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Juvenile exposure to a high fat diet promotes behavioral and limbic alterations in the absence of obesity

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ABSTRACT

The incidence of metabolic disorders including obesity, type 2 diabetes and metabolic syndrome have seriously increased in the last decades. These diseases – with growing impact in modern societies – constitute major risk factors for neurodegenerative disorders such as Alzheimer's disease (AD), sharing insulin resistance, inflammation and associated cognitive impairment. However, cerebral cellular and molecular pathways involved are not yet clearly understood. Thus, our aim was to study the impact of a non-severe high fat diet (HFD) that resembles western-like alimentary habits, particularly involving juvenile stages where the brain physiology and connectivity are in plain maturation. To this end, one-month-old C57BL/6J male mice were given either a control diet or HFD during 4 months. Exposure to HFD produced metabolic alterations along with changes in behavioral and central parameters, in the absence of obesity. Two-month-old HFD mice showed increased glycemia and plasmatic IL1β but these values normalized at the end of the HFD protocol at 5 months of age, probably representing an acute response that is compensated at later stages. After four months of HFD exposure, mice presented dyslipidemia, increased Lipoprotein-associated phospholipase A2 (Lp-PLA2) activity, hepatic insulin resistance and inflammation. Alterations in the behavioral profile of the HFD group were shown by the impediment in nest building behavior, deficiencies in short and mid-term spatial memories, anxious and depressive-like behavior. Regarding the latter disruptions in emotional processing, we found an increased neural activity in the amygdala, shown by a greater number of c-Fos+ nuclei. We found that hippocampal adult neurogenesis was decreased in HFD mice, showing diminished cell proliferation measured as Ki67+ cells and neuronal differentiation in SGZ by doublecortin labeling. These phenomena were accompanied by a neuroinflammatory and insulin-resistant state in the hippocampus, depicted by a reactive phenotype in Iba1+ microglia cells (increased in number and soma size) and an impaired response to insulin given by decreased phosphorylated Akt levels and increased levels of inhibitory phosphorylation of IRS1. Our data portray a set of alterations in behavioral and neural parameters as a consequence of an early-life exposure to a quite moderate high fat diet, many of which can resemble AD-related features. These results highly emphasize the need to study how metabolic and neurodegenerative disorders are interrelated in deep, thus allowing the finding of successful preventive and therapeutic approaches.

Abbreviations: AD, Alzheimer's disease; CD, control diet; DCX, doublecortin; DG, dentate gyrus; EPM, elevated plus maze; GCL, granular cell layer; HFD, high fat diet; HPA, hypothalamic-pituitary-adrenal axis; Iba1, ionized calcium binding adapter molecule 1; IL1β, interleuquin 1 β; IRS, insulin receptor substrate; Lp-PLA2, Lipoprotein-associated phospholipase A2; NB, nest building; NOL, novel object location recognition test; SGZ, subgranular zone; T2D, type 2 diabetes; TNFα, tumor necrosis factor α; TST, tail suspension test.

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1. Introduction

Recent data from the World Health Organization (WHO) revealed an alarming increase of obesity and overweight, responsible for more deaths than underweight in high and middle income countries. The number of obese patients has been duplicated in the last decades in the world and, regrettably, this increase has been even higher within the infant population (WHO, 2015). The over-consumption of industrialized food with a high content of fat is directly implicated, in addition to changes in lifestyle that include low physical activity. During the juvenile period, feeding habits and especially food quality can potentially impact on behavior, emotionality and cognitive capabilities during adulthood, increasing the risk to develop neurodegenerative diseases in both, humans and animal models (Vendruscolo et al., 2010; Andersen, 2003).

Obesity, insulin resistance and type 2 diabetes (T2D) are major consequences of modern feeding habits and their effects on the central nervous system are thought to be mediated through multiple pathways. Obesity and high fat diet consumption are associated with dyslipidemia and alterations in cholesterol levels contributing to inflammation and pro atherogenic status via high oxidative stress. Obesity-associated peripheral and brain inflammatory processes promote the development of emotional and cognitive alterations, (reviewed in Castanon et al., 2015). Furthermore, increased levels of inflammatory cytokines as TNFα can contribute to central and peripheral insulin signaling dysfunction, aggravating the scene (Miller and Spencer, 2014).

As our group and others have shown, neuroinflammation is found in metabolic disorders. Activation of hippocampal astrocytes and microglia are common findings in spontaneous models of type 1 and type 2 diabetes (Beauquis et al., 2006, 2008, 2010; Biessels et al., 1994). Due to its high glucose demand and elevated insulin sensitivity, the hippocampus can participate in central insulin resistance and subsequently promote brain aging and age-related illnesses as Alzheimer's disease (Dineley et al., 2014; Fehm et al., 2006). The amygdala and the hippocampus are recognized as stress-sensitive structures that experience decisive changes during the adolescent period (LeDoux, 2003; Maren, 2005). Thus, metabolic disturbances with a juvenile onset can interfere with limbic maturation and induce lifelong cognitive and emotional alterations.

Multiple lines of evidence from clinical and epidemiological studies indicate that overweight and obesity are growing global public health problems recognizing multifactorial contributions including genetic, behavioral and environmental agents. A number of different risk factors are shared by T2D, cardiovascular pathology and hypertension; moreover, these pathologies are also associated with neurological and psychological outcomes, being among them impulsivity, attention-deficit hyperactivity disorder, depression and anxiety (Puder and Munsch, 2010).

Obesity and insulin signaling defects have been shown to be associated with brain disorders (de la Monte and Tong, 2014; Freeman et al., 2014; Moll and Schubert, 2012) Mice rendered diabetic (Beauquis et al., 2006) and also genetic models show alterations in synaptic transmission, adult neurogenesis, behavior and glial support and reactivity (Beauquis et al., 2008, 2010; Calvo-Ochoa and Arias, 2015; Stranahan et al., 2008). These issues highlight the importance of searching common cellular and molecular pathways in order to design preventive and therapeutic strategies.

The purpose of this work was to study neural and behavioral effects of a non-severe high fat diet in mice – that could be assimilated to a modern western diet – administered during the critical juvenile stage, focusing on limbic structures, cognitive performance and emotionality. Our hypothesis considers that the exposure to a high fat diet during a susceptible period of the early life may influence brain plasticity. In particular, alterations in hippocampal neurogenic capability could potentially increase the risk to develop cognitive failure or anxiety symptoms, common to diseases like depression and dementia.

Our results show that the exposure to a moderately high fat diet during the juvenile period failed to induce significant overweight and hyperglycemia but was sufficient to provoke relevant peripheral and central changes in adulthood. These alterations could represent premature signs of neurological diseases. Though obesity and overt diabetes are frequently diagnosed, other metabolic disorders could have a subclinical course, constituting 'silent' cardiovascular and brain disease risk factors that are usually present since an early age. Therefore, the normoglycemic non-obese mouse model that we describe in the present study may help to improve diagnosis and prevent CNS complications in the context of subclinical metabolic disorders as well as contributing to the study of the pathways involved.

2. Material and methods

2.1. Animals and diets

C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME) were maintained in our animal facility (Institute of Biology and Experimental Medicine, CONICET; NIH Assurance Certificate # A5072-01) and were housed under controlled conditions of temperature (22 °C) and humidity (50%) with 12 h/12 h light/dark cycles (lights on at 7:00 am). All animal experiments followed the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Ethics Committee of the Institute of Biology and Experimental Medicine. All efforts were done to reduce the number of mice used in the study as well as to minimize animal suffering and discomfort.

One month-old male mice were exposed to control or a high fat diet during four months, as the experimental scheme shows (Fig. 1A). Both control diet (CD) and high fat diet (HFD) were provided by Gepsa Feeds (Grupo Pilar, Pilar, Buenos Aires, Argentina). CD pellets provided 2.5 kcal/g energy and were composed by: carbohydrate 28.8%, proteins 25.5%, fat 3.6%, fibers 27.4%, minerals 8.1% and humidity 6.7%. HFD pellets were custom-prepared and provided 3.9 kcal/g energy. HFD pellets' composition included: carbohydrate 22.5%, proteins 22.8%, fat 21.1%, fibers 23.0%, minerals 5.6% and humidity 5.0%. The main fat components of HFD pellets were monounsaturated fatty acids (44.7%), saturated fatty acids (29.8%) and polyunsaturated fatty acids (20.9%), among others as it was reported by Valdivia et al. (2014) using this diet. The ratio between omega 3/omega 6 polyunsaturated fatty acids did not differ comparing HFD to CD and the ratio value was near 17 in both diets.

Thirty five mice were used in total, comprising 15 CD and 20 HFD mice. During the last month of exposure to the diet, the behavioral profile was assessed as described in Section 2.1 and euthanasia was done 5–7 days after the last behavioral task was performed. At end point, the procedure was as follows: CD and HFD mice were fasted for 6-h (fasting started one hour after lights on), weighed, glucose was measured from tail blood, anesthetized with ketamine (IP 80 mg/kg BW, Holliday-Scott, Argentina) and xylazine (IP 10 mg/kg BW, Bayer, Argentina), stimulated for ten minutes with insulin (IP 5 UI/kg BW, Beta Laboratory, Argentina) and then decapitated.

Additionally, a different group of 20 animals was exposed to either CD or HFD (n = 10 for each group) and euthanized at two months of age in order to assess certain parameters, such as weight, glycemia and inflammatory markers.