Trop J Nat Prod Res, February 2025; 9(2): 467 - 472 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Experimental Models Of Metabolic Syndrome In Male Wistar Rats

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ARTICLE INFO

Article history:
Received 10 November 2024
Revised 18 November 2024
Accepted 16 January 2025
Published online 01 March 2025

ABSTRACT

Metabolic syndrome's prevalence ranges from 20-25% of the adult population. It is reported to elevate the risk of cardiovascular diseases, stroke, and several cancers. Establishing an animal model of metabolic syndrome is crucial to evaluating treatment modalities. This research aimed to compare various methods of inducing metabolic syndrome. A study with pre and post-only with a control group design, combined with post-test only with a control group was conducted on 20 male Wistar rats. At the baseline, anthropometric and biochemical parameters were evaluated. Animals were then randomly allocated into four comparable groups, group I (control); group II(dexamethasone), group III: fructose and dexamethasone combination; and hight-fat-diet combined with fructose drinking water (group IV). Dexamethasone was given at 1 mg/kg/day intraperitoneally, fructose was provided ad libitum as 20% fructose drinking water (FDW), while the high-fat diet were given as egg yolk and repeatedly heated vegetable oil (1:1). All treatments were given for 10 days. After interventions, all parameters were re-evaluated and histologic analyses were performed. Group II and III developed systolic hypertension, and elevated glucose and cholesterol levels, but no changes in adipocyte diameter and obesity index. A high-fat diet combined with 20% FDW groups showed weight gain, elevated diastolic blood pressure and glucose levels, and also slight lipohypertrophy. We concluded that treatment with dexamethasone and fructose-dexamethasone combination mimicked the metabolic parameters but not the obesity traits. The high-fat-fructose group was shown to induce lipohypertrophy but not lipohyperplasia.

Keywords: metabolic syndrome, animal model, adipocyte, obesity, dexamethasone

Introduction

Metabolic syndrome (MS) is an accumulation of metabolic risk factors such as obesity, diabetes, dyslipidemia and hypertension. Diagnosis of MS is established when a person has a minimum of three of the five risk factors: obesity; mainly visceral obesity, hypertension, hyperglycaemia, and dyslipidemias.[1,2] The worldwide prevalence of MS tends to increase in recent times (about 20-25% of global adult population).[3] It means that one in four or five adult people have MS. In Asia-Pacific region, the prevalence of MS ranges from 11.9% to 49.0%.[4] In Indonesia, Herningtyas and Ng (2019) reported the MS prevalence from the data of the 4th Indonesian Family Life Survey which involved 27 ethnic groups in 20 provinces. This study reported that the prevalence of MS in Indonesia in 2019 was 21.66% based on the Joint Interim Statement requiring three risk factors: abdominal/visceral obesity, hypertension, decreased level of High-Density Lipoprotein cholesterol or receiving treatment of cholesterol, elevated triacylglycerol (TAG) level or receiving treatment for lowering TAG level, and hyperglycemia or receiving treatment to reduce blood glucose level.[5] The incidence of MS in most countries is about 1/5th of the adult population.

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Citation: Sulistyoningrum E, Hendrawati A, Aulia S.I.D. Experimental Models Of Metabolic Syndrome In Male Wistar Rats. Trop J Nat Prod Res. 2025; 9(2): 467 – 472 https://doi.org/10.26538/tjnpr/v9i2.8

These numbers are also expected to increase along with the obesity prevalence, high-calorie foods and beverage consumptions, and sedentary lifestyles.[2, 6,7] Patients with MS have a 2-3 times elevated risk of having cardiovascular disease and stroke and 5 times elevated risk of type 2 diabetes mellitus.[8] Apart from tending to experience these metabolic diseases, people with metabolic syndrome are also prone to non-alcoholic fatty liver disease, end-organ dysfunctions such as kidney and lung failure, dementia, polycystic ovary syndrome, and sleep apnea.[8–10] There are also several studies that show a correlation between MS and the incidence of morbidity and mortality of several cancers such as hepatic, pancreatic, cervical, and breast cancer.[10–12]

The multifactorial pathological mechanisms of MS have not been completely elaborated. The pathogenesis of MS requires a combination of genetic factors, epigenetic and environmental factors as well.[2] Genetic susceptibilities include insulin signal transduction pathway abnormality, adipocyte signaling mechanism, and defective cytokine secretion. Environmental factors such as several drugs, high-calorie, high-fat diets, reduced physical activity and sedentary lifestyles have exacerbated the pathogenesis of MS.[13]

The magnitude of problems of MS requires the need for research in the field of MS, including prevention and management of MS. Due to ethical issues in human research, studies with animal models are crucial. Among other animals, rodents are the most used as animal models for MS.[14,15] Metabolic syndrome can be investigated in animals with modified genes and non-genetically modified animals (diet and chemical-induced).[15,16] Each method shares its advantages and disadvantages.

The diet-induced animal models, despite their long-term administration, had been widely used to mimic MS associated with the high-fat diet and high-carbohydrate diet or their combination. Among those in high-carbohydrate diets, Mamikutty et al. (2014) delineated administration of 20% fructose in drinking water (FDW) is effective in inducing MS in Wistar rats.[17] A combination of a high-fat diet and high carbohydrate also induces MS phenotypes in experimental rats.[16,18] On

the other hand, in chemical-induced models using glucocorticoids there is adipocyte differentiation, which can lead to insulin resistance.[19] There is a dearth of research comparing these methods of inducing MS. The study endeavored to achieve a comparison of the effectiveness of various methods of inducing metabolic syndrome using metabolic parameters (Lee index, blood pressure, blood glucose, blood cholesterol, and adipocyte histology).

Material And Method

Animals

Twenty healthy male albino rats (*Rattus norvegicus* strain *Wistar*) aged 8-10 weeks (175 + 25 g) were puchased from Animal House of Integrated Research Laboratory, Islamic University of Indonesia. The number of animals was calculated according to the Resource Equation from Festing.[20] Prior treatments, animals were adapted in a cage in typical laboratory conditions (12 hours of dark and light cycle, 22 + 4°C temperature, and 50 + 5% relative humidity) in the Integrated Research Laboratory, Islamic University of Indonesia. Animals were nourished by standard chow for food and tap water during acclimatization and intervention, except for the special-treatment diet.

Animal groupings and treatments

This protocol followed research ethics for experimental animals based on the Declaration of Helsinki and ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. Ethical approval was acquired from Health Research Ethics Committee, Faculty of Medicine, Islamic University of Indonesia (Ethical approval: 11/Ka.Kom.Et/70/KE/I/2024). Anthropometric measurement including body weight and nasoanal length; blood pressure, and biochemical parameters at the baseline were recorded. After that subjects were randomly allocated into four equal groups: control group (I), received a standard diet and tap water; dexamethasone group (II), received a standard diet and tap water + injection of dexamethasone 1 mg/kg/day intraperitoneally;[19] fructose and dexamethasone combination (III), received a standard diet and 20 % FDW ad libitum + injection of dexamethasone 1 mg/kg/day intraperitoneally; and hight-fat-diet combined with fructose drinking water (IV) received an oral gavage of quail egg yolk and repeatedly heated vegetable oil combination (1:1) and 20% fructose-drinking water (FDW) ad libitum.[17] All treatments were given for 10 days. Fructose drinking water was prepared freshly everyday based on the previous studies by diluting 20 g of D-fructose powder (Merck Millipore, Indonesia) in 100 ml tap water and homogenized in a magnetic stirrer for 5 minutes.[17,21,22]

Experimental procedure

Data was taken before the random allocation and treatments and after 10 days of treatments. After overnight fasting, all animals were deeply sedated with ketamine 25 mg/kg intraperitoneally (Sandoz, Canada), after that, anthropometric data including body weight, body length, and blood pressure measurements were recorded. The body length was measured between the nasal to anal region using a calibrated measuring tape, while body mass was weighed using a digital electronic scale (Quattro; Indonesia). Lee indexes were calculated based on the formula: cube root of body weight (g) divided by the nasoanal length (mm). Both systolic and diastolic blood pressures were measured with Panlab non-invasive blood pressure meter LE 5002 (Harvard Apparatus; Canada). Animals were trained for blood pressure measurement for 2 days before the measurements. The training and the measurements were executed in the morning between 8-10 am. For each animal, five measurements of blood pressure were examined and the average was documented as a result. Blood from the tail vein of animals was taken with a sterile lancet to measure the level of glucose and total cholesterol. The cholesterol and glucose levels were determined using glucose and cholesterol meter (EasyTouch GCU; China). After anthropometric and biochemical parameters were measured, all animals were euthanized. After that, a longitudinal incision at the midline at the anterior aspect of the rats' body was performed. Adipose tissue deposition from mesentery, retroperitoneal, and perigonadal was photographed, and fat from perigonadal area as representative of visceral fat were taken for the histological process.[23]

Histological assay

Perigonadal fat tissue was promptly fixed in 10% NBF for 3 x 24 h and after that, the tissue was dehydrated with a serial alcohol mixture and paraffinized. The paraffin-embedded tissue were sliced off to obtain 5 mm thin sections and then processed for Hematoxylin-Eosin (HE) to evaluate morphometry of adipocyte tissue. Adipocyte cell diameter was determined in 10 fields using a photomicrograph from CX22 microscope (Olympus, Japan) provided with Optilab (Miconos, Indonesia). The number of adipocytes (characterized by large lipid droplets, thin cytoplasm, and peripheral-located nuclei) was counted at three measuring areas of 350 μm × 250 μm without counting cells in the borders.[17] The adipocyte size and number were analyzed using ImageRaster software as a slight modification of the previously described method.[24]

Statistical Analyses

Statistical analyses were executed with SPSS Statistics software. All data were analyzed for normality using the Shapiro-Wilk analyses. Normal data were expressed as mean ± standard deviation. Pair t-tests were used to compare baseline and final data, while one-way ANOVA and post hoc tests were used for multiple group comparisons. All parameters were significant if the p-value was < 0.05.

Result And Discussion

Metabolic characteristics including body weight, Lee index, blood pressure, fasting blood sugar levels, and cholesterol levels at the baseline and day 11 were shown in Table 1. In group I, the healthy control, there are no significant changes in the metabolic parameters except the body weight changes which are significantly higher from the baseline, all other metabolic parameters were not changes at day 11. In group II (received dexamethasone 1 mg/kg/day for 10 days injection), there were significant changes in some metabolic parameters such as elevated both systolic and diastolic blood pressure, as well as fasting blood glucose and cholesterol. On the contrary, bodyweight and Lee index of group II decreased significantly. In group III (treated with dexamethasone 1 mg/kg/day for 10 days injection and 20% FDW), systolic blood pressure, blood glucose, and cholesterol levels increased significantly. There's no difference in body weight and Lee index. In the fourth group that received combination of HFD and 20% FDW, there were significant changes in body weight, diastolic blood pressure, and fasting blood sugar levels.

We also compare the metabolic parameter changes among groups on day 11. As described in Figure 1, the mean body weight of group II and III FD groups was significantly lower than groups I and II. The mean of systolic blood pressure group III was significantly higher than group I, while neither group II nor IV was significantly different from the control group. There was no significant difference in the diastolic blood pressure among groups. The average blood glucose levels of the II, III, and IV groups were significantly higher than the control group (group I), while there was no difference in blood cholesterol levels among the groups. Since the body weight of the II and III groups reduced after 10 days, the Lee index of the group II and III groups also showed a reduction (Figure 3B). However, Lee's index of group IV showed no significant difference from the control group.

When observing visceral white adipose tissue distribution, many animals, mostly in group IV received HFD and 20% FDW exhibited fat accumulation in the mesentery, perigonadal (epididymal), and retroperitoneal as shown in Figure 2. Since there was an increased adiposity, we also examined the adipose tissue characteristic further based on the size and number of adipocytes. Figure 3A shows the characteristics of adipocytes among groups. Group IV had a higher diameter of adipocytes compared with normal the control group. However, dexamethasone-treated groups showed smaller adipocyte diameters (Groups II and III). When we examined the number of adipocyte cells, they were not significantly different from the control group. It may be assumed that the increase of adiposity in the group received HFD and 20% FDW was most likely caused by an increase in the size of the adipocyte (adipocyte hypertrophy) rather than hyperplasia. Representative photomicrographs of the epididymal adipocytes showing the diameter were presented in Figure 4. The

Table 1. Metabolic parameters from baseline to day 11

Parameters	Group I (n = 5)		Group II (n = 5)		Group III (n = 5)		Group IV (n = 5)	
	Baseline	Day 11	Baseline	Day 11	Baseline	Day 11	Baseline	Day 11
Body weight (grams)	185.02 ± 2.79	195.88 ± 1.90*	180.52 ± 8.59	166.54 ± 3.54*	174.56 ± 3.93	170.54 ± 4.16	180.44 ± 5.13	198.62 ± 6.95*
Lee index	0.30 ± 0.01	0.30 ± 0.01	0.30 ± 0.01	0.29 ± 0.01*	0.29 ± 0.01	0.29 ± 0.01	0.31 ± 0.01	0.32 ± 0.01
Systolic blood pressure (mmHg)	106.00 ± 4.52	110.40 ± 4.87	110.20 ± 7.46	123.80 ± 14.95*	109.60 ± 6.91	128.60 ± 7.30*	111.00 ± 1.58	112.00 ± 5.57
Diastolic blood pressure (mmHg)	75.20 ± 1.92	76.00 ± 3.53	75.40 ± 5.13	79.60 ± 3.71*	76.00 ± 3.24	77.20 ± 3.49	72.00 ± 4.18	76.20 ± 3.90*
Fasting blood glucose (mg/dl)	98.60 ± 6.27	99.20 ± 5.35	104.40 ± 4.04	127.40 ± 7.23*	102.00 ± 5.24	126.60 ± 4.82*	96.00 ± 7.11	114.60 ± 3.65*
Fasting blood cholesterol(mg/dl)	53.00 ± 1.58	54.00 ± 2.34	52.80 ± 1.48	58.60 ± 3.04*	52.80 ± 2.49	58.40 ± 2.30*	52.60 ± 1.14	54.20 ± 1.64

I: control group; received a standard diet and tap water); II: received a standard diet and tap water + injection of dexamethasone 1 mg/kg/day intraperitoneally); II: received a standard diet and 20 % FDW ad libitum + injection of dexamethasone 1 mg/kg/day intraperitoneally; IV: received an oral gavage of quail egg yolk and repeatedly heated vegetable oil combination (1:1) and as 20% fructose-drinking water (FDW). Values are presented as the mean ± standard deviation, *p < 0.05 compared baseline data with day 11 data using paired sample t-test.

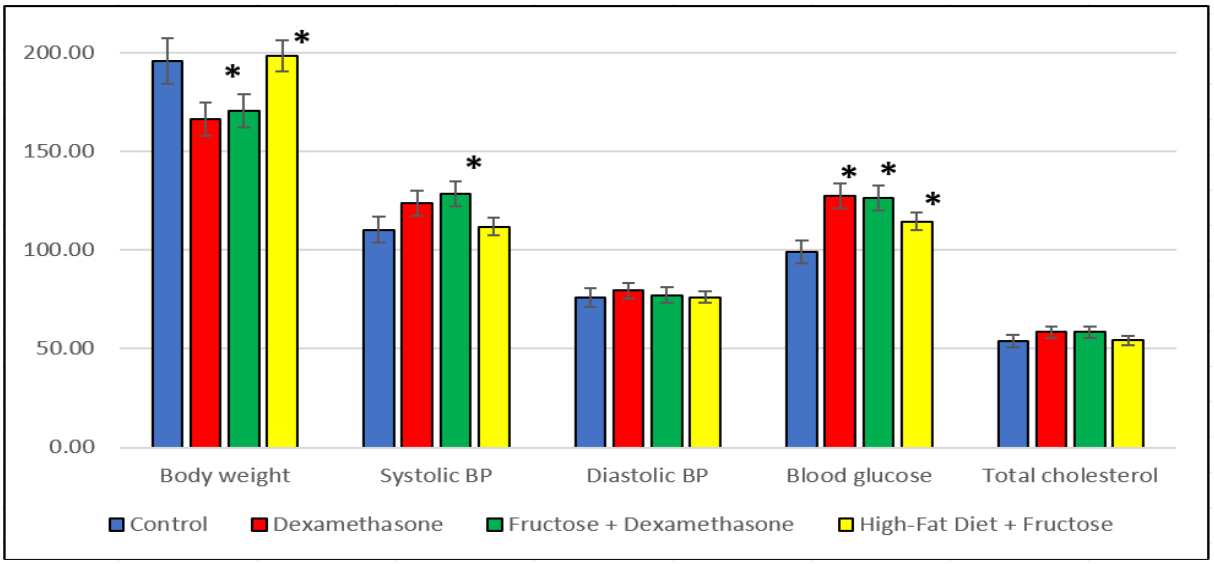

Figure 1. Comparison of metabolic parameters on day 11. n = 5, values as mean ± standard deviation, *p < 0.05 compared with control group, from ANOVA and posthoc analyses. BP: blood pressure

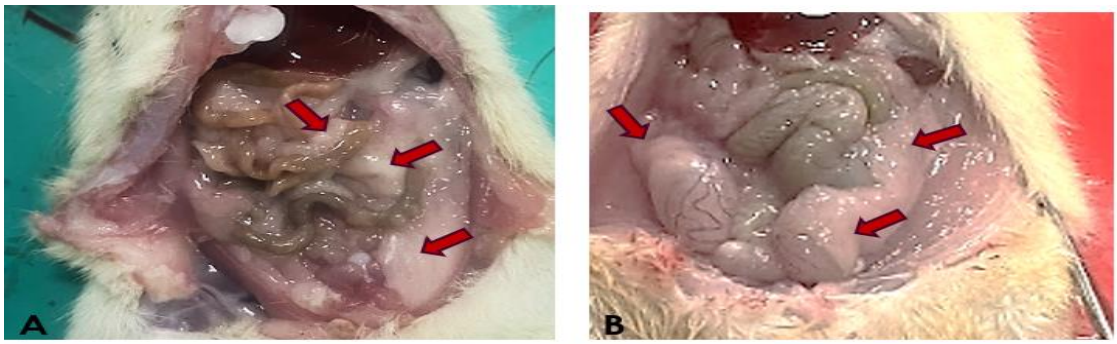

Figure 2. Representative picture of visceral fat accumulation. Adipose tissues (red arrows) accumulate in the mesentery, around the gonads (epididymal or perigonadal) and retroperitoneal; photograph was taken from high fat diet and fructose group

epididymal adipocytes exhibit the mono vacuolar fat cell, characterized by a single large vacuole of fat (appears in a clear large space in the hematoxylin-eosin staining), and distinguished nuclear appearance, single nucleus, flattened in shape and pushed at the peripheral region of the cell.

We have established the validity of some methods utilized to generate MS conditions. Animal models mimicking various aspects of the disease will give an essential contribution to an elaborate comprehensive understanding of the pathogenesis of MS and the possibilities of therapeutic modalities in MS including surgical or

medicinal modalities. From this point of view, we will discuss the effectiveness of the induction methods on each metabolic parameter.
Repeated injections of dexamethasone failed to mimic the weight gain characteristics of metabolic syndrome, as the group that received a 10-day injection of dexamethasone showed a reduced body weight. This result was consistent with Oche et al., which outlined body weight reductions of rats received a one-week injection of dexamethasone 1 mg/kgBB/day in rats.[25] Dexamethasone's impact on body weight depends on the dose. Low doses of dexamethasone can reduce body weight because dexamethasone can modulate gene expression that

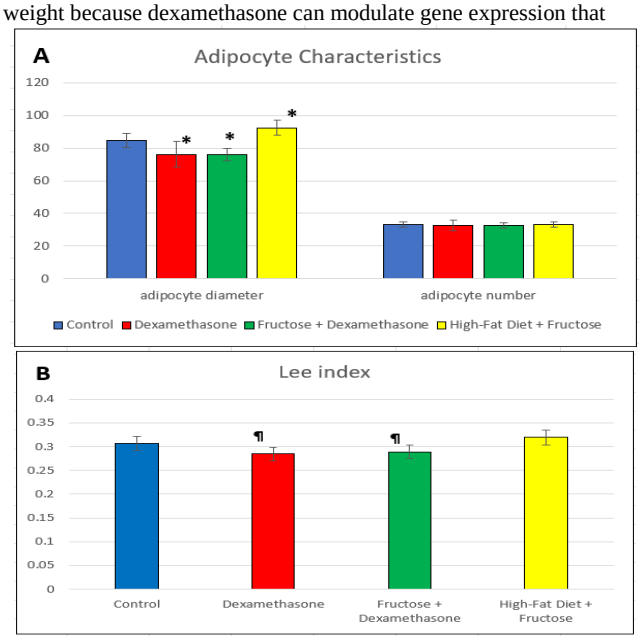

Figure 3. Comparison of adipocye diameter and number and Lee index. n = 5, values as mean ± standard deviation, *p < 0.05 compared with control group, from ANOVA and posthoc analyses, ¶ p < 0.05 compared with high-fat and fructose group, from ANOVA and posthoc analyses

control lipolysis and lipid transporters in brown adipose tissue while at high doses, dexamethasone induces obesity.[26] The common dose for inducing metabolic syndrome in rodents varied from <1 mg/kg to 4 mg/kg in multiple daily doses, ranging from < 7 days to 14 days.[27] Most studies report the weight loss condition related to a single dexamethasone administration is independent of the duration of the exposure, i.e. longer duration of dexamethasone injection up to 42 days

did not change the weight loss condition.[19] In this current research, however, the combination of dexamethasone injection and 20% FDW also failed to demonstrate the obesity parameter of metabolic syndrome. This result is contradictive with previous studies reported a combination of dexamethasone and dietary animal models can lead to the progression of insulin resistance and metabolic syndrome.[19,28,29]
Administration of fructose-dexamethasone combination or high-fat diet and fructose combination have no significant effect on the Lee index. However, dexamethasone injections caused a significant decrease in the Lee index. This result is inconsistent with previous study which stated that the administration of dexamethasone did not affect the Lee index of obese subjects.[30] Low-dose dexamethasone can have a weight-loss effect on rats so that it can potentially reduce the Lee index value because body length does not change.[31] Unlike the impact of dexamethasone on body weight, in this current study, a high-fat diet combined with a high-fructose diet significantly increased body weight (Table 1). This result is similar to Wang et al. (2020), which stated that a high-fat diet, especially for long durations, increases body mass index and induces obesity.[32] A high fructose diet itself can increase body weight by inducing adipogenesis and lipogenesis, thereby accumulating fat in adipose tissue.[33]
We also established the administration of dexamethasone alone and a combination of fructose and dexamethasone increased systolic blood pressure. These results are consistent with previous study outlined dexamethasone effect on both systolic and diastolic blood pressure. The mechanism of increasing blood pressure is by reducing nitric oxide production which has a role as a vasodilator and disrupts endothelial function through glucocorticoid receptors in blood vessels.[34] According to previous studies, a high-fructose diet influences increased blood pressure. Fructose dietary restriction has been reported to reduce diastolic blood pressure.[35] Another study stated that high-carbohydrate and high-fat diets increase both systolic and diastolic blood pressure because these diets can damage blood vessels and increase endothelin which induces vasoconstriction.[36]
In the current study, the administration of dexamethasone, fructose, and dexamethasone, as well as a high-fat diet and fructose increased blood sugar levels. Previous study reported that dexamethasone injection (1mg/kg/day) intraperitoneally in 7-10 days may induce hyperglycemia, hyperinsulinism, and reduced peripheral insulin sensitivity in male Wistar rats.[37] Based on previous studies, administration of low-dose and short-duration dexamethasone has been able to increase blood glucose levels. Dexamethasone increases blood glucose levels by inhibiting the action of insulin.[38] In previous studies, a high-fructose diet can increase blood sugar levels and conversely, a low-fructose diet has been shown to decrease blood sugar levels.[39] A high-fructose diet increases blood sugar levels because it can decrease insulin sensitivity and cause inflammation in the pancreas so insulin production decreases.[40] Based on previous studies, a high-fat diet, high-sugar diet, or high-protein diet has the potential to increase blood sugar levels, especially postprandial blood sugar levels attributable to the high-fat diet increases insulin resistance.[41] Consistent with this study, Oche et al. (2023) also reported hyperglycaemia and insulin resistance in dexamethasone-treated animals as well as abnormalities in pancreatic cells including multifocal nuclear hypochromasia, cytoplasm degeneration, necrosis of pancreatic cells.[25]
Regarding adiposity, we reported that the administration of dexamethasone significantly reduced the diameter of adipose cells. This result is concomitant with the study of Koorneef et al. (2022) which delineated that the size of white and brown fat tissue cells decreased after administration of dexamethasone.[26] The decrease in the size of adipose cells occurs because of the lipid content reduction in adipose cells and increasing levels of lipolysis. In this study, there was a decrease in the diameter of adipose cells in the group that was given a combination of fructose and dexamethasone. This result was probably due to the more dominant effect of dexamethasone. In this research, we also established the role of a high-fat diet and fructose in hypertrophy of adipocytes, indicated by the increased diameter of adipocytes. The higher diameter of adipocytes indicates the hypertrophy of adipocytes which also were reported in the induction of MS using fructose 20% and 25% drinking water in 8 weeks.[17] A high fructose diet induces lipid accumulation in adipose cells, thereby increasing the size and weight of adipose cells.[33] Based on previous studies, high fructose diets increase the size of adipose cells because they increase adipogenesis.[42] The high-fat diet itself can increase the diameter and number of adipose cells because lipid accumulation occurs in the adipose cells and increases adipose cell differentiation. The increase in size and number causes cells to lose function.[43] However, in this current research, we did not report the hyperplasia of adipocytes since the number of the adipocytes did not significantly differ among groups. According to previous studies, dexamethasone increases the proliferation of preadipocyte cells and increases their differentiation into brown adipose cells but inhibits the proliferation of white preadipocyte cells.[44] The impact of a high-fructose diet on the volume of adipocytes depends on their location. The mean adipocyte volume decreases in subcutaneous white adipose tissue but increases in visceral adipose tissue such as in intraabdominal adipose tissue and in the adipose tissue around the uterus (perigonadal).[45,46]
The limitations of this study are the lack of presentation of all metabolic risk factors in metabolic syndrome in a particular model and histopathological analyses were performed only for the end of the study. However, this research highlighted the possibility of elevating systolic blood pressure, increasing body weight, elevated blood glucose, and also lipohypertrophy in some of the studied models.

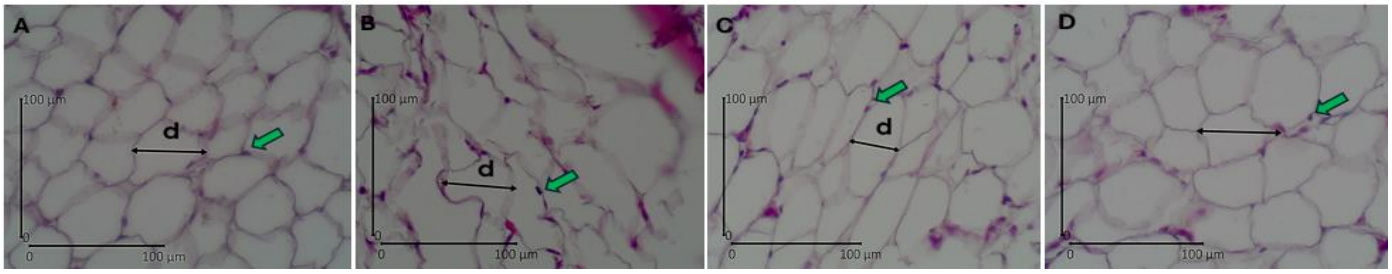

Figure 4. Representative picture of visceral adipocyte histology (a: control; b: dexamethasone; c: dexamethasone + fructose 20%, d: High-fat-diet + fructose 20%); green arrows indicated nuclei of adipocytes, d; diameter. HE-staining, 400x magnifications, bar indicated 100 µm.

Conclusion and Suggestion

Dexamethasone and a combination of fructose and dexamethasone-induced may be useful in inducing metabolic parameters such as increased glucose level, cholesterol levels, and systolic blood pressure but not obesity traits. Future research involving a longer duration of treatment may be useful to investigate the effectiveness of these methods for inducing all traits of metabolic syndrome and a more complete analysis of adipogenesis, including adipocyte hypertrophy.

Conflict Of Interest

The authors declare no conflict of interest

Acknowledgement

The authors would like to acknowledge the Faculty of Medicine, Islamic University of Indonesia for funding and providing facilities for this research.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Authors Contribution

ES: concept and designed the experiments, wrote and revised the manuscript; AH wrote and revised the manuscript, DSIA helped conduct the experiments and collected experimental data.

List Of Abbreviations

MS: metabolic syndrome; TAG: triacylglycerol; FDW: fructose in drinking water; HE: Hematoxylin-Eosin.

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