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Antidiabetic actions of GPR55 agonist Abn-CBD and sitagliptin in obese-diabetic high fat fed mice.

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1.0 Abstract

GPR55 has been recognized as a novel anti-diabetic target exerting positive effects on beta cell function and mass. This study evaluated the metabolic actions and therapeutic efficacy of GPR55 agonist abnormal cannabidiol (Abn-CBD) administered alone and in combination with sitagliptin in diet-induced obese-diabetic mice.

Chronic effects of 21-day oral administration of Abn-CBD (0.1μmol/kg BW) monotherapy and in combination with sitagliptin (50mg/kg BW) were assessed in obese-diabetic HFF mice (n=8). Assessments of plasma glucose, circulating insulin, DPP-IV activity, CRP, amylase, lipids, body weight and food intake were undertaken. Glucose tolerance, insulin sensitivity, DEXA scanning and islet morphology analysis were performed at 21-days.

Sitagliptin, Abn-CBD alone and in combination with sitagliptin attenuated plasma glucose by 37-53% ($p<0.01$ – $p<0.001$) and enhanced circulating insulin concentrations by 23-31% ($p<0.001$). Abn-CBD alone and with sitagliptin reduced bodyweight by 9-10% ($p<0.05$). After 21-days, Abn-CBD in combination with sitagliptin (44%; $p<0.01$) improved glucose tolerance, whilst enhancing insulin sensitivity by 79% ($p<0.01$). Abn-CBD increased islet area (86%; $p<0.05$), beta cell mass ($p<0.05$) and beta cell proliferation (164%; $p<0.001$), whilst in combination with sitagliptin islet area was decreased (50%; $p<0.01$). Abn-CBD alone, in combination with sitagliptin or sitagliptin alone decreased triglycerides by 34-65% ($p<0.001$) and total cholesterol concentrations by 15-25% ($p<0.001$). In addition, Abn-CBD in combination with sitagliptin reduced fat mass by 19% ($p<0.05$) and reduced CRP concentrations (39%; $p<0.05$).

These findings advocate Abn-CBD monotherapy and in combination with sitagliptin as a novel and effective approach for bodyweight control and the treatment of glucose intolerance and dyslipidaemia in type-2-diabetes.

Abbreviations

Abn-CBD	Abnormal cannabidiol
AOC	Area over the curve
AUC	Area under the curve
BMI	Body mass index
BSA	Bovine serum albumin
BW	Body weight
CB1	Cannabinoid receptor 1
CB2	Cannabinoid receptor 2
CRP	C-reactive protein
CVD	Cardiovascular disease
DAPI	4′,6-diamidino-2-phenylindole
DEXA	Dual-energy X-ray absorptiometry
DPP-IV	Dipeptidyl Peptidase IV
ECS	Endocannabinoid system
GIP	Glucose-dependent insulinotropic polypeptide
GLP-1	Glucagon-like peptide-1
GPR55	G-protein coupled receptor 55
GSIS	Glucose stimulated insulin secretion
HDL	High density lipoprotein
HFF	High fat fed
LDL	Low density lipoprotein
LPI	Lysophosphatidylinositol
OEA	Oleoylethanolamide
PBS	Phosphate-buffered saline
PEA	Palmitoylethanolamide

PFA Paraformaldehyde

2.0 Introduction

The endocannabinoid system (ECS) has been shown to exhibit a wide range of physiological functions following activation with small lipid-based retrograde neurotransmitter molecules (Reggio 2010). The ECS is responsible for the pharmacological effects of cannabis, whilst also mediating effects on appetite, pain-sensation, memory, mood and energy metabolism (Manzanares *et al*. 2006, McKillop *et al*. 2013, Donvito *et al.* 2018). The physiological functions of the CB1 and CB2 cannabinoid receptors have been extensively reported, with functionality primarily associated within the brain, central and peripheral nervous systems (Svizenska *et al*. 2018, Anil *et al.* 2022). G-protein coupled receptor 55 (GPR55) has been identified as a novel endocannabinoid receptor and has been hypothesized to be responsible for the non CB1/CB2 physiological effects of cannabinoid ligands (Morales & Reggio 2017, Akimov *et al.* 2021).

GPR55 was first identified and cloned in 1999 (Sawzdargo *et al*. 1999). Later studies revealed GPR55 to be a cannabinoid receptor due to similar amino acid sequence identity in the binding sites of the receptor (Baker *et al*. 2006). Whilst GPR55 has been shown to mediate a range of beneficial effects throughout the body, the entire physiological function of GPR55 is not fully understood, with GPR55 knockout mice displaying no specific phenotype (Johns *et al*. 2007, Carey *et al.* 2017, Morales & Reggio 2017, Janecki *et al.* 2022). Research has intensified on the beneficial actions of the receptor in the regulation of energy metabolism and its potential as a novel pharmacological target for metabolic diseases (McKillop *et al*. 2013, McKillop *et al*. 2016, McCloskey *et al.* 2020).

Activation of GPR55 is mediated by a range of natural and synthetic ligands, including endocannabinoids, phytocannabinoid plant components and long chain fatty acid molecules (McKillop

et al. 2013, Tudurí *et al*. 2017). Lysophosphatidylinositol (LPI) has been generally accepted as the putative endogenous ligand of GPR55, however, studies have also demonstrated LPI to act through GPR55-independent mechanisms, particularly within pancreatic islets (Tudurí *et al*. 2017, Liu *et al*. 2016, Calvillo-Robledo *et al.* 2022). Interestingly, increased circulating concentrations of LPI are observed in obesity and type 2 diabetes with direct correlation to BMI and pro-inflammatory cytokine concentrations in the bloodstream (Moreno-Navarrete *et al*. 2012, Kurano *et al.* 2021). Furthermore, increased plasma LPI concentrations have also been shown to be a prognostic indicator of cardiac arrest and ovarian cancer (Arifin & Falasca 2016). Due to the undefined specificity of LPI and its correlation with poor prognosis of obesity and inflammatory related diseases, studies have favoured LPI as a prognostic biomarker rather than a therapeutic agent (Kim *et al*. 2017, Calvillo-Robledo *et al.* 2022).

GPR55 is expressed widely throughout the body, with abundant expression in the brain, intestine, osteoblasts and endocrine-pancreas (Sawzdargo *et al*. 1999, Ryberg *et al*. 2007, McKillop *et al*. 2013). Recent results from the genome wide association study (GWAS) revealed that a variant of the GPR55 gene displayed the phenotype of type 2 diabetes and coronary artery calcification (Divers *et al*. 2017). The variant displayed a single nucleotide polymorphism (SNP) on an intronic region of the GPR55 gene at chromosome 2q37.1 (Divers *et al*. 2017).

Research is now heavily investigating the therapeutic potential of GPR55 in the treatment of type 2 diabetes and other obesity related diseases (Tudurí *et al*. 2017, Xiang *et al.* 2022, Wang *et al.* 2022). GPR55 has been shown to demonstrate insulinotropic actions in clonal pancreatic beta cells upon activation with a range of endogenous (OEA, PEA) and synthetic (abnormal cannabidiol, AM251) agonists (McKillop *et al*. 2013). In the same study, antagonising the receptor revealed the specificity of several GPR55 ligands, with abnormal cannabidiol (Abn-CBD) potentiating potent and selective GPR55 agonism (McKillop *et al*. 2013). Chronic administration of Abn-CBD was previously reported

to induce glucose lowering and insulinotropic effects in multiple low dose streptozotocin mice, with further improvements of insulin sensitivity and lipid profile also being observed (McKillop *et al*. 2016).

GPR55 is abundantly expressed in both rodent and human pancreatic islet cells (McKillop *et al*. 2013, Liu *et al*. 2016). Interestingly, GPR55 expression was demonstrated to be solely expressed in beta cells in rodent islets, however, one study has shown moderate GPR55 expression in alpha cells of human islets (Liu *et al*. 2016). Studies using a clonal GPR55 knockout beta cell line, developed using innovative CRISPR/Cas9 gene editing, revealed that Abn-CBD induced insulin release is predominately GPR55-dependent, with potential synergistic activity with other cannabinoid receptors such as GPR18 (McCloskey *et al.* 2020).

Given the known insulinotropic actions of Abn-CBD, the present study evaluated the glucoregulatory and anti-obesity effects of Abn-CBD in high fat fed (HFF) induced obese diabetic mice. The glucoregulatory effects exerted by Abn-CBD were previously shown to partly mediate through incretin receptors, with positive effects towards glucose tolerance attenuated in GIP receptor knockout mice (McKillop *et al.* 2016). As GPR55 is also expressed in incretin-releasing enteroendocrine cells, combination therapy was also explored with the DPP-IV inhibitor sitagliptin which acts to prolong the circulating half-life of GLP-1 and GIP hormones. Sitagliptin is an orally active, potent, selective DPP-IV inhibitor. DPP-IV degrades and inactivates incretin hormones GLP-1 and GIP, which have beneficial actions towards beta cell function and glucose stimulated insulin secretion (Herman *et al.* 2005). By inhibiting DPP-IV, sitagliptin prolongs circulating active incretin concentrations and thereby improves the regulation of glucose homeostasis (Herman *et al.* 2005, Karasik *et al.* 2008). Effects of 21-day oral administration were examined on bodyweight, food intake, non-fasting plasma glucose, insulin secretion, insulin sensitivity, lipid profile, pancreatic islet morphology/hormone content, amylase activity, C-reactive protein and DEXA analysis.

3.0 Materials and Methods

3.1 Materials

Abnormal cannabidiol was purchased from Tocris Bioscience (Bristol, UK). Sitagliptin phosphate monohydrate was obtained from Apexbio Technology LLC (Texas, USA). Guinea pig anti-glucagon Molecular Probes (Life Technologies Ltd, Paisley, UK), guinea pig anti-insulin Molecular Probes (Life Technologies Ltd, Paisley, UK) and rabbit anti-Ki67 from Abcam (Cambridge, UK). Multi species GLP-1 total ELISA and rat/mouse GIP total ELISA kits were purchased from Merck Millipore (Watford, UK). Mouse C-reactive Protein ELISA and amylase assay kit (colorimetric) were purchased from Abcam (Cambridge, UK). 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), N, N-Dimethyl-formamide (DMF), Gly-Pro-7-amido-4-methylcoumarin (Gly-pro AMC), 7-amino-4-methylcoumarin (AMC) and acetic acid were purchased from Sigma-Aldrich (Poole, Dorset, UK).

3.2 Animals

All animal experiments were carried out in accordance with the UK Animal (Scientific Procedures) Act 1986. Studies were approved by local Ulster Animal Welfare and Ethical Review Body (AWERB) committee. Male Swiss TO mice (8 wk) were purchased from Envigo (Huntingdon, UK) derived from a nucleus colony originally sourced from the National Institute of Health (Maryland, USA). All mice were individually housed in an air-conditioned room at 22 ± 2 °C with a 12-h light: 12-h dark cycle. Drinking water was supplied *ad libitum*. To induce obesity-diabetes, animals were maintained on a high fat diet (45% fat, 20% protein, 35% carbohydrate; percent of total energy 26.15 kJ/g; Dietex International Ltd., Witham, UK) from 8 weeks of age for a total of 4 months to evoke dietary-induced obesity-diabetes (DIO). Another group of mice was maintained on standard rodent diet (10% fat, 30% protein, 60% carbohydrate; percent of total energy 12.99 kJ/g, Trouw Nutrition, Cheshire, UK) and used as a model of normal controls. Similar high-fat diets, containing a large percentage of energy from

fat, are used routinely in obesity-diabetes research (West *et al*., 1995; Black *et al*., 1998; Buettner *et al*., 2007). Swiss TO mice on a high fat diet exhibited increased body weight, hyperglycaemia, insulin resistance, hyperinsuinaemia, and impaired glucose tolerance when compared to mice on the standard rodent maintenance diet. This form of diet-induced diabetes in mice closely resembles a high fat western diet and several important clinical features identified in human obesity.

3.3 Chronic administration of sitagliptin, Abn-CBD and Abn-CBD in combination with sitagliptin in HFF-induced diabetic mice

Once daily oral administration of Abn-CBD (0.1μmol/kg bodyweight), sitagliptin (50mg/kg bodyweight), Abn-CBD in combination with sitagliptin (0.1μmol/kg bodyweight; 50mg/kg bodyweight) or saline vehicle (0.9% w/v NaCl) were utilised in a long-term study (21 days) examining their effects on HFF-induced obese-diabetic mice. Food intake, body weight, non-fasted plasma glucose and insulin concentrations were monitored every 2 to 4 days as indicated in the figures. At the end of the study, glucose tolerance (18mmol/kg body weight) and insulin sensitivity (40U/kg body weight) were assessed. Dual energy X-ray absorption (DEXA) scanning was performed on all carcasses, after prior calibration and quality control with the aluminium/lucite phantom (0.069 g/cm2, 12.0% fat) using a PIXImus system (software version 1.4x). No adverse effects were noted with any of the treatment regimens.

3.4 Histology

Pancreatic tissues were removed at 21 days for immunohistochemistry or measurement of pancreatic insulin. Pancreatic tissue was fixed in 4% PFA/PBS and embedded in paraffin wax. Longitudinal pancreatic sections were cut at 8 μm thickness on a microtome (Shandon Finesse 325, Thermo Scientific), collected at 250-μm intervals to account for tissue depth variance, and plated onto poly-l-lysine coated glass slides and dried on a hot plate. After incubation, wax was removed and tissue re-

hydrated in ethanol (100%), ethanol (95%), ethanol (80%) and distilled water for 5 min each. Slides were incubated in 50 mM sodium citrate for 20 min at 90 °C for antigen retrieval. BSA (2.5%) was added to each slide (200 µl) for 45 min. Slides were incubated overnight at 4˚C with guinea pig anti-insulin (1:500) or guinea pig anti-glucagon (1:500). Rabbit anti-Ki67 (1:200) was incubated at 37˚C for 2 hr. After washing in PBS, sections were incubated with anti-guinea pig Alexafluor 488nm, anti-guinea pig Alexafluor 594nm and Anti-Rabbit Alexafluor 594nm (1:400; Molecular Probes (Life Technologies Ltd, Paisley, UK) for 45 min at 37˚C and DAPI nuclear stain for 15 min at 37˚C. Finally, slides were washed in PBS, mounted and analysed using a BX51 Olympus microscope equipped with an Olympus XM10 digital camera. Cell^F imaging software (Olympus Soft Imaging Solutions) was used to analyze islet architectural parameters.

3.5 Biochemical analysis

Blood samples were obtained by cut tip from tail vein of conscious mice at the time points indicated in the figures and plasma was separated by centrifugation at 16,060×g for 3 min at 4 °C. Plasma glucose was measured using an automated glucose oxidase procedure with a Beckman glucose analyser (Beckman-Coulter, High Wycome, UK) and plasma insulin was determined by radioimmunoassay (Flatt and Bailey, 1981). Insulin content of resected pancreatic tissue was also assayed after extraction with acid ethanol (HCl 1.5% (v/v), ethanol 75% (v/v), H_2O 23.5% (v/v)). Total-GLP-1 (Millipore), total-GIP (Millipore) and C-reactive protein (Abcam) were assessed using ELISA as per manufacturers' instructions. Amylase Assay Kit (Abcam) was used to quantify plasma amylase activity as per manufacturers' instructions. DPP-IV activity was evaluated by Gly-Pro-AMC cleavage as previously described (Davis *et al.,* 2010, McCloskey *et al.* 2020). Plasma triacylglycerol and cholesterol levels were measured using a Hitachi Automated Analyser 912 (Mannheim, Germany).

3.6 Statistics

All data was analysed with Prism (v.5.0, GraphPad Software Inc. CA, USA) and expressed as mean ± S.E.M. Results were compared using the Student's t test or one-way ANOVA followed by the Bonferroni post-hoc test as appropriate. Area under the curve (AUC) was calculated using trapezoidal rule with baseline correction. $p<0.05$ was considered to be statistically significant.

4.0 Results

4.1 Effects of administration of Abn-CBD alone and with Sitagliptin on body weight, energy intake, non-fasting plasma glucose, insulin, pancreatic insulin content, DPP-IV activity and circulating incretin concentrations.

Once daily oral administration of Abn-CBD alone, in combination with sitagliptin or sitagliptin alone had no significant effect on bodyweight in HFF mice (Fig. 1A). When assessed by percentage weight change, Abn-CBD alone and in combination with sitagliptin resulted in a 9-10% ($p<0.05$-$p<0.01$) decrease in bodyweight (Fig. 1B). No significant changes in cumulative food intake were identified with any of the treatments (Fig. 1C). However, cumulative energy intake of HFF-mice was greater than lean controls from days 3-21 ($p<0.01$-$p<0.001$) consistent with increased body weight (Fig. 1A, D).

HFF-induced hyperglycaemia was reduced by sitagliptin (35%; $p<0.01$), Abn-CBD alone (53%; $p<0.001$) and Abn-CBD in combination with sitagliptin (53%; $p<0.001$) during the 21-day treatment period (Fig. 2A, B). Abn-CBD administered alone surpassed the glucose lowering capabilities of sitagliptin by 18% ($p<0.05$). Corresponding increases in plasma insulin were observed with sitagliptin (31%; $p<0.001$), Abn-CBD alone (23%; $p<0.001$) and Abn-CBD in combination with sitagliptin (31%; $p<0.001$) (Fig. 2C, D). Terminal pancreatic tissue analysis demonstrated that insulin content was reduced by 72% ($p<0.01$) in HFF-induced diabetic mice. Treatment with sitagliptin alone increased insulin content by 90% ($p<0.05$), whilst Abn-CBD alone and in combination with sitagliptin had no effect (Fig. 2E).

Administration of Abn-CBD alone and in combination with sitagliptin enhanced circulating non-fasting GLP-1 concentrations by 36% ($p<0.05$) and 43% ($p<0.01$), respectively (Fig. 3A, B). No effects on circulating GIP were observed (Fig. 3C, D). DPP-IV activity was elevated by 72% ($p<0.01$) in HFF-induced diabetic mice compared with lean mice, whilst administration of sitagliptin alone and in combination with Abn-CBD reduced DPP-IV activity by 26-35% ($p<0.05$) in HFF mice (Fig. 3E, F).

4.2 Effects of administration of Abn-CBD alone and with Sitagliptin on glucose tolerance and insulin sensitivity.

Administration of Abn-CBD in combination with sitagliptin or sitagliptin alone for 21 days improved oral glucose tolerance ($p<0.05$-$p<0.001$) (Fig. 4A). AUC data reveal that the overall improvement was 32-41% ($p<0.01$) (Fig. 4B). Corresponding insulin secretory responses were observed with Abn-CBD plus sitagliptin ($p<0.001$) at 15 min, whilst both sitagliptin and Abn-CBD alone had no obvious effect on insulin release (Fig. 4C, D). Indeed, the Abn-CBD/sitagliptin combination enhanced the insulin secretory response by 42% ($p<0.01$) (Fig. 4D).

Administration of Abn-CBD in combination with sitagliptin for 21 days increased the blood glucose lowering effect of exogenous insulin ($p<0.05$-$p<0.01$) compared to non-treated HFF mice (Fig. 5A). Area over the curve (AOC) data revealed a 79% enhancement of glucose lowering capabilities ($p<0.01$), whilst sitagliptin alone had no effect (Fig. 5B).

4.3 Effects of administration of Abn-CBD alone and with Sitagliptin on DEXA measurements, lipid profile, plasma C-reactive protein and amylase activity.

Administration of Abn-CBD alone, in combination with sitagliptin or sitagliptin alone for 21-days decreased fat mass by 11-19% ($p<0.05$) (Fig. 6A). No effects at all on lean mass were observed (Fig. 6B). DEXA analysis revealed that HFF mice displayed reduced total bone mineral content ($p<0.05$; Fig. 6C), whilst Abn-CBD alone and in combination with sitagliptin had a restorative effect ($p<0.01$; Fig. 6C). Total bone mineral density was the same in all groups (Fig. 6D).

Lipid profile analysis at 21 days revealed that HFF mice displayed increased triglycerides ($p<0.001$), total cholesterol ($p<0.001$) and LDL cholesterol ($p<0.001$) concentrations, with no change in HDL cholesterol (Fig. 7A). Administration of Abn-CBD alone, in combination with sitagliptin or sitagliptin

alone decreased triglycerides by 34-65% ($p<0.001$) and total cholesterol concentrations by 15-25% ($p<0.001$) (Fig. 7A, B). Sitagliptin treated HFF mice demonstrated reduced HDL cholesterol (5%; $p<0.05$) and increased LDL cholesterol (17%; $p<0.01$). Abn-CBD had no effect on HDL cholesterol, however, reduced LDL cholesterol by 15% ($p<0.01$). Abn-CBD in combination with sitagliptin deceased HDL cholesterol by 7% ($p<0.05$), whilst having no effect on LDL cholesterol (Fig. 7C, D).

Mice subjected to a HFF diet displayed 59% elevated plasma CRP concentrations ($p<0.05$), compared to lean controls. Administration of Abn-CBD in combination with sitagliptin reduced plasma CRP by 39% ($p<0.05$), whereas when administered alone Abn-CBD and sitagliptin exerted no effect (Fig. 8A). HFF mice demonstrated an 22% increase in plasma amylase activity ($p<0.01$), whilst Abn-CBD alone, in combination with sitagliptin or sitagliptin alone had no effect (Fig. 8B).

4.4 Effects of administration of Abn-CBD alone and with Sitagliptin on islet morphology.

Immunohistochemistry was utilised to examine islet morphology and staining for insulin and glucagon in lean, HFF and treated mouse pancreatic islets (Fig. 9 A-E). HFF mice displayed a 123% increase in islet size ($p<0.001$). Treatment with Abn-CBD alone increased islet size further by 86% ($p<0.05$) whilst in combination with sitagliptin islet size was paradoxically decreased by 50% ($p<0.01$) (Fig. 9F). HFF mice also demonstrated other islet abnormalities such as increased beta cell mass ($p<0.05$; Fig. 9H). Administration of Abn-CBD alone increased both beta ($p<0.001$; Fig. 9H) and alpha cell mass ($p<0.001$; Fig. 9J). Treatment did not affect percentage beta and alpha cell area (Fig. 9 G, I). Finally, it was demonstrated that the driver behind the positive effects of administration of Abn-CBD on islet architecture was increased beta cell proliferation which was enhanced by 164% ($p<0.001$), as assessed by Ki-67 immunohistochemistry (Fig. 10I).

5.0 Discussion

It has been estimated that ~35% current FDA approved drugs work through GPCRs, with characterization and specific targeting of novel GPCRs revealing a promising approach for future drug discovery (Hauser *et al*. 2018, Cornwell & Feigin 2020). Research has intensified on the potential pharmacological capabilities of novel endocannabinoid receptors in metabolic control, with the CB1 inverse agonist rimonabant once used as an anti-obesity drug (Sam *et al*. 2011). The novel endocannabinoid receptor GPR55 has been identified as a potential therapeutic target for the treatment of obesity and type 2 diabetes (McKillop *et al*. 2016, Tuduri *et al*. 2017, McCloskey *et al.* 2020). However, further investigations are warranted to fully reveal the functionality of GPR55 activation and to evaluate its potential as a novel therapeutic target.

Oral administration of Abn-CBD alone or in combination with sitagliptin for 21 days greatly improved glycaemic control and dyslipidaemia in HFF mice and surpassed the capabilities of therapy with the DPP-IV inhibitor alone. The combination with sitagliptin demonstrated a wide range of anti-diabetic effects, including improvements in glycaemic control, GSIS, bodyweight, insulin sensitivity, dyslipidaemia and islet architecture. The combination also reduced bodyweight by 9% in the obese animal model, whilst terminal DEXA analysis also revealed reduced fat mass with all treatment groups. Previously, Abn-CBD was shown to have no effect on bodyweight in multiple low-dose streptozotocin-induced diabetic mice, with streptozotocin reducing bodyweight in all experimental groups compared to the lean control (McKillop *et al.* 2016). This current study utilizes a DIO model that mimics several human metabolic derangements that are observed in obesity and is more appropriate to evaluate effects towards bodyweight (Wang & Liao 2012). Interestingly, the CB1 and CB2 endocannabinoid receptors share structural similarities with GPR55 and have previously shown to regulate bodyweight, thus clearly revealing the pivotal role of the endocannabinoid system in bodyweight management (Bergholm *et al*. 2013, Verty *et al.* 2015). Although GPR55 shares several cannabinoid ligands with

CB1 and CB2, endocannabinoid receptor signaling pathways are not fully elucidated, with GPR55 signal transduction greatly differing from CB1 and CB2 (Yang *et al.* 2016). With respect to glucose homeostasis, Abn-CBD alone and in combination with sitagliptin improved glucose control well beyond the capabilities of sitagliptin alone, clearly demonstrating the therapeutic efficacy of GPR55 activation. The glucose lowering effect was associated with significant changes in plasma insulin concentrations, with sitagliptin based therapies demonstrating the most potent insulinotropic effect. As anticipated, both sitagliptin therapies decreased circulating DPP-IV activity.

Although marked reductions in bodyweight were induced by Abn-CBD alone and in combination with sitagliptin, no obvious effects on cumulative food intake nor cumulative energy intake were observed, thus indicating an effect on energy expenditure which warrants further investigation. Interestingly, inverse agonism of the structurally related CB1 receptor has also been shown to reduce bodyweight in humans (Sam *et al*. 2011). Post 21-day oral administration of sitagliptin and Abn-CBD in combination with sitagliptin improved oral glucose tolerance. Abn-CBD plus sitagliptin demonstrated the greatest insulin secretory response to the glucose challenge, suggesting beneficial effects of Abn-CBD combination therapy on the restoration of beta cell function. In addition, Abn-CBD in combination with sitagliptin improved insulin sensitivity in HFF mice; a novel finding which may be partly driven by lowering of blood glucose and the anti-inflammatory properties associated with GPR55 activation (Stancic *et al*. 2015).

Lipid profile 21-day post treatment demonstrated that Abn-CBD, sitagliptin and Abn-CBD in combination with sitagliptin improved dyslipidaemia of HFF mice with marked reductions in plasma triglycerides and total cholesterol. Sitagliptin alone reduced HDL cholesterol and increased LDL cholesterol. In contrast, Abn-CBD alone attenuated LDL cholesterol and had no effect on HDL cholesterol, corresponding with previous data demonstrating the cardioprotective effects of Abn-CBD in type 2 diabetes (Su & Vo 2007, Matouk *et al*. 2018). In addition, Abn-CBD plus sitagliptin

ameliorated plasma C-reactive protein concentrations; a biomarker that correlates with cardiovascular disease (CVD) risk and cardiovascular health (Clearfield 2005, Bahadursingh *et al*. 2009). This is a significant finding as there is a pressing need for new therapies that counteract the development of CVD in type 2 diabetes, particularly as CVD is the main cause of death among patients with type 2 diabetes (Ma *et al.* 2022). With ongoing deterioration of glycaemic control and the detrimental impact of diabetic complications, new drug development focused on beta cell and in addition cardiovascular health benefits is needed. Effects on obesity and inflammation are promising but there is a need for greater research to understand the protective role of GPR55 in cardiometabolic disease.

As anticipated, examination of islet architecture revealed that HFF mice demonstrated significant islet abnormalities, including increased islet size and reduced percentage beta cell area. Interestingly, Abn-CBD greatly increased islet area, together with further underlying increases of beta and alpha cell mass. Previous studies have demonstrated the proliferative and anti-apoptotic effect of Abn-CBD in beta cells, which is likely to account for the observed effects (Ruz-Maldonado *et al*. 2018). In harmony with this, Ki-67 staining revealed that Abn-CBD significantly increased beta cell proliferation. Although normal islet architecture was restored, Abn-CBD in combination with sitagliptin reduced islet area to non-diabetic islet levels.

Abn-CBD alone increased beta cell mass, whilst Abn-CBD in combination with sitagliptin attenuated this effect. It has been well reported that increased beta cell mass is observed in obesity and insulin resistance, with neogenesis, transdifferentiation, and not proliferation, found to be the mechanisms responsible in insulin resistant and glucose tolerant subjects (Kahn *et al.* 1993, Hanley *et al*. 2010, Yoneda *et al.* 2013, Mezza *et al.* 2014, Chen *et al.* 2017). Findings from this study suggest that reduced insulin demand due to improved beta cell function and increased insulin sensitivity are the causative factors for the reduction in beta cell mass and the restoration of pancreatic islet architecture following treatment of Abn-CBD in combination with sitagliptin. Together with enhanced GSIS after the 21-day

treatment, these findings suggest that Abn-CBD accompanied with DPP-IV inhibition improves beta cell function and promotes islet cell regeneration.

Collectively, the present study demonstrates that oral administration of GPR55 agonist therapy alone or in combination with sitagliptin exerts potent glucoregulatory and anti-obesity properties in HFF diabetic mice. Our analysis demonstrates that acute and chronic administration of Abn-CBD influences glucose homeostasis through enhancement of both insulin and incretin secretion plus the restoration of islet morphology. In addition, long term administration of Abn-CBD in combination with sitagliptin improved bodyweight, dyslipidaemia, insulin sensitivity and circulating CRP. Overall, this study reveals the efficacy of GPR55 as novel therapeutic target for the treatment of type 2 diabetes and obesity related disorders.

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Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author contribution statement

AGMC conducted the investigation, methodology, validation, formal analysis and writing – original draft. MGM and RAL conducted the investigation. PRF involved in the writing- review and editing and supervision. AMMK involved in the conceptualization, formal analysis, supervision writing – review and editing.

6.0 References

Akimov, M. G., Gamisonia, A. M., Dudina, P. V., Gretskaya, N. M., Gaydaryova, A. A., Kuznetsov, A. S., Zinchenko, G. N., & Bezuglov, V. V. (2021). GPR55 Receptor Activation by the N-Acyl Dopamine Family Lipids Induces Apoptosis in Cancer Cells via the Nitric Oxide Synthase (nNOS) Over-Stimulation. *International journal of molecular sciences*, 22(2), 622.

Anil, S. M., Peeri, H., & Koltai, H. (2022). Medical Cannabis Activity Against Inflammation: Active Compounds and Modes of Action. *Frontiers in pharmacology*, 13, 908198.

Arifin, S.A. and Falasca, M. (2016) Lysophosphatidylinositol Signalling and Metabolic Diseases. *Metabolites,* 6(1), 10.3390/metabo6010006.

Bahadursingh, S., Beharry, K., Maharaj, K., Mootoo, C., Sharma, P., Singh, J., Teelucksingh, K. and Tilluckdharry, R. (2009) C-reactive protein: adjunct to cardiovascular risk assessment. *The West Indian Medical Journal,* 58(6), 551-555.

Baker, D., Pryce, G., Davies, W.L. and Hiley, C.R. (2006) In silico patent searching reveals a new cannabinoid receptor. *Trends in Pharmacological Sciences,* 27(1), 1-4.

Bergholm, R., Sevastianova, K., Santos, A., Kotronen, A., Urjansson, M., Hakkarainen, A., Lundbom, J., Tiikkainen, M., Rissanen, A., Lundbom, N. and Yki-Jarvinen, H. (2013) CB(1) blockade-induced weight loss over 48 weeks decreases liver fat in proportion to weight loss in humans. *International Journal of Obesity (2005),* 37(5), 699-703.

Black, B.L., Croom, J., Eisen, E.J., Petro, A.E., Edwards, C.L., Surwit, R.S. (1998). Differential effects of fat and sucrose on body composition in A/J and C57BL/6 mice. *Metabolism* 47, 1354–1359.

Buettner, R., Schölmerich, J., Bollheimer, L.C. (2007). High-fat diets: modeling the metabolic disorders of human obesity in rodents. *Obesity (Silver Spring)* 15, 798–808.

Calvillo-Robledo, A., Cervantes-Villagrana, R. D., Morales, P., & Marichal-Cancino, B. A. (2022). The oncogenic lysophosphatidylinositol (LPI)/GPR55 signaling. *Life sciences*, 301, 120596.

Carey, L. M., Gutierrez, T., Deng, L., Lee, W. H., Mackie, K., & Hohmann, A. G. (2017). Inflammatory and Neuropathic Nociception is Preserved in GPR55 Knockout Mice. *Scientific reports,* 7(1), 944.

Chen, C., Cohrs, C. M., Stertmann, J., Bozsak, R., & Speier, S. (2017). Human beta cell mass and function in diabetes: Recent advances in knowledge and technologies to understand disease pathogenesis. *Molecular metabolism*, 6(9), 943–957.

Clearfield, M.B. (2005) C-reactive protein: a new risk assessment tool for cardiovascular disease. *The Journal of the American Osteopathic Association,* 105(9), 409-416.

Cornwell, A. C., & Feigin, M. E. (2020). Unintended Effects of GPCR-Targeted Drugs on the Cancer Phenotype. *Trends in pharmacological sciences*, 41(12), 1006–1022.

Davis, J.A., Singh, S., Sethi, S., Roy, S., Mittra, S., Rayasam, G. (2010). Nature of action of Sitagliptin, the dipeptidyl peptidase-IV inhibitor in diabetic animals. *Indian J. Pharmacol.* 42, 229–233.

Divers, J., Palmer, N.D., Langefeld, C.D., Brown, W.M., Lu, L., Hicks, P.J., Smith, S.C., Xu, J., Terry, J.G., Register, T.C., Wagenknecht, L.E., Parks, J.S., Ma, L., Chan, G.C., Buxbaum, S.G., Correa, A., Musani, S., Wilson, J.G., Taylor, H.A., Bowden, D.W., Carr, J.J. and Freedman, B.I. (2017) Genome-wide association study of coronary artery calcified atherosclerotic plaque in African Americans with type 2 diabetes. *BMC Genetics,* 18(1), 105-017-0572-9.

Donvito, G., Nass, S.R., Wilkerson, J.L., Curry, Z.A., Schurman, L.D., Kinsey, S.G. and Lichtman, A.H. (2018) The Endogenous Cannabinoid System: A Budding Source of Targets for Treating Inflammatory and Neuropathic Pain. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology,* 43(1), 52-79.

Drucker, D., Easley, C. and Kirkpatrick, P. (2007) Sitagliptin. *Nature Reviews.Drug Discovery,* 6(2), 109-110.

Flatt, P.R., Bailey, C.J. (1981). Abnormal plasma glucose and insulin responses in heterozygouslean (ob/+) mice. *Diabetologia* 20, 573–577.

Hanley, S. C., Austin, E., Assouline-Thomas, B., Kapeluto, J., Blaichman, J., Moosavi, M., Petropavlovskaia, M., & Rosenberg, L. (2010). {beta}-Cell mass dynamics and islet cell plasticity in human type 2 diabetes. *Endocrinology*, 151(4), 1462–1472.

Hauser, A.S., Chavali, S., Masuho, I., Jahn, L.J., Martemyanov, K.A., Gloriam, D.E. and Babu, M.M. (2018) Pharmacogenomics of GPCR Drug Targets. *Cell,* 172(1-2), 41-54.e19.

Herman, G. A., Stevens, C., Van Dyck, K., Bergman, A., Yi, B., De Smet, M., Snyder, K., Hilliard, D., Tanen, M., Tanaka, W., Wang, A. Q., Zeng, W., Musson, D., Winchell, G., Davies, M. J., Ramael, S., Gottesdiener, K. M., & Wagner, J. A. (2005). Pharmacokinetics and pharmacodynamics of sitagliptin, an inhibitor of dipeptidyl peptidase IV, in healthy subjects: results from two randomized, double-blind, placebo-controlled studies with single oral doses. *Clinical pharmacology and therapeutics*, *78*(6), 675–688.

Janecki, M., Graczyk, M., Lewandowska, A. A., & Pawlak, Ł. (2022). Anti-Inflammatory and Antiviral Effects of Cannabinoids in Inhibiting and Preventing SARS-CoV-2 Infection. *International journal of molecular sciences*, 23(8), 4170.

Johns, D.G., Behm, D.J., Walker, D.J., Ao, Z., Shapland, E.M., Daniels, D.A., Riddick, M., Dowell, S., Staton, P.C., Green, P., Shabon, U., Bao, W., Aiyar, N., Yue, T.L., Brown, A.J., Morrison, A.D. and Douglas, S.A. (2007) The novel endocannabinoid receptor GPR55 is activated by atypical cannabinoids but does not mediate their vasodilator effects. *British Journal of Pharmacology,* 152(5), 825-831.

Kahn, S. E., Prigeon, R. L., McCulloch, D. K., Boyko, E. J., Bergman, R. N., Schwartz, M. W., Neifing, J. L., Ward, W. K., Beard, J. C., & Palmer, J. P. (1993). Quantification of the relationship between insulin sensitivity and beta-cell function in human subjects. Evidence for a hyperbolic function. *Diabetes*, 42(11), 1663–1672.

Karasik, A., Aschner, P., Katzeff, H., Davies, M. J., & Stein, P. P. (2008). Sitagliptin, a DPP-4 inhibitor for the treatment of patients with type 2 diabetes: a review of recent clinical trials. *Current medical research and opinion*, 24(2), 489–496.

Kilkenny, C., Browne, W., Cuthill, I.C., Emerson, M., Altman, D.G., (2010) Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol,* 160:1577–1579

Kim, J., Yin, T., Shinozaki, K., Lampe, J.W. and Becker, L.B. (2017) Potential of lysophosphatidylinositol as a prognostic indicator of cardiac arrest using a rat model. *Biomarkers: Biochemical Indicators of Exposure, Response, and Susceptibility to Chemicals,* 22(8), 755-763.

Kurano, M., Kobayashi, T., Sakai, E., Tsukamoto, K., & Yatomi, Y. (2021). Lysophosphatidylinositol, especially albumin-bound form, induces inflammatory cytokines in macrophages. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 35(6), e21673.

Liu, B., Song, S., Ruz-Maldonado, I., Pingitore, A., Huang, G.C., Baker, D., Jones, P.M. and Persaud, S.J. (2016) GPR55-dependent stimulation of insulin secretion from isolated mouse and human islets of Langerhans. *Diabetes, Obesity & Metabolism,* 18(12), 1263-1273.

Ma, C. X., Ma, X. N., Guan, C. H., Li, Y. D., Mauricio, D., & Fu, S. B. (2022). Cardiovascular disease in type 2 diabetes mellitus: progress toward personalized management. *Cardiovascular diabetology*, 21(1), 74.

Manzanares, J., Julian, M. and Carrascosa, A. (2006) Role of the cannabinoid system in pain control and therapeutic implications for the management of acute and chronic pain episodes. *Current Neuropharmacology,* 4(3), 239-257.

Matouk, A.I., Taye, A., El-Moselhy, M.A., Heeba, G.H. and Abdel-Rahman, A.A. (2018) Abnormal cannabidiol confers cardioprotection in diabetic rats independent of glycemic control. *European Journal of Pharmacology,* 820, 256-264.

McCloskey, A. G., Miskelly, M. G., Flatt, P. R., & McKillop, A. M. (2020). Pharmacological potential of novel agonists for FFAR4 on islet and enteroendocrine cell function and glucose homeostasis. *Eur J Pharm Sci,* 142, 105104.

McCloskey, A. G., Miskelly, M. G., Moore, C., Nesbit, M. A., Christie, K. A., Owolabi, A. I., Flatt, P. R., & McKillop, A. M. (2020). CRISPR/Cas9 gene editing demonstrates metabolic importance of GPR55 in the modulation of GIP release and pancreatic beta cell function. *Peptides*, 125, 170251.

McKillop, A.M., Moran, B.M., Abdel-Wahab, Y.H. and Flatt, P.R. (2013) Evaluation of the insulin releasing and antihyperglycaemic activities of GPR55 lipid agonists using clonal beta-cells, isolated pancreatic islets and mice. *British Journal of Pharmacology,* 170(5), 978-990.

McKillop, A.M., Moran, B.M., Abdel-Wahab, Y.H., Gormley, N.M. and Flatt, P.R. (2016) Metabolic effects of orally administered small-molecule agonists of GPR55 and GPR119 in multiple low-dose

streptozotocin-induced diabetic and incretin-receptor-knockout mice. *Diabetologia,* 59(12), 2674-2685.

Mezza T, Muscogiuri G, Sorice GP, Clemente G, Hu J, Pontecorvi A, Holst JJ, Giaccari A, Kulkarni RN. Insulin resistance alters islet morphology in nondiabetic humans. *Diabetes*. 2014 Mar;63(3):994-1007.

Morales, P. and Reggio, P.H. (2017) An Update on Non-CB1, Non-CB2 Cannabinoid Related G-Protein-Coupled Receptors. *Cannabis and Cannabinoid Research,* 2(1), 265-273.

Morales, P., & Reggio, P. H. (2017). An Update on Non-CB1, Non-CB2 Cannabinoid Related G-Protein-Coupled Receptors. *Cannabis and cannabinoid research*, 2(1), 265–273.

Moreno-Navarrete, J.M., Catalan, V., Whyte, L., Diaz-Arteaga, A., Vazquez-Martinez, R., Rotellar, F., Guzman, R., Gomez-Ambrosi, J., Pulido, M.R., Russell, W.R., Imbernon, M., Ross, R.A., Malagon, M.M., Dieguez, C., Fernandez-Real, J.M., Fruhbeck, G. and Nogueiras, R. (2012) The L-alpha-lysophosphatidylinositol/GPR55 system and its potential role in human obesity. *Diabetes,* 61(2), 281-291.

Reggio, P.H. (2010) Endocannabinoid binding to the cannabinoid receptors: what is known and what remains unknown. *Current Medicinal Chemistry,* 17(14), 1468-1486.

Ruz-Maldonado, I., Pingitore, A., Liu, B., Atanes, P., Huang, G.C., Baker, D., Alonso, F.J., Bermudez-Silva, F.J. and Persaud, S.J. (2018) LH-21 and abnormal cannabidiol improve beta-cell function in isolated human and mouse islets through GPR55-dependent and -independent signalling. *Diabetes, Obesity & Metabolism,* 20(4), 930-942.

Ryberg, E., Larsson, N., Sjogren, S., Hjorth, S., Hermansson, N.O., Leonova, J., Elebring, T., Nilsson, K., Drmota, T. and Greasley, P.J. (2007) The orphan receptor GPR55 is a novel cannabinoid receptor. *British Journal of Pharmacology,* 152(7), 1092-1101.

Sam, A.H., Salem, V. and Ghatei, M.A. (2011) Rimonabant: From RIO to Ban. *Journal of Obesity,* 2011, 432607.

Sawzdargo, M., Nguyen, T., Lee, D.K., Lynch, K.R., Cheng, R., Heng, H.H., George, S.R. and O'Dowd, B.F. (1999) Identification and cloning of three novel human G protein-coupled receptor genes

GPR52, PsiGPR53 and GPR55: GPR55 is extensively expressed in human brain. *Brain Research.Molecular Brain Research,* 64(2), 193-198.

Schicho, R. and Storr, M. (2012) A potential role for GPR55 in gastrointestinal functions. *Current Opinion in Pharmacology,* 12(6), 653-658.

Shore, D.M. and Reggio, P.H. (2015) The therapeutic potential of orphan GPCRs, GPR35 and GPR55. *Frontiers in Pharmacology,* 6, 69.

Stancic, A., Jandl, K., Hasenohrl, C., Reichmann, F., Marsche, G., Schuligoi, R., Heinemann, A., Storr, M. and Schicho, R. (2015) The GPR55 antagonist CID16020046 protects against intestinal inflammation. *Neurogastroenterology and Motility : The Official Journal of the European Gastrointestinal Motility Society,* 27(10), 1432-1445.

Su, J.Y. and Vo, A.C. (2007) 2-Arachidonylglyceryl ether and abnormal cannabidiol-induced vascular smooth muscle relaxation in rabbit pulmonary arteries via receptor-pertussis toxin sensitive G proteins-ERK1/2 signaling. *European Journal of Pharmacology,* 559(2-3), 189-195.

Svizenska, I., Dubovy, P. and Sulcova, A. (2008) Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures--a short review. *Pharmacology, Biochemistry, and Behavior,* 90(4), 501-511.

Tudurí, E., Imbernon, M., Hernandez-Bautista, R.J., Tojo, M., Ferno, J., Dieguez, C. and Nogueiras, R. (2017) GPR55: a new promising target for metabolism? *Journal of Molecular Endocrinology,* 58(3), R191-R202.

Verty, A. N., Stefanidis, A., McAinch, A. J., Hryciw, D. H., & Oldfield, B. (2015). Anti-Obesity Effect of the CB2 Receptor Agonist JWH-015 in Diet-Induced Obese Mice. *PloS one*, 10(11), e0140592.

Wang, C. Y., & Liao, J. K. (2012). A mouse model of diet-induced obesity and insulin resistance. *Methods in molecular biology (Clifton, N.J.)*, 821, 421–433.

Wang, X., Xiang, X., Hu, J., Wu, Y., Li, Y., Jin, S., & Wu, X. (2022). Pharmacological Activation of GPR55 Improved Cognitive Impairment Induced by Lipopolysaccharide in Mice. *Journal of molecular neuroscience* : MN, 10.1007/s12031-022-02020-y. Advance online publication.

West, D.B., Waguespack, J., McCollister, S. (1995). Dietary obesity in the mouse: interaction of strain with diet composition. *Am. J. Physiol.* 268, 658–665.

Xiang, X., Wang, X., Wu, Y., Hu, J., Li, Y., Jin, S., & Wu, X. (2022). Activation of GPR55 attenuates cognitive impairment, oxidative stress, neuroinflammation, and synaptic dysfunction in a streptozotocin-induced Alzheimer's mouse model. *Pharmacology, biochemistry, and behavior,* 214, 173340.

Yang, H., Zhou, J., & Lehmann, C. (2016). GPR55 - a putative "type 3" cannabinoid receptor in inflammation. *Journal of basic and clinical physiology and pharmacology*, 27(3), 297–302.

Yoneda, S., Uno, S., Iwahashi, H., Fujita, Y., Yoshikawa, A., Kozawa, J., Okita, K., Takiuchi, D., Eguchi, H., Nagano, H., Imagawa, A., & Shimomura, I. (2013). Predominance of β-cell neogenesis rather than replication in humans with an impaired glucose tolerance and newly diagnosed diabetes. *The Journal of clinical endocrinology and metabolism*, 98(5), 2053–2061.

Figure legends:

Fig 1. Effects of Abn-CBD or sitagliptin alone, and in combination on (A) bodyweight, (B) percentage weight change, (C) cumulative food intake and (D) cumulative energy intake of high fat fed mice. Bodyweight measurements were obtained before and during Abn-CBD (0.1µmol/kg bw) and sitagliptin (50mg/kg bw) therapies (indicated by the black bar). Values are presented as mean ± SEM (n = 8). *p<0.05, **p<0.01, ***p<0.001, compared to HFF control.

Fig 2. Effects of Abn-CBD or sitagliptin alone and in combination on (A, B) plasma glucose (C, D) plasma insulin and (E) pancreatic insulin content of high fat fed mice. Parameters were obtained before and during Abn-CBD (0.1µmol/kg bw) and sitagliptin (50mg/kg bw) therapies (indicated by the black bar). Values are presented as mean ± SEM (n = 8 for plasma glucose/insulin, n = 4 for insulin content). *p<0.05, **p<0.01, ***p<0.001, compared to HFF control. +p<0.05, +++p<0.001, compared to Abn-CBD monotherapy.

Fig 3. Effects of Abn-CBD or sitagliptin alone or in combination on (A, B) total-GLP-1, (C, D) total-GIP and (E, F) DPP-IV activity of high fat fed mice. Parameters were obtained before and during Abn-CBD (0.1µmol/kg bw) and Sitagliptin (50mg/kg bw) therapies. Values are presented as mean ± SEM (n = 6). *p<0.05, **p<0.01, compared to HFF control.

Fig 4. Oral glucose tolerance (18mmol/kg bw) of high fat fed mice following 21-day treatment with Abn-CBD (0.1µmol/kg bw), sitagliptin (50mg/kg bw) or combination of both agents. (A, B) Plasma glucose and (C, D) plasma insulin were determined. Results are mean ± SEM (n=7). *p<0.05, **p<0.01, ***p<0.001, compared to HFF glucose control. ++p<0.01, +++p<0.001, compared to Abn-CBD monotherapy.

Fig 5. Insulin sensitivity (40U/kg bodyweight; i.p.) of high fat fed mice following 21-day treatment with Abn-CBD (0.1µmol/kg bw), sitagliptin (50mg/kg bw) or a combination of both agents. (A) plasma

glucose and (B) plasma glucose AOC are shown. Results are mean ± SEM (n=8). *p<0.05, **p<0.01, compared to HFF control. +p<0.05, compared to Abn-CBD monotherapy.

Fig 6. Effects of Abn-CBD or sitagliptin alone or in combination for 21-days on (A) fat mass, (B) lean mass, (C) bone mineral content and (D) bone mineral density in high fat fed mice. Values are presented as mean ± SEM (n = 7). *p<0.05, **p<0.01, ***p<0.001, compared to HFF control. +p<0.05, compared to Abn-CBD monotherapy.

Fig 7. Effects of Abn-CBD or sitagliptin alone or in combination for 21-days on plasma (A) triglycerides, (B) total cholesterol, (C) HDL cholesterol and (D) LDL cholesterol. Values are presented as mean ± SEM (n = 8). *p<0.05, **p<0.01, ***p<0.001, compared to HFF control. +p<0.05, ++p<0.01, +++p<0.001, compared to Abn-CBD monotherapy.

Fig 8. Effects of Abn-CBD or sitagliptin alone or in combination for 21-days on plasma (A) C-reactive protein and (B) amylase activity in HFF diabetic mice. Values are presented as mean ± SEM (n = 6). *p<0.05, **p<0.01, compared to HFF control.

Fig 9. Representative images (A-E) for distribution of DAPI (blue), insulin (red) and glucagon (green) in pancreatic islets of high fat fed mice at x 40 magnification, following 21-day treatment with Abn-CBD or sitagliptin alone or in combination on (F) islet size, (G) percentage beta cell area, (H) beta cell mass, (I) percentage alpha cell area and (J) alpha cell mass. Parameters were determined using cell F software. Values are mean ± SEM with 40 islets per group. *p<0.05, **p<0.01, ***p<0.001, compared to HFF. +p<0.05, +++p<0.001, compared to Abn-CBD monotherapy.

Fig 10. Representative images for DAPI (blue), insulin (green) and Ki-67 (red) in pancreatic islets of high fat fed mice at x40 magnification, following 21-day treatment with Abn-CBD alone in (A-D) control and (E-H) Abn-CBD treated mice. Percentage Ki-67 positive beta cells (I), with positive Ki-67

Graphical Abstract

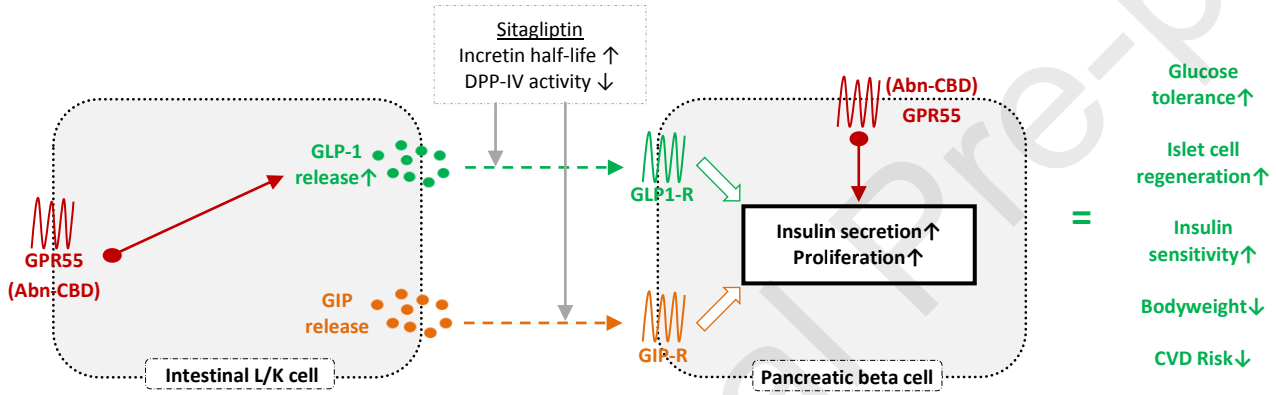


Figure 1

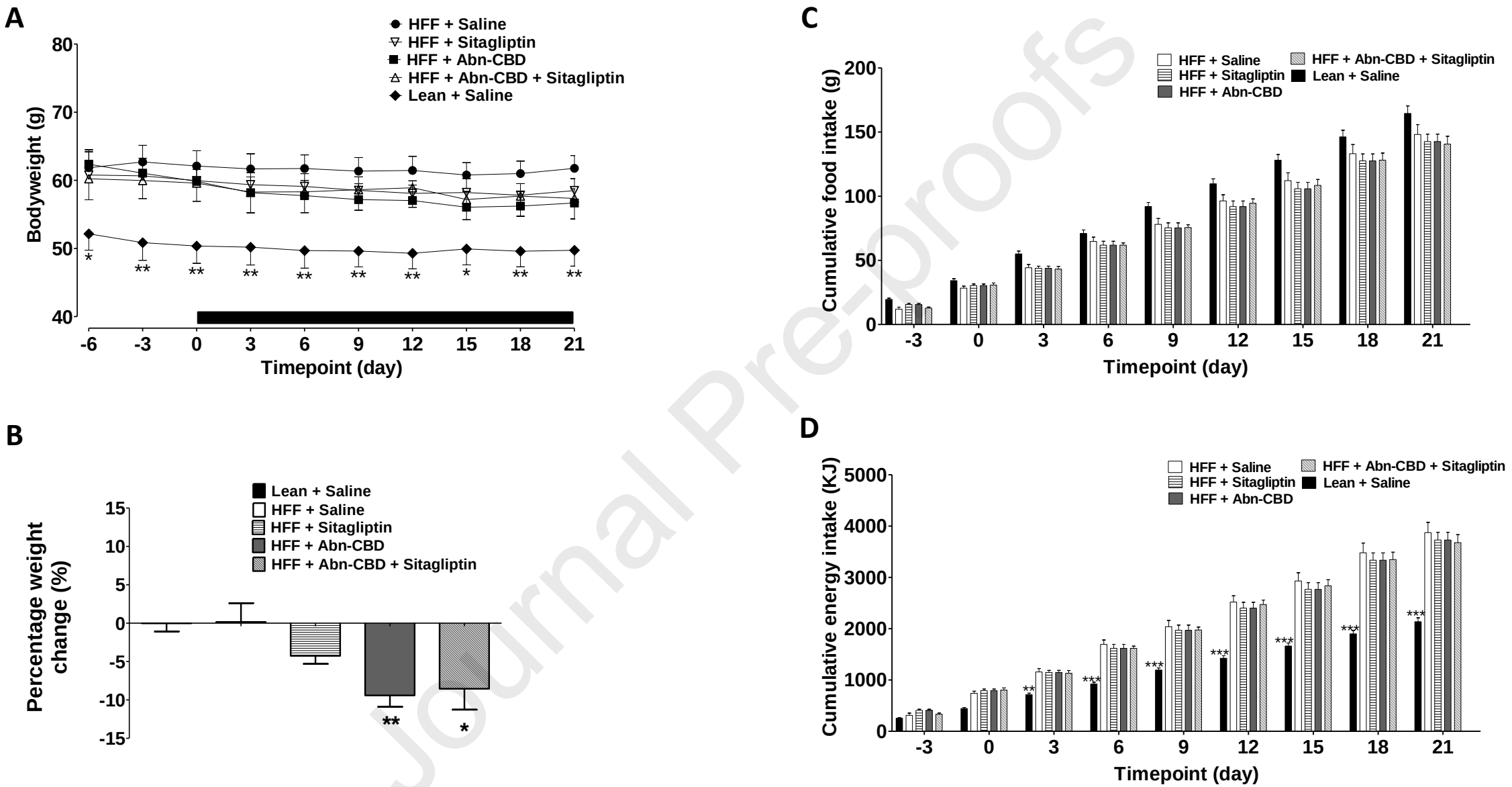
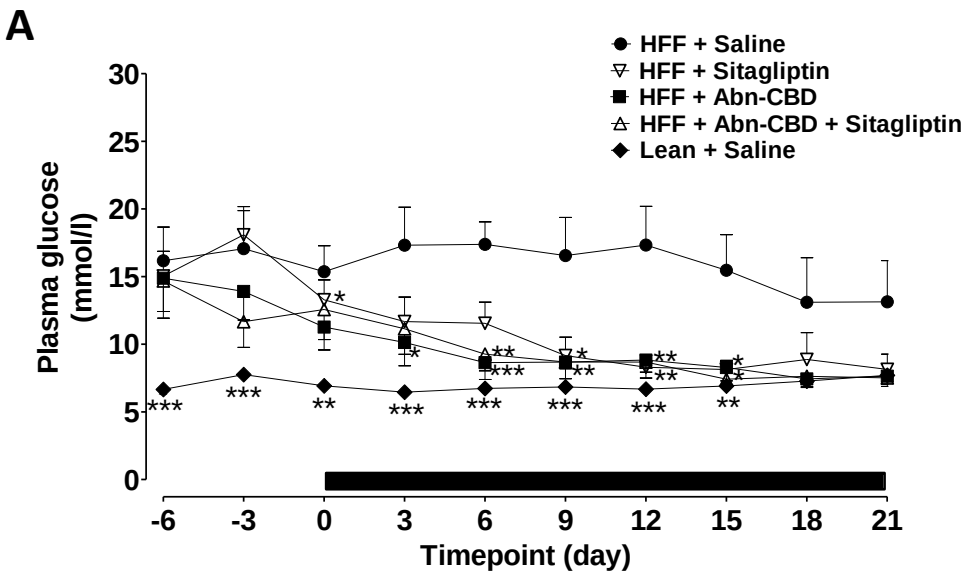
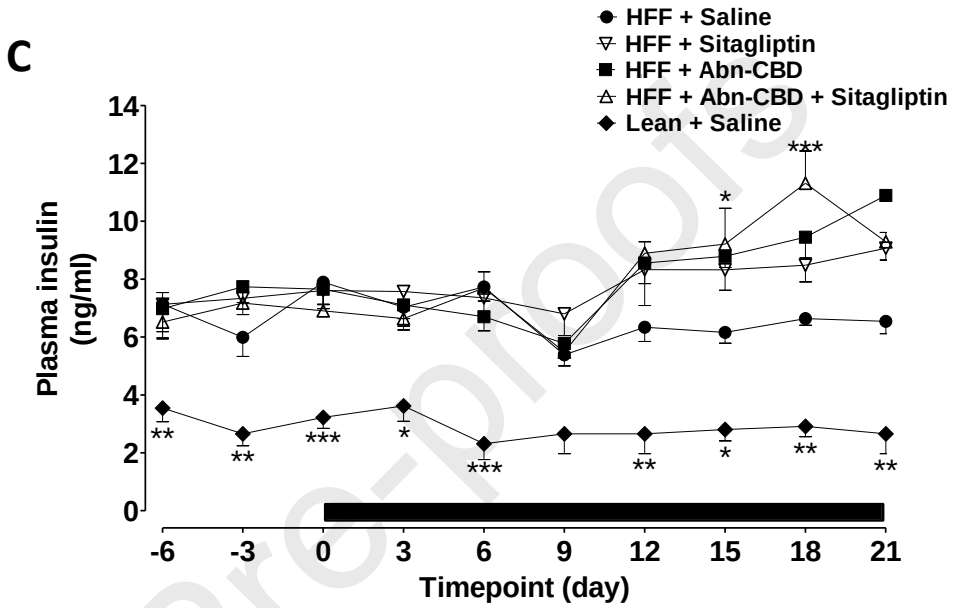
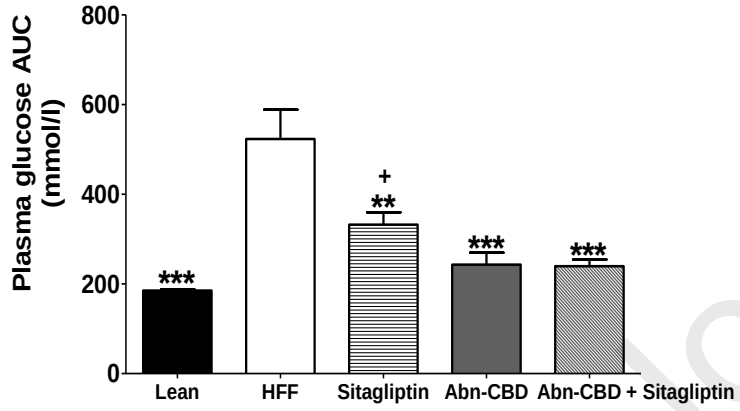
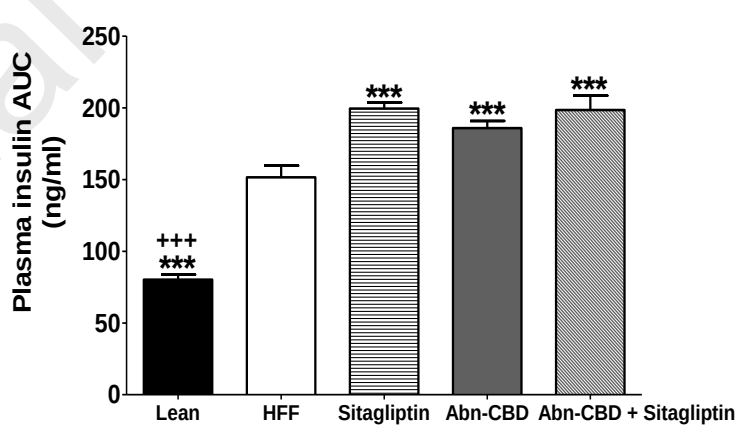


Figure 2









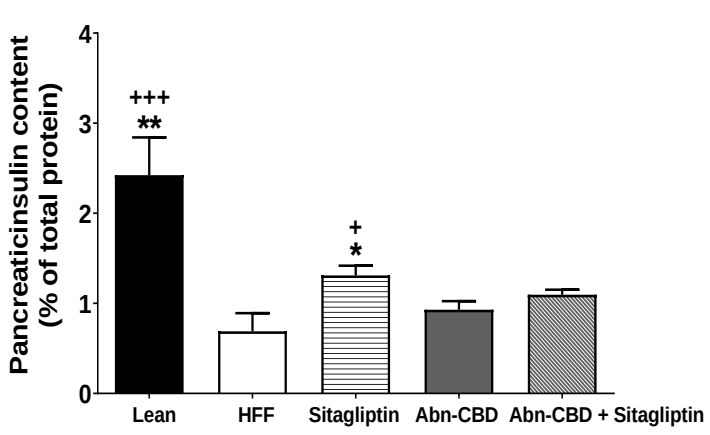
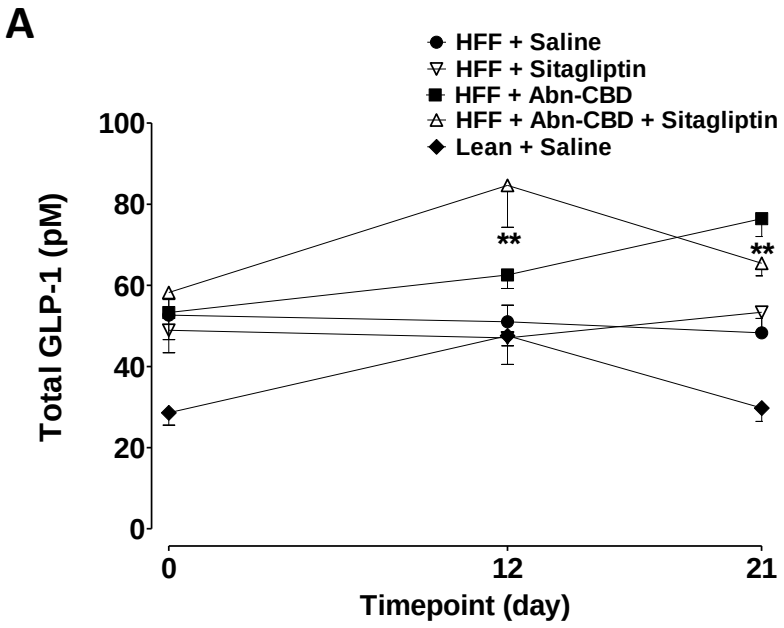
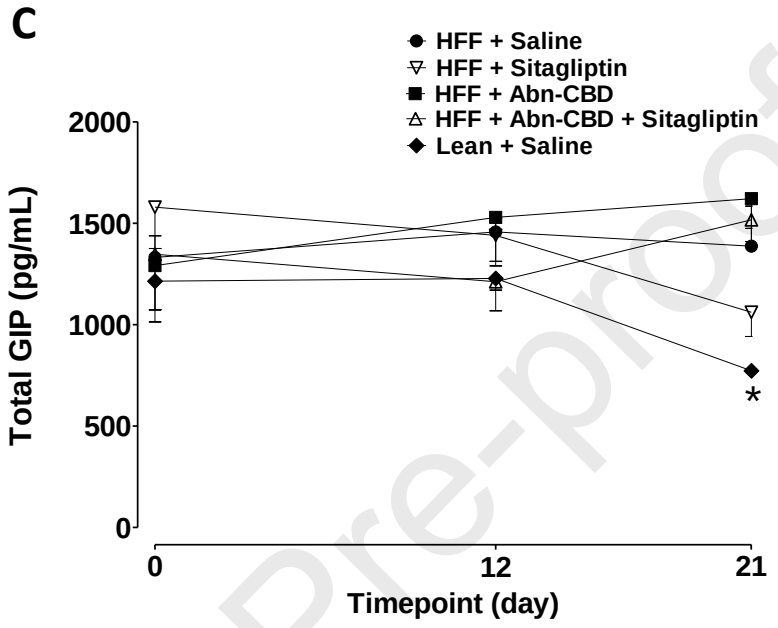
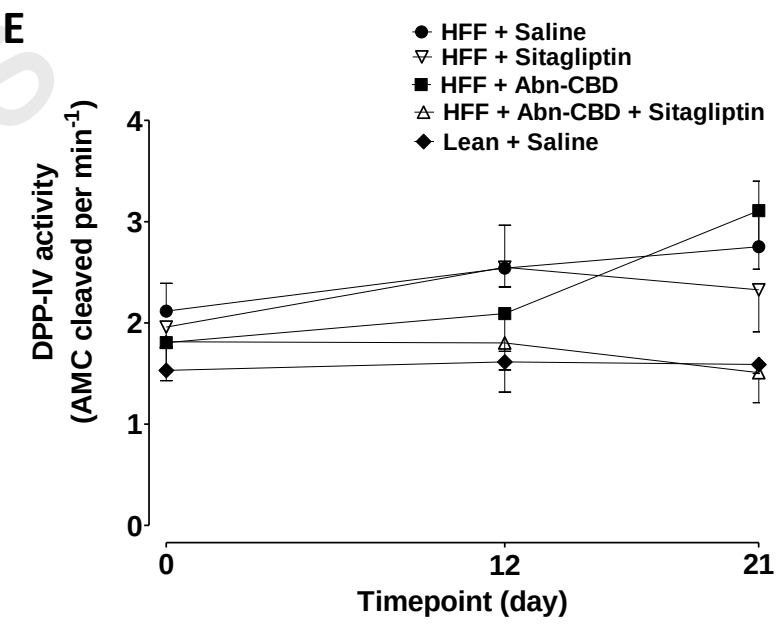
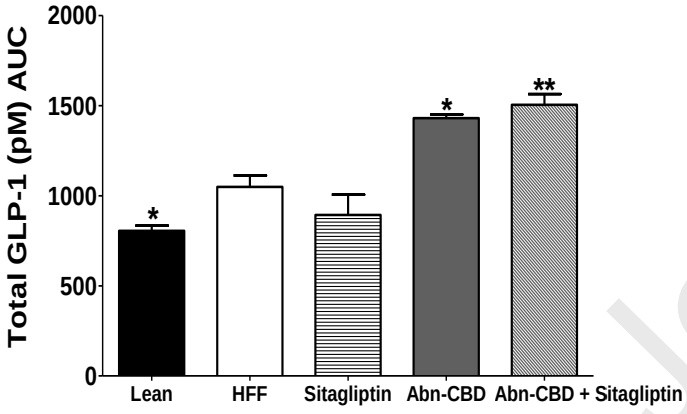


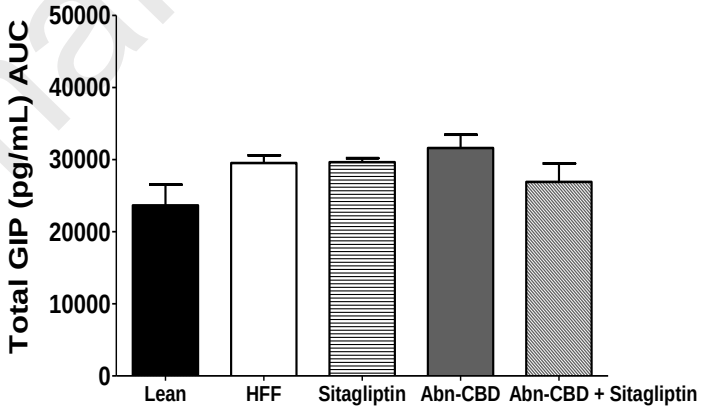
Figure 3











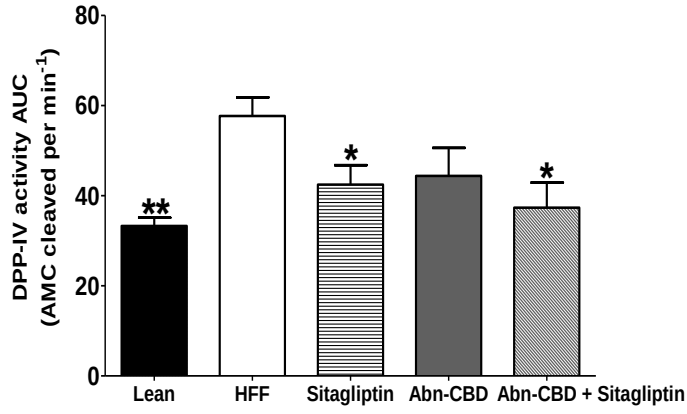


Figure 4

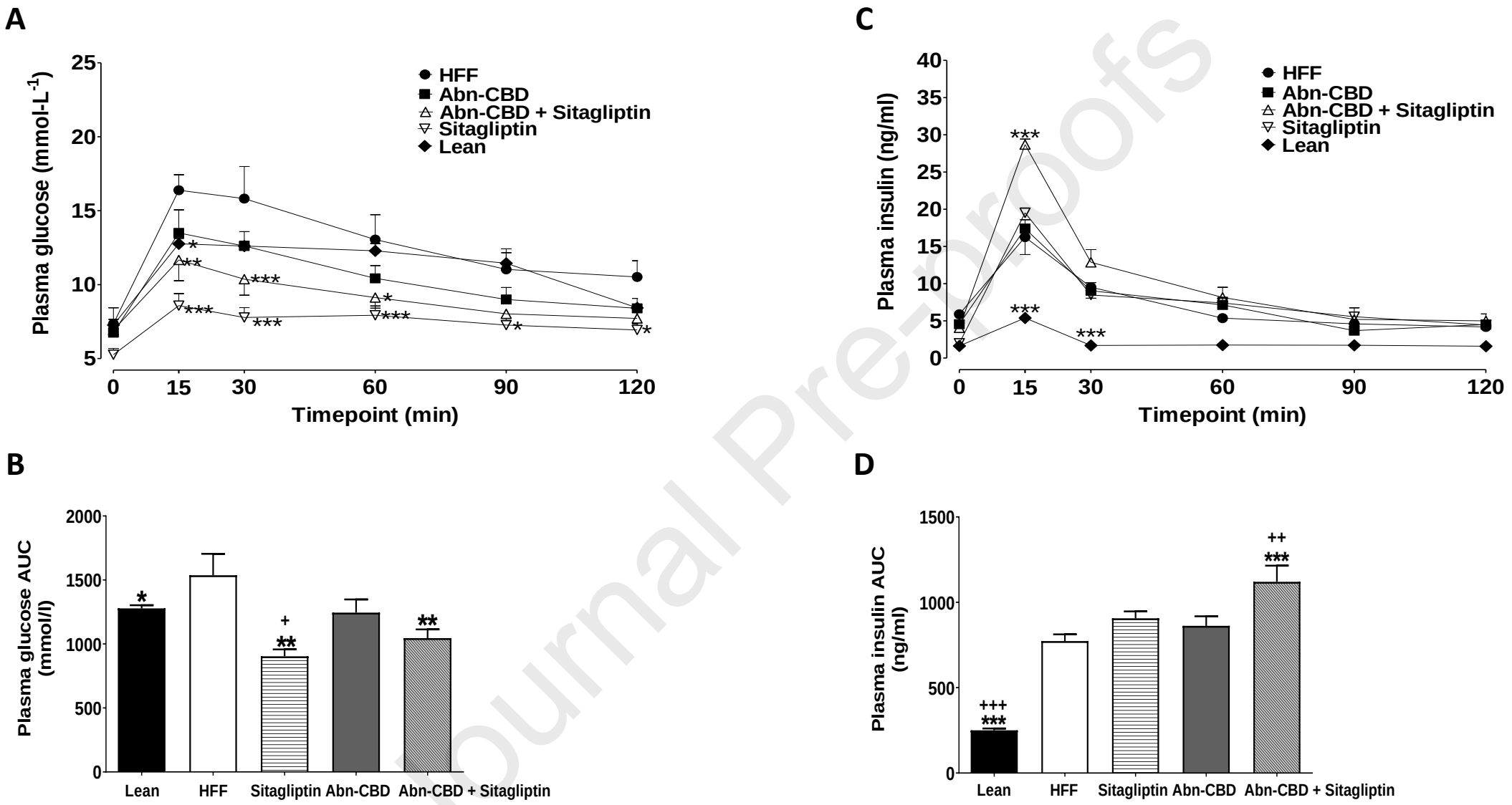


Figure 5

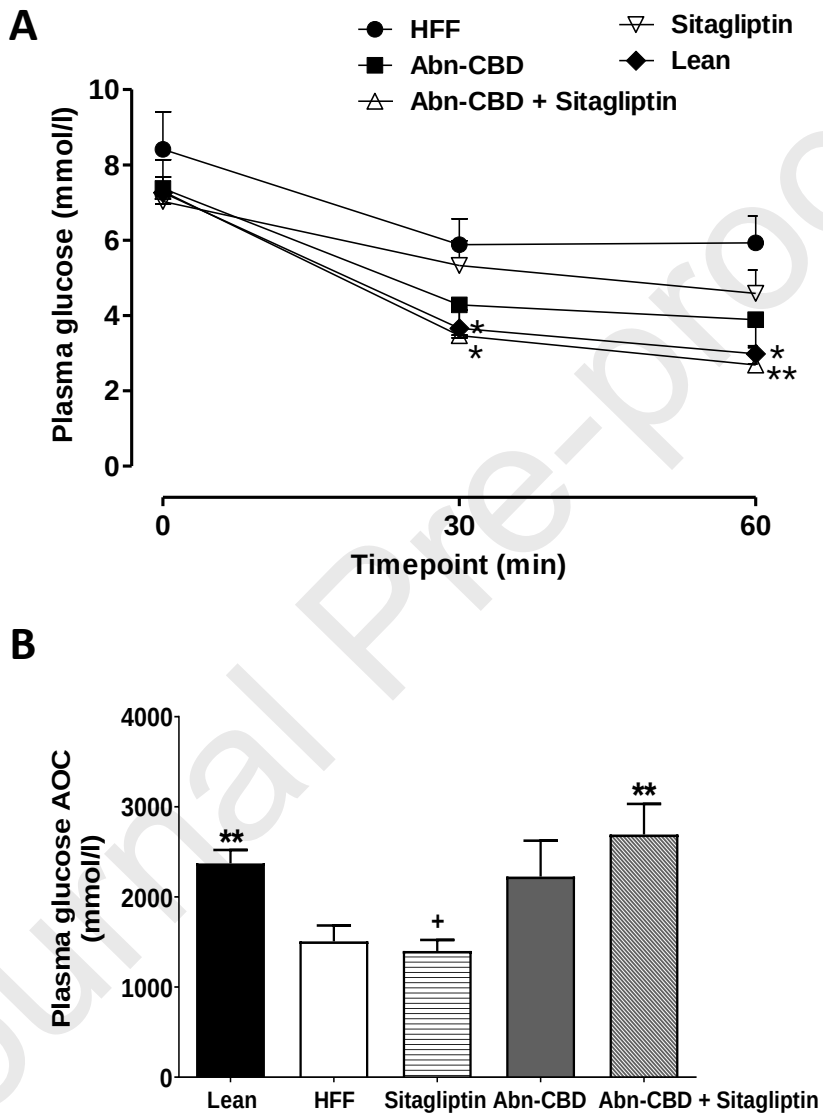


Figure 6

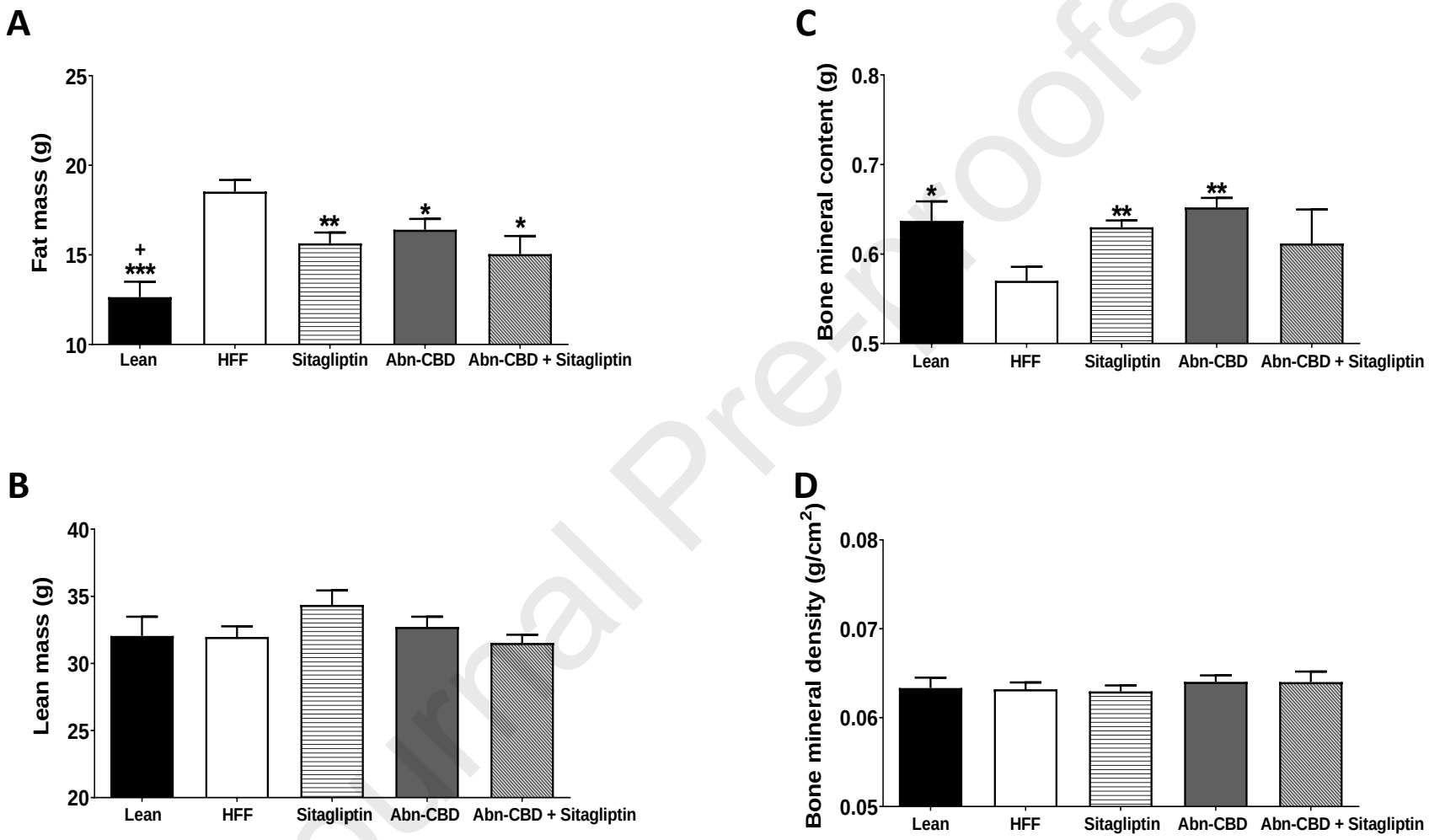


Figure 7

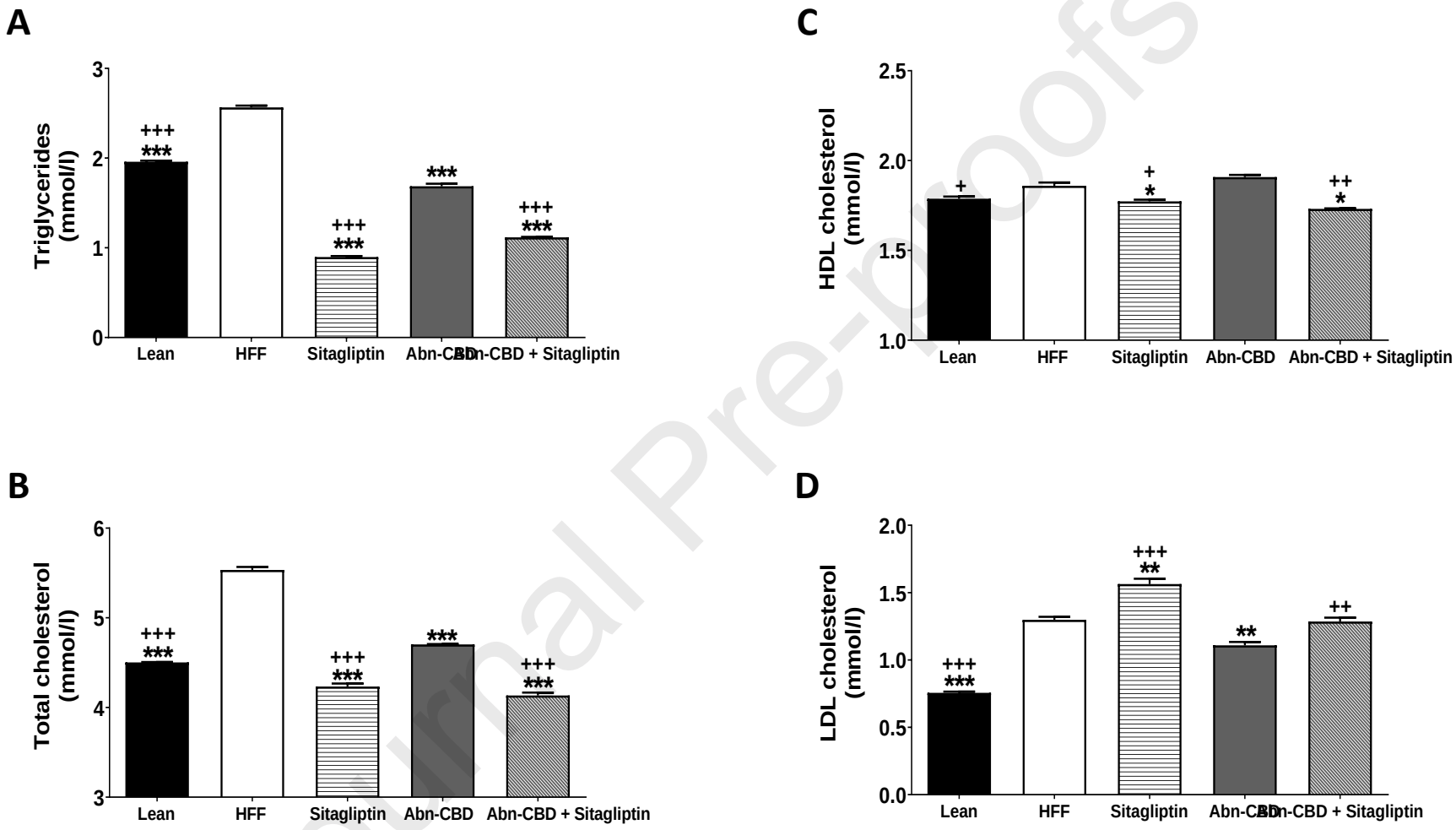


Figure 8

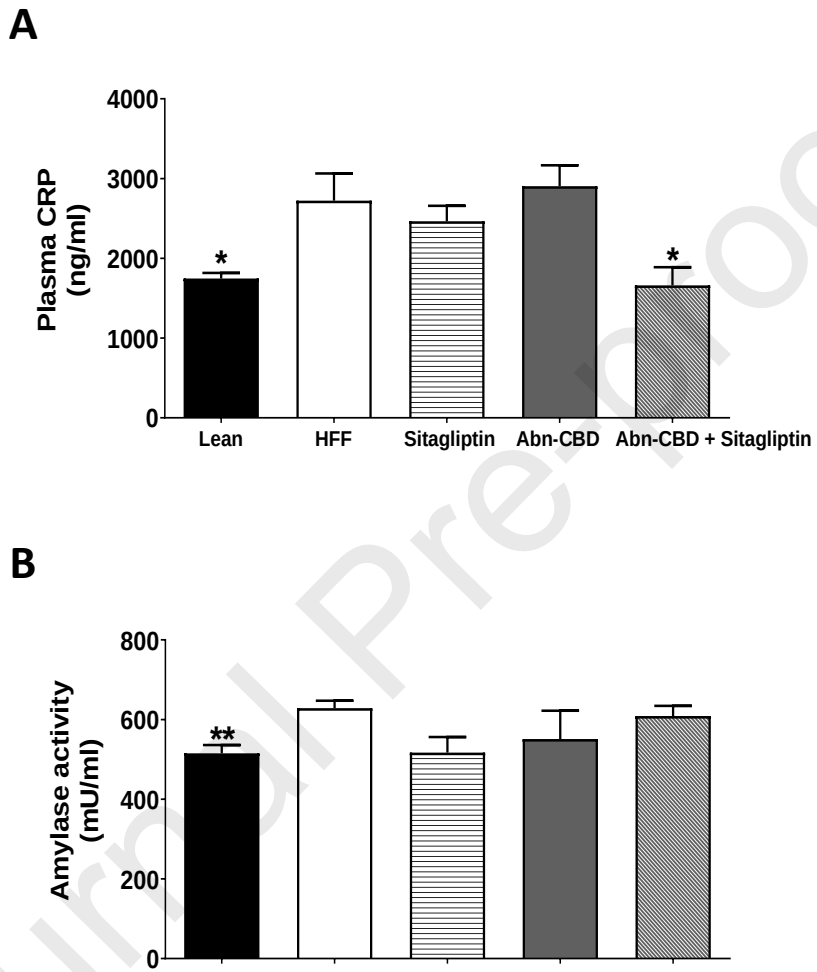
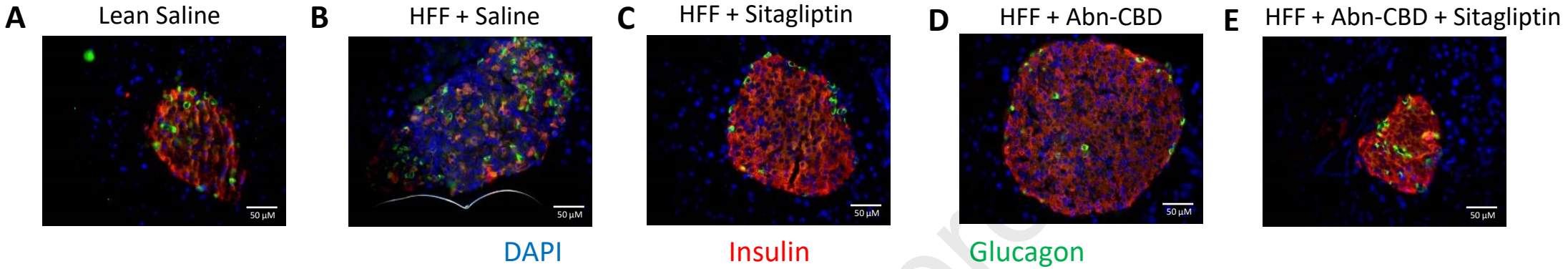
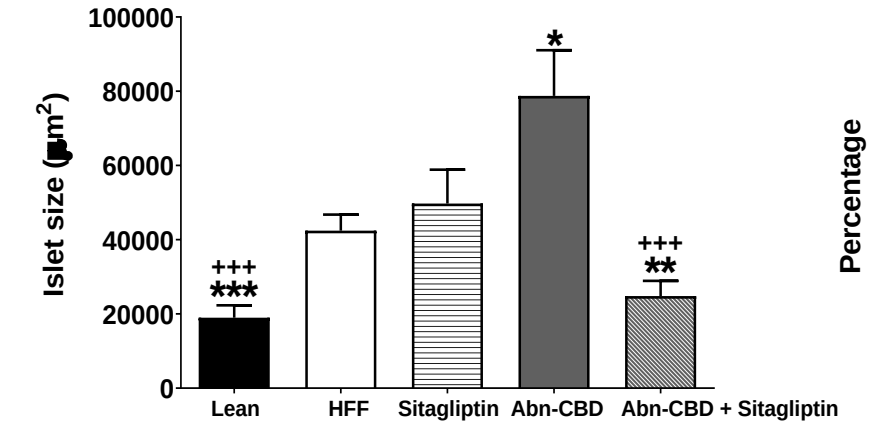
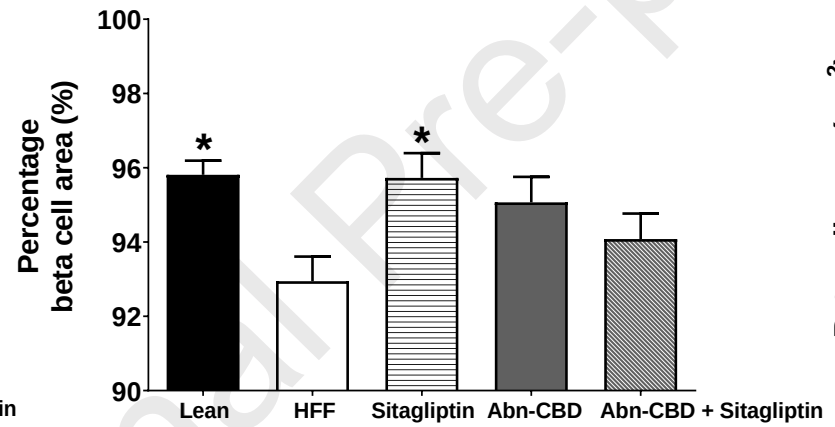
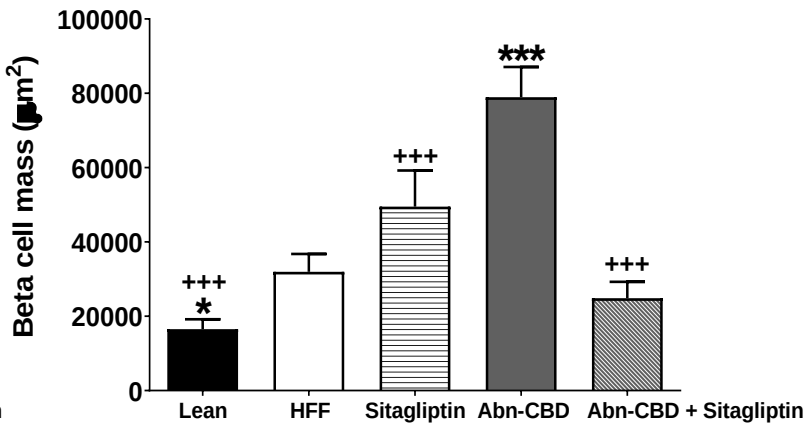


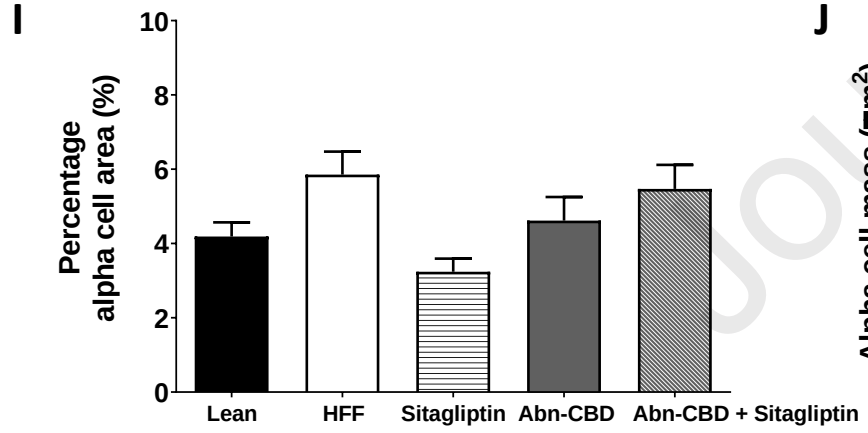
Figure 9











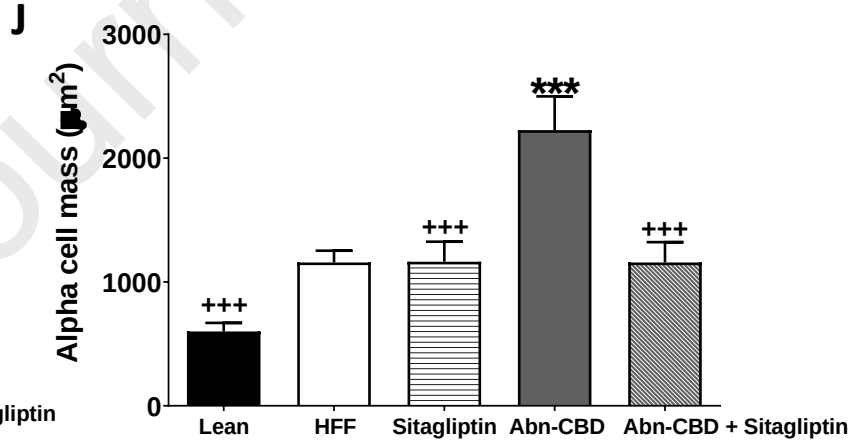
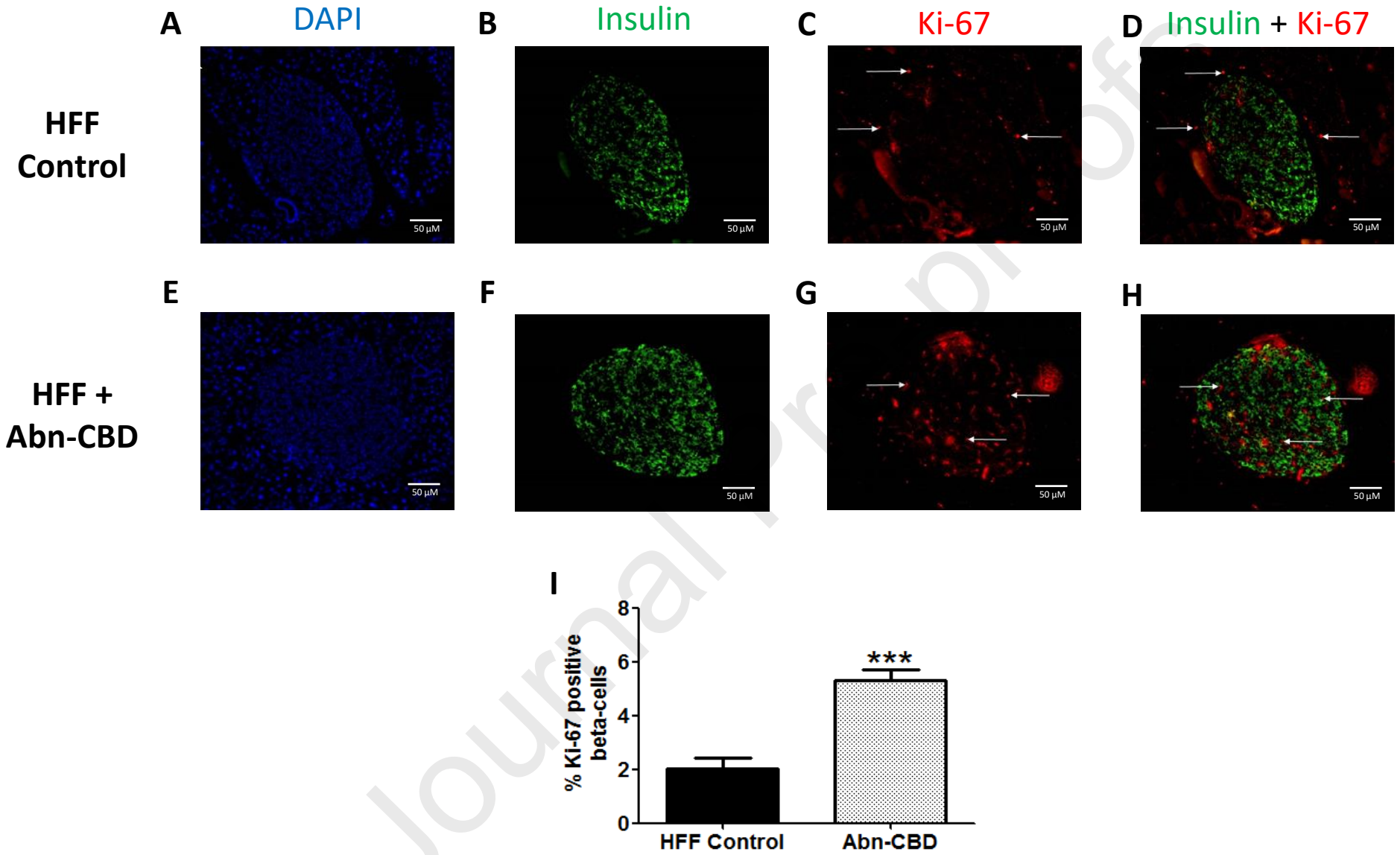


Figure 10



staining indicated by arrows. Values are mean ± SEM with 15 islets per group. ***p<0.001, compared to HFF.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Author contribution statement

AGMC conducted the investigation, methodology, validation, formal analysis and writing – original draft. MGM and RAL conducted the investigation. PRF involved in the writing- review and editing and supervision. AMMK involved in the conceptualization, formal analysis, supervision writing – review and editing.

Author contribution statement

AGMC conducted the investigation, methodology, validation, formal analysis and writing – original draft. MGM and RAL conducted the investigation. PRF involved in the writing- review and editing and supervision. AMMK involved in the conceptualization, formal analysis, supervision writing – review and editing.