Basic Medical Research

Effect of a high-calorie diet on pro- to anti-inflammatory macrophage ratio through fat accumulation in rat lung tissue

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pISSN: 0853-1773 • eISSN: 2252-8083
https://doi.org/10.13181/mji.oa.236991
Med J Indones. 2023;32:212–6

Received: June 01, 2023
Accepted: November 28, 2023
Published online: February 05, 2024

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ABSTRACT

BACKGROUND A high-calorie diet increases the risk of obesity. Accumulation of fat causes inflammation, as seen by the increased ratio of pro- to anti-inflammatory macrophages in a high-calorie diet. The pro-inflammatory shift in macrophage polarization may result in hypoxia, fibrosis, emphysema, and asthma. This study aimed to determine the effect of a high-calorie diet on pro- to anti-inflammatory macrophage ratio through fat accumulation.

METHODS This experimental study used *in vivo* test in 16 male Sprague-Dawley rats aged 10–12 weeks. The rats were divided into high-calorie and normal diet groups for 16 weeks. Obesity in rats was defined as having a body mass index (BMI) of >0.68 g/cm^2. Examination of lung fat accumulation was done through oil red O staining, while pro- to anti-inflammatory macrophage ratio was tested through CD11c and CD206 expressions by immunohistochemical method.

RESULTS The high-calorie diet group had higher BMI (0.72 [0.02] *versus* 0.62 [0.03]; $p<0.001$), lung fat accumulation (32.73 [10.55] *versus* 0.37 [0.38]; $p<0.001$), and pro- to anti-inflammatory macrophage ratio (0.83 [0.02] *versus* 0.24 [0.006]; $p<0.001$). The higher the fat accumulation, the higher the pro- to anti-inflammatory macrophage ratio ($r = 0.933$; $p<0.001$).

CONCLUSIONS The ratio of pro- to anti-inflammatory was higher in the high-calorie diet group, indicating polarization of macrophages toward pro-inflammatory macrophages.

KEYWORDS high-calorie, lung, macrophages

A high-calorie diet increases the risk of obesity. Excess energy in the body from a high-calorie diet is stored as triacylglycerol in the adipose tissue. When this storage becomes excessive, fat can accumulate in various organs, including muscles, pancreas, heart, liver, and lungs.[1] Obesity-associated lung disease plays a role in various pathological lung disorders as it affects disease severity and response to treatment. Fat accumulation causes inflammation, as indicated by the increased ratio of pro- to anti-inflammatory macrophages. Fat accumulation reflects the disruption of normal triglyceride (TG) synthesis and metabolism. The pro-inflammatory shift in macrophage polarization causes lung tissue hypoxia and increased leptin levels.[2] The presence of leptin receptors in bronchial epithelial cells, alveoli, and alveolar macrophages in the lungs can increase cytokine synthesis and promote reactive oxygen species (ROS), hypoxia, and fibrosis. These conditions lead to fibrosis, emphysema, and asthma.[3]

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Obesity also increases the respiratory rate and decreases the forced expiratory volume and lung compliance. These functional changes are frequently associated with lung disease in non-obese individuals. The association between obesity and lung disease may have major health consequences and may be more prevalent in the future.[4] The occurrence of fat accumulation and its effects on visceral organs in obesity have not been widely studied. Therefore, this study determined the occurrence of fat accumulation in the lungs and its effect on macrophage polarization in rats fed a high-calorie diet.

METHODS

This experimental study included male Sprague-Dawley rats (*Rattus norvegicus*) aged 10–12 weeks that were administered 16 weeks of dietary intervention. The rats were divided into high-calorie (n = 8) and normal diet (n = 8) groups. The body weight and length (tip of the nose to the anus) of the rats were used to calculate the body mass index (BMI) (g/cm^2).[5] Active rats weighing 180–220 g that were not disabled were included in the study. This study was approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Brawijaya (No. 10/EC/KEPK-PPDS/01/2023).

Rats in the high-calorie diet group were fed food containing 342.98 kcal/100 g. The high-calorie diet consisted of pars (20 g), wheat flour (10 g), cholesterol (0.8 g), lard oil (1 cc), fructose (4 g), and sufficient water. Rats in the normal diet group were fed food containing 266.12 kcal/100 g. The normal diet consisted of pars (20 g), wheat flour (10 g), and sufficient water.

The maintenance of the experimental animals, treatment processes, and organ harvesting were performed at the Animal House of the Biosains Laboratory, Universitas Brawijaya. Frozen cut slides and paraffin blocks of lung organs were prepared at the Kessima Medika Anatomical Pathology Laboratory. Oil red O staining was performed at the Biomedical Laboratory, Faculty of Medicine, Universitas Brawijaya. The stained slides were examined at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya. Immunohistochemistry (IHC) was used to detect pro- (CD11c) and anti-inflammatory (CD206) macrophages.

IHC of CD11c and CD206 expressions

Paraffin blocks that had been cut and affixed to poly-l-lysine-coated slides were deparaffinized and incubated in Diva Decloacker solution (Biocare Medical, USA) at 95°C for 40 min. The slides were soaked in phosphate-buffered saline (PBS) (Sigma Aldrich, USA) for 5 min. The slide was then incubated with the primary antibodies marker of CD11c (production code sc-398708) and CD206 (production code sc-58986) (Santa Cruz Biotechnology, USA) for 60 min at room temperature. The slides were then rinsed with PBS again and incubated with 3,3-diaminobenzidine (Biocare Medical) for 5 min at room temperature. Then, the slides were rinsed with water, counterstained with hematoxylin for 2 min, and placed in a lithium bicarbonate solution for 30 sec before another rinse with water. Afterward, the slides were soaked in increasing concentrations of alcohol for 3 min each. The samples were then placed in xylol for clearing before they were mounted and covered.

A binocular microscope (400× magnification; CX23 microscope; Olympus, Japan) was used to examine the slides. Cells with positive staining were photographed and manually counted using Indomicro View software (Indomicro, Indonesia). Two experts (anatomical pathology specialists) from the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Brawijaya examined the prepared slides. Pro- and anti-inflammatory macrophages were indicated by irregular morphology with a filopodial processus, darker, grooved, bean-shaped, and eccentric nucleus.[6]

Data analysis

Lung fat accumulation, the presence of pro- and anti-inflammatory macrophages, and the pro- to anti-inflammatory macrophage ratio were compared between the groups. The Shapiro–Wilk test was used to examine the data normality. An independent *t*-test

Table 1. Demographic characteristics of the subjects

Characteristics	High-calorie diet, mean (SD)	Normal diet, mean (SD)	*p*
Body weight (g)	421 (10.54)	360 (27.55)	**<0.001***
Body length (cm)	24 (0.64)	24 (0.53)	0.721†
BMI (g/cm^2)	0.72 (0.02)	0.62 (0.03)	**<0.001***
Lung weight (g)	3.313 (0.211)	2.748 (0.344)	**0.001***

BMI=body mass index; SD=standard deviation
*Independent *t*-test; †Mann–Whitney test

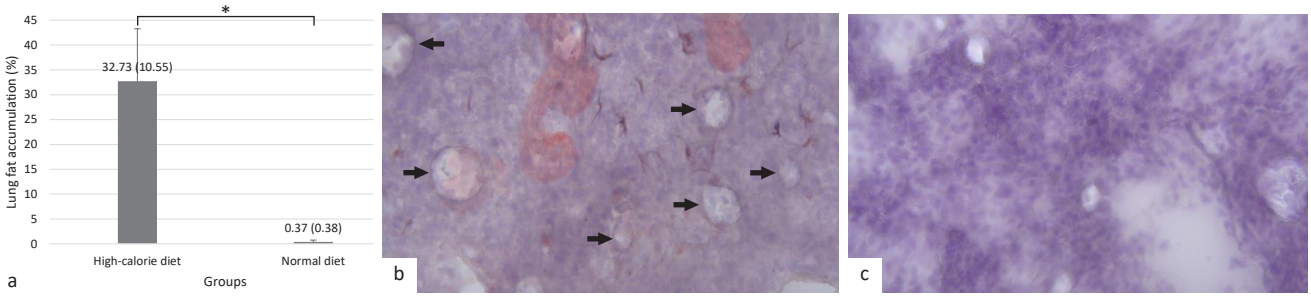

Figure 1. Lung fat accumulation. Lung fat accumulation in the high-calorie and normal diet groups (a) and histology of lung fat accumulation in high-calorie (b) and normal diet (c) groups. Lung fat accumulation shown in fat droplets (arrows) stained red in the tissue and intracellular (oil red O staining in 400× magnification). *Independent *t*-test, $p<0.001$

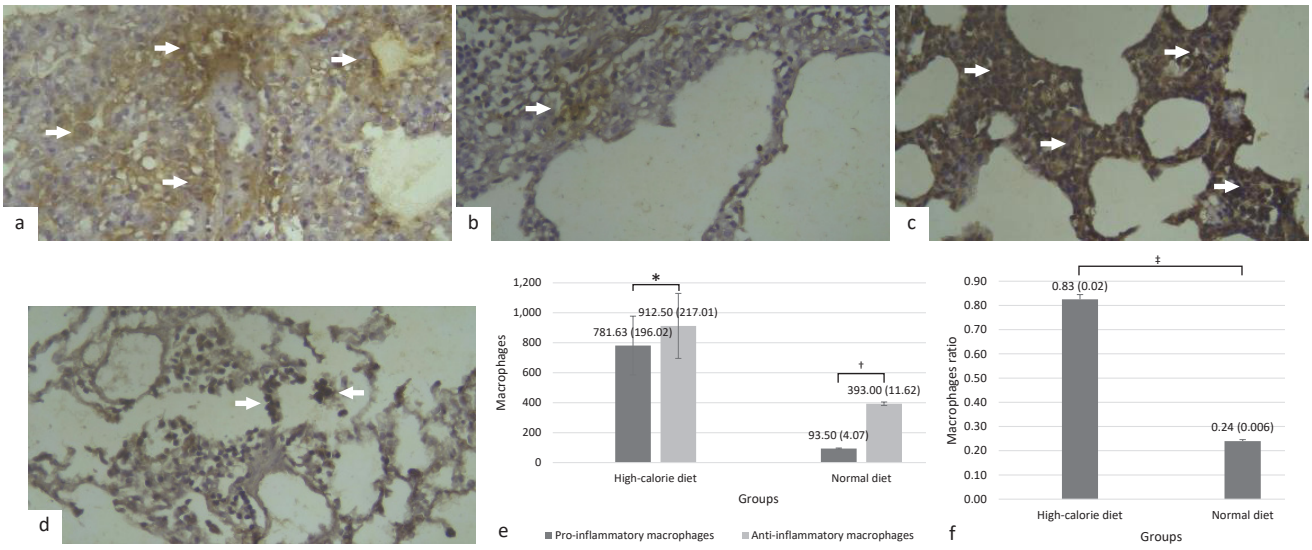

Figure 2. Macrophages and CD11c and CD206 expressions. Pro-inflammatory macrophages identified as positive expression on CD11C in the high-calorie (a) and normal diet (b) groups, anti-inflammatory macrophages identified as positive expression on CD206 in the high-calorie (c) and normal diet (d) groups, the comparison of pro- and anti-inflammatory macrophages (e), and the macrophage ratio analysis (f) between the high-calorie and normal diet groups. The expression of macrophages shown in yellowish-brown color (arrows) on membrane cell and/or cytoplasm from immunohistochemistry (IHC) with 400× magnification. *Independent *t*-test: $p<0.05$; Mann–Whitney: †$p<0.001$, ‡$p<0.001$

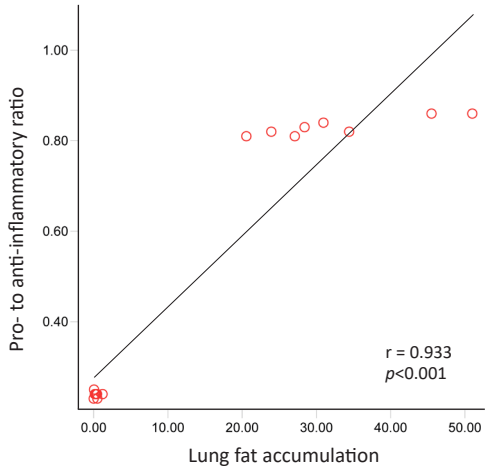

Figure 3. Linear diagram of the relationship between the area of fat accumulation and the ratio of pro- to anti-inflammatory macrophages

was used to examine the differences if the distribution test was normal. The Mann–Whitney test was used if the distribution test was not normal. The Pearson's correlation test was used to identify correlations between the variables. All analyses were conducted using SPSS software 26 (IBM Corp., USA). Statistical significance was set at $p<0.05$.

RESULTS

The high-calorie diet group had a higher average body weight and BMI than the normal diet group (Table 1). Figure 1a shows a higher average area of fat accumulation in lung tissue in the high-calorie diet group than in the normal diet group.

CD11c and CD206 expressions are shown in Figures 2a–d. Pro- and anti-inflammatory macrophages were significantly higher in the high-calorie diet group than in the normal diet group (Figure 2e). The high-calorie diet group had a higher ratio of pro- to anti-inflammatory macrophages (0.83; [1:1.2]) than the normal diet group (0.24; [1:4.2]) (Figure 2f).

A positive relationship was observed between lung fat accumulation and the ratio of pro- to anti-inflammatory macrophages (Figure 3).

DISCUSSION

In this study, a high-calorie diet played an important role in augmenting pro- and anti-inflammatory macrophages. A high-calorie diet increases the macrophage ratio in the lung tissue, inducing chronic inflammation and macrophage recruitment to the lungs. The diet also directly enhances the production of lipopolysaccharides (LPS)-induced tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) by macrophages.[7] The results of this study indicated that 16 weeks of a high-calorie diet resulted in obesity and lung fat accumulation in rats. Obesity is caused by excess TG storage in adipose tissue and an imbalance between energy intake and consumption. Increased glucose uptake into tissues, glycogenesis, and the downregulation of lipolysis and gluconeogenesis lead to increased body mass.[8]

The number of pro- and anti-inflammatory macrophages in the lung tissue was significantly higher in the high-calorie diet group than in the normal diet group, which is consistent with the results of a previous study[9] that reported a pro- to anti-inflammatory ratio of 1:4 in the adipose tissue of normal mice and a ratio of 1:1.2 in obese mice. Obesity induces monocyte chemotactic protein-1 production to recruit macrophages to the lungs, increasing the LPS-induced production of TNF-α and IL-1 by macrophages.[10] This was the first study to report the number and ratio of macrophages in the lung tissue of obese rats through the accumulation of lung fat due to a high-calorie diet. Obesity induces hypoxia of lung tissue through the accumulation of lung fat due to T helper 1 (Th1) activation, increasing leptin, IL-6, TNF-α, and IL-18 and decreasing adiponectin. These changes cause macrophage polarization to be a pro-inflammatory state rather than an anti-inflammatory state.[10]

The percentage of lung tissue fat accumulation is strongly correlated with changes in the ratio of pro- to anti-inflammatory macrophages in the high-calorie diet group in this study. Obesity causes pro-inflammatory mediators that cause macrophages to migrate to the lung tissue. Liu et al[11] reported that a high-calorie diet combined with pneumonia increases the lung organ coefficient, indicating that LPS-induced lung tissue hypertrophy occurred due to the infiltration of macrophages in rat lung tissue. In obese mice, adipocyte cell death, which can be caused by hypoxia, increases in adipose tissue, attracting pro-inflammatory macrophages to adipose tissue. In the obese state, the number of macrophages in adipocytes increases, and the inflammatory phenotype changes due to the destabilization of the balance between pro- and anti-inflammatory macrophages.[12]

Chronic inflammation occurs in the setting of obesity and triggers disturbances in the signal balance of Th1, Th17, and Th2 cells. Polarization of pro-inflammatory macrophages can occur by increasing leptin, ROS, and nitric oxide. Additionally, leptin increases leukotriene biosynthesis. Decreased adiponectin levels and increased adipocytokine levels in the peripheral blood are associated with obesity, asthma, and vascular remodeling in pulmonary hypertension. Decreased adiponectin levels can lead to fibrosis and emphysema.[13]

This study had several limitations. The inflammatory mediators that play a role in macrophage infiltration in the lungs were not investigated. The effects of fat accumulation on lung morphology and function were not examined. The results of this study can be used as a reference for future studies regarding the effects of a high-calorie diet on obesity, inflammation, and lung health.

In conclusion, fat accumulation and the ratio of pro- to anti-inflammatory macrophages were significantly higher in rats fed a high-calorie diet than in those fed a normal diet. The percentage of lung tissue fat accumulation was strongly and positively correlated with an increased pro- to anti-inflammatory macrophage ratio.

Conflict of Interest

The authors affirm no conflict of interest in this study.

Acknowledgment

We would like to thank Iin Noor Chozin and Teguh Rahayu Sartono for providing us with some guidance on writing this article. We also would like to thank Lukman Firmansyah and Hendy Setyo for

reading the prepared slide result. In addition, we thank Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Brawijaya.

Funding Sources

None.

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