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Neurodegeneration Alters Metabolic Profile and Sirt 1 Signaling in High-Fat-Induced Obese Mice

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Abstract Different factors may contribute to the development of neurodegenerative diseases. Among them, metabolic syndrome (MS), which has reached epidemic proportions, has emerged as a potential element that may be involved in neurodegeneration. Furthermore, studies have shown the importance of the sirtuin family in neuronal survival and MS, which opens the possibility of new pharmacological targets. This study investigates the influence of sirtuin metabolic pathways by examining the functional capacities of glucose-induced obesity in an excitotoxic state induced by a quinolinic acid (QA) animal model. Mice were divided into two groups that received different diets for 8 weeks: one group received a regular diet, and the other group received a high-fat diet (HF) to induce MS. The animals were submitted to a stereotaxic surgery and subdivided into four groups: Standard (ST), Standard-QA (ST-QA), HF and HF-QA. The QA groups were given a 250 nL quinolinic acid injection in the right striatum and PBS was injected in the other groups. Obese mice presented with a weight gain of 40 % more than the ST group beyond acquiring an insulin resistance. QA induced motor impairment and neurodegeneration in both ST-QA and HF-QA, although no difference was observed between these groups. The HF-QA group showed a reduction in adiposity when compared with the groups that received PBS. Therefore, the HF-QA group demonstrated a commitment-dependent metabolic pathway. The results suggest that an obesogenic diet does not aggravate the neurodegeneration induced by QA. However, the excitotoxicity induced by QA promotes a sirtuin pathway impairment that contributes to metabolic changes.

Keywords Huntington's disease · Metabolic syndrome · Quinolinic acid · Sirtuin

Introduction

There are more obese and overweight people on the planet than people suffering from malnutrition. Substantial literature has emerged which has found obesity to be a major comorbidity in many common diseases which can lead to further morbidity and mortality, including heart disease, diabetes, and metabolic syndrome [1]. The etiology of obesity is multifactorial and involves many complex social, behavioral, environmental, and genetic factors. While the specific underlying causes of obesity and weight gain are poorly understood, the end result is a positive energy balance, where more energy is consumed than expended [2]. Besides the increase in energy intake, a sedentary lifestyle becomes an important factor in obesity development/exacerbation [3].

Many of the comorbidities of obesity are reflected in the so-called metabolic syndrome (MS) that represents a constellation

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of physical and metabolic abnormalities that include a large waist circumference, type 2 diabetes mellitus, dyslipidemia, and increased blood pressure [4–6]. Currently, up to 30 % of middle-aged people in more developed countries have several features of MS. The prevalence is as high as 60 % among individuals above 70 years of age. Only an estimated 30 % of adults have no features at all [5].

More recently, epidemiological studies have provided further evidence for this link where MS, especially with chronic adherence to diets high in fat, was shown to be associated with dementia, Parkinson's, and Alzheimer's diseases. Indeed, 10 % of the cases of dementia worldwide may be attributable to the metabolic disturbances associated with MS. Thus, one could speculate that metabolic change is associated with neurodegenerative processes [7–11].

Neurodegenerative diseases are devastating conditions in which causes are poorly understood. On the other hand, Huntington's disease (HD), an autosomal and dominantly inherited neurodegenerative disorder characterized by neurological and cognitive symptoms, is accompanied by severe weight loss and metabolic abnormalities [12, 13]. Impaired mitochondrial and energy metabolism, and a consequent negative energy balance, may contribute to the enhanced oxidative damage in HD pathogenesis [14, 15].

One of the animal models used to investigate neurodegenerative disorders is obtained by the administration of quinolinic acid (QA), an *N*-methyl-D-aspartate (NMDA) receptor agonist, in the striatum. High concentrations of this compound leads to an excitotoxic process by excessive increase of glutamate release, influx of calcium ions, and neuronal death [11] as well as an inflammation process and mitochondrial dysfunction with impaired energy metabolism and oxidative stress [16, 17]. This model is considered a chemical model of HD because it mimics the deficits observed in the early stages of neurodegeneration observed in the disease [18, 19], even though no genetic mutation occurs. Thus, this is an interesting model to evaluate the effects of altered metabolism on neurodegeneration and associated pathogenic features [20].

Alteration of cellular metabolism has a crucial role in the pathogenesis of HD and is an important consequence of MS. This raises the possibility of developing therapeutic interventions that activate metabolic defenses [21]. In addition, the study of molecules involved in metabolic activation is an emerging topic in the field. In this context, Sirtuin 1 (Sirt1) is an evolutionarily conserved protein with NAD^+-dependent deacetylase activity that participates in cellular metabolism. Sirt1 has neuroprotective roles in models of neurodegenerative diseases [21, 22]. The central role for sirtuins in nutrient sensing is well established, and there is evidence to indicate that sirtuins are an integrative link between metabolic control and transcriptional regulation [23]. Thus, Sirt1 enhanced the signaling pathway and resulted in increased mitochondrial biogenesis and function, as shown by increased oxidative capacity in skeletal muscle and better resistance to muscle fatigue [24, 25].

In this context, we hypothesize that neurodegenerative disease and obesity are mechanistically linked due to their shared pathophysiology, with obesity acting as a systemic metabolic mediator to increase the body's susceptibility to damage from neurotoxins. Thus, the aim of this study was to determine the main sites of the sirtuin metabolic pathway and to examine the functional capacities of multiple components of the glucose metabolic pathway of obesity on a mouse model for excitotoxicity.

Methods

Animals

The experiment was conducted with 40 male Swiss mice (4-week old) from the Federal University of Minas Gerais (Belo Horizonte, Minas Gerais, Brazil). All activities were performed in accordance with the institution's guide for the care and use of laboratory animals and also with the approval of our internal animal ethics committee. The mice were housed and placed in an air-conditioned room (22 ± 2 °C) with a 12-h light–dark cycle. After a 7-day adaptation period, mice were randomly divided into two groups ($n = 20$) and fed respective experimental diets for 8 weeks: a high-fat diet (HF) and standard diet (ST) until the stereotaxic surgical procedure. After the surgery, the groups were subdivided into four groups: a high-fat diet that received PBS (HF), a high-fat diet that received QA (HF-QA), standard diet that received PBS (ST) and standard diet that received QA (ST-QA). The mice had free access to food and water during the experimental period, and they were acclimated in the experimental room 30 min before the behavioral test. All the experiments were performed between 8 am and 5 pm.

Diets

Obesity was induced in male Swiss mice by feeding them a high-fat diet (HFD) for 8 weeks. A HFD was prepared according to the standards of the Association of Official Analytical Chemists as described previously [26]. Diet macronutrients were weighed and mixed homogeneously to form a soft dough. Diets were stored separately in a refrigerator in separate sealed plastic boxes. Standard diet (Purina–Labina®), which was used for the regular maintenance of our mice, is composed of 66.0 % carbohydrate, 23.0 % protein, and 11.0 % fat, making a total of 2.18 kcal per 1 g of diet. HFD was composed of 24.55 % carbohydrate, 14.47 % protein, and 60.98 % fat, presenting a total of 5.28 kcal per 1 g of diet [6, 26]. All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, SP, Brazil).

Food Intake, Body Weight and Tissue Collection Measurements

Food intake was measured twice per week during the treatment to obtain food efficiency (food intake/body weight). For metabolic parameter analysis, the animals were submitted to an overnight fasting and were subsequently killed by decapitation. Samples of blood, epididymal, retroperitoneal, and mesenteric white adipose tissue were collected, weighed, and immediately frozen in dry ice and stored at −80 °C for subsequent analysis. To analyze adiposity, the sum of epididymal, retroperitoneal, and mesenteric white adipose weights were divided by the body weight.

Insulin Sensitivity Tests

An insulin sensitivity test was performed 8 weeks after the diet started on overnight-fed mice, after intraperitoneal injection of insulin (0.75 units/kg body weight; Sigma–Aldrich®, St. Louis, USA). Tail blood samples were taken at 0, 30, 60, 90 and 120 min time after injection for measurement of blood glucose levels using an Accu-Check glucometer (Roche Diagnostics®, Indianapolis, USA).

Drugs

Quinolinic acid (Sigma-Aldrich, St Louis, Mo USA) was dissolved in phosphate-buffered saline (PBS, pH 7.0) and administered unilaterally in the right striatum (100 nmol in 250 nL).

Intrastriatal Administration of QA

Animals were anesthetized with a solution of ketamine and xylazine (80 and 8 mg/kg, respectively) to implant the cannula guide unilaterally into the right striatum using the following coordinates: anteroposterior +0.6 mm, mediolateral −2.1 mm and dorsoventral −2.2 mm from the bregma based on the atlas as per Paxinos and Watson (2001). After 7 days, QA injections were performed by a 30-gauge stainless steel needle attached to a Hamilton syringe. QA or PBS were injected at a volume of 250 nL at 0.08 μL/min, and the injection needle was left in place for another 1 min to allow diffusion and to avoid reflux of the solution. Animals were divided into four groups, as previously mentioned: ST and HF received PBS; ST-QA and HF-QA received QA.

Rotarod Activity

Motor coordination and balance were assessed using rotarod (Insight®, São Paulo, Brazil). Before the surgical implantation of the guide cannula, all animals were given a prior training session on the rotarod apparatus, which consisted of three consecutive days at low rotational speeds (14, 17, 20, and 24 rpm) for 3 min each. Seven days after the surgery, the mice were placed on the rod starting at 14 rpm and accelerating to 37 rpm within 5 min (Hunter et al., 2009). Three trials were conducted and the time that the animals stayed on the rod before falling off was measured and considered the baseline performance. After 2 days of QA or PBS administration, the latency for the animals to fall off was recorded again for each animal.

Biochemical Assessments

Brain Extraction and Sectioning

Two days after the administration of QA or PBS, mice were anesthetized with a solution of ketamine and xylazine (80 and 8 mg/kg, respectively) and perfused with PBS 1x followed by 4 % paraformaldehyde (PFA). The brains were harvested, fixed with the same solution for 24 h at 4 °C, and then cryoprotected for additional 2 days by immersion in 30 % sucrose at 4 °C. The striatum was sectioned at 30 μm with a cryostat (Leica Biosystem, USA) at −20 °C. A series of four coronal sections of the central striatum was mounted for immunofluorescence analysis and stained with Fluorojade C (FJC).

Assessment of Degenerating Neurons

FJC analysis was used to quantify the number of degenerating neurons in the striatum. Brain sections were mounted on gelatin-coated slides, air-dried, and subjected to FJC staining. Slides were first immersed in a solution containing 1 % NaOH in 80 % ethanol for 5 min. They were rinsed for 2 min in 70 % ethanol and for 2 min in distilled water, and then incubated in 0.06 % potassium permanganate solution for 20 min. Thereafter, the slides were rinsed with water for 2 min and incubated in 0.0001 % FJC solution for 20 min. The 0.0001 % FJC solution was prepared by first making a 0.01 % stock solution of FJC dye in distilled water and then adding 1 mL of the stock solution to 99 mL of 0.1 % acetic acid. Slides were washed two times each for 1 min and then air-dried on a slide warmer at 37 °C. After 10 min, sections were washed, dried, cleared in xylene and cover slipped in DPX mounting media. Sections were imaged in a Zeiss fluorescent microscope (Zeiss, Oberkochen, Germany) using a 488-nm excitation. The number of cell deaths was counted using ImageJ software and the results presented as the number of the cell death divided by area.

Reverse Transcription and qRT-PCR

Total RNA from striatum was prepared using TRIzol reagent (Invitrogen Corp.®, San Diego, CA, USA), treated

with DNAse and reverse transcribed with M-MLV (Invitrogen Corp.®) using random hexamer primers. The endogenous glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (internal control), Sirtuin 1 (Sirt1), peroxisome proliferator-activated receptor-gamma coactivator 1 α (PGC-1α), exchange protein directly activated by cAMP (EPAC1), superoxide dismutase (MnSOD), and nuclear respiratory factor (Nrf-1) complementary DNA (cDNA) were amplified using specific primers and SYBR green reagent (Appllied Biosystems®, USA) in a PlusOne platform (Appllied Biosystems®).

Statistical Analysis

Data are presented as mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc and two-way ANOVA followed by Bonferroni post hoc as appropriate. The level of statistical significance was set at a p value less than 0.05. Graph Pad Prism (Graph Pad Software, San Diego, CA) was used for performing all statistical analysis.

Results

Body Composition

To examine the functional consequences of QA administration, we studied the relationship between fat and body weight. Body weight (g) was different only in animals that received a high-fat (HF) and standard (ST) diet (Fig. 1a). However, the differences between groups, with or without the injection of QA (i.e., HF vs HF-QA) were observed only after the QA administration (Fig. 2a). However, analysis of the adiposity showed that HF mice presented a substantial increase of fat mass in relation to other groups (Fig. 1c). Additionally, a decrease in brown adipose tissue mass was noted in HF-QA groups when compared with HF mice (Fig. 1d)

QA Administration Does Not Affect Glycemic Profile

To understand the molecular mechanisms underlying changes in glucose metabolism in mice submitted to QA administration, we analyzed the levels of insulin sensitivity of these animals. Decreased insulin sensitivity was observed in HF

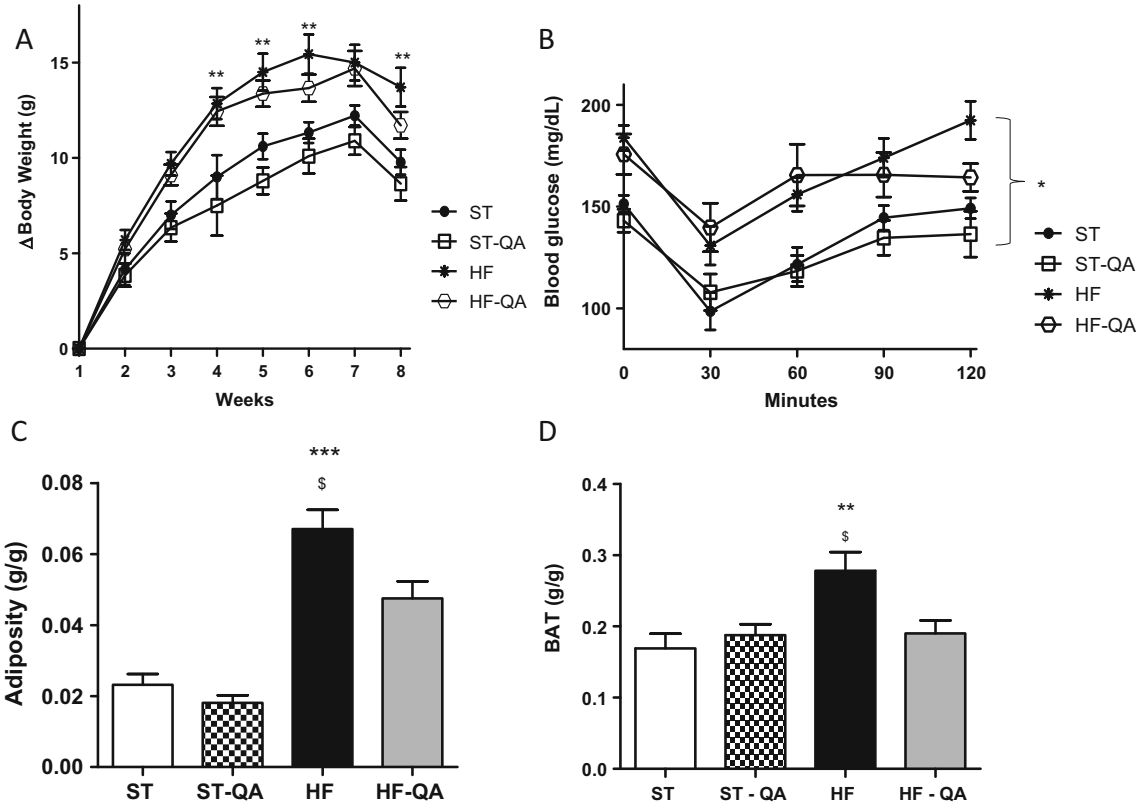


Fig. 1 Body composition and glycemic profile. **a** Time course evaluation of Δ in body weight (g) gain of mice fed for 8 weeks until euthanasia. **b** Insulin sensitivity test after intraperitoneal injection of insulin (0.75 units/kg body weight). Blood samples were collected from the tail at indicated time points and analyzed for glucose concentration ($n = 10$). **c** Adiposity (sum of epididymal, retroperitoneal, and mesenteric adipose tissue weight adjusted by body weight) and brown adipose tissue. Data represent mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs ST. $\$ p < 0.05$ vs HF-QA

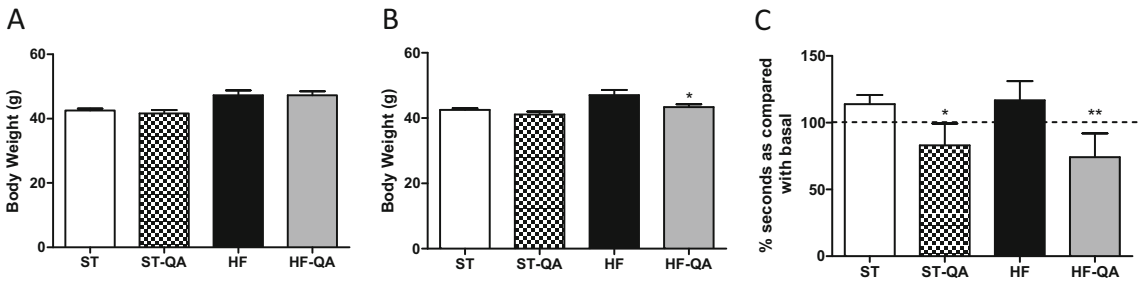


Fig. 2 Effect of the QA administration on body weight and motor activity immediately before stereotaxic surgery, in basal time (**a**), 2 days after QA administration (**b**) and motor activity (**c**). The motor parameters was worse independent of SM ($n = 8–10$ animals/group). Data are expressed as mean ± SEM. $*p < 0.05$ as compared to ST group 2 days $**p < 0.01$ vs HF group

and HF-QA mice when compared with all ST groups (Fig. 1b).

The High-Fat Diet Does Not Modify the Rotarod Performance When Compared with Standard Diet and QA-Treated Animals

QA reduced body weight 2 days after the injection in HF-QA in comparison with the HF group (Fig. 2a, b). Moreover, intrastriatal QA administration significantly impaired the rotarod performance (% of fall off time) as compared to control (Fig. 2c), albeit the diet did not influence this motor behavior in comparison with the ST-QA group.

The High-Fat Diet Does Not Alter the Neurodegeneration of Striatal Neurons When Compared With Standard Diet Group Induced by QA

FluoroJade C analysis was performed to investigate whether diet can enhance the neuronal cell death induced by QA. Both groups injected with QA (ST-QA and HF-QA) significantly increased the neuronal cell death in the striatum as compared with negative control (Fig. 3a, b), although the diet did not alter this pathological parameter.

Metabolic Syndrome Compromises the Central Metabolic Process in QA-Injected Mice

The principal pathway modulating Sirt 1 is the process involving PGC-1α. In this study, we found that Sirt 1 levels decreased significantly in right striatum of the HF-QA mice (Fig. 4c). On the other hand, the left striatum showed high levels in the same groups when compared with other groups, likely in an attempt to compensate the opposite side of striatum (Fig. 4d).

EPAC proteins function as guanine nucleotide exchange factors for small Ras-like GTPases (Rap1 and Rap2), that cycle between an inactive GDP-bound state and an active GTP-bound state. In response to quinolinic acid administration in metabolic pathway, we observe a significant reduction in EPAC 1 dependent on MS. The pathway dependent on Sirt 1, involving PGC-1α, was also compromised secondary to

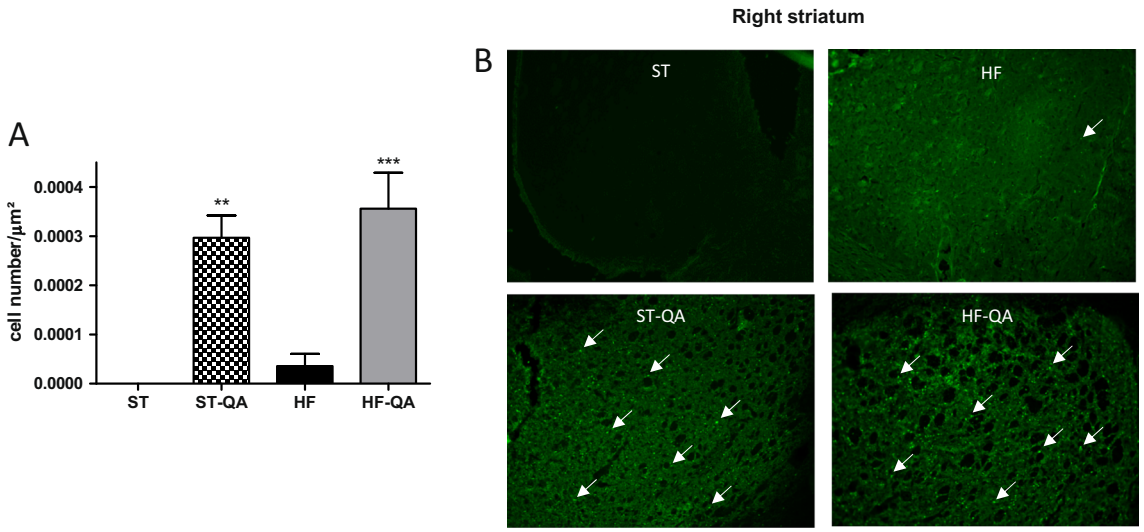
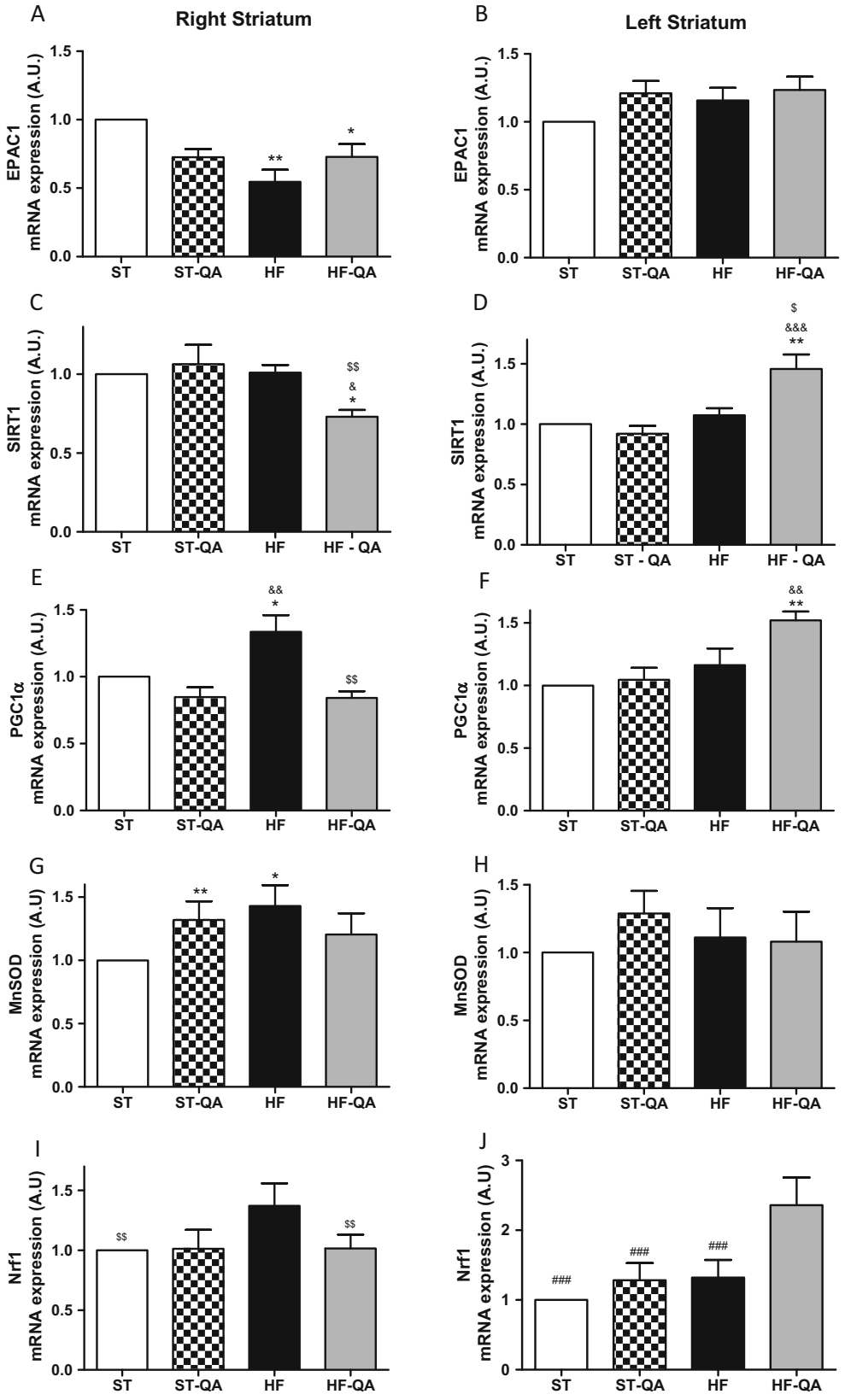


Fig. 3 Effect of metabolic syndrome on neurodegeneration 2 days after QA administration ($n = 4–5$ animals/group). **a** Neuronal death in right striatum. **b** Typical micrographic of FJC of right striatum. *Arrow* indicates a neuronal death. Data are expressed as mean ± SEM. $**p < 0.01$; $***p < 0.001$ vs ST



obesity and QA lesions, suggesting that QA diminishes Sirt 1, EPAC 1 (Fig. 4a) and PGC-1α (Fig. 4e).

NRF1 genes activate the expression of some key metabolic genes that are involved in mitochondrial function. The injured

◀ **Fig. 4** Quinolinic acid worsens metabolic pathway dependent on Sirt 1, even though the left striatum attempts to compensate for this alteration. Analyses of mRNA expression by qRT-PCR. *Right Striatum*: **a** Expression of EPAC-1. **c** Expression of Sirt 1. **e** Expression of PGC1α. **g** Expression of MnSOD. **i** Expression of Nrf-1. *Left Striatum*: **b** Expression of EPAC-1. **d** Expression of Sirt 1. **f** Expression of PGC1α. **h** Expression of MnSOD. **j** Expression of Nrf-1. ($n = 6$) Data represent mean ± SEM, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0001$ vs ST. $\&p < 0.05$, $\&\&p < 0.01$, $\&\&\&p < 0001$ vs ST-QA, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0001$ vs HF, $^{\#\#\#}p < 0001$ vs HF-QA

area did not interfere with this expression, indicating the possibility that the diabetic groups had an augmented NRF 1 expression in the right striatum (Fig. 4i), as reported in literature, which suggest that PGC-1α and NRF 1 are augmented in diabetic conditions [27–29]. However, the combination of MS and quinolinic acid administration stimulates an overexpression of NRF1 in contralateral striatum. Finally, when we evaluated the MnSOD expression, we observed that both MS and neurodegenerative disease stimulate gene expression in the right striatum (Fig. 4g), but when combined, the same does not occur.

Discussion

An increasing number of data recently highlight that metabolic syndrome is correlated with an increased risk for the development of dementia and/or other neurodegenerative diseases, and adiposity has been proposed as an independent factor for development of this disease [30]. Therefore, the present study used a combination of a high-fat diet, to induce MS, combined with excitotoxicity (induced by QA) in mice to evaluate the metabolic commitment when comorbidities are associated.

The major findings in this work are primarily focused on central metabolic abnormality. However, we observed that QA administered in the striatum does not alter the adiposity of euglycemic mice, and, in fact, only has an effect in obese mice. These data were observed when collected to confirm MS. In addition, motor activity was reduced in ST-QA and HF-QA, similar in HD. Neuronal death, as a consequence of QA administration, mimics HD [18, 19]. The high-fat diet has induced some neuronal death, which also likely promotes cognitive impairment [31]. The combination of MS and neurodegenerative lesions induced by QA demonstrates an impaired sirtuin signaling pathway.

Quinolinic acid (QA), a tryptophan metabolite of kynurenine pathway [32], and a well-known excitotoxin, is reported to cause neuroinflammation [33], mitochondrial dysfunction [34, 35], and apoptosis [35, 36]. In fact, inflammation-associated increases in kynurenine pathway levels are commonly observed in obesity. Whether this metabolic disturbance is a risk factor for several obesity-associated disorders including diabetes, neuronal dysfunction, or metabolic syndrome remains to be determined [37]. In addition, QA has been reported to be involved in the pathogenesis of different neurological disorders such as Alzheimer's disease, dementia, hepatic encephalopathy and Huntington's disease itself [38]. Similarly, QA can mimic some symptoms of early stage HD, including the loss of projection of GABAergic neurons with a relative preservation of interneurons, and allows for the study of therapeutics, such as transplantation [39]. The findings of the present study suggest that intrastriatal QA administration significantly altered bodyweight in the high-fat group, with differences in motor performance and neurodegeneration. This data corroborate with the findings of [35] that observed a substantial impairment in locomotor activity followed by weight loss [35].

It is also known that the central nervous system is particularly vulnerable to energetic variations. The main reasons these variations occur is due to the elevated metabolic rate of neuronal cells, and the lower capacity for energy substrate storage. Consequently, the deficiency in energy substrate supplies produces important changes in the intracellular and extracellular ionic concentrations, and, as a result, changes in membrane potential [38]. In contrast, little is known about the effect of QA on brain energy metabolism. In this context, previous investigators have shown that intrastriatal injection of QA provoke a decrease in cellular respiration and ATP levels [40].

Free radicals are now accepted as important mediators of tissue injury in several neurodegenerative states and in some pathological conditions. In fact, free radicals attack membrane lipids, proteins, and nucleic acids, which can cause cell damage or death [41]. The toxic effect on neurons has been partially attributed to excitotoxic damage. Free radicals have also been demonstrated to be responsible for progressive mitochondrial alteration, resulting in the inhibition of complexes II, III, and II–III in the electron transport chain, with a consequent reduction of ATP levels, which in turn may contribute to neurodegeneration. Oxidative stress has been demonstrated as part of its mechanism of toxicity [38]. Mitochondrial disturbances result in an energy deficit and the production of free radicals, which results in disruption of membrane potential and in oxidative stress. Free radicals may lead to protein misfolding or lipid peroxidation, thereby damaging neuronal cells [42].

Distinct neurodegenerative diseases share several common mechanistic pathways, which include oxidative stress, lipid pathway alterations, and increased inflammation. These mechanistic pathways share several elements with the systemic metabolic dysfunction observed in MS that, in this case, could be used to treat both diseases [43].

Two reports by [21, 44] showed that the mammalian Sirt1 can protect against mutant huntingtin neurotoxicity in three different mouse models of Huntington's disease. These studies provide new insights into the neuroprotective functions of

sirtuins and may thus have important implications for the development of neurotherapeutics [23]. Despite the high-fat animals showing an augmented amount of sirtuin in contralateral striatum, it was not sufficient to revert the aggravating metabolic pathway and central damage. Thus, in mouse models of neurodegeneration, the notion exists that sirtuins might represent important therapeutic targets for neurodegenerative disorders. [21] reports that overexpression of Sirt1 is protective in a Huntington's disease mouse model expressing an N-terminal mutant HTT fragment [21].

Despite the significant progress in understanding the molecular basis of neurodegeneration, the lack of known useful molecular targets for effective therapeutic intervention has hindered the drug discovery process. Since the discovery of sirtuin functions in metabolism and aging, these activities were implicated as disease modifiers and as potential therapeutic targets for developing treatments for neurodegenerative disorders [45].

Disrupted metabolic homeostasis is a hallmark of HD. PGC-1α is a transcriptional co-activator that has been implicated in many bioenergetic processes, including energy homeostasis, adaptive thermogenesis, fatty acid oxidation, and glucose metabolism [22, 24]. Recent studies suggest that PGC-1α, a key transcription factor regulating mitochondrial biogenesis and metabolism, is compromised by mutant HTT [46]. PGC-1α knockout mice display neurodegeneration in the striatum and abnormal metabolism as seen in HD [47]. In both the human caudate nucleus and N171-82Q HD mouse striatum, reduced levels of PGC-1α messenger RNA (mRNA) were detected [48]. Recent studies show that administration of a PPAR agonist increases expression of PGC-1α, mitochondrial DNA, and ATP [15]. In addition, we found that intrastriatal QA administration mimics, mainly in obese mice, the PGC-1α profile present in mutant mice.

Within the central nervous system, important tissue with high energetic demand, PGC-1α and NRF1 may have neuroprotective effects in addition to effect on energy and function. Thus, deficiency in this axis leads to certain behavioral abnormalities including profound hyperactivity. These behavioral changes are associated with axonal degeneration in the brain, especially in the striatum, a region known to be very important in the control of movement [28]. This degeneration is similar to QA lesions induced in the present model. Indeed, the molecular basis of this axonal degeneration is not completely understood, though impaired energy homeostasis and reactive oxygen species (ROS) metabolism due to defects in mitochondrial function are the most likely causes [27, 28], as a result of downregulation of ROS defense genes encoding SOD1, SOD2, and glutathione peroxidase [49]. However, we found increased expression of MnSOD in both ST-QA and HF

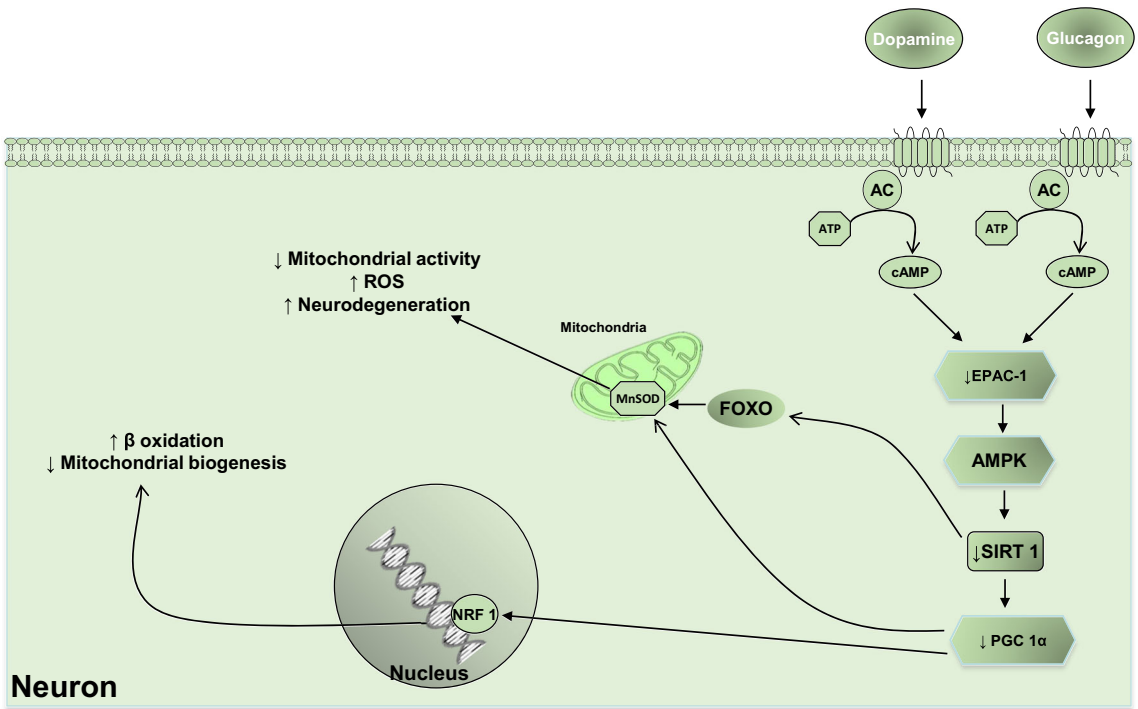


Fig. 5 Sirtuin signaling pathway. Quinolinic acid administration, a drug that mimics some Huntington's diseases symptoms, compromises these same pathways, mainly when associated with metabolic syndrome. Extracellular hormones, such glucagon or dopamine, work inefficiently through receptors to stimulate adenylate cyclase 1 (AC) to convert ATP into cAMP. The poor stimulus does not activate EPAC 1 efficiently, and consequently, the AMPK activation is suboptimal. Thus, the SIRT 1 activation is reduced which leads to deficient PGC-1α activation too. Finally, the endpoints (NRF 1 and MnSOD) in this pathway do not exert the effects properly promoting neurodegeneration, ROS augmentation, mitochondrial activity, and biogenesis impairments

groups. In a similar situation, when the neurotoxicity was induced by QA, we observed a reduction in the evaluated genes (PGC-1α and NRF1) in diabetic mice. In addition, the standard group that received the QA demonstrates a tendency toward PGC-1α and NRF1 reduction when compared with the standard group.

Kalonia et al., in 2010 reported that intrastriatal QA treatment promoted significantly alteration in behavior and reduction of SOD and catalase, beyond mitochondrial enzymes complex (I, II, and IV) activities and TNF-α level alterations in the striatum [50]. In contrast to our protocol, these alterations were observed 3 weeks after QA administration. The variation in SOD levels in our study could be attributed to the experimental time course.

Figure 5 shows a better view of sirtuin metabolic pathways and their importance in neuronal survival. Once these pathways are dysfunctional, the metabolic alterations could contribute to neurodegeneration and aggravation of symptoms, such as motor commitment, as shown in this work. Glucagon and catecholamines are hormones that stimulate metabolic activity. Normally, extracellular hormones work through receptors to stimulate adenylate cyclase 1(AC) to convert ATP into cAMP, which in turn stimulates EPAC 1. Higher EPAC activity indirectly leads to activation of AMP-activated protein kinase (AMPK), a key regulator of cellular energy utilization [51–56] report that resveratrol inhibited the phosphodiesterase (PDE-4) in vitro and stimulated EPAC activity and AMPK phosphorylation in cell culture. In a mouse model of diet-induced obesity, the generic PDE-4 inhibitor increased AMPK activity and Sirt1 activation [56]. PGC-1α deacetylated by Sirt1, combined with NRF1, increases mitochondrial transcription factors; this is essential to maintain mitochondrial function. Stress such as quinolinic acid suppress Sirt1 function and prevent the deacetylation, leading to mitochondrial dysfunction. In addition, Sirt1 leads to the FOXO-dependent activation of the SOD2 gene and the consequently increased MnSOD activity promotes ROS scavenging, which improves mitochondrial activity and prevents neurodegeneration [51–57].

In conclusion, the results suggest that an obesogenic diet does not aggravate the neurodegeneration induced by QA; however, the excitotoxicity induced by QA promotes a sirtuin pathway impairment that contributes to metabolic changes.

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Authors' Contributions Freitas-Lima, LC; Saliba, SW; Andrade, JM; Cunha, ML; Cassini-Vieira, P; Feltenberger, JD; performed the experimental analysis and acquisition of data, analysis and interpretation of the data. Oliveira, ACP; Guimaraes, AL; de-Paula, AM; Barcelos, LS and Santos, SHS contributed to the conception, design, and supervision of the study and interpretation of data. All other authors read, corrected and approved the final version of the manuscript.

Compliance with Ethical Standards All activities were performed in accordance with the institution's guide for the care and use of laboratory animals and also with the approval of our internal animal ethics committee.

Conflict of Interest The authors declare that they have no competing interests.

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