Annals of Medicine and Surgery 65 (2021) 102266

Contents lists available at ScienceDirect

Annals of Medicine and Surgery

journal homepage: www.elsevier.com/locate/amsu

High-fat diet increases the level of circulating Monocyte Chemoattractant Protein-1 in Wistar rats, independent of obesity

Syarif [a,*], Haerani Rasyid [b], Makbul Aman [b], Gatot S. Lawrence [c]

[a] Division of Urology, Department of Surgery, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[b] Department of Internal Medicine, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia
[c] Department of Pathology Anatomy, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia

ARTICLE INFO

Keywords:
High-fat diet
Monocyte chemoattractant Protein-1
Diet-induced obesity
Wistar rat
Experimental animal models

ABSTRACT

Introduction: Low-grade chronic inflammation has emerged as a key pathogenic link between high-fat diet (HFD)–induced obesity and the increased risk of chronic diseases. Evidence has shown that HFDs may induce inflammation in the central nervous system and peripheral tissues. Monocyte Chemoattractant Protein-1 (MCP-1) is a product of various cells that is known to be an inflammatory marker. This study investigated whether a HFD could induce obesity and increase the level of MCP-1 in Wistar rats.

Methods: The Wistar rats were randomized into two groups: normal diet (ND) and HFD (n = 12 per group). Both groups were fed for 8 and 16 weeks, thus dividing the rats into 4 arms: ND8, ND16, HFD8, and HFD16 (n = 6 per sub-group). Obesity in rats was measured using the Lee index. Blood samples were taken to measure the level of MCP-1.

Results: Our results showed that obesity did not occur in the group with a normal diet (ND8 and ND16). However, in the HFD group (HFD8 and HFD16), 4 of the 6 rats became obese. However, MCP-1 was significantly higher among non-obese rats in the HFD group compared with the ND group ($p < 0.001$).

Conclusion: HFDs have been shown to increase the risk of obesity. In addition, increases in circulating MCP-1 were significantly different between Wistar rats given a HFD compared with the ND group.

1. Introduction

The worldwide prevalence of overweight and obesity has doubled since the 1980s, and nearly a third of the world's population is currently classified as overweight or obese [1–3]. Obesity adversely affects most of the physiological functions of the body and has become a major public health problem. Obesity multiplies the risk of the development of chronic diseases, such as diabetes mellitus, cardiovascular disease, several types of cancers, musculoskeletal disorders, and poor mental health—all of which have negative effects on quality of life, work productivity, and healthcare costs [1,4,5].

Genetic predispositions, physical inactivity, and consumption of a high-fat diet (HFD) can all lead to the development of obesity [6,7]. However, given the global acceptance and availability of energy-dense foods, chronic ingestion of a HFD is arguably the main contributor. Consequently, there has been a major emphasis on understanding the link between HFD-induced obesity and the risk of chronic diseases. In this context, low-grade chronic inflammation has emerged as a key pathogenic link [8,9].

Following the ingestion of a HFD, inflammation develops in the central nervous system, hypothalamus, and peripheral tissues, i.e., liver, adipose tissue, skeletal muscle and intestines; however, this inflammation is caused by the diet and not the obesity in itself [10–13].

Monocyte Chemoattractant Protein-1 (MCP-1) is produced by various types of cells, including epithelial, smooth muscle, endothelial, fibroblasts, mesangial, monocytic, astrocytic, and microglial cells [14–16]. However, monocytes and macrophages are largely recognized as the major sources of MCP-1. MCP-1, known to be an inflammatory marker, is responsible for the regulation of the migration and infiltration of monocytes, memory-T lymphocytes, and natural killer cells to sites of infection, inflammation, and injury [17,18].

In this study, we examined whether regular consumption of a HFD

* Corresponding author. Division of Urology, Department of Surgery, Faculty of Medicine, Hasanuddin University Jalan Perintis Kemerdekaan KM 11, Makassar, 90245, Indonesia.
E-mail addresses: syarifbakri@hotmail.com (Syarif), haeraniabdurasyid@yahoo.com (H. Rasyid), makbul_aman@yahoo.com (M. Aman), Gatot.Law@gmail.com (G.S. Lawrence).

https://doi.org/10.1016/j.amsu.2021.102266
Received 10 February 2021; Received in revised form 24 March 2021; Accepted 24 March 2021
Available online 1 April 2021
2049-0801/© 2021 The Authors. Published by Elsevier Ltd on behalf of IJS Publishing Group Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

could induce obesity and increase the level of circulating MCP-1 in Wistar rats. Furthermore, we measured the effect of HFDs on MCP-1 levels by performing a sub-group analysis among the non-obese rats.

2. Materials and methods

2.1. Animal husbandry and diets

All procedures for animal experiments have been approved by the ethics commission in our institution number: 711/UN4.6.4.5.31/PP36/2019. All Wistar rats were treated and cared for in accordance with the ARRIVE guidelines for reporting animal research [19].

We included 24 male Wistar rats aged 10–12 weeks, with body weight between 170 and 220 g. The rats were acclimatized at room temperature (25–30 °C) for two weeks and had access to food and water ad libitum under a 12-h light/dark cycle [20,21]. After the acclimation, the Wistar rats were randomly divided into two groups: normal diet (ND) and high-fat diet (HFD) (n = 12 per group). Both groups were fed for 8 and 16 weeks, so the rats were classified into 4 arms: ND8, ND16, HFD8, and HFD16 (n = 6 per sub-group).

Preparation of the animal diet was carried out by the Division of Animal Food and Nutrition, Faculty of Animal Husbandry, Hasanuddin University, Makassar, Indonesia. ND composition consisted of 3.1% fat, 16.1% protein, 3.9% fiber, and 5.1% ash/mineral, while the HFD contained 21.4% fat, 17.5% protein, 50% carbohydrate, 3.5% fiber, and 4.1% ash/mineral.

The body weight and naso-anal length were measured to obtain the obesity index value. The obesity was measured using the Lee index [22–24].

$$\text{Lee} \times \text{index} * \cdot = \cdot \frac{\sqrt{\text{Body} \times \text{Weight} \times (\text{gram}) \times \cdot 10}}{\text{Naso} - \text{anal} \times \text{lengh} \times (\text{mm})}$$

*Obese rat defined as rat with Lee index >0.3.

2.2. Sample collection

Rats were restrained to control head and body movements. After intraperitoneal anesthesia by using ketamine anesthetic agents (100 mg/kg) and xylazine (10 mg/kg), 2 ml of blood were withdrawn in an intracardiac manner using a 19–21 needle. Blood was inserted into the sample tube. Blood was centrifuged to obtain plasma and stored at −80 °C before the examination. Blood samples were collected at week 8 and 16 to measure the level of circulating MCP-1.

2.3. Sample examination

MCP-1 was measured with the Elisa method using MCP-1 reagents (Rat CCL2/MCP-1 Elisa Kit LS-F37066, LifeSpan BioSciences, Inc.) [20,25]. The circulation MCP-1 level was read by Elisa Reader 270 (Biomeriaux, France), with a wavelength of 450 nm for 30 min in units of ng/ml.

2.4. Statistical analysis

All data were analyzed using SPSS ver.24 for Windows (IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.). Differences on continuous data were obtained using an independent *t*-test with a significance level of <0.05.

3. Results

Obesity in rats was measured using the Lee index (obese if Lee index >0.30). In the present study, none of the rats in the ND group became obese (n = 12), whereas in both HFD arms (HFD8 and HFD16), 4 of the 6 rats became obese. The length of HFD administration had no impact on the obesity incidence in the rats (Table 1).

Normal diet (ND) and HFD administration increased the level of circulating MCP-1. However, the circulation MCP-1 in the HFD arms (HFD8 and HFD16) was significantly higher compared to the ND arms (ND8 and ND16), with $p < 0.001$. Longer HFD feeding significantly increased the MCP-1 level ($p < 0.001$) (Table 2).

In the HFD group, there was no significant difference of MCP-1 level between obese and non-obese rats ($p = 0.401$) (Table 3). However, MCP-1 was significantly higher among the non-obese rats in the HFD group (206.22 ± 16.53) compared with the ND group (166.04 ± 15.08) group ($p < 0.001$) (Table 4).

4. Discussion

In this study, we found that a regular high-fat diet (HFD) for a duration of 8 and 16 weeks may induce obesity and increase the level of circulating MCP-1 in Wistar rats. The longer the HFD feeding, the higher the MCP-1 level. Our findings also show that the MCP-1 level was significantly higher in the HFD group, compared with the ND group, among non-obese rats. It was concluded that a HFD increases circulating MCP-1 levels. Apart from obesity, HFD also induces various diseases such as insulin resistance and type 2 diabetes, cardiovascular disease, gastrointestinal disease, osteoporosis, chronic kidney disease, central nervous system disease, and various types of cancer [9].

With a HFD, inflammation arises in the central nervous system (CNS), hypothalamus, and peripheral tissues, such as liver, adipose tissue, skeletal muscle, and gastrointestinal tract. This systemic inflammation occurs in two stages. Concerning the development of chronic systemic inflammation, alteration in gut microbiota is triggered by the HFD-activated Toll-like Receptor (TLR) signaling pathway, leading to increased intestinal permeability to endotoxins such as lipopolysaccharides (LPS), thus promoting the translocation of LPS into the systemic circulation [26–28]. In addition, increased amounts of free fatty acids (FFAs) present in HFDs may directly act on intestinal cells. Therefore, elevated release of LPS and/or increased FFAs levels lead to an elevated production of pro-inflammatory cytokines [i.e., interleukin (IL)-1β, IL-6, and tumor necrosis factor (TNF)-α] in the gut [9,29,30]. Increased delivery of intestinal LPS, proinflammatory cytokines, and FFAs into the systemic circulation and portal circulation lead to a systemic low-grade inflammation. Elevated plasma FFAs and LPS can upregulate the expression of TLRs in circulating macrophages, enabling macrophages to be activated (M1 phenotype) that, in turn, produce pro-inflammatory cytokines. Before the onset of obesity, these factors in the circulating system triggered inflammatory pathways in many organs [9,10,12,31].

In one study, C57BL/6J mice were assigned to a standard or HFD. After 1 week, mice fed a HFD exhibited an increase in body weight, renal hypertrophy, and a marker of inflammation (urine hydrogen peroxidase/H2O2 and urine MCP-1) and a decrease in circulating adiponectin levels and renal AMP-activating protein kinase (AMPK) activity. After 12 weeks, kidneys of the mice fed a HFD demonstrated a marked increase in markers of fibrosis and inflammation, and AMPK activity remained significantly suppressed. To determine whether inhibition of AMPK activity explained these renal effects, an AMPK activator was administered along with a HFD for 1 week. Although AMPK activation did not abrogate the weight gain, it reduced the renal hypertrophy, urine H2O2, and urine and renal MCP-1, thus suggesting an increase in inflammation

Table 1
Impact of HFD feeding duration on obesity.

Diet	8 weeks		16 weeks	
	Obesity	Non-obese	Obesity	Non-obese
ND	0	6	0	6
HFD	4	2	4	2
Total	4	8	4	8

ND: Normal Diet; HFD: High-Fat Diet.

Table 2
The effect of HFD on circulating MCP-1, based on duration of feeding.

Period	Variable	Group	n	Mean (ng/ml)	SD	p[a]
Week 8	MCP-1	ND	6	156.65	13.19	<0.001
		HFD	6	188.71	7.63	
Week 16	MCP-1	ND	6	175.44	10.69	<0.001
		HFD	6	212.67	10.57	

[a] Independent-t test; MCP-1 = Monocyte Chemoattractant Protein-1; ND: Normal Diet; HFD: High-Fat Diet.

Table 3
The effect of obesity on MCP-1 level in HFD group (n = 12).

Variable	Lee index	N	Mean (ng/ml)	SD	p[a]
MCP-1	Obese	8	197.92	14.98	0.401
	Non-obese	4	206.22	16.53	

[a] Independent-t test; MCP-1 = Monocyte Chemoattractant Protein-1.

Table 4
The effect of HFD on MCP-1 level amongst non-obese rats (n = 16).

Variable	Group	n	Mean (ng/ml)	SD	p[a]
MCP-1	ND	12	166.04	15.08	<0.001
	HFD	4	206.22	16.53	

[a] Independent-t test; MCP-1 = Monocyte Chemoattractant Protein-1.

markers caused by diet via AMPK inhibition and not obesity in itself [32].

In a 6-month randomized controlled-feeding trial, 217 health young adults (aged 18–35 years; body mass index <28 kg/m2; 52% women) who completed the whole trial were included. The three isocaloric diets given to the participants consisted of a lower-fat diet (fat 20% energy), a moderate-fat diet (fat 30% energy), and a higher-fat diet (fat 40% energy). After the 6-month controlled-feeding intervention, all groups lost weight and their waist circumference reduced. However, the lower-fat diet group experienced a significantly greater loss than the higher-fat groups. Plasma concentration of hs-CRP was significantly increased during the higher-fat diet intervention, compared with the moderate-fat and lower-fat diet groups. Likewise, compared with the lower-fat diet, plasma concentration of the inflammatory mediators Thromboxane B2 was increased in the higher-fat diet [12].

This study has limitations, such as not examining inflammatory factors and the degree of inflammation in the prostate tissue. In addition, the length of the study was only 16 weeks, so it is not sufficient to assess the effect of obesity on the incidence of prostate hyperplasia.

5. Conclusion

HFD has been shown to increase the risk of obesity. In addition, increases in circulating MCP-1 were significantly different between Wistar rats given a HFD compared with the ND group.

Provenance and peer review

Not commissioned, externally peer-reviewed.

Annals of medicine and surgery

The following information is required for submission. Please note that failure to respond to these questions/statements will mean your submission will be returned. If you have nothing to declare in any of these categories then this should be stated.

Ethical approval

All procedure for Animal experiment has been approved by Ethics Commission Faculty of Medicine, Hasanuddin University Number: 711/UN4.6.4.5.31/PP36/2019.

Consent

This manuscript does not involve human participants, human data, or human tissue.

Author contribution

Syarif, Haerani Rasyid, Makbul Aman, and Gatot S. Lawrence wrote the manuscript and participated in the study design. Syarif, Haerani Rasyid, Makbul Aman, and Gatot S. Lawrence drafted and revised the manuscript. Syarif took care, feed, and acquired sample of Wistar rat. Syarif and Gatot S. Lawrence performed bioinformatics analyses and revised the manuscript. All authors read and approved the final manuscript.

Registration of research studies

None.

Guarantor

Syarif.

Declaration of competing interest

The authors declare that they have no conflict of interests.

Acknowledgment

We acknowledge Cheria Cahyaningtyas, M.D, and Muhammad Faruk, M.D, for their help in providing us with the linguistic assistance for this experimental study.

References

[1] Y.C. Chooi, C. Ding, F. Magkos, The epidemiology of obesity, Metabolism 92 (2019) 6–10, https://doi.org/10.1016/j.metabol.2018.09.005.
[2] A. Hruby, F.B. Hu, The epidemiology of obesity: a big picture, Pharmacoeconomics 33 (2015) 673–689, https://doi.org/10.1007/s40273-014-0243-x.
[3] J.C. Seidell, J. Halberstadt, The global burden of obesity and the challenges of prevention, Ann. Nutr. Metab. 66 (2015) 7–12, https://doi.org/10.1159/000375143.
[4] Y. Inoue, B. Qin, J. Poti, R. Sokol, P. Gordon-Larsen, Epidemiology of obesity in adults: latest trends, Curr. Obes. Rep. 7 (2018) 276–288, https://doi.org/10.1007/s13679-018-0317-8.
[5] X. Pi-Sunyer, The medical risks of obesity, Postgrad. Med. 121 (2009) 21–33, https://doi.org/10.3810/pgm.2009.11.2074.
[6] L. Qi, Y.A. Cho, Gene-environment interaction and obesity, Nutr. Rev. 66 (2008) 684–694, https://doi.org/10.1111/j.1753-4887.2008.00128.x.
[7] W.W. Cheung, P. Mao, Recent advances in obesity: genetics and beyond, ISRN Endocrinol (2012) 1–11, https://doi.org/10.5402/2012/536905.
[8] R.T. Enos, J.M. Davis, K.T. Velázquez, J.L. McClellan, S.D. Day, K.A. Carnevale, E. A. Murphy, Influence of dietary saturated fat content on adiposity, macrophage behavior, inflammation, and metabolism: composition matters, J. Lipid Res. 54 (2013) 152–163, https://doi.org/10.1194/jlr.M030700.
[9] Y. Duan, L. Zeng, C. Zheng, B. Song, F. Li, X. Kong, K. Xu, Inflammatory links between high fat diets and diseases, Front. Immunol. 9 (2018) 2649, https://doi.org/10.3389/fimmu.2018.02649.
[10] P.S. Dalvi, J.A. Chalmers, V. Luo, D.-Y. Han, L. Wellhauser, Y. Liu, D.Q. Tran, J. Castel, S. Luquet, M.B. Wheeler, D.D. Belsham, High fat induces acute and chronic inflammation in the hypothalamus: effect of high-fat diet, palmitate and TNF-α on appetite-regulating NPY neurons, Int. J. Obes. 41 (2017) 149–158, https://doi.org/10.1038/ijo.2016.183.
[11] O. Guillemot-Legris, J. Masquelier, A. Everard, P.D. Cani, M. Alhouayek, G. G. Muccioli, High-fat diet feeding differentially affects the development of inflammation in the central nervous system, J. Neuroinflammation 13 (2016) 206, https://doi.org/10.1186/s12974-016-0666-8.

[12] Y. Wan, F. Wang, J. Yuan, J. Li, D. Jiang, J. Zhang, H. Li, R. Wang, J. Tang, T. Huang, J. Zheng, A.J. Sinclair, J. Mann, D. Li, Effects of dietary fat on gut microbiota and faecal metabolites, and their relationship with cardiometabolic risk factors: a 6-month randomised controlled-feeding trial, Gut 68 (2019) 1417–1429, https://doi.org/10.1136/gutjnl-2018-317609.
[13] C.-X. Yi, M.H. Tschöp, S.C. Woods, S.M. Hofmann, High-fat-diet exposure induces IgG accumulation in hypothalamic microglia, Dis. Model. Mech. 5 (2012) 686–690, https://doi.org/10.1242/dmm.009464.
[14] J.A. Siddiqui, N.C. Partridge, CCL2/Monocyte chemoattractant protein 1 and parathyroid hormone action on bone, Front. Endocrinol. 8 (2017), https://doi.org/10.3389/fendo.2017.00049.
[15] S.L. Deshmane, S. Kremlev, S. Amini, B.E. Sawaya, Monocyte chemoattractant protein-1 (MCP-1): an overview, J. Interferon Cytokine Res. 29 (2009) 313–326, https://doi.org/10.1089/jir.2008.0027.
[16] B.S. Mulholland, M.R. Forwood, N.A. Morrison, Monocyte chemoattractant protein-1 (MCP-1/CCL2) drives activation of bone remodelling and skeletal metastasis, Curr. Osteoporos. Rep. 17 (2019) 538–547, https://doi.org/10.1007/s11914-019-00545-7.
[17] E.A. Murphy, Novel Adipocytokines: Monocyte Chemotactic Protein-1, Plasminogen Activator Inhibitor-1, Chemerin, 2017, pp. 161–186, https://doi.org/10.1007/978-3-319-41677-9_8.
[18] H. Kanda, S. Tateya, Y. Tamori, K. Kotani, K. Hiasa, R. Kitazawa, S. Kitazawa, H. Miyachi, S. Maeda, K. Egashira, M. Kasuga, MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity, J. Clin. Invest. 116 (2006) 1494–1505, https://doi.org/10.1172/JCI26498.
[19] C. Kilkenny, W.J. Browne, I.C. Cuthill, M. Emerson, D.G. Altman, Improving bioscience research reporting: the ARRIVE guidelines for reporting animal research, PLoS Biol. 8 (2010), e1000412, https://doi.org/10.1371/journal.pbio.1000412.
[20] S.R. Laidding, F. Josh, Francisca, M. Faruk, A.S. Palissei, B. Satria, Warsinggih, A. Bukhari, M.N. Massi, A.A. Islam, Combination of platelet-rich plasma and stromal vascular fraction on the level of transforming growth factor-β in rat subjects experiencing deep dermal burn injury, Ann. Med. Surg. 60 (2020) 737–742, https://doi.org/10.1016/j.amsu.2020.11.088.
[21] R.A. Nasution, A.A. Islam, M. Hatta, Prihantono, A. Turchan, M. Faruk Nasrullah, Role of CAPE in reducing oxidative stress in animal models with traumatic brain injury, Ann. Med. Surg. 57 (2020) 118–122, https://doi.org/10.1016/j.amsu.2020.07.036.
[22] D.C. Damasceno, Y.K. Sinzato, A. Bueno, B. Dallaqua, P.H. Lima, I.M.P. Calderon, M.V.C. Rudge, K.E. Campos, Metabolic profile and genotoxicity in obese rats exposed to cigarette smoke, Obesity 21 (2013) 1596–1601, https://doi.org/10.1002/oby.20152.
[23] A.B. Malafaia, P.A.N. Nassif, C.A.P.M. Ribas, B.L. Ariede, K.N. Sue, M.A. Cruz, Indução de obesidade com sacarose em ratos, ABCD. Arq. Bras. Cir. Dig. (São Paulo). 26 (2013) 17–21, https://doi.org/10.1590/S0102-67202013000600005.
[24] D.N. Stephens, Does the Lee obesity index measure general obesity? Physiol. Behav. 25 (1980) 313–315, https://doi.org/10.1016/0031-9384(80)90222-X.
[25] P. Prihantono, S. Ardi Syamsu, N. Smaradhania, M. Ahmad, N.A. Siagian, K. Lubis, A.S. Umrah, Application of Scaevola taccada (gaertn.) roxb. Reduce pro-inflammatory cytokines interleukin-1β in sprague dawley mice suffering from mastitis, open access maced, J. Med. Sci. 8 (2020) 423–427, https://doi.org/10.3889/oamjms.2020.4363.
[26] R.M. Chakaroun, L. Massier, P. Kovacs, Gut microbiome, intestinal permeability, and tissue bacteria in metabolic disease: perpetrators or bystanders? Nutrients 12 (2020) 1082, https://doi.org/10.3390/nu12041082.
[27] P.D. Cani, M. Osto, L. Geurts, A. Everard, Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity, Gut Microb. 3 (2012) 279–288, https://doi.org/10.4161/gmic.19625.
[28] K. Khoshbin, M. Camilleri, Effects of dietary components on intestinal permeability in health and disease, Am. J. Physiol. Liver Physiol. 319 (2020) G589–G608, https://doi.org/10.1152/ajpgi.00245.2020.
[29] I. Kojta, M. Chacińska, A. Błachnio-Zabielska, Obesity, bioactive lipids, and adipose tissue inflammation in insulin resistance, Nutrients 12 (2020) 1305, https://doi.org/10.3390/nu12051305.
[30] G. Boden, Obesity and free fatty acids, Endocrinol Metab. Clin. N. Am. 37 (2008) 635–646, https://doi.org/10.1016/j.ecl.2008.06.007.
[31] C.B. de La Serre, C.L. Ellis, J. Lee, A.L. Hartman, J.C. Rutledge, H.E. Raybould, Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation, Am. J. Physiol. Gastrointest. Liver Physiol. 299 (2010) G440–G448, https://doi.org/10.1152/ajpgi.00098.2010.
[32] A.-E. Declèves, A. V Mathew, R. Cunard, K. Sharma, AMPK mediates the initiation of kidney disease induced by a high-fat diet, J. Am. Soc. Nephrol. 22 (2011) 1846–1855, https://doi.org/10.1681/ASN.2011010026.