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FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

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Effects of simple sugar consumption on cognitive functions in female rats

Efekt konzumace jednoduchých cukrů na kognitivní funkce potkaních samic

Diploma thesis

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„ I declare, that I have written this master's thesis *Effects of simple sugar consumption on cognitive function in female rats* on my own according to the instructions of my master's thesis supervisors Dra. Marta Alegret Jorda and Mgr. Miloslav Macháček, Ph.D. using the literature and the sources are listed in references. This thesis was not used for achievement of another degree. "

In Hradec Králové……………… Signature………………………

Acknowledgements

At first, I would like to thank my thesis advisor Dr. Marta Alegret Jorda from the Faculty of Pharmacy and Food Sciences, University of Barcelona, for guidance, essential advice, valuable comments and time spent on this thesis. I would also like to thank my supervisor Mgr. Miloslav Macháček, Ph.D. for valuable comments and guidance.

My thanks also belong to my colleagues from the laboratory, for their help and pleasant working environment.

Finally, I must express my profound gratitude to my loving boyfriend Michal for unconditional support and to my parents for their support throughout my years of study.

Abstrakt

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Název diplomové práce: Efekt konzumace jednoduchých cukrů na kognitivní funkce potkaních samic.

Konzumace jednoduchých cukrů, především fruktosy, se za několik uplynulých desetiletí zvýšila. Nadměrný příjem cukrů byl spojen s rozvojem inzulinové rezistence, hypertriglyceridémií a oxidačním stresem, což vede k narušení kognitivních funkcí. Nicméně, stále zůstává nejasné, zda jsou tyto změny v kognitivních funkcích způsobeny vysokým kalorickým příjmem, či zda jsou zapříčiněné příjmem specifického monosacharidu. Mimoto, mechanismus na molekulární úrovni není zatím kompletně rozluštěn.

Naše výzkumná skupina podávala potkaním samicím Sprague-Dawley v dlouhodobé studii po dobu 7 měsíců 10% w/v roztok glukosy ve vodě a 10% roztok fruktosy, což simulovalo chronický příjem cukrů u lidí. Plazmatické parametry a exprese proteinů, které jsou zahrnuty v metabolických drahách, byly zkoumány v prefrontální kůře. Kognitivní funkce byly hodnoceny pomocí testu rozpoznávání nového objektu (NOR) a Morris Water Maze (MWM) testu.

Výsledky získané v naší studii ukázaly významný nárůst v hladině triacylglycerolů nalačno i postprandiálně (1,9x a 1,4x, $p<0,05$) pouze ve skupině, kde byla podávána fruktosa. Mimoto, potkaní samice, které byly krmeny pouze fruktosou, vykazovaly narušený metabolismus inzulinu a glukosový toleranční test. Inzulin degradující enzym byl významně snížen (1,89x, $p<0,05$) a hladiny proteinů IRS2 (0,77x, $p<0,05$) a fosforylovaného proteinu Akt (0,72x, $p<0,05$) byly v prefrontální kůře sníženy. Dále, fruktosou krmené potkaní samice vykazovaly poškození tvorby proteinů účastnících se mitochondriální biogeneze a snížené hladiny BDNF proteinu. Výsledky z MWM testu nevykazovaly žádné rozdíly mezi

skupinami, avšak v NOR testu byl zaznamenán významný pokles diskriminačního indexu pouze u potkaních samic krmených fruktózou.

Závěrem, potkaní samice krmené roztokem fruktosy, na rozdíl od potkaních samic krmených glukosou, vykazovaly významné metabolické změny, narušené molekulární pochody a mitochondriální funkce, což vedlo ke kognitivním dysfunkcím. Díky tomuto zjištění, můžeme škodlivé účinky přisuzovat spíše konzumaci fruktosy než vysokému kalorickému příjmu.

Klíčová slova: kognitivní dysfunkce, jednoduché cukry, prefrontální kúra, narušení metabolismu

Abstract

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Title of diploma thesis: Effect of simple sugar consumption on cognitive functions in female rats

The consumption of simple sugars, especially fructose, increased in the past few decades. Excessive sugar intake has been associated with the development of insulin resistance, hypertriglyceridemia and oxidative stress which lead to the impairment of cognitive functions. Nevertheless, it is still unclear whether these alterations in cognitive functions are exclusively caused by high caloric intake or if they are related to specific characteristics of fructose. Moreover, the mechanisms involved are not yet fully deciphered at the molecular level.

Our research group supplemented Sprague-Dawley female rats with 10% w/v glucose in drinking water or with isocaloric fructose solution in a long-term study (7 months), simulating the chronic consumption of sugars in humans. Plasma parameters and expression of proteins, involved in metabolic pathways, were determined in the frontal cortex. Cognitive functions were evaluated through the novel object recognition (NOR) and Morris Water Maze (MWM) tests.

Results, obtained in our study, showed a significant increase of fasting and postprandial triglyceridemia (1.9- and 1.4-fold, $p<0.05$) only in the fructose group. Moreover, only fructose-supplemented rats displayed impaired insulin signaling and abnormality in the glucose tolerance test. Insulin degrading enzyme was significantly increased (1.86-fold, $p<0.05$) and protein levels of insulin receptor substrate 2 (IRS2) (0.77-fold, $p<0.05$) and phosphorylated protein kinase B (p-Akt) (0.72-fold, $p<0.05$) were reduced in the frontal cortex. Additionally, fructose-drinking rats showed altered expression of proteins involved in mitochondrial biogenesis and decreased levels of brain-derived neurotrophic factor (BDNF). Results from MWM test did not show any differences among all groups, whereas in

NOR test only fructose-supplemented rats showed a significant reduction in the discrimination index, suggesting impaired memory.

In conclusion, liquid fructose-supplemented rats, but not isocaloric glucose-supplemented rats, showed significant alterations in metabolism, impaired molecular pathways and mitochondrial functions leading to cognitive dysfunction. Therefore, deleterious effects can be attributed to fructose consumption and not exclusively to the high caloric intake.

Key words: cognitive dysfunction, simple sugars, frontal cortex, metabolic impairment

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1 Introduction

In the last decades, the consumption of sugars has increased rapidly which brings up a lot of negative effects on our health. According to research which has been performed during the past 3 decades, the consumption of sweetened beverages causes the development of metabolic diseases such as diabetes, obesity, heart diseases and cancer. Moreover, consumption of soft drinks has been associated with approximately 188,000 deaths per year worldwide. This alarming number of deaths, caused by excessive consumption of simple sugars, present global problem to which should be paid attention (Singh *et al.,* 2015). Sugar consumption does not lead only to the failure of metabolism and death, but also to the development of various diseases affecting the brain. This study explains how the excessive consumption of simple sugars, especially fructose, influences metabolic pathways leading to the impairment of cognitive functions.

The World Health Organization recommends that the daily consumption of sugars should not be higher than 10 % of the overall daily caloric intake. Nevertheless, since the food industry started using the high fructose corn syrup as the main sweetener in food (cereals, sweets, jellies) and soft drinks, the consumption of simple sugars, mainly fructose, rapidly increased. It has been found that the increased consumption of simple sugars is associated with metabolic disorders, such as diabetes, hypertriglyceridemia, insulin resistance and obesity development. Importantly, it has been suggested that not only metabolic alterations but also cognitive disorders have been linked to excessive intake of sugars.

Diet-induced metabolic disorders are one of the main reasons of oxidative stress development and inflammation, causing alterations in mitochondrial function in the cortex. In recent studies, it has been suggested, that excessive adiposity, hypertriglyceridemia and insulin resistance, are closely related to the development of cognitive impairment, such as memory loss and learning ability deterioration. The disorders in mitochondrial biogenesis produce the imbalance in the production of reactive oxygen species. These radicals have an impact on various proteins, such as brain derived nuclear factor or synaptophysin, in

signaling pathways in the brain and lead to the loss of synapses and neuronal damage.

This thesis is focused on a research studying the influence of the two most used monosaccharides, glucose and fructose, on the metabolic changes and their impact on cognitive functions. Also, in regard to other recent studies, fructose consumption has been associated with a more harmful effect to the human body than the same caloric intake of glucose. Based on this knowledge, the research was also focused to question whether cognitive alterations are caused due to the high caloric intake or if a specific monosaccharide is the culprit.

2 Research study

2.1 Simple sugars

Simple sugars, or monosaccharides, are essential substances indispensable for life. Their main significance is a usage as a basic nutrient for direct use and secondly, they are stored in the human body as an energy supply.

Monosaccharides play a key role in metabolism, as every cell in human body is dependent on energy supply, especially D-glucose which has the main importance in metabolism. For brain, erythrocytes, retina, embryonal tissue and others, glucose has a predominant position. Other monosaccharides, having different structure and significance in the human body, can be metabolized to glucose as well as glucose can be transformed by metabolic processes to distinctive substances, such as fat (Ledvina *et al.*, 2009).

In this thesis, the research conducted about the effects of the two most common monosaccharides - glucose and fructose – on cognitive functions in female rats, will be explained.

2.2 Glucose

Glucose, a monosaccharide also known as dextrose with formula $C_6H_{12}O_6$, is an essential organic compound for humans. Glucose is an enantiomer, but only D-glucose is useful for the human body since the L-isomer does not occur in the human body naturally and cannot be metabolized (Britannica.com, 2009).

Glucose is a hexose, specifically an aldose, which can be synthesized in the body or obtained in food. In nature, glucose is a terminal product of synthesis in process of photosynthesis in plants. After food intake, the level of glucose is increased through hydrolysis of polysaccharides to oligosaccharides and later to monosaccharides and in this form is used as a main source of energy for cells through aerobic respiration in mitochondria (FAO, 2003). Glucose can be metabolized in the human body through various metabolic pathways depending on the presence of air – aerobic respiration, anaerobic respiration and fermentation (Ledvina *et al.,* 2009).

The normal blood level of glucose is 3,9 – 5,6 mmol/l. When the level is lower or higher, it results in metabolic impairment which leads to various complications, such as hypoglycemia, when mental effort is disturbed, or hyperglycemia, which is linked to insulin resistance (IR) when cells do not respond to the insulin presence causing accumulation of glucose in the blood stream (Craft, 2009; Gailliot *et al.,* 2016).

In addition, hyperglycemia can be the promotor of accumulation of advanced glycation end (AGE) products, which are harmful to cells, due to enhanced generation of reactive oxygen species (ROS) causing cell damage (de la Monte et Wands, 2008). Also, enhanced production of ROS causes an imbalance between antioxidants (superoxide dismutase - SOD, catalase - CAT, glutathione peroxidase - GSH-Px) and pro-oxidants, which results in oxidative stress, linked to diabetes mellitus (DM) type 2 and Alzheimer disease (AD) (Kandimalla *et al.,* 2017). AGE proteins are neurotoxic as influence formation of amyloid oligomer aggregation (Woltjer *et al.,* 2003).

Disease based on an imbalance in glucose metabolism, characterized by high levels of blood sugar and insulin resistance (IR) is called DM type 2, while deficiency of insulin characterizes DM type 1 (Kandimalla *et al.,* 2017). In the case of a normal metabolic function, after food ingestion, beta-pancreatic cells release insulin, which is responsible for transfer of glucose from the blood to the muscles, adipose tissue, liver and other tissues. Moreover, insulin stimulates glycogenesis and inhibits gluconeogenesis (Guyton *et al.,* 2005).

Insulin signaling pathway starts with the binding of insulin to insulin receptor associated with tyrosine kinase activity, and insulin receptor substrate (IRS1 and IRS2) triggers downstream signaling cascades of followed steps. IRS activates phosphatidylinositide 3-kinase (PI3K), which has one of the most important roles in insulin signaling. Due to the phosphorylation of this enzyme, PI3K transforms phosphatidylinositol 4,5 bisphosphate (PIP2) to phosphatidylinositol 3,4,5 trisphosphate (PIP3) which stimulates PDK1 (3'-phosphoinositide-dependent kinase 1) activity. This kinase phosphorylates and activates Akt (also known as protein kinase B – PKB). One of the targets of Akt is glycogen synthase kinase 3 beta (GSK3β) and by this mechanism, insulin influences

and regulates metabolism of glycogen from the cell surface (Kandimalla *et al.,* 2017).

Glucose is the main energy supply to the brain. Brain cells express transporters for glucose (GLUTs) which are represented in various isoforms. In the cerebral cortex, the main transporters are GLUT1 (in astrocytes and endothelial cells) and GLUT3 (in neurons). GLUT4 is mainly expressed in hippocampal neurons (Cisternas *et al.,* 2017). After transport to cell cytoplasm, glucose can be used in wide spectrum of pathways which includes glycolysis, where the obtained end product is pyruvate entering into the Krebs cycle (KC). Glycolysis is regulated by various enzymes as hexokinase, phosphofructokinase (PFK-1) as well as pyruvate kinase, thereby the regulation of glycolysis is sufficiently controlled (Brekke *et al.,* 2015). Moreover, other pathways, where glucose enters, are the pentose phosphate pathway (PPP), glycogen synthesis and oxidative phosphorylation. If all these processes are disturbed, mitochondrial metabolism expresses dysfunction, deregulation of KC, insulin resistance, oxidative phosphorylation and decreased expression of key enzymes and transporters (GLUT1-4), the utilization of glucose is lower which is associated with various alterations in cognitive function (Cisternas *et al.,* 2017).

2.3 Fructose

Fructose, a monosaccharide, also known as levulose, is a chemical substance with the same chemical formula as glucose, $C_6H_{12}O_6$. These two positional isomers differ in the position of oxygen, bound with a double bond. Fructose possesses the keto group at the second carbon in the carbon chain while glucose possesses an aldehyde group at the first carbon.

Moreover, glucose and fructose, bound together with α-1,4-*O*-glycosidic bond, form disaccharide called sucrose (Figure 1), mainly obtained from sugar cane and beets. Not only sucrose is present in natural products, but also fructose and glucose are found separately in fruits and honey (Tappy at Le, 2010).

In the last few decades, a trend towards higher intake of fructose as a sweetener has been observed. Nevertheless, not only consumption of fructose but also higher intake of calories in total increased. All these changes in lifestyle and

nutrients intake led to an increase of appearance of diseases related to metabolic syndrome as dyslipidemia, obesity, diabetes mellitus type 2 and others (Gross *et al.,* 2004; Welsh *et al.,* 2010).

2.3.1 Fructose and its use as a sweetener

The most common sweetener, used in last century till 1960's, was mainly sucrose. Industry development and the use of new technologies permitted the production of new sweeteners by the food industry. Due to the extraction of corn starch, which was hydrolyzed to glucose, part of it was converted into fructose through isomerization catalyzed by enzymes. Therefore, the product resulted in new corn-derived sweeteners and the most important is high fructose corn syrup (HFCS) (Tappy at Le, 2010).

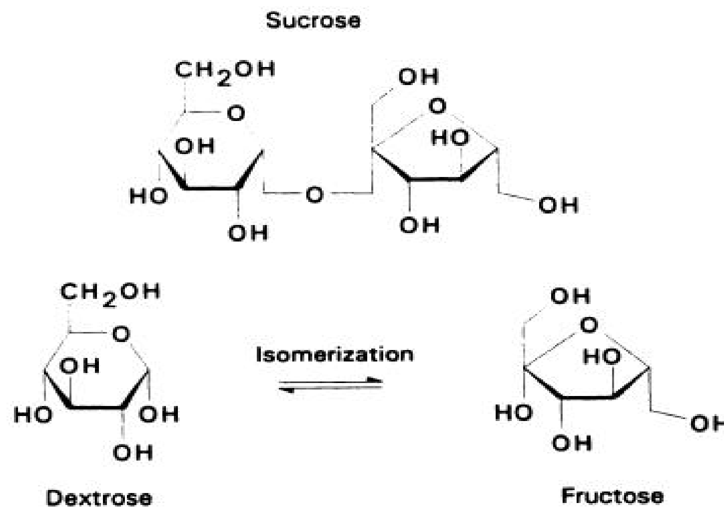


Figure 1 *Chemical structure of disaccharide sucrose and his monosaccharide components: dextrose (glucose) and due to the isomeric conversion, fructose (Hanover et White, 1993).*

The first HFCS introduced to the food industry in 1967, was the HFCS-42 (42% of fructose). The second one, HFCS-55 (55% of fructose) was developed in 1977. The consumption of these sweeteners rapidly increased between the years 1970 and 2000 (Figure 2). During these three decades, the use of HFCS reached 42% of sweeteners consumed in the United States in total (Bray *et al.,* 2004). Since

1980, especially the HFCS-55 represented 61% of all HFCSs used. This growth was induced by a global integration of fructose in the food industry. Specifically, HFCS started to be added in cereals, soft drinks, jellies, dairy desserts, juices, sweets and sweetened beverages (White *et al.,* 2015).

Nowadays, the replacement of sucrose with fructose is popular due to its sweet potency, stability, functional properties and low cost. A graphical summary of sucrose and HFCS consumption in the US between the years 1970 and 2006 is shown in Figure 2. Also, as comparative studies have shown, if we set sucrose to have a sweetness of 100, an equivalent of this sample made of fructose would reach sweetness of 173 while glucose would have 74 (Bray *et al.,* 2004).

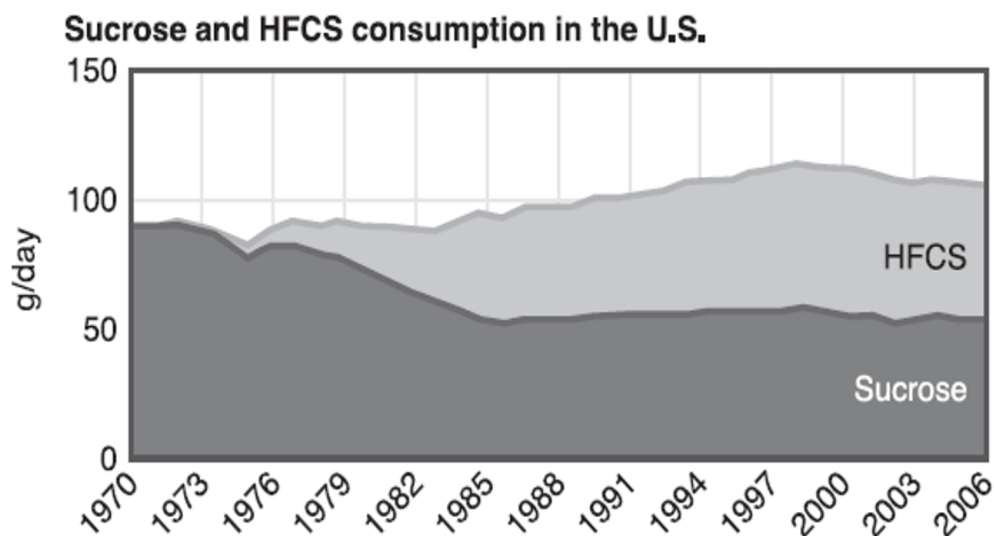


Figure 2 *Consumption of sucrose and HFCS in the United States (Tappy et Le, 2010)*

On the other hand, not just in the United States but also in the rest of the world, the consumption of simple sugars has increased during last decades. The International Sugar Organization is the only official source available, providing every year worldwide statistics (Figure 3 and 4) (Tappy et Le, 2010).

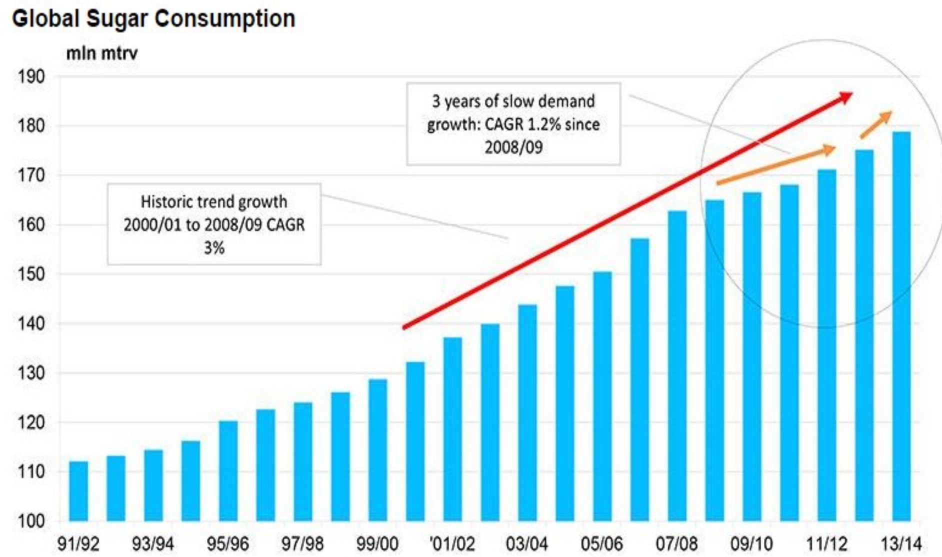


Figure 3 *Development of global sugar consumption (Czarnikow.com, 2014).*

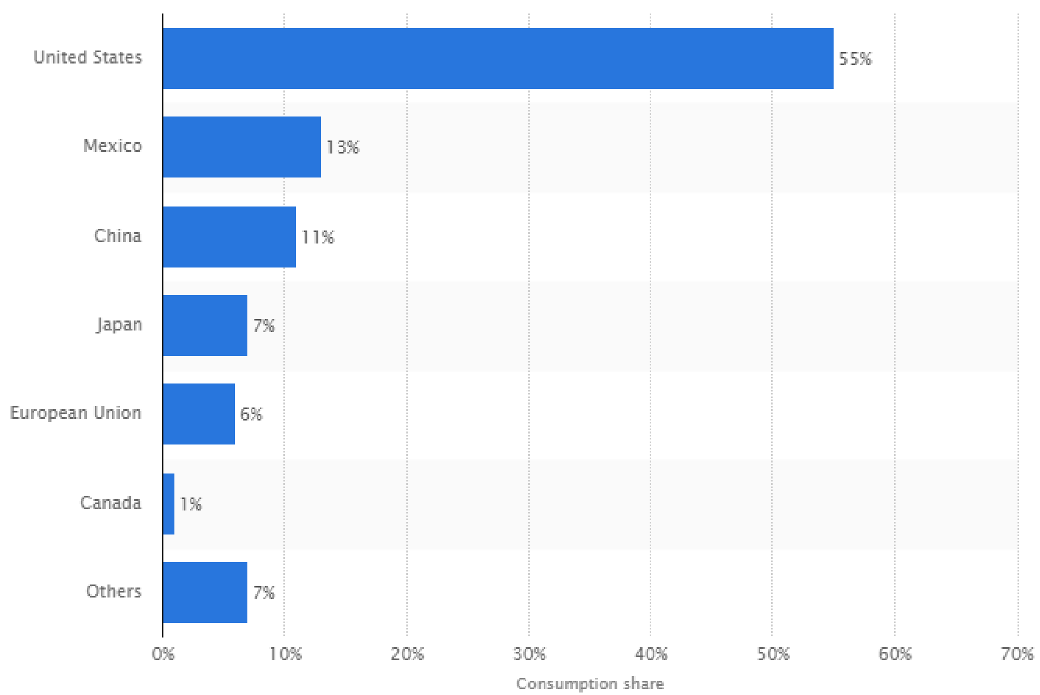


Figure 4 *Comparison of consumption of HFCS in different countries in 2013 (statista.com, 2017).*

2.3.2 Fructose for diabetic patients

At the beginning, fructose as a sweetener was meant to be very beneficial for patients suffering from DM due to its low glycemic index. Various studies compared fructose-containing meals with meals with isocaloric intake based on glucose, sucrose and starch. The results revealed the lower postprandial rise of glucose levels in plasma (Bantle, 2009).

Besides, another advantage was a good economic benefit because fructose has high sweet potency and is very low caloric comparing to glucose (respectively, 19 and 100), that means lower caloric intake (Tappy et Le, 2010).

Another aspect beneficial for diabetic patients, using fructose as a sweetener, could be the effect of fructose on metabolism and insulin levels after consumption in comparison to glucose. Fructose does not have any effect on secretion of insulin after the consumption since insulin is not required to metabolize fructose (Curry, 1989). Fructose has also different metabolic way than glucose which is the reason why levels of sugars in blood do not increase after ingestion of fructose (Rebollo *et al.,* 2012).

Anyway, despite all these benefits, which at the beginning seemed to be rewarding, various alterations in energy metabolism were observed. The liver is the main organ able to metabolize fructose from the monosaccharide to acyl parts and glycerol. Consumption of fructose is associated with metabolic disorders such as an enhanced rate of triglyceride synthesis and lipogenesis. Other alterations associated with fructose consumption are insulin resistance, hyperuricemia, an increase of body weight and abdominal fat. Consequently, all these metabolic disturbances are important causative factors leading to the development of metabolic syndrome (Basciano *et al.,* 2005; Tappy et Le, 2010).

2.4 Metabolism of fructose and glucose

Sucrose is a disaccharide formed by the two different monosaccharides glucose and fructose bound by *O*-glycosidic bond, as mentioned before (2.3.).

Metabolic paths of fructose and glucose are different, so are the effects on the human body. After entering the intestines, the absorption of fructose does not require ATP hydrolysis and is insulin-independent. In the apical part of enterocytes in jejunum and duodenum, fructose enters the enterocytes through its

own specific transporter GLUT5 (Tappy et Le, 2010). This transporter is expressed also in brain, fat tissue, skeletal muscle or kidney (Douard et Ferraris, 2008). Glucose has a transportation system dependent on sodium, which requires ATP as a form of energy. Once monosaccharides enter the enterocytes, on the basolateral part is located transporter GLUT2 common for glucose and fructose. In enterocyte, fructose can be partly converted to lactate or to glucose. However, the major part of fructose reaches the liver (Douard et Ferraris, 2008; Tappy et Le, 2010). Thus, after entering the portal blood, fructose is transferred to the liver, the main organ where 50-75% of fructose is metabolized while only a small amount of fructose is released to the circulatory system (Álvarez *et al.,* 2012). In contrast, glucose can be metabolized in the liver as well as in other extrahepatic tissues such as brain, muscles or fat tissues (Schaefer *et al.,* 2009).

Once fructose is transported inside the hepatocyte, the enzyme fructokinase phosphorylates the fructose to form of fructose-1-phosphate. Next, aldolase cleaves fructose-1-phosphate into trioses glyceraldehyde and dihydroxyacetone phosphate, used for triacylglycerol synthesis and for phospholipid formation. At the end, phosphorylated glyceraldehyde can enter in the glucose pathway and can be transformed into pyruvate (Figure 5). The biochemical synthesis of triacylglycerol (TAG) is more efficient in metabolic path of fructose than glucose as suggested by Bray *et al.* (2004) or Tappy et Le (2010).

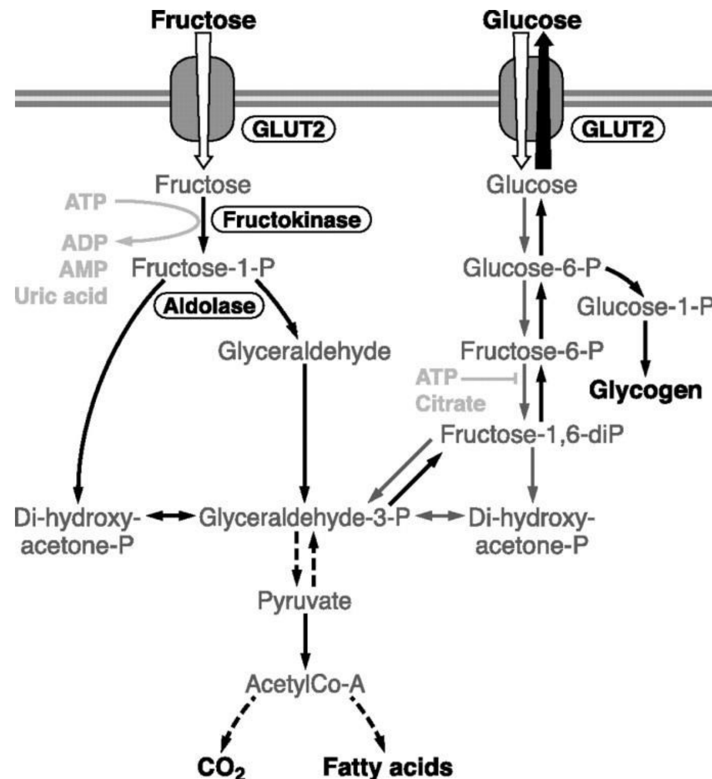


Figure 5 *Glucose and fructose metabolism in the hepatic cell (Tappy et Le, 2010).*

The metabolic pathway of glucose is different from that of fructose and can be controlled at various levels. Phosphorylation of glucose depends on concentration of glucose in plasma and with increased concentration of glucose in plasma or increased activity of insulin, the phosphorylation increases (Iynedjian, 1993). Glucose is phosphorylated by the enzyme glucokinase to glucose-6-phosphate. Next, due to the phosphohexoisomerase, glucose-6-phosphate is converted to fructose-6-phosphate and then to fructose-1,6-bisphosphate through the enzyme phosphofructokinase. This enzyme is inhibited by high concentration of intracellular ATP and citrate, therefore phosphofructokinase is a key regulatory node in the metabolism of glucose. In the last step in the metabolizing process, glucose and fructose metabolism converge: fructose-1,6-biphosphate is converted into trioses dihydroxyacetone-phosphate and glyceraldehyde-3-phosphate (Figure 5) (Tappy et Le, 2010).

Moreover, one of the biggest differences in the metabolism of monosaccharides is the regulation by insulin. Insulin regulates the uptake of glucose through the GLUT4 transporter, and the conversion of glucose into

pyruvate through the stimulation of the expression of the enzyme glucokinase. In contrast, the metabolism of fructose is insulin-independent. In addition, while the metabolism of glucose is tightly controlled at the level of the enzyme glucokinase, the enzyme fructokinase is not inhibited by ATP or citrate. Thus, the metabolism of fructose is unrestricted and originates triose-phosphates can be converted to glucose and glycogen via glyconeogenesis, or continue through the glycolytic pathway facilitating the synthesis of TAG (Tappy et Le, 2010).

As a result, it can be observed how easily fructose can be metabolized into TAG and induce hypertriglyceridemia, a part of metabolic syndrome.

2.5 Problems related to fructose intake

2.5.1 Weight and abdominal fat

An excessive consumption of fructose represents one of the main reasons of overweight and obesity development. Moreover, the variation in metabolism of fructose leads to fat storing due to the production of TAG (Elliot *et al.,* 2002).

Unlike glucose, fructose intake does not promote the stimulation of insulin secretion from pancreatic beta cells. Liberation of insulin stimulates secretion of leptin which gives signals to the brain to inhibit food intake. Since fructose does not induce secretion of this hormone, concentrations of leptin are lower. People who lack leptin are massively obese and their brain never has a signal of saturation by food. Therefore, fructose consumption causes an enhanced intake of food (Bray *et al.,* 2004).

Also, fructose produces an increase of fat tissue, especially the visceral type. Visceral fat is considered riskier than subcutaneous fat, since visceral fat promotes liberation of proinflammatory cytokines. Consequently, abdominal fat is considered to be responsible for developing metabolic syndrome (Anuradha *et al.,* 2012). Storage of lipids in visceral fat may depend on lipoprotein lipase (LPL), an enzyme which is involved in the uptake of TAG from circulating lipoproteins. It has been suggested, that LPL associated with subcutaneous fat tissue is more sensitive to insulin, than visceral LPL. As a result, since fructose does not stimulate secretion of insulin, consumption of fructose invokes increased storage of lipids in visceral

fat, compared to glucose, which stimulates excretion of insulin and causes activation of subcutaneous lipid storage (Stanhope *et al.,* 2012).

2.5.2 Fructose and NAFLD

As it was mentioned before, HFCS is one of the most used sweeteners in the food industry (2.3.1.). The daily average intake of fructose in the USA was estimated 49 g/day per person based on collected data between 1999-2004 by NHANES (Nutrition Health and Nutrition Examination Survey) (Tappy et Le, 2010). The World Health Organization (WHO) recommends that the consumption of free sugars should be less than 10% of the daily caloric intake (Waxman *et al.,* 2004).

High consumption of fructose seems to be one of the most important factors causing Non-Alcoholic Fatty Liver Disease (NAFLD) (Akar *et al.,* 2012). Mainly in developed countries, more than 30% of population suffers from NAFLD which is associated with insulin resistance (Asrih *et al.,* 2013). NAFLD is a clinical-pathological state defined as a large spectrum of liver damage including accumulation of lipids in the liver, especially triglycerides, advanced fibrosis and cirrhosis. In addition, this disease is not considered to be caused by a higher intake of alcohol, including the maximum daily intake limits of ethanol <20g per day for woman and <30g of ethanol per day for men (Alba *et al.,* 2003).

Excess of adiposity and insulin resistance play a big role in facilitating the lipid influx to the liver and enhancement of hepatic lipogenesis *de novo*, especially promoting hepatic triglyceride accumulation (Jung et Choi, 2014). Moreover, this hepatic accumulation of lipids causes increased vulnerability of liver to many damaging factors leading to progression of the pathology to fibrosis/cirrhosis. Inflammation, apoptosis, insulin resistance, increased chemokines/cytokines and increased oxidative stress have been proposed as damaging factors promoting liver disease (Buzzetti *et al.,* 2016).

Oxidative stress is associated with inflammation and its occurrence is caused by misbalance between ROS and the capacity for damage repair. For patients with hyperglycemia, oxidative stress was found to be the major factor in appearance of insulin resistance. In addition, it has been described, how signal production of insulin provides the inhibition of transduction via activation of kinases phosphokinase C (PKC) and JNK (C-Jun N-terminal kinase) (Geda *et al.,*

2013). Due to severity of steatosis, lipid peroxidation tends to increase. Malondialdehyde is end-figure in lipid peroxidation, stimulates production of collagen and fibrogenesis. In addition, malondialdehyde can also assist to inflammation due to promoting nuclear factor κB (NFκB). Proinflammatory cytokines, especially tumor necrosis factor α (TNFα) and interleukin-8, are regulated by this nuclear factor (Alba *et al.,* 2003). Moreover, TNF-α has own leading role in inflammation which is closely connected to ROS.

2.5.3 Insulin resistance and glucose homeostasis disturbance

IR is a condition when tissues change the susceptibility to insulin and become insensitive. This unresponsive state can affect actions of insulin on adipose tissue, muscle, liver or brain selectively. IR is usually associated with compensatory mechanism in periphery – hyperinsulinemia - which also has independently harmful effect (Craft, 2009).

For a long time it has been considered, that development of DM type 2 is caused by insulin resistance, the main key in etiology of DM. Mediators of oxidative stress, pro-inflammatory cytokines such as TNF-α, IL1, IL6 and other chemokines and adipokines, are related to the IR-development. Although at the beginning IR causes compensatory hyperinsulinemia, the chronic exposition to inflammatory mediators, results progressively in damaging β pancreatic cells, and the release of insulin is blocked (Rehman *et al.,* 2016).

As it was mentioned before, consumption of fructose promotes only very low secretion of insulin, probably due to absence of receptor GLUT5 in pancreatic beta cells (Curry, 1989). Consequently, lower concentration of leptin leads to increased body weight and increased levels of circulating non-esterified fatty acids (NEFA), which may be responsible for reduced insulin sensitivity. Moreover, the increased exposure of NEFA in the portal blood increases production of glucose in the liver, which may lead to further impairment in carbohydrate metabolism. Not only carbohydrate metabolism is impaired, but also beta cell function may be affected after long-term exposure to NEFA (Elliot *et al.,* 2002). This may be one of the mechanisms causing insulin resistance since reduced binding of insulin and insulin resistance was reported in hypertriacylglycerolemic patients (Bieger *et al.,* 1984). Disorders of lipid metabolism, linked to higher deposition of lipids, may

produce toxic lipid-derived metabolites, such as ceramides, fatty acyl-CoA and diacylglycerol. These metabolites, which have presence intracellularly, causing higher serine/threonine phosphorylation of insulin IRS1, which is related to lower insulin signaling.

Both IRS1 and IRS2, contain 40 potential serine/threonine sites, these sites are phosphorylated by p38α, mTOR, JNK, MAPK, and PKC, which stimulate IRS protein inhibition or degradation of IRS, which is associated with PI3K activation, while some pathological impairment in cell is occurring (Figure 6) (Guo, 2014).

Mammalian target of rapamycin (mTOR) may be also related to insulin resistance. The mTOR is a serine/threonine protein kinase which appears in two different complexes mTORC1 and mTORC2. Each complex has distinct functions. The mTORC1 is activated by nutrients, such as amino acids, growth factors and hormones like insulin, and controls cell growth, protein synthesis, cell proliferation and metabolism. The mTORC2 is activated as mTORC1 by growth factors and insulin, and participates in the control of the organization of actin cytoskeleton, cell size and eventually cell cycle progression. In addition, mTORC2 takes part in insulin signaling pathway. Akt, also known as protein kinase B, was the first recognized substrate for mTOR2 (Foster et Fingar, 2010). Although Akt activates mTOR through phosphorylation, the chronic activation of mTOR can promote IRS2 degradation and impairs IRS1 signaling through increasing serine phosphorylation, thus leads to insulin resistance.

Insulin sensitivity may also be modified through alterations in the gut microbial flora or changes of permeability in intestines. Diets, rich in fat may lead to higher permeability of intestines and changes of bacterial flora, which result in enhanced concentrations of bacterial lipoproteins or endotoxins in plasma. Endotoxins promote activation of inflammatory pathways and disrupt action of insulin, contributing to insulin resistance development (Tappy et Le, 2010).

Another hormone having a role in insulin resistance is adiponectin. Adiponectin is a protein synthesized in adipocytes, which increases the sensitivity of the tissues to the action of insulin. Therefore, decreased concentration of adiponectin potentially causes insulin resistance (Esfahani *et al.,* 2015).

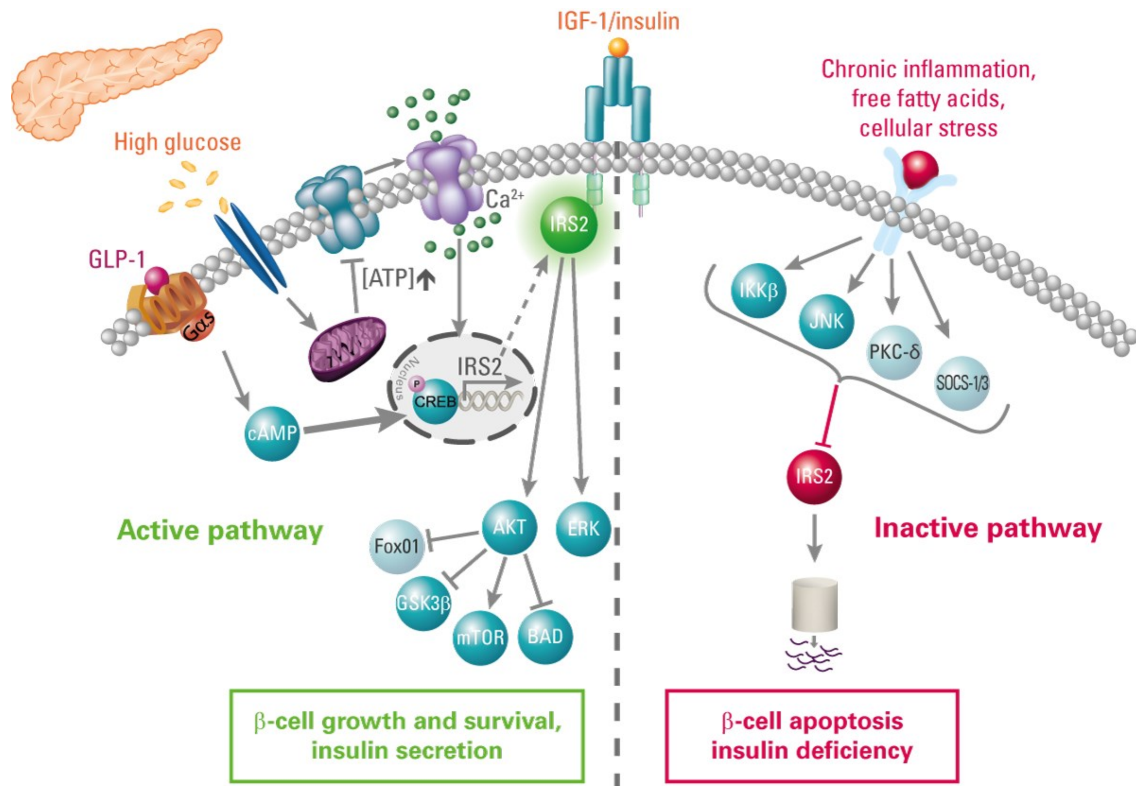


Figure 6 *Description of insulin signaling pathway and insulin resistance. Expression of IRS2 is stimulated by glucose and GLP-1 in pancreatic β-cells. The production of ATP is induced by glucose and then* Ca^{2+} *entry into the cell. In addition, GLP-1 induces the production of cAMP. It leads to increased expression of IRS2, which is necessary for keeping functional IGF-1/Insulin signaling. Another step is the activation of AKT which promotes function, β-cell growth and survival. TNF-α, IL-6, and IL-1β are pro-inflammatory adipocytokines which play a key role in oxidative stress. Cellular stress and free fatty acids are responsible for the activation of the JNK, IKKβ, PKC-δ and SOCS-1/3. Activation of these proteins leads to degradation of IRS2, which consequently inhibits the IGF-1/Insulin signaling pathway. Finally, the lower levels of IRS2 cause natural β-cell apoptosis, which causes reduced β-cell mass, disorders in insulin signaling and DM type 2 (Cisbio.com, 2016).*

2.6 Cognitive functions

By definition, cognition is a mental action or process of acquiring knowledge and understanding through thought, experience, and the senses. Processes which are involved in cognition are attention, knowledge, memory, decision making, comprehension, etc.

Between normal cognitive function and one of the most prevalent dementias - Alzheimer disease, a transitive state known as mild cognitive impairment has been described. People with mild cognitive impairment suffer from memory disorders without any impact on their daily activities (Luchsinger, 2008).

Connection between cognitive impairment and insulin resistance, induced by diets rich in sugars, has been already suggested (Stranahan *et al.,* 2008). Also, a higher risk of neurodegenerative diseases is linked to long-term diabetes (Mastrocola *et al.,* 2016).

A group of risk factors which include insulin resistance, altered cholesterol levels and hypertriglyceridemia, mainly caused by the impairment in carbohydrate and lipid metabolism, is defined as metabolic syndrome (MS) which is also the main precursor of vascular brain diseases and DM type 2. Due to an unhealthy lifestyle, with diets rich in sugars and fats together with low physical activity, MS is observed already in people under 30 years of age. Considering recent studies, MS is currently assumed as a risk factor for development of cognitive decline and memory impairment in senescence, such as Alzheimer disease (AD). One of the main evidence of mild cognitive disorders and AD are decreased synaptic markers, which lead to synaptic degeneration, according to studies on animals with glucose-impaired metabolism (Duarte *et al.,* 2009, Treviňo *et al.,* 2017). Hyperglycemia may also influence brain function and may be responsible for modification of neurochemical processes and loss of synapses, which can also affect synaptic plasticity and further lead to death of neurons.

In subsequent chapters, other metabolic alterations, which are caused by excessive consumption of sugars and at the same time, these alterations are related to development of neurodegenerative disorders, will be revised.

2.6.1 Adiposity

Excessive adiposity is related to an increased risk of insulin resistance, hypertension, dyslipidemia and cardiovascular diseases. Body mass index and wider waist circumference (men and women, 102 and 88 cm, respectively) (Janssen *et al.,* 2004) are also associated with increased risk of development of diabetes, dyslipidemia and other factors of MS. Increased adiposity is also related to higher caloric intake and intake of sugars and fats which are the most potent dietary risk factors for development of AD (Luchsinger *et al.,* 2002; Luchsinger, 2008).

The fatty tissue is active and produces a lot of metabolites which are important in metabolic processes and inflammation, such as adipokines and cytokines, respectively. The adipokines include leptin, adiponectin and resistin. Moreover, inflammatory factors are cytokines as TNF-α, IL-6, IL-1. All these substances are related to hyperinsulinemia and insulin resistance (Esfahani *et al.,* 2015).

Middle-aged obese patients belong to a group which has a higher risk of development of dementia in the future, independently on cardiovascular system (CVS), comorbidities and diabetes, whereas in older age the risk of development of dementia due to obesity is more variable (Whitmer *et al.,* 2008).

Free fatty acids (FFAs) have been proposed as critical factor linking IR and obesity. Normally, insulin is responsible for the inhibition of lipase activity on adipocytes, thus the release of FFAs from adipose tissue is decreased. This action is affected when some imbalance in glucose metabolism, such as insulin resistance or obesity, is occurring, then it leads to persistent elevations of FFAs. Higher concentrations of FFAs related to the development of DM type 2 are confirmed by findings, that patients having normal levels of glycemia, which have a higher risk of diabetes due to family history, show higher levels of FFAs in plasma; therefore, higher FFAs levels predict development of diabetes.

FFAs inhibit insulin degrading enzyme (IDE), which has a significant role in amyloid beta (Aβ) clearance. Moreover, FFAs stimulate not only the gathering of tau filaments and amyloid, but also induce inflammation via interactions with cytokine TNF-α (Craft, 2009). As mentioned before, TNF-α is one of the substances

which are linked to tissular IR, and it is responsible for inhibition of Aβ transport from CNS to the periphery. Patients suffering AD or mild cognitive impairment report increased concentrations of TNF-α in cerebrospinal fluid (CSF) and in the brain (Craft, 2009).

As a result, higher accumulation of Aβ in the brain may be the product of increased levels of TNF-α related to IR, obesity and hyperinsulinemia.

2.6.2 Hyperinsulinemia and insulin resistance

Hyperinsulinemia and insulin resistance have been proposed as key factors for the development of cognitive impairment (Kim et Feldman, 2015).

The regulation of glucose metabolism by insulin is supposed to be the most indispensable step for the modulation of glucose-dependent neurons, which are closely associated with glucose metabolism, regulated by insulin, which is a critical point for brain function. In the brain, transporters GLUT4 and GLUT8 (insulin-dependent glucose transporters) are co-localized with receptors for insulin. A number of these receptors are reduced in the case of presence of DM type 2. The permanent glucose supply to the brain is essential for the brain function and is supposed to be a critical regulator of cognitive processes, as the location of GLUT receptors is on the surface of neurocytes, especially in the hippocampus (Craft, 2009; McNay et Recknagel, 2011).

Cognitive and neural deficits, related to insulin resistance, are associated with DM type 2. As it was described before, insulin signaling pathway begins with the interaction between insulin and insulin receptor. Insulin receptors have been found in multiple tissues, including the brain. Decreased components of the insulin pathway (Figure 6) may be related to AD development (Jolivalt *et al.,* 2008).

For reaching the CNS, insulin must be transported by a saturable receptor-mediated process across the blood brain barrier (BBB). High concentration of these insulin receptors has been found in neuronal synapses and astrocytes, especially in the cerebral cortex, hippocampus, amygdala, hypothalamus and olfactory bulb. The localization of insulin in cortex and hippocampus strongly suggests that insulin can influence memory (Craft, 2009). Moreover, insulin performs multiple functions in the brain including regulatory processes of

memory, neuronal plasticity and in synaptic region activates NMDA receptors (Ríos *et al.,* 2014). Thus, deficiency of energy in neurons, caused by impairment in insulin signaling, increases the neuronal vulnerability to damage caused by oxidative stress and other injuries.

In addition, administration of insulin causes in the hippocampus a rapid and permanent increase in local glycolysis, but in an animal model with DM type 2, this process was completely reduced (McNay *et al.,* 2010). This strongly supports the theory that metabolic processes of insulin in the hippocampus may be the one key element for the cognitive impairment observed in DM type 2.

2.6.3 Effect on blood-vessels

DM type 2 may also affect blood vessels and this may be related also to the development of AD. In the brain, persistent high concentrations of glucose lead to malfunctions in macro and microvessels. Hyperglycemia has damaging effects on endothelium, which have been correlated with cognitive impairment (Ríos *et al.,* 2014).

In addition, IR has many negative consequences on vascular functions since insulin has many direct effects on hemodynamic functions and vasoreactivity. Normally, insulin regulates vasoconstriction via endothelin-1 and dilates vessels by production of nitrogen oxide (NO). Therefore, in the presence of IR vessels are constricted, NO is decreased, activity of endothelin-1 increased and capillary recruitment is also reduced. Hence, because of the endothelial dysfunction, the transport of insulin to the brain is reduced as well as the blood flow and capillary recruitment.

Neural activity is correlated with enhanced blood flow. Consequently, decreased capillary recruitment and vasoconstriction have the impact on the neurovascular function, interaction of neurons and astrocytes (Craft, 2009).

2.6.4 Relation between insulin and Aβ

Insulin is transported to the brain through the BBB by an active transport and may be produced also directly in the brain (Schulingkamp *et al.,* 2000). Long lasting peripheral hyperinsulinemia related to the insulin resistance decreases the transport of insulin through the BBB, resulting in lower levels of insulin and brain activity (Craft, 2009).

Insulin receptors are profusely localized in cerebral cortex and hippocampus. Due to their regulation of neurotransmitter release and recruitment of receptor at synapses, insulin receptors modulate neuronal plasticity and therefore play a key role in learning and memorization (Kandimalla *et al.,* 2017). In IGF/insulin signaling pathway, PKB (Akt) have the key role in neuroprotective function, thereby phosphorylate directly apoptotic regulators, such as FOXO or pro-apoptotic Bcl-2-associated death promoter (BAD), but also as well as the pro-survival transcription factors CREB and NF-kB.

In the brain, decreased concentrations of insulin in cerebrospinal fluid and lack of markers of insulin-signaling may be associated with impaired balance in glucose metabolism (Craft, 2009). A study performed on diabetic mice (DM induced by streptozocin) by Jolivalt *et al.* (2008) showed that this effect is also associated with higher Aβ levels and tau phosphorylation. Furthermore, Watson *et al.* (2003) investigated in a human study how the administration of insulin through intravenous infusion caused an increase of the peptide Aβ42 in CSF. In addition, followed processes, such metabolism of APP and amyloid beta, are also controlled by insulin. Aβ increases microglial release of TNF-α in the brain, which in turn activates neuronal TNF-α receptors and instigates other proteins (e.g. c-Jun N-terminal kinase – JNK1, interferon-inducible protein kinase - PKR, IκB kinase- IKK).

Patients with AD have increased activity of the enzyme glycogen synthase kinase 3β (GSK-3β). In metabolic pathway of insulin, when insulin interacts with IRS, it leads to the activation of PI3K and further Akt, followed by the inhibition of GSK-3β (Figure 7). In addition, GSK-3β play a role in binding the tau protein to neuronal microtubules and has been described as a part contributing to the formation of Aβ and neurofibrillary tangles (NFTs), neuronal plasticity and neuroendocrine functions. Normally, GSK-3β is phosphorylated by Akt which

consequently inhibits activation of glycogen synthase. In the case of insulin resistance dephosphorylated GSK-3β is activated and can promote the binding of tau protein to microtubules (Ríos *et al.,* 2014; Kandimalla *et al.,* 2017).

AD patients with higher BMI had significantly more deleterious results than control patients. In conclusion, there is the evidence of a link between obesity and IR that is supposed to have notable implications for Alzheimer disease and vascular dementia. In late origin of AD may be important especially mechanisms regulating clearance of Aβ.

IDE is an enzyme which is responsible for clearance of Aβ. If the activity of IDE is decreased, Aβ accumulation occurs, leading to senile plaques formation. Insulin and Aβ compete for IDE, and due to the high affinity of IDE to insulin, the degradation of Aβ is suppressed (Malito *et al.,* 2008). In addition, it has been shown that Aβ reduces the insulin binding to its receptors, which leads to insulin resistance (Xie *et al.,* 2002).

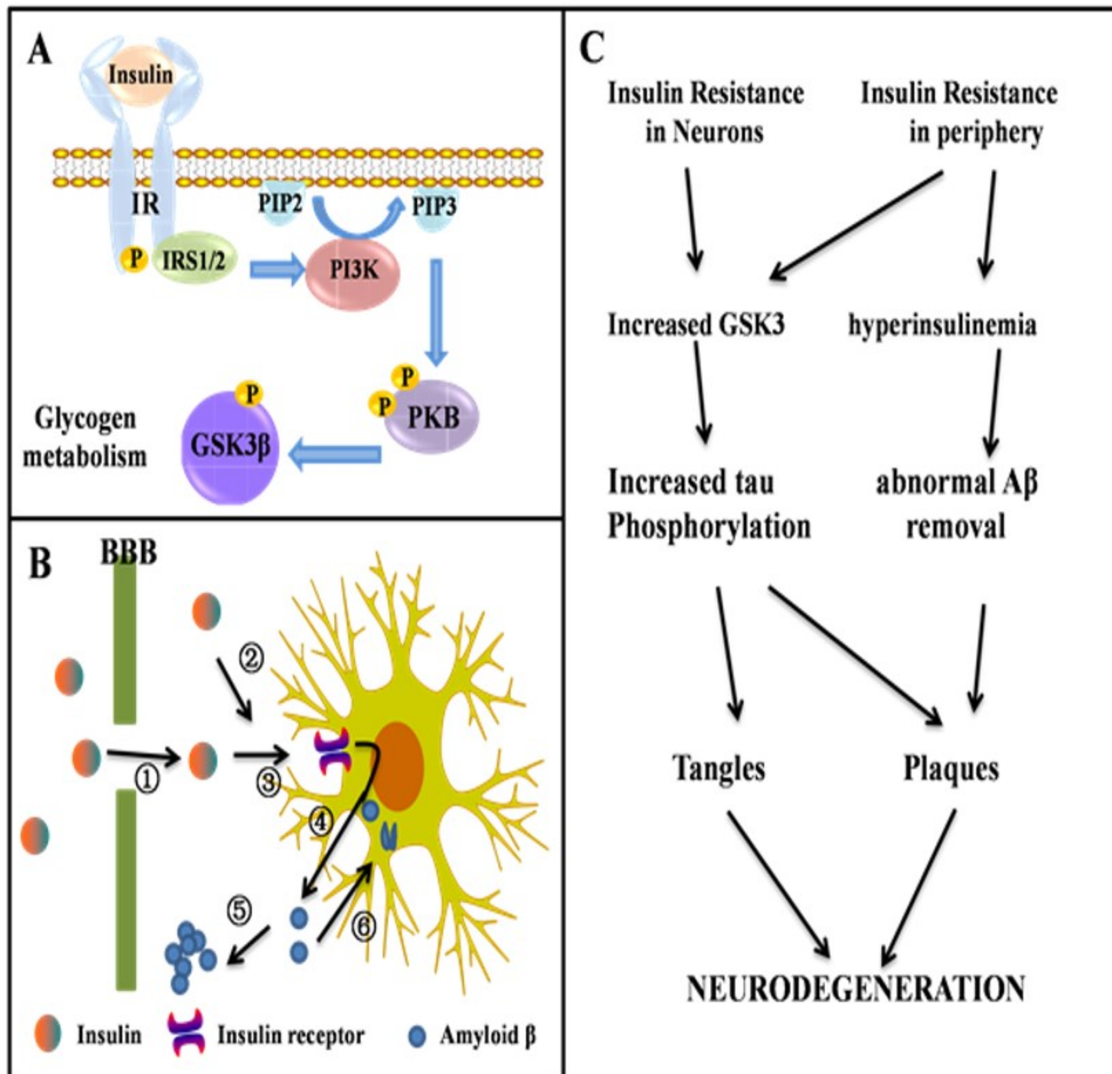


Figure 7 *Scheme of insulin pathway and phosphorylation of GSK-3β – part A. In part C is in detail explained the process of neurodegeneration due to the accumulation of Aβ in neurites in the case of insulin resistance (Lin et Zhang, 2014).*

2.6.5 Mitochondrial function

Mitochondria are vital organelles which have a fundamental role in cell survival and energy metabolism. Neurons are notably vulnerable to some mitochondrial dysfunction, which is also one of the main features of AD.

Mitochondria play a key role in plasticity and synaptic development, thus the impairment in the function or distribution of these organelles may result in dysfunction and/or loss of synapses (Wang *et al.,* 2009).

One of the risk factors being responsible for oxidative stress is chronic hyperglycemia. Decreased use of glucose and insulin resistance associated with aging and Alzheimer disease could be linked to mitochondrial oxidative phosphorylation (OXPHOS) disorders (Palomera-Avalos *et al.,* 2017).

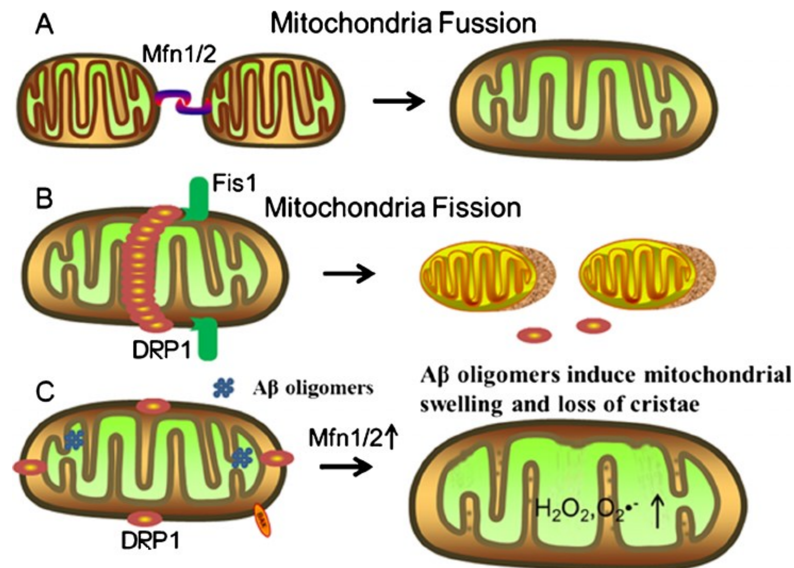
Mitochondria are dynamic organelles which cycle between two processes – fission and fusion. Fusion is the process by which two mitochondria merge in one compartment, so that the synthesis of ATP is increased for maximizing respiration of the cell. Otherwise, fission is the process leading to disintegration of mitochondria to fragmented parts (Figure 8). This occurs when the cell is in resting phase and when high respiratory activity is not needed.

The mitochondrial bioenergetic state regulates directly the mitochondrial dynamics. Some of the proteins involved in mitochondrial dynamics are optic atrophy-1 protein (OPA1), mitofusin 1 (Mfn1) and mitofusin 2 (Mfn2), fission protein-1 (Fis1), dynamic-related protein 1 (Drp1) and others (Hall *et al.,* 2014). Fission is regulated by proteins Mfn1, Mfn2 and OPA1; however, fusion is under control of Fis1 and Drp1.

The location of Mfn1, Mfn2 and Fis1 is in the outer membrane of mitochondria and on the other hand, OPA1 is located in the inner membrane (Wang *et al.,* 2009).

A correct balance between the processes of fission/fusion is critical for neuronal survival and function. Oxidative stress, which occurs in neurodegenerative disorders and aging, alters this equilibrium (Wang *et al.,* 2009; Zhang *et al.,* 2016).

Elevated fission, when mitochondria are split into abnormal fragmented parts, subsequently causes the elimination of these organelles by autophagy processes. Otherwise, fusion is needed in case of energetic efficiency, due to formation of round and larger mitochondria, which directly influence the maintenance and development of synapses.



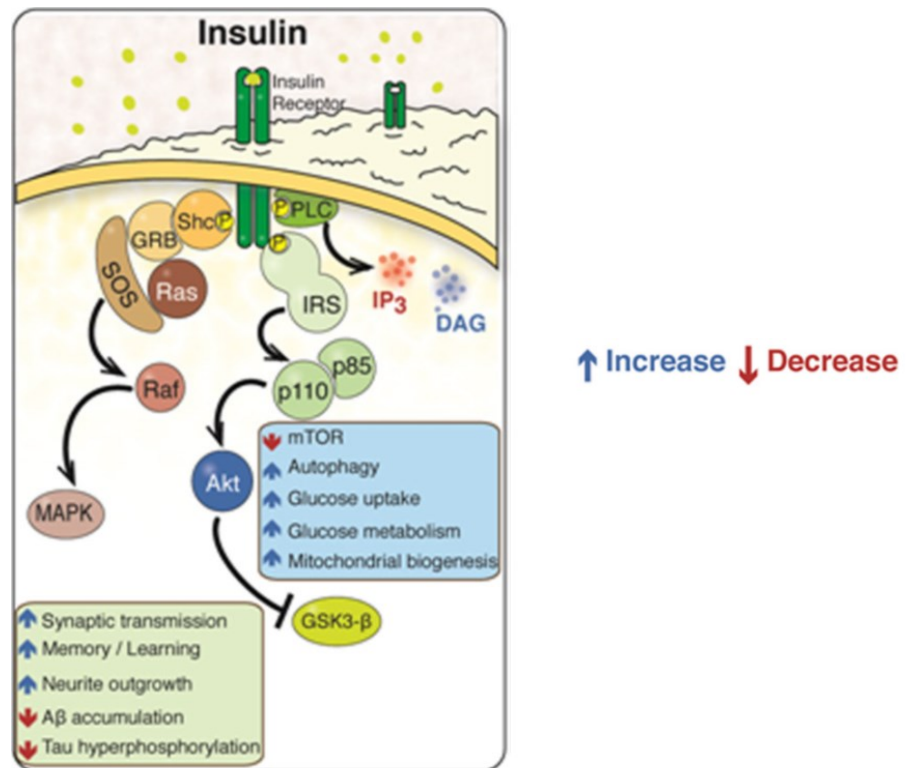


Figure 8 *In the top part of Figure 8 is shown mitochondrial biogenesis with specific proteins for fission (Fis1 and Drp1), fusion (Mfn1/2) and effect of Aβ on mitochondria (Wu et al.,2014). The bottom part of Figure 8 explains how insulin enhances synaptic transmission, memory, growth of neuron and on the other hand decreases accumulation of plaque and tau phosphorylation (Ríos et al., 2014).*

In addition, another significant protein with influence on mitochondrial biogenesis is peroxisome proliferator-activated receptor gamma coactivator 1 alpha (PGC-1α). PGC-1α is mainly expressed in tissues dependent on high energetic intake such as liver, adipose tissue, muscles and brain. PGC-1α occurs as coactivator associated with PPARα and has an important function in energy metabolism of cells through various interactions with transcription factors (Besseiche *et al.,* 2015; Wu *et al.,* 2016). NRF1 and TFAM are transcription factors which play the key role in regulation of mitochondrial biogenesis and are notably downregulated in dorsal root ganglion (DRG) neurons (Choi *et al.,* 2014).

Energy metabolism is essential for many cellular processes mentioned before such as gluconeogenesis, fatty acid oxidation, glucose transport and mitochondrial respiration and biogenesis (Corona et Duchen, 2015). Furthermore, PGC-1α regulates various metabolic adaptations and also takes part in development of DM type 2 and neurodegeneration.

PGC-1α is involved in insulin signaling: When insulin is released from beta-pancreatic cells, binds to insulin receptor leading to Akt phosphorylation and downstream to GSK3β. GSK3β subsequently phosphorylates PGC-1α which results in inhibition of this protein by enhancing the proteasomal degradation localized in the nucleus during oxidative stress (Fernandez-Marcos et Auwerx, 2011). PGC-1α is strongly correlated with serine/threonine kinase AMP (AMPK). AMPK is responsible for lipid and glucose metabolism in different organs and it is a critical point in regulation. AMPK enhances PGC-1α transcriptional activity. Overexpression of PGC-1α was associated with translocation of transporter GLUT4 to the membrane in muscle cell, important process avoiding hyperglycemia. Due to this evidence, PGC-1α is an interesting target for treatment of DM type 2 (Wu *et al.,* 2016).

In addition, PGC-1α affects mitochondrial metabolism in beta-pancreatic cells, which plays a role in metabolic signals and union between insulin secretion and recognition of glucose. Thus, when this mechanism is impaired, this dysfunction leads to enhanced production of ROS and results in beta cell dysfunction and oxidative stress, therefore it is significantly related to DM type 2 and eventual complications. In result, oxidative stress promotes formation of ROS

in mitochondria, lower PGC-1α expression and secretion of insulin was reduced as well (Li *et al.,* 2009; Wu *et al.,* 2016).

Furthermore, in the human brain and in other neurons, PGC-1α has been distinctly shown and is related to neurodegenerative diseases, including impairment in energy metabolism and creation of great vacuoles in a dense network formed by nerve fibers, synapses and glial filaments (Choi *et al.,* 2014).

Oxidative stress provoked by high glucose levels can be prevented by enhanced adenoviral expression of PGC-1α, as was reported in a study, realized by Choi and his associates (2014), on dorsal root ganglion neurons. However, complete mechanisms and regulation of PGC-1α in the nervous tissue are still unclear.

2.6.6 BDNF and synapsin

Brain-derived neurotrophic factor (BDNF) is a protein which belongs to family of neurotrophins and has a key role in survival of neurons, axonal guidance and synaptic plasticity in CNS (Jiao *et al.,* 2016). BDNF is expressed mostly in CNS, especially in synapses and acts locally in the brain (Siuda *et al.,* 2017). Recent evidence comes up with theory that reduced levels of BDNF and its polymorphism is closely linked to pathogenesis of neurodegenerative diseases, such as Alzheimer disease (Kunugi *et al.,* 2001; Tsai *et al.,* 2004). Patients with AD have reduced levels of BDNF protein respect to control group, moreover higher expression of this protein has positive effects on cognitive decline and could be used as a novel marker for diagnosis of AD. In addition, cytotoxic effects and problems with learning, induced by storage of Aβ can be alleviated due to the neuroprotective effect on synapses and cell loss provided by BDNF (Jiao *et al*., 2016). Moreover, BDNF protein has been recognized as a strong inhibitor of neurodegeneration caused by neurotoxins and apoptosis (Siuda *et al.,* 2017).

Another family of proteins, which has been associated with regulation of neurotransmitters and their release at neuronal synapses, are proteins called synapsins. These phosphoproteins, which are present in CNS, are significantly involved in regulation of quantity of synaptic vesicles which are responsible for neurotransmital release via exocytosis (Evergreen *et al.,* 2007). Synaptic vesicles

(SV) are relatively simple small organelles, highly specialized to transport of neurotransmitters and providing the propagation of nerve impulses (Ikeda et Bekkers, 2009).

In mammals have been recognized among others, 4 most abundant members of synapsin protein family, synapsin (SYN) 1-3 and synaptophysin, also known as protein p38, due to its molecular weight of 38 kDa (Evergreen *et al.,* 2007). Synaptophysin is the integral membrane protein, which is not indispensable for neurotransmission, however, is important for modulation and function of synaptic vesicles. Knockout mice, lacking synaptophysin, showed impaired brain function due to damaged synaptic plasticity (Schmitt *et al.,* 2009).

Insulin has a large impact on neuronal function, especially via PI3-K/Akt signaling pathway, thereby can influence expression of proteins and insertion of receptors localized at the postsynaptic membrane. Moreover, insulin can enhance and establish memory through influence on growth, survival and synaptic modulation of neurons (Yin *et al.,* 2013; Calvo-Ochoa *et al.,* 2014).

In summary, the evolution of cognitive impairment is a complex process (Figure 9). The link between DM type 2 and cognitive impairment, such as AD, is due to common association with IGF, GSK3β mechanism, oxidative stress, IR, accumulation of Aβ, APP and NFT formation. Therefore, for this metabolic impairment and link between DM and AD, researchers used the term DM type 3 (Kandimalla *et al.,* 2017).

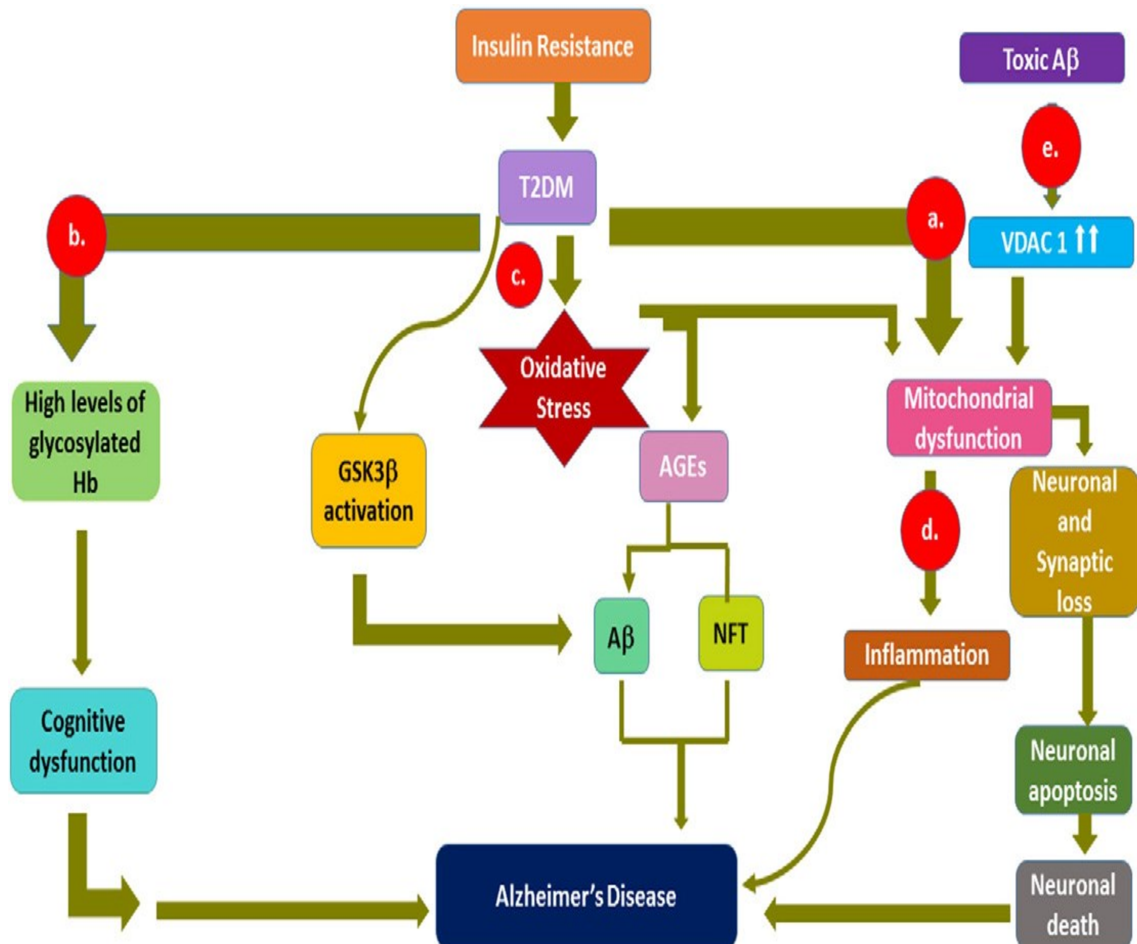


Figure 9 *Scheme of various pathways aiming to the development of AD. Insulin resistance is the hallmark of DM type 2, which consequences are oxidative stress (c), glycosylation (b) and mitochondrial dysfunction (a). During occurring state of oxidative stress are produced AGEs, which cause storing of amyloid beta leading to the development of AD. Toxic Aβ damages voltage-dependent anion channel (VDAC), which is responsible for correct function of mitochondrial membrane and in the case of some disruption provoke mitochondrial dysfunction. Mitochondrial dysfunction is characterized by inflammation (d) and impairment of neuronal and synaptic loss which leads to neuronal apoptosis, consequently neuronal death and finally to the AD (Kandimalla et al., 2017).*

2.7 Experimental model: studies on female rats

Nowadays, rats are the most frequently used experimental model. In subsequent experiments, our research group uses female rats due to previous studies and findings that female rats are more susceptible to metabolic alterations than male rats (Vilá *et al.,* 2011). Rats are the most convenient model to study the mechanism of sugar metabolism due to the absence of the enzyme glucose-6-phosphatase (G6P). Due to this lack of enzyme, which humans also lack, rats are not able to transform fructose to glucose in intestines (Mayes *et al.,* 1993).

All factors including metabolic syndrome, world-widely spread series of diseases such as hypertriglyceridemia, insulin resistance, hyperglycemia, were developed similarly in rats as well as in humans. In addition, due to the metabolism, all effects observed in studies wholly appertain to the investigated sugar (Nagai *et al.,* 2002).

The effects of sugar intake expressed by rats are dependent on the form of sugar supply. If the diet is based on 50-60% fructose intake in solid form, it provokes in body various disorders such as insulin resistance and hypertriglyceridemia (Nagai *et al.,* 2002; Nagata *et al.,* 2004). However, when fructose is consumed in liquid form at low % (10 % w/v), it provokes hypertriglyceridemia and liver steatosis, as well as in solid form, but does not influence glycemia and sensitivity to insulin, at least in short-term studies (Roglans *et al.,* 2007).

The second model of administration, when 10% fructose is consumed in liquid form, is equivalent to the superior quartile of the human population which is high fructose consumers (Alegret *et al.,* 2011).

3 Materials and methods

3.1 Object of study

For our study, Sprague-Dawley female rats (n=36), from Charles River Laboratories (Barcelona, Spain), were provided. These rats were maintained housed at the animal facility in the Faculty of Pharmacy and food Sciences of the University of Barcelona in constant humidity (40-60%) conditions and constant temperature (20-24 °C), with 12 hours light/dark cycle and whole-day access to food and drink. After two weeks of acclimatization, rats were split into 3 groups: control, glucose and fructose. The rats from fructose group were supplemented by 10% solution of fructose (Applichem) in potable water and the concentration of glucose (AppliChem) in the group of glucose-supplemented rats was periodically adjusted so that the intake was isocaloric in comparison with fructose group. All animals were fed the standard diet (Harlan Teklad). Duration of study was 28 weeks. For the evaluation of the effect of sugar consumption on cognitive functions and memory, two tests were performed– Morris Water Maze test (MWM) and Novel Object Recognition (NOR) test.

At the end of 28 weeks study, after 12 hours fast, blood samples were obtained from the tail, and glucose, cholesterol and TAG levels were determined using a glucometer with specific strips. Then rats were anesthetized with ketamine/xylazine (9 mg and 40 μg/100 g body weight, respectively) and sacrificed by decapitation. The brains were dissected and the frontal cortex and hippocampus were separated. Samples intended for protein and RNA extraction were collected, frozen immediately in liquid nitrogen and stored at -80 °C until their use.

3.2 Methods

3.2.1 Morris water maze test

The water maze test known as the Morris water maze test is designed especially for rodents in order to study and evaluate learning and spatial memory. This test consists of the rats searching for a specific location of a submerged platform in a pool, with various reference objects. Learning is assessed through repetition of several experimental tests. In case of reference memory, the preference must be given to the area where the submerged platform is located. The test was performed in a circular pool of 160 cm in diameter and 45 cm in height, which contained water at a temperature of 22 ± 1 °C up to a height of 25 cm. The pool was divided into four imaginary quadrants differentiated by the four cardinal points. In each cardinal point, a bulky object, very different from the rest, was placed. In the center of one of these quadrants stood a platform of 11 cm in diameter, 1 cm submerged in the water. The water solution contained non-toxic latex, so due to the provided opacity, the platform was not visible for rats. The whole pool remained isolated by black curtains to minimize any part of a room that could serve as an orientation point.

The learning phase consisted of four experimental tests per day during 6 consecutive days. The starting point of each test was determined randomly, so the animal could not learn a specific route to reach the platform. Additionally, the most distant position from the platform is not used in the learning phase since it is reserved for the actual test. The rats were placed in the water facing the wall of the pool and given 90 seconds to find the platform. If after these 90 seconds the rats did not locate the platform, the animals were guided or placed on the platform, where they remained for 30 seconds to memorize the location.

After 24 h of the learning phase, the memory test was performed. During this test, the platform was removed from the pool. The animals were placed in the pool to the point most distant from the previous platform position. During the first 90 seconds, we analyzed different parameters such as the time they spent in each quadrant of the pool, time spent in the area, where the platform used to be, numbers of entries in the certain quadrants and the latency time to reach the previous platform position. The route of each animal was recorded through a

camera located above the center of the pool and the results were analyzed using appropriate software (Smart 3.0 Panlab). It should be mentioned that the location of the platform was different in tests performed at the beginning and at the end of the study, to prevent any interferences in the second test due to the possible knowledge acquired in the first test.

3.2.2 Cognitive tests of novel object recognition

The object recognition test, called Novel Object Recognition test, evaluates the memory of animals, mainly rodents, based on their preference of exploring new rather than familiar objects.

To carry out the test, a box of 30×70×70 cm was used. After every single test, the box was properly cleaned with ethanol to avoid any interference caused by the previous animal. The test consisted of three phases: acclimatization, familiarization and the actual test.

In the phase of acclimatization, the animal was placed inside the box and was left for 10 min freely to explore the entire area without the presence of any object. This phase is called exploring the open-field and was conducted for 2 consecutive days.

The next day, the familiarization phase was carried out. The animal was left for 10 minutes inside the box that contained two identical objects (A + A), placed symmetrically. Since the box was spacious, the animal could move freely and explore the whole space.

After two hours of familiarization phase, the actual test, which allows studying a short-term memory, was carried out. Thus, one of the old objects (A) was replaced by a new object (B). The rats were placed again inside the box for 10 minutes so they could freely explore the objects.

After 24 hours of familiarization phase, we performed the test that allows studying a long-term memory. As a part of this test, the object from the familiarization phase (A) remained in the box and the object from the short-term memory test (B) was replaced by a second new object (C). Again, the animals were left 10 min inside the box (Figure 10).

All procedures were recorded by a camera located above the box. Once all the tests were finished, the videos were analyzed and the times, which each rat dedicated to explore the new object (TN) and the familiar object (TF), were calculated. From these values, discrimination index (DI) defined as (TN - TF) / (TN + TF) to permit an assessment report of each animal, was determined.

Importantly, exploration was defined as the animal oriented to the object at a distance ≤ 1 cm or showed signs of active sniffing, or both. However, the scan of the animal climbing or sitting on the object was not recognized as exploration.

The analysis of these videos was conducted in collaboration with the Research Group Aging and Neurodegeneration led by Dra. Mercè Pallàs, Faculty of Pharmacy and Food Sciences, University of Barcelona.

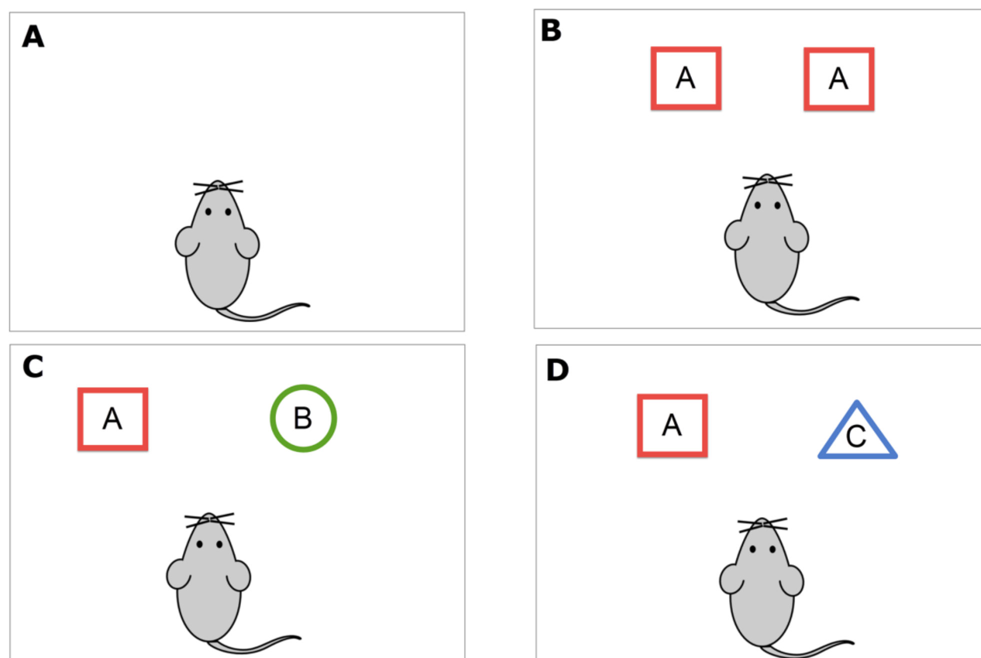


***Figure 10** Test Object Recognition: phase of habituation (A), familiarization (B), short-term memory test (C) and long-term memory test (D).*

3.2.3 Glucose tolerance test

Glucose tolerance test (GTT) is based on evaluating the response of the body to administration of certain quantity of glucose. This test was performed 3 weeks before the end of this study. After 6 hours of fasting, 2 g of glucose/kg body weight were administrated to the animals intraperitoneally, using a solution which contained 0.4 g of glucose/ml in NaCl 0,9% (w/v).

The blood concentrations of glucose were determined using a glucometer Accutrend® Plus (Roche Farma) and specific strips at 0, 15, 30, 60, 90 and 120 min after the administration of glucose, while the plasmatic levels of insulin were measured after 0, 15 and 120 min of the administration of the glucose solution. The blood was obtained from the tail by a small cut.

3.2.4 ELISA

The plasmatic concentrations of insulin, adiponectin, and AGEs were determined by the method called ELISA (Enzyme-Linked ImmunoSorbent Assay). This immune enzymatic assay is based on the detection of connected antigens and antibodies through the colorimetric activity produced by an enzyme linked to one of the immunoreactants.

In this study, we used the following commercial sets.

- Insulin: EZRMI-13K (Millipore)
- Leptin: EZRL-83K (Millipore)
- Adiponectin: EZRADP-62K (Millipore)
- AGEs : MBS261131 (MyBioSource)

In all cases, we followed instruction of producer.

3.2.5 Immune detection of total proteins extracted

The extraction of total and nuclear proteins was performed by the Helenius method (Helenius *et al.,* 1996). Micronized samples were homogenized with lysis buffer containing proteases, phosphatases and acetylase inhibitors (Table 1) and incubated in constant agitation at 4 °C for 1.5 h. Further, samples were placed in a centrifuge process of 15,000×g for 15 min at 4 °C. Next, the supernatant was collected and kept in aliquots at -80 °C until their use.

Table 1 *Reactants and its concentrations used for protein extraction.*

Reactants	Concentration
Tris-HCl pH = 8 (AppliChem)	50 mM
NaCl (Sigma-Aldrich)	150 mM
Igepal (Sigma – Aldrich)	0.010
NaF (Sigma – Aldrich)	10 mM
EDTA (Sigma – Aldrich)	1mM
EGTA (Sigma – Aldrich)	1 mM
Nappi (Sigma – Aldrich)	2 mM
PMSF (Sigma – Aldrich)	1 mM
Aprotinin (Sigma – Aldrich)	2 µg/mL
Leupeptin (Sigma – Aldrich)	2 µg/mL
Na_3VO_4 (Sigma – Aldrich)	1 mM
NaM (Sigma – Aldrich)	10 mM
TSA (Sigma – Aldrich)	1 µM
Water bidistilled	q.s.

3.2.6 Bradford method

To determine the protein concentration of the extracted samples, the method described by Bradford (1976) adapted to microplate reading was used. This method is based on the fact, that the maximum absorbance of the acid solution Coomassie Blue G-250 changes from 465 to 595 nm, which is the moment, when it binds to proteins and the color turns from brown to blue. Therefore, reading the absorbance at 595 nm is directly proportional to the concentration of protein, which is determined by interpolation on a calibration line.

The technique was performed in 96-well microplates. Each well contained volume of 10 ml either of the standard solution or sample to be analyzed. The calibration line was elaborated with known concentrations (0.1 to 0.6 mg/ml) of a standard solution of bovine albumin serum (BSA, Sigma-Aldrich). Then, 190 ml of Bradford reagent (Bio-Rad) were added, after dilution with bidistilled water 1:5. After leaving reagents for 10 minutes to react, the absorbance at 595 nm was determined using a plate spectrophotometer (Bio-Rad Benchmark Plus).

3.2.7 Western Blot analysis

The Western Blot technique allows to determine levels of specific proteins in several samples. It consists of different phases: electrophoresis, which enables the separation of proteins in acrylamide gel according to their molecular weight due to denaturalization in presence of SDS (Sodium Dodecyl Sulfate); transfer, where proteins transfer from gel to membrane of PVDF (polyvinyl of fluoride); and immune detection, allowing identification of proteins by specific antibodies. The detailed procedure is described below.

3.2.7.1 Electrophoresis

Electrophoresis was performed in polyacrylamide gels; this method is called SDS-PAGE (Sodium PolyAcrylamide - Dodecyl Sulfate Gel Electrophoresis). These gels originate due to the polymerization of acrylamide and bisacrylamide, which begins when persulfate ammonium (APS) and tetramethylethylendiamine (TEMED) are added. The gels consist of two phases: phase concentrator with a low percentage of acrylamide-bisacrylamide, which allows the concentration of protein sown; and phase separator, where the higher percentage of acrylamide-bisacrylamide separates proteins. The percentage depends on the molecular weight of the analyzed proteins and determines their speed and their migration grade of separation. Generally, the most used concentrations are 3-5 % in gels concentrators and between 8-12 % in gels separators.

Table 2 *Specific reagents necessary for the production of two SDS-PAGE gels, in this case, 4 % and 8 %.*

Reactants	Concentration gel (4%)	Separator gel (8%)
	Volume	Volume
Acrilamid-bisacrilamid 40% (AppliChem)	750 µl	4,50 ml
Tris-HCl 1,5 M, pH 8,8 (AppliChem)	---	5,63 ml
Tris-HCl 1 M, pH 6,8 (AppliChem)	938 µl	---
SDS 10% (Sigma-Aldrich)	75 µl	255 µl
APS 10% (Sigma-Aldrich)	45 µl	300 µl
TEMED (Sigma-Aldrich)	12 µl	30 µl
Water bidistilled	q.s.p. 7,5 ml	q.s.p. 22,5 ml

For the preparation of the samples, proteins must be denaturalized and the samples must contain the same volume of total proteins. Based on previously calculated concentrations, in order to obtain the same amount of total protein in each sample (10-30 µg), different volumes of mother samples were used. Then the half of final volume of loading buffer 2X and q.s. Tris-HCl 20 mM pH 7.5, were added. The loading buffer 2X contains 125mM Tris-HCl pH 6.8, 4 % (w/v) SDS, 20 % (v/v) glycerol, 10% (v/v) β-mercaptoethanol, 0.012 % (w/v) blue bromophenol and q.s. bidistilled water.

Once prepared, the samples were boiled for 5 minutes at 100 °C in a dry bath (Techne DRI-BLOCK DB-2A), to denature the proteins.

Previously prepared gels were placed in MiniProtean III® system (Bio-Rad). After that migration buffer, adjusted to pH = 8, which contains 25 mM Tris base (Applichem), 192 mM glycine (AppliChem), 0.1% SDS (Sigma-Aldrich) and q.s. bidistilled water, was added.

Then the samples were loaded along with a molecular weight marker (Benchmark ™ Pre-Stained Protein Ladder (Invitrogen) or Dual Precision Plus Protein ™ Color Standards (Bio-Rad), which permit the identification of protein of interest depending on its height. Electrophoresis was carried out at a constant voltage of 100 V and ambient temperature.

3.2.7.2 Transfer

After electrophoresis, proteins were transferred from the gel to a PVDF membrane (Millipore) using the Mini Trans-Blot® system (Bio-Rad) containing transfer buffer. This buffer contained 25 mM Tris base (AppliChem), 192 mM glycine (AppliChem), 20% (v/v) methanol (Fischer Chemical) and q.s. bidistilled water. The transfer was performed at 4 °C and a constant intensity 200 mA for 100-120 min.

3.2.7.3 Immunodetection

The immunodetection consists of the detection of the chemiluminescent signal produced by secondary antibody which coupled with a primary antibody, specific for the protein of interest (Table 3).

To continue the immunodetection, the obtained membranes from transfer were washed in a solution called TBS, containing TBS-T (Tris-buffered saline 20mM Tris base, 150 mM NaCl and q.s. bidistilled water) with 0.1% (v/v) of Tween®20 detergent (Sigma-Aldrich).

Then the membranes were incubated with blocking solution containing 5% milk in TBS-T for 1 h at a room temperature. Consequently, the membranes were incubated overnight at 4 °C with a solution containing the primary antibody, specific for the protein of interest and diluted in TBS-T with 5% BSA (Sigma-Aldrich). After that, the procedure continued as the membranes were incubated for 1 h at the room temperature with a solution containing the secondary antibody, conjugated to an enzyme peroxidase, diluted in TBS-T.

Table 3 *Specific primary antibodies, references and their source for each protein.*

Antibodies	Reference	Source
β aktin	Sigma-Aldrich (A5441)	Mouse
β tubuline	Sigma-Aldrich (T4026)	Mouse
Akt	Cell Signaling (#9272)	Rabbit
Phospho-Akt (Ser473)	Cell Signaling (#9271)	Rabbit
DRP1	Abcam (ab56788)	Mouse
Phospho-GSK3β (Ser9)	Cell Signaling (#9336)	Rabbit
IDE	Abcam (ab32216)	Rabbit
IRS2	Cell Signaling (#2382)	Rabbit
MFN2	Abcam (ab56889)	Mouse
OPA1	Bioscience (612606)	Mouse
PGC1α	Cayman (101707)	Rabbit

Finally, the protein of interest was detected by a reaction of chemiluminescence generated between peroxidase enzyme of the secondary antibody and reagent of detection (Peroxide Solution® Immobilon Western HRP substrate (Millipore) or enhanced chemoluminiscence (ECL) (GE Healthcare). The revealed chemiluminescent signal carried out by Chemidoc™ XRS apparatus (Bio-Rad) and quantified using the software (ImageLab, Bio-Rad).

Among the various steps (blocking, incubation with primary antibodies, incubation with secondary antibodies and addition of detection reagent) 3 washes of the membrane in TBS-T solution, for five minutes each, were performed.

To normalize the results, we used the protein β-actin and β-tubulin, to reveal the membrane protein of interest (Table 4).

Table 4 *Secondary antibodies used for β-actin and β-tubulin detection.*

Antibody	Reference
Anti-Mouse IgG HRP	Amersham (NA931)
Anti-Rabbit IgG HRP	Amersham (NA934)

4 Hypothesis and object of the study

Hypothesis: Chronic intake of sweetened beverages, especially by fructose and glucose, have damaging effects on metabolism, causing metabolic syndrome and impairment of cognitive functions such as memory and learning ability. Not only the high intake of calories but also the type of simple sugar ingested is responsible for these deleterious effects.

The objectives of this master's thesis are:

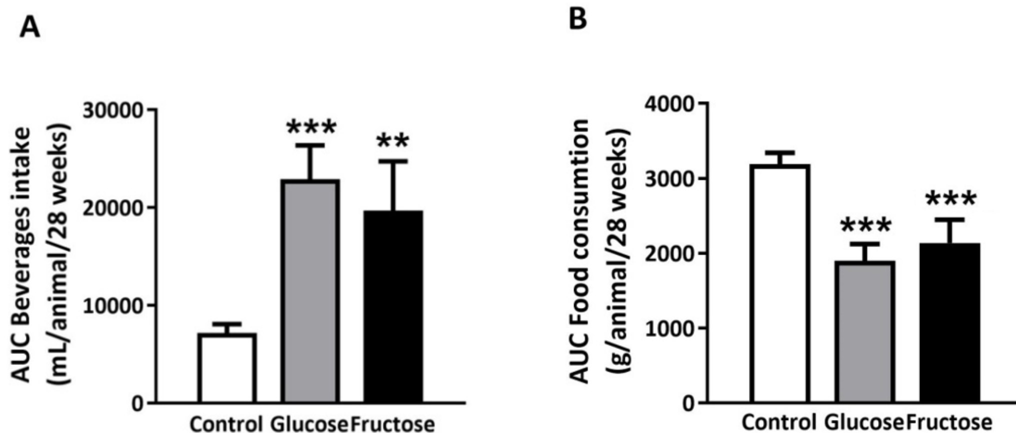
- To study the effects of chronic consumption of simple sugars (glucose, fructose) on cognitive functions, such as memory and learning, examined by various memory tests.
- To study the effects of chronic consumption of simple sugars (glucose, fructose) on plasmatic levels of proteins and parameters related to the metabolic syndrome.
- To determine whether the alterations induced by sugar intake are related to the specificities of each sugar or exclusively due to increased calorie consumption.

5 Results

5.1 Body weight and caloric intake

During the whole 28 weeks study, the data about the food consumption and beverages intake were obtaining periodically, so it was observed that rats supplemented with the solution of 10% glucose and 10% fructose had a significantly higher consumption of liquids than the control group. In the graphic, the area under the curve (AUC) of beverages intake in the glucose group is 3.2-fold higher than the control group, while the consumption of fructose-supplemented rats was 2.7-fold higher than the control group (Figure 11A). On the other hand, the consumption of solid food was reduced in both groups supplemented with sweetened beverages, 40% and 33% in glucose and fructose groups, respectively (Figure 11B).

However, despite the fact that the consumption of solid food was reduced, the total caloric intake was almost similar in the glucose and fructose group and was 1.6-fold and 1.5-fold higher than the control group, respectively (Figure11C). The total caloric intake during the 7 months study was calculated taking into account the caloric contents of solid food (2.9 kcal/g) and of sweetened solutions, glucose and fructose (4 kcal/g).



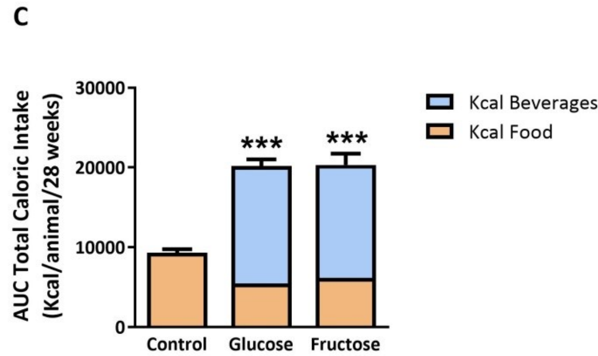
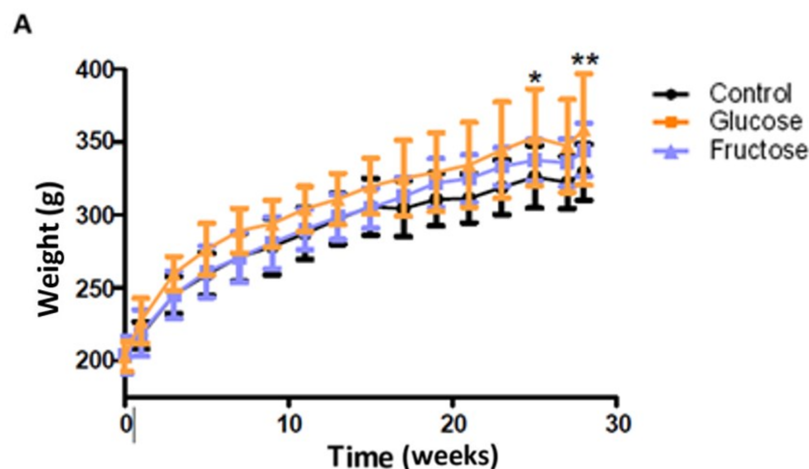


Figure 11 *Bar plots represent the area under the curve (AUC) of liquid consumption expressed in ml/rat (A), the food consumption expressed in g/rat (B) and the total caloric intake corresponding to the food (orange bars) and the beverages (blue bars) consumed by rats expressed in kcal/g (C). All results are presented as the mean ± SD. Statistical analysis One-way ANOVA followed by Bonferroni post-test (***p<0.001 vs control).*

Along the 7 months of the study, the body weight of rats increased, especially in the fructose group. The evolution of weight of rats is shown in Figure 12 (A), as absolute values and in Figure 12 (B) as the AUC. Despite the similar caloric intake in the glucose and fructose supplemented groups, in the case of fructose, the AUC is significantly higher (1.06-fold) compared to control group. The weight at the end of the treatment was also significantly higher (1.1-fold) in the fructose group respect to the control group (Figure 12C).



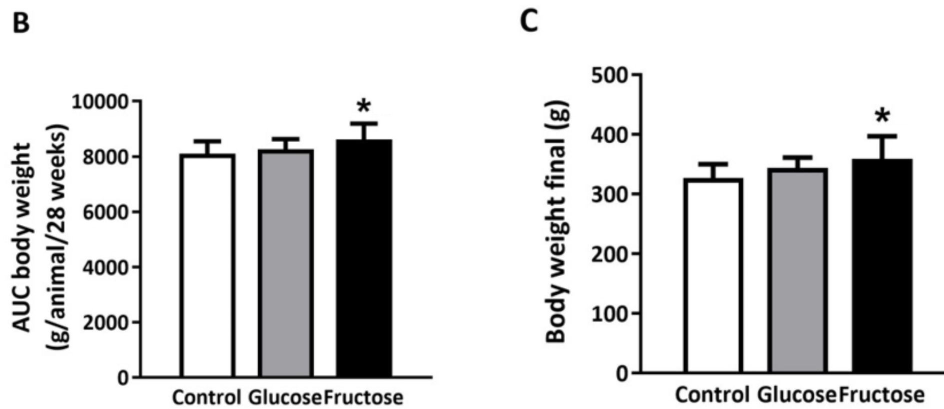


Figure 12 *Graphic representation of the progress of body weight gain in rats during the study expressed in g (A), the area under the curve expressed in g/rat/28weeks (B) and the weight in 28th week, expressed in g (C). Each bar represents the mean ± SD. Two-way ANOVA statistical analysis (A) and One-way ANOVA statistical analysis (B and C) followed by Bonferroni post-test (*p< 0.05; **p< 0.01 vs control).*

5.2 Organs weights

At the end of the study, the organs were weighed, and the weights were standardized respect to femur length. In the fructose-supplemented group, the weights of livers were 1.41-fold higher vs control group, but also 1.18-fold higher vs glucose group. Moreover, the weight of adipose tissue of fructose-supplemented rats was 1.6-fold higher vs control group.

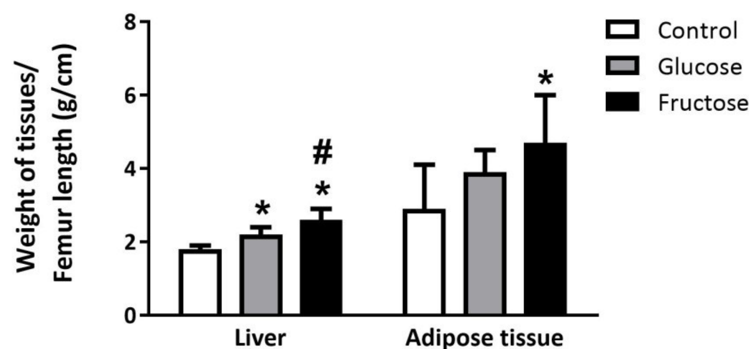


Figure 13 *Weight of liver and adipose tissue expressed in g/cm respect to femur length. Each bar represents the mean ±SD. Statistical analysis One-way ANOVA followed by Bonferroni post-test *p<0.05 and ***p<0.001 vs control, ##p<0.01 vs glucose.*

5.3 Glucose tolerance test

At the end of the study, a glucose tolerance test was performed. During the test, the blood glucose levels were not altered, except for the last time point (120 min after the administration of glucose), when the group of fructose-supplemented rats showed a level of glucose 1.09-fold higher than the control group (Figure 14A). However, the area under the curve of blood glucose did not show any significant differences between groups (Figure 14B).

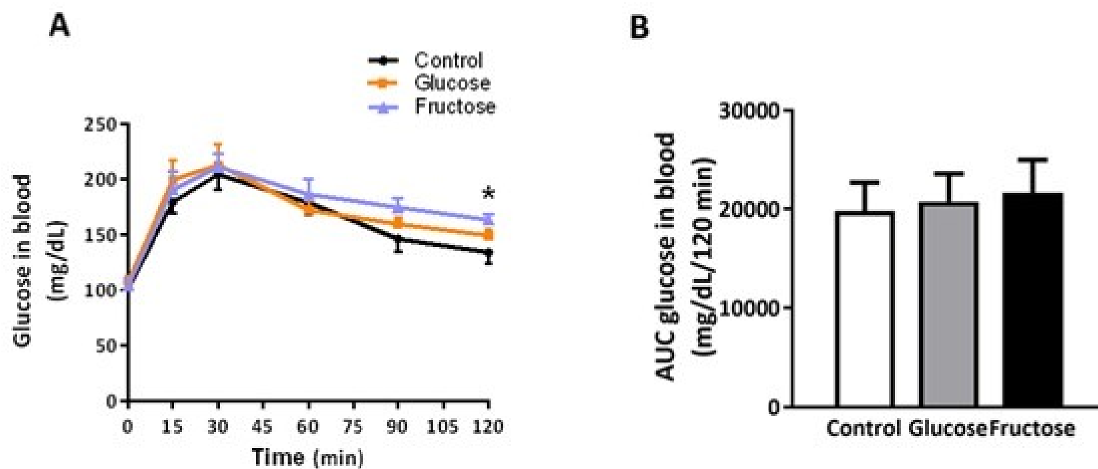


Figure 14 *Blood glucose levels during the glucose tolerance (Figure 14A), expressed the mean ± SD. Statistical analysis One way ANOVA followed by Bonferroni post-test for each time (*p < 0.05). Figure 14B represents the area under the curve (AUC) of glucose concentration (mg/dl/120 minutes). Statistical analysis One-way ANOVA followed by Bonferroni post-test.*

During the GTT, insulin plasma levels were also determined. At the initial point, the plasmatic concentration of insulin in the fructose group was 1.8-fold higher than in control and glucose group. After 15 min, significant differences of fructose group in comparison to control and glucose were still observed, 1.53-fold and 2.43-fold higher, respectively. However, at the end of the test (120 min), the plasmatic concentrations of insulin did not show significant differences, although in the fructose group was still higher than the rest of the groups (Figure 15A). Nevertheless, the AUC of plasmatic levels of insulin was significantly higher in fructose group than in groups control and glucose, 1.54-fold and 1.95-fold, respectively (Figure 15B).

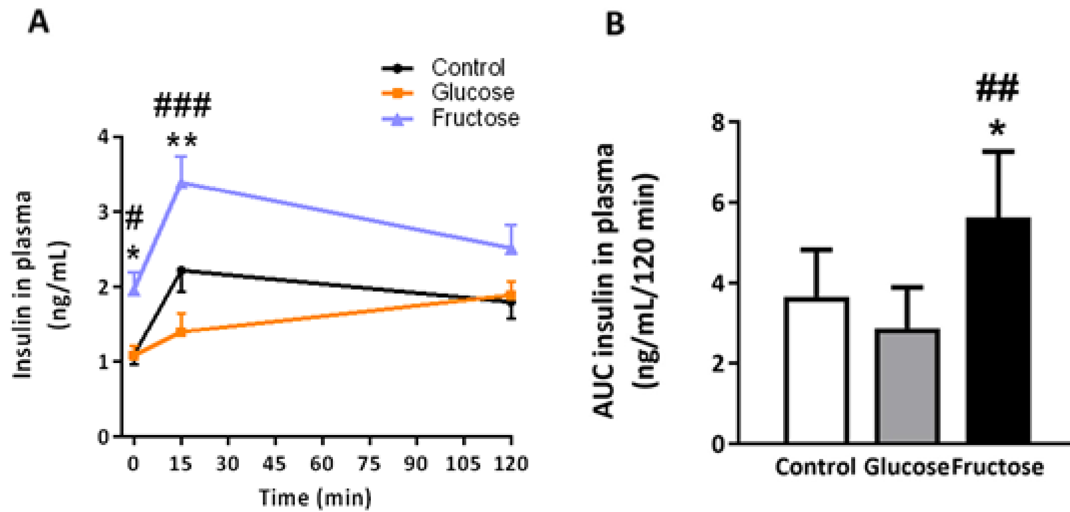


Figure 15 *Plasma insulin levels during the glucose tolerance test. The results represent the mean ± SD. One-way ANOVA statistical analysis followed by Bonferroni post-test for each time (*p < 0.05 and **p < 0.01 vs control; #p < 0.05 and ###p < 0,001 vs glucose) (Figure 15A). In Figure 15B is represented the AUC of insulin concentration. One way ANOVA statistical analysis followed by Bonferroni post-test (*p < 0.05 vs control; ##p < 0.01 vs glucose).*

5.4 Blood levels of TAG, cholesterol and glucose

Blood levels of cholesterol (Figure 16A) and glucose (Figure 16B) were not significantly changed between groups after 12 hours of fasting, even though levels of triglycerides were significantly higher in the fructose-supplemented group. Triglycerides in fructose group, after 12 hours fasting, reached 1.88-fold and 1.48-fold higher levels than control and glucose group, respectively (Figure 16C).

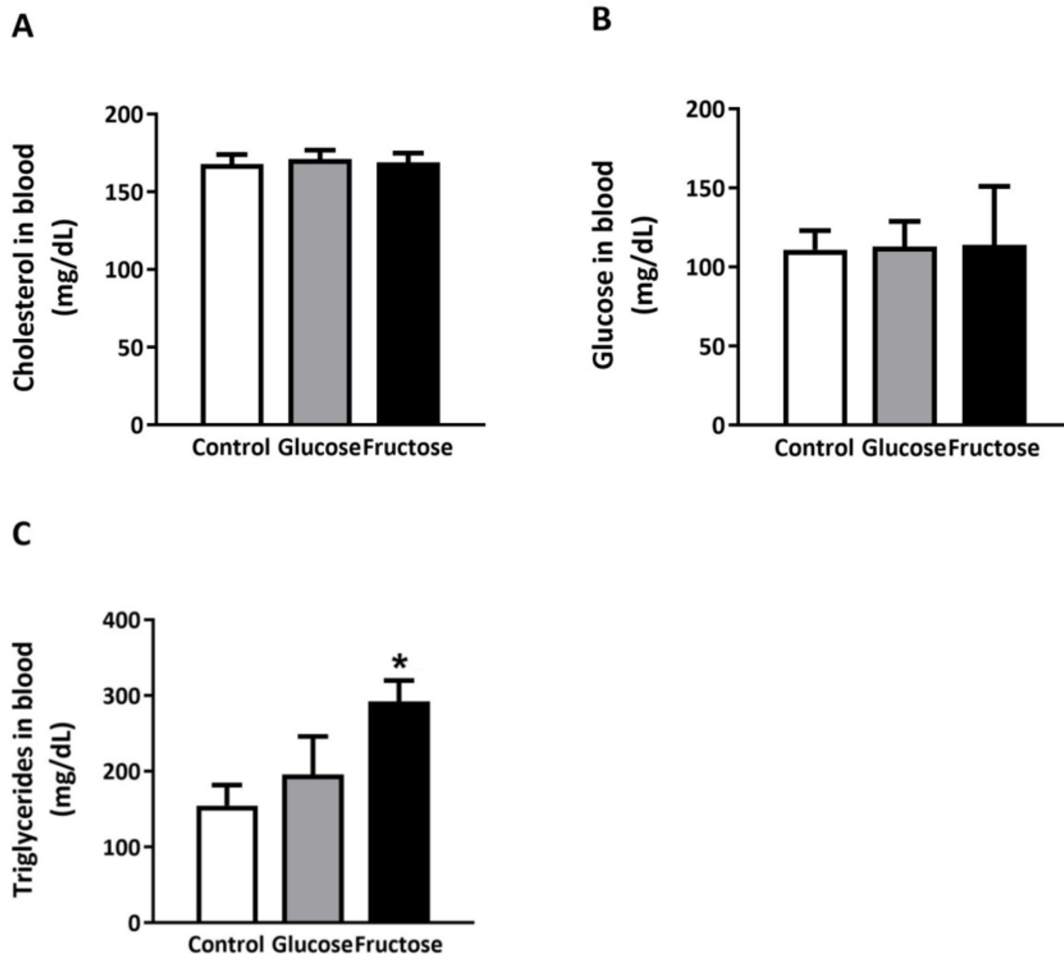


Figure 16 *Levels of plasma cholesterol (A), glucose (B), triglycerides after 12 hours of fasting (C). Each bar represents the mean ± SD. One-way ANOVA statistical analysis followed by Bonferroni post-test (* p < 0.05 vs control).*

5.5 Insulin, adiponectin and leptin plasma levels

Results obtained from ELISA showed the increase of levels of insulin and leptin, especially in the group of fructose-supplemented rats. Plasma insulin concentration was 1.79-fold higher in fructose-supplemented rats with respect to the control group (Figure 17A), and plasma leptin levels were 2.03-fold higher in fructose-supplemented rats with respect to the control group (Figure 17B). Adiponectin levels were significantly increased in glucose-supplemented rats (1.58-fold higher than the control group and 1.29-fold higher with respect to the fructose group (Figure 17C).

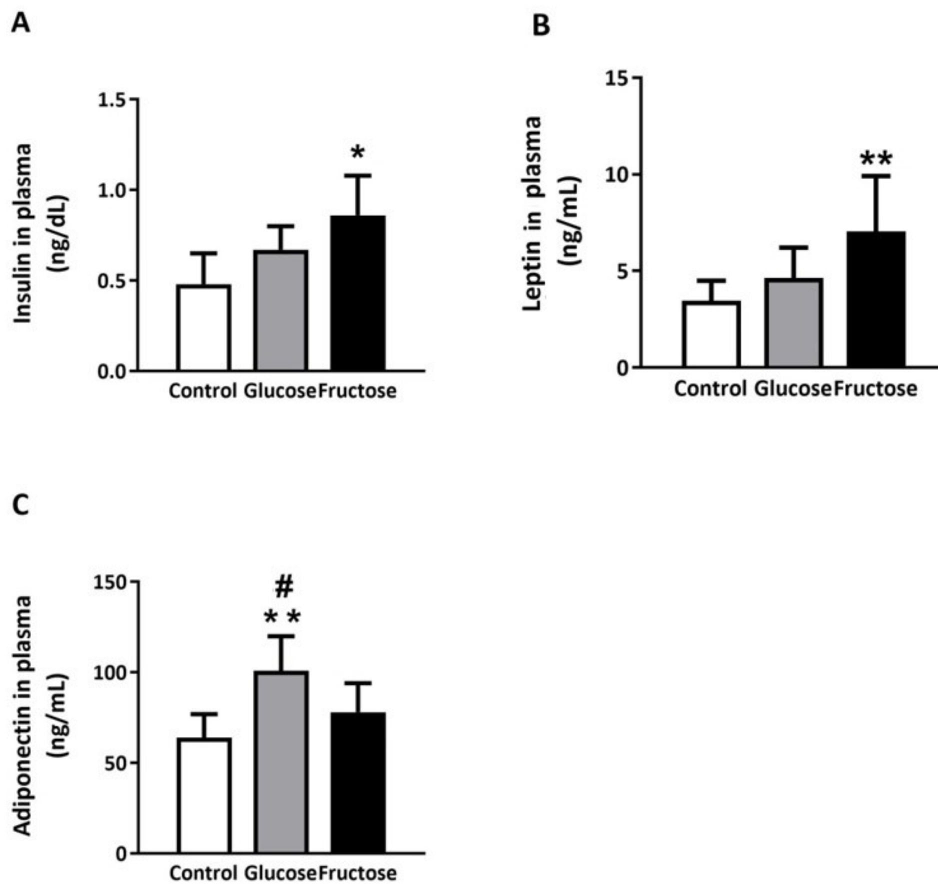


Figure 17 *Plasma levels of insulin (A), leptin (B) and adiponectin (C) measured by ELISA test. Each bar represents the mean ± SD. One-way ANOVA statistical analysis followed by Bonferroni post-test (* $p < 0.05$ and ** $p < 0.001$ vs Control; #$p < 0.05$ vs Fructose).*

5.6 Morris water maze test

The Morris water maze test was used to study the effects of short- and long-term supplementation of sweetened beverages on memory. Thus, the first test performed after two weeks of sugar supplementation and the second at the end of the study (28 weeks). During the six days of trials of the learning phase, the velocity of swimming did not show any differences between groups neither at the beginning nor at the end of the study (Figure 18A-B). Learning curves (Figure 18C-D) represent the time needed for each group of animals to reach the hidden platform in the pool. The curves during the six days of trials of the learning phase

exhibit a digressive tendency and show how animals are able to learn and they need less time for locating the platform at last days than at the beginning. Nevertheless, the results show no differences between groups.

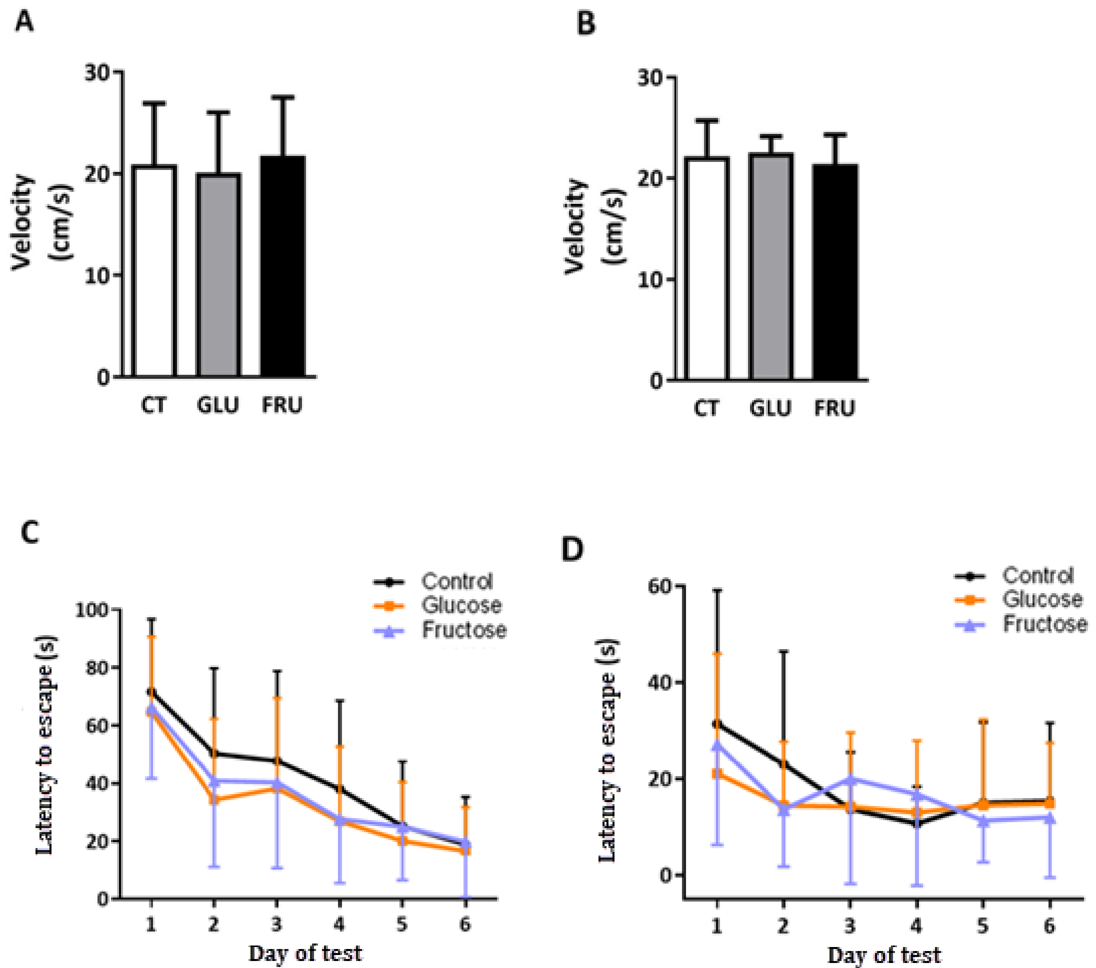
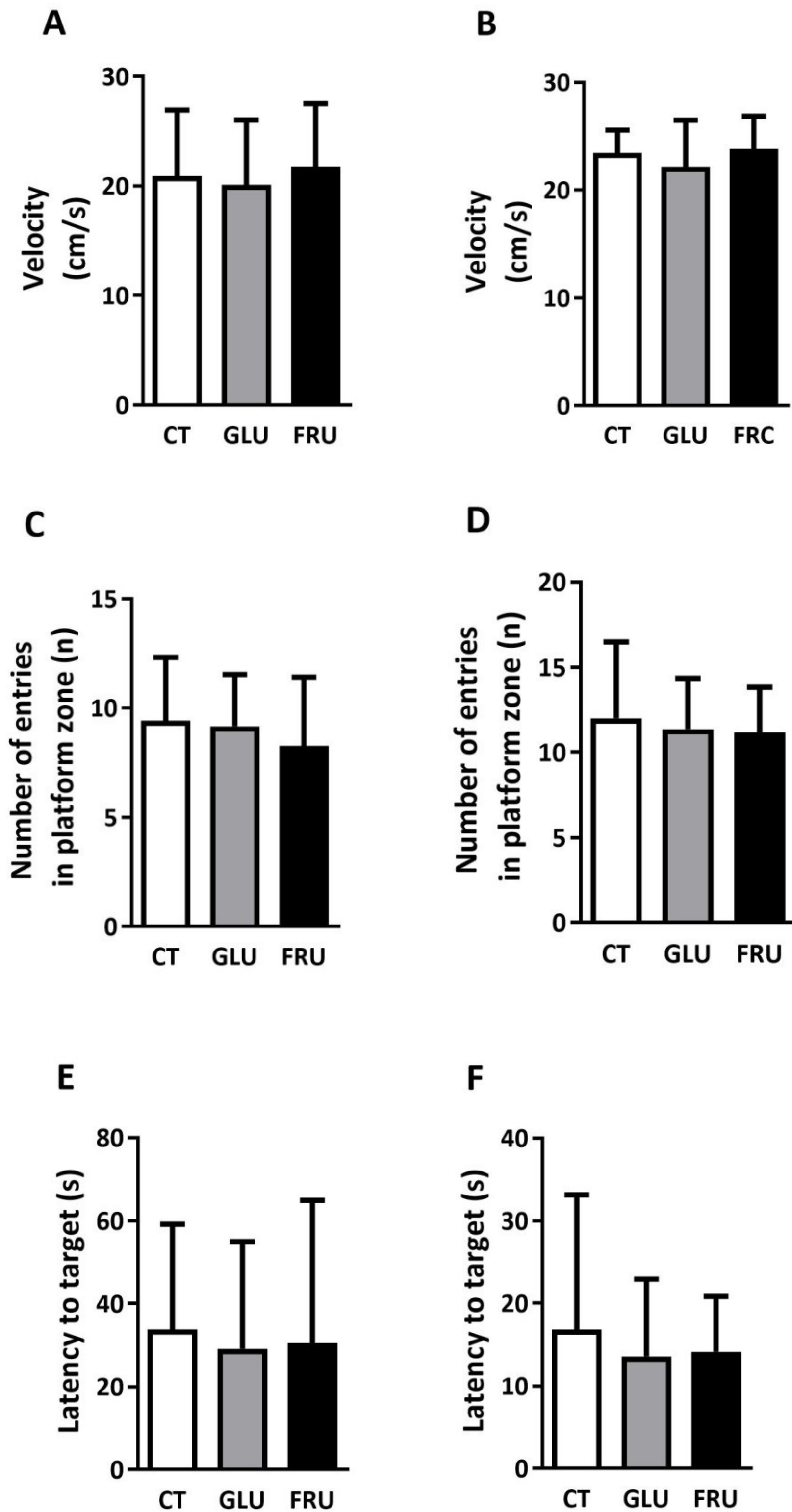
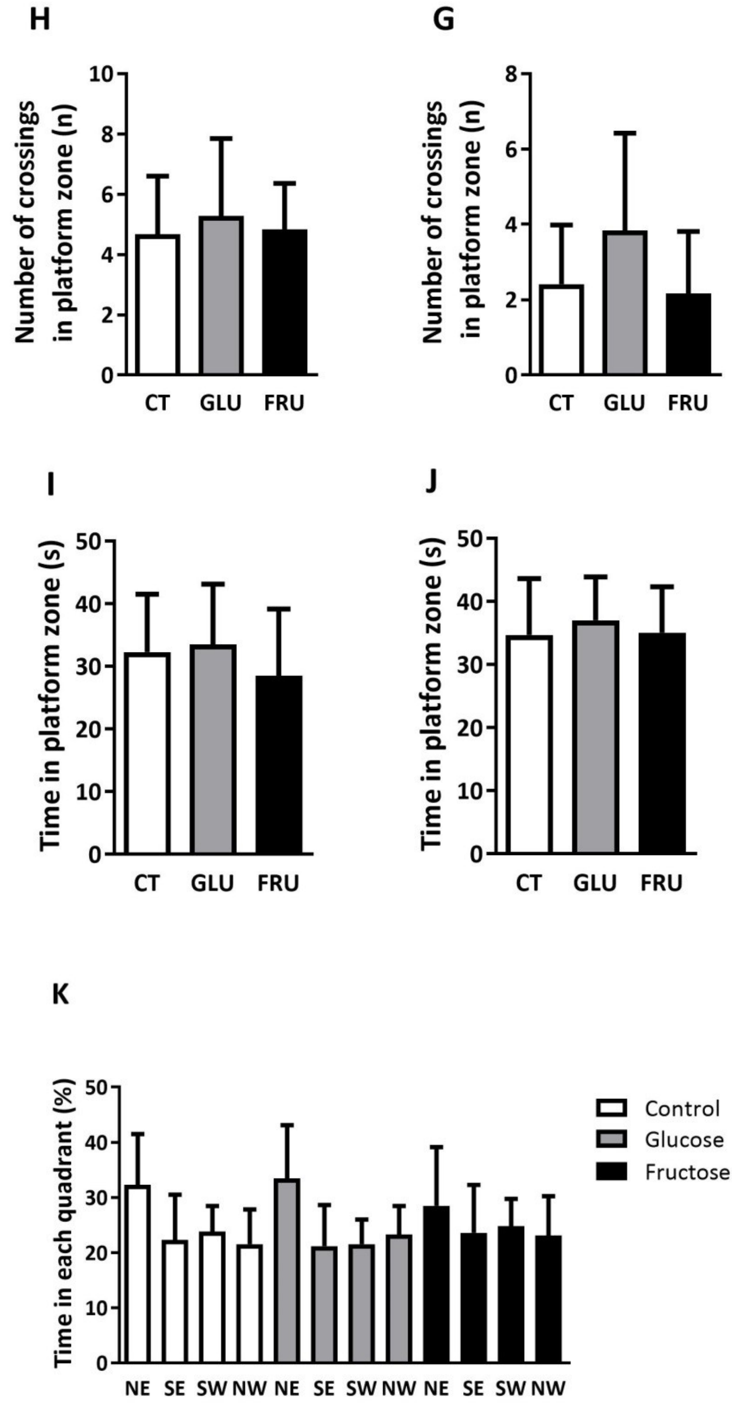


Figure 18 *Graphic representation of velocity of swimming at the beginning (A) and at the end of the trial (B), expressed in cm/s. (C) represents learning curve at the beginning and after 28 weeks (D), expressed in s. Each bar represents the mean ± SD. Statistical analysis One-way ANOVA (A and B), Two-way ANOVA (C and D), followed by Bonferroni post-test.*

A memory test was performed on day 7 whereby the platform was removed and a series of parameters were determined: the swimming velocity, number of entries in the platform zone, latency to the target (which means time needed to reach the platform), number of crossings in platform zone (number of times when the rat crossed the imaginary frontier of the quadrant where the platform was

located), time spent in quadrant and time spent in each quadrant. None of these parameters were altered by sugar consumption, neither at the beginning nor at the end of the supplementation period.




H
Number of crossings in platform zone (n)
CT GLU FRU
G
Number of crossings in platform zone (n)
CT GLU FRU
I
Time in platform zone (s)
CT GLU FRU
J
Time in platform zone (s)
CT GLU FRU
K
Time in each quadrant (%)
NE SE SW NW NE SE SW NW NE SE SW NW
Control
Glucose
Fructose

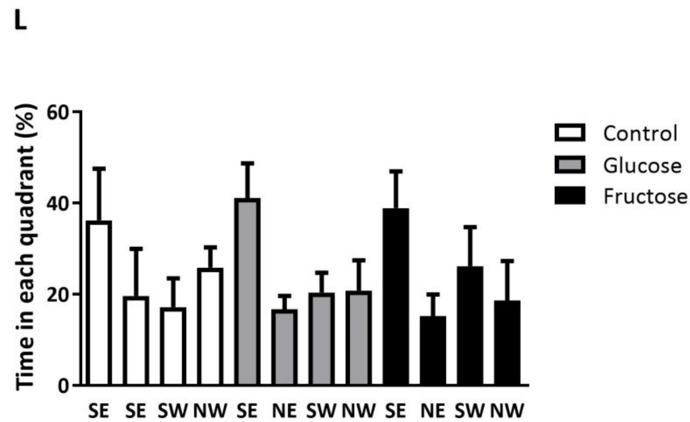


Figure 19 *Graphic representation of swimming velocity (A and B), number of entries in the platform zone (C and D), latency to the target (E and F), number of crossings in platform zone (G and H), time spent in platform zone (I and J) and time spent in each quadrant (K and L), at the beginning and at the end of the study, respectively. Each bar represents the mean ± SD. Statistical analysis One-way ANOVA followed by Bonferroni post-test.*

5.7 Novel object recognition test

The results of the Novel object recognition test are expressed as a discrimination index (DI), which corresponds to the following formula:

$$DI=\frac{\text{time spent with novel object} - \text{time spent with familiarized object}}{\text{time spent with novel object} + \text{time spent with familiarized object}}$$

Figure 20 (A) and (B) show the results of the short-term memory tests, which were performed after 2 hours of familiarization at the beginning and at the end of the study. Despite there were no differences between groups after 2 weeks of sugar supplementation (Figure 20A), the DI of the fructose group decreased significantly to 51 % and 57 % with respect to control and glucose groups at the end of the study (Figure 20B).

The same test was performed after 24 hours of familiarization phase, to study the effects on long-term memory. Similarly to the previous test, only after 28 weeks of sugar-supplementation, significant differences were shown in the fructose group, which showed a 50 % decrease in the DI with respect to glucose-supplemented rats (Figure 20 C and D).

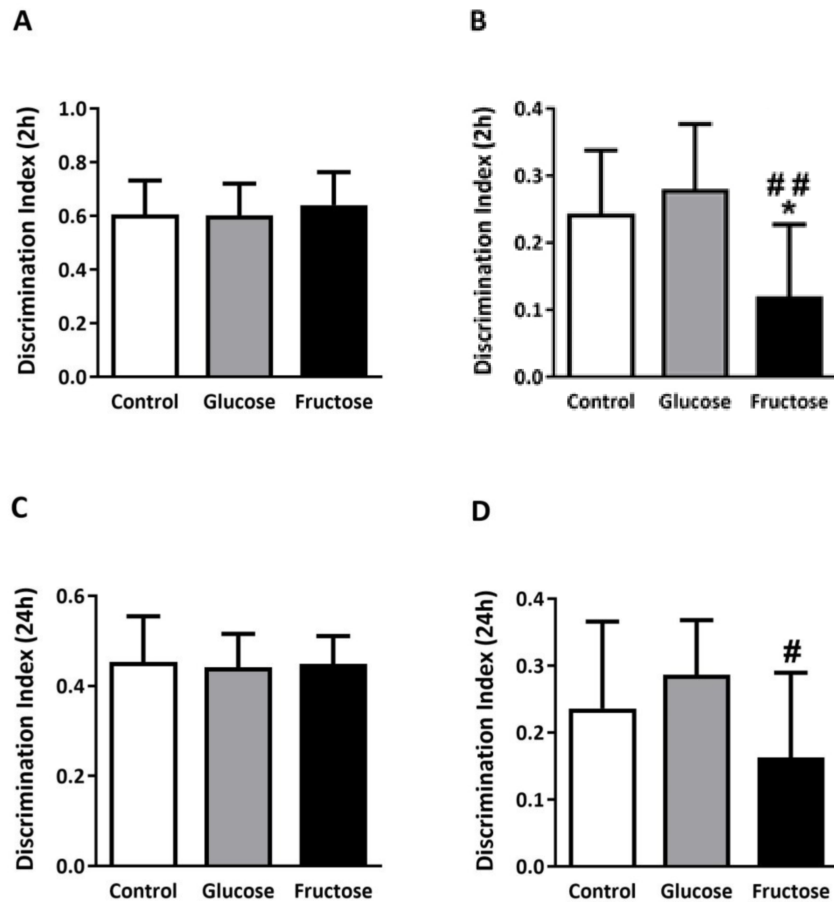


Figure 20 *Graphics represent the decrease of discrimination index (DI) in the test after 2 hours (A) at the beginning and at the end (B) of the study and after 24 hours of the study at the beginning (C) and at the end (D). Each bar represents the mean ± SD. Statistical analysis One-way ANOVA, followed by Bonferroni post-test (*p < 0.05 vs control) and (#p < 0.05, ##p < 0.01 vs glucose).*

5.8 Determination of AGEs

Advanced glycation end products (AGEs) showed significantly increased levels only in fructose-supplemented rats (1.99-fold and 2.25-fold higher compared to control and glucose group, respectively).

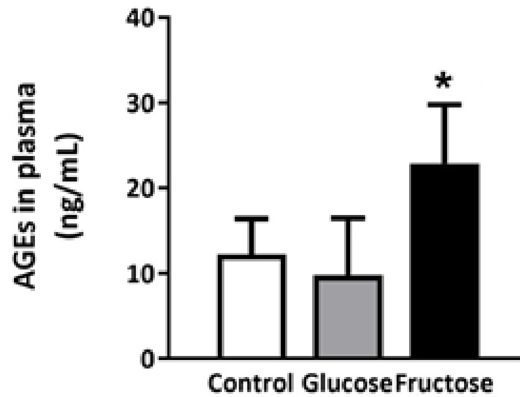


Figure 21 *Plasma AGEs levels expressed in ng/μL. Each bar represents the mean ± SD. Statistical analysis One-way ANOVA followed by Bonferroni post-test (*p<0.05 vs glucose).*

5.9 Determination of proteins in the cerebral cortex

5.9.1 Proteins involved in insulin signaling

We determined various proteins which play a key role in insulin signaling in the cortex of rats from the control, glucose and fructose groups. Expression of IRS2 protein was significantly reduced only in the fructose group (Figure 22A). We also observed a significant reduction of p-Akt levels only in fructose-supplemented rats with respect to control and glucose groups (Figure 22B). However, the Akt-total protein levels were similar in all groups (Figure 22C). Phosphorylated glycogen synthase kinase (GSK3β)-p, which is downstream of Akt in insulin signaling, was not influenced by consumption of sweetened beverages (Figure 22D). In Figure 22 (E), the levels of PGC-1α were 1.27-fold higher than the control group.

We also determined the expression of insulin degrading enzyme (IDE). IDE protein levels showed a significant reduction only in the fructose-supplemented rats, 1.8-fold and 1.4-fold higher than in control and glucose group, respectively (Figure 22F).

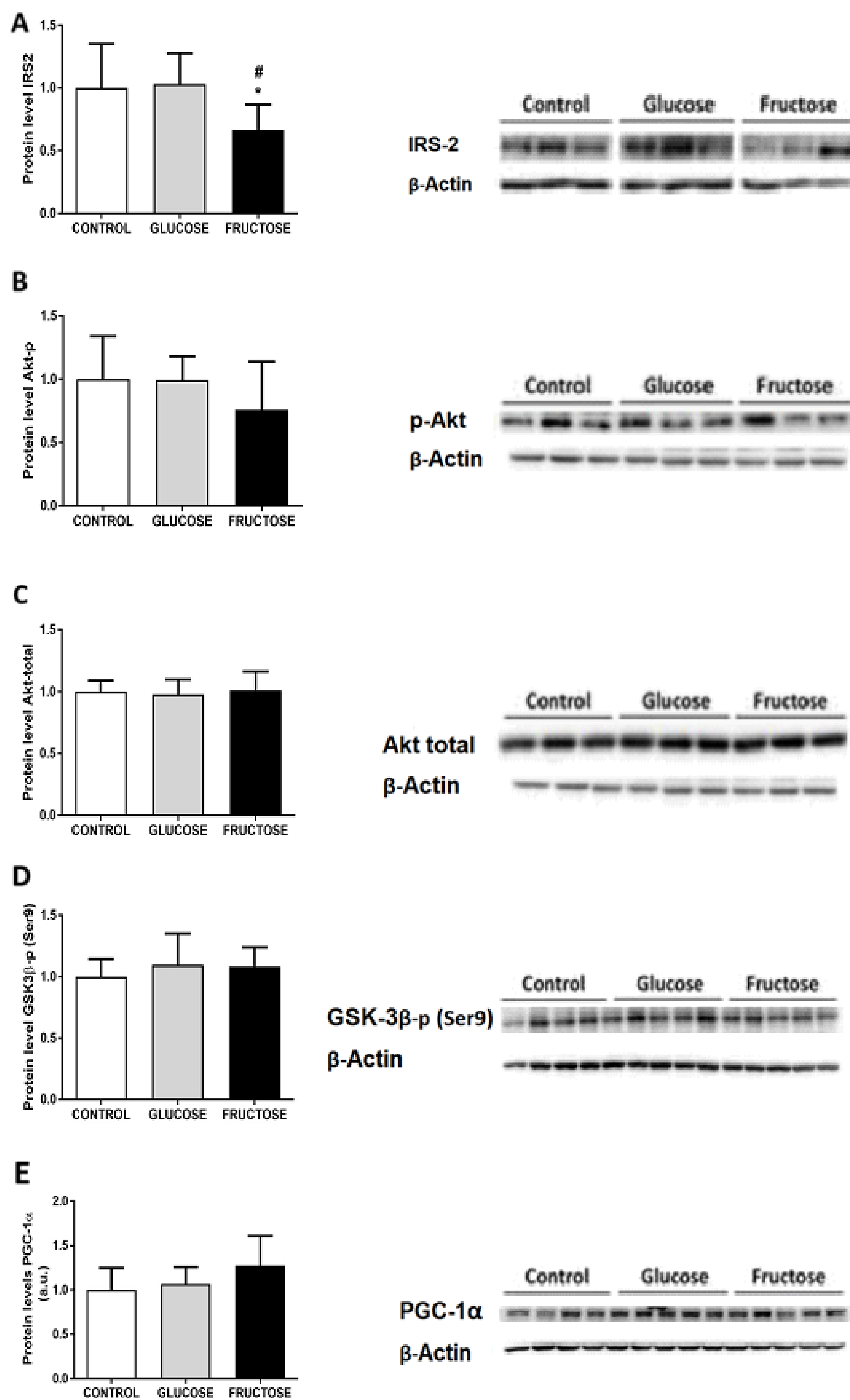

A
Protein level IRS2
CONTROL
GLUCOSE
FRUCTOSE
Control
Glucose
Fructose
IRS-2
β-Actin
B
Protein level Akt-p
p-Akt
C
Protein level Akt-total
Akt total
D
Protein level GSK3β-p (Ser9)
GSK-3β-p (Ser9)
E
Protein levels PGC-1α (a.u.)
PGC-1α

Figure 22 *Protein expressions of IRS2 (A), p-Akt (B), Akt-total (C), GSK3β-p (D), PGC-1α (E) and IDE (F) determined by Western Blot. Each bar represents the level of proteins expressed as the mean (a.u.) ± SD of the values obtained from 4-5 rats (*p < 0.05, ***p<0.001 vs control values; #p < 0.05, ##p <0.01 vs glucose group values).*

5.9.2 Mitochondrial proteins

We also determined the expression of proteins involved in mitochondrial dynamics. Regarding the proteins responsible for mitochondrial fusion, the expression of Optic atrophy protein 1 (Opa1) was significantly reduced only in fructose-supplemented rats (Figure 23A) whereas mitofusin (Mfn2) expression did not show any significant changes between groups (Figure 23B). Dynamic related protein 1 (Drp1), responsible for fission, was significantly increased in fructose group, compared to control (Figure23C).

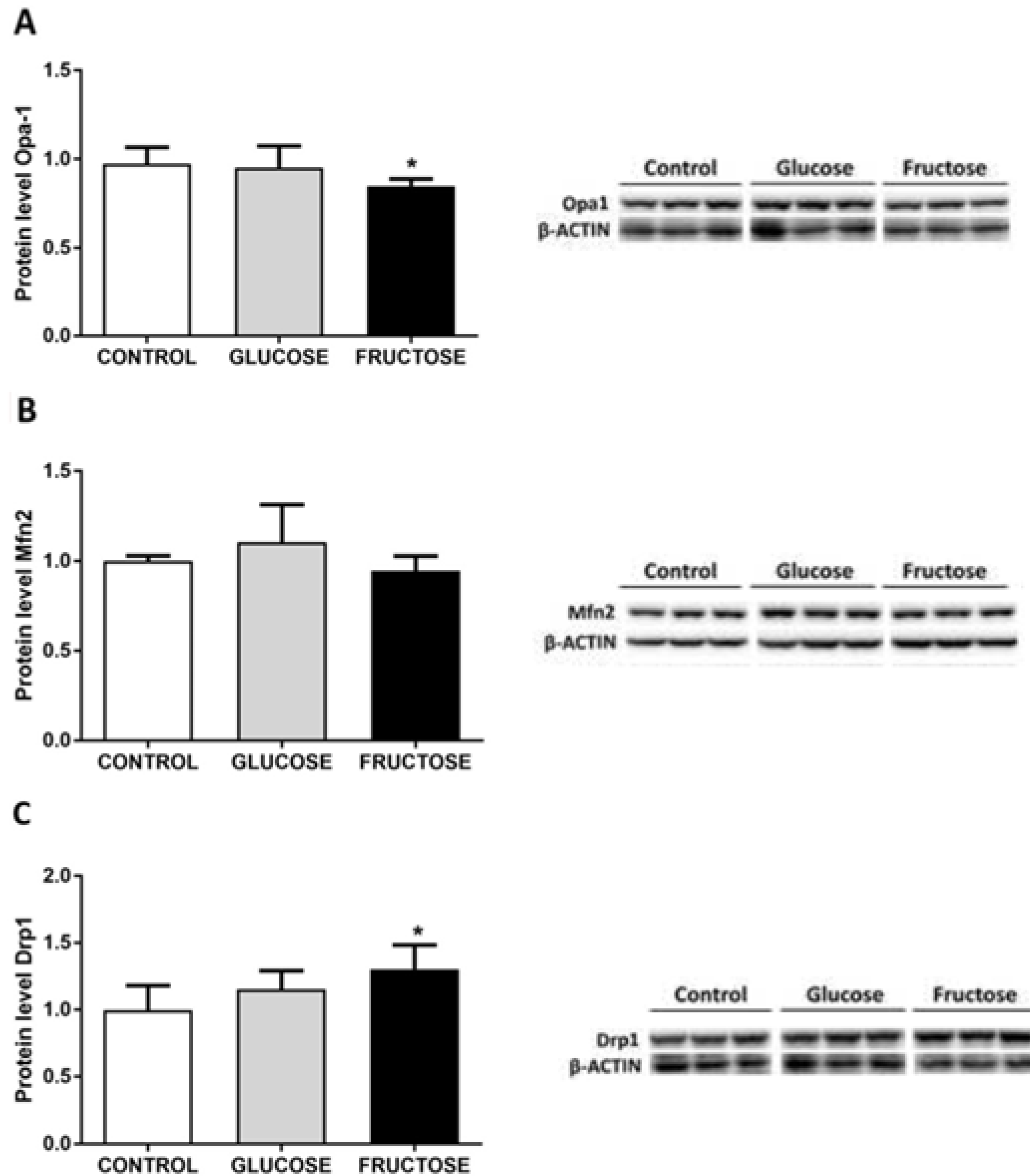


Figure 23 *Graphic representation of protein levels of Opa1 (A), Mfn (B) and Drp1 (C). Each bar is expressed as the mean ± SD. Statistical analysis One-way ANOVA followed by Bonferroni post test. (*p < 0.05 vs control values). Opa1 t-test *p < 0.02 Fructose vs control values.*

5.9.3 Expression of BDNF, synaptophysin

Levels of BDNF decreased in both groups of sugar supplemented rats with respect to control (33 % and 51 % for glucose and fructose groups, respectively, Figure 24A). Expression of synaptophysin was not altered (Figure 24B).

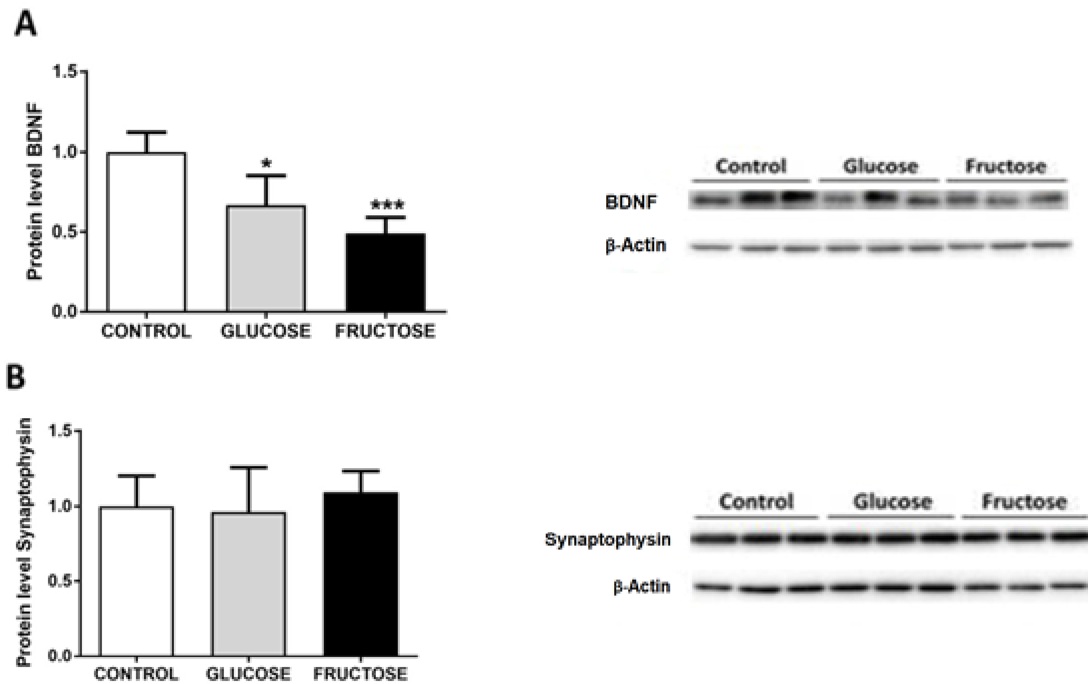


Figure 24 *Graphic representation of protein levels - BDNF (A) and synaptophysin (B). Each bar represents the mean ± SD. Statistical analysis One-way ANOVA followed by Bonferroni post-test (*p<0.05, *p< 0.001 vs control). Representative bands shown in the right part of the figure correspond to 3 different rats in each group.*

6 Discussion

The number of people suffering from metabolic syndrome has increased significantly in the last few decades. Metabolic syndrome is a complex disease which is linked to unhealthy lifestyle, low physical activity and high caloric intake. Moreover, all these metabolic alterations, induced by diet, have been also connected with cognitive deficits. The main objective of the present research is to determine if chronic simple sugar consumption causes cognitive alterations in female rats and, in this case, whether the high amount of calories is the only reason for the impairment of cognitive functions or whether the type of nutrients plays a key role in these alterations. Moreover, we aimed to investigate the molecular mechanisms involved.

The consumption of simple sugars, especially of fructose in form of HFCS, significantly increased in past 4 decades. The introduction of HFCS, as the main sweetener, into the food industry was the main intervention to the human diet, while naturally, fructose occurred only in small amounts as a part of honey or fruits. Sweetened food and soft drinks provide to the body huge caloric intake and due to the differences in metabolic pathways, cause the metabolic imbalance and negative energy homeostasis (Tappy et Le, 2010).

Our research was focused on metabolic and cognitive alterations in rats after 7 months of supplementation with sweetened beverages, 10 % (w/v) liquid glucose and 10 % (w/v) liquid fructose, which imitates the ingestion of simple sugars in the superior quartile of human population (Alegret *et al.,* 2012).

The rats belonging to groups supplemented with sugars in liquid form presented a significantly higher consumption of beverages than the control group. Despite the fact that the fructose and glucose groups had a lower intake of solid food, the calories coming from the beverages were not fully compensated, and sugar-supplemented rats had significantly higher caloric intake than the control group, despite the reduced food intake. This phenomenon was observed already in previous studies performed by our research group (Rebollo *et al.,* 2014). Due to the similar caloric intake in both sugar groups, the possible metabolic alterations found in this study cannot be attributed exclusively to the high caloric intake, but to specific sugar properties.

The body weight was constantly increasing during the study and the results were comparable in all groups, but at the end of the study, the weight of fructose-supplemented rats (despite the caloric intake similar to glucose group) was significantly increased. Although there are a lot of studies presenting the same results (Tappy et Le, 2010; Wu *et al.,* 2014), on the other hand, various studies did not notice any changes in body weight (Lee *et al.,* 2015). The cause of different obtained results can be either the form of sugar supplementation (liquid or solid) (Ross *et al.,* 2009) but also the duration of the study.

The increased total body weight is linked to a weight increase of various organs. We studied the gain of weight of adipose tissue and liver. The final weight of both organs was significantly higher in the fructose group respect to the control group; moreover, the increase in adipose tissue in fructose-supplemented rats was also higher respect to the glucose group. These results confirm the theory that consumption of fructose leads to the fat storage more than the consumption of glucose, which stimulates liberation of insulin and activates LPL, so the fat is not stored so easily (Stanhope *et al.,* 2012).

Although the levels of cholesterol were not altered, fructose-supplemented rats presented a significant hypertriglyceridemia, which may be related to the weight gain in adipose tissue.

High fructose consumption has been linked to the development of NAFLD, which may lead to insulin resistance (Akar *et al.,* 2012; Asrih *et al.,* 2013). Our results from the study showed hyperinsulinemia, which is a marker of insulin resistance. Insulin resistance is a hallmark of DM type 2, and has not been linked only with diabetes, but also with the development of dementia and cognitive decline (Biessels et Reagan, 2015).

In addition, in fructose-supplemented rats, the levels of leptin were higher respect to the other groups. Normally, levels of leptin should be lower respect to the other group, because leptin is a hormone which is released due to the liberation of insulin, which fructose does not promote (Bray *et al.,* 2004). In contrast, our research group has observed in several studies of different duration that fructose causes hyperleptinemia accompanied by a state of hepatic leptin resistance (Roglans *et al.,* 2007).

Adiponectin is supposed to be a protective factor in the development of IR. This hormone stimulates beta-oxidation of lipids, enhances sensibility to insulin and reduces the storage of TAG in adipose tissue (Esfahani *et al.,* 2015). Significantly higher levels of adiponectin in the glucose group, and not in the fructose group, may be a protective factor. In accordance, glucose-supplemented rats did not present hypertriglyceridemia, hyperinsulinemia and they had a better response to insulin even though the caloric intake was the same than the control group.

The curve (Figure 15A), obtained from the glucose tolerance test shows, that blood levels of glucose were similar at the beginning. However, at the end of the test, the curve of the fructose group presented significantly higher levels of blood glucose than the glucose and control groups. Therefore, this result can be indicative of the developing insulin resistance, which is confirmed with persistent hyperinsulinemia during the whole 120 min test respect to the glucose and control groups. These results are in accordance with the observed relation between higher intake of fructose and an elevated prevalence of DM type 2 (Elliot *et al.,* 2002; Gross *et al.,* 2004).

The MWM test was performed 2 weeks after the beginning of the study and then at the end of 7 months study. Although some studies in humans report temporal enhancement in cognitive abilities after consumption of glucose in liquid form (Messier, 2004; Stollery et Christian, 2016), our sugar-supplemented groups of rats did not show any improvement in cognitive abilities after short-term supplementation, nor after long-term supplementation. On the other hand, no cognitive alterations were observed in this test. The reason why in other studies cognitive alterations in the MWM test were observed can be the sexual dimorphism, which results in cognitive impairment in male rats (Ross *et al.,* 2012; Wu *et al.,* 2016).

After 2 weeks of sugar-supplementation, we did not observe any alteration in cognitive abilities assessed by the NOR test, as the DI was comparable in all groups after 2 hours as well as after 24 hours. However, when the test was performed and evaluated after 7 months of the study, fructose-supplemented rats presented significant cognitive impairment respect to the control and glucose

groups, showed by a lower DI. The NOR test is a cognitive test which is used to study the animal ability to recognize new objects, and therefore to evaluate memory alterations (Antunes et Biala, 2012). Based on previous studies, it has been suggested that results obtained in memory tests may be different depending on the type of the performed test (Kanoski et Davidson, 2011). It is important to say that the MWM test is a method which assesses spatial learning and memory, and it is useful for evaluating the damage in the hippocampus because this tissue is mainly involved in spatial memory (Sharma *et al.,* 2010). In contrast, non-spatial memory is better evaluated with the NOR test, which usually is not affected by lesions in the hippocampus. Thus, results from our study suggest that the memory deficits provoked by fructose supplementation, which were observed only in the NOR test, are mainly due to effects in the cortex, although we cannot rule out a contribution of hippocampal tissue.

With reference to cognitive decline, important markers of synaptic plasticity, function and neuronal growth are BDNF, synapsin 1 and synaptophysin. In our study, results obtained from Western Blot analysis showed significantly reduced levels of BDNF in sugar-supplemented groups, especially in the fructose group. The cognitive decline caused by diets rich in fats and simple sugars is linked to decreased levels of BDNF, as has been suggested in various studies performed on laboratory animals (Molteni *et al.,* 2002; Stranaham *et al.,* 2008). BDNF reduction is already observed before the clinical development of AD in humans (Jiao *et al.,* 2016). Thus, BDNF may be a preclinical marker. Although levels of BDNF were significantly reduced respect to the control group, downstream proteins involved in BDNF pathway, such as synaptophysin, were not altered by sugar supplementation.

It is interesting to notice that the reduction of the discrimination index and hypertriglyceridemia occurs only in fructose-supplemented rats. These results support the theory that high TAG levels may be responsible for the development of the cognitive alterations (Farr *et al.,* 2008). On the other hand, only fructose-supplemented rats showed hyperinsulinemia and decreased ISI, suggesting a state of whole-body insulin resistance which has also been related to cognitive impairment (Drew *et al.,* 1998; Agrawal *et al.,* 2012). In this regard, we

examined also various components of an insulin signaling pathway in the cortex and we studied possible changes in this brain region.

Referring to the insulin signaling cascade, protein levels of IRS2 were significantly reduced as well as Akt phosphorylation, which indicates a lower activation of this pathway. However, protein levels of phosphorylated GSK3β were not altered. On the other hand, we also examined protein levels of IDE. Insulin competes with Aβ for IDE which is responsible for clearance of both proteins (Malito *et al.,* 2008). Insulin resistance has been related to decreased levels of IDE (Bedse *et al.,* 2015). Despite this association, our results showed increased levels of IDE in fructose-supplemented rats. Hyperinsulinemia developed in fructose-drinking rats, could be the cause of this increase, as insulin positively regulates IDE expression. It is also possible that IDE increase is a compensatory mechanism triggered to reduce high levels of insulin due to its insulin-degrading activity. Hypothetically based on these findings, high levels of IDE could reduce the concentration of insulin in the cortex, therefore it would contribute to reduced insulin signaling and cognitive impairment.

Oxidative stress is another factor which can be involved in reduced cognitive functions and neurodegeneration. One hallmark of oxidative stress is AGEs, which binds to the receptor for AGEs (RAGE) and then can activate other mediators of inflammation, such as TNF-α, IL-6 and others, caused by oxidative stress (Münch *et al.,* 2012). Increased levels of AGEs were observed in the group of fructose-drinking rats. This could explain the difference in results from cognitive tests between glucose- and fructose-supplemented groups. The glucose group did not show any significant changes in NOR test, even though the levels of BDNF were altered. In comparison, the plasma levels of AGEs did not increase in the glucose group as they did selectively in the fructose group. The exclusive increase of plasma level of AGEs only in the fructose group, not in the glucose group, can be due to the higher reactivity of fructose to form AGEs due to its specific mechanisms (Gugliucci, 2017).

Oxidative stress is closely related to neurodegenerative diseases (Seo *et al.,* 2010; Itoh *et al.,* 2013). Small organelles, responsible for the maintenance of balance in energy metabolism, are mitochondria. Any dysfunctions occurring in

mitochondrial dynamic processes, such as fission and fusion, have a large impact on cognitive alterations because neurons are vulnerable to mitochondrial dysfunction and the damage can result in loss of synapses (Benard *et al.,* 2007; Wang *et al.,* 2009). The results obtained in our study showed an increase of Drp1 protein, which is involved in mitochondrial fusion, and a decrease in Opa1 protein, which is involved in process of mitochondrial fission. Both significant alterations of protein levels were observed only in fructose-supplemented rats. The imbalance in mitochondrial dynamics has been observed only in the fructose-supplemented group, which indicates metabolic alterations occurring in frontal cortex in the brain. This impairment can derive from the oxidative stress, which is linked to hypertriglyceridemia, insulin resistance and all these circumstances lead to the cognitive dysfunction.

The protein PGC-1α, which plays a key role in insulin signaling and is a master regulator of mitochondrial biogenesis, is closely associated with mitochondrial dysfunction and insulin resistance. Therefore, PGC-1α is said to be a bidirectional regulator. Overexpression of protein PGC-1α increases insulin signaling and moreover has a positive effect on mitochondrial function. On the other hand, in the case of insulin resistance, the expression of PGC-1α is lower which leads to impaired mitochondrial metabolism (Pagel-Langenickel *et al.,* 2008). In our study, protein levels of phosphorylated PGC-1α were altered especially in fructose-supplemented rats, concretely the levels were 27 % higher respect to the control group. Higher expression of PGC-1α would be the signal that function of insulin-mediated signaling is correct or improved, however, our other results show the insulin resistance and impaired mitochondrial functions. Nevertheless, it has been suggested that a modest increase of PGC-1α, about approximately 25 %, enhances insulin sensitivity in the body and is beneficial (Benton *et al.,* 2008). Therefore, the activation of PGC-1α can be seen as a compensatory mechanism aimed to restore insulin sensitivity and to regulate energy metabolism of mitochondria.

There is a strong association between metabolic alterations caused by the supplementation by liquid fructose and cognitive impairment which could lead to neurodegenerative diseases, such as Alzheimer disease. From our study, we

observed that metabolic alterations which can lead to cognitive impairment, such as hyperinsulinemia, hypertriglyceridemia, changes in protein levels of various markers of metabolic pathways, mitochondrial dynamics and oxidative stress, are only observed in the fructose group. Therefore, we can conclude that these effects are not exclusively related to the high caloric intake, which was similar in comparison with the glucose group.

7 Conclusions

Our results support the hypothesis that long-term sugar supplementation with liquid fructose causes cognitive decline and metabolic alterations, whereas glucose consumption, which leads to equal caloric intake, did not cause these effects.

- Simple sugar supplementation in liquid form results in alterations in energy compensatory mechanism and leads to higher total caloric intake compared to control group.

- Fructose supplementation leads to increased visceral fat and body weight. Even though the total caloric intake was identical with the glucose group, these rats did not develop obesity.

- The consumption of fructose leads to the development of hypertriglyceridemia and hyperinsulinemia, which is linked to insulin resistance. In the glucose group, these alterations were observed, but plasma adiponectin levels were increased.

- Simple sugar consumption did not affect memory or spatial learning evaluated in MWM test.

- The consumption of fructose, but not glucose, for seven months, produced alterations in the Novel Object Recognition test.

- The consumption of fructose reduced levels of BDNF and increased levels of AGEs in the cortex, which may be linked to oxidative stress. In line with this, we observed changes in mitochondrial dynamics in fructose-supplemented rats, in which protein levels responsible for fusion (Drp1) decreased and for fission (Opa1) increased.

- The consumption of fructose increased levels of phosphorylated PGC-1α, maybe as a compensatory system to insulin resistance and mitochondrial function impairment.

8 Abbreviations

ACTH	Adrenocorticotropic hormone
AD	Alzheimer disease
AGEs	Advanced Glycation End products
Akt	Protein kinase B (PKB)
AMPK	AMP-activated protein kinase
APP	β-amyloid precursor protein
APS	Ammonium persulfate
ATP	Adenosine triphosphate
a.u.	Arbitrary units
AUC	Area under the curve
BAD	Bcl-2-associated death promoter
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
BSA	Bovine albumin serum
cAMP	Cyclic adenosine monophosphate
CAT	Catalase
CoA	Coenzyme A
CREB	Cyclic AMP response element-binding protein
CSF	Cerebrospinal fluid
CVS	Cardiovascular
DM	Diabetes mellitus
Drp1	Dynamic-related protein 1

ECL	Enhanced chemoluminiscence
ELISA	Enzyme-Linked ImmunoSorbent Assay
FFAs	Free fatty acids
Fis1	Fission protein 1
Foxo1	Forkhead box protein O1
G6P	Glucose 6-phosphatase
GLUT	Glucose transporter
GSH-Px	Glutathione peroxidase
GSK3β	Glycogen synthase kinase 3 beta
GTT	Glucose tolerance test
HFCS	High Fructose Corn Syrup
IDE	Insulin degrading enzyme
IGF	Insulin like growth factor
IKK	IκB kinase
IL-1	Interleukin 1
IL-6	Interleukin 6
IR	Insulin resistance
IRS	Insulin receptor substrate
JAK-2	Janus kinase 2
JNK1	c-Jun N-terminal kinase
KC	Krebs cycle
LPL	Lipoprotein lipase
MAP	Microtubule associated proteins
MAPK	Mitogen-activated protein kinases
Mfn	Mitofusine

mTOR	Mammalian target of rapamycin
MS	Metabolic syndrome
MWM	Morris water maze
NAFLD	Non-alcoholic fatty liver disease
NFA	Non-esterified fatty acids
NFTs	Neurofibrillary tangles
NFκB	Nuclear factor κB
NO	Nitrogen oxide
NOR	Novel object recognition
NRF	Nuclear respiratory factor 1
OPA1	Optic atrophy-1 protein
OXPHOS	Oxidative phosphorylation
PBS	Phosphate buffered saline
PI3K	Phosphatidylinositide 3-kinase
PIP2	Phosphatidylinositol 4,5 bisphosphate
PIP3	Phosphatidylinositol 3,4,5 trisphosphate
PFK-1	Phosphofructokinase 1
PGC-1α	Peroxisome proliferator-activated receptor gamma coactivator 1 alpha
PKB	Protein kinase B
PKC	Protein kinase C
PKR	Interferon-induced protein kinase
PPARα	Peroxisome proliferator-activated receptor alpha
PPP	Pentose phosphate pathway
PVDF	Polyvinyl of fluoride

RAGE	Receptor for AGEs
RNA	Ribonucleic acid
RH	Recurring hypoglycemia
ROS	Reactive oxygen species
SD	Specified deviation
SDS	Sodium Dodecyl Sulfate
SDS-PAGE	Sodium Polyacrylamide - Dodecyl Sulfate Gel Electrophoresis
SOD	Superoxide dismutase
STAT-3	Signal transducer and activator of transcription 3
TAG	Triacylglycerol
TBS	Tris buffered saline
TEMED	Tetramethylethylenediamine
TFAM	Mitochondrial transcription factor A
TNF-α	Tumor necrosis factor alpha
WHO	World Health Organization

9 References

AGRAWAL, Rahul a Fernando GOMEZ-PINILLA. Metabolic syndrome' in the brain: deficiency in omega-3 fatty acid exacerbates dysfunctions in insulin receptor signaling and cognition. The Journal of Physiology [online]. 2012, 590 (10), 2485-2499 [cit.2017-07-28]. DOI: 10.1113/jphysiol.2012.230078. ISSN 00223751. Available from: http://doi.wiley.com/10.1113/jphysiol.2012.230078

AKAR, Fatma, Orhan ULUDAĞ, Ali AYDIN, Yasin Atacan AYTEKIN, Sehri ELBEG, Mehmet TUZCU a Kazim SAHIN. High-fructose corn syrup causes vascular dysfunction associated with metabolic disturbance in rats: Protective effect of resveratrol. Food and Chemical Toxicology [online]2012, 50 (6), 2135-2141 [cit. 2017-08-17]. DOI: 10.1016/j.fct.2012.03.061.ISSN 02786915. Available from: https://www.ncbi.nlm.nih.gov/pubmed/22465803

ALBA, L. M. a K. LINDOR. Non-alcoholic fatty liver disease. Alimentary Pharmacology and Therapeutics [online]. 2003, 17(8), 977-986 [cit. 2017-07-28]. DOI: 10.1046/j.1365-2036.2003.01493.x. ISSN 0269-2813. Available from: http://doi.wiley.com/10.1046/j.1365-2036.2003.01493.x

ALEGRET, Marta. Opposite fates of fructose in the development of metabolic syndrome. World Journal of Gastroenterology [online]. 2012, 18(33), 4478- [cit. 2017-07-27]. DOI: 10.3748/wjg.v18.i33.4478. ISSN 1007-9327. Available from: http://www.wjgnet.com/1007-9327/full/v18/i33/4478.htm

ÁLVAREZ Gómez, A., ROSALES, L. C., RODRÍGUEZ, G. P. (2012). Consumo elevado de fructosa y su posible influencia sobre el metabolismo lipídico. Rev Cubana Aliment Nutr, 22(2), 287–300.

ANTUNES, M. a G. BIALA. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cognitive Processing [online]. 2012, 13(2), 93-110 [cit. 2017-08-11]. DOI: 10.1007/s10339-011-0430-z. ISSN 1612-4782. Available from: http://link.springer.com/10.1007/s10339-011-0430-z

ANURADHA R. The Waist Circumference Measurement: A Simple Method for Assessing the Abdominal Obesity. Journal of Clinical and Diagnostic Research [online]. 2012 [cit. 2017-08-17]. DOI: 10.7860/JCDR/2012/4379.2545. ISSN 2249782x. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3527782/

ASRIH, M. a F. R. JORNAYVAZ. Inflammation as a potential link between nonalcoholic fatty liver disease and insulin resistance. Journal of Endocrinology [online]. 2013, 218(3), R25-R36 [cit. 2017-07-27]. DOI: 10.1530/JOE-13-0201. ISSN 0022-0795. Available from: http://joe.endocrinology-journals.org/cgi/doi/10.1530/JOE-13-0201

BANTLE, J. P. Dietary Fructose and Metabolic Syndrome and Diabetes. Journal of Nutrition [online]. 2009, 139(6), 1263S-1268S [cit. 2017-07-27]. DOI: 10.3945/jn.108.098020. ISSN 0022-3166. Available from: http://jn.nutrition.org/cgi/doi/10.3945/jn.108.098020

BASCIANO, Heather, Lisa FEDERICO a Khosrow ADELI. Fructose, insulin resistance, and metabolic dyslipidemia. Nutrition & Metabolism [online]. 2(1), 5- [cit. 2017-07-27]. DOI: 10.1186/1743-7075-2-5. ISSN 17437075. Available from: http://nutritionandmetabolism.biomedcentral.com/articles/10.1186/1743-7075-2-5

BEDSE, Gaurav, Fabio DI DOMENICO, Gaetano SERVIDDIO a Tommaso CASSANO. Aberrant insulin signaling in Alzheimer's disease: current knowledge. Frontiers in Neuroscience [online]. 2015, 9, - [cit. 2017-08-08]. DOI: 10.3389/fnins.2015.00204. ISSN 1662-453x. Available from: http://journal.frontiersin.org/Article/10.3389/fnins.2015.00204/abstract

BENARD, G., N. BELLANCE, D. JAMES, P. PARRONE, H. FERNANDEZ, T. LETELLIER a R. ROSSIGNOL. Mitochondrial bioenergetics and structural network organization. Journal of Cell Science [online]. 2007, 120(5), 838-848 [cit. 2017-08-17]. DOI: 10.1242/jcs.03381. ISSN 0021-9533. Available from: http://jcs.biologists.org/cgi/doi/10.1242/jcs.03381

BENTON, David. The influence of children's diet on their cognition and behavior. European Journal of Nutrition [online]. 2008, 47 (S3), 25-37 [cit. 2017-08-17]. DOI: 10.1007/s00394-008-3003-x. ISSN 1436-6207. Available from: http://link.springer.com/10.1007/s00394-008-3003-x

BIEGER, Wilfried P., Gerd MICHEL, Dieter BARWICH, Karin BIEHL a Alfred WIRTH. Diminished insulin receptors on monocytes and erythrocytes in hypertriglyceridemia. Metabolism [online]. 1984, 33(11), 982-987 [cit. 2017-08-17]. DOI: 10.1016/0026-0495(84)90224-5. ISSN 00260495. Available from: http://linkinghub.elsevier.com/retrieve/pii/0026049584902245

BIESSELS, Geert Jan and Lawrence P. REAGAN. Hippocampal insulin resistance and cognitive dysfunction. Nature Reviews Neuroscience[online]. 2015, 16(11), 660-671 [cit. 2017-08-11]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/26462756

BRADFORD, Marion M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* [online]. 1976, **1976**(1-2), 248-254 [cit. 2017-08-18]. DOI: 10.1016/0003-2697(76)90527-3. Available from: http://www.sciencedirect.com/science/article/pii/0003269776905273

BRAY, GA, SJ NIELSEN a BM POPKIN. Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. The American Journal of Clinical Nutrition [online]. 2004, 79(4), 537-543 [cit. 2017-08-11]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/15051594

BREKKE, Eva, Tora Sund MORKEN a Ursula SONNEWALD. Glucose metabolism and astrocyte–neuron interactions in the neonatal brain. Neurochemistry International [online]. 2015, 82, 33-41 [cit. 2017-08-17]. DOI: 10.1016/j.neuint.2015.02.002. ISSN 01970186. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0197018615000224

BUZZETTI, Elena, Massimo PINZANI a Emmanuel A. TSOCHATZIS. The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). Metabolism [online]. 2016, 65 (8), 1038-1048 [cit. 2017-08-17]. DOI: 10.1016/j.metabol.2015.12.012. ISSN 00260495. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0026049515003832

CALVO-OCHOA, E., HERNÁNDEZ-ORTEGA, K., FERRERA, P., MORIMOTO, S., & ARIAS, C. (2014). Short-term high-fat-and-fructose feeding produces insulin signaling alterations accompanied by neurite and synaptic reduction and astroglial activation in the rat hippocampus. Journal of Cerebral Blood Flow & Metabolism, 34(6), 1001–1008. http://doi.org/10.1038/jcbfm.2014.48 Available from: https://www.ncbi.nlm.nih.gov/pubmed/24667917

CHOI, Joungil, Krish CHANDRASEKARAN, Tatsuya INOUE, Anjaneyulu MURAGUNDLA a James W. RUSSELL. PGC-1α regulation of mitochondrial degeneration in experimental diabetic neuropathy. Neurobiology of Disease [online]. 2014, 64, 118-130 [cit. 2017-07-28]. DOI: 10.1016/j.nbd.2014.01.001. ISSN 09699961. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0969996114000084

Cisbio*.com. Total IRS2 Cellular Assay Kit.* [online]. Codolet: Cisbio Bioassays, 2016 [cit. 2017-08-19]. Dostupné z: http://www.cisbio.com/japan/drug-discovery/total-irs2-cellular-assay-kit

CISTERNAS, Pedro a Nibaldo C. INESTROSA. Brain glucose metabolism: Role of Wnt signaling in the metabolic impairment in Alzheimer's disease. Neuroscience & Biobehavioral Reviews [online]. 2017, 80, 316-328 [cit. 2017-07-27]. DOI: 10.1016/j.neubiorev.2017.06.004. ISSN 01497634. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0149763416305991

CORONA, Juan Carlos a Michael R. DUCHEN. PPARγ and PGC-1α as Therapeutic Targets in Parkinson's. Neurochemical Research [online]. 2015, 40 (2), 308-316 [cit. 2017-08-17]. DOI: 10.1007/s11064-014-1377-0. ISSN 0364-3190. Available from: https://www.ncbi.nlm.nih.gov/pubmed/25007880

CRAFT, Suzanne. The Role of Metabolic Disorders in Alzheimer Disease and Vascular Dementia. Archives of Neurology [online]. 2009, 66(3), - [cit. 2017-07-28]. DOI: 10.1001/archneurol.2009.27. ISSN 0003-9942. Available from: http://archneur.jamanetwork.com/article.aspx?doi=10.1001/archneurol.2009.27

CURRY, DL. Effects of mannose and fructose on the synthesis and secretion of insulin. *Pancreas* [online]. 1989, **4**(1), 2-9 [cit. 2017-08-18]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/2654926

Czarnikow.com. *Outlook for the Global Sugar Economy*. [online]. London: Czarnikow, 2014 [cit. 2017-08-10]. Available from: http://www.czarnikow.com/news/outlook-global-sugar-economy

DE LA MONTE, Suzanne M. a Jack R. WANDS. Alzheimer's Disease is Type 3 Diabetes—Evidence Reviewed. Journal of Diabetes Science and Technology [online]. 2008, 2(6), 1101-1113 [cit. 2017-07-28]. DOI: 10.1177/193229680800200619. ISSN 1932-2968. Available from: http://journals.sagepub.com/doi/10.1177/193229680800200619

DOUARD, V. a R. P. FERRARIS. Regulation of the fructose transporter GLUT5 in health and disease. AJP: Endocrinology and Metabolism[online]. 2008, 295(2), E227-E237 [cit. 2017-08-11]. DOI: 10.1152/ajpendo.90245.2008. ISSN 0193-1849. Available from: http://ajpendo.physiology.org/cgi/doi/10.1152/ajpendo.90245.2008

DREW, P.A, E SMITH a P.D THOMAS. Fat distribution and changes in the blood brain barrier in a rat model of cerebral arterial fat embolism. Journal of the Neurological Sciences [online]. 1998, 156(2), 138-143 [cit. 2017-08-08]. DOI: 10.1016/S0022-510X(98)00039-2. ISSN 0022510x. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0022510X98000392

DUARTE, João M. N., Rui A. CARVALHO, Rodrigo A. CUNHA a Rolf GRUETTER. Caffeine consumption attenuates neurochemical modifications in the hippocampus of streptozotocin-induced diabetic rats. Journal of Neurochemistry [online]. 2009, 111 (2), 368-379 [cit. 2017-08-17]. DOI: 10.1111/j.1471-

4159.2009.06349.x. ISSN 00223042. Available from: http://doi.wiley.com/10.1111/j.1471-4159.2009.06349.x

ELLIOT, Sharon S., Nancy L. KEIM, Judith S. STERN, Karen TEFF a Peter J. HAVEL. Fructose, weight gain, and the insulin resistance syndrome. The American Journal of Clinical Nutrition [online]. 2002, 76(5), 911-922 [cit. 2017-08-11]. Available from: http://ajcn.nutrition.org/content/76/5/911.full

ESFAHANI M, MOVAHEDIAN A, BARANCHI M, GOODARZI MT. Adiponectin: an adipokine with protective features against metabolic syndrome. Iran J Basic Med Sci 2015; 18:430-442. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4475650/

EVERGREN, E., F. BENFENATI a O. SHUPLIAKOV. The synapsin cycle: A view from the synaptic endocytic zone. Journal of Neuroscience Research [online]. 2007, 85(12), 2648-2656 [cit. 2017-08-02]. DOI: 10.1002/jnr.21176. ISSN 03604012. Available from: http://doi.wiley.com/10.1002/jnr.21176

FARR, Susan A., Kelvin A. YAMADA, D. Allan BUTTERFIELD, H. Mohammad ABDUL, Lin XU, Nicole E. MILLER, William A. BANKS a John E. MORLEY. Obesity and Hypertriglyceridemia Produce Cognitive Impairment. Endocrinology [online]. 2008, 149(5), 2628-2636 [cit. 2017-08-12]. DOI: 10.1210/en.2007-1722. ISSN 0013-7227. Available from: https://academic.oup.com/endo/article-lookup/doi/10.1210/en.2007-1722

FERNANDEZ-MARCOS, P. J. a J. AUWERX. Regulation of PGC-1, a nodal regulator of mitochondrial biogenesis. American Journal of Clinical Nutrition [online]. 2011, 93(4), 884S-890S [cit. 2017-08-01]. DOI: 10.3945/ajcn.110.001917. ISSN 0002-9165. Available from: http://www.ajcn.org/cgi/doi/10.3945/ajcn.110.001917

FOSTER, Kathryn G. a Diane C. FINGAR. Mammalian Target of Rapamycin (mTOR): Conducting the Cellular Signaling Symphony. Journal of Biological Chemistry [online]. 2010, 285(19), 14071-14077 [cit. 2017-07-28]. DOI: 10.1074/jbc.R109.094003. ISSN 0021-9258. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.R109.094003

GAILLIOT, Matthew T. a Roy F. BAUMEISTER. The Physiology of Willpower: Linking Blood Glucose to Self-Control. Personality and Social Psychology Review [online]. 2016, 11(4), 303-327 [cit. 2017-08-10]. DOI: 10.1177/1088868307303030. ISSN 1088-8683. Available from: http://journals.sagepub.com/doi/10.1177/1088868307303030

GEDA, Y. E., RAGOSSNIG, M., ROBERTS, L. A., ROBERTS, R. O., PANKRATZ, V. S., CHRISTIANSON, T. J. H., PETERSEN, R. C. (2013). Caloric intake, aging, and mild cognitive impairment: A population-based study. *Journal of Alzheimer's Disease*, *34*(2), 501-507. DOI: 10.3233/JAD-121270 Available from: https://www.ncbi.nlm.nih.gov/pubmed/23234878

Glucose. *Encyclopædia Britannica* [online]. Chicago,USA: Encyclopædia Britannica, 2009 [cit. 2017-08-19]. Available from: https://www.britannica.com/science/glucose

GROSS, Lee S., Li LI, Earl F. FORD a Simin LIU. Increased consumption of refined carbohydrates and the epidemic of type 2 diabetes in the United States: an ecologic assessment. The American Journal of Clinical Nutrition [online]. 2004, 79(5), 774-779 [cit. 2017-08-11]. Available from: http://ajcn.nutrition.org/content/79/5/774.full.pdf+html

GUGLIUCCI, Alejandro. Formation of Fructose-Mediated Advanced Glycation End Products and Their Roles in Metabolic and Inflammatory Diseases. Advances in Nutrition [online].2017, 8(1), 54-62 [cit. 2017-08-11]. doi: 10.3945/an.116.013912.54 Available from: http://advances.nutrition.org/content/8/1/54.abstract

GUO, S. Insulin signaling, resistance, and the metabolic syndrome: insights from mouse models into disease mechanisms. Journal of Endocrinology [online]. 2014, 220(2), T1-T23 [cit. 2017-07-28]. DOI: 10.1530/JOE-13-0327. ISSN 0022-0795. Available from: http://joe.endocrinology-journals.org/cgi/doi/10.1530/JOE-13-0327

GUYTON AC, HALL JE (2005). "Chapter 78: Insulin, Glucagon, and Diabetes Mellitus". Textbook of medical physiology (11th ed.). Philadelphia: W.B. Saunders. pp. 963–68. ISBN 978-0-7216-0240-0.

HALL, AR, N BURKE, R K DONGWORTH a D J HAUSENLOY. Mitochondrial fusion and fission proteins: novel therapeutic targets for combating cardiovascular disease. British Journal of Pharmacology [online]. 2014, 171(8), 1890-1906 [cit. 2017-07-28]. DOI: 10.1111/bph.12516. ISSN 00071188. Available from: http://doi.wiley.com/10.1111/bph.12516

HANOVER, LM a JS WHITE. Manufacturing, composition, and applications of fructose. *The American Journal of Clinical Nutrition* [online]. 1993, **58**(5), 7245-7325 [cit. 2017-08-18]. Available from: http://ajcn.nutrition.org/content/58/5/724S.abstract

HELENIUS, M., HÄNNINEN, M., LEHTINEN, S.K., SALMINEN, A. (1996). Aging-induced up-regulation of nuclear binding activities of oxidative stress responsive NFκB transcription factor in mouse cardiac muscle. J. Mol. Cell. Cardiol. 28 (1996) 487–498.

IKEDA, K. a J. M. BEKKERS. Counting the number of releasable synaptic vesicles in a presynaptic terminal. Proceedings of the National Academy of Sciences [online]. 2009, 106(8), 2945-2950 [cit. 2017-08-02]. DOI: 10.1073/pnas.0811017106. ISSN 0027-8424. Available from: http://www.pnas.org/cgi/doi/10.1073/pnas.0811017106

ITOH, Kie, Ken NAKAMURA, Miho IIJIMA a Hiromi SESAKI. Mitochondrial dynamics in neurodegeneration. Trends in Cell Biology[online]. 2013, 23(2), 64-71 [cit. 2017-08-11]. DOI: 10.1016/j.tcb.2012.10.006. ISSN 09628924. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0962892412001845

IYNEDJIAN, P B. Mammalian glucokinase and its gene. Biochemical Journal [online]. 1993, 293(1), 1-13 [cit. 2017-08-17]. DOI: 10.1042/bj2930001. ISSN 0264-6021. Available from: http://biochemj.org/lookup/doi/10.1042/bj2930001

JANSSEN, I, PT KATZMARZYK a R ROSS. Waist circumference and not body mass index explains obesity-related health risk. *American Journal of Clinical Nutrition* [online]. 2004, **79**(3), 379-383 [cit. 2017-08-18]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/14985210 JIAO, S-S, L-L

SHEN, C ZHU, et al., Brain-derived neurotrophic factor protects against tau-related neurodegeneration of Alzheimer's disease. Translational Psychiatry [online]. 2016-10-4, 6(10), e907- [cit. 2017-08-17]. DOI: 10.1038/tp.2016.186. ISSN 2158-3188. Available from: http://www.nature.com/doifinder/10.1038/tp.2016.186

JOLIVALT, C.G., C.A. LEE, K.K. BEISWENGER, J.L. SMITH, M. ORLOV, M.A. TORRANCE a E. MASLIAH. Defective insulin signaling pathway and increased glycogen synthase kinase-3 activity in the brain of diabetic mice: Parallels with Alzheimer's disease and correction by insulin. Journal of Neuroscience Research [online]. 2008, 86(15), 3265-3274 [cit. 2017-07-27]. DOI: 10.1002/jnr.21787. ISSN 03604012. Available from: http://doi.wiley.com/10.1002/jnr.21787

JUNG, Un a Myung-Sook CHOI. Obesity and Its Metabolic Complications: The Role of Adipokines and the Relationship between Obesity, Inflammation, Insulin Resistance, Dyslipidemia and Nonalcoholic Fatty Liver Disease. International Journal of Molecular Sciences [online]. 2014, 15(4), 6184-6223 [cit. 2017-08-17]. DOI: 10.3390/ijms15046184. ISSN 1422-0067. Available from: http://www.mdpi.com/1422-0067/15/4/6184/

KANDIMALLA, Ramesh, Vani THIRUMALA a P. Hemachandra REDDY. Is Alzheimer's disease a Type 3 Diabetes? A critical appraisal. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease [online]. 2017, 1863(5), 1078-1089 [cit. 2017-07-28]. DOI: 10.1016/j.bbadis.2016.08.018. ISSN 09254439. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0925443916302150

KANOSKI, Scott E. a Terry L. DAVIDSON. Western diet consumption and cognitive impairment: Links to hippocampal dysfunction and obesity. Physiology & Behavior [online]. 2011, 103(1), 59-68 [cit. 2017-08-08]. DOI: 10.1016/j.physbeh.2010.12.003. ISSN 00319384. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0031938410004506

KIM, Bhumsoo a Eva L FELDMAN. Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. Experimental & Molecular Medicine [online]. 2015-3-13, 47(3), e149- [cit. 2017-08-17]. DOI: 10.1038/emm.2015.3. ISSN 2092-6413. Available from: http://www.nature.com/doifinder/10.1038/emm.2015.3

KUNUGI, H, A UEKI, M OTSUKA, et al., A novel polymorphism of the brain-derived neurotrophic factor (BDNF) gene associated with late-onset Alzheimer's disease. Molecular Psychiatry [online]. 2001, 6 (1), 83-86 [cit. 2017-08-11]. Available from: http://www.nature.com/mp/journal/v6/n1/full/4000792a.html

LEDVINA, M., A. STOKLASOVÁ a J. CERMAN. Biochemie pro studující medicíny. Vyd. 2. Praha: Karolinum, 2009, 94 s. ISBN 9788024614144.

LEE, Jai Sun, Dae Won JUN, Eun Kyung KIM, Hye Joon JEON, Ho Hyun NAM a Waqar Khalid SAEED. Histologic and Metabolic Derangement in High-Fat, High-Fructose, and Combination Diet Animal Models. The Scientific World Journal [online]. 2015, 2015, 1-9 [cit. 2017-08-17]. DOI: 10.1155/2015/306326. ISSN 2356-6140. Available from: http://www.hindawi.com/journals/tswj/2015/306326/

LI Ning, Thierry BRUN, Miriam CNOP, Daniel A. CUNHA, Decio L. EIZIRIK a Pierre MAECHLER. Transient Oxidative Stress Damages Mitochondrial Machinery Inducing Persistent β-Cell Dysfunction. Journal of Biological Chemistry [online]. 2009, 284(35), 23602-23612 [cit. 2017-07-28]. DOI: 10.1074/jbc.M109.024323. ISSN 0021-9258. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M109.024323

LIN, Xiaoqian a Qingzhu ZHANG. The Drug Development Based on Pathogenetic Research in Alzheimer's Disease. *Advances in Alzheimer's Disease* [online]. 2014, **03**(02), 55-63 [cit. 2017-08-19]. DOI: 10.4236/aad.2014.32007. ISSN 2169-2459. Dostupné z: http://www.scirp.org/journal/doi.aspx?DOI=10.4236/aad.2014.32007

LUCHSINGER, José Alejandro, MX TANG, S SHEA a R MAYEUX. Caloric intake and the risk of Alzheimer disease. *Arch. Neurol* [online]. 2002, **59**(8), 1258-1263 [cit. 2017-08-18]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/12164721

LUCHSINGER, José Alejandro. Adiposity, hyperinsulinemia, diabetes and Alzheimer's disease. European Journal of Pharmacology [online]. 2008, 585(1), 119-129 [cit. 2017-08-11]. DOI: 10.1016/j.ejphar.2008.02.048. ISSN 00142999. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0014299908002239

MALITO, E., R. E. HULSE a W.-J. TANG. Amyloid β-degrading cryptidases: insulin degrading enzyme, presequence peptidase, and neprilysin. Cellular and Molecular Life Sciences [online]. 2008, 65(16), 2574-2585 [cit. 2017-07-27]. DOI: 10.1007/s00018-008-8112-4. ISSN 1420-682x. Available from: http://link.springer.com/10.1007/s00018-008-8112-4

MASTROCOLA, Raffaella, Debora NIGRO, Alessia S. CENTO, Fausto CHIAZZA, Massimo COLLINO a Manuela ARAGNO. High-fructose intake as risk factor for neurodegeneration: Key role for carboxy methyllysine accumulation in mice hippocampal neurons. Neurobiology of Disease [online]. 2016, 89, 65-75 [cit. 2017-07-27]. DOI: 10.1016/j.nbd.2016.02.005. ISSN 09699961. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0969996116300262

MAYES, PA. Intermediary metabolism of Fructose. *American Journal of Clinical Nutrition* [online]. 1993, **58**(5), 754S-765S [cit. 2017-08-18]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/8213607

MCNAY, Ewan C. a Andrew K. RECKNAGEL. Brain insulin signaling: A key component of cognitive processes and a potential basis for cognitive impairment in type 2 diabetes. Neurobiology of Learning and Memory [online]. 2011, 96(3), 432-442 [cit. 2017-07-27]. DOI: 10.1016/j.nlm.2011.08.005. ISSN 10747427. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1074742711001493

MCNAY, Ewan C., Cecilia T. ONG, Rory J. MCCRIMMON, James CRESSWELL, Jonathan S. BOGAN a Robert S SHERWIN. Hippocampal memory processes are modulated by insulin and high-fat-induced insulin resistance. Neurobiology of

Learning and Memory [online]. 2010, 93(4), 546-553 [cit. 2017-08-17]. DOI: 10.1016/j.nlm.2010.02.002. ISSN 10747427. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1074742710000419

MESSIER, Claude. Glucose improvement of memory: a review. European Journal of Pharmacology [online]. 2004, 490(1-3), 33-57 [cit. 2017-08-11]. DOI: 10.1016/j.ejphar.2004.02.043. ISSN 00142999. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0014299904002006

MOLTENI, R, R.J BARNARD, Z YING, C.K ROBERTS a F GÓMEZ-PINILLA. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. Neuroscience [online]. 2002, 112(4), 803-814 [cit. 2017-08-11]. DOI: 10.1016/S0306-4522(02)00123-9. ISSN 03064522. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0306452202001239

MÜNCH, Gerald, Bernadette WESTCOTT, Teresita MENINI a Alejandro GUGLIUCCI. Advanced glycation endproducts and their pathogenic roles in neurological disorders. Amino Acids [online]. 2012, 42(4), 1221-1236 [cit. 2017-08-17]. DOI: 10.1007/s00726-010-0777-y. ISSN 0939-4451. Available from: http://link.springer.com/10.1007/s00726-010-0777-y

NAGAI, Yoshio, Yoshihiko NISHIO, Takaaki NAKAMURA, Hiroshi MAEGAWA, Ryuichi KIKKAWA a Atsunori KASHIWAGI. Amelioration of high fructose-induced metabolic derangements by activation of PPARα. American Journal of Physiology - Endocrinology And Metabolism [online]. 2002, 282(5), E1180-E1190 [cit. 2017-07-27]. DOI: 10.1152/ajpendo.00471.2001. ISSN 0193-1849. Available from: http://ajpendo.physiology.org/lookup/doi/10.1152/ajpendo.00471.2001

NAGATA, Ryoko, Yoshihiko NISHIO, Osamu SEKINE, Yoshio NAGAI, Yasuhiro MAENO, Satoshi UGI, Hiroshi MAEGAWA a Atsunori KASHIWAGI. Single Nucleotide Polymorphism (–468 Gly to Ala) at the Promoter Region of Sterol Regulatory Element-binding Protein-1c Associates with Genetic Defect of Fructose-induced Hepatic Lipogenesis. Journal of Biological Chemistry [online]. 2004, 279(28), 29031-29042 [cit. 2017-07-28]. DOI: 10.1074/jbc.M309449200. ISSN 0021-9258. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M309449200

PAGEL-LANGENICKEL, Ines, Jianjun BAO, Joshua J. JOSEPH, Daniel R. SCHWARTZ, Benjamin S. MANTELL, Xiuli XU, Nalini RAGHAVACHARI a Michael N. SACK. PGC-1α Integrates Insulin Signaling, Mitochondrial Regulation, and Bioenergetic Function in Skeletal Muscle. Journal of Biological Chemistry [online]. 2008, 283(33), 22464-22472 [cit. 2017-08-17]. DOI: 10.1074/jbc.M800842200. ISSN 0021-9258. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M800842200

PALOMERA-AVALOS, V., C. GRIÑÁN-FERRÉ, D. PUIGORIOL-ILAMOLA, A. CAMINS, C. SANFELIU, A. M. CANUDAS a M. PALLÀS (2017). Resveratrol Protects SAMP8 Brain Under Metabolic Stress: Focus on Mitochondrial Function and Wnt Pathway. Molecular Neurobiology[online]., 54(3), 1661-1676 [cit. 2017-07-27]. DOI: 10.1007/s12035-016-9770-0. ISSN 0893-7648. Available from: http://link.springer.com/10.1007/s12035-016-9770-0

REBOLLO, Alba. Way back for fructose and liver metabolism: Bench side to molecular insights. World Journal of Gastroenterology [online]. 2012, 18(45), 6552- [cit. 2017-07-27]. DOI: 10.3748/wjg.v18.i45.6552. ISSN 1007-9327. Available from: http://www.wjgnet.com/1007-9327/full/v18/i45/6552.htm

REBOLLO, A., ROGLANS, N., BAENA, M., PADROSA, A., SÁNCHEZ, R. M., MERLOS, M., LAGUNA, J. C. (2014). Liquid fructose down-regulates liver insulin receptor substrate 2 and gluconeogenic enzymes by modifying nutrient sensing factors in rats. Journal of Nutritional Biochemistry, 25(2), 250–258. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24445051

REHMAN, Kanwal a Muhammad Sajid Hamid AKASH. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? Journal of Biomedical Science [online]. 2016, 23(1), - [cit. 2017-07-28]. DOI: 10.1186/s12929-016-0303-y. ISSN 1423-0127. Available from: http://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-016-0303-y

RÍOS, Juvenal A., Pedro CISTERNAS, Marco ARRESE, Salesa BARJA a Nibaldo C. INESTROSA. Is Alzheimer's disease related to metabolic syndrome? A Wnt signaling conundrum. Progress in Neurobiology [online]. 2014, 121, 125-146 [cit.

2017-07-27]. DOI: 10.1016/j.pneurobio.2014.07.004. ISSN 03010082. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0301008214000872

ROGLANS, Núria, Laia VILÀ, Mireia FARRÉ, Marta ALEGRET, Rosa María SÁNCHEZ, Manuel VÁZQUEZ-CARRERA a Juan Carlos LAGUNA. Impairment of hepatic Stat-3 activation and reduction of PPARα activity in fructose-fed rats. Hepatology [online]. 2007, 45(3), 778-788 [cit. 2017-07-27]. DOI: 10.1002/hep.21499. ISSN 02709139. Available from: http://doi.wiley.com/10.1002/hep.21499

ROSS, A.P., T.J. BARTNESS, J.G. MIELKE a M.B. PARENT. A high fructose diet impairs spatial memory in male rats. Neurobiology of Learning and Memory [online]. 2009, 92(3), 410-416 [cit. 2017-08-17]. DOI: 10.1016/j.nlm.2009.05.007. ISSN 10747427. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1074742709001257

SCHAEFER, E. J., J. A. GLEASON a M. L. DANSINGER. Dietary Fructose and Glucose Differentially Affect Lipid and Glucose Homeostasis. Journal of Nutrition [online]. 2009, 139(6), 1257S-1262S [cit. 2017-08-17]. DOI: 10.3945/jn.108.098186. ISSN 0022-3166. Available from: http://jn.nutrition.org/cgi/doi/10.3945/jn.108.098186

SCHMITT, U., N. TANIMOTO, M. SEELIGER, F. iyEFFEL a R.E. LEUBE. Detection of behavioral alterations and learning deficits in mice lacking synaptophysin. Neuroscience [online]. 2009, 162(2), 234-243 [cit. 2017-08-02]. DOI: 10.1016/j.neuroscience.2009.04.046. ISSN 03064522. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0306452209006538

SCHULINGKAMP, R.J, T.C PAGANO, D HUNG a R.B RAFFA. Insulin receptors and insulin action in the brain: review and clinical implications. Neuroscience & Biobehavioral Reviews [online]. 2000, 24(8), 855-872 [cit. 2017-08-11]. DOI: 10.1016/S0149-7634(00)00040-3. ISSN 01497634. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0149763400000403

SEO, A. Y., A.-M. JOSEPH, D. DUTTA, J. C. Y. HWANG, J. P. ARIS a C. LEEUWENBURGH. New insights into the role of mitochondria in aging: mitochondrial dynamics and more. Journal of Cell Science [online]. 2010, 123(15), 2533-2542 [cit. 2017-08-11]. DOI: 10.1242/jcs.070490. ISSN 0021-9533. Available from: http://jcs.biologists.org/cgi/doi/10.1242/jcs.070490

SHARMA, Sunita, Sharlene RAKOCZY a Holly BROWN-BORG. Assessment of spatial memory in mice. Life Sciences [online]. 2010, 87(17-18), 521-536 [cit. 2017-08-08]. DOI: 10.1016/j.lfs.2010.09.004. ISSN 00243205. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0024320510003796

SINGH, Anurag Kumar. Fructose-induced hypertension: essential role of chloride and fructose absorbing transporters PAT1 and Glut5. Kidney International [online]. 2008, 74(4), 438-447 [cit. 2017-08-10]. Available from: http://www.sciencedirect.com/science/article/pii/S008525381553336X

SINGH, Gitanjali M., Renata MICHA, Shahab KHATIBZADEH, Stephen LIM, Majid EZZATI a Dariush MOZAFFARIAN. Estimated Global, Regional, and National Disease Burdens Related to Sugar-Sweetened Beverage Consumption in 2010CLINICAL PERSPECTIVE. Circulation [online]. 2015, 132(8), 639-666 [cit. 2017-08-10]. DOI: 10.1161/CIRCULATIONAHA.114.010636. ISSN 0009-7322. Available from: http://circ.ahajournals.org/lookup/doi/10.1161/CIRCULATIONAHA.114.010636

SIUDA, Joanna, Maja PATALONG-OGIEWA, Weronika ŻMUDA, Magdalena TARGOSZ-GAJNIAK, Ewa NIEWIADOMSKA, Iwona MATUSZEK, Halina JĘDRZEJOWSKA-SZYPUŁKA a Monika RUDZIŃSKA-BAR. Cognitive impairment and BDNF serum levels. Neurologia i Neurochirurgia Polska [online]. 2017, 51(1), 24-32 [cit. 2017-08-01]. DOI: 10.1016/j.pjnns.2016.10.001. ISSN 00283843. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0028384316301608

STANHOPE, Kimber L. Role of Fructose-Containing Sugars in the Epidemics of Obesity and Metabolic Syndrome. Annual Review of Medicine [online]. 2012, 63(1), 329-343 [cit. 2017-08-17]. DOI: 10.1146/annurev-med-042010-113026. ISSN

0066-4219. Available from: http://www.annualreviews.org/doi/10.1146/annurev-med-042010-113026

Statista.com. *Consumption share of high-fructose corn syrup (HFCS) worldwide in 2013, by country.* [online]. Hamburg: Statista, 2017 [cit. 2017-08-19]. Available from: https://www.statista.com/statistics/495946/consumption-share-of-high-fructose-corn-syrup-worldwide-by-country/

STOLLERY, Brian a Leonie CHRISTIAN. Glucose improves object-location binding in visual-spatial working memory. Psychopharmacology [online]. 2016, 233(3), 529-547 [cit. 2017-08-11]. DOI: 10.1007/s00213-015-4125-5. ISSN 0033-3158. Available from: http://link.springer.com/10.1007/s00213-015-4125-5

STRANAHAN, Alexis M., Eric D. NORMAN, Kim LEE, Roy G. CUTLER, Richard S. TELLJOHANN, Josephine M. EGAN a Mark P. MATTSON. Diet-induced insulin resistance impairs hippocampal synaptic plasticity and cognition in middle-aged rats. Hippocampus [online]. 2008, 18(11), 1085-1088 [cit. 2017-07-27]. DOI: 10.1002/hipo.20470. ISSN 10509631. Available from: http://doi.wiley.com/10.1002/hipo.20470

TAPPY, L. a K.-A. LE. Metabolic Effects of Fructose and the Worldwide Increase in Obesity. Physiological Reviews [online]. 2010, 90(1), 23-46 [cit. 2017-07-27]. DOI: 10.1152/physrev.00019.2009. ISSN 0031-9333. Available from: http://physrev.physiology.org/cgi/doi/10.1152/physrev.00019.2009

THE STATE OF FOOD INSECURITY. *Fao.org* [online]. Food and Agriculture Organization of the United Nations, 2003 [cit. 2017-08-18]. Available from: http://www.fao.org/docrep/006/j0083e/j0083e00.htm

TREVIÑO, Samuel, Rubén A. VÁZQUEZ-ROQUE, Gustavo LÓPEZ-LÓPEZ, et al., Metabolic syndrome causes recognition impairments and reduced hippocampal neuronal plasticity in rats. Journal of Chemical Neuroanatomy [online]. 2017, 82, 65-75 [cit. 2017-07-28]. DOI: 10.1016/j.jchemneu.2017.02.007. ISSN 08910618. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0891061816302253

TSAI, Shih-Jen, Chen-Jee HONG, Hsiu-Chih LIU, Tsung-Yun LIU, Li-En HSU a Ching-Hua LIN. Association Analysis of Brain-Derived Neurotrophic Factor Val66Met Polymorphisms with Alzheimer's Disease and Age of Onset. Neuropsychobiology [online]. 2004-1-1, 49(1), 10-12 [cit. 2017-08-10]. DOI: 10.1159/000075332. ISSN 0302-282x. Available from: http://www.karger.com/?doi=10.1159/000075332

VILÀ, Laia, Núria ROGLANS, Victoria PERNA, Rosa M. SÁNCHEZ, Manuel VÁZQUEZ-CARRERA, Marta ALEGRET a Juan C. LAGUNA. Liver AMP/ATP ratio and fructokinase expression are related to gender differences in AMPK activity and glucose intolerance in rats ingesting liquid fructose. The Journal of Nutritional Biochemistry [online]. 2011, 22(8), 741-751 [cit. 2017-07-31]. DOI: 10.1016/j.jnutbio.2010.06.005. ISSN 09552863. Available from: http://linkinghub.elsevier.com/retrieve/pii/S095528631000152X

WANG, X., B. SU, H.-g. LEE, X. LI, G. PERRY, M. A. SMITH a X. ZHU. Impaired Balance of Mitochondrial Fission and Fusion in Alzheimer's Diseases. Journal of Neuroscience [online]. 2009, 29(28), 9090-9103 [cit. 2017-07-27]. DOI: 10.1523/JNEUROSCI.1357-09.2009. ISSN 0270-6474. Available from: http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1357-09.2009

WATSON, GS, ER PESKIND, S ASTHANA, et al. Insulin increases CSF Abeta42 levels in normal older adults. *Neurology* [online]. 2003, **60**(12), 1899-1903 [cit. 2017-08-18]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/12821730

WAXMAN, Amalia. The WHO Global Strategy on Diet, Physical Activity and Health: The controversy on sugar. Development [online]. 2004, 47 (2), 75-82 [cit. 2017-08-17]. DOI: 10.1057/palgrave.development.1100032. ISSN 1011-6370. Available from: http://link.springer.com/10.1057/palgrave.development.1100032

WELSH, Jean A. Caloric Sweetener Consumption and Dyslipidemia Among US Adults. JAMA [online]. 2010, 303(15), 1490- [cit. 2017-08-17]. DOI: 10.1001/jama.2010.449. ISSN 0098-7484. Available from: https://www.ncbi.nlm.nih.gov/pubmed/20407058

WHITE, J S, L J HOBBS a S FERNANDEZ. Fructose content and composition of commercial HFCS-sweetened carbonated beverages. International Journal of Obesity [online]. 2015, 39(1), 176-182 [cit. 2017-08-17]. DOI: 10.1038/ijo.2014.73. ISSN 0307-0565. Available from: http://www.nature.com/doifinder/10.1038/ijo.2014.73

WHITMER, R. A., D. R. GUSTAFSON, E. BARRETT-CONNOR, M. N. HAAN, E. P. GUNDERSON a K. YAFFE. Central obesity and increased risk of dementia more than three decades later. Neurology [online]. 2008, 71 (14), 1057-1064 [cit. 2017-08-17]. DOI: 10.1212/01.wnl.0000306313.89165.ef. ISSN 0028-3878.

WOLTJER, RL, I MAEZAWA, KS MONTINE a TJ MONTINE. Advanced glycation endproduct precursor alters intracellular amyloid-beta/A beta PP carboxy-terminal fragment aggregation and cytotoxicity. *Journal of Alzheimer Disease*[online]. 2003, **5**(6), 467-476 [cit. 2017-08-18]. Available from: https://www.ncbi.nlm.nih.gov/pubmed/14757937

WU, Zhaofei, Yushan ZHU, Xingshui CAO, Shufeng SUN a Baolu ZHAO. Mitochondrial Toxic Effects of Aβ Through Mitofusins in the Early Pathogenesis of Alzheimer's Disease. *Molecular Neurobiology*[online]. 2014, **50**(3), 986-996 [cit. 2017-08-19]. DOI: 10.1007/s12035-014-8675-z. ISSN 0893-7648. Available from: http://link.springer.com/10.1007/s12035-014-8675-z

WU, Haijiang, Xinna DENG, Yonghong SHI, Ye SU, Jinying WEI a Huijun DUAN. PGC-1α, glucose metabolism and type 2 diabetes mellitus. Journal of Endocrinology [online]. 2016, 229(3), R99-R115 [cit. 2017-07-28]. DOI: 10.1530/JOE-16-0021. ISSN 0022-0795. Available from: http://joe.endocrinology-journals.org/lookup/doi/10.1530/JOE-16-0021

XIE, Ling, Erik HELMERHOST, Kevin TADDEI, Brian PLEWRIGHT, Wilhelm VAN BRONSWIJK a Ralph MARTINS. Alzheimer's β-Amyloid Peptides Compete for Insulin Binding to the Insulin Receptor. Journal of Neuroscience [online]. 2002, 2002(Vol. 22, 10), 5 [cit. 2017-08-10]. Available from: http://www.jneurosci.org/content/22/10/RC221

YIN, Q.-Q. ,PEI, J.-J., XU, S., LUO, D.-Z., DONG, S.-Q., SUN, M.-H., … LIU, X.-P. (2013). Pioglitazone Improves Cognitive Function via Increasing Insulin Sensitivity and Strengthening Antioxidant Defense System in Fructose-Drinking Insulin Resistance Rats. PLoS ONE, 8(3), e59313. http://doi.org/10.1371/journal.pone.0059313

ZHANG, Liang, Sergey TRUSHIN, Trace A. CHRISTENSEN, et al., Altered brain energetics induces mitochondrial fission arrest in Alzheimer Disease. Scientific Reports [online]. 2016, 6(1), - [cit. 2017-07-27]. DOI: 10.1038/srep18725. ISSN 2045-2322. Available from: http://www.nature.com/articles/srep18725