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Δ9-Tetrahydrocannabinol Treatment Modifies Insulin Secretion in Pancreatic Islets from Prediabetic Mice Under Hypercaloric Diet

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Abstract

Background: The endocannabinoid system over-activation is associated with type-2 diabetes mellitus onset, involving physiological, metabolic, and genetic alterations in pancreatic islets. The use of Δ9-Tetrahydrocannabinol (THC) as treatment is still controversial since its effects and mechanisms on insulin secretion are unclear. The aim of this study was to evaluate the effects of THC treatment in pancreatic islets from prediabetic mice.

Methods: Prediabetes was induced in mice by hypercaloric diet, and then treated with THC for 3 weeks. Blood glucose and body weight were determined, after behavior tests. Histological changes were evaluated in whole pancreas; in isolated islets we analyzed the effect of THC exposure in glucose-stimulated insulin secretion (GSIS), gene expression, intracellular cyclic adenosine monophosphate (cAMP), and cytosolic calcium changes.

Results: THC treatment in prediabetic mice enhanced anxiety and antidepressive behavior without changes in food ingestion, decreased oral-glucose tolerance test, plasma insulin and weight, with small alterations on pancreatic histology. In isolated islets from healthy mice THC increased GSIS, cAMP, and CB1 receptor (CB1r) expression, meanwhile calcium release was diminished. Small changes were observed in islets from prediabetic mice.

Conclusions: THC treatment improves some clinical parameters in prediabetic mice, however, in isolated islets, modifies GSIS, intracellular calcium and gene expression, suggesting specific effects related to diabetes evolution.

Keywords: THC; endocannabinoid system; pancreatic islets; diabetes; cannabinoid signaling; insulin secretion

Introduction

Pharmacological modulators of endocannabinoid system (ECS) have been proposed as therapeutic alternatives for obesity and type-2 diabetes, after significant improvements in glucose metabolism, insulin sensitivity, and weight loss.[1–3] Previously we reported significant improvements of islet function, in prediabetic and diabetic mice treated with a novel rimonabant analog.[1] Zucker diabetic fatty rats treated with Ibipinabant presented weight loss, reduction in oral glucose tolerance test (OGTT), and increase in islet area and insulin content.[3] ECS is involved in food intake and metabolism regulation, however, its overactivity has been associated to metabolic syndrome, obesity, and type-2 diabetes, developing impaired insulin sensitivity, glucose intolerance, and dyslipidemia.[4–7] These evidences suggest ECS modulation could be a therapeutic solution to restore the metabolic imbalance.

Therapeutic use of Δ9-Tetrahydrocannabinol (THC) has demonstrated effective reduction of fasting glucose and insulin resistance. The chronic consumption of *Cannabis sativa* in obese and diabetic patients decreased fasting insulin and HOMA-IR, waist circumference, and body mass, despite the increase in caloric intake.[8] In human volunteers, chronic exposure to THC induced transitory glucose intolerance, desensitization and decrease of CB1 receptor (CB1r) activity, ameliorated diabetic neuropathy, and mechanical

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allodynia.[9] More specific in isolated pancreatic islets, THC treatment increased glucose-stimulated insulin secretion (GSIS) and improves beta cell function.[10] Rats under cafeteria diet treated with THC 5 mg kg/day for 8 weeks reduced body weight, glucose intolerance, and islets dysfunction.[11] In a different rat model with streptozotocin-induced type-2 diabetes, THC 15 mg/kg for 28 days evoked protective changes in islets, with a brief decrease in hyperglycemia.[12,13]

ECS function in pancreatic islets has been studied under different approaches, through interactions of agonists and antagonists with the cannabinoid receptors CB1r, CB2r, GPR55, and TRPV, heterology expressed in mouse, rat, and human islets.[14–26] Many evidences propose CB1r is coupled to Gαi proteins, therefore decrease cyclic adenosine monophosphate (cAMP) production by adenylyl cyclase inhibition and reduce intracellular calcium release, leading to the negative modulation of GSIS and beta cell apoptosis.[21–26] On the other hand, GPR55 receptor is coupled to Gα12/13 and Gαq proteins, closely related to Rho family GTPases and FAK activity, so agonists induce intracellular Ca^{2+} release, extracellular signal-regulated kinase 1/2 (ERK) phosphorylation, secretory vesicle exocytosis by integrin signaling, improving glucose tolerance, increased plasma insulin levels, and a maintaining-cell mass effect.[26–29] Until today, the specific interaction of THC with cannabinoid receptors in pancreatic islets is unclear, according to dose and exposure.

Another controversial detail regarding the therapeutic use of THC is related to psychiatric disorders. Rats under hypercaloric diet (HCD) display anxiety and depression-like behavior, without memory alterations.[30] Mice treated with THC from 1.25 to 5 mg/kg improved their performance in the forced swimming test, indicating amelioration of depression-like behavior.[31] Many studies support the use of pharmacological drugs that modify behavioral components present in diabetes and obesity, suggesting THC as a therapeutic option.[32]

In this study, we evaluated the effects of THC treatment in pancreatic islets from prediabetic (prediabetes mellitus [pDM]) mice, considering the animal model behavior studies, glucose control, insulin secretion, food ingestion, and histological alterations. *In vitro*, we analyzed changes on gene expression and intracellular signaling by cAMP and calcium.

Materials and Methods

Reactives

THC was kindly provided by Dr. Itzell Gallardo, obtained from ethanolic extract of 10 g *Cannabis sativa*: leaves were macerated and contained in 96% ethyl alcohol for 48 h at room temperature, and 10 mL of sample was quantified and purified by HPLC with Infinity 1260 Agilent equipment. The final vial contained 9.5 mg/mL of 90% THC. Antibodies for immunofluorescence were obtained from Santa Cruz Biotechnology CB1(C-11) sc-518035, insulin B(C-12) sc-377071 and Invitrogen goat anti-mouse IgG AlexaF546 A-11030, goat anti-rabbit IgG AlexaF488 A-11034. Anandamide and AM251 were purchased from Tocris Bioscience. Commercial cannabidiol was obtained from HempsMed Mexico (Mexico). Dulbecco's modified eagle medium (DMEM) low glucose, SBF, penicillin/streptomycin, L-glutamine, collagenase Type V, Histopaque 1077, isobutylmethylxanthine (IBMX), nifedipine, glucagon, cAMP ELISA Kit CA201-1KT, and PCR primers for CB1r and 18s rRNA were purchased from Sigma sch (St. Louis, MO).

Preproinsulin, preproglucagon, and Pdx-1 primers were obtained from Qiagen (West Sussex, UK).[33] Real-time PCR master mix and reagents were purchased from Fermentas/Thermo Scientific (Madison, WI), and insulin ELISA Kits 80-INSMS-E01 were obtained from Alpco (Salem, NH).

Animal models

Male 4-week-old CD-1 mice were obtained from the Facultad de Estudios Superiores Iztacala animal facility, and maintained under controlled conditions of light/-dark cycles from 07:00 to 19:00, temperature, humidity, and air, according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health NOM-062-ZOO-1999. All experiments were approved by the local Institutional Ethics Committee of the Universidad Nacional Autonoma de Mexico. Prediabetes (pDM) was induced with a modified HCD of 4.9–5.1 kcal/g: 30% added fat (lard) and 20% sucrose solution intake, for 8 weeks. Control groups were fed with standard rodent chow food of 3.9–4.1 kcal/g. Mice received orally 5 mg/kg THC, diluted in 0.2 mL coconut oil, daily for 3 weeks at noon; one group was treated with 5 mg/kg THC during 2 weeks before change to HCD. Coconut oil as vehicle was administered to control groups. Each group contained $n = 10$ animals, selected randomly.

Open field test

To recognize the conduct effects of THC treatment on mice, locomotor activity and anxiety-like behavior were determined by the open field test (OFT) according

to previous reports.[34,35] On a white plexiglass surface we defined the central zone area (20 × 20 cm), corners (10 × 10 cm), and walls (10 × 20 cm) as periphery area, with a digital video camera collocated 90 cm over the arena. The test was performed between 7:00 and 10:00 am inside an isolated noise room. Control and pDM mice received orally 5 mg/kg of THC or cannabidiol (CBD) oil before OFT. Individual mouse was located on the central zone, and the ambulatory activity was recorded on video for 15 min. After each trial, the arena was cleaned with 70% ethanol to remove residues. Videos were processed with ImageJ plug in Animal Tracker software[35] to analyze time spent in center zone (sec) and distance (cm). Data were normalized to the proportional percentage of each parameter.

Forced swim test

Depressive-like behavior probably induced by THC in mice was evaluated with the forced swim test, as previously reported.[36] Clear glass cylinders (30 × 20 cm) were filled with water (15 cm from the bottom) at 24°C ± 1°C. A digital video camera was positioned 50 cm in front of the cylinders. The forced swim test was carried out 2 days after the evaluation of OFT, with administration of 5 mg/kg THC or CBD as control. Each mouse individually was collocated in the cylinders and recorded for 6 min. At the end, the animal was removed from water, dried with towel paper, and moved back to cages. Immobility time was determined during the last 4 min, using the Kinoscope application.[35] Data of immobility time were normalized as described for OFT.

OGTT and body weight

OGTT was performed after 3 weeks of THC treatment. Mice received orally 2 g/kg glucose after 12 h fasting period. Blood samples were obtained from tail vein at 0, 30, 60, 90, and 120 min and glucose levels were measured with OneTouch UltraMini Glucometer (Johnson & Johnson). Body weight was registered weekly and food ingestion every day. Animals were euthanized with isoflurane and pentobarbital overdose for the next procedures.

Histological analysis

Pancreas from five mice per group were fixed with Bouin's solution, horizontal oriented for paraffin inclusion, and cut in 7 μm sections, from body to tail. Islet contrast from acinar tissue was performed with Hematoxylin–Eosin standard stain, and islets area was determined on 20,000 μm^2 of tissue, using a Microscope Digital Eyepiece MDE-130 coupled to ScopeImage 9.0 software. Immunodetection for insulin and CB1r were conducted using a polyclonal anti-insulin antibody and monoclonal anti-CB1r antibody (Santa Cruz Biotechnology H-86 sc-9168 and C-11 sc-518035), with secondary antibodies anti-rabbit IgG Alexa 488 and anti-mouse IgG Alexa 549 (Invitrogen A11008, 571716 and A11030, 134546) used as previously reported.[1,37] Nuclear DNA was detected with Hoechst (Vector laboratories) as control. Images and intensity signal were obtained in a Leica TCS SP2 inverted confocal laser Scanning microscope (Leica, Leidemberg, Germany).

Islet isolation for GSIS, gene expression analysis, cAMP, and Ca^{2+} determination

Pancreatic islets from control and pDM mice were isolated as previously reported[1,33,37] by collagenase digestion, density gradient, handpicked and size matched, and maintained 24 h in low-glucose DMEM, 10% FBS, 2 mM L-glutamine, and 3% antibiotics.

Static insulin secretion was evaluated in five islets preincubated 1 h at 37°C in modified Krebs buffer with 2.8 mM glucose. Islets were stimulated 1 h with 16 mM glucose, in the presence of 100 pg/mL glucagon, 1 μM anandamide (AEA), 10 μM AM251, and 10 μM THC, and insulin release was determined by ELISA (Alpco).

Determination of cAMP content was performed in 50 islets preincubated with 10 mM IBMX for 30 min to inhibit phosphodiesterase. Treatments were added for 15 min: vehicle 20% DMSO, 100 pg/mL glucagon, 1 μM AEA, 10 μM AM251, and 10 μM THC. Islets were sonicated and cAMP content was determinate by ELISA Kit (Sigma). All experiments were performed by triplicate in islets from control and pDM mice.

For gene expression analysis, RNA was collected from 50 islets of each mice group, using TRIzol reagent, isopropanol, and quantified by spectrometer. Conversion to cDNA was obtained from 0.5 μg of total RNA by reverse transcription reaction and real-time PCR was performed with 20 ng/μL of cDNA. Primers and amplification conditions have been reported previously[1,33] Relative expression was determined after normalization against 18s rRNA, and calculated by the $2^{-\Delta\Delta Ct}$ method.[38]

Effects of THC on Ca^{2+} influx: 150 isolated islets from control and pDM mice were dispersed with 1 × EDTA-trypsin solution (Sigma) for 10 min, and

washed in Krebs solution with 0.1% BSA. After 8 h incubation in DMEM, cells were preloaded with 1 μL/mL FURA-2 for 1 h at 37°C. Intracellular calcium measurement was performed in a PerkinElmer spectrofluorometer, with 50,000 cells per experiment, under stir and temperature control. After setting a basal 5 mM glucose exposure, cells were exposed to 1 μM AEA, 10 μM AM251, 10 μM THC, and 15 mM glucose for 400 sec, and 1 μM nifedipine was applied at the end, as negative control to block calcium influx. Fluorescence intensity is measured at 340–380 nm excitation (F340/F380) and 510 nm emission.

Statistical data analyses

Data are expressed as mean ± standard error of the mean obtained from 3 to 10 individual observations per experiment. Tukey–Kramer test, one-way and two-way analysis of variance with Bonferroni's *post hoc* tests were used for analyses; differences between treatments were considered statistically significant at $p < 0.05$.

Results

THC modifies behavior/depression activities under different diets

We evaluated the possibility of THC-induced behavior changes in control and pDM mice, compared with CBD, another cannabinoid with lower psychoactive effect. Administration of THC decreased the time in central zone in all mice, evoking anxiety response (Fig. 1A) Unexpected, CBD decrease of static behavior in pDM mice as well. Regarding traveled distance, THC increased it significantly in pDM mice, suggesting a hyperactivity response (Fig. 1B). The forced swim test suggests a depression-like behavior, and only CBD reduced 25% immobility time in control mice (Fig. 1C). THC treatment presented a nonsignificant effect.

THC treatment improves glucose tolerance in prediabetic mice, but not as preventive therapy

Prediabetic mice (light gray line and bars) showed a significant increase in OGTT (Fig. 2A), over 100% increase of area under the curve (Fig. 2B), and a 10% increase in body weight (Fig. 2C), with respect to control mice: 34.4 to 36.9 g in control, 32.25 to 36.3 g in pDM mice. THC treatment for 3 weeks (medium gray lines and bars) normalized OGTT, decreased area under the curve, and decreased significantly body weight, from 35.2 to 33.4 g. In healthy mice, THC administration did not alter glucose but increased body weight from 36.8 to 39.6 g (dark gray line and bars), however, food ingestion was not modified between groups (data not shown). The group of mice with THC administrated previously and after HCD (blue line and bars) presented a modified OGTT curve associated to an increased area under the curve, without effect in body weight.

Histological analysis of pancreas showed expected increase in the islet area and insulin production from pDM mice (Fig. 3). THC treatment induced discrete nonsignificant changes in islet size, insulin, and CB1r expression. The number of islets per area, in body and tail of pancreas, remain constant between groups,

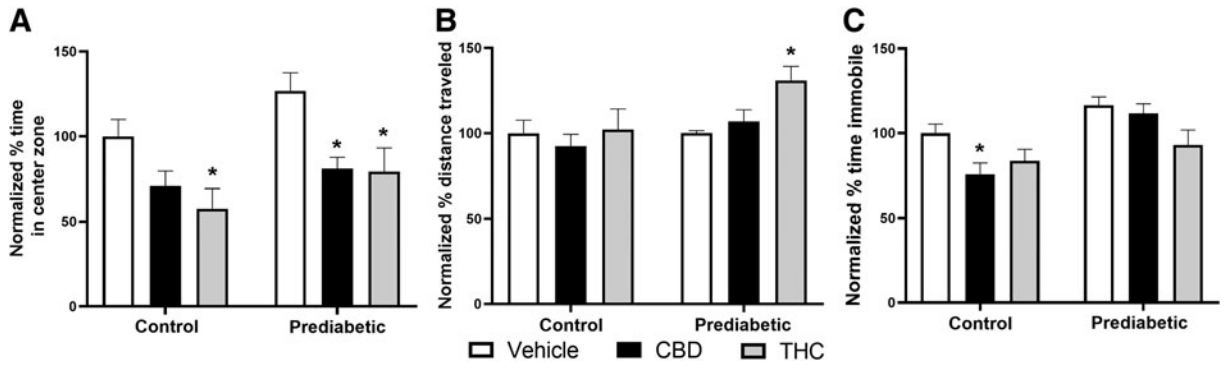


FIG. 1. **(A, B)** Open field and **(C)** forced swim tests on control and prediabetic mice, with a single dose of CBD (black bars) or THC (gray bars), or vehicle (white bars). Data normalized regarding control, presented as mean ± SEM, $n = 10$ mice per group. *$p < 0.05$ versus control by two-way ANOVA. ANOVA, analysis of variance; CBD, cannabidiol; SEM, standard error of the mean; THC, Δ9-Tetrahydrocannabinol.

THC alters insulin secretion through changes in cAMP, gene expression, and calcium

Basal insulin secretion was significantly increased in isolated islets from healthy mice, after AEA, inverse agonist AM251, and THC exposure. GSIS at 16 mM was decreased with glucagon and in the presence of cannabinoid receptor agonists (Fig. 4A). Islets from pDM mice increased basal secretion with AEA, AM251, and THC treatment, but glucose stimuli were abolished in all conditions (Fig. 4B). Since cAMP production can be promoted by glycolytic metabolism, we observed a significant effect on islets exposed to 16 mM glucose. Glucagon, AEA, AM251, and THC treatments increased significantly cAMP content at low glucose (Fig. 4C), but this effect was diminished in islets under glucose stimuli. In isolated islets from pDM mice, only THC exposure increased basal cAMP production (Fig. 4D).

The quantity and quality of RNA isolated was only satisfactory for the islets obtained from healthy mice; the results of gene expression obtained from islets of pDM mice were inconclusive and not reported. Insulin and Pdx-1 mRNA increased after AEA treatment; AM251 elevated insulin expression and decreased CB1r, meanwhile THC exposure induced the opposite effects (Fig. 4E).

Calcium influx in dispersed cells showed a slight decrease in the presence of AEA after 16 mM glucose, either from control (Fig. 5A) or pDM mice (Fig. 5B). AM251 induced a significant increase in calcium current in islet cells from both groups (Fig. 5C, D), meanwhile THC treatment significantly reduced calcium influx, similar to nifedipine inhibitory effect (Fig. 5E, F).

Discussion

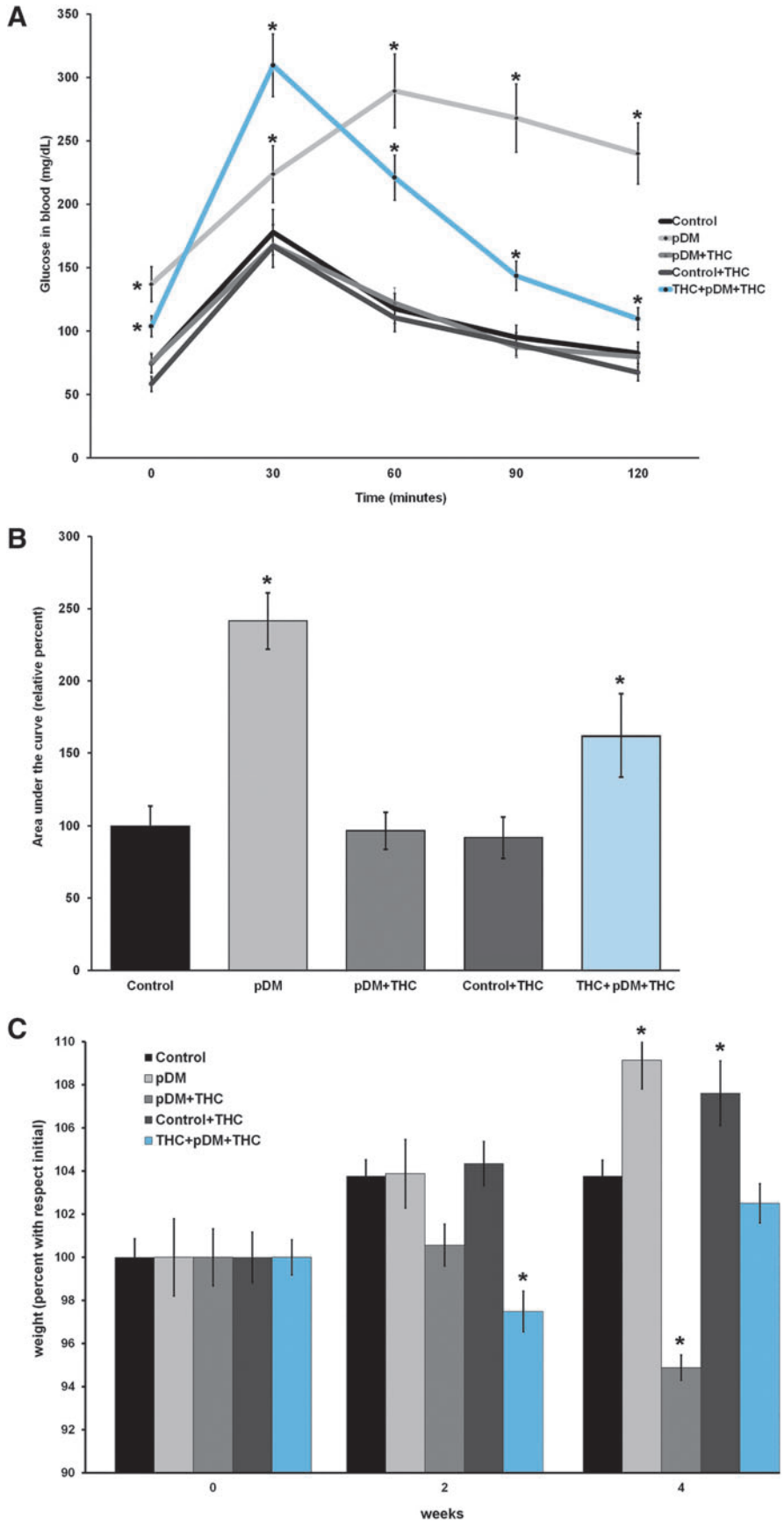
We evaluated the effects of THC treatment in a mouse model of prediabetes, induced by HCD. It is well known that consuming THC increases food ingestion, mostly carbohydrates and fatty food, suggesting a potential risk factor for developing diabetes, but paradoxically, several evidences suggest its beneficial use as modulator of the ECS.[8–13] The psychoactive effects of THC ameliorate anxiety and depression-like symptoms in mice, evaluated by forced swimming and tail suspension tests.[31] Our OFT results suggest THC treatment induces hyperactivity and anxiety-like behavior since mice increase changes in their positions without alterations in their walking velocity, independently of the metabolic state. The antidepressive behavior observed in mice under forced swim support the effect of THC as emotional motivator with potential therapeutic use for specific cases of obesity and diabetes, considering that consumption of high sugar diet increases the risk for anxiety and depression.[5,8,30]

Prediabetic mice treated with THC reduced OGTT and body weight without changes in food ingestion and discrete effects on pancreatic islet area, however, when THC is administrated before HCD, modifications are less evident regarding prediabetic mice, except body weight. Similar results were observed in streptozotozin-induced diabetic rats treated with 3 mg/kg/day THC, small reduction in blood glucose after 3 weeks, not significant changes in body weight, and improvements in oxidative stress response, reflected in the insulin-positive cell area.[12,13]

We assume that THC treatment could moderate oxidative and metabolic stress induced by HCD, however, a deeper analysis will be required to determine long-term effects and molecular mechanisms, with detailed analysis of the particular size of islets, and consider potential differences between males and females. Another potential effect of THC could include the secretion and mechanisms of action of intestinal hormones (GLP-1, GIP) that modifies glucose adsorption and metabolism involved in OGTT.[39]

In diet-induced obese mice and Zucker fatty rats, chronic treatment with rimonabant reduced OGTT, correlated with 60% reduction on islet-cell surface and 50% less disorganized islets, food intake, and evoked diet resistance, and another metabolic processes involved in body weight loss.[40–42] In our pDM mice, we assumed THC chronic administration could saturate CB1r in pancreatic islets and maybe in other tissues, such as intestine, liver, muscles, and adipose, and induce a similar improvement in glucose absorption and metabolism.[5] Another possible effect is THC activation of different cannabinoid receptors.[43] CB1r

FIG. 2. **(A)** OGTT in control mice (black), prediabetic mice with vehicle (light gray), treated with 5 mg/kg THC (medium gray), control mice with 5 mg/kg THC (dark gray), and THC-treated mice previous and after prediabetes induction with HCD (blue). **(B)** Area under the curve was analyzed for each group, and **(C)** body weight percent during 3 weeks of treatment. Data are presented as mean ± SEM, $n = 10$ mice per group. *$p < 0.05$ versus control. HCD, hypercaloric diet; OGTT, oral glucose tolerance test.



antagonists, SR141716A and AM251, increased beta cell proliferation in isolated human and mouse islets.[44] CB1r and CB2r agonists at high concentrations enhance GSIS,[45] as well as THC at 20 μM increases insulin secretion.[10]

In isolated pancreatic islets, we evaluate the effects of THC on insulin secretion, gene expression, cAMP generation, and intracellular calcium changes, compared with well-known actions of AEA as CB1r agonist, and AM251 as CB1r inverse agonist. THC increased

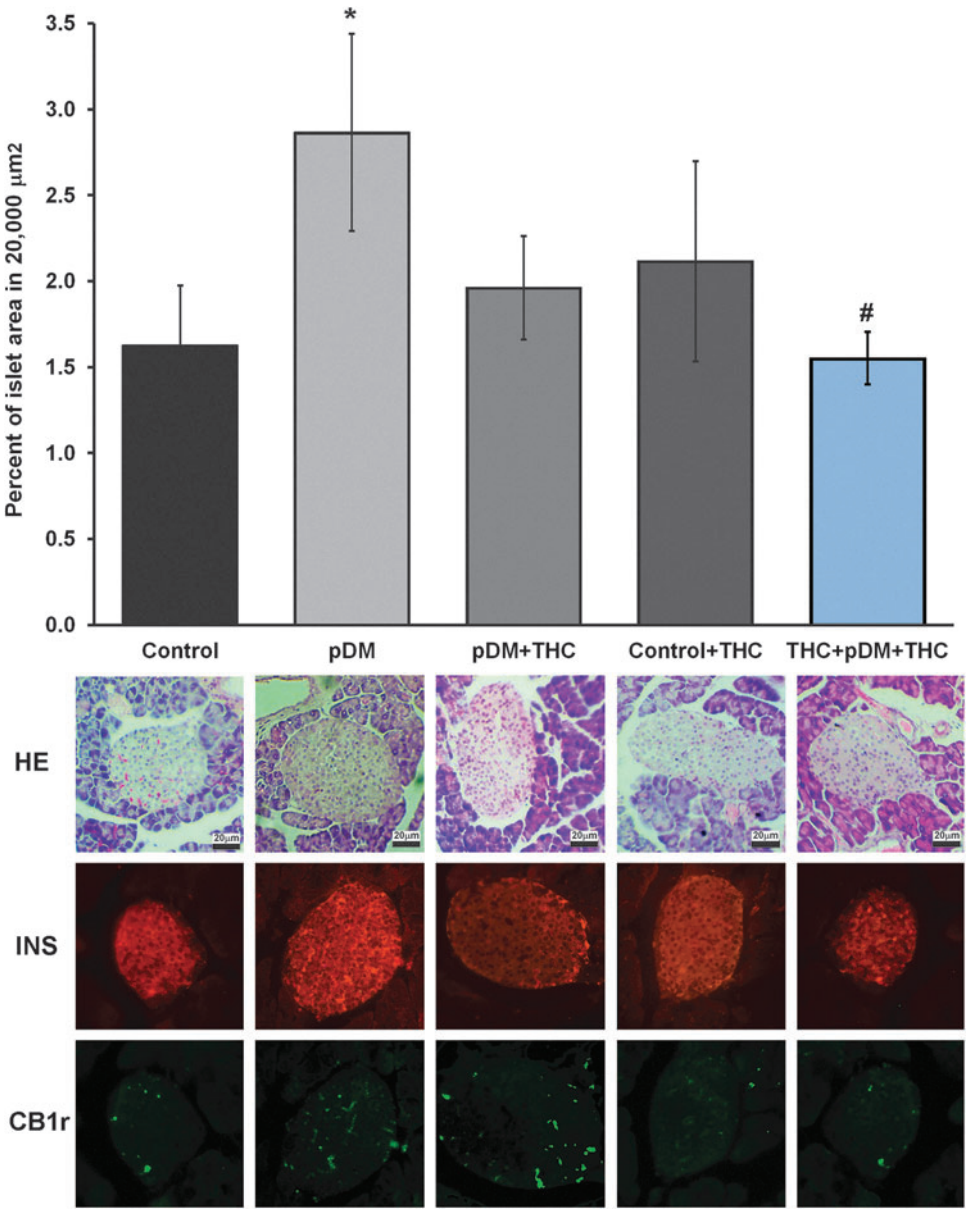


FIG. 3. Histological analysis of pancreas obtained from control mice (black bar), prediabetic mice with vehicle (light gray); prediabetic with 5 mg/kg THC (medium grey); control treated with THC (dark gray), and prediabetic with previous administration of THC and 3 weeks of treatment (blue bar). H&E and immunofluorescence are represented from 10 different cuts at 5 μm. Relative area was determined from 10 different observations under 20,000 μm^2, presented as mean ± EM; $n = 6$ mice per group. *$p < 0.05$ versus control. #$p < 0.05$ versus pDM. pDM, prediabetes mellitus.

basal insulin secretion, as previously reported for AEA and AM251 under similar conditions.[10–13,33,37] As an orexigenic factor, it is expected that THC increases insulin secretion probably as an indirect action on glucagon secretion.[33,37,42,45] After high-calorie meals, endocannabinoid synthesis rates are increased in humans and rodents, along with changes in islet structure.[4,41,42] It is possible that our diet-induced prediabetic model promotes a local ECS overstimulation, with altered cannabinoid receptor activity and expression; THC administration could modify this physiological adaptation through interactions as CB1r partial agonist and with other receptors.

Previous reports have demonstrated the close relationship among ECS, glucose metabolism, and gene expression in pancreatic islets. Glucose metabolism in islets is indirectly mediated by CB1r through interaction with insulin receptor signaling, regulating the expression of insulin, glucokinase, and GLUT2.[1,2,33,37,43] In pDM mice, we observed that THC decreased insulin and PDX-1 mRNA, but increased CB1r, opposite effects to AEA and AM251. Considering that ECs can be synthesized in islets during high-fat/sugar feeding and in diabetic patients,[4,12,13,41,42] THC treatment could induce structural and physiological changes in islets, overthrowing the activity and saturation of the local ESC. In future, we will evaluate THC effects on alpha cells, and implications in glucagon secretion.

Hormones like glucagon and GLP-1 promote the increase of cAMP in beta cells, to potentiate insulin secretion in response to glucose (GSIS). Previous evidences have demonstrated that CB1r activation inhibits adenylate cyclase (AC), therefore, cAMP generation is decreased and GSIS.[15–17,21–23] In isolated islets from healthy mice, AEA, AM251, and THC treatments increased cAMP, probably as a paracrine effect on the alpha cell, enhancing glucagon secretion by stimulation of different cannabinoid receptors.[1,37,45] Another explanation is, under certain circumstances of oversaturation, CB1r can be coupled to Gαs, leading to AC activation and increase in GSIS.[46–48] Independently of glucose metabolism and CB1r binding, AEA, AM251, and THC can interact with different receptors, like GPR55, coupled to the activation of AC or inactivation of phosphodiesterase. This receptor can be related to Gα13 or Gq, and is activated by AM251 and synthetic CBD analogs, even THC has greater affinity as agonist (Emax¼ 92%) compared with CB1r or CB2r (Emax¼ 61 and 67%, respectively).[24,26–29]

Changes in intracellular calcium concentration highly contribute to insulin secretion, meanwhile cAMP and protein kinase A (PKA) activity have a regulatory role to potentiate glucose stimuli. Dispersed islet cells, from healthy or pDM mice, decreased intracellular calcium and enhanced nifedipine inhibition after AEA exposure; CB1r antagonist and GPR55 agonist AM251 significantly increased calcium currents and moderated nifedipine effect.

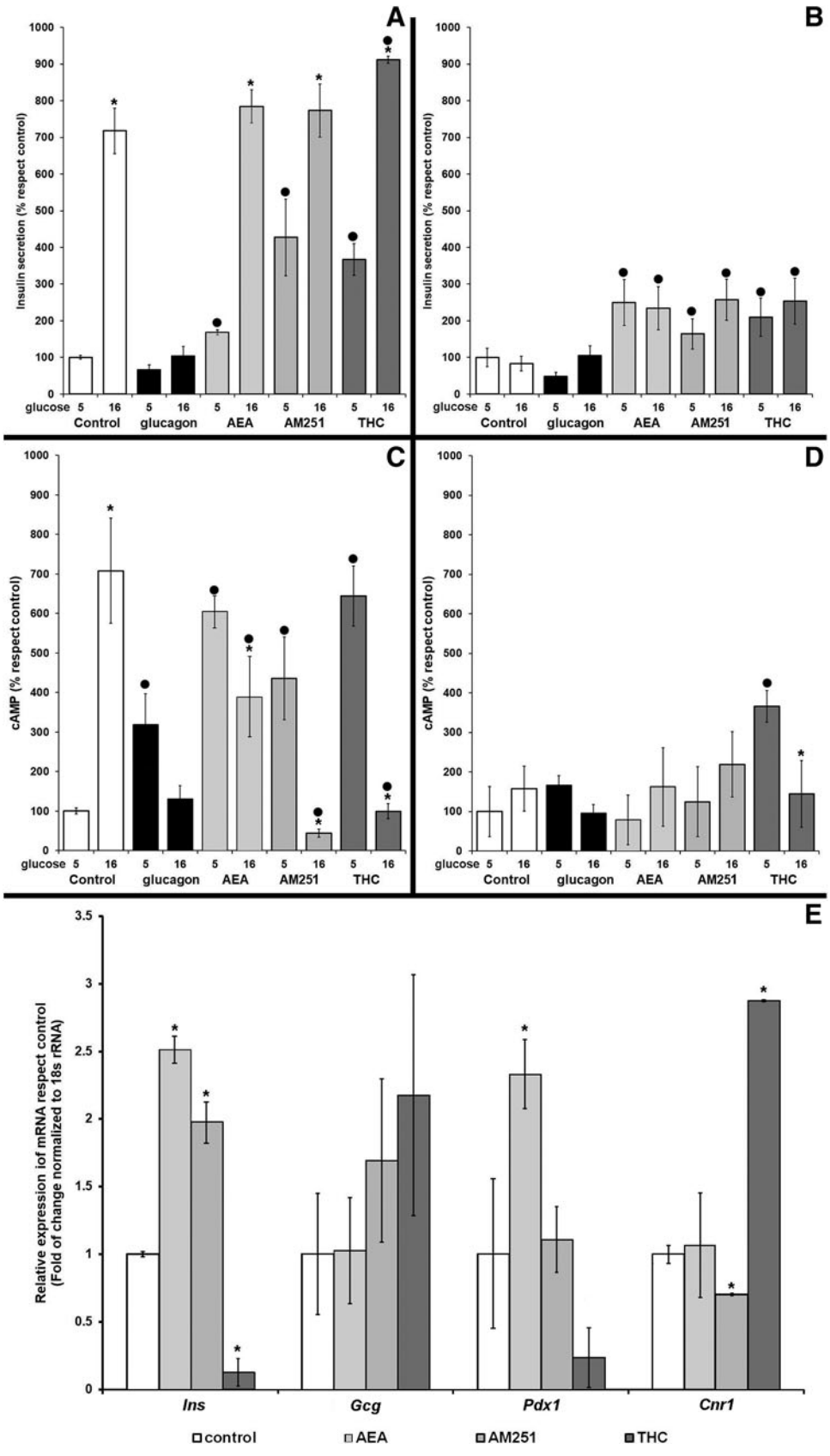
These results are in concordance with previous reports involving calcium channels with cannabinoid receptor activity and the variable results in islet cells[16,17,19,20,49,50]: AEA interacts with CB1r coupled to Gi/o,Gαi, inhibits voltage-gated L-, N-, and P/Q-type calcium channels and reduce intracellular oscillations in 54% of isolated beta cells, but in intact islets produce significant increase in calcium current. AM251 can induce calcium changes as CB1r antagonist and as GPR55 agonist. In MIN6 cells, CB1r and CB2r are coupled to elevations in calcium to potentiate GSIS, but inhibit cAMP generation; in RINm5F cells, CB1r and TRPV1 antagonists elevate intracellular calcium-activating ion channels in the membrane.

Cells from dispersed islets, isolated either from healthy or pDM mice, presented a significant inhibition of calcium release after THC exposure, and enhanced nifedipine inhibitory effect. We assume that THC evoked a strong agonist effect in CB1r and saturation of GPR55, inducing increase in cAMP and insulin secretion, by an independent calcium mechanism.

Conclusion

THC treatment induces small changes on clinical parameters in prediabetic mice. In isolated islets from lean and under HCD, acute THC exposure modifies GSIS and gene expression, involving signaling mechanisms by cAMP and intracellular calcium.

FIG. 4. GSIS and cAMP content in isolated islets from healthy mice **(A, C),** and prediabetic mice **(B, D)**. Islets were treated with vehicle (white bar), 100 pM glucagon (black), 1 μM AEA (light gray), 10 μM AM251 (medium gray), and 10 μM THC (dark gray bar). **(E)** Gene expression of insulin, glucagon, Pdx-1, and CB1r mRNAs, in isolated islets from control mice, treated with AEA, AM251, and THC. Data are presented as mean ± SEM, $n = 3$ of groups of 5 islets for secretion, 50 for cAMP and qPCR. *$p < 0.05$ versus 5 mM glucose, black dot versus control group. AEA, anandamide; cAMP, cyclic adenosine monophosphate; CB1r, CB1 receptor; GSIS, glucose-stimulated insulin secretion.



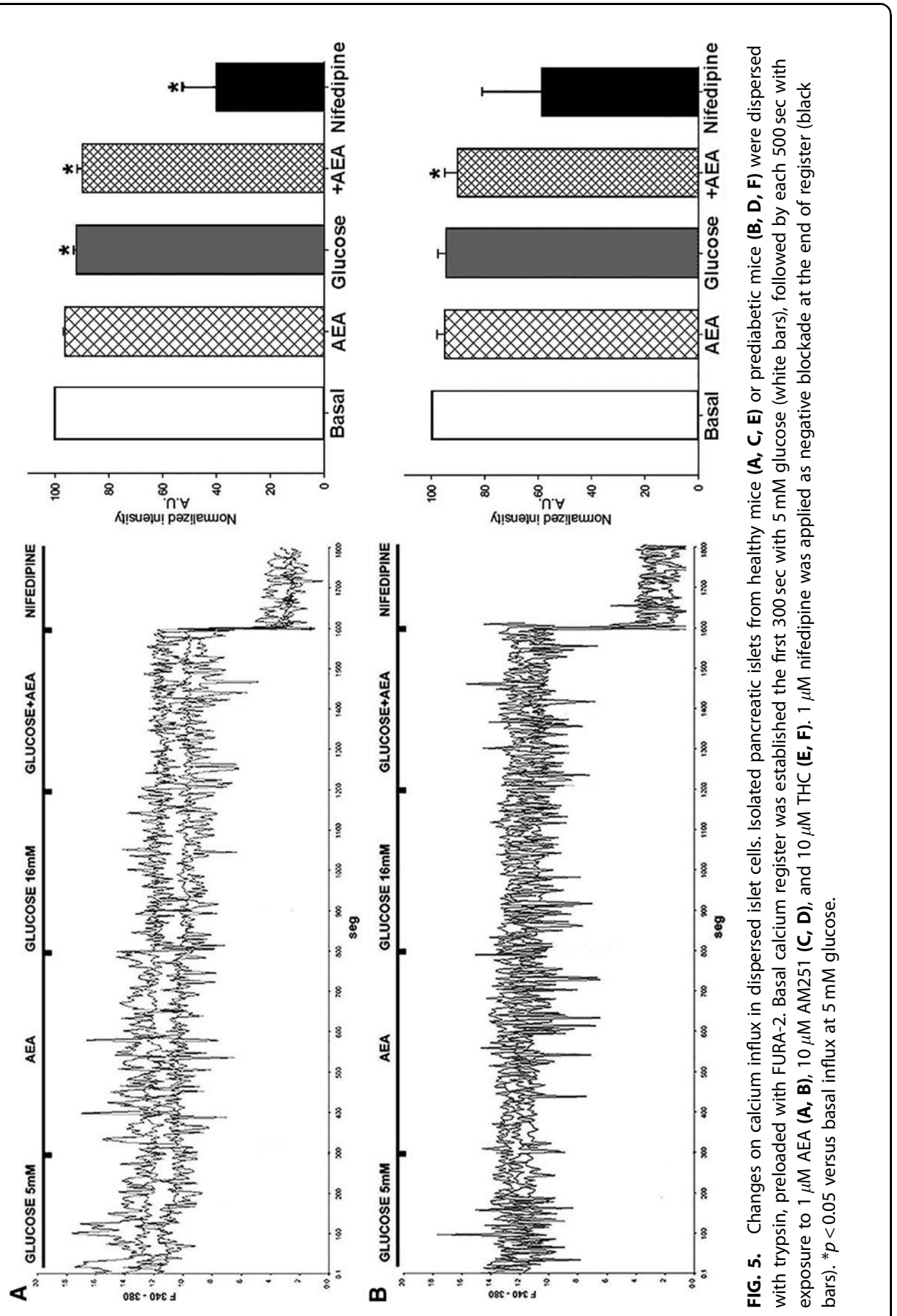


FIG. 5. Changes on calcium influx in dispersed islet cells. Isolated pancreatic islets from healthy mice **(A, C, E)** or prediabetic mice **(B, D, F)** were dispersed with trypsin, preloaded with FURA-2. Basal calcium register was established the first 300 sec with 5 mM glucose (white bars), followed by each 500 sec with exposure to 1 μM AEA **(A, B)**, 10 μM AM251 **(C, D)**, and 10 μM THC **(E, F)**. 1 μM nifedipine was applied as negative blockade at the end of register (black bars). $^*p < 0.05$ versus basal influx at 5 mM glucose.

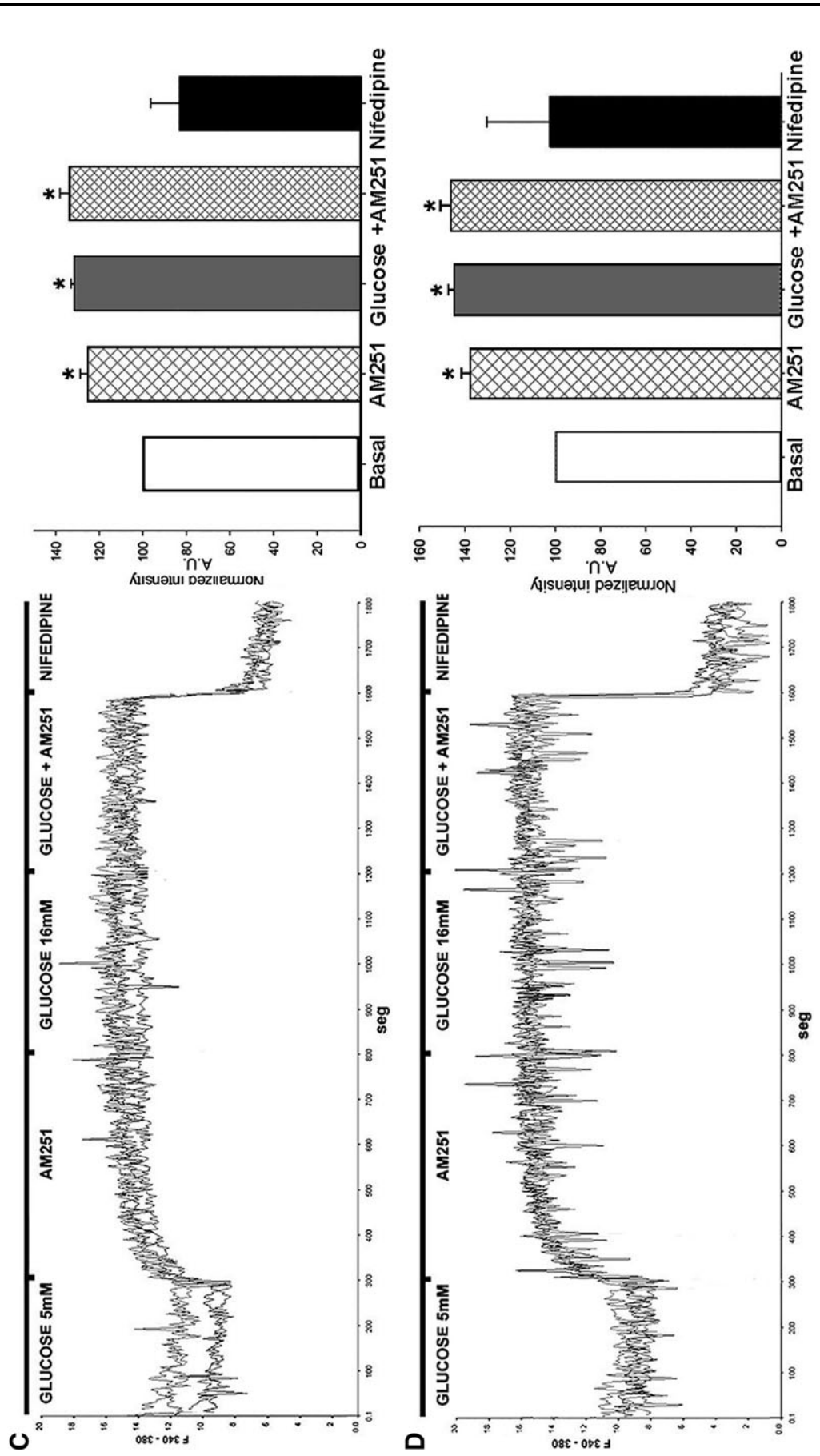


Fig. 5. (*Continued*).

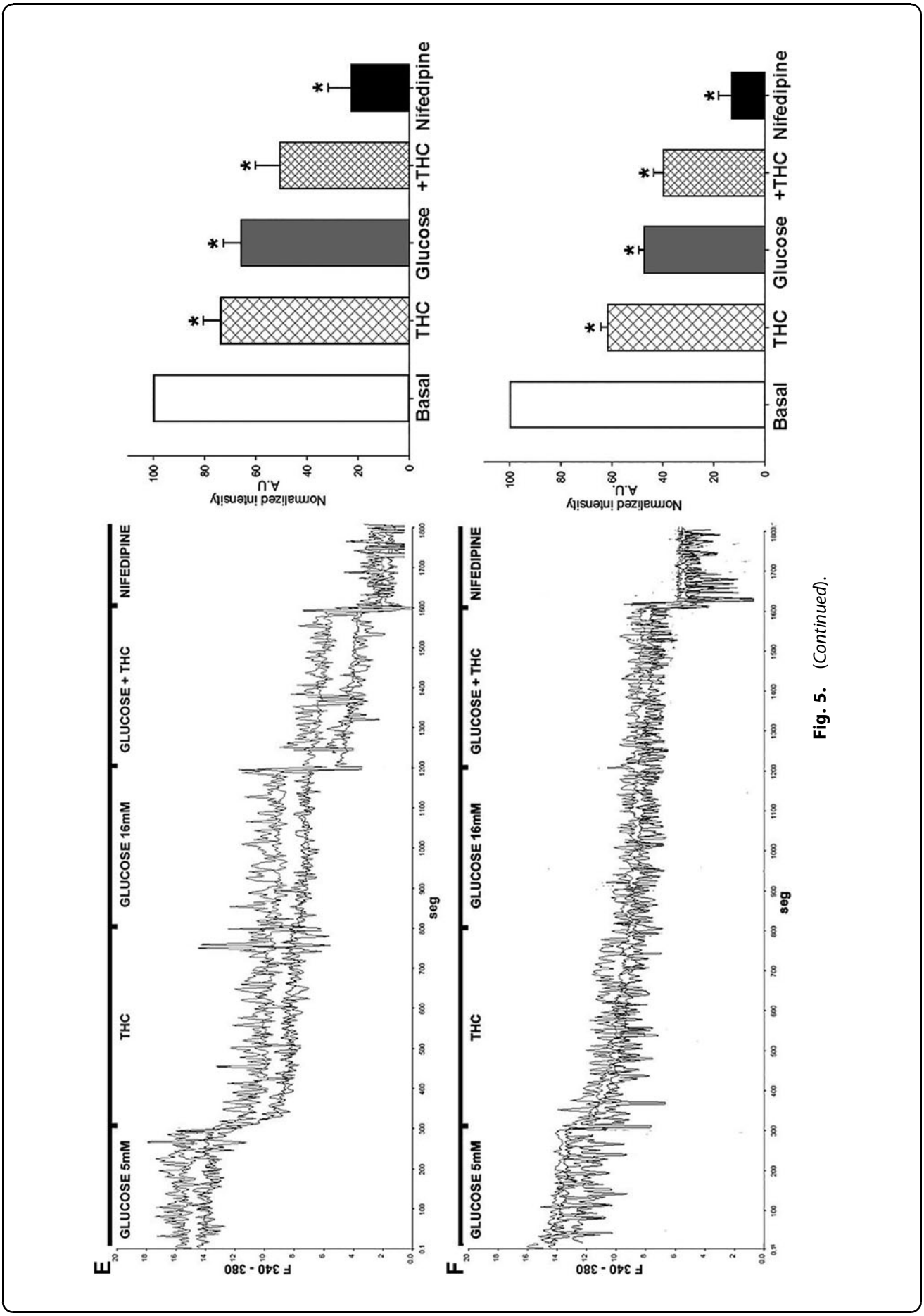


Fig. 5. (*Continued*).

Authors' Contributions

Experimental procedures, data analysis, and draft writing: G.M.G.-L., J.D.B.-C., S.H.-C., S.H.G.-R., and J.O.S.-O. Study design, funding, article writing and review: G.M.G.-L., D.D.-U., A.V.V., R.V.-M., and A.V.-F.

Author Disclosure Statement

All authors declare no conflicts of interest. Original data are available anytime through the corresponding author.

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Abbreviations Used

AEA = anandamide
ANOVA = analysis of variance
cAMP = cyclic adenosine monophosphate
CB1r = cannabinoid receptor 1
CB2r = cannabinoid receptor 2
CBD = cannabidiol
DMEM = Dulbecco's modified eagle medium
ECS = endocannabinoid system
ERK = extracellular signal-regulated kinase 1/2
GPR55 = G-protein-coupled receptor 55
GSIS = glucose-stimulated insulin secretion
HCD = hypercaloric diet
OFT = open field test
OGTT = oral glucose tolerance test
pDM = prediabetes mellitus
Pdx-1 = pancreatic homeobox transcription factor 1
THC = Δ9-tetrahydrocannabinol