Research Article

Prevention of Elevation in Plasma Triacylglycerol with High-Dose Bezafibrate Treatment Abolishes Insulin Resistance and Attenuates Glucose Intolerance Induced by Short-Term Treatment with Dexamethasone in Rats

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Received 18 April 2018; Revised 7 August 2018; Accepted 27 August 2018; Published 8 November 2018

Academic Editor: Ludwik K. Malendowicz

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Objective. Fibrates are used as lipid-lowering drugs and are well tolerated as cotreatments when glucose metabolism disturbances are also present. Synthetic glucocorticoids (GCs) are diabetogenic drugs that cause dyslipidemia, dysglycemia, glucose intolerance, and insulin resistance when in excess. Thus, we aimed to describe the potential of bezafibrate in preventing or attenuating the adverse effects of GCs on glucose and lipid homeostasis.

Methods. Male Wistar rats were treated with high-dose bezafibrate (300 mg/kg, body mass (b.m.)) daily for 28 consecutive days. In the last five days, the rats were also treated with dexamethasone (1 mg/kg, b.m.).

Results. Dexamethasone treatment reduced the body mass gain and food intake, and bezafibrate treatment exerted no impact on these parameters. GC treatment caused an augmentation in fasting and fed glycemia, plasma triacylglycerol and nonesterified fatty acids, and insulinemia, and bezafibrate treatment completely prevented the elevation in plasma triacylglycerol and attenuated all other parameters. Insulin resistance and glucose intolerance induced by GC treatment were abolished and attenuated, respectively, by bezafibrate treatment.

Conclusion. High-dose bezafibrate treatment prevents the increase in plasma triacylglycerol and the development of insulin resistance and attenuates glucose intolerance in rats caused by GC treatment, indicating the involvement of dyslipidemia in the GC-induced insulin resistance.

1. Introduction

Glucose and lipid metabolism is a process continuously regulated by several humoral and neural factors that guarantee the constant physiological supply of circulating energetic substrates (i.e., glucose and free fatty acids). Disruption of such a fine process can lead to dyslipidemia or dysglycemia, which are both risk factors for cardiovascular and metabolic diseases (i.e., hypertension and diabetes mellitus) [1]. The worldwide increasing prevalence of metabolic-related diseases, including obesity, hypertension, and diabetes mellitus type 2 (DM2), is associated with numerous factors, such as genetic background, epigenetics (i.e., intrauterine influences), and lifestyle (i.e., physical inactivity and hypercaloric diets) as well as indirect ingestion of endocrine disruptor compounds (i.e., pesticides and bisphenol A) [1].

In addition to these well-known risk factors, exposure to prolonged treatment or high dosages of glucocorticoids (GCs) may also be a concern of glucose and lipid metabolism.
GC-based treatments are typically associated with glucose and lipid disturbances (i.e., glucose intolerance, reduction in insulin sensitivity, dyslipidemias of varied degrees, and hyperinsulinemia), which are well reproducible in both rodents [3–5] and in humans [6, 7]. GCs exert such an effect upon the reduction of glucose disposal and increased plasma triacylglycerol levels, which typically result from the attenuation of insulin-stimulated glucose uptake in skeletal muscles, attenuation of insulin suppression on liver glucose output, and attenuation of insulin-suppressive action on adipose tissue lipolysis [2]. These low responses to insulin in peripheral tissues, also termed as peripheral insulin resistance, may be related to increased plasma lipid abundance, which ultimately can be accumulated ectopically in nonadipose tissues [8].

A few preclinical [9] and clinical studies [10] have demonstrated the positive impact of glucose-lowering drugs (i.e., metformin, pioglitazone) coadministered together or prior to treatment with GCs. However, none of these glucose-lowering drugs were developed to act primarily on lipid metabolism. Considering that the elevation of plasma free fatty acids may be involved with the genesis of insulin resistance [8], the coadministration of lipid-lowering drugs with GCs could offer insight. In this sense, bezafibrate is an ideal candidate. Fibrates are a class of drugs with better results for the management of elevation in plasma triacylglycerol than statins (statins are primarily indicated to reduce LDL cholesterol levels), especially when glucose metabolism is also impaired [11].

Considering that dexamethasone treatment induces marked hypertriacylglycerolemia together with reduced insulin sensitivity, glucose intolerance, and hyperinsulinemia in adult rats [3–5], we explored the potential of bezafibrate treatment in preventing the elevation of plasma triacylglycerol and other adverse metabolic actions induced by dexamethasone treatment. In view of GCs in excess induces robust genomic-mediated adverse effects [2] that may surpass the possible therapeutic actions of fibrates (if it is introduced after GC), our study design resides in a hypothetical context where fibrates are being used prior to GCs (e.g., a patient that is on continuous bezafibrate treatment and needs to be exposed to a therapy based on GC action). Furthermore, we expect that with this design, the GC-induced elevation in plasma triacylglycerol will be minimal. We hypothesized that previous treatment with bezafibrate will attenuate the elevation in triacylglycerol levels caused by GC treatment and protect rats from the GC-induced side effects on glucose metabolism. Our main results demonstrate that the introduction of high-dose bezafibrate treatment prior to GC treatment prevented the increase in plasma triacylglycerol and advancement of insulin resistance and attenuated the glucose intolerance in rats, indicating the involvement of dyslipidemia in GC-induced insulin resistance.

2. Materials and Methods

2.1. Ethical Approval. The experiments with rats were approved by the Institutional São Paulo State University Committee for Ethics in Animal Experimentation (approval ID: 2150/46/01/09) in accordance with the National Council for Animal Experimentation Control (CONCEA).

2.2. Materials. Dexamethasone phosphate (Decadron®) was purchased from Aché (Campinas, SP, Brazil), and Bezafibrate was purchased from Pharma Nostra (Campinas, SP, Brazil). Gum Arabic was obtained from Sigma (St. Louis, MO, USA). Regular human recombinant insulin (Biohulin®) was acquired from Biobrás (Montes Claros, MG, Brazil). Sodium thiopental (Thiopental) was purchased from Cristália (Itapira, SP, Brazil). The 125I-labelled insulin (human recombinant) for RIA assay was purchased from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK). The reagents were used in the glucose tolerance test, and the hepatic glycochen, insulin secretion, and RIA protocols were acquired from LabSynth (Diadema, SP, Brazil) and Sigma. The enzymatic colorimetric assay for the quantification of nonesterified fatty acid (NEFA) was obtained from Wako Chemicals USA Inc. (Richmond, VA, USA). The enzymatic colorimetric assays for the quantification of triacylglycerol and total cholesterol (T-Chol) from detection kits were purchased from InVitro (Itabira, SP, Brazil). Plasma insulin was quantified by RIA using a gamma alpha counter (Perkin Elmer, Waltham, MA, USA) as previously described [4, 5].

2.3. Animals. Experiments were performed on 4 groups of 40 male Wistar rats (defined as sets 1, 2, 3, and 4), accounting for a total of 160 rats. The rats were obtained from the UNESP Animal Care Unit, Campus of Botucatu, and maintained at 22 ± 2°C on a 12 h light-dark cycle (lights on at 0600, lights off at 1800). The rats had access to food (commercial standard chow for rats, BIOBASE® 9301; Águas Frias, SC, Brazil) and water *ad libitum*.

2.4. Bezaflibrate and Dexamethasone Treatments. According to Figure 1, rats aged 28 days were housed for acclimatization until they reached 3 months old and subsequently separated in four groups: bezafibrate group (Beza) received a daily oral gastric (gavage) administration of bezafibrate ((300 mg/kg of body mass (b.m.)), between 0700 and 0800 h, diluted in 5% gum Arabic, for 28 consecutive days; control group (Ctl) received a daily gavage administration of vehicle alone (5% gum Arabic), between 0700 and 0800 h, equivalent to 1 ml/kg of b.m., for 28 consecutive days; bezafibrate plus dexamethasone group (BezaDexa), in the last 5 days of bezafibrate treatment, half of rats from the Beza group received daily intraperitoneal (i.p.) injection of dexamethasone phosphate (equivalent to 1 mg/kg of dexamethasone, b.m., diluted in saline solution), between 0700 and 0800 h; dexamethasone group (Dexa), in the last 5 days of vehicle treatment, half of rats from the Ctl group received daily i.p. injection of vehicle (0.9% NaCl, at 1 ml/kg of b.m.), between 0700 and 0800 h. The administration of bezafibrate reduces plasma triacylglycerol in normal rats [12] and hyperglycemic rat models (OLETF rats) [13, 14]. Although the dose of bezafibrate used in the present study was 2 to 3 times higher than that used in previous studies, this dose was still 25% lower than the dose where only mild and transitional...
hepatic toxicity was observed in rats [15]. We opted for this high dose since there is evidence that low dosages have no persistent effect on reducing plasma triacylglycerol and NEFA values in a rat model of dyslipidemia [14]. With this high bezafibrate dose, we generated a rat model where the influence of circulating triacylglycerol was not present among the outcomes produced by dexamethasone treatment. This pharmacological intervention permitted us to observe how much plasma triacylglycerol could be involved in the dexamethasone adverse effects on glucose homeostasis.

Dexamethasone, at the dose and period administered, produces significant adverse effects on glucose metabolism, typical of prediabetes, including glucose intolerance, reduction of insulin sensitivity, augmented fasting blood glycemia and plasma triacylglycerol, and increased pancreatic beta cell mass, which makes this compound a valuable tool for challenging glucose metabolism experimentally [16, 17].

2.5. Metabolic Measurements (Set 1). Body mass was measured daily from the beginning of bezafibrate treatment until the day of euthanasia using a conventional electronic balance (Tecnal, Piracicaba, Brazil). The growth rate was determined based on the formula \( \text{final b.m.} - \text{initial b.m.} \times 100 \). Food intake was measured only during the period of dexamethasone administration to verify the reproducibility of its anorexigenic action [5] and how bezafibrate impacts on it. For this, the remaining chow after a 24 h period was normalized to the total body mass from each cage as previously described [5, 17].

The day after the last day of dexamethasone treatment, separate groups of fasted (12–14 h) and fed rats had blood collected from the tail to measure blood glucose levels with a glucometer (One Touch, Johnson & Johnson, NJ, USA). Immediately after blood collection, the rats were euthanized (exposure to CO₂ followed by decapitation), and the trunk blood was collected in EDTA-NaF-containing tubes (Glistab-Labtest; Lagoa Santa, MG, Brazil) to obtain the plasma. The plasma, obtained after blood centrifugation (600 \( \times g \)), was stored at \(-80^\circ C\) in several aliquots for posterior measurement of insulinemia, plasma triacylglycerol, total cholesterol, and circulating NEFA with commercial kits according to the manufacturer’s instructions. The organs (listed in Table 1) were gently withdrawn and weighed.

2.6. Liver Glycogen (Set 1). Determination of hepatic glycogen content was performed as previously described [4, 5, 16]. Glycogen was determined by a phenol-based assay using a spectrophotometer.

2.7. Intraperitoneal Glucose Tolerance Test (ipGTT) and In Vivo Glucose-Stimulated Insulin Secretion (Set 2). On the day after the last day of dexamethasone treatment, separate groups of fasted (12–14 h) and fed rats had blood collected from the tail to measure blood glucose levels with a glucometer (One Touch, Johnson & Johnson, NJ, USA). Immediately after blood collection, the rats were euthanized (exposure to CO₂ followed by decapitation), and the trunk blood was collected in EDTA-NaF-containing tubes (Glistab-Labtest; Lagoa Santa, MG, Brazil) to obtain the plasma. The plasma, obtained after blood centrifugation (600 \( \times g \)), was stored at \(-80^\circ C\) in several aliquots for posterior measurement of insulinemia, plasma triacylglycerol, total cholesterol, and circulating NEFA with commercial kits according to the manufacturer’s instructions. The organs (listed in Table 1) were gently withdrawn and weighed.

FIGURE 1: Experimental design. At 3 months old, rats ingested bezafibrate ((300 mg/kg of body mass (b.m.)) once a day via oral gastric gavage administration between 0700 and 0800 h, diluted in 5% gum Arabic, for 28 consecutive days, while control group received a daily gavage administration of vehicle alone (equivalent to 1 ml/kg of b.m.). In the last 5 days of bezafibrate treatment, half of rats under bezafibrate treatment received daily intraperitoneal (i.p.) injection of dexamethasone phosphate (equivalent to 1 mg/kg of dexamethasone, b.m., diluted in saline solution), between 0700 and 0800 h, while half of rats from the control group received daily intraperitoneal injection of vehicle (0.9% NaCl, at 1 ml/kg of b.m.). A day after the last dexamethasone or vehicle administration, separate groups of rats were submitted to ipGTT, ipITT, and euthanasia for biochemical data analyses and ex vivo experiments. ipGTT: intraperitoneal glucose tolerance test; ipITT: intraperitoneal insulin tolerance test.
solution prewarmed at 36°C (2 g/kg, b.m., i.p.) was immediately administered, and blood samples were collected from the tail tip at 15, 30, 60, 90, and 120 min for blood glucose measurements [16, 17]. Area under curve (AUC) was obtained after normalization by the initial blood glucose value. At 0, 15, and 120 minutes, additional blood volumes (80 μl) were collected in anticoagulant-containing tubes for plasma separation and the posterior quantification of insulin by RIA as a measure of the in vivo glucose-stimulated insulin secretion [4, 16].

2.8. Intrapretoperitoneal Insulin Tolerance Test (ipITT) (Set 3). On the day after the last day of treatment in each group, fed rats were used for ipITT experiments. The rats were anesthetized as for ipGTT and tail tips were cut for blood collection. The first drop of blood was discarded, and the second drop was used for the determination of glycemia (time 0) using a glucometer. Immediately, human recombinant insulin prewarmed at 36°C (equivalent to 2 IU/kg b.m., i.p.) was administered. Additional samples were collected at 10, 20, and 30 min for blood glucose measurement. The constant rate for glucose disappearance (KITT) was calculated from the slope of the regression line obtained with log-transformed glucose values between 0 and 30 min after insulin administration (linear phase of glucose decay) [4, 16, 17].

2.9. Isolation of Islet and Glucose-Stimulated Insulin Secretion (Set 4). The islets were isolated by collagenase digestion of the pancreas as previously described [4, 5, 16, 17]. For static incubation, groups of five islets were first incubated for 1 h at 37°C in 1 ml Krebs bicarbonate buffer solution of the following composition (in mM): 115 NaCl, 5 KCl, 2.56 CaCl2, 1 MgCl2, 24 NaHCO3, 15 HEPEs, and 5.6 glucose, supplemented with 0.1% of bovine serum albumin and equilibrated with a mixture of 95% O2:5% CO2, pH 7.4. The medium was subsequently replaced with fresh buffer containing 2.8 or 16.7 mM glucose and further incubated for 1 h. At the end of the incubation, the samples were stored at −20°C for subsequent measurement of the insulin content by RIA. Total islet insulin content was determined in separate pools of islets (four different pools consisting of 50 islets each) after extraction in acid ethanol solution (12 mM HCl in 70% ethanol). The average insulin content, obtained from these pools, was used for estimation of insulin secretion in each group based on total islet insulin content as previously described [4]. All islets hand-picked for insulin secretion experiments were selected based in their spherical uniformity, integrity, and size to avoid any bias of islet aspect and size.

2.10. Statistical Analysis. The results are expressed as the mean ± SEM (points and connecting line) and the mean ± SD or the median and interquartile range (scatter plot with bar) of the indicated number (n) of animals. The symmetry of the data was tested by Kolmogorov-Smirnov and Shapiro-Wilk’s normality tests. Analysis of variance (ANOVA) (two-way ANOVA) for unpaired groups, followed by Tukey’s post hoc test, was utilized for multiple comparisons. The extreme studentized deviate method was applied to determine whether any of the values was a significant outlier (Grubb’s test from online available GraphPad QuickCalculcs). References in the text to “their respective control groups” means differences among Dexa and BezaDexa groups vs. Ctl and Beza groups, respectively (effect of dexamethasone), or Beza and BezaDexa groups vs. Ctl and Dexa groups, respectively (effect of bezafibrate). Significance was set at p < 0.05.

3. Results

3.1. Bezafibrate Treatment Had a Slight Impact on the Anorexigenic Effect of Dexamethasone Treatment. Before initiating bezafibrate treatment, the rats in all groups exhibited similar body masses, and bezafibrate administration for 28 consecutive days changed neither the body mass (Figure 2(a)) nor the estimated growth rate (based on 1 and 23 days) (Figure 2(b)). Dexamethasone treatment resulted in a reduced final body mass (~15%) in the Dexa group compared with that in the Ctl group (Figure 2(c)) (n = 8–10, p < 0.05), but the 10% reduction was not significant in the BezaDexa group compared with that in the Beza group. Relative food intake was similar among the four groups prior to the initiation of dexamethasone treatment (morning of the first dexamethasone injection) but was significantly lower in the Dexa and BezaDexa groups vs. their respective controls, after 1 and 2 days of dexamethasone treatment, respectively (Figure 2(d)) (n = 8–10, p < 0.05), which remained consistent until the end of treatment in the Dexa group. The estimation of food intake between the final and initial dexamethasone treatment

Table 1: The relative organ and tissue masses and hepatic glycogen data at the day of euthanasia.

|                     | Ctl     | Dexa    | Beza    | BezaDexa |
|---------------------|---------|---------|---------|----------|
| Retroperitoneal fat | 1.1 ± 0.3 | 1.1 ± 0.4 | 1.0 ± 0.4 | 1.1 ± 0.4 |
| Epididymal fat      | 1.1 ± 0.2 | 1.3 ± 0.3 | 1.1 ± 0.3 | 1.2 ± 0.3 |
| Visceral fat (both) | 2.2 ± 0.4 | 2.4 ± 0.7 | 2.1 ± 0.7 | 2.3 ± 0.6 |
| Spleen              | 193 ± 17 | 124 ± 9* | 200 ± 22 | 133 ± 16* |
| Hepatic glycogen    | 0.9 ± 0.5 | 4.6 ± 1.1* | 0.6 ± 0.2 | 0.7 ± 0.4* |

1(g/100 g, b.m.); 2(mg/100 g, b.m.; 3(mg/g tissue), read methods for details. Results are expressed as mean ± SD. *Significantly different vs. the respective control group (dexamethasone effect) and significantly different vs. the respective control group (bezafibrate effect) using two-way ANOVA with Tukey’s post hoc test. n = 7–8, p < 0.05. 1 and 2 are equal to (g/b.m) × 100 or (mg/b.m.) × 100, respectively.
revealed no negative or positive impact of bezafibrate administration on the anorexigenic effect of GC (Figure 2(d)). Altogether, treatment with bezafibrate before and during the administration dexamethasone had no major impact on the anorexigenic action of high doses of GC treatment.

3.2. Bezafibrate Treatment Abolished the GC-Induced Elevation in Plasma Triacylglycerol and Improved Basal Glycemic Values. To verify the efficacy of bezafibrate treatment, we quantified circulating triacylglycerol, NEFA, and T-Chol both at fasted and fed states. In fact, bezafibrate administration prevented any increase in the
plasma triacylglycerol caused by GC treatment in both metabolic states (Figures 3(a) and 3(f)) \( (n = 7-11, p < 0.05) \). Bezafibrate treatment exerted no significant impact on the elevated fasting NEFA values but prevented the increase in fasting blood glucose as well as plasma insulin caused by dexamethasone administration (Figures 3(b)–3(e)) \( (n = 7-10, p < 0.05) \). In the fed state, bezafibrate treatment had no major impact on the elevated plasma T-Chol values but abolished the rise in NEFA and blood glucose values and attenuated the elevation in plasma insulin values induced by GC treatment (Figures 3(g)–3(j)) \( (n = 7-12, p < 0.05) \). Bezafibrate had no negative impact per se on any of the parameters evaluated when compared with the Ctl group (Figures 3(a)–3(j)). Evaluation of relative visceral fat mass revealed no impact of dexamethasone nor from bezafibrate treatment (Table 1).
3.3. Beza  

Responsive to High Glucose. To verify whether beza insulin sensitivity during exposure to a high GC dose was associated with improved glucose tolerance and prevention of elevation in plasma triacylglycerol levels the Ctl group. These data suggested that pharmacological leading to an enhanced insulin sensitivity compared with fl islets from dexamethasone-treated rats and suprathreshold (16.7 mM) glucose was augmented in lin secretion in response to both subthreshold (2.8 mM) and glucose values at min 30 were 210 ± 8, and 227 ± 18, and 247 ± 12 mg/dl for the Ctl, Dexa, Beza, and BezaDexa groups, respectively. Treatment with bezaibrate had no effect per se (in relation to the Ctl group) but attenuated the effect of dexamethasone treatment on glucose tolerance as observed during GTT experiment (Figure 4(a)) (30 min for the BezaDexa group) and by the AUC values (Figure 4(b)) (n = 7–8). This positive impact of bezaibrate treatment upon glucose tolerance in rats treated with GC was associated with enhanced beta cell function (see insulin secretion in response to glucose along the GTT experiment in the BezaDexa group related to the Beza group) (Figure 4(c), n = 6–8) and the complete prevention of peripheral insulin resistance, as demonstrated in the insulin tolerance test (Figure 4(d), n = 6–8) and the estimation for the constant rate for glucose disappearance (KITT) (Figure 4(e), n = 6–8). Bezaibrate treatment for 28 days had an effect per se, leading to an enhanced insulin sensitivity compared with the Ctl group. These data suggested that pharmacological prevention of elevation in plasma triacylglycerol levels was associated with improved glucose tolerance and insulin sensitivity during exposure to a high GC dose.

3.4. Islets from Rats Treated with Beza  

Were More Responsive to High Glucose. To verify whether bezaibrate treatment influenced beta cell function, we evaluated the glucose-stimulated insulin secretion in isolated islets. Insulin secretion in response to both subthreshold (2.8 mM) and suprathreshold (16.7 mM) glucose was augmented in islets from dexamethasone-treated rats (n = 10–12 wells, p < 0.05) and bezaibrate treatment did not affect these insulin responses (Figures 5(a) and 5(b)). Bezaibrate exerted a positive effect per se on islet response to 16.7 mM glucose. This effect is better described by the insulin responsiveness to high glucose as can be observed by the higher insulin release ratio between 16.7 mM and 2.8 mM glucose (Figure 5(c)). Thus, ex vivo insulin release was not downregulated in bezaibrate-treated rats.

4. Discussion

Elevation in plasma triacylglycerol/NEFA levels is commonly associated with a context of reduced insulin sensitivity [18, 19]. There is evidence that an increase in circulating NEFA is one of the causal factors of such peripheral insulin insensitivity [8, 20]. GC in excess exerts diabetogenic actions that include the increase in plasma triacylglycerol and NEFA levels and the reduction in peripheral insulin sensitivity [2–5, 16, 17], accompanied by an increase in blood glucose levels and the presence of glucose intolerance [2–7, 16, 17]. Here, we have provided the first demonstration that previous treatment with high-dose bezaibrate in adult male rats abolished the increase in plasma triacylglycerol levels caused by dexamethasone administration. The effect of bezaibrate on plasma triacylglycerol was associated with the prevention or attenuation of the increase in plasma NEFA and insulin, as well as blood glucose levels caused by GC administration. This normalization of plasma triacylglycerol in the BezaDexa group also paralleled the attenuation of glucose intolerance and prevention of any alterations in the peripheral insulin sensitivity caused by GC treatment.

Long-term treatment with bezaibrate exerted no impact on body mass gain and relative food intake, which were both reduced by the action of dexamethasone treatment (Figures 2(a)–2(e)). The reduction in body mass gain caused by GC results from reduced food intake [17, 21] and a negative hydric balance (increased water and sodium excretion without affecting water intake) [22]. This reduction in food intake is partially attributable to the anorexic effect of insulin and leptin on the hypothalamus, which is upregulated in dexamethasone-treated rats as they develop hyperinsulinemia and hyperleptinemia [3–5, 16, 17, 23]. Although the BezaDexa group had lower plasma insulin levels than in the Dexa group, it is feasible that these animals are more insulin sensitive (as our ITT experiments demonstrated), which favors the action of insulin in the hypothalamus (insulinemia in the BezaDexa group was between the Ctl and Dexa values).

Previous administration of bezaibrate was efficient in preventing any increase in plasma triacylglycerol levels caused by GC in the BezaDexa group, a result accompanied by a reproductive correction in plasma NEFA (especially in the fed state) and in blood glucose levels, with a milder effect on the insulinemia (Figures 3(a)–3(j)). This action of bezaibrate on the reduction of plasma triacylglycerol has been well documented in both rodents (rats and mice) and humans. The mechanisms by which bezaibrate acts include the increase in plasma triacylglycerol clearance through increased lipoprotein lipase and hepatic lipase activities [24, 25]. This increased plasmatic clearance of triacylglycerol is also associated with augmented mitochondrial performance and mass, energy expenditure, and a better metabolic flexibility [26, 27], which altogether support, at least in part, the disposal and oxidation of glucose and NEFA. The improvement in insulin sensitivity caused by bezaibrate administration, as discussed hereafter, may also explain the improvement of the insulin-suppressive effect on fat lipolysis and the hepatic gluconeogenesis (not directly investigated in
Figure 4: Glucose tolerance and insulin sensitivity. (a) The average blood glucose values during an intraperitoneal glucose tolerance test (ipGTT) (2 g/kg, body mass), (b) the average values of area under curve (AUC), and (c) the plasma insulin in response to glucose load. Bezaﬁbrate treatment attenuated the glucose intolerance caused by the dexamethasone treatment in the Beza/Dexa group. (d) The average of normalized blood glucose values (as % from min 0) during an intraperitoneal insulin tolerance test (ipITT) (2 IU/kg, body mass) and (e) the respective constant for glucose disappearance ($K_{ITT}$). Bezaﬁbrate treatment prevented the insulin resistance caused by the dexamethasone treatment in the Beza/Dexa group. Results are expressed as mean ± SEM in (a, c, and d) and as mean ± SD in (b) and (e). In (a, c, and d), the variances were expressed as standard error of the mean (SEM) for esthetic reasons. *Signiﬁcantly different vs. the respective control group (dexamethasone effect) and #signiﬁcantly different vs. the respective control group (bezaﬁbrate effect) using two-way ANOVA with Tukey’s post hoc test. n = 6–8, p < 0.05.
the present study), favoring a better control of blood glucose and plasma lipids levels. There are numerous laboratory evidences demonstrating the positive impact of bezafibrate treatment on lipid and glucose metabolism in rodents, including diet-induced metabolic dysfunctions [28, 29] and monogenic/polygenic phenotypes of obesity/diabetes, such as db/db mice [30], TallyHo mice [27], OLEFT rats [13, 14], and models of diabetes induced by streptozotocin [26]. Studies with dyslipidemic nonobese humans [31] who are overweight [32] and have diabetes [33–35] also revealed the positive impact of bezafibrate treatment on lipid and glucose metabolism. Thus, bezafibrate may have a good spectrum of action and is well tolerated as a cotreatment [11].

One striking observation in the BezaDexa group was the fact that these animals did not present any alteration in the insulin sensitivity and had an improved glucose tolerance (Figures 4(a)–4(d)). There is evidence associating enhanced plasma NEFA values with reduction in peripheral insulin sensitivity [18, 19]. Among the free fatty acids (FFAs), palmitic acid is the most abundant in circulation and is a substrate for the synthesis of diacylglycerol (DAG) and ceramides, lipidic molecules that impair insulin signaling in peripheral tissues [8, 20]. Considering that rats submitted to the same protocol of GC treatment applied in the present study have decreased plasma levels of proinflammatory cytokines (i.e., tumor necrosis factor alpha, interleukin 1 beta) and normal plasma levels of interleukin 6 [36], we cannot attribute the insulin resistance in the Dexa group by an effect of such cytokines, which typically cause the downregulation of insulin signaling [37]. The reduction in insulin sensitivity in rats treated with dexamethasone includes decreased insulin signaling activation in the liver, skeletal muscle, and adipose tissue [3, 36, 38]. Thus, the activation of atypical and novel protein kinases C, which are lipid-mediated (i.e., by ceramides and DAG, respectively), may underlie this downregulation of insulin signaling [39]. There is evidence that bezafibrate treatment reduces the ectopic lipid content in skeletal muscle from rats fed with high fructose plus lard [28] and in the livers of normal rats [12]. Thus, we could consider that the prevention of lipids elevation in the circulation of the BezaDexa group might contribute to prevent a reduction in peripheral insulin sensitivity that
could be related to the enhanced content of lipid in peripheral tissues, suggesting a contribution of excessive free fatty acids in the GC-induced insulin resistance.

The improvement in the glucose tolerance in the BezaDexa group may result from normal insulin sensitivity combined with enhanced islet insulin response to glucose (Figures 4(a)–4(e)). By the interpretation of the disposition index (the product of insulin sensitivity by the insulin secretion), we would not expect enhanced insulin secretion in vivo during the GTT experiment since these rats (Beza-Dexa) were not insulin-resistant. This benefit of bezafibrate on glucose tolerance is also reproducible in patients with DM2 after combined treatment of pioglitazone and bezafibrate [40]. In this study, authors observed that this attenuation of glucose tolerance is due to a reduction in postmeal-induced insulin secretion rather than reduction in glucagon secretion, rendering a diminished plasma insulin/glucagon ratio. Similar results on insulin/glucagon ratio, due to reduced insulin secretion rather than increase glucagon secretion, after stimulation with arginine, were observed in health subjects [41] and hypertriglyceridemic patients [42] treated with 2 grams of clofibrate for 7 and 2–4 months, respectively. These findings support our results in the BezaDexa rats that had decreased insulinemia (Figures 3(e) and 3(j)) and insulin secretion during the ipGTT (Figure 4(c)). However, there is evidence for a direct effect of bezafibrate on glucose-stimulated insulin secretion in islets isolated from rats [43], consistent with the results of insulin secretion in response to 16.7 mM glucose in islets isolated from the Beza and BezaDexa groups (Figures 5(b) and 5(c)). Notably, the highest plasma insulin levels observed in the Dexa group (under fasting and fed conditions) involved not only the endocrine pancreas adherence to the disposition index (that demand increased islet function) but also a reduction in insulin clearance. A previous study involving rats treated with the same dexamethasone protocol demonstrated that the insulin-degrading enzyme activity in the liver is reduced in GC-treated rats, explaining, in part, the hyperinsulinemia observed in these animals [44]. Nonetheless, we cannot rule out the direct action of GCs on gene transactivation of gluconeogenic enzymes (i.e., phosphoenolpyruvate carboxykinase and glucose-6-phosphatase) that results in augmented hepatic glucose output when upregulated by dexamethasone treatment [36, 45], an action that likely outweighs the bezafibrate benefit.

5. Conclusions

In conclusion, prevention of the elevation of plasma triacylglycerol levels through high-dose bezafibrate treatment abolishes the insulin resistance and attenuates the glucose intolerance caused by dexamethasone administration in rats. These observations open the possibility of a combination of lipid-lowering drugs with GCs therapies in clinical practice, aiming to reduce the most prevalent adverse effects of GC on metabolism, insulin resistance, and glucose intolerance [46].

Data Availability

The datasets generated to support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

Natália Moretti Violato’s present address is ULB Center for Diabetes Research, Brussels, Belgium.

Conflicts of Interest

The authors declare no conflict of interest.

Authors’ Contributions

J.R.B. participated in the research design. M.D., N.A.P.C., P.T., P.M.P.V., and N.M.V. conducted the experiments. A.R. contributed with the analytic tools and data analysis. A.R. and M.D. performed the data collection and interpretation. J.R.B. provided the reagents/materials. A.R. wrote the paper. A.R. and J.R.B. contributed to the discussion. All the authors read and approved the manuscript’s final format. Maiara Destro Inácio and Alex Rafacho contributed equally to this study.

Acknowledgments

The following people received fellowships from the São Paulo Research Foundation (FAPESP) during the period of study conduction: M.D.I., N.A.P.C., P.T., P.M.P.V., and N.M.V. A.R. was sponsored by the National Council for Scientific and Technological Development (CNPq) (302261/2014-1). This work was supported by the São Paulo Research Foundation (FAPESP) (grants 2009/12128-6 and 2010/11872-0).

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