Adiponectin Reverses β-Cell Damage and Impaired Insulin Secretion Induced by Obesity

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Abstract

Obesity is known to impair β-cell insulin secretion, an effect typically related to damage promoted by nutrient overload in prior studies. Here, we show that diluted plasma from obese male and female human donors acutely impairs β-cell integrity and insulin secretion relative to plasma from lean subjects, in the absence of nutrient overload. Similar results were observed with diluted sera from obese rats fed *ad libitum*, when compared to sera from lean, calorically-restricted, animals. This suggests that β-cell function is affected by changes in circulating, non-nutrient, factors in obese blood. We demonstrate that increased levels of adiponectin, as found in the lean plasma, protect β-cell function, surprisingly even in the absence of other serological components. Mechanistically, this protection involves the preservation of glucose-supported mitochondrial oxidative phosphorylation and ATP production, which is completely abrogated in the absence of this hormone. Furthermore, the addition of adiponectin, at concentrations seen in lean individuals, to cells treated with plasma from obese donors completely restored β-cell integrity and glucose-stimulated insulin secretion. Our results demonstrate that adiponectin, at levels circulating in lean individuals, has highly robust protective effects on β-cells, supporting physiological insulin secretion and protecting against damage promoted by obese plasma, thus indicating a new target pathway for the protection against β-cell dysfunction.

Keywords: adiponectin, β-cell, diabetes, obesity, oxidative phosphorylation

Introduction

About half a billion persons currently live with type 2 diabetes mellitus, and this number is expected to increase by 51% until 2045 [1] as a result of population aging and rising obesity rates. This chronic disease is characterized by an inability to control blood glucose homeostasis, either by a decrease in insulin action (insulin resistance) or inadequate insulin release by pancreatic β-cells. β-cells are susceptible to damage caused by excess circulating glucose and lipids (glucolipotoxicity), which is common in obese individuals. Indeed, a high calorie obesogenic diet, favoring glucolipotoxic conditions, is associated with type 2 diabetes and increased mortality in humans and rodents [2], while caloric restriction (CR), preventing nutrient overload, is one of the most studied dietary interventions known to extend health and longevity [2-6].

In a previous study [7], we found that circulating factors in the sera from lean CR rats were highly protective against nutrient overload (glucolipotoxicity) for pancreatic islets, primary β cells, and insulin-secreting cell lines when added as a 10-fold diluted part of the incubation media, substituting commercial culture sera. Glucolipotoxicity leads to β-cell dysfunction in a manner dependent on changes in mitochondrial structure and function [8]. Indeed, mitochondria are central players in the loss of β-cell function related to type 2 diabetes [9]; in addition to their role regulating intracellular ATP production in response to glucose levels (and consequent insulin secretion), changes in mitochondrial genetics, bioenergetics, redox state, structure, and dynamics are involved in β-cell functional regulation [9]. During glucolipotoxicity, extensive mitochondrial fission occurs, promoting a fragmented and poorly interconnected mitochondrial phenotype and causing decreased mitochondrial ATP synthesis and β-cell dysfunction [7,10]. Inhibiting this fragmentation prevents the loss of cell viability, demonstrating that the change in mitochondrial morphology and dynamics is causative of β-cell dysfunction [8]. Indeed, the protective effects of diluted CR sera on β-cells are related to changes in mitochondrial dynamics and function, as it increases mitochondrial networking and respiratory rates, without overt changes in mitochondrial mass [8]. CR sera also increases the expression of proteins involved in mitochondrial fusion (mitofusin-2, Mfn- 2; optic atrophy 1, OPA-1), and reduces those involved in

mitochondrial fission (dynamin related protein 1, DRP-1), a result consistent with the presence of a more filamentous and interconnected mitochondrial network [7].

While previous work demonstrated the intracellular mechanisms in which CR sera promotes the preservation of β-cell function under conditions of nutrient overload, the molecular factors from CR sera involved in this protection have not yet been identified. The dilution of the sera as well as the fact it was collected from overnight-fasted animals and used in the presence of nutrient-rich culture media implies that changes in the amounts of substrates such as glucose, fatty acids, and amino acids are not involved in these results. Rather, the effects are most likely associated with hormones or other circulatory signaling components.

Here, we investigated possible β-cell-protective factors in the sera of lean rats and humans, and identified adiponectin as a strong promoter of mitochondrial oxidative phosphorylation, insulin secretion, and β-cell preservation. Strikingly, adiponectin alone was able to promote β-cell function in the absence of any other serological component. It was also able to protect against β-cell dysfunction promoted by incubation with the serum or plasma from obese subjects, indicating a new therapeutic potential for this pathway in the prevention of insulin secretion failure in obesity.

Experimental procedures

Animals, diets and serum collection

All experiments were approved by the animal use committee (*Comissão de Ética em Uso de Animais do Biotério de Produção e Experimentação da Faculdade de Ciências Farmacêuticas e Instituto de Químcia da USP* #109/18). Male 8-week-old Sprague Dawley rats were divided into two groups: AL, fed *ad libitum* with an AIN-93-M [11] diet prepared by Rhoster (Campinas, Brazil) and CR rats, fed 60% of the AL group's intake, using a diet from the same supplier supplemented with micronutrients to reach the same vitamin and mineral levels [4]. The animals were housed in groups of three per cage in 12 h light/dark cycles and given water *ad libitum*. The weight of the animals and food intake were recorded weekly to adjust the CR group diet to 60% of the intake of the AL group. At 34 weeks of age (after 26 weeks of the diet), rats were euthanized after 12 h fasting, blood was collected by cardiac puncture, and serum was obtained after clotting at room temperature for 30 min and centrifugation for 20 min at 300 x g. The supernatant was collected and stored at -20°C. Serum samples were thawed and heat-inactivated at 56°C for 30 min prior to use.

Pancreas histology

Whole pancreases from AL and CR animals were removed and immersed in 20 mL of 10% formalin for 24 hours. The tissue was then PBS-washed and dehydrated through a series of graded ethanol solutions (70, 80, 95 and 100%), followed by a clearing process with xylol, and finally embedded into paraffin blocks. The paraffin-embedded tissues were sectioned (5 µm thick) with a semi-automated rotary microtome (Leica Microsystems, Wetzlar, Germany) and placed on microscope glass slides coated with poly-L-lysine. Slides were stained with hematoxylin and eosin (H&E) and scanned using the TissueFAXS iPLUS (TissueGnostics, Wien, Austria) system under a magnification of 20. Three areas of each slide were blindly analyzed by two independent examiners using the ImageJ Fiji Software. Islets were categorized according to areas into very small (<1000 μm^2), small (1000-5,000 μm^2), medium (5,001-10,000 μm^2), large (10,001-50,000 μm^2) or very large (>50,000 μm^2).

Human plasma samples

Human plasma samples were obtained from the A.C. Camargo Cancer Center Biobank, and all experiments were carried out in accordance with the A. C. Camargo Cancer Center Institutional Review Board under registration nº. 3117/21. Samples include healthy donors who signed a free and informed consent form and authorized the institution to store and use their biological material for future studies. For the selection of research participants, data from medical records were examined and a balance in gender, age, eating habits, physical activity, and height was sought, separating subjects into lean and obese groups according to their body mass index (BMI). Subjects with pathologies, elevated blood pressure, smokers, regular alcohol consumption, sexually transmitted diseases, bariatric surgery and chronic diseases were eliminated (Table 1). The overall selection includes patients of both genders, equal in all quantifiable parameters except BMIs, producing four distinct groups: Lean women (BMI 22.0 ± 0.9, n=4), Obese women (BMI 31.0 ± 1.4, n=6), Lean men (BMI 23.3 ± 0.4, n=8) and Obese men (BMI 31.2 ± 1.4, n=10). Their blood was collected in a sterile vacuum tube containing 4.45 mmol/mL of the anticoagulant ethylenediaminetetraacetic acid dipotassium salt (EDTA). The blood was then centrifuged at 300 x g and 4ºC for 20 min. The supernatant was collected, heat-inactivated at 56°C for 30 min and stored at -20°C until use. Sample analysis and cellular stimuli were performed with the pooled samples from the four groups mentioned.

Cell cultures and incubations

INS-1E cells (a rat insulin-secreting β-cell line) were cultured with 100 IU/mL penicillin/streptomycin in RPMI-1640 medium (11.1 mM glucose, 10% bovine serum, 1 mM pyruvate, 10 mM HEPES, 2 mM glutamine and 0.1% β-mercaptoethanol) at 37°C and 5% CO_2. Plating was done at 60,000 cells for all experiments. After 24 h, media was substituted for RPMI-1640 containing 10% fetal bovine serum (FBS), 10% serum from AL or CR animals; or 10% inactivated plasma from human volunteers. All experiments were performed 24 h after this medium change. In adiponectin supplementation experiments, cells were incubated with medium containing 10 µg/mL recombinant human adiponectin (SRP4901, Sigma-Aldrich), in the absence or presence of other serum or plasma, as indicated.

Cellular oxygen consumption

On the day of the experiment, cells were incubated in 500 μL RPMI-1640 without HEPES or FBS, containing 11.1 mM glucose for 1 h at 37°C, without CO_2. During these 60 min, the ports of the cartridge containing the oxygen probes were loaded with the compounds to be injected during the assay (75 μL/port). The 24-well plate was then introduced into the Seahorse Bioscience XF24 analyzer (Billerica, MA, USA). Oxygen consumption was recorded for 30 min, at 5 min intervals, until system stabilization. Oligomycin was then injected at a final concentration of 1 μM, followed by carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP) used at a final concentration of 10 μM, and antimycin (AA) and rotenone, both used at final concentrations of 2 μM. All respiratory modulators were used at optimal titrated concentrations, determined in preliminary experiments.

Western blots

Serum and plasma samples were diluted in Laemmli buffer at a concentration of 1 μg/μL (human samples) or 7 μg/μL (animal samples), and proteins were separated using a 12% denaturing polyacrylamide gel. Proteins were transferred to nitrocellulose membranes and incubated with anti-adiponectin antibody diluted 1:1000 (ab22554, Abcam). Ponceau staining throughout the membrane was used as a loading control. Fluorescent Secondary Anti-Rabbit Antibody diluted 1:10000 was added to the membranes and bands were visualized using an Odyssey infrared system. Bands were semi-quantified by densitometric analysis using ImageJ software.

Cultured cell insulin secretion

The cells were plated as described above, and 24 h later they were incubated with different serum or plasma samples, as indicated. After 24 h, they were pre-incubated for 30 min in Krebs-Henseleit (KH) solution containing 0.1% albumin and 5.6 mM glucose. Then, they were incubated for 1 hour at 37ºC in the presence of 5.6 mM, 11.3 mM or 16.7 mM glucose. The supernatant was collected and stored at -20ºC for subsequent measurements of secreted insulin. In addition, cells were lysed with acid-alcohol solution (52 mL ethanol, 17 mL water, 1 mL hydrochloric acid) to disrupt the cells and collect the intracellular insulin content.

The determination of the amounts of secreted and intracellular insulin was performed following the Elisa Insulin Quantitation Kit protocol (Milipore, Billerica, MA, USA). Glucose-stimulated insulin secretion (GSIS) was calculated by dividing the concentration in the supernatant (secreted) by the remaining intracellular insulin (content).

Pancreatic islet isolation and insulin secretion

Male Wistar rats (10 to 12 weeks) were deeply anesthetized with ketamine and xylazine, followed by decapitation. The abdomen was dissected, and the pancreas was inflated with 20 mL collagenase type V (0.7 mg/mL) in KH buffer. After full inflation, pancreases were removed and incubated for 25 min at 37ºC, shaken manually, washed with KH buffer, and centrifuged three times at 1,000 rpm for 5 min. Islets were collected with a micropipette under a stereomicroscope and cultured for 24 h in RPMI-1640 medium containing 10 mM glucose, 1% penicillin/streptomycin and 10% FBS before receiving treatments. After 24 h, islets were randomly divided into wells under different conditions in 10 mM glucose RPMI: without FBS, with FBS, with 10 µg/mL adiponectin, or with FBS + adiponectin. After the 24 h incubation, the media were collected to check for insulin release over 24 hours, as well as LDH release (see below).

Islets were also checked for acute GSIS. Batches of 5 islets were collected in fresh tubes containing KH buffer with 5.6 mM glucose and incubated at 37ºC for 30 min for stabilization. Supernatants were discarded and replaced by KH buffer with low (5.6 mM) or high (16.7 mM) concentrations of glucose and incubated at 37ºC for 60 min. Insulin release in the medium over 24 hours and in the supernatant after acute stimulation with glucose was measured blindly by the Provet Institute (São Paulo, Brazil), by radioimmunoassay. Insulin concentrations are expressed as ng/mL.

LDH quantification

Cultured INS-1E cells were plated and after 24 h treated with 10% plasma from lean and obese men and women for an additional 24 h, with or without 10 µg/mL of recombinant human adiponectin. Rat islets were incubated in 10 mM glucose for 24 h, with or without FBS and adiponectin, as described above. The culture medium supernatant was collected for lactate dehydrogenase (LDH)

quantification, an enzyme that is released into the medium when there is damage to the plasma membrane. LDH activity was measured colorimetrically measuring NADH absorbance, following the protocol of a commercial quantification kit (Labtest, Lagoa Santa, MG, Brazil).

Dada analysis

GraphPad Prism 7 was used for statistical evaluations. Data were expressed as means ± standard error of the mean (SEM) and statistically analyzed by unpaired Student's t-tests or one-way ANOVA tests, with Tukey posttests. The minimum limit of significance was $p < 0.05$.

Results

In order to investigate the effects of circulating factors in the sera from lean versus mildly obese rats, we established a colony of animals in which a 60% caloric restriction (CR) diet, enriched with micronutrients to avoid malnutrition [4], was introduced in early adulthood. These animals were compared to *ad libitum* (AL)-fed animals, which develop obesity, insulin resistance and other characteristics of the metabolic syndrome over time [12]. Fig. 1A shows that the animals on the CR diet gained significantly less weight over the course of 15 weeks, but did not lose mass (which, if present, could be indicative of malnutrition). At the end of the intervention, the pancreases from both groups were collected, stained, and their islets quantified. We observed that pancreas from CR rats displayed an increased percentage of large islets compared to AL (Fig. 1B, $p = 0.024$), without changes in total islet area and average circularity (not shown). This is a moderate change in islet morphological distribution, compatible with the fact that the animals were obese, but not diabetic.

Next, sera from both groups were collected to be used on cultured INS-1E β-cells, under physiologically relevant conditions, and in the presence of glucose as a substrate. As β-cell maintenance and its role in insulin secretion are dependent on oxidative phosphorylation [9], we tested the effects of culture media containing 10% serum from CR versus AL animals on oxygen consumption rates (OCR) in intact β-cells (Fig. 1C), measured by extracellular flux analysis. We found that 24 hours incubation with sera from lean CR animals results in higher OCRs relative to sera from obese AL animals, and also results in higher OCRs relative to commercial fetal bovine serum (FBS). Interestingly, this increase in mitochondrial electron transport rates occurs under physiological culture conditions, without nutrient overload [7], and indicates that lean animal sera have factors that not only protect against damage, but enhance baseline β-cell metabolism.

Enhanced OCRs were observed under basal conditions (Fig. 1D), which reflect normal mitochondrial oxygen consumption before the addition of any modulator (in traces such as shown in Fig. 1C), as well as conditions in which OCRs were maximized by uncoupler FCCP (Fig. 1E), which reflect the limits of mitochondrial electron transport chain capacity. Finally, CR sera increased ATP-linked OCR, which represents the difference between oxygen consumption under basal conditions and in the presence of ATP synthase inhibitor oligomycin (Fig.

1F), and is directly associated with ATP production and insulin secretion in these cells [7,8]. Overall, these findings confirm that serum from lean animals enhances oxidative phosphorylation activities in β-cells, an effect known to be linked to glucose-stimulated insulin secretion.

As the effects of sera from lean animals are seen with 24 h incubations, they suggest the exciting idea that β-cell function, and hence their dysfunction in type 2 diabetes, can be acutely altered by the presence of circulating factors in the blood. Given the importance of this concept, we evaluated if the same was seen with human circulating factors. Human plasma samples stored in the Biobank of the *A. C. Camargo Cancer Center* were used. Although differences in clotting factors and nutrients exist between plasma and serum samples [13-15], human plasma samples from healthy donors were readily available in the Biobank, together with extensive metabolic and nutritional information (Table 1), allowing for more directed sample selection. Importantly, circulating hormonal factors do not differ much between plasma and serum samples (e.g. leptin [16]). Selected donors did not present chronic health conditions, smoke, or drink alcohol. They were within the same age range (Table 1), but were clearly distinct in body mass indexes (BMI), which separated them into lean and obese groups: lean women (BMI 22 ± 0.9, Fig. 2B), obese women (BMI 31.0 ± 1.4), lean men (BMI 23.2 ± 0.3), and obese men (BMI 31.2 ± 1.3).

We measured the effects of diluted inactivated human plasma on β-cell metabolic fluxes in Fig. 2A. Typical OCR traces (Fig. 2A) were conducted under the same conditions as Fig. 1, and basal (Fig. 2B), maximal (Fig. 2C) and ATP-linked (Fig. 2D) OCRs were again calculated. Samples incubated in the presence of plasma from lean women presented metabolic fluxes similar to those incubated in commercial FBS. On the other hand, plasma from obese women significantly suppressed basal, maximal, and ATP-linked OCRs. Plasma samples from lean men also resulted in lower oxygen consumption compared to women's samples, and OCRs were further suppressed in cells incubated with plasma from obese men. Overall, these results show a clear modulatory effect of circulating blood factors on metabolic fluxes in β-cells, which are stimulated by factors present in samples from lean and female subjects.

We were interested in identifying the circulating blood factors responsible for these robust effects on β-cell metabolic responses. In peripheral tissues and

vascular cells, enhanced mitochondrial electron transport capacity promoted by CR has been linked to adiponectin-activated eNOS signaling [17]. Indeed, adiponectin is a hormone that modulates numerous metabolic processes, and is secreted by the adipose tissue in a manner increased by low body weights [18] and decreased by central adiposity [19]. We quantified adiponectin in our rat serum samples (Fig. 3A), and found that the hormone was significantly increased in CR serum compared to AL. Indeed, prior work [20] has shown that animals under caloric restriction show a two-fold increase in circulating adiponectin levels. We also quantified adiponectin in human plasma (Fig. 3B) and found that both male and obese donors had decreased adiponectin, also consistent with prior work [19,21]. The levels of adiponectin in the blood therefore closely mirror the metabolic flux effects we observed in β-cells (Figs. 1 and 2).

Given previous findings that adiponectin can increase mitochondrial respiratory activity in vascular cells [17], we investigated the effects of this hormone on β-cell metabolic fluxes (Fig. 4A-D). Interestingly, we found that while robust OCRs are present in FBS, oxygen consumption in these cells was strongly suppressed, reaching undetectable levels, in the absence of serum. Impressively, in the absence of any other serological factor, 10 μg/mL adiponectin (a quantity compatible with levels present in CR sera [20]) promoted metabolic fluxes close to those seen in the presence of full commercial FBS. This shows that adiponectin induces a strong and previously undescribed effect promoting oxidative phosphorylation in β-cells in the absence of any other circulating factor.

Given the strong adiponectin effects on OCRs, we questioned if it could also promote glucose-stimulated insulin secretion (GSIS) in INS-1E β-cells, which are known to depend on the presence of serum [22]. Fig. 4E compares insulin secretion with different glucose concentrations in cells incubated in FBS or adiponectin in the absence of FBS. Our data show that 10 μg/mL adiponectin alone was able to promote GSIS patterns similar to those seen in full serum. Indeed, responses were not significantly different from those seen in FBS controls. This demonstrates that adiponectin´s metabolic effect increasing ATP-linked OCRs is accompanied by the expected increase in insulin release from β-cells. It also confirms that adiponectin is a soluble serological factor with a pronounced impact on β-cell function.

The finding that adiponectin strongly protects cultured β-cells on its own, in the absence of any other serological factor, is highly surprising, and prompted us verify whether primary β-cells are also able to respond to adiponectin. Figures 5A and 5B show measurements of insulin release from primary rat islets in the presence of low and high glucose, respectively. As expected, insulin was secreted in high glucose (Fig. 5B) when FBS was present, but not in its absence. Strikingly, the presence of recombinant adiponectin alone restored islet secretory function, in a manner that was not additive to the presence of FBS (Fig. 5B), indicating that adiponectin itself is the main protective circulating factor.

The lack of insulin release in media with high glucose and no serum is a consequence of islet damage, as indicated by the fact that 24 h insulin secretion (due to β-cell membrane disruption, leading to intracellular insulin release independent of glucose stimulation, Fig. 5C) and lactate dehydrogenase (LDH) release (Fig. 5D) were augmented in islets incubated with no sera. Once again, adiponectin alone was protective against these insults, in a manner that was not additive with the effect of FBS, demonstrating that this hormone is strongly protective toward primary islets, as well as cultured β-cells.

Next, we sought to verify if adiponectin supplementation in the plasma from obese donors was capable of reversing the metabolic flux limitations observed in Fig. 2. We monitored OCRs in samples in which 10 μg/mL adiponectin was added together with the plasma of obese men and women (Fig. 6). Once again, the effect of added adiponectin was very pronounced, stimulating OCRs under all conditions, in both male and female obese plasma-treated β-cells. These results indicate that samples from male and obese subjects do not primarily decrease metabolic fluxes in β-cells due to the presence of damaging circulating molecules, but instead as a result of the lack of the stimulatory factor adiponectin.

Given the strong protective effect on metabolic fluxes, we also tested the effect of added adiponectin on GSIS. In samples incubated for 24 h in plasma from lean women, an expected increase in insulin release was observed with increasing glucose concentrations (Fig. 7A). Incubation in plasma from obese women lead to very high levels of insulin secretion, without any effect of glucose concentrations. This could be due to the presence of ruptured cells, releasing insulin into the supernatant. Indeed, when we measured LDH release (Fig. 7B), we found that the sera of obese women promoted significant cell damage. Strikingly, the addition of

adiponectin to the samples incubated with plasma of obese women completely prevented LDH release (Fig. 7B) and restored functional GSIS (Fig. 7A). Adiponectin also prevented LDH release from cells incubated in plasma from obese male donors (Fig. 7D), and restored expected insulin secretion patterns (Fig. 7C), which were not observed in the plasma from obese male donors alone. Overall, these results demonstrate that the presence of adiponectin is capable of completely abrogating the damaging effects of obese plasma on β-cells.

Discussion

Previous studies by our group found that serum from CR rats diluted in culture media is capable of increasing the expression of proteins involved in mitochondrial fusion (mitofusin-2, Mfn-2; optic atrophy 1, OPA-1) and reducing DRP-1, involved in mitochondrial fission. This results in increased mitochondrial length and connectivity in β-cells, an effect that protects against nutrient overload and promotes glucose-stimulated ATP production and insulin secretion [7]. Knowing that the sera could not contribute significantly to the nutrient pool at the dilution used, we hypothesized that the results were due to the presence of hormonal circulatory components.

Other studies have tested the *in vitro* effects of sera from calorically-restricted animals. In normal human diploid fibroblasts, serum from CR animals delayed senescence and significantly increased longevity compared to serum from AL animals [23]. In human hepatoma cells, serum from calorie-restricted volunteers increased longevity markers such as Sirtuin 1 and PGC-1α, and enhanced stress resistance [24]. In vascular cells, rat CR sera activates the insulin pathway and phosphorylation of endothelial nitric oxide synthase [17], which modulates mitochondrial biogenesis, a process believed to have a central role in the longevity effects of CR [25]. The beneficial effects of CR sera on vascular cells were eliminated when the sera were depleted of adiponectin [17], suggesting this is an important hormonal factor modulating oxidative phosphorylation in lean animals.

In the present study, we not only corroborate the results observed previously, but also shed light on adiponectin as a protective component of sera from lean subjects on β-cells. Indeed, we found that adiponectin itself (even in the absence of sera) is a strong determinant of β-cell metabolic fluxes and GSIS, in both cell lines and primary rat β-cells. Additionally, supplementation of this hormone reverses the damaging effects of obese human plasma on β-cells, demonstrating that its low levels, rather than the presence of damaging signaling molecules, are the reason for the β-cell damage.

Adiponectin acts on cells by binding to adiponectin receptors and modulating the phosphorylation of protein kinase B (AKT) [14,17,26]. In pancreatic

β-cells, adiponectin regulates insulin gene expression, in addition to increasing cell viability and decreasing apoptosis via AKT phosphorylation [27], results compatible with the protective effects of adiponectin we observed in this study. Consistently, circulating adiponectin is lower in individuals with type 2 diabetes compared to healthy individuals, and well as those with risk factors such as obesity [28-32]. Furthermore, transgenic mice overexpressing human adiponectin and fed a high-fat/high-sucrose diet present increased longevity as well as reduced morbidity and mortality [33]. These animals also showed reduced body weight, and less accumulation of subcutaneous and visceral fat, with smaller adipocytes in both tissues. This, added to an increase in oxygen consumption associated with equal caloric consumption compared to control animals, suggests an increase in energy expenditure [33]. On the other hand, adiponectin-deficient animals exhibit higher body mass, impaired glucose tolerance, and more triacylglycerol accumulation than control animals [34-37]. Adiponectin also acts through the hypothalamus to inhibit appetite and increase energy expenditure [38,39], promoting fatty acid oxidation in muscle and liver, and leading to weight loss [40,41]. Overall, these studies indicate that this hormone is a major regulator of energy metabolism in various organs, and are in line with our added finding of its strong metabolic and functional effects directly on β-cells.

In addition to adiposity, gender also affects the amount of adiponectin, with more of this hormone circulating in women than in men [32,42-45]. The reduction in men appears to be linked to the presence of higher concentrations of testosterone, as this hormone reduced adiponectin concentrations in mice, and castration induced an increase in plasma adiponectin associated with a significant improvement in insulin sensitivity [42]. In fact, although obesity is more common in women, type 2 diabetes is more often diagnosed at a lower age and with lower body mass indexes in men [1,46,47]. It is tempting to speculate that this may be related to the lack of adiponectin and its β-cell-protective effects, as uncovered in this study.

Conclusions

We show that adiponectin, at quantities present in the blood of lean animals and lean women donors, is a strong stimulatory factor necessary to maintain metabolic fluxes and physiological insulin secretion in β-cells. The presence of adiponectin alone, in the absence of any other serological factor, sustains ATP-linked respiration and associated glucose-stimulated insulin release in primary and cultured β-cells. Addition of adiponectin to plasma-supplemented media also rescues β-cell function compromised by incubation with samples from obese donors. Overall, our results suggest this hormone, its receptors, and the signaling pathways it activates are robust potential targets for treatment in obesity-related disruption of glucose-stimulated insulin secretion.

Conflict of interest

The authors declare no conflicts of interest.

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Data sharing

Raw data will be provided upon request.

Abbreviations

Antimycin A (AA); *ad libitum* (AL); adiponectin (Adipo); body mass index (BMI); calorie restricted (CR); carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP); ethylenediaminetetraacetic acid dipotassium salt (EDTA); fetal bovine serum (FBS); glucose-stimulated insulin secretion (GSIS); lactate dehydrogenase (LDH); oxygen consumption rate (OCR); standard error of the mean (SEM)

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Table 1: Plasma Donor Characteristics

Group	Age (years)	Systolic blood pressure	Diastolic blood pressure	Height (m)	Weight (kg)	BMI (kg/m²)	Bariatric surgery	Physical activity	Eating habits	Tobacco use	Alcohol use	STDs	Total hysterectomy	Hepatitis B (HBsAg)
Lean women	43	118	73	150	45	20	No	Sedentary	Diet high in vegetables, fiber and white meat	No	Drinks socially	No	No	No
Lean women	29	110	70	168	60	21	No	Walking (>5 hours/ week)	Diet high in red meat and fat, few vegetables	No	No		Yes	No
Lean women	33			165	62	23	No	Sedentary	Diet high in vegetables, fiber and white meat	No	No	No	No	
Lean women	36	148	88	170	70	24	No	Sedentary	Diet high in vegetables, fiber and white meat	No	No	No	No	No
Averages Lean Women	**35.2 ± 3.0**	**125 ± 10**	**77 ± 5**	**163 ± 5**	**59 ± 5**	**22.0 ± 0.9**								
Obese women	38	142	78	168	75	27	No	Walking (>2.5 hours/ week)	Diet high in red meat and fat, few vegetable	No	No	No	No	No
Obese women	34			162	70	27	No	Walking (>2.5 hours/ week)	Diet high in vegetables, fiber and white meat	No	No	No	No	No
Obese women	39			157	78	32	No	Sedentary	Diet high in red meat and fat, few vegetables	No	No	No	No	No
Obese women	40			156	80	33	No	Sedentary	Diet high in vegetables, fiber and white meat	No	No	No	No	No
Obese women	30			161	89	34	No	Sedentary	Diet high in red meat and fat, few vegetables	No	Drinks socially	No	No	Yes
Obese women	36	140	90	156	86	35	No	Sedentary	Diet high in red meat and fat, few vegetables	No	No	No	No	No
Averages Obese Women	**36.1 ± 1.5**	**141 ± 1**	**84 ± 3**	**160 ± 2**	**80 ± 3**	**31.3 ±1.4**								

Lean men	35			172	64	22	No	Sedentary	Diet high in vegetables, fiber and white meat	No	Drinks socially	No	Not applicable	No
Lean men	37	120	62	170	65	22	No	Sedentary	Diet high in vegetables, fiber and white meat	No	No	No	Not applicable	No
Lean men	32	124	79	184	78	23	No	Walking (>5 hours/ week)	Diet high in vegetables, fiber and white meat	No	Drinks socially	Yes	Not applicable	No
Lean men	37	110	70	179	74	23	No	Walking (>5 hours/ week)	Diet high in red meat and fat, few vegetables	No	Drinks socially	No	Not applicable	No
Lean men	38	113	65	184	79	23	No	Walking (>2.5 hours/ week)	Diet high in vegetables, fiber and white meat	No	Drinks socially	No	Not applicable	No
Lean men	32			170	68	24	No	Sedentary	Diet high in red meat and fat, few vegetables	No	No	Yes	Not applicable	No
Lean men	36			174	74	24	No	Walking (>5 hours/ week)	Diet high in red meat and fat, few vegetables	No	Drinks socially		Not applicable	No
Lean men	32			180	81	25	No	Walking (>5 hours/ week)	Diet high in red meat and fat, few vegetables	No	Drinks socially	No	Not applicable	
Averages Lean Men	**35 ± 1**	**117 ± 1**	**69 ± 3**	**177 ± 2**	**73 ± 2**	**23.3 ± 0.4**								
Obese men	38	110	70	184	91	27	No	Sedentary	Diet high in red meat and fat, few vegetables	No	No	Yes	Not applicable	No
Obese men	34	116	73	178	87	27	No	Sedentary	Diet high in vegetables, fiber and white meat	No	Drinks socially	No	Not applicable	No
Obese men	37	110	85	170	80	28	No	Walking (>5 hours/ week)	Diet high in red meat and fat, few vegetables	No	Drinks socially	No	Not applicable	No
Obese men	40			170	80	28	No	Sedentary	Diet high in vegetables, fiber and white meat	No	No	No	Not applicable	No
Obese men	38	117	80	174	92	30	No	Sedentary	Diet high in red meat and fat, few vegetables	No	No	No	Not applicable	No
Obese men	35			165	83	30	No	Sedentary	Diet high in red meat and fat, few vegetables	No	No	No	Not applicable	No

Obese men	34			175	94	31	No	Walking (>2.5 hours/ week)	Diet high in vegetables, fiber and white meat	No	Drinks socially	No	Not applicable	No
Obese men	34			180	115	35	No	Walking (>5 hours/ week)	Diet high in vegetables, fiber and white meat		Drinks socially	Yes	Not applicable	No
Obese men	35			187	130	37	No	Sedentary	Diet high in red meat and fat, few vegetables	No	No	No	Not applicable	No
Obese men	34	137	108	171	113	39	No	Sedentary	Diet high in red meat and fat, few vegetables	No	No	No	Not applicable	No
Averages Obese Men	**36 ± 1**	**118 ± 4**	**83 ± 5**	**175 ± 2**	**97 ± 5**	**31.2 ± 1.4**								

Figure Legends

Figure 1 – Sera from lean rats increases β-cell mitochondrial oxygen consumption. A. Weight progression of eight-week-old male rats on CR (blue) or AL (red) diets; *p < 0.05, **p < 0.005 compared to AL, unpaired t-student test, n = 6. **B.** Frequency (%) of islets from AL and CR rats versus area, in μm^2. **C.** Typical INS1-E cell oxygen consumption rate (OCR) traces. Cells were incubated in media in the presence of the sera indicated, with 11.1 mM glucose. 1 µM oligomycin, 10 µM carbonyl cyanide-4-(trifluoromethoxy) phenylhydrazone (FCCP), and 2 µM antimycin plus rotenone were added when indicated. **D.** Basal OCR, quantified as initial minus antimycin plus rotenone-insensitive respiration, from traces such as those in panel B. **E.** Maximal OCR, quantified as FCCP-stimulated minus antimycin plus rotenone-insensitive respiration. **F.** ATP-linked OCR, quantified as initial minus oligomycin-insensitive respiration. Results are presented as means ± SEM, with individual dots representative of biological replicates. *p < 0.05, **p < 0.005, as indicated by one-way ANOVA with Tukey posttest; n = 15-19.

Figure 2 – Female lean donor plasma increases β-cell mitochondrial oxygen consumption. A. Typical OCR traces, conducted under the same conditions as Fig. 1, with plasma samples from male, female, lean, and obese donors, as indicated. **B-D.** Basal, maximal and ATP-linked OCR, calculated as described in Fig.1. Results are presented as means ± SEM. *p <0.05, **p < 0.005***, p < 0.0005, ****p < 0.00005, one-way ANOVA test, with Tukey posttest, n = 8-13.

Figure 3 – Gender and nutritional status alter the amount of circulating adiponectin. A. Quantification of adiponectin in serum samples from AL and CR rats; means ± SEM, *p < 0.05, unpaired t-student test, n = 12-14. **B.** Quantification of adiponectin in plasma samples from lean or obese men and women; means ± SEM; ***p < 0.0005, ****p < 0.00005, one-way ANOVA test with Tukey posttest, n = 9.

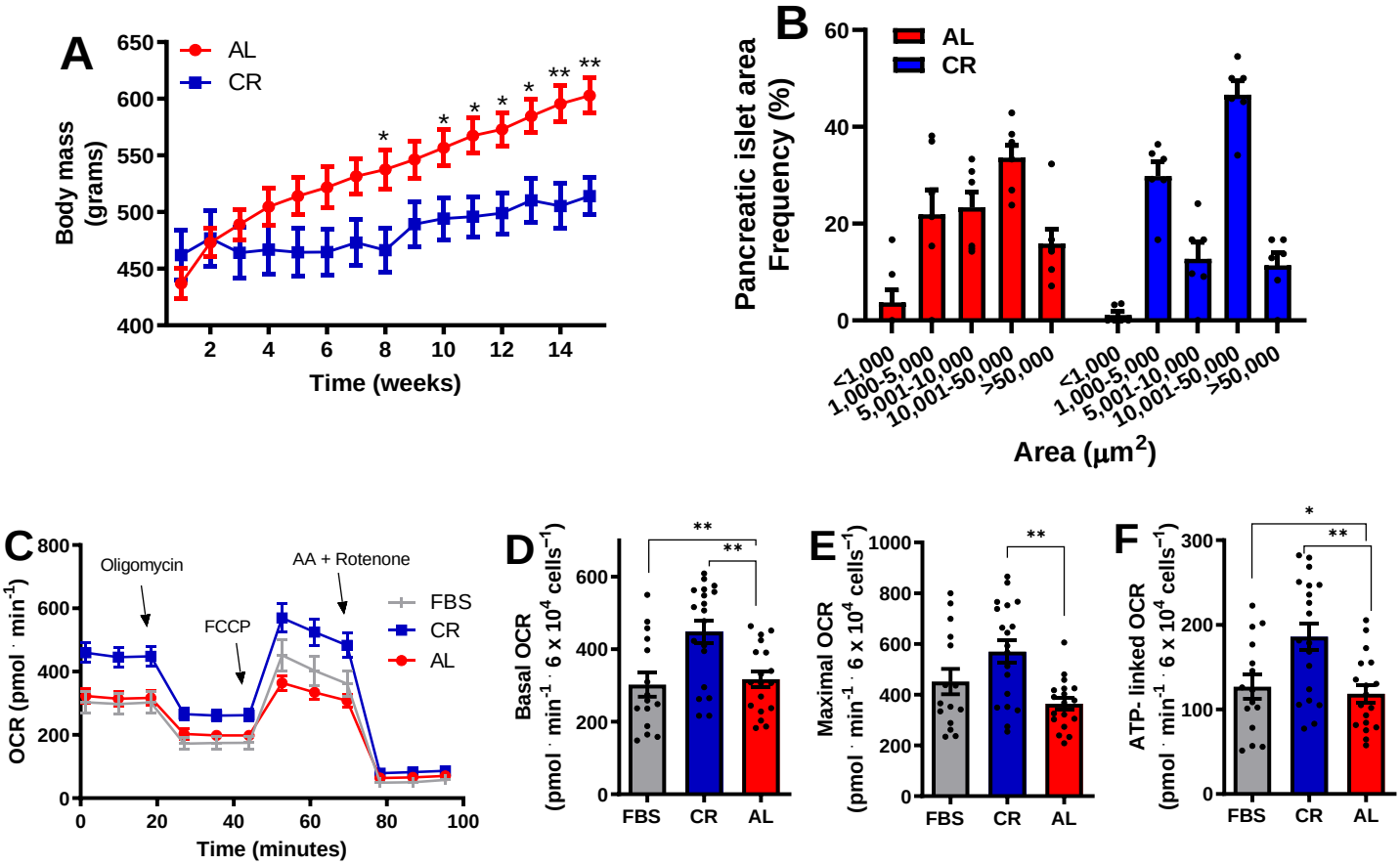
Figure 4 – β-cell metabolic fluxes are strongly induced by adiponectin at levels present in lean sera. Experiments were conducted under similar conditions to Fig. 1, in the presence of FBS, no sera, and/or 10 µg/mL adiponectin, as indicated. **A.** Typical traces. **B-D.** Basal, maximal, and ATP-linked OCR. **E.** Insulin secretion corrected for cellular insulin content was measured after one hour of incubation with 5.6, 11.3, or 16.7 mM glucose, in the presence of FBS or adiponectin, as indicated. Results are presented as means ± SEM. *p <0.05, ****p < 0.00005, one-way ANOVA with Tukey posttest, n = 3-5.

Figure 5 – Serum adiponectin is necessary to maintain islet function and integrity. Islet insulin secretion was measured after 60 min as described in the Methods section with low (5.6 mM, Panel **A**) or high (16.7 mM, Panel **B**) glucose in the presence of FBS, no serum, or 10 µg/mL adiponectin, as indicated. **C.** Insulin secretion collected from islets incubated in 10 mM glucose over a 24 h period, as an indication of cell integrity loss. **D.** LDH activity measured in the culture media. *p <0.05, one-way ANOVA with Tukey posttest, n = 3-4.

Figure 6 – Low β-cell metabolic fluxes in obese and male sera are reversed by adiponectin. A. Typical OCR traces, conducted under the same conditions as

Fig. 2, with plasma samples from male, female, lean, and obese donors, in the presence of 10 µg/mL adiponectin, where indicated. **B-D.** Basal, maximal, and ATP-linked OCR. Results are presented as means ± SEM. ****$p < 0.00005$, one-way ANOVA test, with Tukey posttest, n = 4-13.

Figure 7 – Adiponectin protects β-cell function and integrity against damage promoted by plasma from obese donors. A, C. Insulin secretion corrected for cellular insulin content after one hour of incubation with 5.6, 11.3, or 16.7 mM glucose, as indicated. Mean ± SEM, **$p < 0.005$, ***$p < 0.0005$, ****$p < 0.00005$, one-way ANOVA test with Tukey posttest, n = 5. **B, D.** Lactate dehydrogenase (LDH) in culture media after incubation with 10% FBS or plasma from lean or obese men and women, with or without adiponectin, as shown. Mean ± SEM, *$p < 0.05$, ***$p < 0.0005$, one-way ANOVA with Tukey posttest, n = 5-12.



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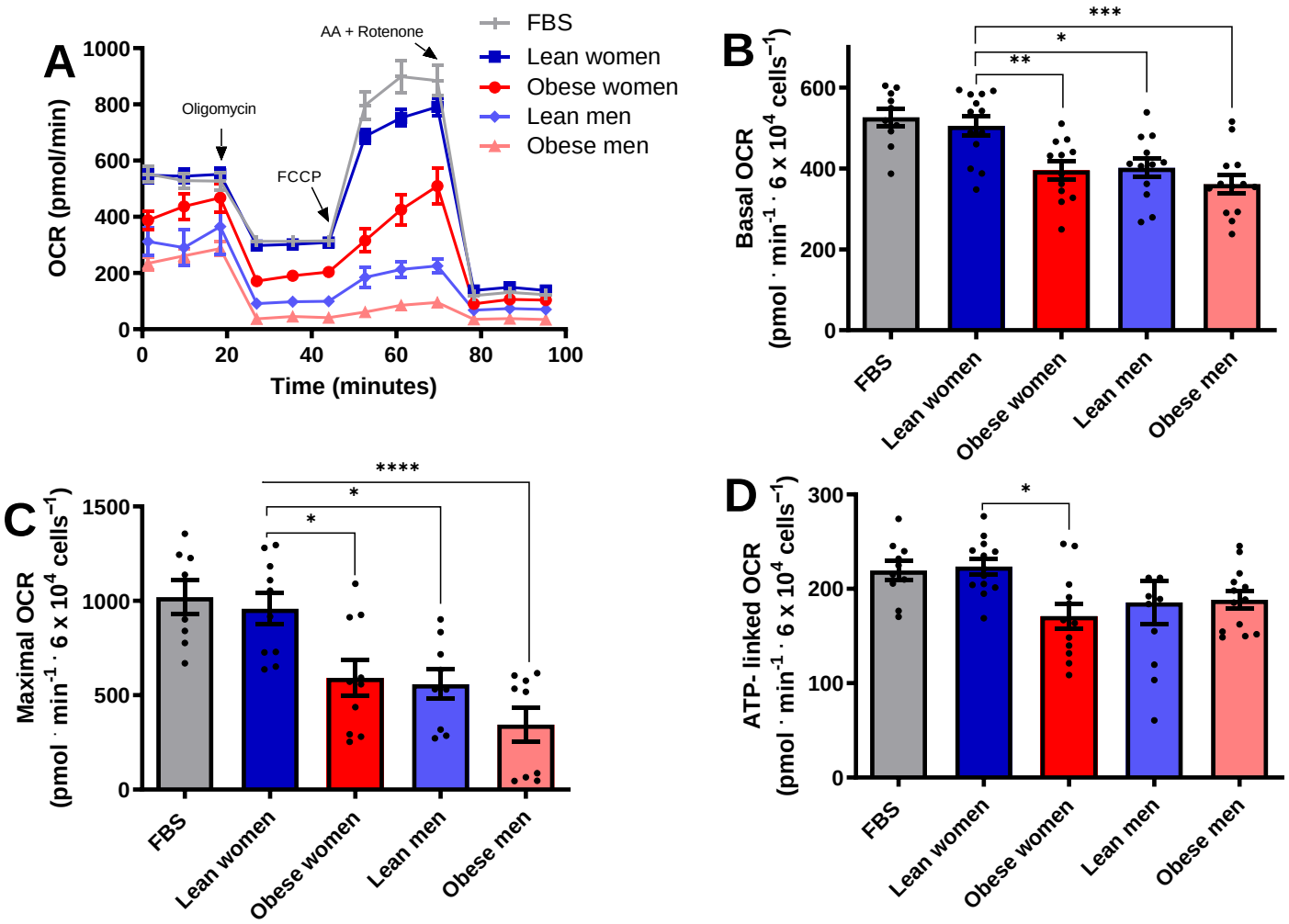

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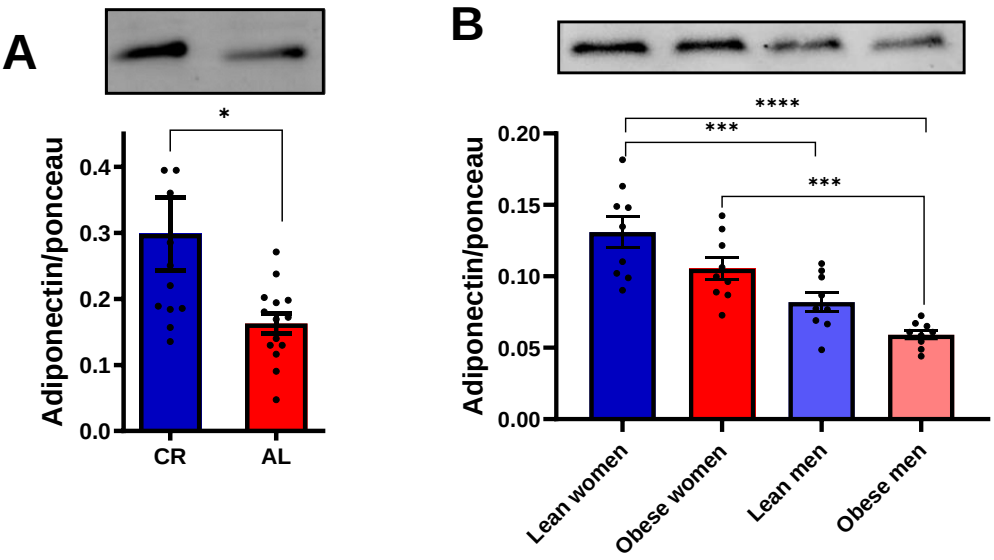

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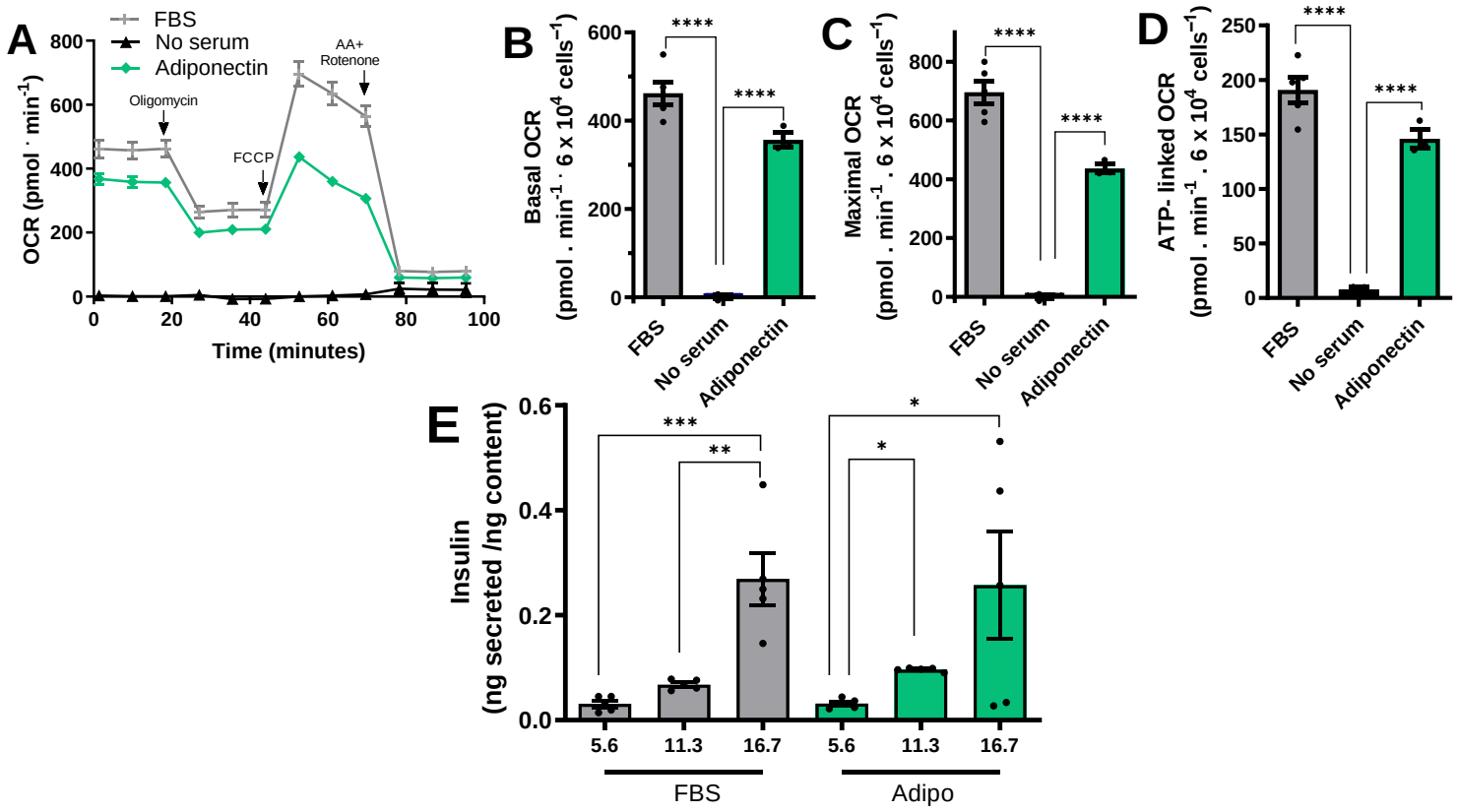

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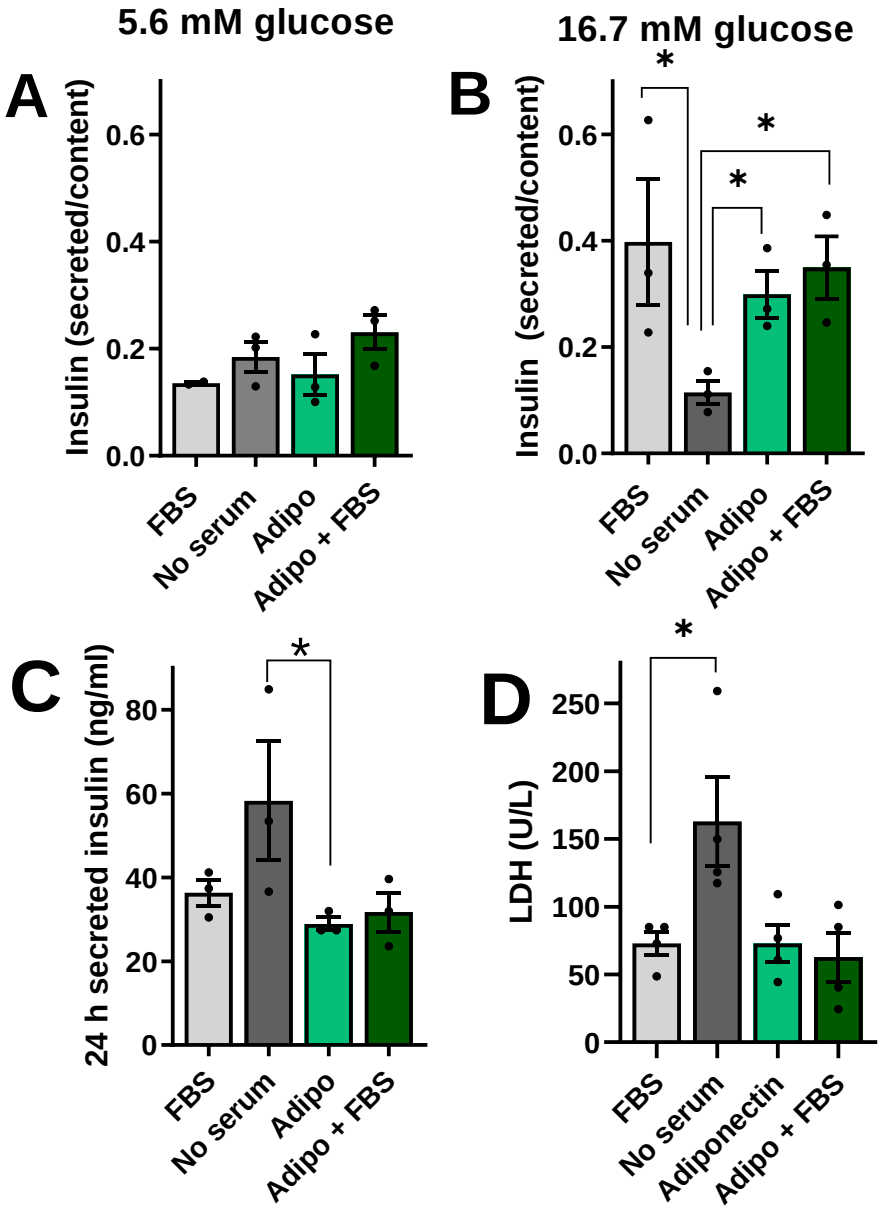

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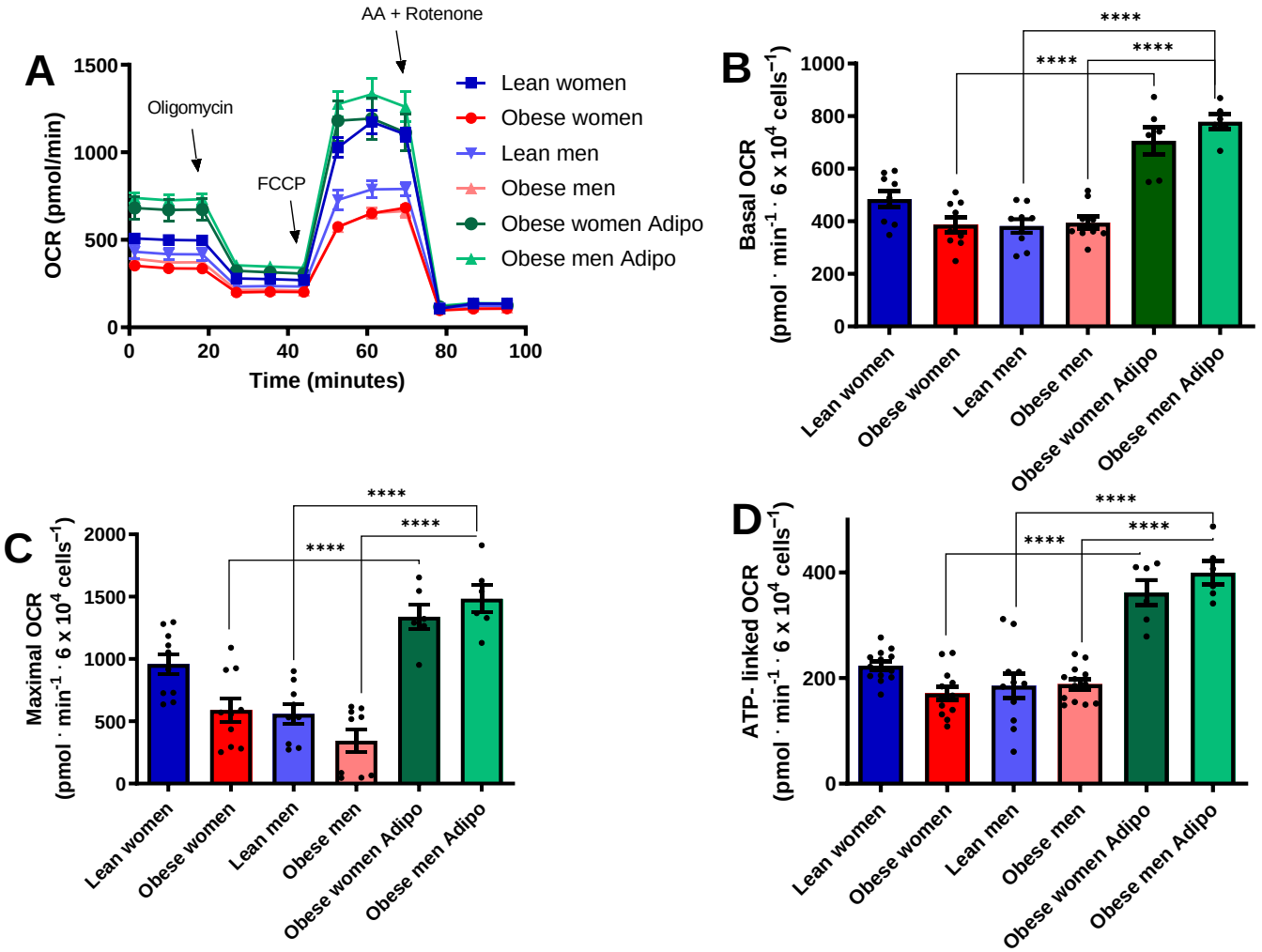

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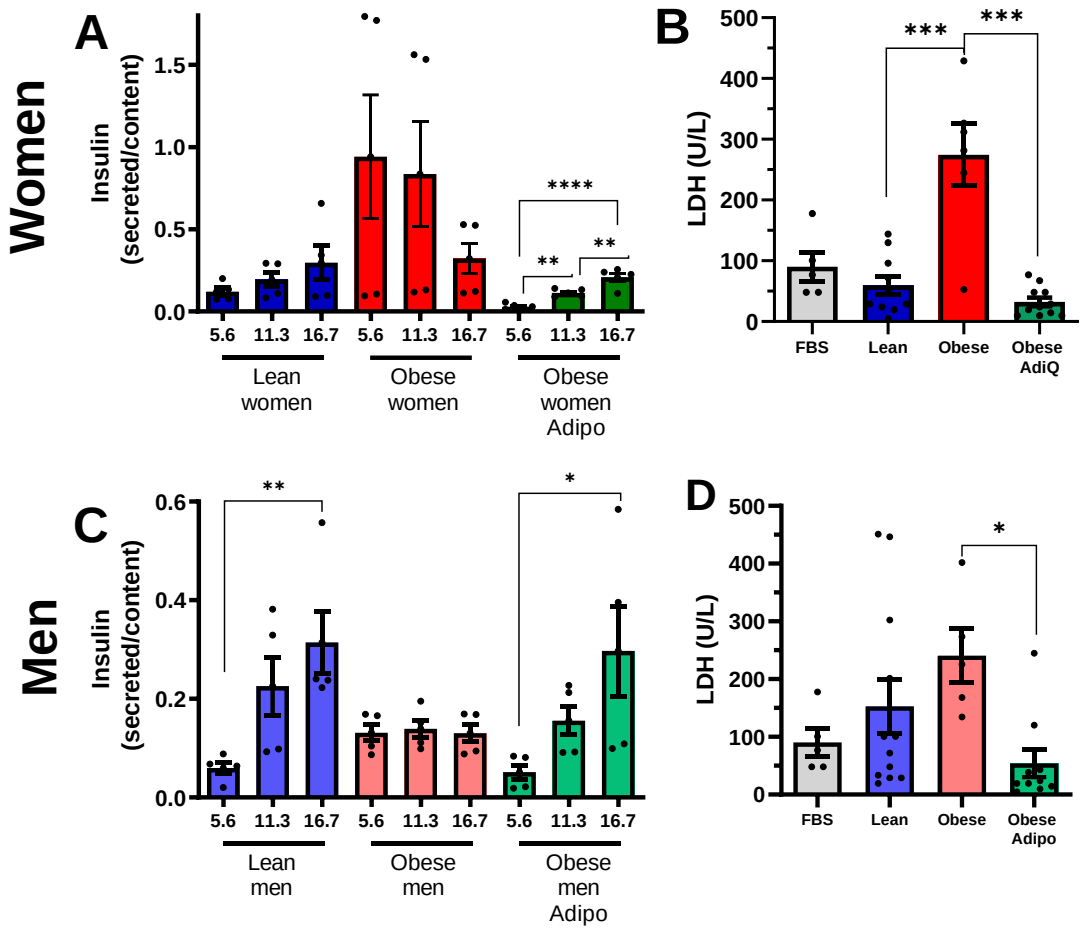

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