

Chronic sucrose intake increases expression of SREBP-1c and inflammatory response genes in rat kidneys without significant changes in long chain polyunsaturated fatty acid content

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ABSTRACT

We studied the influence of long-term treatment with sucrose in drinking water on the kidney histology, inflammation gene expression and lipogenesis. Male Wistar rats were supplemented with 30% sucrose (w/v) in water (MetSyn group) or with plain water (Control group). Kidney histology showed that the sucrose treated rats had increased renal lipid content and glomerulosclerosis. TGF- β 1 and TNF- α gene expression was also increased in the MetSyn group indicating low-grade inflammation. The fatty acid profile was characterized by increased saturated and monounsaturated fatty acids and a decrease in C18 essential fatty acids. The content of arachidonic and docosahexaenoic acids did not decrease despite the decrease in the content of their precursors. Neither the expression nor the protein quantity of delta-5 desaturase increased, indicating that renal delta-5-desaturation is not important for the preservation of the kidney arachidonic and docosahexaenoic acid content in metabolic syndrome. The expression of SREBP-1c transcriptional factor increased two-fold in the MetSyn group, indicating increased renal lipogenesis in the sucrose treated rats. In conclusion, the results show that chronic sucrose intake induces glomerulosclerosis, low-grade inflammation, increases saturated and monounsaturated fatty acids and decreases C18 fatty acids without further decrease in arachidonic and docosahexaenoic fatty acids content.

Key words: chronic inflammation; kidney; lipogenesis; glomerulosclerosis

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Introduction

Nowadays the increase in simple sugar consumption via sweetened beverages is connected to metabolic syndrome and diabetes type 2 (MALIK et al., 2010). Several types of cells, including: capillary endothelial cells in the retina, mesangial cells in the renal glomerulus, and neurons and Schwann cells in peripheral nerves, are particularly sensitive to hyperglycemia (BROWNEE, 2005). The connection between sugar intake and kidney damage is very complex. There are several potential pathways linking excessive sugar consumption with developing of kidney diseases including: increase in uric acid and development of hypertension, development of obesity and diabetes, and other possible factors independent of uric acid, diabetes and obesity (KARALIUS and SHOHAM, 2013). In many metabolic diseases, such as diabetes and obesity, lipogenesis and consequent fatty acid concentrations are modified in different organs. The kidney is not an exception and the high sugar or fat intake or diabetes disturbs lipogenesis and lipolysis in the kidney, which results in renal lipid accumulation and renal injury (PROCTOR et al., 2006, KUME et al., 2007).

The fatty acids in tissues originating from food or synthesized *de novo* are further metabolized into fatty acids with more carbon atoms and/or more unsaturations. These processes are highly regulated by different transcriptional factors, metabolites, hormones, dietary factors and other factors, including sex (WANG et al., 2006, TU et al., 2010, ALESSANDRI et al., 2012, JUMP et al., 2013). Kidney tissue is able to desaturate and elongate fatty acids, as evidenced by the expression of key lipogenic enzymes: delta-5 desaturase ($\Delta 5D$) (IRAZÚ et al., 1993, HAGVE et al., 2001, LIABØ et al., 2003), delta-9 desaturase ($\Delta 9D$), (ZHANG et al., 2006) and elongase 6 (elovl6) as well as key transcriptional factors (sterol regulatory element-binding protein-1c, SREBP-1c and carbohydrate response element binding protein, ChREBP) (STRABLE and NTAMBI, 2010). Nevertheless, it has not been completely established to what extent kidney lipogenesis is important for kidney fatty acid profile in comparison to the fatty acids obtained by circulation from lipogenic tissues, such as liver and adipose tissue. The fatty acid profile of kidney tissue is specific because it is characterized by a high level of arachidonic acid (20:4n-6). Linoleic acid, which is a direct precursor for the synthesis of arachidonic acid, is strongly depressed in animal models with chronic sucrose intake via drinking water (EL HAFIDI et al., 2001, MAŠEK et al., 2017, MAŠEK and STARČEVIĆ, 2017a).

The purpose of this study was to investigate renal lipid metabolism in a model of metabolic syndrome based on the chronic intake of sucrose via drinking water (SDW). We first examined how SDW affects renal histology and the expression of profibrotic growth factor, transforming growth factor- $\beta 1$ (TGF- $\beta 1$) and proinflammatory cytokines, tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6). Furthermore, we investigated

the influence of SDW on the fatty acid profile of total kidney lipids and phospholipids as an important constituent of the cell membrane, as well as desaturase enzyme expression, to determine the extent to which desaturase activity contributes to the kidney lipid composition in pathological conditions.

Materials and methods

Animals and experimental design. Two month old male Wistar rats (approximate body weight 200 g) were assigned to two experimental groups: control rats given tap water for drinking (Control, n = 10), and metabolic syndrome rats given 30% sucrose in their drinking water for 20 weeks (MetSyn, n = 10). The drinking solutions were prepared daily. All animals were fed ad libitum with high-fiber rodent feed containing 18% crude protein, 5% crude fat and 20% crude fiber. The rats were maintained in pairs in polycarbonate cages at a room temperature of approximately 22 °C with a 12 h light: dark cycle. All animals were weighed at the beginning and at weekly intervals during the study, and non-fasting blood glucose levels were determined at the same time using an Accu-Chek Go (HAWKINS, 2005). The experiments were approved by the Croatian National Ethics Committee and Veterinary Directorate, Ministry of Agriculture, Republic of Croatia (authorization EP 13/2015).

Sample collection and preparation. At the end of the experiment (20th week), the rats were sacrificed under Narketan/Xylapan anesthesia (Narketan[®], 80 mg/kg b.m. + Xylapan[®], 12 mg/kg b.m., i.p., Vétoquinol, Bern, Switzerland). Immediately after the animals were sacrificed, the kidneys were divided into four parts which were: a) stored in Zamboni's fixative (4% paraformaldehyde and 0.20% picric acid in 0.1 M phosphate-buffered saline at pH 7.4) for histological assessment, b) stored in RNA preserving agent (RNAlater, Thermo Fisher Scientific, Waltham, MA, USA) for extraction and quantification of mRNA, c) frozen at -80 °C for fatty acid analyses and d) homogenized for further protein extraction.

Kidney histology. After washing in PBS, transverse sections of the kidney were dehydrated and embedded in paraffin wax. Five µm thick sections were made by microtome and mounted on microscopic slides (Histobond+, Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany). Sections were subsequently deparaffinised, rehydrated and stained by trichrome Mallory staining, as described previously (AGNIĆ et al., 2014). Kidney sections were viewed and photographed using a microscope (BX61) equipped with a digital camera (DP27; both Olympus, Tokyo, Japan). Four microphotographs were randomly captured at 10x lens magnification for analysis of damaged tubules and the entire surface of the section was photographed at ×4 magnification for analysis of damaged glomeruli. Image J software (National Institutes of Health, Bethesda, MD, USA) was used for analyses. The entire surface of the tissue on the figures was analyzed

and the percentage of damaged tubuli or glomeruli was expressed per total number of tubuli or glomeruli on the surface area. Data are presented as mean \pm standard deviation.

Analysis of kidney gene expression by real-time quantitative PCR (RTqPCR). The isolation of total RNA was performed from 50 mg kidney using the SV Total RNA Isolation System (Promega GMBH, Mannheim, Germany). Final preparation of RNA was considered DNA and protein-free if the ratio between readings at 260/280 nm was ≥ 1.8 (BioDrop μ LITE, BioDrop, Cambridge, UK). Isolated mRNA was reverse transcribed and quantified in a one step reaction using One-Step SYBR PrimeScript RT-PCR Kit II (Perfect Real Time, TaKaRa Bio Inc. Shiga, Japan). The quantitative real-time PCR was performed in Stratagene MxPro3005 thermocycler (Agilent Technologies, US and Canada) with the specific primers listed in Table 1. Expression was relatively quantified using the $\Delta\Delta C_t$ method ($2^{\Delta\Delta C_t}$) after normalization to β -actin (housekeeping gene). Data are represented as fold change in gene expression relative to the control group.

Table 1. List of specific primers used in this study

Gene	Forward	Reverse
$\Delta 5D$	TGGAGAGCAACTGGTTTGTG	GTTGAAGGCTGACTGGTGAA
$\Delta 6D$	TGTCACACAAGTTTGTCCATTGG	ACACGTGCAGGCTCTTTATG
$\Delta 9D$	ACATTCAATCTCGGGAACA	CCATGCAGTCGATGAAGAAC
TGF- $\beta 1$	GTAGCTCTTGCCATCGGG	GAACGTCCCGTCAACTCG
TNF- α	CACCACGCTCTTCTGTCTACTGAAC	CCGGACTCCGTGATGTCTAAGTACT
IL-6	TGATGGATGCTTCCAAACTG	GAGCATTGGAAGTTG GGGTA
SREBP-1c	GATTGCACATTTGAAGACATGCTT	GTCCCAGGAAGGCTTCCAGAGA
β -actin	ACTATTGGCAACGAGCGGTT	TGTCAGCAATGCCTGGGTACA

$\Delta 5D$: Delta-5 desaturase; $\Delta 6D$: Delta-6 desaturase; $\Delta 9D$: delta-9 desaturase; TGF- $\beta 1$: Transforming growth factor beta 1; TNF- α : tumor necrosis factor alpha; IL-6: interleukin-6; Sterol regulatory element-binding protein-1c: SREBP-1c.

Immunoblotting. The kidney tissue was homogenized in lysis buffer (RIPA lysis buffer, EMD Millipore, Billerica, MA, USA) with added protease inhibitors (SIGMAFASTTM Protease Inhibitor Tablets, Sigma Aldrich, Germany). Lysates were then centrifuged for 5 min at 15000g at 4 °C, and the supernatants collected to clear tubes. Quantification of proteins was performed by BCA assay (Sigma Aldrich, Taufkirchen, Germany). Afterwards, the samples (50 μ g of total protein) were boiled for 5 min in Laemmli SDS loading buffer and loaded on 10% SDS polyacrylamide gel electrophoresis and then transferred to a nitrocellulose membrane. The membranes were blocked for 1 h at room temperature in 1% non-fat milk dissolved in tris-buffered saline (TBS) with 0.5% (v/v) Tween (TBST). Subsequently, membrane was incubated at 4 °C with primary antibody for PUFA desaturase ($\Delta 5D$, 1:500 in 0.5% milk-TBST; from Santa Cruz Biotchnology, Heildeberg, Germany). Membrane was then washed three times with TBST buffer and

incubated with secondary antibody (rabbit anti-goat from Santa Cruz Biotechnology, 1:1500). Reactive bands were detected by chemiluminescence (Odyssey Fc, LI-COR, Bad Homburg, Germany). The β -actin (dilution, 1:5000; Santa Cruz Biotechnology, Heidelberg, Germany) was used as loading control. The expression levels of $\Delta 5D$ were quantified relative to β -actin chemiluminescence and expressed relative to the Control group.

Fatty acid analyses. Total lipids (TL) were extracted from homogenized kidney tissue using chloroform/methanol (2:1, v/v) in the presence of the antioxidant butylated hydroxytoluene (30 mg/100 ml, Sigma-Aldrich, St. Louis, MO) (FOLCH et al., 1957). The separated chloroform layer was transferred to a tube, evaporated to dryness under nitrogen, redissolved in 100 μ l of chloroform-methanol (2:1, v/v) and stored at -80 °C. Total phospholipids (PL) were isolated by solid-phase extraction on a 500 mg aminopropyl bonded silica cartridge (Supelclean, Supelco, Bellefonte, PA, USA) (PÉREZ-PALACIOS et al., 2007). Phospholipid isolation was confirmed by 1-D TLC using H_3BO_3 impregnated (2.3%) 20 \times 20 cm silica gel 60 plates (Merck KGaA, Darmstadt, Germany) with chloroform, ethanol, triethylamine and water (30:35:35:7, v/v) in the first development and hexane, diethyl-ether (50:50, v/v) in the second development. Fatty acids from TL and PL were transmethylated using 2M KOH in methanol. The analysis of fatty acid composition was performed as described previously (STARCEVIC et al., 2015, MAŠEK et al., 2017). All the experimental measurements were performed in triplicates and the average values reported. Nonadecanoic fatty acid (C19:0) was used as an internal standard. The results of fatty composition were expressed as mole percentage of total fatty acids. Desaturase activity was calculated as the product/precursor ratio for $\Delta 5D$ (20:4n-6/20:3n-6), $\Delta 9D$ (18:1n-9 + 16:1n-7/18:0 + 16:0) and $\Delta 5D$, $\Delta 6D$ and Elov1 (20:4n-6/18:2n-6). De novo lipogenesis index was calculated as [(C16:1n-7)+(C18:1n-7)+(C20:3n-9)]/[(total fatty acids)] (LAMAZIERE et al., 2013).

Statistical analyses. Data were analyzed using the Statistica 2010 program (STATISTICA 2010 program, Tulsa, OK, USA) and visualized using the GraphPad Prism 7 program. Normality of distribution was tested with Shapiro-Wilks test. T-test test was applied in order to determine statistical differences between group means. Significant differences were considered at $P < 0.05$.

Results

Sections of the kidneys of the Control group of rats showed normal morphology (Fig. 1a, b, c). Some of the glomeruli and tubuli are showing signs of decay, which is consistent with the age of the animals. On the contrary, in the sections from the MetSyn group substantially larger number of damaged tubuli can be seen, specifically distal tubuli are more damaged, along with signs of glomerular sclerosis (Fig. 1d, e, f). A loss of structures

of the loops of Henley and collecting ducts could also be seen in renal medulla of the rats from the MetSyn group (Fig. 1f).

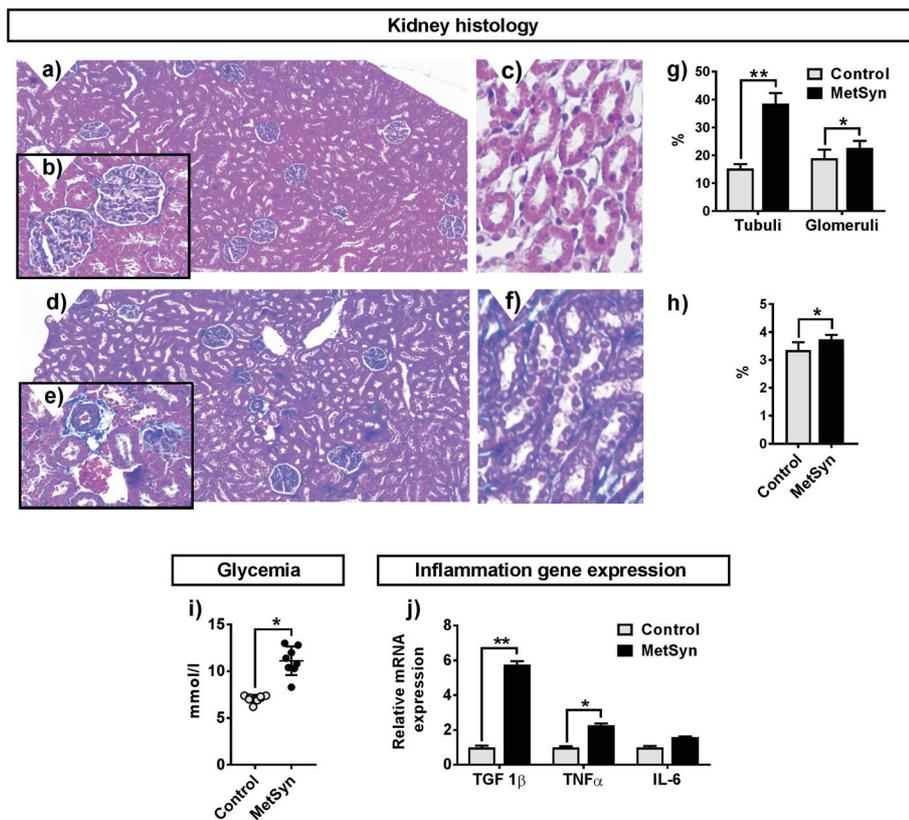


Fig. 1. Histological sections of the kidney stained by Mallory trichrome staining of the Control group rats (a, b, c) and the MetSyn group (d, e, f). Panels a and d show histological sections at $\times 10$ objective magnification, while the panels b and e show the details of the glomeruli from the a and d (respectively), photographed at $\times 40$ objective magnification. Panels c and f show histological sections of the renal medulla of the corresponding groups of rats photographed at $\times 40$ objective magnification. The percentages of damaged tubuli and glomeruli was expressed per total number of tubuli or glomeruli on the surface area (g). Total lipid content (h). The non-fasting blood glucose value (i). The expression of inflammatory response genes was determined by RTqPCR. Control, untreated group (n = 10); MetSyn, sucrose treated group (n = 10). Values are means \pm SD. *P<0.05, **P<0.01.

Significantly higher percentages of damaged tubuli ($P<0.01$) and glomeruli ($P<0.05$) were observed in the sections of the kidney in the MetSyn group in comparison to the Control group (Fig. 1g). The percentage of total lipids was significantly higher in the MetSyn group, compared to the Control (Fig. 1h).

The non-fasting blood glucose values, measured at the 20th week, were significantly increased in the MetSyn group (Fig. 1i, $P<0.05$).

Comparing the sucrose treated with the untreated rats, TGF- β 1 and TNF- α expression showed a statistically significant up-regulation in the kidney tissue after the 20 weeks of high sucrose intake via the drinking water ($P<0.01$, $P<0.05$, for the TGF- β 1 and TNF- α , respectively; Fig. 1j). IL-6 mRNA levels were not significantly different compared to the Control group (Fig. 1j).

The proportions of fatty acids in the TL and PL of the kidney tissue are indicated in Fig. 2a, b. Sucrose treatment increased the proportion of 16:0, 16:1n-7 and 18:1n-7 and decreased the proportions of 18:0, 18:2n-6 and 18:3n-3 in TL and PL. Sucrose treatment also decreased 20:3n-6 and increased 18:1n-9 content in the TL and increased values for 20:4n-6 in the PL.

Desaturation indices including Δ 5D activity (20:4n-6/20:3n-6 and 20:4n-6/18:2n-6), were significantly increased after sucrose treatment (Fig. 2c) and to further explain that increase we quantified Δ 5D mRNA and protein quantity. The relative quantity of Δ 5D mRNA determined by RTqPCR was significantly lower in the MetSyn group than in the Control group (Fig. 2d). The expression of SREBP-1c increased two-fold in the MetSyn group ($P<0.05$, Fig. 2d). The protein content of Δ 5D enzyme was determined using western blotting and the difference was not significant between the experimental groups (Fig. 2e).

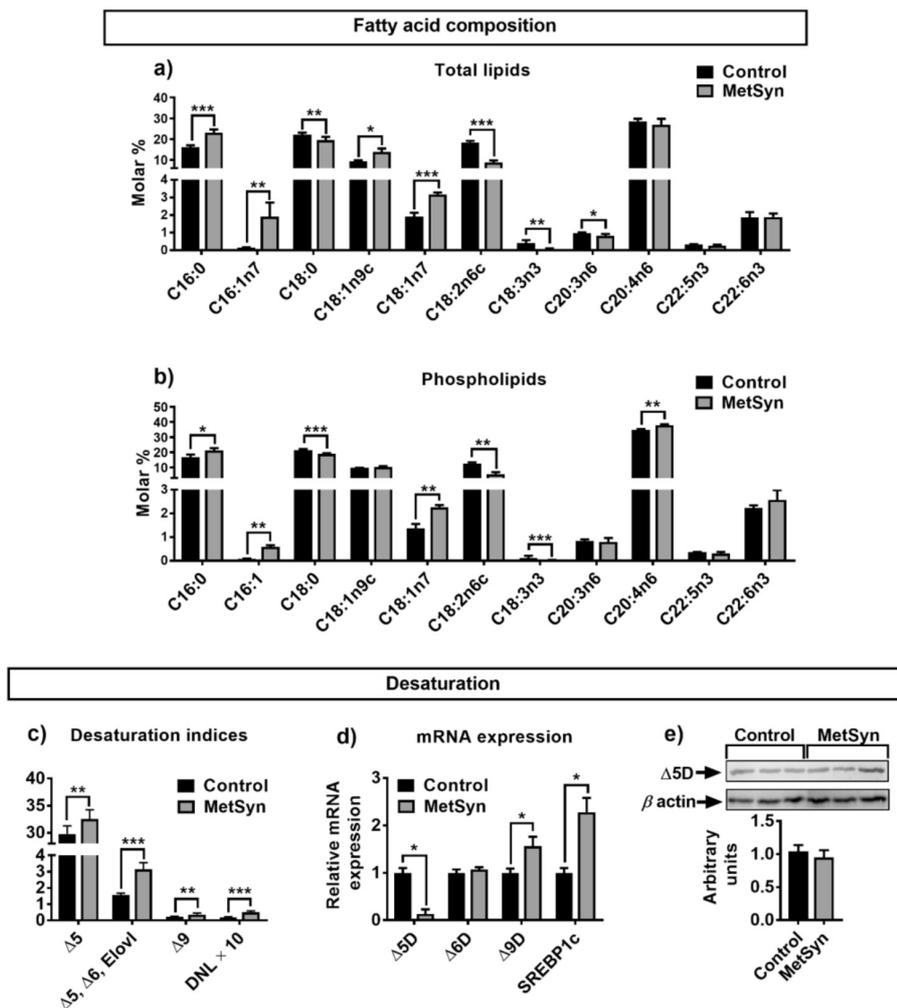


Fig. 2. Concentrations of fatty acids in the kidney total lipids (a) and phospholipids (b). Desaturase activity was calculated as the product/precursor ratio for $\Delta 5D$ (20:4n6/20:3n6) and $\Delta 5D$, $\Delta 6D$ and Elovl1 (20:4n6/18:2n6) (c). Relative expression of Δ desaturases and SREBP-1c in the kidney of control and sucrose treated rats was determined by RTqPCR (d). Representative western blotting images of the $\Delta 5D$ (upper blot) and the gel-loading control protein (β -actin, lower blot). Relative protein levels were normalized to gel-loading control protein (e). Control, untreated group (n = 10); MetSyn, sucrose treated group (n = 10). Values are means \pm SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Discussion

Metabolic syndrome is defined by different factors that increase the risk of cardiovascular diseases and diabetes mellitus type 2 with adiposity and dyslipidemia as important features (KAUR, 2014). In recent years, it has been confirmed that the ectopic accumulation of lipids in organs like pancreatic β cells, liver or muscle is associated with different diseases such as insulin resistance, metabolic syndrome and type 2 diabetes (FERRÉ and FOUFELLE, 2007). That abnormal lipid metabolism and lipid accumulation in kidneys plays an important role in the pathogenesis of diabetic nephropathy (PROCTOR et al., 2006). The relation between the consumption of sugar and particularly sugar-sweetened beverages and the development of obesity gained significant interest (LUDWIG et al., 2001, BRAY et al., 2004), therefore, the animal models that include treatment with different sugars via the drinking water could be very useful in the investigation of the metabolic syndrome.

The results of histological study show that the prolonged sucrose treatment, as a model for metabolic syndrome, resulted in the pathological changes in kidneys of rats, that resemble to changes characteristic for diabetic nephropathy including the destruction of the glomeruli with progressive sclerosis, thickening of the glomerular basement membrane, along with the oedema and damage of the proximal convoluted tubuli (DRONAVALLI et al., 2008).

Our trial showed two-fold increase in the expression of kidney SREBP-1c after the chronic treatment with sucrose in the drinking water which points to disturbance in kidney lipogenesis. SREBP-1c is a transcription factor which responds to insulin and is essential for the lipogenesis and fatty acid homeostasis. The expression of SREBPc1 strongly decreased in the hepatic tissue of streptozotocin treated rats (model of insulin dependent diabetes mellitus, IDDM), nevertheless in the kidney tissue expression is increased (SUN et al., 2002, PROCTOR et al., 2006). These findings indicate tissue specific regulation of the SREBP-1c expression by hyperglycemia and/or insulin deficiency. Increased renal SREBP-1 contributes to nephropathy through increase in lipid synthesis and the increase in profibrotic growth factors (SUN et al., 2002, KUME et al., 2007). During our study important pathological features were observed: increased renal mRNA SREBP-1c expression and increased lipid accumulation in kidney, indicating regulation of SREBP-1c by hyperglycemia and increased lipogenesis.

Today, it is evident that obesity is associated with a state of chronic low-level inflammation (WELLEN and HOTAMISLIGIL, 2005). Profibrotic growth factors (angiotensin II, TGF- β and vascular endothelial growth factor, VEGF) and proinflammatory cytokines (TNF- α and IL6) are frequently increased in diabetes-related renal disease (PROCTOR et al., 2006). Our results showed increase of TGF- β 1 as profibrotic growth factor which stimulates the synthesis of extracellular matrix proteins and inhibits matrix degradation and TNF- α , an inflammatory cytokine which is increasingly produced in

obesity (WELLEN and HOTAMISLIGIL, 2005). These results indicate the presence of low grade chronic inflammation during the chronic sucrose treatment.

Fatty acid profile of rat renal tissue in our trial was characterised by increased palmitic acid content indicating increased kidney lipogenesis and/or increased renal uptake of fatty acids from the circulation. In contrast, the content of essential C18 fatty acids, linoleic and linolenic was strongly decreased. Linoleic and linolenic acids must be supplied by the food and therefore, their decrease in metabolic syndrome is explained by partitioning into oxidation (EL HAFIDI et al., 2001, PEREZ et al., 2009, MAŠEK et al., 2017, MAŠEK and STARČEVIĆ, 2017b). In different models of metabolic syndrome the content of arachidonic acid (20:4n6) is usually decreased in hepatic tissue and serum (WANG et al., 2006, PEREZ et al., 2009). Decrease in the kidney tissue content of arachidonic acid was not observed in our trial, despite the high decrease in the content of linoleic acid which is the precursor for the synthesis of arachidonic acid. Moreover, delta-5-desaturation index calculated as product (20:4n-6) / precursor (20:3n-6) ratio was increased. To further elucidate the contribution of kidney delta-5-desaturation to the kidney arachidonic acid content we examined the mRNA expression of desaturase genes and protein quantity of $\Delta 5D$. Because neither gene expression nor protein quantity of $\Delta 5D$ were increased, it could be concluded that delta5-desaturation in kidney does not play significant role in homeostasis of arachidonic acid in metabolic syndrome. Considering the fact that the content of arachidonic acid was decreased in the total lipids, but increased in the phospholipids, partitioning of arachidonic acid from neutral lipids into phospholipids should not be neglected.

Conclusions

In summary, our findings in rats chronically treated with sucrose in drinking water, show renal lipid accumulation, glomerulosclerosis and low-grade inflammation. Lipid metabolism shows increased fatty acid synthesis of saturated and monounsaturated fatty acids mediated by SREBP-1c. Decrease in essential C18 fatty acids does not induce decrease in arachidonic and docosahexaenoic fatty acid. Although delta-5-desaturation is active in kidney, it does not contribute to the preservation of important long chain polyunsaturated fatty acids. Therefore, other mechanisms like partitioning into phospholipids and oxidation as well as uptake from circulation should be further evaluated.

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Conflicts of interest

The authors have declared no conflict of interest.

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MAŠEK, T., N. FILIPOVIĆ, A. GALAN, K. STARČEVIĆ: Kronični unos saharoze povećava ekspresiju SREBP-1c i gena upalnog odgovora u bubrezima štakora bez znatnih promjena u količini višestruko nezasićenih masnih kiselina. Vet. arhiv 88, 497-510, 2018.

SAŽETAK

Istražili smo učinak dugotrajnog tretmana saharozom putem pitke vode na histologiju bubrega, ekspresiju gena upalnog odgovora te lipogenezu. Wistar-štakori muškog spola tretirani su s 30 % saharoze u vodi (skupina MetSyn) ili su dobivali čistu vodu (kontrola). Histologija bubrega pokazala je povećanu količinu lipida i glomeruloskleroze. Ekspresija TGF- β 1 i TNF- α gena bila je povišena u skupini MetSyn, pokazujući upalu. Profil masnih kiselina pokazivao je povišenu razinu zasićenih i jednostruko nezasićenih te smanjenje masnih kiselina s 18 C atoma. Koncentracija aradidonske i dokozaheksaenske kiseline nije bila smanjena unatoč smanjenju koncentracije njihovih prekursora. Ni ekspresija delta-5-desaturaze ni količina proteina delta-5-desaturaze nisu bile povišene, iz čega je vidljivo da delta-5-desaturacija nije bitna za održavanje koncentracije aradidonske i dokozaheksaenske kiseline u bubregu u metaboličkom sindromu. Ekspresija SREBP-1c transkripcijskog faktora porasla je dvostruko u skupini MetSyn, što upućuje na povećanje bubrežne lipogeneze kod štakora tretiranih saharozom. Rezultati pokazuju da kronični tretman saharozom uzrokuje glomeruloskleroze, upalu, porast koncentracije zasićenih i jednostruko nezasićenih masnih kiselina te smanjenje C18 masnih kiselina, no nema daljnjeg smanjenja koncentracije aradidonske i dokozaheksaenske kiseline.

Ključne riječi: kronična upala; bubrezi; lipogeneza; glomeruloskleroza
