Effects of high glucose, egg yolk intake, calorie restriction, and air pollution on rats’ fat cell

Diniwati Mukhtar1, Aan Royhan1, Hasna Luthfiyah Fitriani1, Melasari Marantika1, Dita Safira Salsabila1, Lena Fitriyana1, Febri Irwansyah1

1Faculty of Medicine, Yarsi University, Jakarta, Indonesia

*Corresponding author: diniwati.mukhtar@yarsi.ac.id

**ABC:** Obesity is one of the cardiovascular disease risk factors that cause hypertrophy or hyperplasia of adipocytes. Changes in fat cells have the potential to increase inflammation and insulin resistance.

**Objective:** This study aims to determine the effects of high glucose, egg yolk consumption, calorie restriction, and air pollution on body weight, cell size, and fat tissue weight in rats.

**Methods:** The type of study is experimental and conducted for six weeks. Thirty white male rats were divided into five groups: sugar-treated group, egg yolk-treated group, restricted calorie intake-treated group, air pollutant-treated group, and control group. The cell size and weight of adipose tissue were obtained from abdominal and supraclavicular fat samples.

**Results:** High glucose, egg yolk consumption, air pollution exposure, and calorie restriction were affected by increases in body weight, cell size, and adipose tissue mass (p < 0.05). The cell size and weight of adipose tissue have a negative effect on body mass index. Nevertheless, dietary intervention studies have shown that the size of fat cells decreases after calorie restriction-induced weight loss.

**Conclusion:** The body weight, cell size, and adipose tissue weight of the rats were affected by a high sugar intake, egg yolk consumption, calorie restriction, and exposure to air pollution.

**INTRODUCTION**

Obesity is among the most significant factors associated with an increased risk of metabolic diseases, particularly type 2 diabetes mellitus (T2DM) and cardiovascular disease. According to Michele et al., in the world, there will be an increase...
in the prevalence of obesity, and continue it will reach 18% in men and 21% in women by 2025. Numerous risk factors contribute to the worsening of obesity, including diet rich in processed and preserved foods, lack of sleep, and lack of exercise. The pathological mechanism that has the greatest impact on cardiometabolic diseases is insulin resistance (IR), which is caused by obesity and affects white adipose tissue (WAT), liver, and skeletal muscle, in combination with impaired insulin secretion. Hypertension and coronary heart disease are the major effects of metabolic imbalance supported by obesity-induced IR in cardiovascular diseases.\(^1,2\)

WAT is a complex organ that plays a major role in energy homeostasis regulation. The functions of brown adipose tissue (BAT) are to release energy and act as an anti-obesity agent because it can oxidise fatty acids and glucose to maintain thermogenesis. WAT is a complex organ that plays a major role in energy homeostasis regulation. By contrast, obesity causes WAT to become dysfunctional and impair its ability to store excess energy, thereby causing ectopic fat deposition in other tissues, such as the liver, skeletal muscle, and heart, which regulate glucose homeostasis. This condition is commonly referred to as lipotoxicity. In the presence of excessive nutrition, mature adipocytes accumulate a great deal of fat and develop cellular hypertrophy. Hypertrophic adipocytes induce adipose tissue remodelling, which then induces cytokine dysregulation in adipose tissue, resulting in systemic inflammation and impaired precursor cell adipogenesis. Both ectopic fat accumulation and adipose cell hypertrophy contribute to the increased incidence of insulin resistance and the release of inflammatory mediators. According to a clinical study by Michele et al., excess fat in cardiac perivascular adipose tissue (PVAT) has functional significance and implications for CVD. In obese individuals, dysfunctional PVAT causes an increase in the release of vasoconstrictors and pro-inflammatory molecules, leading to alterations in vascular homeostasis.\(^1,3\)

Sugar is a nutrient that affects the accumulation of body fat. Sugar has high in calories but low in vitamins and minerals content. When glucose levels increase, adipocyte tissue in humans develops hypertrophy, which is a risk factor for obesity.\(^4\) In addition, a high-fat diet affects the quantity and structure of fat cells. Approximately 32% of the fat in an egg is contained in the yolk. A high-fat diet increases the amount of adipose tissue fat deposition. Every stored fat is stored as triglycerides under the skin instead of being directly used as an energy source. The accumulation of excessive triglycerides will result in hypertrophic obesity, which facilitates the occurrence of obesity. Appropriately restricting calorie intake is one of the methods used to reduce the risk of metabolic diseases. This has been reported by intervention studies, showing that fat cell size is reduced following weight loss.\(^5\)

Several studies have also demonstrated that air pollution is an obesity risk factor. The adverse effect of increased exposure to particulate matter (PM) 2.5 is linked to a higher risk of ischaemic heart disease, heart failure, and cerebrovascular disease. Experimental data have shown that early exposure to ambient particles increases the risk of weight gain due to increased insulin resistance, adiposity, and inflammation.\(^6\) Some environmental pollutants acting as endocrine disruptors (EDCs), such as dichlorodiphenyltrichloroethane (DDT) and its metabolite dichlorodiphenyldichloroethylene (DDE), as well as some traffic pollutants, are associated with increased obesity risk pollutants. Its metabolite DDE has been shown to induce NF-κB activation and pro-inflammatory cytokines production. Cytokines and other mediators that ‘spill over’ into the systemic circulation. Inflammation after exposure to particle pollution can increase sympathetic tone and changes in vasomotor regulation. The increased blood pressure is the most likely physiological change to explain an increased sympathetic tone. Particulate air pollutants, as the major mediator of cardiovascular, can affect the elevations in blood pressure and acute coronary syndromes. All of that at the population level can have important public health implications.\(^6–8\)

The role of fat cell size that affect cardiometabolic may be urgent to study. Fat cell size has a strong correlation with pro-inflammatory adipocytokine secretion. Increased FCS is strongly associated with AT leptin expression, production, secretion, and plasma leptin level. Fat cell size represents in response to excess energy and pollutant.\(^9,10\) Therefore, this study aims to determine the impact of a high-sugar diet, egg yolk intake, calorie restriction, and exposure to air pollution on the
body weight, cell size, and fat tissue weight of rats.

METHODS

The experimental study employs a pretest-posttest control group design approach. The Research Ethics Committee of the YARSI University Research Institute has approved the ethics of this study with letter number No: 006-009/KEP-UY/BIA/I/2020.

Animals preparation

Male Wistar rats with a mean body weight of 100–200 g were purchased from LITBANGKES, the Ministry of Health Indonesia. Before treatment, rats required behavioural and physiological adaptations for one week. The rats would then receive standard food three times daily, ad libitum water, and one week of acclimatisation. The rats were placed in an environment with good conditions to adapt and be healthy during the study. Conditions before treatment were the same in all groups.

Animals experiment

A total of thirty male Wistar rats were included in this study, and they were allocated into five groups, with five rats in each group. The groups were classified as follows: 1) the high glucose intake group; 2) the egg yolk intake group; 3) the calorie restriction group; 4) the air pollution exposure group; and 5) the control group. Additionally, five rats were kept as replacements to ensure the validity of the results in case some rats in each group died during the experiment. The administration of high glucose and egg yolk intake was conducted through a nasogastric tube (NGT). The high glucose intake group was fed ad libitum with an amount of sugar of 1.8 g/200 g body weight in 20 ml of distilled water. The egg yolk group was fed ad libitum with egg yolk emulsion of as much as 5 g of egg yolk/200 g of body weight control. For the calorie restriction group, food intake, except for the water, was off for the alternate day for six weeks. Throughout the experiment, all rats were housed at 23 ± 1°C and 60% humidity with a day-night-cycle of 12 h and wood shavings as bedding material. The air pollution exposure group was fed ad libitum and placed in a special room at the parking area near the bus public transportation station, Simpang Lima Senen, where the mean of PM2.5 is 104.7 g/m³. The control group was fed ad libitum with standard rat food. All groups received treatment for six weeks in order to observe a statistically significant difference between the treated and control groups.

At the end of the treatment, the rats were weighed using laboratory scales, and they were then anaesthetised with 75–100 mg/kg intraperitoneally of ketamine–xylazine for 10–30 min. While under anaesthesia, the rats underwent surgery to remove abdominal (WAT) and subcutaneous (BAT) fat cells. The fat cell size was measured using a microstepper for the largest diameter and a software image raster. The fat was then measured with a Sartorius scale.

Histological and biochemical analysis

Histological examinations were carried out to observe the enlargement of adipose tissue fat cells. Using an objective microscope lens with a 10× magnification, the entire field of view was observed during the preparation of the fat cells. The fat cells’ size was examined using a light microscope at 40× magnification in the area with the largest fat cells after hematoxylin and eosin staining. Two methods were used to analyse the adipose tissue. Firstly, the histopathological image of the fat tissue between the negative control group and the treatment group were assessed descriptively. Secondly, fat tissue was removed from each rat in two locations, and 100 cells were counted for each of the two preparations to measure the fat tissue.

Statistical analysis

According to Connelly et al., the hypothesis regarding the difference in mean between more than two independent variables or populations was analysed using ANOVA. In this study, a one-way ANOVA was used to determine the real or significant mean difference between the independent variables or treatment groups in influencing the dependent variable. Data in this study were analysed using IBM SPSS Statistic for Windows, Version 23. After determining differences between each independent variable or treatment group, a Bonferroni post hoc test was conducted to identify the significant difference between each group with a p-value of < 0.005.
RESULTS
Mean body weight in control, high glucose, egg yolk intake, calorie restriction, and air pollution exposure groups for six weeks

A high-glucose intake variable with a mean value of (231.357) causes an increase in body weight. Conversely, calorie restriction causes the smallest mean weight gain, with a mean value of 134.743, which is smaller than the other independent variables. Consequently, it can be assumed that a high glucose intake has a greater impact on weight gain. ANOVA revealed a significant value (less than the cut-off value, \( p = 0.05 \)). Body weight has a significance value of 0.005. Thus, it can be concluded that the mean effect of the independent variable (treatment) on the dependent variable differs significantly (Table 1).

Mean fat cell size in control, high glucose, egg yolk intake, calorie restriction, and air pollution exposure groups for six weeks

The measurements were taken using a 10×-magnification light microscope. Table 2 shows that air pollution exposure has the highest mean value in the size of fat cells, which is 58.9420. Moreover, calorie restriction has the smallest mean value, with a mean value of 27.0400. Thus, it can be assumed that air pollution exposure has a greater potential to increase fat cell size than calorie restriction, egg yolk intake, and high glucose intake. The one-way ANOVA statistical test results demonstrated that the size of fat cells has a significance value of 0.000. Thus, it can be concluded that there is a significant difference in the mean effect of the independent variable (treatment) on the dependent variable.

Furthermore, as shown in Figure 1, the measurement using a light microscope with a 10× magnification found that the control group had a mean white fat cell diameter of 46.48 m. Conversely, the air pollution exposure group had a mean diameter of white fat cells of 58.77 m.

Mean weight of adipose tissue in control, high glucose intake, egg yolk intake, calorie restriction, and air pollution exposure groups after six weeks of treatment

Table 3 shows that the weight of white fat tissue in the control group has lower than the treatment group, and the largest mean value is the air pollution variable, with a mean value of 7.9120. By contrast, the calorie restriction variable has the smallest mean value, with a mean value of 2.9760. Thus, it can be interpreted that exposure to air pollution can increase the weight of white fat tissue more than calorie restriction. Furthermore, in increasing brown fat tissue weight, the variable with the largest mean value was calorie restriction, with a mean value of 2.1620. Conversely, the variable with the smallest mean value is the provision of egg yolks,

<table>
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<tr>
<th>Table 1. Mean body weight in control, high glucose, egg yolk intake, calorie restriction, and air pollution exposure</th>
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<tbody>
<tr>
<td><strong>Dependent variable</strong></td>
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<tr>
<td>Body weight</td>
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<tr>
<th>Table 2. Mean fat cell size in control, high glucose, egg yolk intake, calorie restriction, and air pollution exposure</th>
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<tbody>
<tr>
<td><strong>Dependent variable</strong></td>
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<tr>
<td>Fat cell size</td>
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with a mean value of 0.7280, which is smaller than the other independent variables. This raises the possibility that calorie restriction has the greatest influence on the weight gain of brown tissue. The one-way ANOVA statistical test results demonstrated that the weight of white fat tissue and brown fat has a significance value of 0.000. Thus, it can be concluded that there is a significant difference in the mean effect of the independent variable (treatment) on the dependent variable.

**DISCUSSION**

The mean body weight increased in the treatment group (sugar administration) due to fructose-containing sugar, which is believed to increase body weight and insulin resistance. The incidence of dyslipidemia will be affected by an excessive accumulation of fructose in the liver. Consumption of fructose decreases circulating levels of polypeptide 1 (GLP-1) satiety hormones such as glucagon but does not attenuate the levels of ghrelin, an appetite-stimulating hormone.\(^{12}\)

A high glucose intake increases adipose tissue fat deposition. Any excess carbohydrates not immediately used will be stored as triglycerides in adipose tissue.\(^{13}\) This is supported by a study by Stanhope et al. in which men and women who consumed a beverage sweetened with sucrose up to 1 L/day for six months along with an ad libitum diet experienced an increase in liver triglyceride and fasting plasma triglyceride concentrations compared to those who consumed an ad libitum diet with low-fat or isovolumetric milk in isocaloric amounts of aspartame-sweetened beverages or

![Figure 1. The histological structure of fat cells, HE staining, 10x. Comparison between white fat cells in the control group (a) and the air pollution exposure group (b)](image)

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>Weight of white adipose tissue</td>
<td>Control</td>
<td>5.0940</td>
<td>1.05940</td>
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<tr>
<td></td>
<td>High glucose intake</td>
<td>7.4120</td>
<td>0.66556</td>
<td></td>
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<tr>
<td></td>
<td>Egg yolk intake</td>
<td>7.4800</td>
<td>0.98321</td>
<td>0.000</td>
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<tr>
<td></td>
<td>Calorie restriction</td>
<td>2.9760</td>
<td>0.53252</td>
<td></td>
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<tr>
<td></td>
<td>Air pollution exposure</td>
<td>7.9120</td>
<td>0.25193</td>
<td></td>
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<tr>
<td></td>
<td>Total</td>
<td>6.1748</td>
<td>2.03898</td>
<td></td>
</tr>
<tr>
<td>Weight of brown adipose tissue</td>
<td>Control</td>
<td>1.4920</td>
<td>0.14360</td>
<td></td>
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<tr>
<td></td>
<td>High Glucose Intake</td>
<td>0.8740</td>
<td>0.06229</td>
<td></td>
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<tr>
<td></td>
<td>Egg Yolk Intake</td>
<td>0.7280</td>
<td>0.16022</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Calorie Restriction</td>
<td>2.1620</td>
<td>0.24098</td>
<td></td>
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<tr>
<td></td>
<td>Air Pollution Exposure</td>
<td>0.8140</td>
<td>0.11589</td>
<td></td>
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<tr>
<td></td>
<td>Total</td>
<td>1.2140</td>
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In fact, sucrose consumption increased visceral fat volume relative to milk consumption, despite the comparable weight gain, and it increased plasma cholesterol concentrations relative to aspartame and water consumption.\textsuperscript{14}

According to the study's results, a high glucose intake was associated with weight gain rather than calorie restriction. The high glucose intake group was fed ad libitum with an amount of sugar of 1.8 g/200 g body weight in 20 ml of distilled water. The egg yolk group was fed ad libitum with egg yolk emulsion of as much as 5 g of egg yolk/200 g of body weight control. After six weeks, a high glucose intake was associated with weight gain rather than calorie restriction, with a mean value of 231.357 for the high glucose intake group. Malik et al. conducted a meta-analysis of cohort studies and randomised controlled trials of beverages high in sugar, both fructose and glucose, and weight gain in children and adults. According to seven cohort studies involving 174,252 adults, an increase of one daily serving of high-sugar beverages was associated with a 0.12 kg weight gain over one year. Despite the apparent simplicity of this estimate, adult weight gain in the general population is a gradual process that occurs over decades and means approximately 0.45 kg per year. Moreover, according to an analysis of NHANES III cohort data, consumption of added sugar and high-sugar beverages was associated with an increased risk of cardiovascular disease-related mortality (CVD). In one study with a mean of 14.6 years of follow-up, consumption of added sugar was associated with a twofold increased risk of CVD mortality.\textsuperscript{15}

Increased adipocyte size correlates positively with impaired insulin sensitivity and human glucose tolerance. T2DM is one of the conditions that correlate with adipocyte size. Larger adipocytes are less insulin-sensitive and exhibit impaired glucose uptake. Lior et al. found that air pollution is associated with increased adipose tissue inflammation and that PM exposure can increase insulin resistance and ultimately lead to obesity and T2DM. According to an experiment, chronic PM2.5 exposure was associated with an increase in the mean content of fatty macrophages. In a previous study, it was reported that mice exposed to PM2.5 for ten months exhibited an increase in the number of macrophages and accumulation of white adipose tissue, resulting in larger fat cells.\textsuperscript{16,17}

In a study of diet-induced obesity, AdipoChaser mice showed signs of adipose tissue hypertrophy and hyperplasia within four weeks and two months, respectively. This result supports the findings of our study, which demonstrated the second-largest effect, namely, the impact of egg yolk consumption on fat cell size. We found that subcutaneous fat tissue only exhibited hypertrophy in response to a high-fat diet for two months and limited adipogenesis. Additionally, a greater increase in adipocyte fraction size following a high-fat diet predicted a decrease in insulin-stimulated glucose uptake in insulin-sensitive obese individuals. Changes in the plasticity of adipose tissue are a major contributor to obesity-related metabolic complications.\textsuperscript{18} The absence of egg yolk administration is not equivalent to studying only egg yolk consumption. In fact, egg yolk intake has been associated with an increased risk of dyslipidemia. Our study showed that egg yolk induced adipose tissue accumulation and impaired glucose metabolism.

A change in cell size and spread represents the expansion of fat tissue. An experimental study reported that early exposure to ambient particles increases the risk of weight gain (BB) due to increased insulin resistance, adiposity, and inflammation. Recent reports of multiple interactions between adipose tissue and persistent organic pollutants (POPs) indicate that these tissues play a significant role in the kinetics and toxicity of POPs. Based on substantial epidemiological evidence, PM2.5 air pollution is a significant risk factor with severe consequences for human health and a risk factor for obesity.\textsuperscript{7} Chen et al. found that long-term PM2.5 exposure (a mean of one year) can reduce muscle mass in the upper extremities and trunk but not in the lower extremities. By contrast, exposure to PM2.5 air pollution was also associated with increased body fat mass in the same body parts. In addition, Chen et al. reported that the increase in body fat mass was affected by exposure to PM2.5 air pollution. Previous animal studies have demonstrated a direct effect of PM2.5 exposure on adipose tissue, with the result that brown adipose tissue was damaged by PM2.5 air pollution exposure. The gene expression pattern was also changed from brown adipocytes to white. Consequently, it decreased brown adipose tissue and increased white adipose tissue, influencing insulin resistance.
and cardiometabolic disease incidence.\textsuperscript{19} Gregorio et al. suggested that air pollutants and their metabolite DDE have been shown to induce NF-κB activation and pro-inflammatory cytokines production. Pollutants are a trigger of systemic inflammation and an increase in visceral adiposity. Increased fat mass in the abdominal would be the major site of increased release of cytokines like NF-κB.\textsuperscript{7}

Calorie restriction (CR) is one of the most prevalent and least invasive weight loss methods.\textsuperscript{20} According to this study’s findings, calorie restriction significantly affects the metabolism of rats, particularly in weight loss and brown adipose tissue weight increase. CR may delay the onset of age-associated cardiovascular and neurodegenerative diseases and may induce T2DM remission, as studies in rodents, primates, and humans reported. In rats, CR for 12–20 weeks decreased metabolic rate, BAT mass, and core body temperature, whereas in mice, 40% CR for 6 and 26 months caused BAT hypertrophy without affecting Ucp1 gene expression. BAT activity is not necessarily affected by changes in BAT mass. In rats, calorie restriction induces the development of beige fat in subcutaneous and visceral adipose tissue, which may be required during feeding to increase body temperature. A study by Suchacki et al. indicated that the effect of CR on BAT activity in normal-weight individuals is unknown, but in obese subjects, CR may lead to BAT activation, thereby preventing BAT mass reduction.\textsuperscript{21}

CONCLUSION
In this study, we investigated the effects of a high-sugar diet, egg yolk consumption, air pollution exposure, and calorie restriction on body weight, fat cell size, and the weight of white and brown fat tissue in mice. Our findings revealed significant differences between the control group and the treatment groups. High glucose and egg yolk consumption, as well as exposure to air pollution, resulted in increased body weight, fat cell size, and adipose tissue mass. Conversely, calorie restriction interventions led to a decrease in fat cell size. It is important to comprehensively understand the molecular mechanisms underlying adipose tissue dysfunction to identify potential therapeutic targets to improve adipose tissue function and expansion. The mechanisms by which bioactive components in high glucose, egg yolk consumption, and air pollution exposure affect fat cell size and adipose tissue weight need to be further characterised and studied. To better understand the pathological conditions and the efficacy of treatments involving high sugar intake, egg yolk consumption, air pollution exposure, and calorie restriction, future studies should have longer durations and more precise levels of study. Additionally, optimising image capture in the microscope could allow for more accurate measurement of the number of fat cells.

CONFLICT OF INTEREST
The authors declare no competing interest.

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