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Effect of Bromocriptine in improving Non-alcoholic Fatty Liver Disease in obese animal model of Type 2 Diabetes

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Effect of Bromocriptine in improving Non-alcoholic Fatty Liver Disease in obese animal model of Type 2 Diabetes

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ABSTRACT

The D2 agonist Bromocriptine was approved by the FDA to improve insulin sensitivity and Type 2 Diabetes and may have beneficial effects in associated pathologies like Non-alcoholic Fatty Liver Disease (NAFLD). Although the underlying mechanisms remain unclear, Bromocriptine may have a direct effect on various insulin-sensitive organs. This study evaluated the improvement of insulin sensitivity and reversion of hepatic steatosis in an animal model of obese type 2 diabetes. Wistar (W) rats fed a normal diet and non-obese type 2 diabetic Goto-Kakizaki (GK) rats were divided into 4 groups: GK with normal diet, GK with obesity induced by a high fat and sucrose diet, GK obese treated with Bromocriptine 10 mg/kg/day for 30 days and obese GK treated with vehicle. The glycaemic and lipid profiles and insulin and dopamine signalling in liver were evaluated at fasting. Phosphorylated forms of IR and AMPK were also evaluated 1h after a mixed diet ingestion. In addition, Hematoxylin-Eosin staining of liver was performed, as well as the liver weight and hepatic triglycerides quantification. Rats maintained on a fat diet revealed a worsening of fasting glycemia and plasma triglycerides which were reverted with the administration of Bromocriptine. In the liver, there was an evident reduction of hepatic steatosis and hepatic triglycerides, increase IR levels in fasting, decrease of IRβ(Tyr1361) in post prandial period and decrease of GLUT2 levels in Bromocriptine-treated rats, suggesting a remodelling of glucose and fatty acid metabolism. Moreover, there was an increase in D1R and TH levels and no alterations were observed in D2R and DARPP32 levels. Our results suggest that Bromocriptine acts directly in the liver modulating dopamine signalling, which is associated with an increase of IR and a reduction of hepatic steatosis. Although further studies are still required, our results suggest that Bromocriptine improves glucose and lipid metabolism and may be effective in reducing hepatic lipotoxicity.

Keywords: Type 2 Diabetes, Non-alcoholic Fatty Liver Disease, Bromocriptine, Dopamine.

RESUMO

A Bromocriptina, agonista D2 da dopamina, foi aprovada pela FDA para melhorar a sensibilidade à insulina e a Diabetes tipo 2 e pode ter benefícios nas patologias associadas como a Doença do Fígado Gordo não Alcoólico (NAFLD). Embora os mecanismos subjacentes ainda não sejam conhecidos, a Bromocriptina pode ter um efeito direto sob vários órgãos insulino-dependentes. Este estudo avaliou a melhoria da sensibilidade à insulina e a reversão da esteatose hepática num modelo animal obeso e diabético tipo 2. Os ratos Wistar (W) foram alimentado com uma dieta normal e os ratos Goto-Kakizaki (GK) diabéticos não obesos foram divididos em 4 grupos distintos: GK com dieta normal, GK com obesidade induzida por dieta calórica, GK obesos tratados com Bromocriptina 10mg/kg/dia durante 30 dias e GK obesos tratados com veículo. Os perfis glicémico e lipídico assim como a sinalização da insulina e dopaminérgica no fígado foram avaliados em jejum. As formas fosforiladas do IR e da AMPK foram também avaliadas 1h após a ingestão de uma dieta mista. Para além disto, também foi realizada a coloração do tecido hepático com Hematoxilina-Eosina (HE), assim como registado o peso do fígado e quantificados dos triglicerídeos hepáticos. Os ratos alimentados com dieta calórica revelaram níveis maiores de glicémia em jejum e trigliceridémia, os quais foram revertidos após a administração da Bromocriptina. No fígado verificou-se uma evidente redução da esteatose hepática e dos triglicerídeos hepáticos, aumento dos níveis de IR em jejum, diminuição do IRβ(Tyr1361) no período pós-prandial e diminuição do GLUT2 nos ratos tratados com Bromocriptina, sugerindo uma alteração no metabolismo da glucose e dos lípidos. Além disso, houve um aumento dos níveis de D1R e da TH em jejum e não se verificaram alterações nos níveis de D2R e DARPP32. Os nossos resultados sugerem que a Bromocriptina atua diretamente no fígado modulando a sinalização dopaminérgica, a qual está associada a um aumento dos níveis de IR em jejum e à reversão da esteatose hepática. Embora sejam necessários mais estudos, estes resultados indicam que a Bromocriptina altera o metabolismo da glicose e dos lípidos, o que pode ser eficaz na redução da lipotoxicidade hepática.

Palavras Chave: Diabetes tipo 2, Doença do Fígado Gordo não Alcoólico, Bromocriptina, Dopamina.

INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is characterized by chronic hyperglycaemia due to peripheral insulin resistance and insufficient insulin secretion caused by progressive pancreatic β-cell failure. From all diabetic patients, 90-95% are type 2 diabetic, being linked to overweight or obesity [1]. The increased prevalence of T2DM worldwide is associated to the increase of obesity in adult and paediatric ages. Visceral fat has been related to insulin resistance and with increased risk for metabolic syndrome development [2]. According to the American Heart Association, this syndrome comprises 3 or more of the following metabolic risk factors: increased waist circumference, hypertriglyceridemia, low levels of high-density lipoprotein (HDL) cholesterol, hypertension and fasting hyperglycaemia, being insulin resistance the central event [3].

The liver is constituted mostly by hepatocytes and it is an important metabolic organ in processing nutrients, detoxification and regulating glucose, fat and protein blood levels. Non-alcoholic Fatty Liver Disease (NAFLD), the most common chronic liver disease, consists in an increased (more than 5%) hepatic lipid storage. In up to 30% of people with NAFLD, this fat origins hepatocyte's injury, including inflammation and fibrosis which leads to cell death and development of Non-alcoholic Steatohepatitis (NASH). In advanced stages, after years or decades, around 20% of people with NASH can develop cirrhosis, with a high risk of liver failure or cancer [4]. Hepatic fatty acids are mostly originated from the diet, adipose tissue lipolysis and de novo lipogenesis [5]. Adipose tissue is the physiological local to store them, avoiding their ectopic deposition in other tissues. However, metabolic syndrome is characterized by adipose tissue dysfunction, with upregulation of proinflammatory cytokines and adipokines and suppression of insulin signalling , leading to lipolysis and ectopic deposition of non-esterified fatty acids (NEFA) in liver and skeletal muscle [6,7]. Despite liver TGs-enriched lipid droplets are considered harmless and suggested to protect from fatty acid-induced insulin resistance, NEFA are known to be a trigger for inflammatory pathways and insulin resistance. The activation of F4/80-expressing Kupffer cells creates the inflammatory environment to NAFLD progression to NASH [8].

Age, diet, male gender and genetics were suggested to contribute to the development of NAFLD. However, this disease is strongly associated with T2DM, obesity, features of metabolic syndrome, such as insulin resistance and high blood pressure. NAFLD diagnosis is still made by non-specific methods such ultrasound or serum transaminases measurement. In advanced stages, NASH diagnosis may require liver biopsy [6]. Approximately 70% of T2DM patients have NAFLD [9,10]. Hazim and colleagues demonstrated the influence of insulin resistance in the NAFLD development in T2DM patients [11]. On the other hand, the role of NAFLD in insulin resistance and T2DM development has also been recognized, given the

decrease of T2DM incidence with NAFLD improvement [12]. Almost all of the existing studies indicate a positive and bidirectional relation between T2DM and NAFLD, promoting a “vicious circle” [12,13].

Previous studies aiming the treatment of patients with NAFLD commonly used drugs originally developed to T2DM. Beyond lifestyle changes, some drugs which have shown a positive effect include Pioglitazone, glucagon-like peptide 1 (GLP-1) receptor agonists, dipeptidyl peptidase-4 inhibitors (DPP4i), sodium-glucose cotransporter 2 inhibitors (SGLT2i) and combination of atorvastatin with metformin [13,14,15]. Furthermore, there is not a medication which acts directly in liver to reduce fat content. In this sense, future investigations are necessary to create new therapeutic strategies for this pathology.

Bromocriptine is a dopaminergic D2 receptor (D2R) agonist able to inhibits prolactin secretion by anterior pituitary. In clinical practice, it is indicated for endocrine and neurological diseases, including prolactinomas, supress lactation, acromegaly and Parkinson’s disease [16]. Given its effects in improving insulin sensitivity, Bromocriptine was also approved by the Food and Drug Administration (FDA) for the treatment of type 2 diabetes in USA [17]. Dopamine function in central nervous system is well known but the presence of dopamine receptors in the eye, cardiovascular system, kidney, gastrointestinal tract and endocrine pancreas has also been shown [18]. However, the role of peripheral dopamine in insulin sensitivity and reduction of hepatic steatosis remains poorly understood [19,20]. The actions of Bromocriptine are believed to be caused by improved insulin secretion and insulin sensitivity. Bromocriptine is known to regulate glucose-stimulated insulin secretion on pancreatic beta cells, avoiding long-term beta cell exhaustion [21]. On the other hand, regulation of the hypothalamic sympathetic output and prolactin have been proposed to be involved in the positive effects on insulin sensitivity [22]. Nevertheless, the peripheral actions of Bromocriptine on insulin-sensitive tissues should be better elucidated since they Bromocriptine have never been addressed before.

In this study, our objective was to address the role of Bromocriptine in reducing liver triglycerides content, regulating the mechanisms of glucose uptake in liver, as well as fatty acid oxidation and storage in hepatic tissue, besides its effects in the amelioration of the glycemic and lipid profile in an animal model of type 2 diabetes with diet-induced dyslipidemia.

MATERIALS AND METHODS

Reagents and Antibodies: Salts and organic solvents used in solutions were purchased to Fisher scientific (Leicestershire, UK), Sigma Chemicals (United States of America - USA) or Merck Darmstad (Germany), with the highest grade of purity commercially available. Antibodies used were targeted to AMPK and AMPK(Thr172) (#2532, #2535, Cell Signaling, USA), GLUT2, (Tyr1361)IRβ, DARPP-T, D1R, D2R (ab54460, ab60946, ab40801, ab81296 and ab21218 Abcam, UK), Tyrosine Hydroxylase (T1299, Sigma, USA) and IRβ (sc-57342, Santa Cruz Biotechnology, USA). Calnexin was used as loading control (AB0037, Sicgen, Portugal).

Animal Models and Maintenance: The experimental protocol was approved by the local Institutional Animal Care and Use Committee and all the procedures were performed by licensed users of Federation of Laboratory Animal Science Associations (FELASA). We studied 24-week-old male Wistar and non-obese type 2 diabetic Goto-Kakizaki rats, from our breeding colonies (Faculty of Medicine, University of Coimbra). Animals were kept under standard conditions: light (12h light and 12h darkness), temperature (22-24ºC), humidity (50-60%) and ventilation with free access to food and water (standard diet A03, SAFE, Barcelona).

Experimental groups: Male Wistar rats were fed the standard diet A03 (5% triglycerides and 45% carbohydrates, SAFE, France) and used as controls. Type 2 diabetic Goto-Kakizaki (GK) were divided into two groups, the first maintained with the same standard diet (GK), while the other was fed a high-fat high-sucrose diet (A03 high-fat, 20% fat, 20% sucrose, SAFE, France). The group fed the high-caloric diet was randomly divided into three groups: the first without no further treatment (GKHFD), the second with bromocriptine treatment in the last month (GKHFDBr) and the third with vehicle administration during the same period (GKHFDVh).

Diet and bromocriptine administration: Rats were fed the high-caloric diet for 5 months (1 to 6 months old). Bromocriptine (10mg/Kg/day) was administered daily during the last month through subcutaneous injection. In the vehicle group, the same volume of the vehicle (DMSO) was administered also subcutaneous during the same period (V<100ul).

Body weight and blood and liver collection: Animals were anesthetized, weighted and serum and plasma were collected as described before [23,24]. After sacrifice by cervical displacement, liver was collected, washed in an isotonic solution (0.9% NaCl), weighted and frozen in liquid nitrogen, being stored at -80ºC. Liver collection was performed in different sets of animals in two different times: after 6h fasting and 1h after intake (3ml) of a mixed diet by gavage (Fortimel, Nestle).

Blood analyses: Before the sacrifice, glycemia and triglyceridemia (n=10) were recorded after a 6h fasting in the tail using a glucometer (Glucometer, Bayer, Germany) and a portable analyser (Accutrend Plus, Roche, Germany) with reactive test stripes.

Liver Triglycerides: 100mg of each liver sample was prepared in homogenization solution (1mL of 5% NP-40/ddH2O solution) and triglycerides were quantified by the colorimetric method Triglyceride Quantification Assay Kit (Abcam 65336, UK).

Western Blotting: Liver sections of 100mg were homogenized in a lysis buffer [25mM Tris, 150mM NaCl, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 10 mM PMSF and 40 μl/g tissue of proteases inhibitor cocktail (Sigma, USA), pH = 7.7]. These homogenates were centrifuged at 14000 rpm for 20 minutes at 4ºC. The supernatant portion was collected and centrifuged again at 14000 rpm for 15 min at 4ºC. Supernatants were then collected, ultra-sonicated for 10 seconds (70 Hz) and aliquoted. The BCA (Bicinchoninic Acid) method (Pierce, USA) was used to determine protein concentration. SDS-PAGE (Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis), with 8% acrylamide gels, was used to separate the loaded samples. Then, they were transferred to PVDF (Polyvinylidene Difluoride) membranes. Membranes were blocked with TBST-0,1% solution [25mM Tris-HCl, 150mM NaCl, 0.1% Tween, pH = 7.6] supplemented with 5% of BSA (Bovine Serum Albumin). The membranes were then incubated overnight at 4°C with the primary antibodies. The secondary antibodies (anti-mouse, GE Healthcare, UK; anti-rabbit and anti-goat, Bio-Rad, USA) were placed on membranes during 2h at room temperature. After each procedure, membranes were washed with TBST solution. After, they were revealed using ECL (Enhanced Chemiluminescence) substrate in a Versadoc system (Bio-Rad, USA) and analyzed with the software Image Quant® (Molecular Dynamics, USA). Calnexin was used as a loading control and it was also quantified.

Histology: Hepatic tissue was sectioned (4μm) and embedded in paraffin (n=5/group). It was stained with Hematoxylin-Eosin (HE). Images were obtained in a Zeiss microscope with incorporated camera (Germany).

Statistical analysis. Results are presented as mean ± SEM. Given the small sample number, the non-parametric Kruskal-Wallis test (all pairwise multiple comparisons) was applied to determine statistical differences between the groups, using the SPSS software (IBM, NY, USA). $p<0.05$ was considered significant.

RESULTS

Weight Gain and Metabolic Profile

Regarding body weight (Figure 1. A), WSD rats were significantly heavier than GKSD ($p<0.001$). Also, GKHCD and GKHCDVh were heavier than age-matched GKSD rats ($p<0.05$), also having a higher percentage of weight gain in the last month (Figure 1. A and B) than its GKSD control rats ($p<0.05$). On the other hand, rats treated with Bromocriptine presented a body weight similar to GKSD (Figure 1. A), which as associated with a significantly lower percentage of weight gain in the last month (Figure 1. B), having been significantly different from GKHCD rats ($p<0.001$). Also, rats injected with DMSO showed differences in relation to GKHCD group ($p<0.05$) (Figure 1. B).

Regarding glycaemic and lipid profiles, GKHCD rats presented higher fasting glycemia and triglycerides levels (Figure 1. C and D). Bromocriptine significantly improved both parameters when compared with the animals fed the high caloric diet (Figure 1. C and D). GKHCDBr still had significantly higher fasting glycemia as compared to WSD ($p<0.05$), but lower than GKHCD ($p<0.01$). All the groups revealed statistical differences in fasting glycemia (Figure 1. C) to WSD rats ($p<0.001$), except Bromocriptine-treated rats which showed the slightest difference ($p<0.05$). As expected, higher triglyceridemia (Figure 1. D) was observed in GKHCD and vehicle groups, showing a significant increase when comparing with its control GKSD and WSD rats ($p<0.001$). On the other hand, it (Figure 1. D) was significantly decreased in GKHCDBr when compared to GKSD ($p<0.01$) and GKHCD ($p<0.001$) group (Figure 1. D). Rats administered only with vehicle were still different from GKHCD ($p<0.05$) group (Figure 1. D).

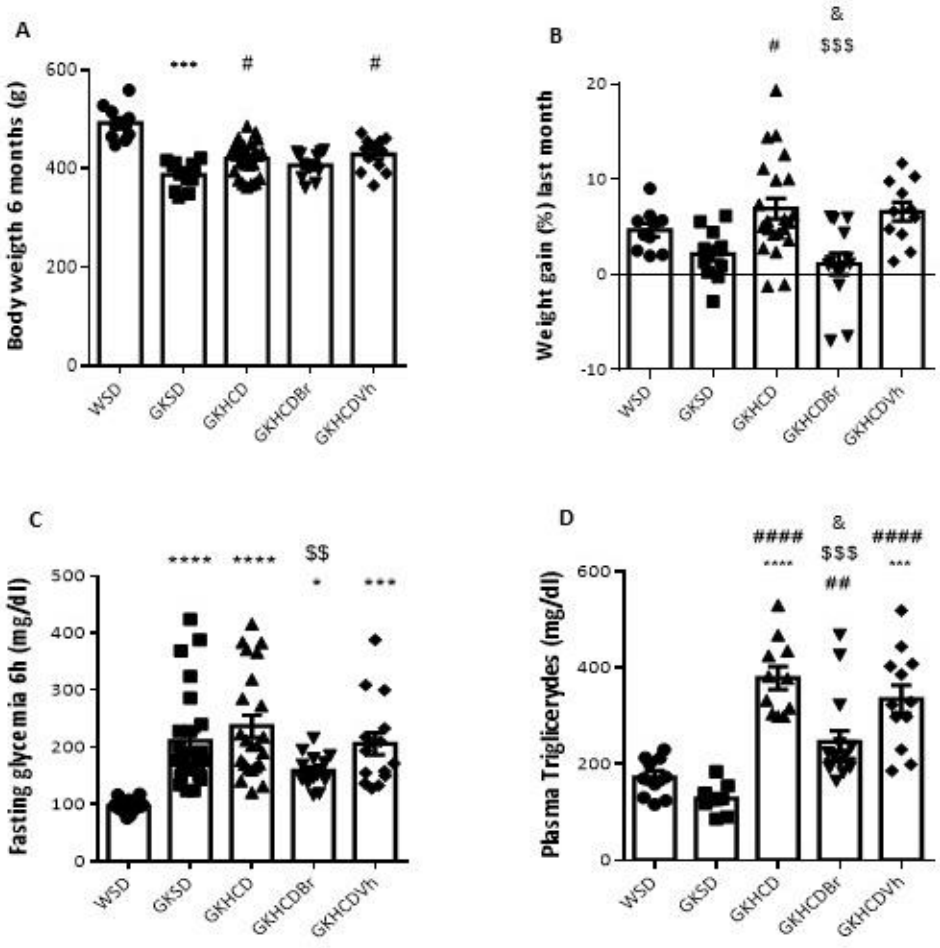


Figure 1: Evaluation of body weight at the end of experience (A), percentage of weight gain in last month (B), fasting glycemia at 6h (C) and plasma triglycerides levels (D). High caloric diet increases the body weight of rats during experimental period. However, it was not observed in rats treated with Bromocriptine. Glycaemic and lipid profiles were improved with Bromocriptine treatment in relation to rats fed the high caloric diet.

WSD - Wistar with standard diet, 6 months of age; GKSD - Goto-Kakizaki with standard diet, 6 months of age; GKHCD – GK with diet enriched in fat and sucrose 1-6 months of age; GKHCDBr – GK with diet enriched in fat and sucrose 1-6 months, submitted to administration of Bromocriptine (5-6 months); GKHCDVh – GK with diet enriched in fat and sucrose 1-6 months, submitted to vehicle administration (5-6 months).

Data is presented as mean ± SEM. * Different from WSD at the same age. # Different from GKSD. $ Different from GKHCD. & Different from GKHCDBr. 1 symbol $p<0.05$; 2 symbols $p<0.01$; 3 symbols $p<0.001$.

Hepatic Morphology

Liver weight (Figure 2. A) and hepatic triglyceride levels (Figure 2. B) were higher in rats fed the diet enriched with fat and sucrose. Livers of GKHCD rats were statistically heavier than its controls GKSD and WSD ($p<0.001$) and similar findings were observed in the GKHCDVh group GKSD ($p<0.001$ vs GKSD and $p<0.01$ vs WSD) (Figure 2. A). The liver weight of GKHCDBr group (Figure 2. A) showed a significant reduction in relation to GKHCD ($p<0.001$) and GKSD ($p<0.01$). GKHCD and GHHCDVh groups showed a significant increase of hepatic triglycerides levels comparing to WSD and GKSD ($p<0.001$) rats (Figure 2. B). This was confirmed by Hematoxylin-Eosin (HE) staining of hepatic tissue (Figure 2. C), which has shown more fat droplets in GKHFD and GHHCDVh groups, corresponding to a higher hepatic steatosis degree. On the other hand, Bromocriptine treatment significantly improved hepatic triglycerides levels (Figure 2. B) in comparison to the other rat groups fed with caloric diet. Bromocriptine-treated rats showed a significant reduction in relation to GKHCD and GKHCDVh ($p<0.01$), although still higher than WSD ($p<0.05$) (Figure 2. B). Such results were confirmed in the histological analysis, where a reduction of lipid droplets is observed, suggesting that Bromocriptine improves hepatic steatosis in these rats.

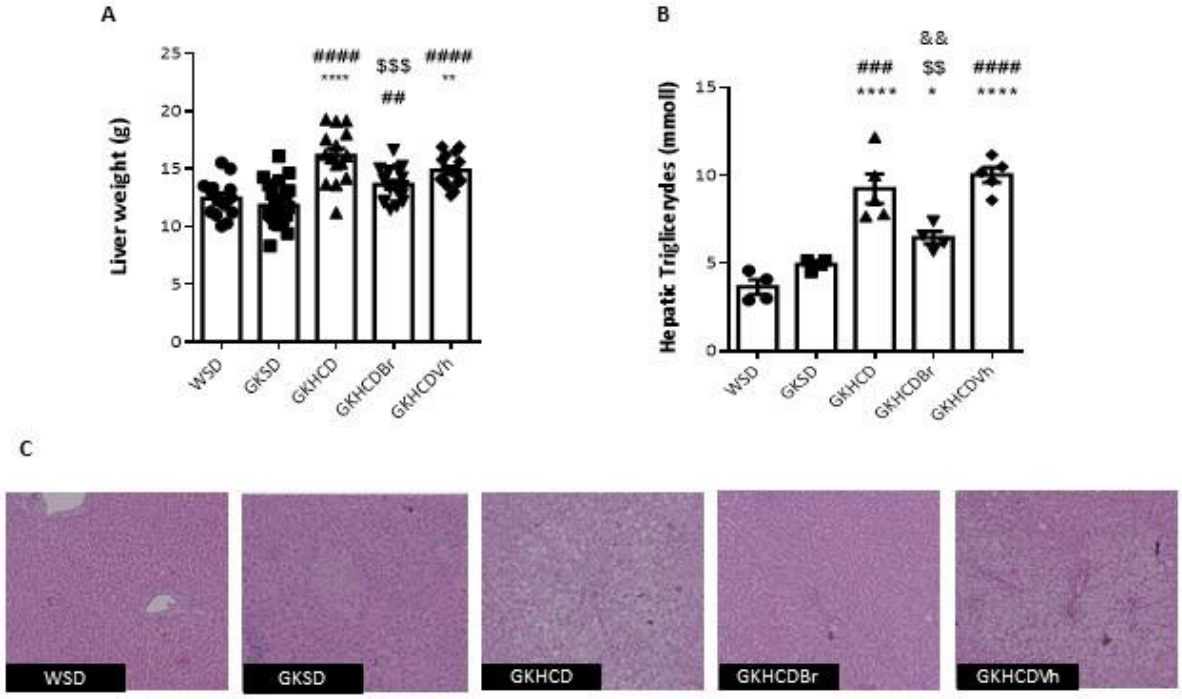


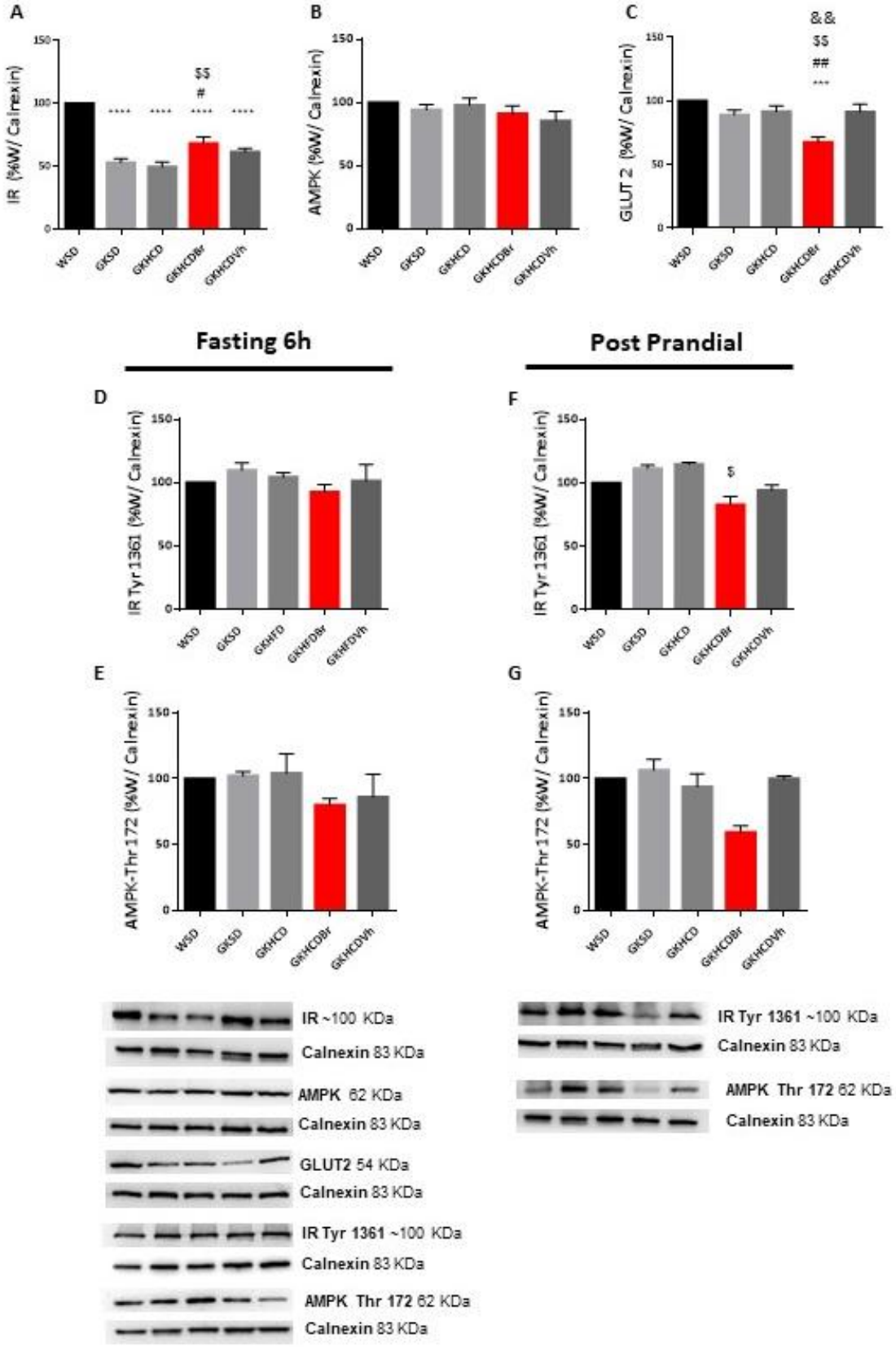
Figure 2: Evaluation of liver weight (A), hepatic triglycerides quantification (nmol) (B) and HE staining of hepatic tissue (C). The liver of the GKHFD rats presented a higher weight, as well as a higher degree of hepatic steatosis in relation to any other study group. The administration of Bromocriptine reduced hepatic steatosis in these rats, which resulted in reduction of the liver weight.
WSD - Wistar with standard diet, 6 months of age; GKSD - Goto-Kakizaki with standard diet, 6 months of age; GKHCD – GK with diet enriched in fat and sucrose 1-6 months of age; GKHCDBr – GK with diet enriched in fat and sucrose 1-6 months, submitted to administration of Bromocriptine (5-6 months); GKHCDVh – GK with diet enriched in fat and sucrose 1-6 months, submitted to vehicle administration (5-6 months).
* Different from WSD; # different from GKSD; $ different from GKHCD; & different from GKHCDVh. 1 symbol, $p < 0.05$; 2 symbols, $p < 0.01$; 3 symbols, $p < 0.001$.

Liver Pathways of Glucose and Lipid Metabolism

In order to evaluate the hepatic pathways involved in the improvement of glycaemic and lipid metabolism by Bromocriptine administration, we have quantified insulin receptor (IR), Glucose Transporter-2 (GLUT2) and AMP-activated Kinase (AMPK), a key enzyme in metabolism (Figure 3. A-C). The phosphorylated forms of IRβ(Tyr1361) and AMPK(Thr172) were also studied at fasting and post prandial period (Figure 3. D -G).

In what concerns to total levels of IR (Figure 3. A), a significant decrease was observed in all the groups in relation to WSD rats ($p<0.001$). Bromocriptine treatment increased IR levels (Figure 3. A), in relation to GKSD ($p<0.05$) and GKHCD ($p<0.01$) rats. No differences were observed in total AMPK levels between the groups (Figure 3. B), while GLUT2 levels were significantly decreased in GKHCDBr rats when compared to all the other studied groups ($p<0.01$ vs WSD and $p<0.001$ vs all the other groups) (Figure 3. C).

Regarding to phosphorylated forms of IR and AMPK (Figure 3. D and E), no statistical differences between the groups were observed in the fasting period. In the post prandial period, 1h after the ingestion of a mixed meal, IRβ(Tyr1361) levels were decreased in rats treated with Bromocriptine, having been statistically different from GKHCD group ($p<0,05$) (Figure 3. F). Moreover, although AMPK(Thr172) did not have shown significant differences, a trend to decrease in Bromocriptine-treated rats was also observed when compared to the other experimental groups (Figure 3. G).


A
IR (%W/ Calnexin)
$$
#
****
B
AMPK (%W/ Calnexin)
C
GLUT 2 (%W/ Calnexin)
&&
$$
##
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WSD
GKSD
GKHCD
GKHCDBr
GKHCDVh
Fasting 6h
Post Prandial
D
IR Tyr1361 (%W/ Calnexin)
GKHFD
GKHFDBr
GKHFDVh
F
$
E
AMPK-Thr172 (%W/ Calnexin)
G
IR ~100 KDa
Calnexin 83 KDa
AMPK 62 KDa
GLUT2 54 KDa
IR Tyr 1361 ~100 KDa
AMPK Thr 172 62 KDa

Figure 3: Western Blot detection of IR (A), AMPK (B), GLUT2 (C), IRβ(Tyr1361) (D) and AMPK(Thr172) (E) in the liver of fasted rats and IRβ(Tyr1361) (F) and AMPK(Thr172) (G) 1h after intake of caloric diet. Bromocriptine treated rats showed an increase of IR and a reduction in GLUT2 during fasting. In post prandial period, there was a significant decrease in IRβ(Tyr1361) and AMPK(Thr172) with Bromocriptine treatment.

WSD - Wistar with standard diet, 6 months of age; GKSD - Goto-Kakizaki with standard diet, 6 months of age; GKHCD – GK with diet enriched in fat and sucrose 1-6 months of age; GKHCDBr – GK with diet enriched in fat and sucrose 1-6 months, submitted to administration of Bromocriptine (5-6 months); GKHCDVh – GK with diet enriched in fat and sucrose 1-6 months, submitted to vehicle administration (5-6 months).

* Different from WSD; # different from GKSD; $ different from GKHCD; & different from GKHCDVh. 1 symbol, $p < 0.05$; 2 symbols, $p < 0.01$; 3 symbols, $p < 0.001$.

Dopaminergic Signalling

For the comprehension of dopaminergic signalling we evaluated dopamine receptors 1 and 2 (D1R and D2R), Tyrosine Hydroxylase (TH) and dopamine and cAMP-regulated phosphoprotein Mr 32,000 (DARPP-32). Regarding the dopaminergic receptors (Figure 4. A and B), D1R showed a significant increase in Bromocriptine-treated rats in comparison to WSD ($p<0.05$) and GKHCD ($p<0.05$) groups. On the other hand, Bromocriptine treatment did not significantly affect D2R levels, having been the levels similar in all the groups. Similar to D1R, Bromocriptine treatment increased TH levels (Figure 4. C) when comparing to WSD, GKSD and GKHCDVh ($p<0.05$) rats. No significant differences were found in DARPP32 levels (Figure 4. D).

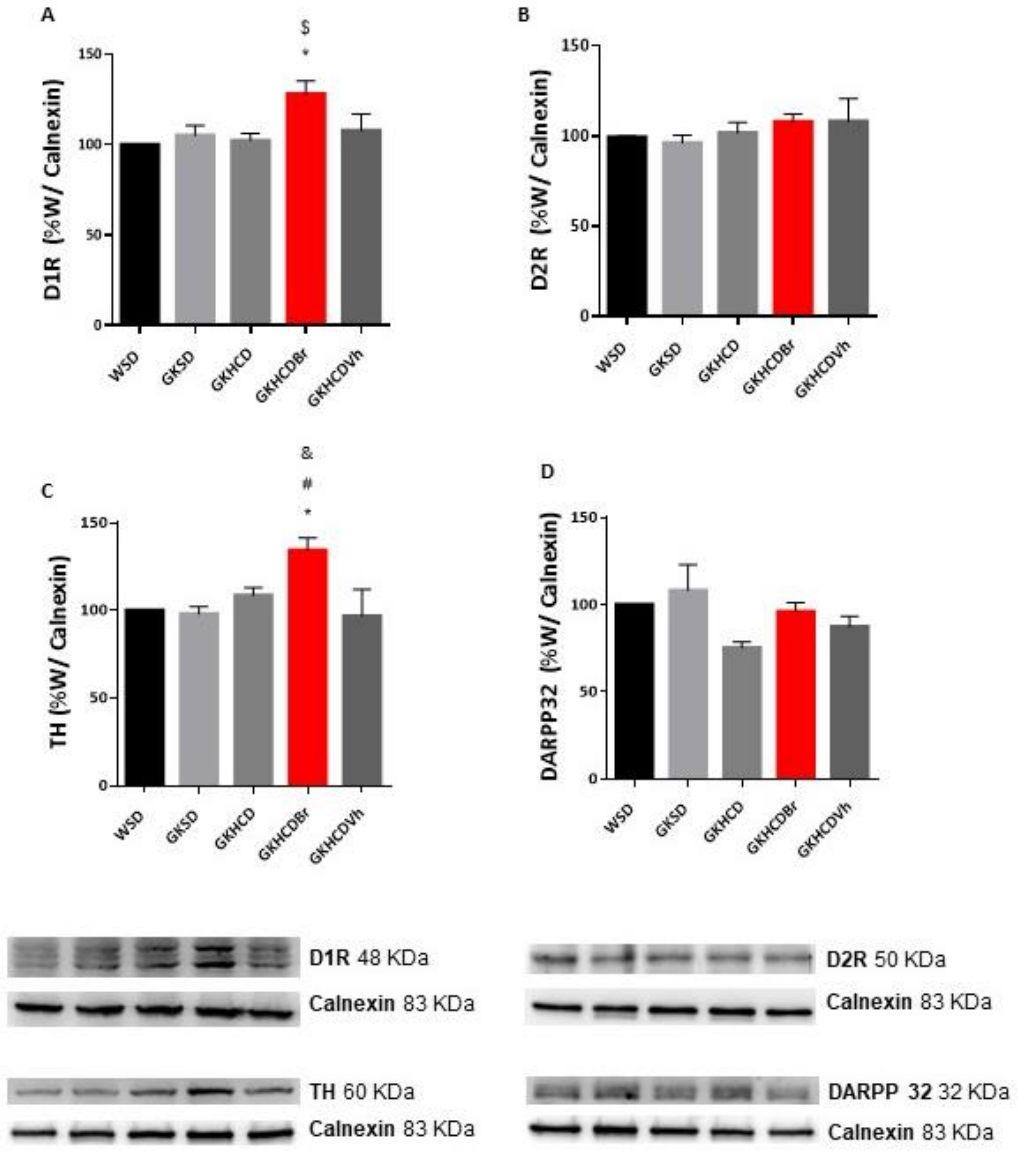

Figure 4: Western Blot detection of the dopaminergic receptors (A and B), Tirosine Hydroxilase (TH) and DARPP (D). An increase of D1R and TH were found after Bromocriptine treatment. However, there were no differences between the groups in D2R expression as well as in DARPP32.
WSD - Wistar with standard diet, 6 months of age; GKSD - Goto-Kakizaki with standard diet, 6 months of age; GKHCD – GK with diet enriched in fat and sucrose 1-6 months of age; GKHCDBr – GK with diet enriched in fat and sucrose 1-6 months, submitted to administration of Bromocriptine (5-6 months); GKHCDVh – GK with diet enriched in fat and sucrose 1-6 months, submitted to vehicle administration (5-6 months).
* Different from WSD; # different from GKSD; $ different from GKHCD; & different from GKHCDVh. 1 symbol, p <0.05; 2 symbols, p <0.01; 3 symbols, p <0.001.

DISCUSSION

In this study we demonstrate that Bromocriptine administration to high-fat high-sucrose diet-fed T2DM rats improves the glycemic profile, insulin sensitivity and plasma triglycerides levels through the modulation of liver pathways involved in glucose uptake and fatty acid oxidation.

T2DM is caused by genetic and environmental factors such as obesity that may play a major role in the development of insulin resistance and alterations on the pancreatic β cells [25,26]. Insulin resistance is associated with the excessive deposition of fatty acids in their non-esterified form. The consequent activation of inflammatory pathways inhibits the phosphorylation and activation of the insulin receptor, glucose uptake by the adipocyte and the storage of fatty acids in the form of triglycerides. Such alterations lead to an increase on the FFA plasmatic levels and the ectopic deposition on insulin sensitive tissues such as the liver [7,27]. Bromocriptine, a dopaminergic agonist of D2, commonly used for Parkinson and prolactinomas treatment, was recently approved by FDA, accompanied by a healthy diet and exercise, as a drug appropriate to treat T2DM due to its insulin sensitizing properties [19,28].

Our goal was to evaluate the therapeutic potential of Bromocriptine on the modulation of the metabolic function in the liver and its repercussions on hepatic steatosis, insulin resistance and on the glycemic and lipid profiles in T2DM. We used the GK rats, a non-obese type 2 diabetic animal model and their controls Wistar rats. For this purpose, besides fasting glycemia, triglyceridemia and hepatic triglycerides, we also analysed the activation of pathways involved in glucose and lipid metabolism (IR, AMPK and GLUT2), as well as dopaminergic signalling (D1R, D2R, TH and DARPP32).

GK rats are a known model of impaired insulin secretion with central and peripheral insulin resistance [29], which develop mild fasting hyperglycaemia and marked insulin intolerance. Our group as previously described GK rats type 2 diabetic-like systemic alterations and vascular complication, with higher visceral adiposity, leptin, hypoadiponectinemia and lipid dysmetabolism [24]. However, GK rats do not exhibit hepatic steatosis at 6 nor at 12 months old [15,30].

Both glucose and lipid metabolism are affected by dopaminergic signalling, having been previously reported that antipsychotic drugs impair the metabolism, by increasing weight gain, promoting insulin resistance and dyslipidaemia [17]. Despite previous studies have shown no body weight loss on T2DM obese individuals to whom Bromocriptine was administrated [31,32], our results showed that Bromocriptine treatment prevents diet-induced weight gain [20] partially by reduction of food intake (data not shown). A recent study conducted by Peixoto Silva and collaborators verified that Bromocriptine administration to rats prevents overweight in adulthood by reducing hyperphagia, visceral fat and hepatic

triglycerides [22]. Also, another study revealed an improvement of BMI in prolactinoma's patients after treatment with this dopamine agonist [33]. Moreover, Bromocriptine was able to improve some pathologies related to the metabolic syndrome in hypertensive and obese rats, such as reduced systolic and diastolic blood pressure, body fat mass, plasma insulin and glucose levels, suggesting an improvement of insulin sensitivity and a reduction of the gluconeogenic activity in the liver [34]. Accordingly, we have also observed that Bromocriptine improves fasting glycemia, which is in accordance with other data from our laboratory showing improvement of HOMA and AUC during the IPITT (unpublished data). These results may support the possibility of a higher glucose uptake by peripheral tissues on animals treated with Bromocriptine, which is also in accordance with previous studies [28,32-34]. Particularly, the study from Pijl and colleagues (2010) on T2DM individuals subjected to Bromocriptine treatment for 16 weeks has shown lower glycaemia, both at fasting and during the glucose tolerance test [32]. A recent case report performed by Oshige and collaborators confirmed a beneficial Bromocriptine effect in glucose-lowering in patient with prolactinoma complicated with T2DM [36]. Furthermore, Bromocriptine was also shown to decreases insulin secretion by beta cells [21], which has been hypothesized as a protective effect against long-term beta cell exhaustion [19,34]. Although such mechanisms may be involved in the variation of post prandial glucose, the effects of Bromocriptine on its uptake by adipose tissue, liver and skeletal muscle cells are unknown, as well as the mechanisms involved.

Other studies describe Bromocriptine as a drug able to improve triglyceride and FFA levels by diminishing their synthesis in the liver and their mobilization from adipose tissue [36]. In fact, our results show that, decreased plasma triglycerides levels were obtained in animals treated with Bromocriptine, as well as liver triglycerides and weight. This is in concordance with the decrease in food intake and suggests alterations on lipid mobilization, as reported in the study performed by Davis and his colleagues [20].

In order to understand the molecular mechanisms involved in glucose and triglycerides improvement, insulin signaling pathways were evaluated in the hepatic tissue. We have observed an increase on the expression of the IR with Bromocriptine treatment, while its phosphorylated form was not changed during the fasting period and was decreased 1h after a mixed meal ingestion. Furthermore, it was visible a decrease of GLUT2, suggesting lower glucose uptake by the liver. Although the liver plays an important role in glucose uptake and storage, together with lower fasting glycemia, such results may denote increased peripheral instead of hepatic glucose uptake after Bromocriptine treatment. Such hypothesis is in line with other results from our group showing increased GLUT4 levels in white and brown adipose tissues (unpublished data). We have evaluated markers of lipid oxidation, due to their known regulation by insulin and, possibly, by Bromocriptine. Previous studies mention Bromocriptine as a drug able to reduce the accumulation of lipids through modulation of lipolysis and

lipogenesis, [37,19]. In the present study, no differences were verified in AMPK activation. However, tendentially lower AMPK(Thr172) was observed in rats administrated with Bromocriptine in post prandial period. Although this protein is diminished in cases of obesity and insulin resistance [38,39], such results may derived from lower insulin signaling in the same conditions. Nevertheless, our results showed an improvement in hepatic steatosis of Bromocriptine-treated rats [20]. Together with other results of our laboratory, such results suggest changes in fatty acid oxidation, storage or synthesis resulting in their lower hepatic accumulation which is reflected by an improvement in hepatic steatosis. Thereby, this data, apparently contradictory, raise new questions about the role of Bromocriptine in what concerns its effects on fatty acids oxidation, synthesis and storage in different insulin-sensitive tissues. The absence of studies in this area and, particularly, in the regulation of these mechanisms on the adipose tissue makes it difficult to understand the obtained results and reinforce the need of further studies.

Several previous studies have indicated that the peripheral role of Bromocriptine involves its action in hypothalamic dopaminergic pathway which, consequently, impacts in the improvement of metabolic outcomes [27,33]. However, antidiabetic effect of Bromocriptine was shown to only partially dependent on decreased serum prolactin levels [41]. However, there are few studies exploring the underlying mechanisms in peripheral tissues by which Bromocriptine improves glycaemic and lipid profiles. As mentioned above, it has been shown that Bromocriptine directly activates the α2-adrenergic receptors in β cells, inhibiting glucose-stimulated insulin secretion and preventing β cell failure [21]. Recently, it was been shown that anti-diabetic mechanisms of Bromocriptine in skeletal muscle of diabetic rats could affect different pathways including Leptin-IL-6/JAK2/p-STAT3/SOCS3, p-IR/p-AKT/GLUT4, PPAR-γ/Adiponectin, Nrf2/PARP-1, and GLP-1 [42]. In our study, Bromocriptine administration promoted an increase of D1R and TH levels in liver while no changes were observed in D2R and DARPP32. The higher D1R levels can be explained by a compensatory/physiological mechanism in response to D2R saturation after administration of its agonist. Moreover, in fact it was observed an increase of TH, the enzyme responsible for conversion of amino acid L-tyrosine to L-DOPA, which suggests an increase of intrinsic dopamine synthesis. Thus, we hypothesize a consequent increase of adenyl cyclase stimulating catabolic pathways which could explain the enhancement of metabolic parameters and hepatic steatosis. Thus, these findings suggest that Bromocriptine action seems to be dependent of D1R and TH increase.

Taken together, our results strengthen the role of Bromocriptine in weight loss and food intake decrease, improving glycaemia and hepatic steatosis through changes in the dopaminergic signalling. However, it is important to discern the central effects of Bromocriptine and its possible peripheral effects to understand the mechanisms by which an improvement in glycaemia and lipid profiles and hepatic steatosis are observed.

CONCLUSION

Our findings suggest that agonist D2 Bromocriptine improves metabolic outcomes and hepatic steatosis by reduction of hepatic triglycerides and GLUT2 levels which may be modulated by dopamine signalling in T2DM animal model. The mechanisms involved should be disclosed in the future in order to improve insulin sensitivity in type 2 diabetes.

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