Use of high-fat high-fructose diet for a model of metabolic syndrome in Wistar rats: challenges remain

Marija Paunovic[1], Vesna Vucic[1], Maja Milosevic[2], Danijela Ristic-Medic[1], Aleksandra Arsic[1], Milica Kojadinovic[1], Snjezana Petrovic[1]

University of Belgrade, National Institute of Republic of Serbia, Institute for Medical Research, [1]Centre of Research Excellence in Nutrition and Metabolism, Group for Nutritional Biochemistry and Dietology, [2]Group for Neuroendocrinology, Belgrade, Serbia

Received January 16, 2023
Accepted October 25, 2023

Abstract

Metabolic syndrome (MetS) is a global public health challenge and one of the main risk factors for cardiovascular diseases. Its pandemic prevalence created a demand for developing a relevant model system for deep insight into the molecular basis of MetS. Animal models, especially Wistar rats, are commonly used for that purpose. However, there are no standardized protocols in terms of the diet, strain, or age of rats used for the development of MetS. Studies have mostly used a high-fat high-fructose (HFHF) diet in Wistar rats but have reported inconsistent results; thus the main aim of this study was to examine the effects of the HFHF diet on inducing MetS in Wistar rats. We used two different sub-strains of Wistar rats – Hannover and Kyoto – of two different age groups (8 weeks and 4 months). Animals were placed on a modified diet, standard chow diet enriched with 25% fat and 20% fructose. Following 8 weeks of treatment, all groups were tested for indicators of MetS and the treatment was extended to 16 weeks for groups that developed some of the required parameters. None of the tested groups developed MetS after 16 weeks of HFHF diet, suggesting that the HFHF diet is not sufficient to develop at least three out of five (visceral obesity, high fasting glucose, high triglyceride, low HDL-cholesterol, high blood pressure) needed parameters. Based on our results, the addition of some pharmacological agents (e.g., cholic acid) is necessary for establishing a rat model system of MetS.

Dysmetabolic syndrome, high-fat high-fructose diet, Kyoto strain, Hannover strain, rat model

Metabolic syndrome (MetS) represents a cluster of several physiological and metabolic abnormalities such as abdominal obesity, hypertension, hyperglycaemia, dyslipidaemia (low level of high-density lipoprotein cholesterol [HDL-C] and high triglyceride levels [TG] (O'Neill and O'Driscoll 2015), all of them known to be diet related. The International Diabetes Federation (IDF) estimates that around 25% of the world's population has MetS, which is commonly labelled as a pandemic (Saklayen 2018). Metabolic syndrome increases the risk of developing cardiovascular diseases, the leading global cause of mortality (Sturgeon et al. 2019). In addition, it contributes to an increased risk of stroke, type 2 diabetes, non-alcoholic fatty liver disease, and some types of cancer (Ziki and Mani 2016).

Multiple studies have been directed toward the prevention and treatment of this condition. Irrespective of genetic predisposition (Ziki and Mani 2016), two leading causes for developing MetS are increased consumption of high-calorie low-dietary fibre food and a sedentary lifestyle (Pitsavos et al. 2006).

Although studies in humans are the most valuable, ethical restrictions prevent invasive procedures and detailed insights into the molecular basis of diseases. Rat models are often used for these studies as they are genetically similar to humans, easy to breed, low cost, and able to develop MetS as a result of a dietary modification (Wong et al. 2016).

Address for correspondence:
Marija Paunovic
Institute for Medical Research
National Institute of Republic of Serbia
University of Belgrade
Dr Subotica 4, 11000 Belgrade, Serbia

Phone: +381 11 303-1997
E-mail: marija.paunovic@imi.bg.ac.rs
http://actavet.vfu.cz/

The approaches to developing MetS in rodents have incorporated sugar-rich diets (fructose or sucrose), high-fat diets (HF), or a combination of diets with a high proportion of both sugar and fat. Such diets with a high proportion of sugar and fat, especially those with a high fructose content (HFHF), faithfully copy an unhealthy human dietary model known as the "Western diet" (Brown and Panchal 2011). However, despite numerous studies, there is no universal model to follow, and induction of MetS in rats by dietary intervention is still challenging. Moreover, authors have reported different results even for the same dietary protocols, obtaining an increase in different indicators of MetS, or being unsuccessful in inducing MetS in rats (Wong et al. 2016; Kwitek 2019). Therefore, the development of a relevant MetS animal model is urgently needed.

Besides precise dietary protocols, recommendations on the strain and age of the animals are also lacking. Thus, the present study aimed to compare model systems for dietary-induced MetS in Wistar rats using two different sub-strains – Kyoto and Hannover, and two age groups – young (8 weeks old) and adult (4 months old) rats.

Materials and Methods

Experimental protocols

The experimental protocol was carried out in accordance with the National Law of Animal Welfare ("Sl.gl.RS" 41/09 and 39/10) and the Directive 2010/63/EU. The study protocol was approved by the Ethics Committee of the Institute for Medical Research, National Institute of Republic of Serbia, University of Belgrade, Serbia, and Veterinary Directorate, Ministry of Agriculture and Environmental Protection, Republic of Serbia (No. 323-07-06069/2019-05), 26. 6. 2019.

Two sub-strains of male Wistar rats were used in the experiment – Kyoto and Hannover. Two age groups were included from each strain – 8 weeks old and 4 months old. Animals from the same group were randomly assigned to a standard diet or a HFHF diet. Thus, we formed eight groups: a group of young Kyoto rats on a standard chow diet (KyC); a group of young Kyoto rats on a HFHF diet (KyHFHF); a group of adult Kyoto rats on a standard chow diet (KaC); a group of adult Kyoto rats on a HFHF diet (KaHFHF); a group of young Hannover rats on a standard chow diet (HyC); a group of young Hannover rats on a HFHF diet (HyHFHF); a group of adult Hannover rats on a standard chow diet (HaC); and a group of adult Hannover rats on a HFHF diet (HaHFHF). Each group was composed of 6 animals. Our decision to use only six rats per group stemmed from the principles of 3Rs (replacement, reduction, and refinement) and animal welfare (Hubrecht and Carter 2019). After 8 weeks of treatment, all groups were tested for indicators of MetS and the treatment was extended to 16 weeks for groups that developed some of the required parameters. All rats were kept under standard conditions: room temperature (21 ± 2 ˚C); humidity (55 ± 10%); 12 h light/dark cycles, with *ad libitum* access to food and tap water. Three rats were kept per cage. The rats were weighed once per week.

Following a 7-day adaptation period, the animals were placed on specific diets. The HFHF diet was prepared weekly by mixing 55% powder chow diet, 25% sunflower oil, and 20% fructose. The mixture was pelleted. Composition of the standard (Veterinarski Zavod Subotica, Serbia) and the HFHF diets are presented in Table 1.

For food intake measurement, twice per week, the leftovers in each cage (three rats per cage), were measured on a specific day and subtracted from the pre-measured amount of food that was given the previous measurement. Energy intake was calculated from the amount of food consumed.

Table 1. Composition of the standard and the HFHF diets.

Composition	Diet	
	Standard chow diet (%)	HFHF diet (%)
Proteins	20	11
Fats	4.2	27.3
Carbohydrate	49.3	47.4
Cellulose	8	4.4
Starch	38	20.9
Sugars	3.3	22.1
Moister	13	7.1
Ash	10	5.5
Energy (kJ/100 g)	1100	1813

HFHF – high-fat high-fructose

Sample collection and biochemical analysis

After 8 and 16 weeks of the experiment, 1 ml of blood sample was taken by heart puncture from anaesthetized animals (zoletil 150 mg/kg), to measure biochemical indices. Blood was collected in ethylene-diamine-tetra-acetic acid disodium salt (EDTA) vacutainer tubes. Prior to blood sampling, the rats were placed on overnight fasting.

Plasma was separated by centrifugation at 2,600 *g* at 4 °C for 20 min. Biochemical indices triglyceride (TG), total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), urea, and liver enzymes aspartate

aminotransferase (AST) and alanine aminotransferase (ALT) were analysed on the same day the samples were collected using Clinical chemistry analyser (Cobas c111, Roche Diagnostics, Basel, Switzerland) and Roche Diagnostics kits, according to the manufacturer's instructions. Blood glucose concentrations were determined after overnight starvation with a glucometer (Contour Plus, Bayer, Germany) using blood drops obtained from the tail vein, without anaesthesia, three days before blood sampling for other biochemical analysis. Although only Glc, TG, and HDL-C values are relevant for MetS, other indices were determined to follow the effects of the HFHF diet on liver and kidney function.

Statistical analysis

The data are presented as means ± SD. For statistical analysis, the data distribution was checked by Shapiro-Wilk test. Student's *t*-test and one-way analysis of variance (ANOVA) with Tukey's *post hoc* test were used for normally distributed variables. Mann-Whitney U test was used for comparisons between non-normally distributed variables for independent samples, while Wilcoxon's test was used for comparing variations between the same groups following 8 and 16 weeks of treatment. P values < 0.05 were considered significant. IBM SPSS software Version 20.0 for Windows was used for statistical analysis.

Results

To reveal the effect of a HFHF diet on physiological indices in Wistar rats, we analysed the food and energy intake in 0–8 weeks and 9–16 weeks periods, as well as body mass at the start (0 weeks), in the middle (8 weeks), and at the end (16 weeks) of dietary treatment. As shown in Tables 2 and 3, all HFHF groups had a lower average food intake than the rats on standard chow. At the same time, HFHF-feeding resulted in a significantly increased estimated energy intake compared to the consumption of the standard chow diet ($P < 0.001$ for KyHFHF vs KyC, KaHFHF vs KaC, HyHFHF vs HyC, and HaHFHF vs HaC, respectively), with the energy intake being the highest in the HaHFHF group.

Despite a higher energy intake, young Kyoto and Hannover rats had a significantly lower body weight than the control groups after 8 weeks ($P < 0.01$ for KyHFHF vs. KyC, and HyHFHF vs. HyC, respectively), but the differences were lost after 16 weeks of the treatment (Tables 2 and 3).

In contrast to our expectations, the HFHF diet induced very limited changes in the metabolic indices necessary to develop MetS in Wistar rats. Fasting plasma Glc increased only in KyHFHF rats after 8 weeks of dietary treatment ($P < 0.001$ for KyHFHF vs. KyC) (Table 4), but decreased after 16 weeks, while TG levels were increased in the same experimental group after 16 weeks of HFHF-feeding (Plate XI, Fig. 1). Furthermore, in KyHFHF a significant increase in HDL-C was found. Considering the Hannover sub-strain, after 16 weeks of treatment, HaHFHF rats had significantly lower ($P < 0.01$)

Table 2. Food intake, energy intake, and body weight in control rats and HFHF-fed Kyoto rats.

	KyC	KyHFHF	KaC	KaHFHF
Food intake 0–8 weeks (g/day/cage)	45.1 ± 1.7	32.7 ± 1.7***	61.6 ± 1.1	50.6 ± 4.6***
Food intake 9–16 weeks (g/day/cage)	58.7 ± 1.0	41.0 ± 0.6***	/	/
Energy intake 0–8 weeks (kJ/day/cage)	495.8 ± 19.0	592.9 ± 31.3***	677.3 ± 12.4	916.5 ± 84.3***
Energy intake 9–16 weeks (kJ/day/cage)	646.0 ± 11.6	742.4 ± 10.9***	/	/
0-week initial body weight (g)	104 ± 11.4	97 ± 5.9	235 ± 11	235 ± 9.8
8-week body weight (g)	230 ± 7.8	193 ± 11.6**	280 ± 12.8	267 ± 17.8
16-week body weight (g)	280 ± 12.4	261 ± 16.5	/	/

KyC - young Kyoto on standard chow, KyHFHF - young Kyoto on a HFHF diet, KaC - adult Kyoto on standard chow, KaHFHF - adult Kyoto on a HFHF diet. Missing data in the table for the adult Kyoto group are due to these rats being excluded for further treatment since the results after 8 weeks were non-significant.

The data are presented as means ± SD (n = 6; 3 rats per cage). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 3. Food intake, energy intake, and body weight in control rats and HFHF-fed Hannover rats.

	HyC	HyHFHF	HaC	HaHFHF
Food intake 0–8 weeks (g/day/cage)	63.6 ± 2.6	40.4 ± 8.3***	69.2 ± 5.2	65.4 ± 8.5
Food intake 9–16 weeks (g/day/cage)	67.6 ± 5.3	49.2 ± 2.7***	98.7 ± 9.7	82.7 ± 4.6***
Energy intake 0–8 weeks (kJ/day/cage)	699.6 ± 28.9	731.6 ± 150.9	761.5 ± 56.9	1185.7 ± 154.7***
Energy intake 9–16 weeks (kJ/day/cage)	744.2 ± 58.6	892.0 ± 49.0***	814.3 ± 80.0	1124.6 ± 62.6***
0-week initial body weight (g)	221 ± 18	216 ± 16.5	391 ± 18.9	398 ± 13.8
8-week body weight (g)	366 ± 8.7	302 ± 22**	488 ± 28.8	491 ± 29.3
16-week body weight (g)	415 ± 20.9	384 ± 34.1	520 ± 33.6	561 ± 31.6

HyC - young Hannover on standard chow, HyHFHF - young Hannover on a HFHF diet, HaC - adult Hannover on standard chow, HaHFHF - adult Hannover on a HFHF diet.

The data are presented as means ± SD (n = 6; 3 rats per cage). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Table 4. Changes in biochemical indices in Kyoto rats following 8 and 16 weeks of treatment.

	KyC	KyHFHF 8 weeks	KyHFHF 16 weeks	KaC	KaHFHF 8 weeks
Glc (mmol/l)	5.54 ± 0.93	8.86 ± 1.02***	6.30 ± 0.50##	6.05 ± 0.55	5.71 ± 1.01
Urea (mmol/l)	8.06 ± 1.35	5.88 ± 0.93**	6.85 ± 1.83**	6.86 ± 0.44	5.00 ± 0.54*
AST (IU/l)	177.6 ± 23.8	125.6 ± 15.3	131.2 ± 14.1	88.5 ± 24.7	76.8 ± 13.3
ALT (IU/l)	71.7 ± 21.6	86.6 ± 32.6	49.4 ± 6.0	68.6 ± 23.3	45.5 ± 6.6

KyC - young Kyoto on standard chow, KyHFHF - young Kyoto on s HFHF diet, KaC - adult Kyoto on standard chow, KaHFHF - adult Kyoto on a HFHF diet. Glc - glucose, AST - aspartate aminotransferase, ALT - alanine aminotransferase.

The data are presented as means ± SD (n = 6). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, when compared with the control group. ## $P < 0.01$ when compared with 8 weeks of the HFHF diet.

Table 5. Changes in biochemical indices in Hannover rats following 8 and 16 weeks of treatment.

	HyC	HyHFHF 8 weeks	HyHFHF 16 weeks	HaC	HaHFHF 8 weeks	HaHFHF 16 weeks
Glc (mmol/l)	7.22 ± 1.90	6.75 ± 1.17	6.25 ± 1.49	6.65 ± 0.80	6.95 ± 1.17	6.87 ± 1.67
Urea (mmol/l)	8.25 ± 0.92	5.54 ± 1.08**	3.90 ± 0.45***	6.41 ± 0.76	4.73 ± 1.01*	3.84 ± 0.48***
AST (IU/l)	184.37 ± 50.26	177.31 ± 18.08	161. 60 ± 8.91	112.44 ± 46.05	119.89 ± 63.87	154.09 ± 101.9
ALT (IU/l)	56.20 ± 14.76	57.34 ± 12.14	60.40 ± 2.40	42.54 ± 5.87	37.08 ± 7.22	38.44 ± 9.84

HyC - young Hannover on standard chow, HyHFHF - young Hannover on HFHF diet, HaC - adult Hannover group on standard chow, HaHFHF - adult Hannover on HFHF diet. Glc - glucose, AST - aspartate aminotransferase, ALT -alanine aminotransferase.

The data are presented as means ± SD (n = 6). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

HDL-C compared to normal fed control (0.63 ± 0.19 vs. 1.13 ± 0.22), while no significant changes of HDL-C in young Hannover rats were found (Fig. 1). However, HyHFHF manifested a marked decrease of LDL-C, at the end as well as in the middle of the study, while this indicator was elevated in HaHFHF at 8 weeks. The observed decreases in urea values in all HFHF-fed groups are due to the reduced intake of proteins in the modified diet. No significant differences in AST and ALT values in the examined HFHF-fed groups in compared to normal fed controls were found (Table 5).

Discussion

In this study, we compared the suitability of two sub-strains of Wistar rats, Kyoto and Hannover, as well as the age of the rats, for the development of dietary-induced MetS. The intrastrain differences in Wistar rats' susceptibility to the onset and progression of diseases have already been reported, in relation to neurological disorders and pharmacological response, kidney function and disease (Drábková et al. 2020), and vascular impairment and hypertension (Zhang-James et al. 2013). To the best of our knowledge, there is no data on the intrastrain difference in the onset and development of MetS in the Wistar strain so far. Moreover, only a minority of research articles on MetS in Wistar rats denoted the sub-strain which was used (Sousa et al. 2018).

No consensus has been reached on the optimal age of rats to be used for the development of MetS markers. Previously published studies used rats aged between 3 weeks (Dupas et al. 2017; La Russa et al. 2019; Du Preez et al. 2020) and 3 months (Reynés et al. 2014). In this study, we used both Wistar sub-strains at two different ages at the beginning of the study, young (8 weeks old) and adult (4 months old). At the age of 8 weeks, rats are still in a period of intensive growth and body weight accumulation, thus being considered adolescents in transition to adulthood. Conversely, 4-month-old rats are adults that have reached full maturity and entered a period of slower body growth (Sengupta 2013). Our goal was to investigate the developmental phases which are more susceptible to MetS induction by HFHF diet in Wistar rats.

After 16 weeks of HFHF diet, neither Kyoto nor Hannover young rats manifested higher body weight gains compared to their littermates on the standard diet. The decreased protein content in HFHF might not meet the physiological requirements of the intensive growing period, contributing to reduction of growth performance in young animals (Even et al. 2021). In addition, during the whole period of HFHF feeding, young rats significantly decreased the average food intake compared to the control groups on the standard diet. Therefore, despite the diet they consumed being energetically denser than the standard diet, the total food intake was insufficient to lead to increased body weight and visceral fats, as one of the components of MetS (Arsic et al. 2020). Dissard et al. (2013) and Zhao et al. (2020) found a decreased mean daily intake of HFHF diets containing 45% fat and 30% or 15% fructose solution, respectively, but an increased caloric intake, as well as body and fat weight in HFHF-fed young rats and mice, while 60 days of a fructose diet failed to increase body mass in 30-day-old male Wistar rats compared to rats on a normal diet (De Castro et al. 2013). Considering the adult rats examined in our study, results on food and energy intake were similar to those in young rats, with lower food intake but higher intake of energy. Unlike young rats, this intake did not affect the body weight significantly. Recent results obtained in mice showed that the higher energy intake led to elevated energy expenditure, which at least partially contributes to the prevention of fat and weight accumulation due to energy overconsumption (Chaumontet et al. 2019).

Dyslipidaemia comprising mainly high TG levels and low HDL-C is one of the typical features of MetS. In our experimental setting, impaired TG clearance resulted in significantly higher levels of plasma TG observed only in KyHFHF rats after 16 weeks of dietary treatment. The elevation in TG due to HFHF could be expected, due to the abundant fat present in the diet and *de novo* lipogenesis from dietary fructose (Stanhope 2016), and many studies reported increased TG levels in rats after HFHF dietary intervention, such as those by Gancheva and Zhelyazkova-Savova (2020) and Theodoro et al. (2021) who detected elevated TG in rats fed HFHF diets containing 30% fat and 20% fructose, as well as 20% fat and 10% fructose solution, respectively. However, our HyHFHF group as well as both adult groups HaHFHF and KaHFHF showed no significant changes in TG induced by the HFHF diet, suggesting that the induction of abnormally high TG levels in rats by diet only is challenging.

Although hypertriglyceridaemia is an important factor of MetS, other components such as reduced HDL-C and increased plasma Glc levels are also involved in MetS development (Mogane et al. 2019). In our study, the changes in HDL-C were found in KyHFHF and HaHFHF groups. After 8 weeks, our HaHFHF rats manifested a noticeable decrease in HDL-C concentration, which became significant when the HFHF diet was prolonged for 16 weeks. Considering the role of HDL-C in transporting excessive cholesterol from peripheral tissues to the liver, the dietary-induced decrease of HDL-C may affect the overall cholesterol metabolism, directing it toward the pathogenesis of atherosclerosis (Zhou et al. 2018). On the other hand, increased HDL-C in the the KyHFHF group and reduced LDL-cholesterol in HyHFHF were unexpected results that further emphasize the unreliability of HFHF intervention in establishing MetS. The literature data on HDL-C also vary. In accordance with our results, a significant reduction in HDL-C was noted in adult male Wistar rats fed a HFHF diet containing 20% fat and 17% fructose (Ojetola et al. 2021). In contrast, 12-week-old animals in a study by De Castro et al. (2013) did not develop lower HDL-C concentration by high-fat and high-fructose diets, while the decrease was significant in young 4-week-old rats.

When considering hyperglycaemia, KyHFHF rats were the only group that manifested elevated Glc after 8 weeks, however, the trend did not persist throughout the period of 16 weeks. Also, no change of Glc levels in the HyHFHF group was found. Our results are in line with those of Gancheva and Zhelyazkova-Savova (2020) who detected no increase in fasting blood Glc levels in young HFHF-fed Wistar rats. Moreover, when checked after 8 weeks, both dietary intervened adult groups HaHFHF and KaHFHF also failed to develop hyperglycaemia. Therefore, at that time point, KaHFHF rats were excluded from the rest of the study, as we detected no significant changes in biochemical indices related to the MetS cluster in this experimental group. Fasting blood Glc concentrations did not increase in 9 weeks old Wistar rats when a high-carbohydrate high-fat (HCHF) diet was applied for 8–16 weeks, still, the possible progress to hyperglycaemia after prolonged feeding of the HCHF containing 20% fat and 17.5% fructose together with 25% fructose in drinking water was assumed (Panchal et al. 2011).

However, based on the findings of the present study, we can conclude that prolonging the intervention period does not affect the development of various MetS markers.

Another component of MetS is hypertension. El-Bassossy and Shaltout (2015) reported elevated blood pressure in rats induced by HFHF diet containing 28% fat together with 10% fructose solution rats. However, MetS are developed if at least three indices out of five (fasting blood Glc, HDL-C, TG, hypertension, and abdominal obesity) are altered. Since none of the experimental groups developed alterations in even two parameters, elevated blood pressure would not change the situation. Therefore, we measured blood pressure (the tail-cuff non-invasive method BP blood pressure system) in two to three rats per group and found no significant increase in systolic blood pressure compared to normal-fed control (data not shown).

Among biochemical indices, the HFHF diet significantly decreased plasma urea levels in all rat groups. Urea is a metabolic end-product of protein-rich foods and appears in blood soon after the dietary intake of protein (Bilancio et al. 2019). Thus, we assume that our finding is due to the lower protein content in the HFHF diet compared to the normal rat diet.

Before conclusion, the authors need to emphasize that there is still little consensus regarding the characteristics and compositions of diets used to induce MetS in rats. Therefore, the right diet design as well as comparison of data among different groups might be difficult (Rodríguez-Correa et al. 2020).

In summary, our HFHF (25% sunflower oil, 20% fructose) diet failed to induce more than one component of MetS in any of the investigated groups of Wistar rats (Kyoto and

Hannover, young and adult). This is important since there is inconsistency in the literature data on the effects of HFHF in developing MetS in rats, and further unsuccessful studies could be avoided by the addition of some pharmacological agents (e.g., cholic acid) in the HFHF diet for the MetS development in Wistar rats. Ultimately, the results presented in this study may be highly useful to prevent redundant, inefficient, and non-economic studies.

Acknowledgements

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Contract No. 451-03-47/2023-01/200015).

References

Arsic A, Takic M, Kojadinovic M, Petrovic S, Paunovic M, Vucic V, Ristic-Medic D 2020: Metabolically healthy obesity: is there a link with polyunsaturated fatty acid intake and status? Can J Physiol Pharmacol **99**: 64-71

Bilancio G, Cavallo P, Ciacci C, Cirillo M 2019: Dietary protein, kidney function and mortality: review of the evidence from epidemiological studies. Nutrients **11**: 196

Brown L, Panchal SK 2011: Rodent models for metabolic syndrome research. J Biotechnol Biomed **2011**: 351982

Chaumontet C, Azzout-Marniche D, Blais A, Piedcoq J, Tomé D, Gaudichon P, Even PC 2019: Low protein andmethionine, high starch diets increase energy intake and expenditure, increase FGF21, decrease IGF-1, and has little effect on adiposityin mice. Am J Physiol Regul Integr Comp Physiol **316**: R486-R501

De Castro UGM, dos Santos RAS, Silva ME, de Lima WG, Campagnole-Santos MJ, Alzamora AC 2013: Age-dependent effect of high-fructose and high-fat diets on lipid metabolism and lipid accumulation in liver and kidney of rats. Lipids Health Dis **12**: 136

Dissard R, Klein J, Caubet C, Breuil B, Siwy J, Hoffman J, Sicard L, Ducassé L, Rascalou S, Payre B, Buléon M 2013: Long term metabolic syndrome induced by a high fat high fructose diet leads to minimal renal injury in C57BL/6 mice. PloS one **8**: e76703

Drábková N, Hojná S, Zicha J, Vaněčková I 2020: Contribution of selected vasoactive systems to blood pressure regulation in two models of chronic kidney disease. Physiol Res **69**: 405-414

Dupas J, Feray A, Goanvec C, Guernec A, Samson N, Bougaran P, Guerrero F, Mansourati J 2017: Metabolic syndrome and hypertension resulting from fructose enriched diet in Wistar rats. Biomed Res Int **2017**: 2494067

Du Preez R, Paul N, Mouatt P, Majzoub ME, Thomas T, Panchal SK, Brown L 2020: Carrageenans from the red seaweed *Sarconema filiforme* attenuate symptoms of diet-induced metabolic syndrome in rats. Mar Drugs **18**: 97

El-Bassossy HM, Shaltout HA 2015: Allopurinol alleviates hypertension and proteinuria in high fructose, high salt and high fat induced model of metabolic syndrome. Transl Res **165**: 621-630

Even PC, Gehring J, Tomé D 2021: What does self-selection of dietary proteins in rats tell us about protein requirements and body weight control? Obes Rev **22**: e13194

Gancheva S, Zhelyazkova-Savova M 2020: Are bisphosphonates associated with adverse metabolic and cognitive effects? A study in intact rats and rats fed high-fat high-fructose diet. Calcif Tissue Int **107**: 41-51

Hubrecht RC, Carter E 2019: The 3Rs and humane experimental technique: implementing change. Animals **9**: 754

Kwitek AE 2019: Rat Models of Metabolic Syndrome. In: Hayman G, Smith J, Dwinell M, Shimoyama M (Eds): Rat Genomics. Methods in Molecular Biology. Vol 2018. Humana, New York, NY, USA

La Russa D, Giordano F, Marrone A, Parafati M, Janda E, Pellegrino D 2019: Oxidative imbalance and kidney damage in cafeteria diet-induced rat model of metabolic syndrome: effect of bergamot polyphenolic fraction. Antioxidants **8**: 66

Mogane C, Mokotedi LP, Millen AME, Michel FS 2019: Increased systolic blood pressure associated with hypertriglyceridemia in female Sprague-Dawley rats. Can J Physiol Pharmacol **97**: 971-979

Ojetola AA, Adeyemi WJ, David UE, Ajibade TO, Adejumobi OA, Omobowale TO, Oyagbemi AA, Fasanmade AA 2021: D-ribose-L-cysteine prevents oxidative stress and cardiometabolic syndrome in high fructose high fat diet fed rats. Biomed Pharmacother **142**: 112017

O'Neill S, O'Driscoll L 2015: Metabolic syndrome: A closer look at the growing epidemic and its associated pathologies. Obes Rev **16**: 1-12

Panchal SK, Poudyal H, Iyer A, Nazer R, Alam A, Diwan V, Kauter K, Sernia C, Campbell F, Ward L, Gobe G, Fenning A, Brown L 2011: High-carbohydrate high-fat diet–induced metabolic syndrome and cardiovascular remodeling in rats. J Cardiovasc Pharmacol **557**: 51-64

Pitsavos C, Panagiotakos D, Weinem M, Stefanadis C 2006: Diet, exercise and the metabolic syndrome. Rev Diabet Stud **3**: 118-126

Reynés B, García-Ruiz E, Díaz-Rúa R, Palou A, Oliver P 2014: Reversion to a control balanced diet is able to restore body weight and to recover altered metabolic parameters in adult rats long-term fed on a cafeteria diet. Food Res Int **64**: 839-848

Rodríguez-Correa E, González-Pérez I, Clavel-Pérez PI, Contreras-Vargas Y, Carvajal K 2020: Biochemical and nutritional overview of diet-induced metabolic syndrome models in rats: what is the best choice? Nutr Diabetes **10**: 24

Saklayen MG 2018: The global epidemic of the metabolic syndrome. Curr Hypertens Rep **20**: 12

Sengupta P 2013: The laboratory rat. Relating its age with human's. Int J Prev Med **4**: 624-630

Sousa RM, Ribeiro NL, Pinto BA, Sanches JR, da Silva MU, Coêlho CF, França LM, de Figueiredo Neto JA, Paes AM 2018: Long-term high-protein diet intake reverts weight gain and attenuates metabolic dysfunction on high-sucrose-fed adult rats. Nutr Metab **15**: 1-3

Stanhope KL 2016: Sugar consumption, metabolic disease and obesity: The state of the controversy. Crit Rev Clin Lab Sci **53**: 52-67

Sturgeon KM, Deng L, Bluethmann SM, Zhou S, Trifiletti DM, Jiang C, Kelly SP, Zaorsky NG 2019: A population-based study of cardiovascular disease mortality risk in US cancer patients. Eur Heart J **40**: 3889-3897

Theodoro JMV, Martinez ODM, Grancieri M, Toledo RCL, Binoti ML, Martins AMD, Carvalho CWP, Lisboa PC, Martino HSD 2021: Germinated millet flour (*Pennisetum glaucum* (L.) R. BR.) improves adipogenesis and glucose metabolism and maintains thyroid function *in vivo*. Food Funct **12**: 6083-6090

Wong SK, Chin KY, Suhaimi FH, Fairus A, Ima-Nirwana S 2016: Animal models of metabolic syndrome: a review. Nutr Metab **13**: 1-12

Zhang-James Y, Middleton FA, Faraone SV 2013: Genetic architecture of Wistar-Kyoto rat and spontaneously hypertensive rat substrains from different sources. Physiol Genomics **45**: 528-538

Zhao Q, Li L, Zhu Y, Hou D, Li Y, Guo X, Wang Y, Olatunji OJ, Wan P, Gong K. Kukoamine B 2020: Ameliorate insulin resistance, oxidative stress, inflammation and other metabolic abnormalities in high-fat/high-fructose-fed rats. Diabetes Metab Syndr Obes **13**: 1843-1853

Zhou JM, Wang HM, Lv YZ, Wang ZZ, Xiao W 2018: Anti-atherosclerotic effect of Longxuetongluo Capsule in high cholesterol diet-induced atherosclerosis model rats. Biomed Pharmacother **97**: 793-801

Ziki MDA, Mani A 2016: Metabolic syndrome. Genetic insights into disease pathogenesis. Curr Opin Lipidol **2**: 162-171

Plate XI
Paunovic M. et al.: Use of high-fat ... pp. 389-396

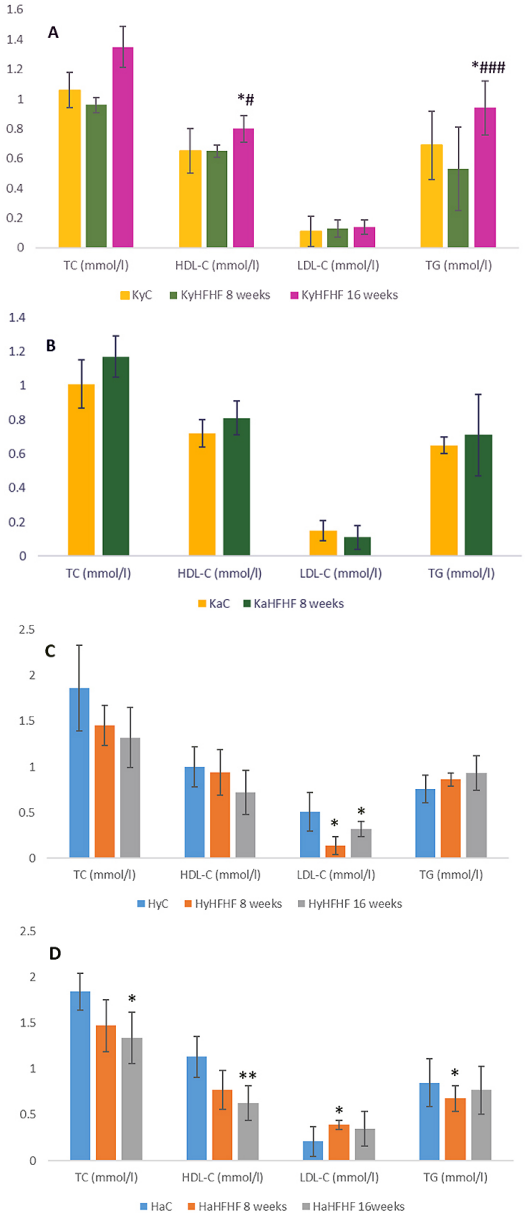

Fig. 1. Changes in the lipid status in young and adult Kyoto and Hannover rats following 8 and 16 weeks of treatment.

KyC - young Kyoto on standard chow, KyHFHF - young Kyoto on a HFHF diet, KaC - adult Kyoto on standard chow, KaHFHF - adult Kyoto on a HFHF diet, HyC - young Hannover on standard chow, HyHFHF - young Hannover on a HFHF diet, HaC - adult Hannover on standard chow, HaHFHF - adult Hannover on a HFHF diet. TC - total cholesterol, HDL-C - high density lipoprotein cholesterol, LDL-C - low density lipoprotein cholesterol, TG - triglycerides.

The data are presented as means ± SD (n = 6). *$P < 0.05$, **$P < 0.01$ when compared with the control group; # $P < 0.05$; ### $P < 0.05$ when compared with 8 weeks of the HFHF diet.