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Corticosterone values and blood biochemistry in a model of streptozotocin-induced diabetes: the influence of dietary n6/n3 ratio

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ABSTRACT

The study was carried out to investigate the influence of dietary n6/n3 ratio in streptozotocin (STZ) induced diabetes on serum biochemistry and corticosterone values in blood in 15 Wistar male rats assigned into three experimental groups, two diabetic and a control group (STZ-N3, STZ-N6, CON) fed with different diets (n6/n3 ratio: ≈ 1, n6/n3 ratio: ≈60, CON n6/n3 ratio: ≈7). Significantly higher values of alkaline phosphatase (AP) and beta-hydroxybutyrate (BHB) and lower levels for triglyceride and albumin were noticed in both STZ treated groups compared to the control. The values for blood urea nitrogen (BUN) were increased in only the STZ-N3 group compared to the control ($P<0.05$). The values for the total bilirubin ($P<0.05$) and alanine aminotransferase (ALT) ($P<0.05$) were higher in only the STZ-N6 group compared to the control. In addition,

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the STZ-N3 group had lower albumin values compared to the STZ-N6 group ($P < 0.001$). Corticosterone values did not significantly differ among all three experimental groups indicating similar levels of stress. In conclusion, the n6/n3 ratio significantly influences blood biochemical parameters in STZ-treated rats. Nevertheless, neither the n6/n3 ratio nor blood sampling and handling influences corticosterone values, which proved the validity of the STZ-induced rodent model of diabetes mellitus in the experimental conditions described.

Key words: diabetes mellitus; stress; corticosterone; n6/n3 ratio; streptozotocin model

Introduction

Streptozotocin (STZ) induced diabetes in rats is characterized by hyperglycemia and a decrease in serum insulin, as well as an increase in serum immunoreactive glucagon concentration (KHADOURI et al., 1987), and it is a valuable model for investigation of diabetes mellitus type 1 (high dose) or type 2 (low dose). It is also associated with an increase in glucocorticoids (cortisone and corticosterone) values in serum (UNGER, 1971). Diabetes mellitus (DM) is a metabolic disorder which leads not only to abnormalities in carbohydrate, but also in protein and lipid metabolism because of the dysfunction and failure of vital organs (ZIMMET et al., 1994; MERAL et al., 2004; THOMAS and RAMPERSAD, 2004).

Animals used in laboratory research are subjected to routine monitoring procedures including handling by humans, cage movement and cleaning, body weight measuring, physical examination, injections, and the collection of blood or other samples. All of these procedures are incidental in nature and, in laboratory conditions, may cause significant stress to animals (BALCOMBE et al., 2004; CASTELHANO-CARLOS and BAUMANS, 2009). One of the primary endocrinological systems involved in the stress response is the hypothalamic-pituitary-adrenal (HPA) axis, which reacts to stress by releasing glucocorticoids. Glucocorticoid levels, usually corticosterone in rats and mice, as well as cortisol in other species, are used as indicators of the strength and impact of a stressor, and they are typically measured in blood serum or plasma (ABELSON et al., 2005; SISWANTO et al., 2008). Consequently, hormonal and biochemical changes may compromise the immune system and lead to disease, and influence the results of experiments (BALCOMBE et al., 2004).

There are two series of the polyunsaturated fatty acids (PUFA) classified as n3 or n6 fatty acids on the basis of the location of the last double bond relative to the terminal methyl end of the molecule. The most important n3 and n6 fatty acids are alpha linolenic acid (ALA, C18:3n3) and linoleic acid (LA, C18:2n6), which represent the precursors for synthesis of fatty acids with more C atoms and more double bonds (MOON et al., 2009; WALL et al., 2010; UGBAJA et al., 2013). Neither ALA nor LA can be synthesized within the organism in mammals, and they must be obtained through diet (WALL et al., 2010; TOTSCH et al., 2015). There are significant differences between these two lines of

fatty acids in their metabolic effects as well as the metabolic effects of their products. Due to the differences in their physiological and metabolic properties, a balanced n6/n3 ratio is a critical factor for health throughout the life cycle (SIMOPOULOS, 2016).

In recent decades, numerous studies have evaluated the potential beneficial effects of n3 PUFA on inflammatory, autoimmune and renal diseases (WALL et al., 2010). Consequently, the potential beneficial influence of particular fatty acids on diabetes pathology has been extensively investigated (SURESH and DAS, 2003). The present study was undertaken to examine the interaction between the dietary n6/n3 ratio, STZ-induced diabetes and stress, to validate the existing rodent model of diabetes mellitus.

Materials and methods

Animals and diet. All procedures were approved by the National Ethics Committee (EP 13/2015) and Veterinary Directorate, Ministry of Agriculture, Republic of Croatia. The study was performed at the Institute for Medical Research and Occupational Health (IMI), Zagreb, Croatia. Fifteen, eight-week old, Wistar rats (male, approximate body weight 160-180 g) were used during the 20 weeks of this study. The rats were housed in polycarbonate cages and placed in rooms under controlled microclimatic conditions. The air temperature was 22 ± 1 °C and relative humidity 50 ± 10%. The diurnal rhythm was regulated with 12 h of light from 7 a.m. to 7 p.m: and 12 h dark cycle. Wood shavings were used as bedding material. The animals passed through a 7 day accommodation period before the trial began. The animals fasted for 12 hours before the induction of diabetes. Diabetes mellitus type 1 (DM1) was induced using a single intraperitoneal injection of 55 mg/kg of STZ, as previously described (MAŠEK et al., 2014). After 3 days of STZ injection, blood was obtained from the tail vein and glucose levels were determined. Rats with glucose levels higher than 15 mmol/l were considered diabetic.

The animals were assigned into three experimental groups, fed with different diets: the control group (CON, n6/n3 ratio: ≈7), the diabetic group that received a n3 enriched diet (STZ-N3, n6/n3 ratio: ≈ 1) and the diabetic group that received a n6 enriched diet (STZ-N6, n6/n3 ratio: ≈60). The rats were fed *ad libitum* with standard rodent feed whose composition has previously been described (STARČEVIĆ et al., 2018).

In all rats, standard laboratory-handling procedures were performed including lifting the animal, and cleaning or moving the animal's cage, blood collection and body weight measuring (BALCOMBE et al., 2004).

Glucose and body mass. The rats were weighed weekly at 8.00 hours using an electronic balance. The nonfasting blood glucose levels were determined weekly using an Accu-Chek Go.

Blood biochemistry. Before blood sample collection, the animals were anesthetized. The quantity of 2 mL of blood samples was taken slowly from the ventricle to avoid

collapse of the heart, and collected into cooled tubes containing EDTA. The blood samples were centrifuged at 3000 g for 10 minutes at 4 °C and the plasma obtained was stored at -20 °C until analysis. The biochemical parameters were measured photometrically by the recommended methods on an automated clinical chemistry analyser Olympus AU640 Beckman Coulter Biomedical K. K. (Beckman Coulter, Mishima K. K, Tokyo, Japan).

Corticosterone determination. The corticosterone levels in the blood were determined according to enzyme immunoassay method previously published (EIA) (PALME and MÖSTL, 1997). Briefly, plasma samples were extracted with diethyl ether (10 times the amount of plasma), then the ether phase was dried down and resuspended in EIA buffer. The aliquot was analysed by corticosterone EIA. The results were obtained from the standard curve and expressed as ng/mL plasma.

Statistical analyses. Data were analyzed using the statistical program GraphPad Prism 7. Normality of distribution was tested by the Shapiro-Wilks test. ANOVA and the post-hoc Tukey test were applied in order to determine statistical differences between the group means. Significant differences were considered at $P<0.05$.

Results

The characteristic signs of STZ-induced diabetes, including increased blood glucose concentrations ($P<0.001$) and a decrease in body weight ($P<0.01$), were observed (Fig. 1). The results of measured biochemical parameters are presented in Fig. 1. Statistically significantly higher values of AP ($P<0.05$) and BHB ($P<0.05$) and lower values for triglyceride and albumin were observed in both STZ treated groups compared to the control. Values for BUN were increased ($P<0.05$) in only the STZ-N3 group compared to the control, while the values for the total bilirubin ($P<0.05$) and ALT ($P<0.05$) were higher only in the STZ-N6 group compared to the control. Additionally, the STZ-N3 group had lower albumin values compared to the STZ-N6 group ($P<0.001$).

Corticosterone values did not differ significantly between the experimental groups, indicating a similar level of stress (Fig. 2).

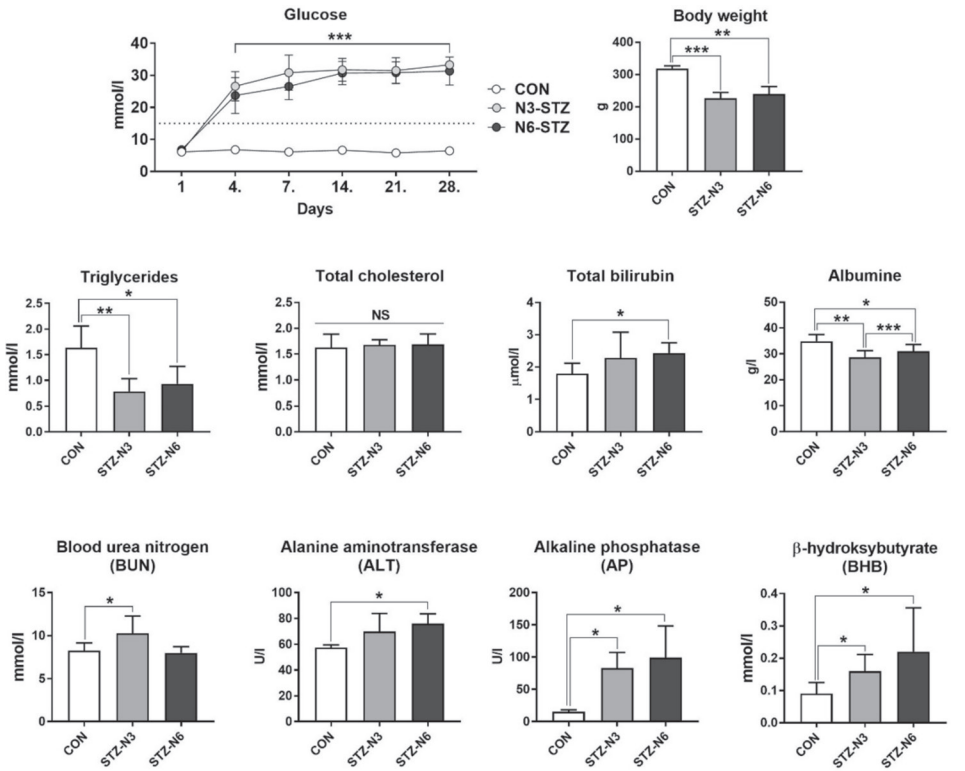

Fig. 1. The effects of different n6/n3 ratios and STZ treatment on body mass and biochemical parameters of STZ-N3, STZ-N6 and control (CON) groups of rats. *P< 0.05, **P< 0.01, ***P< 0.001.

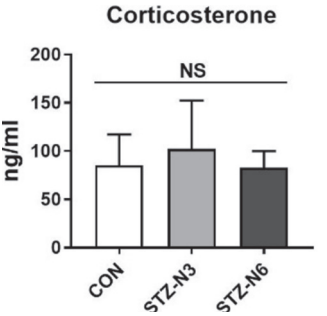

Fig. 2. The effects of different n6/n3 ratios and STZ treatment on plasma corticosterone values in the STZ-N3, STZ-N6 and control (CON) groups of rats

Discussion

STZ is a toxic agent for the B-cells of the islets of Langerhans, and it is widely used for induction of DM (BOLZÁN and BIANCHI, 2002). STZ in rats causes degeneration in the Langerhans islet beta cells, and induces DM in 2-4 days (AKBARZADEH et al., 2007). In our study, DM occurred within 3 days after a single intraperitoneal dose of STZ (55 mg/kg) which resulted in an increase in glucose level in all STZ treated groups.

BHB and cholesterol share a common intermediate, acetoacetyl-CoA, which causes the synthesis of both to be regulated by changes in the intracellular concentrations of acetyl-CoA. Consequently, a long-term rise in glucose could result in increased concentrations of BHB and cholesterol values, and indeed, we found that the concentration of BHB in the plasma of diabetic rats was higher than that of the healthy control rats (AVOGARO et al., 1996). Concerning the total cholesterol level, there was no significant difference noticed between the groups, and these findings are opposite to a previous study (MENSINK et al., 2003).

One of the indicators of hepatocyte injury is the release of intracellular enzymes, such as ALT and AP, into the circulation after STZ administration (SAEED et al. 2008; SENTHIL et al., 2003). In the present study, STZ increased the serum level of AP in both STZ-treated groups and ALT values in only the STZ-N6 group. Similar results were observed by other authors (ZAFAR et al., 2009; OMONKHUA et al., 2014; GHANBARI et al., 2016). The normal ALT values in the STZ-N3 group could be the result of the beneficial influence of a higher content of n3 fatty acids, mainly linolenic acid, originating from the linseed oil present in the experimental feed. Indeed, the beneficial effect of n3 fatty acids on liver and kidney function measured by ALT was previously reported by AL-BISHRI (2013). This result implies that treatment with n3 FA could ameliorate the hepatocyte damage caused by the administration of STZ. Similar to the ALT values, bilirubin was also increased in only the STZ-N6 group. Bilirubin is the product of heme catabolism in the systemic circulation. The connection of the heme catabolic pathway with DM, metabolic syndrome, and obesity has been discussed in numerous studies (VÍTEK, 2012).

The total serum protein level as well as protein profile could be altered in various conditions, such as various infectious diseases, liver disorders, acute inflammatory and proliferative cases, tissue damage, such as trauma, and many other physiological disorders (RAGBETLI et al., 2017). A decrease in total protein level and albumin was also previously reported in STZ-treated rats (GHADGE et al., 2016; GHANBARI et al., 2016). We also observed the same significant decrease in albumin values in the STZ-treated groups in comparison to the control.

Corticosterone levels in blood are valuable markers of stress in rodents, indicating if routine handling procedures are stressful. High levels of stress can seriously influence

the outcome of experimental results. In our trial, corticosterone values were in the normal range for rats and not significantly different between the experimental groups, implying the absence of handling stress. The frequency of blood sampling significantly influences corticosterone values (ABELSON et al., 2005). Therefore, the possible explanation for the corticosterone values in our trial could be the low frequency of blood sampling and manual handling used in our trial (weekly). An additional factor that must be considered are sex differences in corticosterone values during stress. The rats used in our trial were males, which have lower corticosterone values compared to females (ABELSON et al., 2005).

On the basis of our results, we may conclude that the n6/n3 ratio influences certain biochemical blood parameters in STZ-treated rats. Nevertheless, neither the n6/n3 ratio nor blood sampling and handling influenced corticosterone values which proved the validity of the STZ-induced rodent model of diabetes mellitus in the experimental conditions described.

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SAŽETAK

Pokus je proveden kako bi se istražio utjecaj udjela n6/n3 u hrani na biokemijske pokazatelje i vrijednost kortikosterona u serumu štakora kojima je primjenom streptozotocina uzrokovan dijabetes. Istraživanjem je obuhvaćeno 15 mužjaka štakora Wistar podijeljenih u tri skupine: dvije skupine dijabetičara i kontrolnu skupinu (STZ-N3, STZ-N6, CON). Svaka je skupina hranjena različitim udjelom n3/n6: STZ-N3 udjelom n6/n3 ≈ 1, STZ-N6 udjelom n6/n3 ≈ 60, CON udjelom n6/n3 ≈ 7. U objema pokusnim skupinima utvrđene su znakovito više vrijednosti alkalne fosfataze (AP) i betahidroksimaslačne kiseline (BHB) te niže vrijednosti triglicerida i albumina u odnosu na kontrolnu skupinu. Vrijednosti glukoze (BUN) bile su povišene samo u skupini STZ-N3 u odnosu na kontrolnu ($P < 0,05$). Vrijednosti ukupnog bilirubina ($P < 0,05$) i alanine-transferaze (ALT) ($P<0,05$) bile su više samo u skupini STZ-N6 u odnosu na kontrolnu skupinu. Skupina STZ-N3 imala je nižu vrijednost albumina u odnosu na skupinu STZ-N6 ($P < 0,001$). Vrijednost kortikosterona nije se znakovito razlikovala među skupinama posredno dokazujući da među njima postoji slična razina stresa. Zaključno, omjer n3/n6 u hrani utjecao je na određene biokemijske pokazatelje u krvi štakora tretiranih streptozotocinom. No omjer n3/n6, postupak uzorkovanja krvi i baratanje štakorima nisu utjecali na vrijednosti kortikosterona čime je dokazana valjanost animalnog modela sa streptozotocinom uzrokovanom šećernom bolešću u opisanim uvjetima pokusa.

Ključne riječi: dijabetes melitus; šećerna bolest; stres; kortikosteron; omjer n6/n3; model streptozotocin