Biochimica et Biophysica Acta 1850 (2015) 80–87

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Magainin-AM2 improves glucose homeostasis and beta cell function in high-fat fed mice

O.O. Ojo, D.K. Srinivasan, B.O. Owolabi, J.M. Conlon, P.R. Flatt, Y.H.A. Abdel-Wahab *

SAAD Centre for Pharmacy & Diabetes, School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, UK

ARTICLE INFO

Article history:
Received 10 July 2014
Received in revised form 22 September 2014
Accepted 13 October 2014
Available online 20 October 2014

Keywords:
Magainin-AM2
Amphibian peptide
Type 2 diabetes
Obesity
Insulin-release

ABSTRACT

Background: Magainin-AM2, a previously described amphibian host-defense peptide, stimulates insulin- and glucagon-like peptide-1-release in vitro. This study investigated anti-diabetic effects of the peptide in mice with diet-induced obesity and glucose intolerance.
Methods: Male National Institute of Health Swiss mice were maintained on a high-fat diet for 12-weeks prior to the daily treatment with magainin-AM2. Various indices of glucose tolerance were monitored together with insulin secretory responsiveness of islets at conclusion of study.
Results: Following twice daily treatment with magainin-AM2 for 15 days, no significant difference in body weight and food intake was observed compared with saline-treated high fat control animals. However, non-fasting blood glucose was significantly ($P < 0.05$) decreased while plasma insulin concentrations were significantly ($P < 0.05$) increased. Oral and intraperitoneal glucose tolerance and insulin secretion following glucose administration via both routes were significantly ($P < 0.05$) enhanced. The peptide significantly ($P < 0.001$) improved insulin sensitivity as well as the beta cell responses of islets isolated from treated mice to a range of insulin secretagogues. Oxygen consumption, CO_2 production, respiratory exchange ratio and energy expenditure were not significantly altered by sub-chronic administration of magainin-AM2 but a significant ($P < 0.05$) reduction in fat deposition was observed.
Conclusion: These results indicate that magainin-AM2 improves glucose tolerance, insulin sensitivity and islet beta cells secretory responsiveness in mice with obesity-diabetes.
General significance: The activity of magainin-AM2 suggests the possibility of exploiting this peptide for treatment of type 2 diabetes.

1. Introduction

Use of natural products and biologically active peptides from exogenous sources for the treatment of human disease has been well documented [1,2]. Many such agents are currently being evaluated for their ability to stimulate insulin secretion [3–6], delay glucose absorption [7], promote weight loss [8], improve insulin sensitivity [9], retard gastric emptying [10], promote incretin activity [11] and enhance glucose homeostasis. Exendin-4, isolated from the saliva of Gila monster lizard, is a classic example of a naturally occurring biologically active peptide successfully developed for the treatment of type 2 diabetes [12].

While the structural similarity between exendin-4 and the endogenous incretin hormone, glucagon-like peptide 1 (GLP-1) accounts for their similar biological effects, our previous studies revealed that a number of structurally diverse, amphibian host-defense peptides exhibited incretin-like effects [5]. Magainin-AM2, a 23 amino acid peptide isolated from the skin secretion of the Volcano clawed frog, *Xenopus amieti* on the basis of its antimicrobial activities [13], is one such example. This peptide is an ortholog of magainin-2, a cationic, amphipathic, α-helical antimicrobial peptide first isolated from *Xenopus leavis* [14]. In addition to antimicrobial effects, peptides in magainin family or their analogs have been shown to possess wound-healing [15], anti-inflammatory [16,17], anti-malarial [18], anti-viral [2] and anti-cancer [19] effects. In addition, magainins have been reported to decrease plasma levels of endotoxin and TNF-alpha in a rat model of sepsis [20], affect respiratory control and inhibit mammalian spermatozoa motility [21].

We recently reported that magainin-AM1 and -AM2 significantly stimulated the release of GLP-1 from GLUTag cells [22]. Our more recent investigations of the in vitro actions of magainin-AM2 revealed a significant stimulation of insulin-release from clonal BRIN-BD11 cells and isolated mouse islets [23]. This was associated with membrane depolarization and increased intracellular calcium

* Corresponding author at: School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, Northern Ireland, UK. Tel.: +44 2870324354.
E-mail address: y.abdel-wahab@ulster.ac.uk (Y.H.A. Abdel-Wahab).

http://dx.doi.org/10.1016/j.bbagen.2014.10.011
0304-4165/

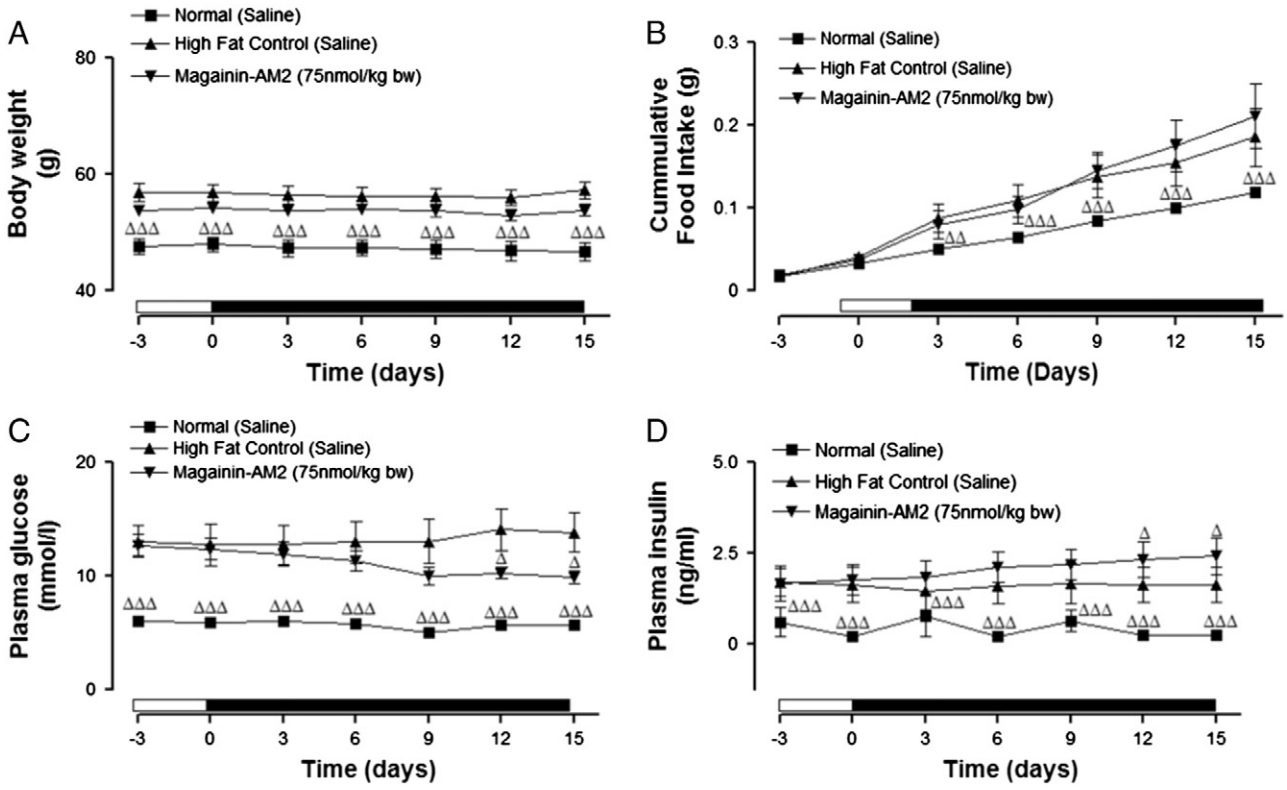

Fig. 1. Effects of magainin-AM2 on body weight (A), food intake (B), plasma glucose (C) and insulin (D) in normal meal and high-fat fed mice. Parameters were measured 3 days prior to, and every 72 h during treatment (indicated with black bar) with saline or magainin-AM2. Values are means ± SEM for 8 mice. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ compared to high fat fed control mice.

in the BRIN-BD11 beta cell line [23]. These positive in vitro results stimulated our interest to investigate in vivo antidiabetic effects of magainin-AM2 in an animal model of type 2 diabetes. The present study has examined the biological effects of sub-chronic (15 days) administration of magainin-AM2 on energy balance, glucose homeostasis, insulin secretion and insulin sensitivity in mice with diet-induced obesity, insulin resistance and glucose intolerance.

2. Materials and methods

2.1. Peptide synthesis and purification

Magainin-AM2 (GVSKILHSAGKFGKAFLGEIMKS) was supplied in crude form by GL Biochem Ltd (Shanghai, China). Purification to near homogeneity (>98% purity) was performed by reversed-phase HLPC

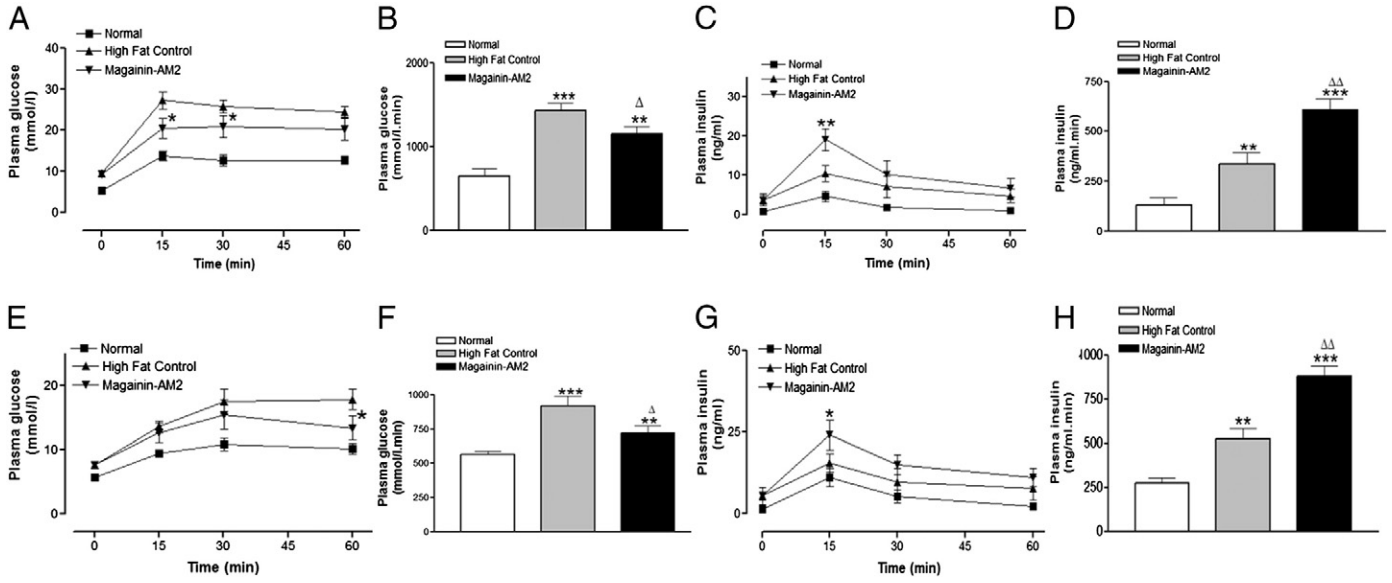

Fig. 2. Effects of magainin-AM2 on plasma glucose and insulin concentrations following intraperitoneal (A–D) and oral (E–H) glucose administration in normal and high-fat fed mice. Tests were conducted after twice-daily treatment of mice with magainin-AM2 (75 nmol/kg body weight/day) for 15 days. Values are means ± SEM for 8 mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared with saline treated lean mice. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ compared with high-fat fed control mice.

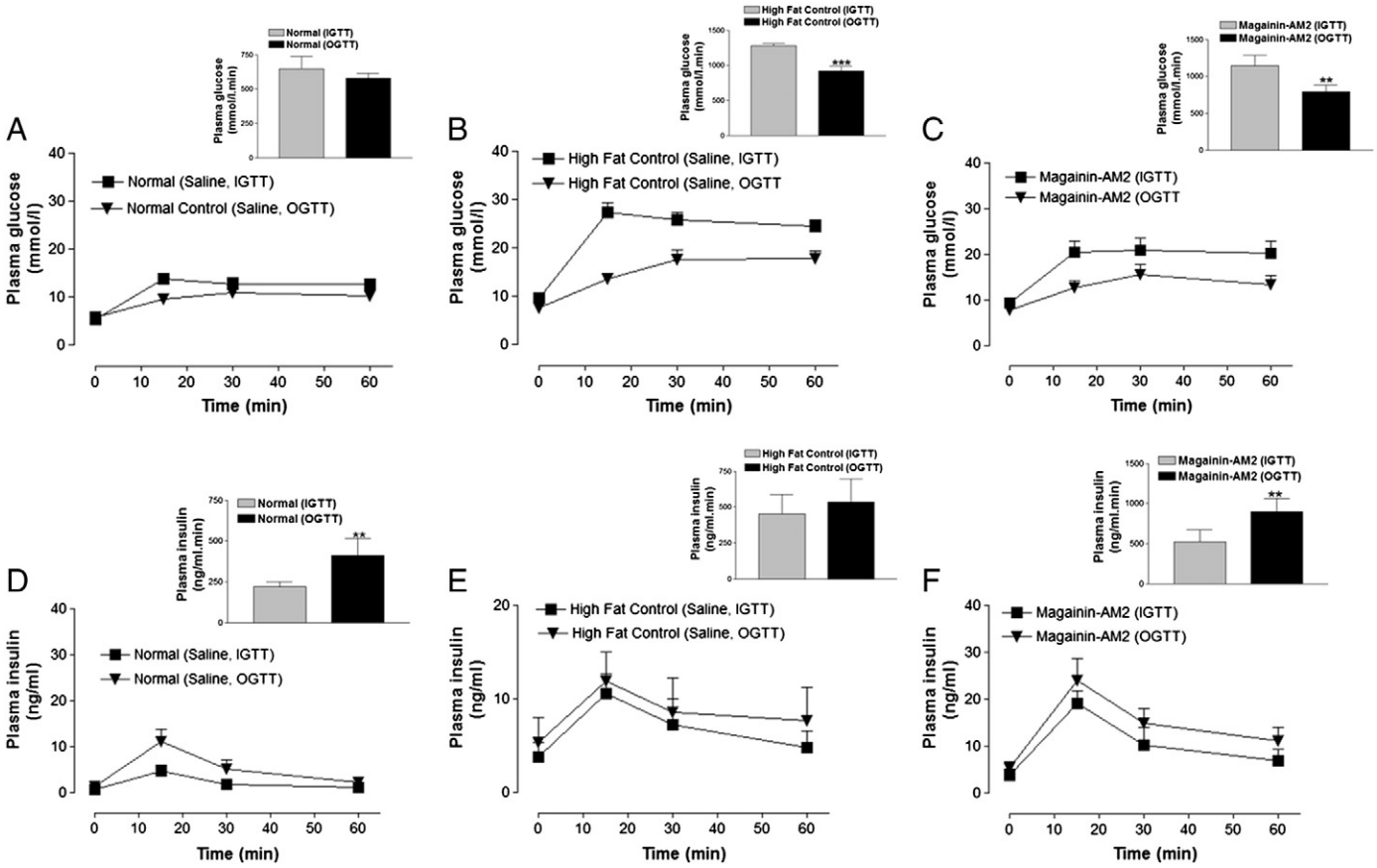

Fig. 3. Effects of magainin-AM2 on oral and intraperitoneal glucose tolerance and insulin secretion in normal and high-fat fed mice. Plasma glucose (A–C) and insulin (D–F) were measured prior to and after oral or intraperitoneal administration of glucose (18 mmol/kg) to high-fat fed mice pre-treated with saline or magainin-AM2 (75 nmol/kg bw) for 15 days. Area under the curve (AUC) graphs, for each group of mice, is included as inserts. Values are means ± SEM for 8 mice. $^{**}P < 0.01$, $^{***}P < 0.001$ compared with oral glucose tolerance test (OGTT).

on a (2.2 cm × 25 cm) Vydac 218TP1022 (C18) column equilibrated with acetonitrile/water/triflouroacetic acid (21/78.9/0.1 v/v) at a flow rate of 6 ml/min. The concentration of acetonitrile was raised to 56% (v/v) over 60 min. Identity of the peptide was confirmed by electrospray mass spectrometry.

2.2. Laboratory animals

National Institutes of Health (NIH) Swiss mice (male, 8 weeks old, Harlan Ltd, UK), housed individually in an air-conditioned room (22 ± 2 °C) with a 12-h light:12-h dark cycle (08:00–20:00 h) were used. Mice were maintained for 3 months prior to the experiment on a high-fat diet consisting of 45% kcal from fat, 20% kcal from protein, and 35% kcal from carbohydrate (total energy 19.5 kJ/g, Dietex International Ltd, Witham, UK) or standard rodent diet. Weight-matched mice with clearly manifested obesity, insulin resistance and glucose intolerance together with their age matched lean controls were used. All animal experiments were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and EU Directive 2010/63EU for animal experiments.

2.3. In vivo experimental procedure

High-fat fed mice (n = 8) received twice daily injections of either saline vehicle (0.9% (w/v)) or magainin-AM2 (75 nmol/kg body weight) for 15 days. This peptide dose was selected from results of a pilot study that investigated doses of 37, 75 and 150 nmol/kg body weight. Twice daily injection of magainin-AM2 was selected based on our previous results which show that the peptide is active within 12 h of administration. This dose can be expected to yield circulating concentrations of magainin-AM2 considerably lower than the minimum inhibitory concentration to induce antimicrobial effects. Energy intake, body weight, non-fasting blood glucose and plasma insulin concentrations were measured 3 days prior to and every 72 h during the study. Glucose tolerance (18 mmol/kg body weight, i.p. or oral) and insulin sensitivity tests (25 U/kg bw) were carried out after 15 days in overnight fasted mice as previously described [24]. Indirect calorimetry and energy expenditure in high-fat fed mice were measured using the Comprehensive Laboratory Animal Monitoring System (CLAMS) metabolic chambers (Columbus Instruments, Columbus, OH, USA) [25]. Terminal analysis included measurement of total body lean and fat mass, bone mineral density and bone mineral content by DXA scanning (Piximus Densitometer, USA). In addition, pancreatic tissue was excised for islet isolation and analysis of insulin content following acid–ethanol extraction [26]. No adverse effects were observed in mice treated with magainin-AM2.

2.4. Insulin-release studies from isolated islets

Insulin secretory function of islets isolated from mice receiving magainin-AM2 or saline was assessed in response to established insulin secretagogues and incretin hormones, including 1.4–16.7 mM of glucose, 1 μM of GLP-1, 1 μM of GIP, 10 mM of alanine, 10 mM of arginine and 30 mM of KCl. The latter 5 agents were tested in the presence of 16.7 mM of glucose. Islets isolated by collagenase digestion [27,28] were cultured overnight in RPMI 1640 prior to a 40 min pre-incubation (5–10 islets per tube) at 37 °C in KRB buffer supplemented with 1.4 mM of glucose. After this period, islets were incubated for 1 h in KRB buffer containing 5.6 mM of glucose or 16.7 mM of glucose and the agents as indicated in Fig. 7. Insulin release was measured by

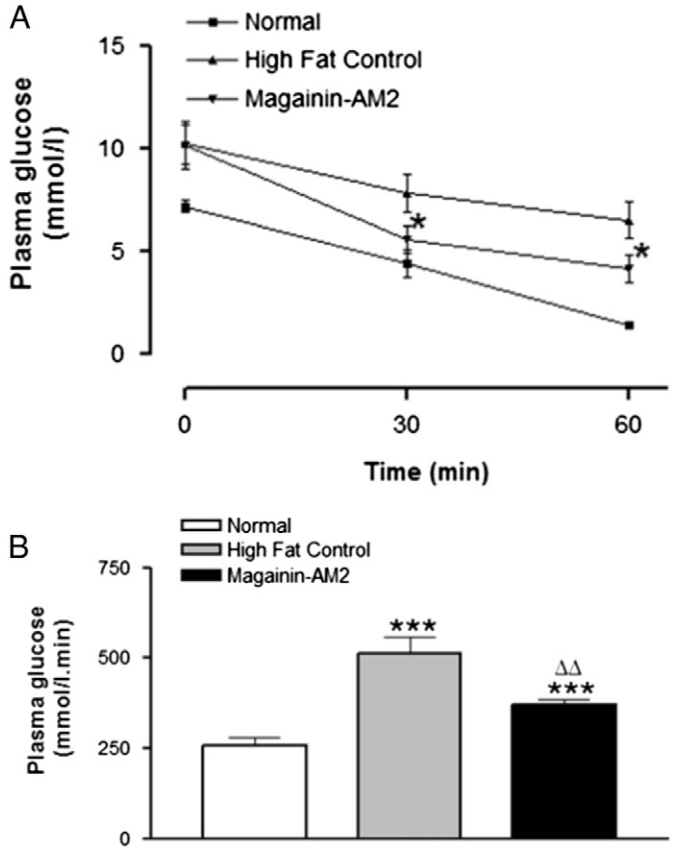

Fig. 4. Effects of magainin-AM2 on insulin sensitivity in normal and high-fat fed mice. Plasma glucose levels were measured prior to, and after, intraperitoneal injection of insulin (25 U/kg bw) in normal and high-fat fed mice treated with twice-daily injections of saline or magainin-AM2 (75 nmol/kg body weight) for 15 days. Values are means ± SEM for 8 mice. $^{*}P < 0.05$, $^{***}P < 0.001$ compared with saline-treated lean mice. $^{\Delta\Delta\Delta}P < 0.001$ compare with high-fat fed control mice.

radioimmunoassay as described previously [29] and results were expressed as a percentage of total insulin content of islets.

2.5. Biochemical measurements

Blood samples (approximately 150 μl) were collected from the cut tip of the tail vein of conscious mice at intervals indicated in the figures and plasma was separated and stored as described previously [30]. Blood glucose was measured using a hand-held Ascencia Contour meter (Bayer Healthcare, UK). Plasma and pancreatic insulin concentrations were determined by radioimmunoassay [29].

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. Values are compared using one-way ANOVA followed by Student–Newman–Keuls post hoc test. Area under the curve (AUC) analysis was performed using the trapezoidal rule with baseline correction. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effects of magainin-AM2 on energy intake, body weight, non-fasting blood glucose and plasma insulin

Energy intake, body weight as well as non-fasting glucose and insulin concentrations were significantly higher in mice fed a high-fat diet compared to lean controls. Treatment of the high-fat fed mice with twice-daily injections of magainin-AM2 for 15 days had no significant effect on energy intake or body weight compared with saline treated high-fat fed mice (Fig. 1A, B). However, non-fasting blood glucose levels were gradually reduced by magainin-AM2 with significantly reduced values ($P < 0.05$) on days 12 and 15 compared to saline controls (Fig. 1C). Non-fasting plasma insulin concentrations were similarly increased ($P < 0.05$) on days 12 and 15 of magainin-AM2 treatment (Fig. 1D).

3.2. Effects of magainin-AM2 on glucose tolerance, insulin secretion and insulin sensitivity

Treatment with magainin-AM2 (75 nmol/kg body weight) for 15 days significantly ($P < 0.05$) improved i.p. glucose tolerance (18 mmol/kg body weight) compared to high-fat fed controls (Fig. 2A, B). A rapid increase in insulin secretion was observed in treated animals within 15 min of glucose administration (Fig. 2C), with integrated insulin responses being increased 1.8-fold ($P < 0.01$) compared to untreated high-fat fed control mice (Fig. 1D). Both groups of high-fat fed mice exhibited significantly higher plasma glucose ($P < 0.001$) and insulin ($P < 0.001$) levels compared to lean mice fed a normal diet (Fig. 2A–D). Magainin-AM2 treated mice also showed improved glucose tolerance and insulin secretion following oral glucose administration (18 mmol/kg body weight) (Fig. 2E–H).

Metabolic responses to oral and intraperitoneal glucose administration were compared in order to assess incretin-like effects of magainin-AM2 (Fig. 3). The difference in plasma glucose levels following oral or intraperitoneal glucose administration in magainin-AM2 treated mice was significantly lower ($P < 0.05$) compared to high-fat fed control mice (Fig. 3A–D). Conversely, the difference in plasma insulin levels after oral or intraperitoneal glucose administration in magainin-AM2 treated mice was significantly greater ($P < 0.01$) compared to high-fat fed controls. Moreover, differential insulin-release observed in high-fat fed control mice was lower than that of mice maintained on standard rodent diet (Fig. 3E–H). As shown in Fig. 4, twice-daily treatment with magainin-AM2 also significantly ($P < 0.05$) improved insulin sensitivity compared with untreated control mice (Fig. 4).

3.3. Effects of magainin-AM2 on indirect calorimetry, energy expenditure and body composition

Mice fed a high-fat diet increased their rate of O_2 consumption by 30% (Fig. 5, $P < 0.001$) compared to a basal rate of 6.9 ± 0.2 ml/kg per h observed for mice fed a standard rodent diet. Treatment with magainin-AM2 had no effect on O_2 consumption. Similarly, basal rate of CO_2 production and energy expenditure were increased by 22% ($P < 0.001$) and 28% ($P < 0.001$) respectively in mice fed a high-fat diet. Treatment with magainin-AM2 had no effect on these parameters and the respiratory exchange ratio was similar (19.9 ± 0.2) in all groups. Body fat increased by 34% in mice fed a high-fat diet compared with mice maintained on a standard rodent diet (Fig. 6). Treatment with magainin-AM2 significantly ($P < 0.05$) prevented fat deposition in high-fat fed mice (Fig. 6A, C), and produced a 13% decrease in body fat compared to high-fat fed control mice. Feeding a high-fat diet and treatment with magainin-AM2 had no effect on lean body mass or bone mineral density. A slight but statistically insignificant increase in bone mineral content and bone area were observed in magainin-AM2 treated mice compared to high fat controls.

3.4. Effects of magainin-AM2 on pancreas weight, insulin content and islet secretory responses

Feeding a high-fat diet did not affect pancreas weight (Fig. 7A) but significantly ($P < 0.001$) decreased pancreatic insulin (Fig. 7B) without affecting islet insulin content (Fig. 7C). Treatment with magainin-AM2 prevented the observed reduction in pancreatic insulin content

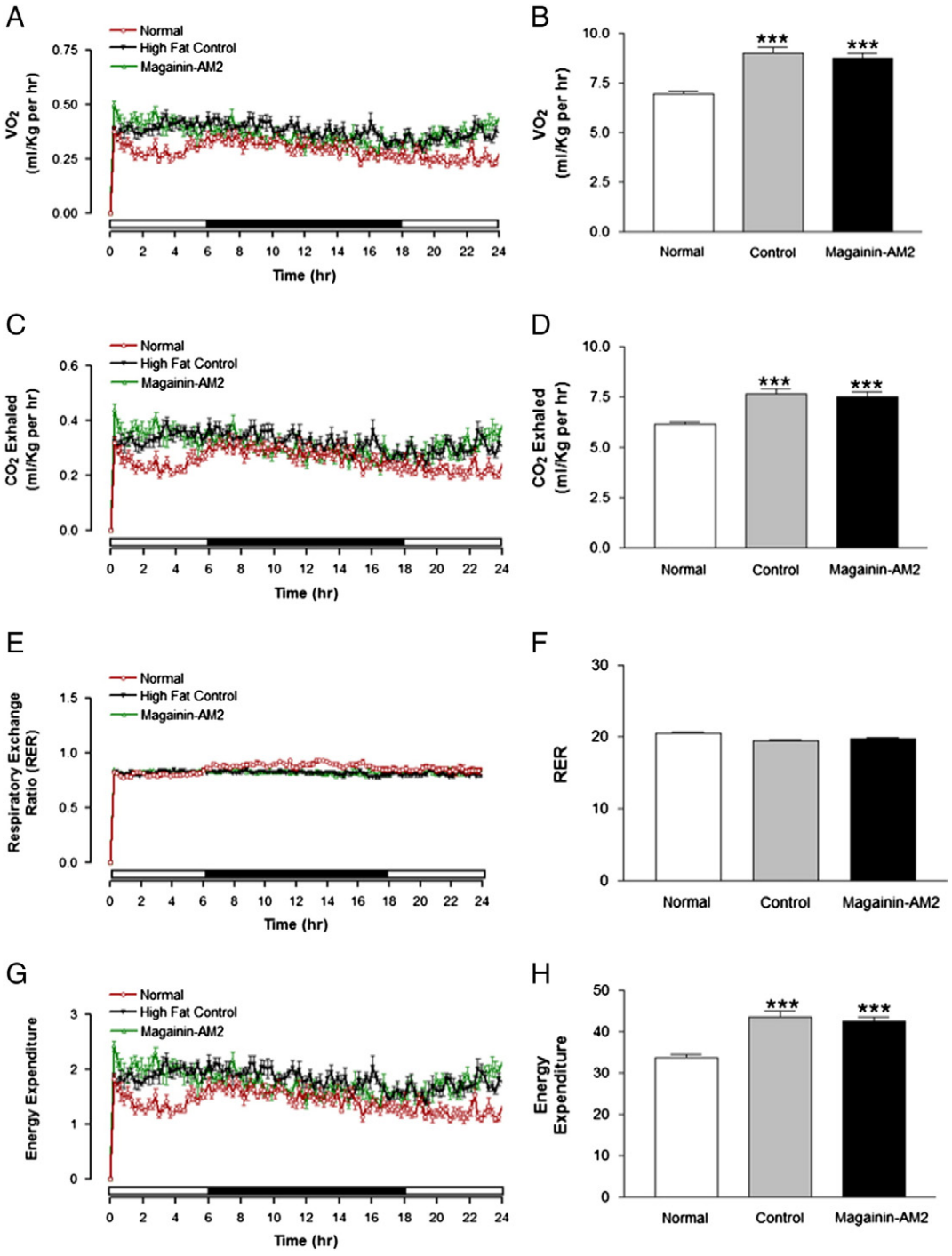

Fig. 5. Effects of magainin-AM2 on O_2 consumption (A, B), CO_2 production (C, D), respiratory exchange ratio (E, F) and energy expenditure (G, H) in normal and high-fat fed pre-treated with saline or magainin-AM2 (75 nmol/kg bw) for 15 days. Mice were placed in CLAMS metabolic chambers, and O_2 consumption or CO_2 production were measured for 30 s at 15 min intervals. RER was calculated by dividing VCO_2 by VO_2. Energy expenditure was computed using the formula $(3.815 + 1.232 \times \text{RER}) \times VO_2$. Values are means ± SEM for 6 mice. ***$P < 0.001$ compared with saline-treated lean mice. Shaded bar indicates dark phase.

($P < 0.01$). Moreover, insulin secretory responses of islets isolated from magainin-AM2-treated mice to 5.6 mM or 16.7 mM glucose, GLP-1, GIP and other established insulin secretagogues including alanine, L-arginine and KCl were significantly improved compared with high-fat fed controls (Fig. 7D). This latter group exhibited impaired insulin secretory responses compared with mice fed normal diet whereas high-fat fed magainin-AM2 treated mice did not (Fig. 7E).

4. Discussion

The increasing global incidence of type 2 diabetes and the challenges associated with the management of the disease have stimulated the search for novel agents for the treatment of the disease. A growing body of evidence suggests beneficial effects of certain amphibian host defense peptides in the management of glucose homeostasis and type 2 diabetes [2,5]. In common with several other host-defense peptides,

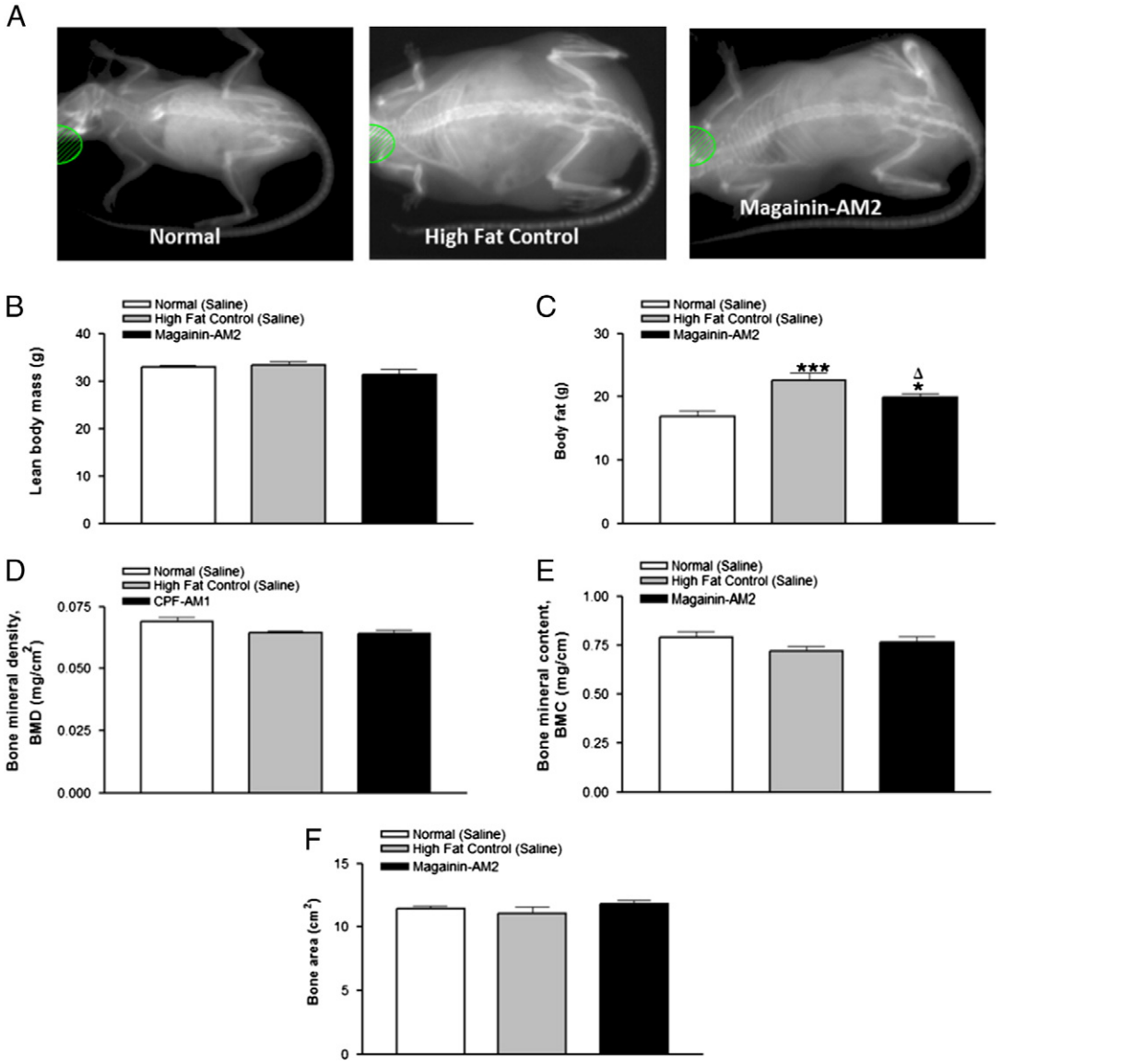

Fig. 6. Effects of magainin-AM2 on body composition in normal or high-fat fed mice. Mice were treated with twice-daily injections of saline or magainin-AM2 (75 nmol/kg body weight) for 15 days prior to DEXA scan (A) and computation of data on lean body mass (B), body fat (C), bone mineral density (D), bone mineral content (E) and bone area (F). Values are means ± SEM ***P < 0.001, P compared with saline-treated lean mice, ΔP < 0.01 compared with high-fat fed control mice.

in vitro data revealed that magainin-AM2 demonstrates potent in vitro insulinotropic effects that are mediated largely via the beta cell K-ATP-dependent pathway [23]. However, data on sub-chronic and long-term in vivo anti-diabetic effects of magainin-AM2 and other amphibian skin peptides are generally lacking.

Prolonged feeding of a high fat diet has been shown to induce characteristic features of obesity and type 2 diabetes in mice [31,33]. Consequently, these animals, which exhibit obesity, hyperglycemia and insulin resistance, have become a useful model for the investigation of anti-diabetic effects of new therapeutic agents, including amphibian skin peptides [30,32,34]. In the present study, twice-daily administration of magainin-AM2 for 15 days in high-fat fed mice produced no visible adverse effects and resulted in significantly improved glucose tolerance. The peptide did not affect the feeding, ruling out the possibility that the improved glycemic status was simply a consequence of nausea and decreased food intake. This contrasts with a number of agents where appetite suppression results in weight loss, including exendin-4, obestatin, pancreatic polypeptide, amylin, CCK, peptide YY, oxyntomodulin and GLP-1 [35,36]. In the present study, total body weight was not changed by magainin-AM2 but body fat was significantly decreased which can be expected to contribute to improvements in blood glucose control. Defective insulin secretion and islet dysfunction is a major consequence of consumption of a high-fat diet in mice [33]. In this study, high-fat fed mice showed a marked reduction in the insulin secretory responses of islets to a range of agents including glucose, GLP-1, GIP, L-alanine, L-arginine and membrane depolarization with KCl. Treatment with magainin-AM2 improved insulin secretion and glucose tolerance, the differential insulin-secretory response following oral versus i.p. glucose administration and extrapancreatic insulin-sensitivity. The marked potentiation of the incretin effect in magainin-AM2 treated mice might indicate a positive effect on secretion of insulinotropic gut hormones. In this respect, it is worthwhile to note that recent studies have shown positive in vitro effects of magainin-AM2 on GLP-1 secretion [22]. Further, we cannot rule out a positive effect of magainin-AM2 on inflammation of adipose tissues to improve insulin sensitivity. Studies have also shown that prolonged feeding on a high fat diet could lead to endoplasmic reticulum stress and beta apoptosis [37], leading to reduced pancreatic

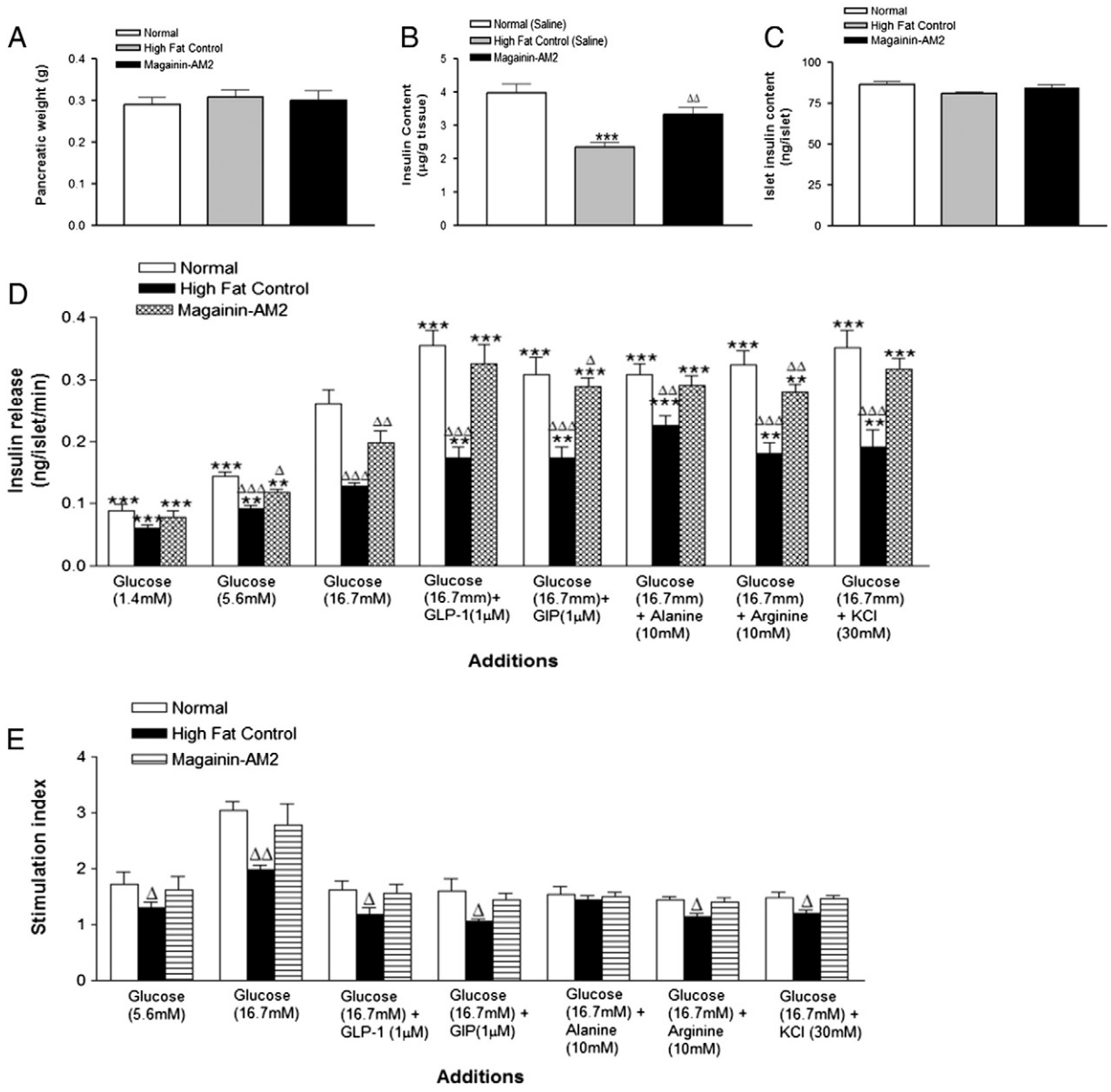

Fig. 7. Effects of magainin-AM2 on pancreatic weight, pancreatic insulin content and insulin-secretory responses of isolated islets in normal and high-fat fed mice treated with saline or magainin-AM2 (75 nmol/kg body weight) for 15 days. Values are means ± SEM for 8 mice. For B, $^{***}P < 0.001$ compared with saline treated lean (normal) mice, $^{\Delta\Delta}P < 0.01$compared with high-fat fed control mice. For D, $^{*}P < 0.05$,$^{**}P < 0.01$, $^{***}P < 0.001$ compared with the response of islets isolated from each group of mice at 16.7 mM glucose; $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$, $^{\Delta\Delta\Delta}P < 0.001$ compared with the response of islets isolated from mice fed standard diet (normal) to each secretagogue or glucose concentration. For E, stimulation index refers to fold-increase in insulin secretion from 1.4 mM glucose to 16.7 mM glucose or from 16.7 mM glucose to insulin secretion observed in the presence of each secretagogue in the presence of 16.7 mM glucose. $^{\Delta}P < 0.05$, $^{\Delta\Delta}P < 0.01$ compared the stimulation index of normal mice.

insulin content as observed in this study. Therefore, the observed increase in beta cell function and pancreatic insulin content in magainin-AM2-treated mice suggests that the peptide is able to reverse abnormalities induced by high-fat feeding. Further investigations are needed to examine underlying mechanisms.

Oxygen consumption and CO_2 production were measured to assess effects of high-fat diet and magainin-AM2 on whole-body energy metabolism and fuel selection (adjudged by RER). Consistent with previous studies [25], prolonged consumption of a high-fat diet resulted in elevated whole-body energy metabolism while fuel selection was unchanged. These parameters were not altered by treatment with magainin-AM2. Energy expenditure, on the other hand, was not affected by feeding a high-fat diet or treatment with magainin-AM2. A recent study with pioglitazone, an insulin-sensitizing agent that acts as a peroxisome proliferator-activated receptor-γ (PPARγ) agonist [38], resulted in a reduction of whole-body metabolism accompanied by unaltered fuel selection in rats, suggesting a mechanism that involves reduction in beta-cell metabolism in vivo. Thus, PPARγ does not appear to be a target for magainin-AM2.

An increasing body of evidence indicates that skeletal muscle fat deposition arising from chronic feeding of high-fat diets may interfere with normal insulin-mediated signaling and alter cellular, as well as whole-body glucose metabolism [39]. This is consistent with the clearly manifested features of obesity and insulin resistance observed in this study and other studies involving mice maintained chronically on high-fat diets [25]. Although lean mass and body weight in this study were not affected by magainin-AM2, the observed reduction in fat deposition may play an important role in the improved insulin-sensitivity exhibited by treated mice. Moreover, small positive effects on bone mineral content and bone area observed in this study may

have compensated for the decline in fat mass, resulting in no change in total body weight. In conclusion, the present study has revealed significant beneficial metabolic effects of magainin-AM2 in mice with diet-induced obesity-diabetes. In addition to improving glucose tolerance and glucose stimulated insulin secretion, the peptide enhanced pancreatic beta cell function as well as insulin sensitivity. These observations encourage further investigations of the antidiabetic potential of magainin-AM2.

Acknowledgement

Funding for this study was provided by a project grant from Diabetes UK (Project Number: 12/0004457) and award of a University of Ulster Vice Chancellor's Research Studentship to DKS.

References

[1] T.L. Pukala, J.H. Bowie, V.M. Maselli, I.F. Musgrave, M.J. Tyler, Host-defence peptides from the glandular secretions of amphibians: structure and activity, Nat. Prod. Rep. 23 (2006) 368–393.
[2] J.M. Conlon, M. Mechkarska, M.L. Luki, P.R. Flatt, Potential therapeutic applications of multifunctional host-defense peptides from frog skin as anti-cancer, anti-viral, immunomodulatory, and anti-diabetic agents, Peptides 57 (2014) 67–77.
[3] J.M. Conlon, G.J. Power, Y.H.A. Abdel-Wahab, P.R. Flatt, H. Jiansheng, L. Coquet, J. Leprince, T. Jouenne, H. Vaudry, A potent, non-toxic insulin-releasing peptide isolated from an extract of the skin of the Asian frog, *Hylarana guntheri* (*Anura*:*Ranidae*), Regul. Pept. 151 (2008) 153–159.
[4] Y.H.A. Abdel-Wahab, S. Patterson, P.R. Flatt, J.M. Conlon, Brevinin-2-related peptide and its [D4K] analogue stimulate insulin release in vitro and improve glucose tolerance in mice fed a high fat diet, Horm. Metab. Res. 42 (2010) 652–656.
[5] O.O. Ojo, P.R. Flatt, Y.H.A. Abdel-Wahab, J.M. Conlon, Insulin-releasing peptides, Handbook of Biologically Active Peptides, Elsevier, Cambridge, 2013, pp. 364–370.
[6] D. Srinivasan, O.O. Ojo, Y.H.A. Abdel-Wahab, P.R. Flatt, L. Guilhaudis, J.M. Conlon, Insulin-releasing and cytotoxic properties of the frog skin peptide, tigerinin-1R: a structure–activity study, Peptides 55 (2014) 23–31.
[7] A.D. Mooradian, J.E. Thurman, Drug therapy of postprandial hyperglycaemia, Drugs 57 (1999) 19–29.
[8] S. Manning, A. Pucci, N. Finer, Pharmacotherapy for obesity: novel agents and paradigms, Ther. Adv. Chronic Dis. 5 (2014) 135–148.
[9] R. Padiya, T.N. Khatua, P.K. Bagul, M. Kuncha, S.K. Banerjee, Garlic improves insulin sensitivity and associated metabolic syndromes in fructose fed rats, Nutr. Metab. 8 (2011) 53.
[10] A.M. Vinagre, E.F. Collares, Evidence for the involvement of peripheral β-adrenoceptors in delayed liquid gastric emptyinginduced by dipyrone, 4-aminoantipyrine, and antipyrine in rats, Braz. J. Med. Biol. Res. 46 (2013) 735–738.
[11] Y. Yamashita, M. Okabe, M. Natsume, H. Ashida, Cinnamtannin A2, a tetrameric procyanidin, increases GLP-1 and insulin secretion in mice, Biosci. Biotechnol. Biochem. 77 (2013) 888–891.
[12] M. Tokuda, T. Katsuno, F. Ochi, K. Miyakoshi, Y. Kusunoki, K. Murai, M. Miuchi, T. Hamaguchi, J. Miyagawa, M. Namba, Effects of exenatide on metabolic parameters/control in obese Japanese patients with type 2 diabetes, Endocr. J. 61 (2014) 365–372.
[13] J.M. Conlon, N. Al-Ghaferi, E. Ahmed, M.A. Meetani, J. Leprince, P.F. Nielsen, Orthologs of magainin, PGLa, procaerulein-derived, and proxenopsin-derived peptides from skin secretions of the octoploid frog *Xenopus amieti* (Pipidae), Peptides 31 (2010) 989–994.
[14] M. Zasloff, B. Martin, H.C. Chen, Antimicrobial activity of synthetic magainin peptides and several analogues, Proc. Natl. Acad. Sci. U. S. A. 85 (1998) 910–913.
[15] M. Cannon, Antimicrobial peptides: a family of wound healers, Nature 328 (1987) 478.
[16] O. Cirioni, A. Giacometti, R. Ghiselli, F. Mocchegiani, A. Fineo, F. Orlando, M.S. Del Prete, M. Rocchi, V. Saba, G. Scalise, Single-dose intraperitoneal magainins improve survival in a gram-negative-pathogen septic shock rat model, Antimicrob. Agents Chemother. 46 (2002) 101–104.
[17] J.M. Conlon, M. Mechkarska, Host-defense peptides with therapeutic potential from skin secretions of frogs from the family Pipidae, Pharmaceuticals 7 (2014) 58–77.
[18] R.W. Gwadz, D. Kaslow, J.Y. Lee, W.L. Maloy, M. Zasloff, L.H. Miller, Effects of magainins and cecropins on the sporogonic development of malaria parasites in mosquitoes, Infect. Immun. 57 (1989) 2628–2633.
[19] L. Jacob, M. Zasloff, Potential therapeutic applications of magainins and other antimicrobial agents of animal origin, CIBA Found. Symp. 186 (1994) 197–223.
[20] A. Giacometti, R. Ghiselli, O. Cirioni, F. Mocchegiani, G. D'Amato, F. Orlando, V. Sisti, W. Kamysz, C. Silvestri, P. Naldoski, J. Lukasiak, V. Saba, G. Scalise, Therapeutic efficacy of the magainin analogue MSI-78 in different intra-abdominal sepsis rat models, J. Antimicrob. Chemother. 54 (2004) 654–660.
[21] A. de Waal, A.V. Gomes, A. Mensink, J.A. Grootegoed, H.V. Westerhoff, Magainins affect respiratory control, membrane potential, and motility of hamster protozoa, FEBS Lett. 293 (1991) 219–233.
[22] O.O. Ojo, J.M. Conlon, P.R. Flatt, Y.H.A. Abdel-Wahab, Frog skin peptides (tigerinin-1R, magainin-AM1, AM2, CPF-AM1 and PGla-AM1) stimulate secretion of glucagon-like peptide 1 (GLP-1) by GLUTag cells, Biochem. Biophys. Res. Commun. 431 (2013) 14–18.
[23] O.O. Ojo, D.K. Srinivasan, O.O. Owolabi, P.R. Flatt, Y.H.A. Abdel-Wahab, Magainin-related peptides stimulate insulin-release and improve glucose tolerance in high fat fed mice, 2014. (submitted for publication).
[24] M.A.C. Martin, V.A. Gault, S. McClean, P.R. Flatt, N. Irwin, Degradation, insulin secretion, glucose-lowering and GIP additive actions of palmitate-derivatised analogues of xenin-25, Biochem. Pharmacol. 84 (2012) 312–319.
[25] V.A. Gault, D.W. Porter, N. Irwin, P.R. Flatt, Comparison of sub-chronic metabolic effects of stable forms of naturally occurring GIP(1–30) and GIP(1–42) in high-fat fed mice, J. Endocrinol. 208 (2011) 265–271.
[26] F.P. O'Harte, M.H. Mooney, C.M. Kelly, A.M. McKillop, P.R. Flatt, Degradation and glycemic effects of His(7)-glucitol glucagon-like peptide-1(7–36)amide in obese diabetic ob/ob mice, Regul. Pept. 96 (2001) 95–104.
[27] P.E. Lacy, M. Kostinovsky, Method for the isolation of intact islets of Langerhans from the rat pancreas, Diabetes 16 (1967) 35–39.
[28] M. Gotoh, T. Maki, T. Kiyoizumi, S. Satomi, A.P. Monaco, An improved method for isolation of mouse pancreatic islets, Transplantation 40 (1985) 437–438.
[29] P.R. Flatt, C.J. Bailey, Abnormal plasma glucose and insulin responses in heterozygous lean (ob/+) mice, Diabetologia 20 (1981) 573–577.
[30] O.O. Ojo, Y.H.A. Abdel-Wahab, P.R. Flatt, M. Mechkarska, J.M. Conlon, Tigerinin-1R: a potent, non-toxic insulin-releasing peptide isolated from the skin of the Asian frog, *Hoplobatrachus rugulosus*, Diabetes Obes. Metab. 13 (2011) 1114–1122.
[31] V.A. Gault, W.D. Porter, P.R. Flatt, C. Holscher, Actions of exendin-4 therapy on cognitive function and hippocampal synaptic plasticity in mice fed a high-fat diet, Int. J. Obes. 34 (2010) 1341–1344.
[32] N. Irwin, P.L. McClean, K. Hunter, P.R. Flatt, Metabolic effects of sustained activation of the GLP-1 receptor alone and in combination with background GIP receptor antagonism in high fat-fed mice, Diabetes Obes. Metab. 11 (2009) 603–610.
[33] M.S. Winzell, B. Ahre'n, The high-fat diet-fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes, Diabetes 53 (2004) S215–S219.
[34] Y.H.A. Abdel-Wahab, G.J. Power, P.R. Flatt, D.C. Woodhams, L.A. Rollins-Smith, J.M. Conlon, A peptide of the phylloseptin family from the skin of the frog *Hylomantis lemur* (Phyllomedusinae) with potent in vitro and in vivo insulin-releasing activity, Peptides 29 (2008) 2136–2143.
[35] J.C. Halford, Pharmacology of appetite suppression: implication for the treatment of obesity, Curr. Drug Targets 2 (2001) 353–370.
[36] T.H. Moran, Gut peptide signalling in the controls of food intake, Obesity 14 (2006) 250S–253S.
[37] I. Mathijs, D.A. Da Cunha, E. Himpe, L. Ladriere, N. Chellan, C.R. Roux, E. Joubert, C. Muller, M. Cnop, J. Louw, L. Bouwens, Phenylpropenoic acid glucoside augments pancreatic beta cell mass in high-fat diet-fed mice and protects beta cells from ER stress-induced apoptosis, Mol. Nutr. Food Res. (2014) 1–11.
[38] J. Lamontagne, E. Jalbert-Arsenault, E. Pepin, M. Peyot, N.B. Ruderman, C.J. Nolan, E. Joly, S.R.M. Madiraju, V. Poitout, M. Prentki, Pioglitazone acutely reduces energy metabolism and insulin secretion in rats, Diabetes 62 (2013) 2122–2129.
[39] M.P. Corcoran, S. Lamon-Fava, R.A. Fielding, Skeletal muscle lipid deposition and insulin resistance: effect of dietary fatty acids and exercise, Am. J. Clin. Nutr. 85 (2007) 662–677.