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Effect of high-fat high fructose diet and carbon tetrachloride on high sensitivity C reactive protein (hsCRP) levels male Wistar rat

Delvina Tandiari[1*], Ika Yustisia[2], Arif Santoso[3], Husni Cangara[4], Firdaus Hamid[5], Nu'man AS Daud[6]

[1]Aging and Regenerative Medicine Concentration, Biomedical Magister Program, Universitas Hasanuddin, Makassar, Indonesia
[2]Department of Biochemistry, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia
[3]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia
[4]Department of Pathology Anatomy, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia
[5]Department of Microbiology, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia
[6]Department of Internal Medicine, Faculty of Medicine, Universitas Hasanuddin, Makassar, Indonesia

*Corresponding to:
Delvina Tandiari; Aging and Regenerative Medicine Concentration, Biomedical Magister Program, Universitas Hasanuddin, Makassar, Indonesia.
delvinatandiari@hotmail.com

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ABSTRACT

Background: The consumption of a high-fat diet affects fat metabolism in the body, and it gets progressively worse by adding high fructose to the diet. Similarly, the chemical compound carbon tetrachloride (CCl4) can cause toxic effects in multiple organs. High sensitivity C reactive protein (hsCRP) is a micro-inflammatory marker used as a primary preventive tool against several diseases. This study aimed to determine the impact of giving a high-fat, high-fructose diet (HFHFD) and CCl4 on hsCRP levels of Wistar rats.

Methods: An experimental study among 24 male Wistar rats aged 8-12 weeks divided into four groups. Rats were fed an HFHFD and were either administered CCl4 biweekly (0.08 mL/kg, peritoneal) or not. The other two groups were fed with standard chow meal with CCl4 injection, and the un-injected group stands as a control group. All groups were monitored for eight weeks, and serum parameters were examined. Data were analyzed using SPSS version 17 for Windows. P-value <0.05 was significant.

Results: The intake of a high fat and high-fructose diet for eight weeks was proven to have higher levels of hsCRP than those without the diet. Similar to the administration of 0.8 ml/kg peritoneal CCl4 biweekly shown to have higher levels of hsCRP compared to the control group. This study also showed increased body weight except for the group that received a standard meal and CCl4 peritoneal injection.

Conclusion: High-fat high fructose diet and carbon tetrachloride had a significant effect on hsCRP level.

Keywords: high-fat, high-fructose diet (HFHFD), carbon tetrachloride (CCl4), high sensitivity C reactive protein (hsCRP), Wistar.
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INTRODUCTION

Ever since many health complications caused by food habits, many studies have found various diets to make similar animal models for identifying the main factors contributing to its development. High-fat diets are known to lead to adipose mass accumulation.[1] An excess of body fat tissue may closely be associated with several non-communicable diseases.[2] Dietary fructose has been implicated in risk factors for cardiovascular disease (CVD) with increased plasma triglycerides.[3] A high-fat, high fructose diet (HFHFD) can also trigger oxidative stress in tissue and plasma, metabolic syndrome, and obesity-related-disease such as nonalcoholic fatty liver disease (NAFLD).[4] The critical factors in developing metabolic diseases, including obesity, are chronic low-grade inflammation triggered by nutrients and metabolic signals, also known as metabolic inflammation.[5]

Carbon tetrachloride (CCl4) has been a frequently used chemical to experimentally induced hepatic fibrosis depending on the dose and duration.[6] Some studies also use CCl4 to accelerate and aggravate the progression of NAFLD in rats.[7] Kubota et al. developed a combined HFD feeding and CCl4 administration; this study demonstrates that multiple administration of CCl4 to HFD-fed obese mice induces chronic oxidative stress that triggers inflammation apoptosis and leads to the development of fibrosis in the liver, resulting in progression from fatty liver to steatohepatitis.[8]

C-reactive protein (CRP) is commonly associated with inflammatory reactions, high-sensitivity CRP (hsCRP), has been linked to several chronic low-grade inflammatory processes leading to cardiovascular disease (CVD).[9] High-sensitivity CRP levels report having a significant correlation with obesity indices.[10] Various inflammatory markers have been assessed and evaluated in NAFLD; hsCRP has also been part of the scoring system in a Japanese study that predicts the disease progression.[11] Besides, hsCRP may be used as a predictor of NAFLD and other obesity-associated diseases.[12] Therefore, the present study aimed to determine the effect of a high-fat, high fructose diet and carbon

tetrachloride on hsCRP levels. Moreover, to determine hsCRP as a preventive tool in short-term HFHFD feeding and minor induced oxidative stress by microdose CCl4 administration as a novelty of this study.

METHODS

Experimental animals

This study's sample was Wistar rats (Rattus norvegicus) Wistar aged 8-12 weeks with a weight between 200-250 grams characterized by active and healthy movement. Wistar rats were placed in a room with adequate air circulation and standard temperature room (28±2^{0}C) with 50%±10% humidity to maintain a stable environmental atmosphere. Room lights were set in a cycle of 12 hours of darkness and 12 hours of light.

Preparation of diet and CCl4

Standard food was prepared in the Laboratory of Animal Food Chemistry, formulated based on NutriSurvey® software to calculate the calorie intake and the percentage of macro and micronutrients per gram pellet. A standard meal's nutritional composition consists of 3.1% fat, 16.1% protein, 3.9% fiber, 5.1% ash/mineral with tap water. High-fat meals consist of 40% fat, 11.6% protein, 4.5% fiber, and 4.5% ash/minerals with 20% fructose in drinks. Fat percentage is 42% of total calories.

Rats will receive CCl4 by peritoneal injection using a microdose of 1.5ml/kg BW of rats. Pure CCl4 solution was diluted into corn oil with the final volume of 10cc to obtain a final concentration of 0.08 ml/kg. The solution is injected intraperitoneally (IP) twice a week.[7]

Experimental design

Twenty-four rats were randomly divided into four groups with the same amount in each group. Group A includes rats with HFHFD. Group B is a group of rats given HFHFD and received a peritoneal injection of CCl4. Group C as a control group with standard chow meal. Group D includes rats given a standard chow meal with CCl4 injection. During the treatment for eight weeks, experimental animals' weight weighed every week to see body weight changes. Blood samples were taken at the end of treatment to measure the serum. Blood was collected into a red-cap vacutainer tube and centrifuged at 3000 rpm for fifteen minutes to obtain serum. Rat blood serum was studied using a Bioassay Technology Laboratory kit with the ELISA method in Anonymous University Medical Research Center. The test was entirely done according to the manufacturer's guidance.

Data analysis

An independent sample T-test was used to evaluate the significant difference between control and experimental groups. Differences in body weight changes levels between the analysis groups were tested with one-way ANOVA and showed the significance of body weight changes with sample paired T-test. To show statistically significant differences, a p-value <0.05 was determined. The data were processed using SPSS software version 17 for Windows.

Ethical Clearance

This study was approved by the Committee of Health Research Ethics, Anonymous, under letter number 75/UN4.6.4.5.31/PP36/2021.

RESULTS

The weighing results showed an increase in rat's body weight in three groups during treatment, including both the high-fat high-fructose diet group (with or without CCl4 injection) and also in the control group. Rats' body weight in the control group significantly increased (p=0.007), followed by HFHFD with the CCl4 injection group (p=0.035). In contrast, the CCl4 injection group with a standard meal food showed a static change in body weight and decreased at the final week. Statistically, there is a significant change in body weight between the sample groups (p=0.004) (Table 1).

After eight weeks of treatment, hsCRP levels in all three groups (Figure 1) increased. The increase in hsCRP levels in the high-fat, high-fructose diet group was higher than the rats with the standard meal group (p=0.004) (Table 2). The hsCRP level also increased in the group given CCl4 injection (p=0.039) (Table 4) and groups that received both CCl4 injection and high-fat and high-fructose diet (p=0.018) (Table 3). Data have shown that the increase in hsCRP levels in each treatment group increased significantly compared to the control group.

DISCUSSION

After eight weeks of treatment, the control group experienced the most significant increase in body weight (p=0.007) compared to the group with a high-fat high fructose diet. Some other studies may support our findings; a study shows that

Table 1. The changes in body weight (g) after eight weeks of treatment

Sample	Initial weight	Final weight	Weight Changes	**p-value
Group A	278.7 ±54.24	357.3 ± 21.08	78.7 ± 33.25	0.055
Group B	251.5 ±49.13	309.8 ± 55.15	58.3 ± 31.64	**0.035**
Group C	215.3 ± 11.15	304.0 ± 23.39	88.7 ± 12.50	**0.007**
Group D	254.7 ±4.63	246.0 ± 8.54	-8.7 ± 4.73	0.086
p-value*			**0.004**	

Notes: Significance was tested with *one way ANOVA (p<0.05) and **paired sample T-test (p<0.05)
Group A (HFHFD); Group B (HFHFD+CCl4 injection); Group C (control with standard meal); Group D (standard meal+CCl4 injection)

Table 2. Comparison value of the hsCRP (ng/mL) serum between HFHFD group and control group

Sample Group		Mean	SD	p-value*
HFHFD	Group A	1.503	0.0067	0.004
control	Group C	1.062	0.1272	

Notes: *Significance was tested with Independent Samples T-test (p<0.05)

Table 3. Comparison value of the hsCRP (ng/mL) serum between HFHFD with CCl4 injection group and control group

Sample Group		Mean	SD	p-value*
HFHFD + CCl4	Group B	1.861	0.3347	0.018
control	Group C	1.062	0.1272	

Notes: *Significance was tested with Independent Samples T-test ($p<0.05$)

Table 4. Comparison value of the hsCRP (ng/mL) serum between CCl4 group and control group

Sample Group		Mean	SD	p-value*
CCl4	Group D	1.796	0.4341	0.039
control	Group C	1.062	0.1272	

Notes: *Significance was tested with Independent Samples T-test ($p<0.05$)

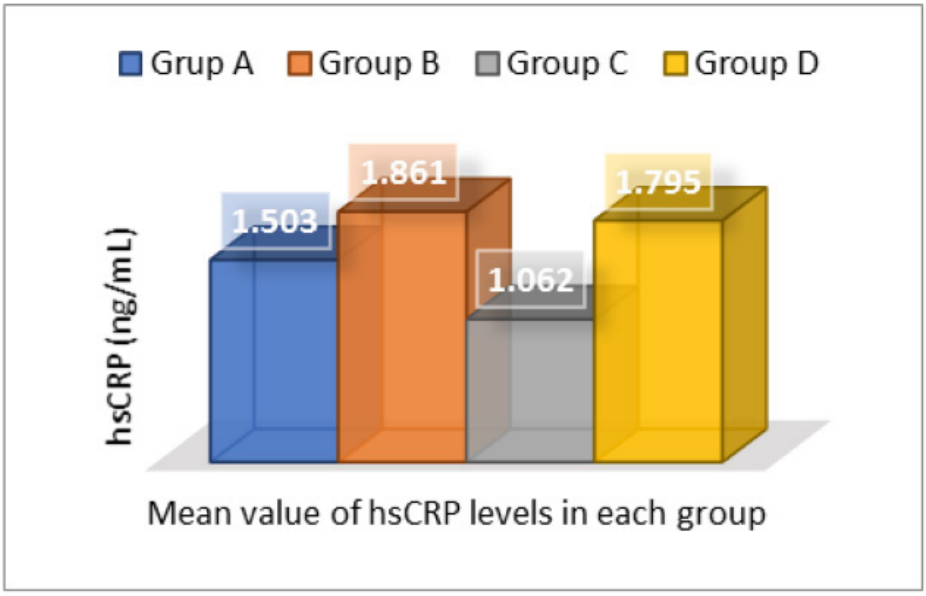

Figure 1. Mean of hsCRP levels between experimental and control groups

Notes: Group B, which was given HFHFD and CCl4 injection, produced the highest mean value of hsCRP levels, followed by group D with a standard meal and CCl4 injection. Group A with HFHFD alone also had a higher hsCRP level than the control group (Group C)

a ketogenic diet by giving 60% of fat on rats for 60 days significantly affects weight loss by around 100 g from their baseline body weight.[13] Buettner et al. found a different high-fat source on the diet result varying body weight changes.[14] A study shown rats fed high fructose did not differ body weight among the control group but shown a significantly higher grade of liver steatosis.[15]

Both groups that received a CCl4 injection biweekly were found to have lighter body weight. The group with a standard meal and CCl4 injection tend to reduce body weight in the final week. Similar to Nhung et al., rats administered with CCl4 (1.0 mL/kg) caused significant weight loss in mice after eight weeks of treatment.[16] Two subsequent studies showed that a combination of a high-fat diet with CCl4 leads to prominent fibrosis after 24 weeks of protocol yet induced body weight increases.[17,18]

The present study found that the group with HFHFD had increased hsCRP levels. The same finding was reported by Hou et al.[19] Rats were fed with a high-fat diet after 24 weeks resulting in elevated plasma triglyceride and hsCRP levels ($p<0.05$). Opposite to the study, Shahi et al. showed that restriction of calorie intake caused weight loss and subsequently led to a significant decrease in hsCRP levels ($p<0.001$).[20] Another study on humans states that body mass index and triglyceride are significantly higher in subjects with the highest hsCRP levels in 38.6% of participants NAFLD. Therefore, the risk for NAFLD increased as the hsCRP level increased ($p<0.001$).[12]

For several years, carbon tetrachloride has been known as a classic toxin to induce liver damage. CCl4 intoxication of the liver affected the lipoproteins' morphology and function, which drastically impaired their ability to act as transport vehicles for lipids from the liver to the circulation.[21] A study using active smokers populations shown lipid peroxide toxicity causes oxidative stress, and systemic inflammation plays a role in producing hsCRP. The study concludes a relationship between total cholesterol level and hsCRP in active smokers with a solid correlation level.[22]

In this study, rats with CCl4 injection biweekly were proven to have higher hsCRP levels than the other groups without CCl4 injection, followed by the group with HFHFD alone. Indicates significant liver damage occurred unto the group—similar findings from Kubota et al.[8] However, all three experimental groups produce higher hsCRP levels than the control group. Nevertheless, further studies of organ involvement in hsCRP levels were a limitation of this study; we also propose other studies to examine liver steatosis levels to assess the fatty liver disease.

CONCLUSION

This study demonstrated high-fat, high fructose diet and carbon tetrachloride significantly affected hsCRP levels compared to the control group. Based on statistical tests, HFHFD, HFHFD with CCl4, and CCl4 groups have raised hsCRP levels.

DISCLOSURE

Conflict of Interest

The authors state no conflicts of interest associated with this manuscript.

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AUTHOR CONTRIBUTION

All authors have contributed in drafting, conducting the experiment, data analyzing, and publication writing.

REFERENCES

1. Coelho DF, Pereira-Lancha LO, Chaves DS, Diwan D, Ferraz R, Campos-Ferraz PL, et al. Effect of high-fat diets on body composition, lipid metabolism and insulin sensitivity, and the role of exercise on these parameters. *Brazilian J Med Biol Res*. 2011;44(10):966–72. Available from: http://dx.doi.org/10.1590/s0100-879x2011007500107
2. Thomas EL, Fitzpatrick JA, Malik SJ, Taylor-Robinson SD, Bell JD. Whole body fat: Content and distribution. *Prog Nucl Magn Reson Spectrosc*. 2013;73:56–80. Available from: http://dx.doi.org/10.1016/j.pnmrs.2013.04.001
3. Rizkalla SW. Health implications of fructose consumption: A review of recent data. *Nutr Metab (Lond)*. 2010;7:82. Available from: https://pubmed.ncbi.nlm.nih.gov/21050460
4. Engel MMS, Kusumastuty I, Anita KW, Handayani D. The Effect of High Fat High Fructose Diet (Modification of AIN-93M) on Nuclear Factor Kappa Beta Expression in the Liver Tissue of Male Sprague Dawley Rats. *J Phys Conf Ser*. 2019;1374:12042. Available from: http://dx.doi.org/10.1088/1742-6596/1374/1/012042
5. Fabbrini E, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology*. 2010;51(2):679–89. Available from: https://pubmed.ncbi.nlm.nih.gov/20041406
6. El Boshy ME, Abdelhamidb F, Richab E, Ashshia A, Gaitha M, Qustya N. Attenuation of CCl4 Induced Oxidative Stress, Immunosuppressive, Hepatorenal Damage by Fucoidan in Rats. *J Clin Toxicol*. 2017;07(03). Available from: http://dx.doi.org/10.4172/2161-0495.1000348
7. Zhang G, Wang X, Chung T-Y, Ye W, Hodge L, Zhang L, et al. Carbon tetrachloride (CCl(4)) accelerated development of nonalcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH) in MS-NASH mice fed western diet supplemented with fructose (WDF). *BMC Gastroenterol*. 2020;20(1):339. Available from: https://pubmed.ncbi.nlm.nih.gov/33059584
8. Kubota N, Kado S, Kano M, Masuoka N, Nagata Y, Kobayashi T, et al. A high-fat diet and multiple administration of carbon tetrachloride induces liver injury and pathological features associated with nonalcoholic steatohepatitis in mice. *Clin Exp Pharmacol Physiol*. 2013;40(7):422–30. Available from: http://dx.doi.org/10.1111/1440-1681.12102
9. Salazar J, Martínez MS, Chávez-Castillo M, Núñez V, Añez R, Torres Y, et al. C-Reactive Protein: An In-Depth Look into Structure, Function, and Regulation. *Int Sch Res Not*. 2014;2014:653045. Available from: https://pubmed.ncbi.nlm.nih.gov/27433484
10. CD S, J A, PM R. Relation between high sensitivity C reactive protein to obesity among indians. *Int J Med Sci Public Heal*. 2015;4(11):1523. Available from: http://dx.doi.org/10.5455/ijmsph.2015.280420153141
11. Kumar R, Porwal YC, Dev N, Kumar P, Chakravarthy S, Kumawat A. Association of high-sensitivity C-reactive protein (hs-CRP) with nonalcoholic fatty liver disease (NAFLD) in Asian Indians: A cross-sectional study. *J Fam Med Prim care*. 2020;9(1):390–4. Available from: https://pubmed.ncbi.nlm.nih.gov/32110624
12. Lee J, Yoon K, Ryu S, Chang Y, Kim H-R. High-normal levels of hs-CRP predict the development of nonalcoholic fatty liver in healthy men. *PLoS One*. 2017;12(2):e0172666–e0172666. Available from: https://pubmed.ncbi.nlm.nih.gov/28234943
13. Arsyad A, Idris I, Rasyid AA, Usman RA, Faradillah KR, Latif WOU, et al. Long-Term Ketogenic Diet Induces Metabolic Acidosis, Anemia, and Oxidative Stress in Healthy Wistar Rats. *J Nutr Metab*. 2020;2020:3642035. Available from: https://pubmed.ncbi.nlm.nih.gov/32685205
14. Buettner R, Parhofer KG, Woenckhaus M, Wrede CE, Kunz-Schughart LA, Schölmerich J, et al. Defining high-fat-diet rat models: metabolic and molecular effects of different fat types. *J Mol Endocrinol*. 2006;36(3):485–501. Available from: http://dx.doi.org/10.1677/jme.1.01909
15. Kawasaki T, Igarashi K, Koeda T, Sugimoto K, Nakagawa K, Hayashi S, et al. Rats Fed Fructose-Enriched Diets Have Characteristics of Nonalcoholic Hepatic Steatosis. *J Nutr*. 2009;139(11):2067–71. Available from: http://dx.doi.org/10.3945/jn.109.105858
16. Truong HN, Nguyen HN, Nguyen TKN, Le MH, Tran HG, Huynh N, et al. Establishment of a standardized mouse model of hepatic fibrosis for biomedical research. *Biomed Res Ther*. 2014;1(2). Available from: http://dx.doi.org/10.7603/s40730-014-0009-2
17. Tsuchida T, Lee YA, Fujiwara N, Ybanez M, Allen B, Martins S, et al. A simple diet- and chemical-induced murine NASH model with rapid progression of steatohepatitis, fibrosis and liver cancer. *J Hepatol*. 2018/03/21. 2018;69(2):385–95. Available from: https://pubmed.ncbi.nlm.nih.gov/29572095
18. Maeso-Díaz R, Boyer-Diaz Z, Lozano JJ, Ortega-Ribera M, Peralta C, Bosch J, et al. New Rat Model of Advanced NASH Mimicking Pathophysiological Features and Transcriptomic Signature of The Human Disease. *Cells*. 2019;8(9):1062. Available from: https://pubmed.ncbi.nlm.nih.gov/31510105
19. Hou N, Han F, Wang M, Huang N, Zhao J, Liu X, et al. Perirenal fat associated with microalbuminuria in obese rats. *Int Urol Nephrol*. 2014;46(4):839–45. Available from: http://dx.doi.org/10.1007/s11255-014-0656-7
20. Shahi MM-, Rafiei H, Karandish M, Omidian K, Haidari F. Effect of Calorie Restriction Supplemented with Genistein on Serum Levels of Glucose, Lipid Profile and Inflammatory Markers (Resistin and hsCRP) in Obese Rats. *Asian J Biochem*. 2012;7(2):98–105. Available from: http://dx.doi.org/10.3923/ajb.2012.98.105
21. Boll M, Weber LWD, Becker E, Stampfl A. Hepatocyte Damage Induced by Carbon Tetrachloride: Inhibited Lipoprotein Secretion and Changed Lipoprotein Composition. *Zeitschrift für Naturforsch C*. 2001;56(3–4):283–90. Available from: http://dx.doi.org/10.1515/znc-2001-3-419
22. Alima S, Kusdinar G, Rokim MA. Hubungan Antara Kadar Kolesterol Total Dengan High Sensitivity C-Reactive Protein (hsCRP) Pada Perokok Aktif Di Dusun Gambirejo RW 03 Desa Warujayeng. In: Prosiding SINTESIS (Seminar Nasional Sains, Teknologi dan Analisis). 2018. p. 42–6.