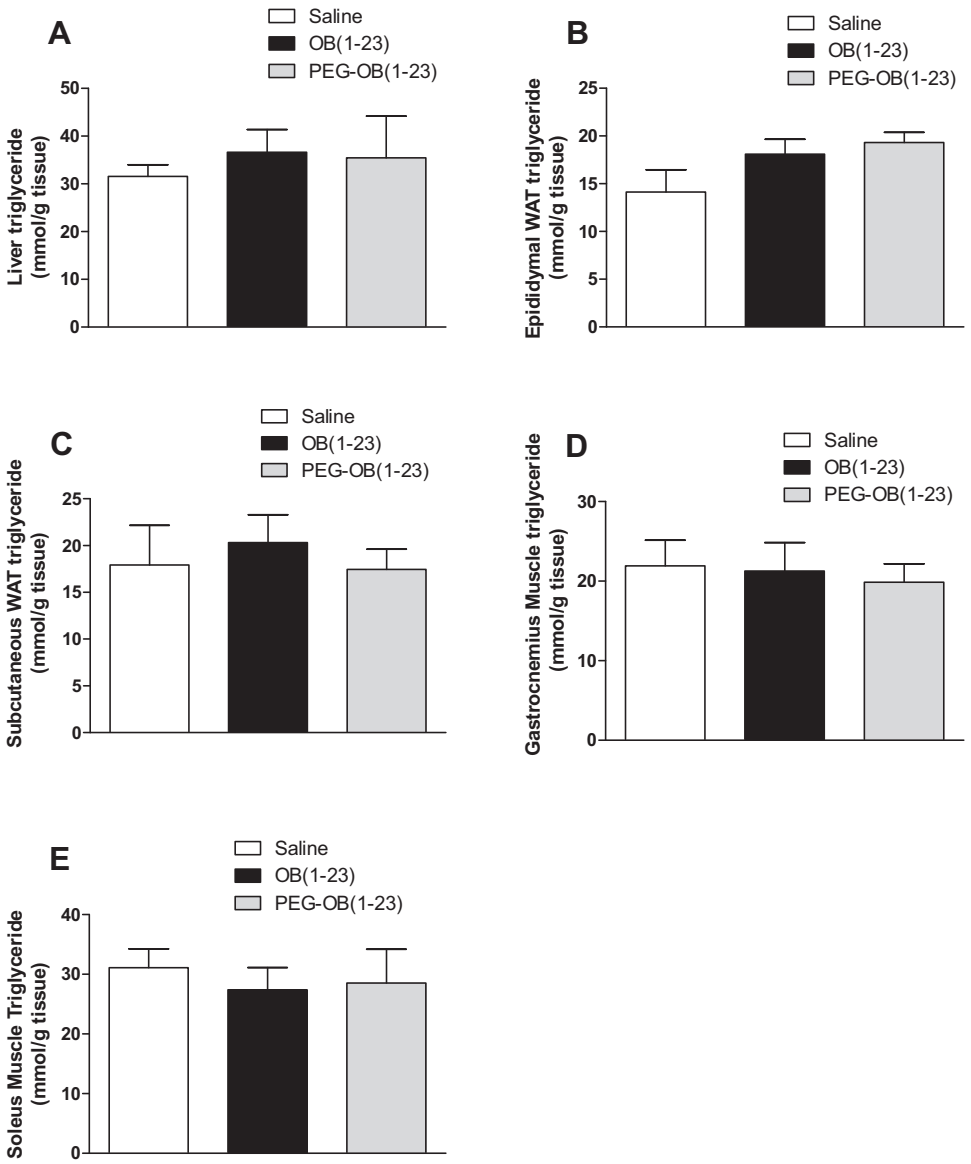

Fig. 5. Effects of chronic OB(1-23) or PEG-OB(1-23) treatment on triglyceride levels in (A) liver, (B) epididymal WAT, (C) subcutaneous WAT, (D) gastrocnemius muscle and (E) soleus muscle. Sprague–Dawley rats (5 week old) were implanted with osmotic mini-pumps for a period of 14 days which contained either saline (white bar), OB(1-23) (50 nmol/kg/day; black bar) or PEG-OB(1-23) (50 nmol/kg/day; gray bar). After the treatment period rats were sacrificed, tissues were dissected and snap frozen prior to extraction. Triglycerides were obtained by tissue homogenization and $CHCl_3$:HCl extraction. Data are expressed as mmol/g of tissue. All values are mean ± SEM ($n = 6$).

ber of studies have failed to find such actions [1,6,11,28,31,35,36]. A number of possible explanations for these discrepancies have been investigated. For example, a U-shaped dose–response effect for obestatin was proposed [21], but this suggestion like many others has been contested [35]. The present study investigated the *in vivo* effects of chronic infusion of rats with native OB(1-23) or an N-terminally modified peptide analog, PEG-OB(1-23). We examined global measures of food/fluid intake, body weight, morphometric fat distribution, and lipid concentrations in both plasma and tissue depots. Interestingly, we failed to observe any changes in food intake, fluid intake, body weight or body weight gain with either OB(1-23) or PEG-OB(1-23). Previously we reported that acute OB(1-23) injection (1000 nmol/kg) significantly reduced the meal-related responses of plasma glucose and insulin in normal mice [15] and high-fat fed mice [16,32]. However, these bolus doses were 20-fold higher than those in the present study where OB(1-23) was infused at a continuous and constant rate of 50 nmol/kg/day. Our findings agree those of Unniappan et al. who found that 7 days continuous OB(1-23) infusion did not alter food intake, fluid intake or weight gain [35]. It should also be noted that physiological obestatin concentrations typically range from 15 to 40 pmol/l in rodent plasma [23] and the concentrations infused here are vastly higher. If either of the tested peptides did possess biological actions pertaining to food intake/body weight then they should be apparent at these supra-physiological doses.

Previous studies suggest that OB(1-23) is involved in lipid metabolism. In humans Vicennati et al. reported significant positive correlations between OB(1-23) concentrations and total cholesterol and triglyceride levels [38]. Grala et al. demonstrated that prolonged infusion of OB(1-23) in cows significantly decreased the expression of ATP-binding cassette A1 in adipose tissue, indicating possible changes in cholesterol transport [12]. Nagaraj et al. reported that 8-day OB(1-23) treatment led to a small rise in total cholesterol levels (approximately 4%) and decreased triglyceride levels (around 22%) [26]. A later study by the same group reported a 32% reduction in plasma triglycerides and a 13% reduction in total cholesterol following 8-days of OB(1-23) treatment [25]. The findings here partially agree. We did not observe any

changes in fasting cholesterol levels following 14-day continuous infusion with either OB(1-23) or PEG-OB(1-23), however PEG-OB(1-23) led to a significant 40% reduction in plasma triglyceride levels.

Changes in epididymal or subcutaneous WAT were not detected in the present study. As far as we are aware, there are no studies which have measured subcutaneous WAT after chronic OB(1-23) treatment, although there are two reports of decreases in epididymal (18–20%) and perirenal (30–31%) WAT [25,26]. This study does not support the notion that OB(1-23) influences body weight or composition. This is evidenced by the lack of any changes in body weight or body weight gain during or after treatment, the absence of any variations in the proportions of liver, adipose or muscle tissues, and also by the fact that there were no alterations in the amount of tissue triglycerides. Since treatment led to reductions in plasma triglycerides without any accompanying adjustments in tissue triglycerides it could speculated that OB(1-23) influences intestinal triglyceride absorption. Such effects on plasma triglycerides warrant further investigation.

The mechanisms by which OB(1-23) might exert the triglyceride-lowering action remain to be elucidated. OB(1-23) was previously purported to be the cognate ligand for the orphan G-coupled receptor GPR39 [40,41] and this receptor appears to be expressed in human adipose and intestinal tissue [4]. It should be noted that some studies have cast doubt on the assertion that OB(1-23) binds to or activates GPR39 [5,19,22]. There appear to be two variants of GPR39, designated 1a and 1b [7]. GPR39-1a is expressed selectively throughout the gastrointestinal tract, liver, pancreas, kidney and adipose tissue, whereas GPR39-1b is more broadly expressed. Whether either of these receptor variants are involved in lipid metabolism is unknown but it is known, that GPR39-1a is expressed in WAT but not in brown adipose tissue [7]. It has been contended that OB(1-23) might bind to the glucagon-like peptide-1 receptor [13], but as with GPR39, this finding has been disputed [35].

Derivatising peptides with polyethylene glycol, fatty acids or various chemical linkers is a strategy that has been successfully employed to prolong the half-life of other peptides (e.g. glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide and exendin-4) [14,20,42]. Such strategies are advantageous because they can prevent enzymatic degradation but they may also facilitate the adhesion of peptides to plasma proteins, thus circumventing renal filtration [17,34]. Renal excretion is a factor strongly affecting the half-life of peptides and it is possible that PEG-OB(1-23) is retained within the circulation for an extended period of time.

In conclusion, this study demonstrated that PEG-OB(1-23) is a novel OB(1-23) analog with enhanced stability. Chronic and continuous infusion of PEG-OB(1-23) substantially lowered fasting plasma triglyceride levels. The fact that OB(1-23) did not bring about such changes is further evidence of PEG-OB(1-23)'s improved physiological stability and extended duration of action. Although chronic treatment with obestatin peptides did not result in changes in food intake or body weight, the probable effects of obestatin on lipid metabolism should be further investigated.

Conflict of interest

The authors have no conflicts of interest to declare.

Contributions

A. Agnew – *in vivo* data collection and analysis. D. Calderwood – analytical measurementsand analysis. O.P. Chevallier and B. Greer – conducted degradation studies and LC-MS studies. D.J. Grieve & B.D. Green–Academic Supervisors of A.A. and D.C. Designed studies, interpreted data and co-wrote the manuscript.

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