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Impact of Two Different Feeding Regimens for Eight Weeks on Lee Index, Triglyceride Glucose Index and Atherogenic Index of Rats

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Abstract

Background Obesity, driven by excess calorie and fat intake, contributes to diabetes and cardiovascular disease. In rats, simple indices such as the Lee Index (LI) for obesity, triglyceride-glucose index (TyGI) for insulin resistance, and atherogenic index of plasma (AIP) for cardiovascular risk provide cost-effective tools to assess metabolic dysfunction and evaluate dietary or therapeutic interventions.

Objective To show the effect of eight weeks of two types of feeding on the LI, TyGI and AIP in rats.

Methods A comparative study involved 36 rats, divided into two groups; group 1 received an ordinary lab diet of 20 g per day and group 2 consumed a modified diet of 20 g per day. The studied parameters were the LI, TyGI, and AIP. LI was dependent on weight, and length from nose to anus, TyGI was dependent on triglyceride and glucose, while AIP was dependent on triglyceride and high-density lipoprotein cholesterol. All parameters were measured at 2 intervals (0 and 8) weeks. The study was done at Animal House at the College of Veterinary, University of Baghdad.

Results The results showed that the LI did not significantly increase with no differences between the two groups conversely TyGI and AIP increased significantly in the modified and ordinary groups within 8-week intervals.

Conclusion Eight weeks of feeding of ordinary and modified diet in rats causes significant rise in TyGI and AIP but not enough to cause a significant change in LI.

Keywords Obesity, Lee index, triglyceride glucose index, atherogenic index

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List of abbreviations: HFD = High-fat diet, LI = Lee Index, TyGI = Triglyceride-glucose index, AIP = Atherogenic index of plasma

Introduction

Obesity is a growing health concern worldwide, leading to an increased risk of chronic diseases such as type 2 diabetes and cardiovascular disease (1).

The regulation of body weight is greatly influenced by the intake of calories, when there is an excessive intake of calories, regardless of their source, it leads to a state of positive energy balance this, in turn, results in the accumulation of fat and the development of metabolic dysfunction (2).

The role of dietary fat composition in body weight regulation has garnered considerable attention. Butter and soybean oil impact various dimensions of physiological and metabolic responses in both lean and obese rat models. The findings yield insights into the intricate interplay between dietary fat composition and body weight regulation [3,4].

Lee index (LI) is an anthropometric technique to determine rat obesity [5]. LI can be used as a fast and accurate way to determine obesity in rats subjected to a treatment weight gain. Stephens described the determination of obesity in rats proposed by Lee in 1928 [6]; it consists of dividing the cube root of the weight in grams and national length in millimeters and multiplying by 1000. The result sets the nutritional content, LI, as a measure of obesity. The results below 0.300 are considered normal [7,8].

The triglyceride glucose index (TyGI) is a biomarker that combines fasting triglyceride and glucose levels to assess insulin resistance and predict various health outcomes. Several studies have investigated the association between TyGI and different conditions. Research has shown that a high TyGI is significantly associated with an increased risk of acute kidney injury (AKI) in critically ill patients [9,10]. In patients with coronary heart disease (CHD) and hypertension, an elevated TyGI is also associated with adverse prognosis and increased risk of cardiovascular events [11].

The atherogenic index of Plasma (AIP) is a valuable tool used in studies with rats to assess their risk of developing atherosclerosis, a major contributor to cardiovascular disease (CVD) [12,13]. AIP is a simple calculation that compares two key cholesterol values in a blood lipid profile total cholesterol and high-density lipoprotein cholesterol (HDL-C) (good cholesterol) [14,15]. Studies might induce hyperlipidemia (high cholesterol) in rats with a cholesterol-rich diet and monitor changes in AIP to assess the atherogenic effect [16,17]. Researchers might evaluate the effectiveness of potential therapeutic agents or dietary modifications by measuring their impact on AI levels in rats [18,19].

LI, TyG, and AIP are costless bedside parameters that can be easily calculated. These parameters can predict the severity of disease in first-diagnosed patients. Besides, LI is a parameter for obesity and weight gain, TyGl and AIP parameters can guide our treatment in the patient population.

This study aimed to show the effect of eight weeks of two types of feeding on the LI, TyGl and AIP in rats.

Methods

Experimental animals

The study was conducted from July 27th to September 30th, 2023 at the Animal House of the College of Veterinary Medicine, University of Baghdad, Iraq and involved male rats' group of 36, aged 7-9 weeks and within a range of 110-150 grams. Ethical considerations were taken From the College of Pharmacy, Al-Nahrain University, Iraq, (approval ID: nah.co.pha.24) regarding dealing with rats.

The rat's acclimatization period familiarized them with their new environment and dietary regime before a one-week study began. The rats received standard food pellets and fresh tap water. Accommodation in clean polypropylene cages in a controlled setting with a 12/12 light-dark cycle and a constant temperature of 23±2°C. Continuous monitoring of these vital parameters by temperature and humidity meter within the room. The 36 male rats were divided randomly into two groups of 18 each: Group 1 included rats that received an ordinary lab diet of 20 g per day, and Group 2 included rats that consumed a modified diet (high-fat mix) of 20 g per day. Each rat was housed in a separate plastic cage within designated carts.

Diet preparations

The ordinary lab diet for rats was low in calories. It contained various ingredients such as wheat, barley, corn, dried milk, sunflower seeds, fish, meat, vegetable meal, chicken, egg, dietary salt mix, and gelatin (≈14.4% protein and ≈7.6% fat). While, the modified diet includes high-fat, as well as micronutrients. It is prepared using butter, soy, peanuts, starch, tomato, potato, egg, chicken, cooked meat, and potato chips (≈ 11.2% protein and ≈ 43.4% fat). The modified diet is produced by a specialized company "Altagi Feeders Company".

Body weight and length measurement

Healthy rats weighing above 110 grams were involved in this study. Their body weight and length were tracked exactly, with week 0, and week 8 measurements documented using a calibrated electronic scale and Flexible tape for [20].

Blood sampling and collection

Blood sampling at baseline, and in week 8, the rats were carefully anesthetized using a precise intramuscular injection of ketamine (60 mg/kg body weight) and Xylazine (40 mg/kg body weight) [21]. Using 23G syringe, specially chosen for its fine gauge to minimize tissue disruption, was used to gently draw 2 mL of blood from the rat's heart. The blood was then transferred to a clot activator tube, promoting efficient serum separation, the collected blood samples were centrifuged at 4000 rpm for 15 minutes, then the isolated serum was carefully extracted and stored at -20°C. This freezing step preserves the sample's integrity, ensuring reliable lipid profile measurements at a later stage.

Assessment of lipid profile levels

By Rat ELISA Kit of Sun Long Biotech Co. LTD and the procedure used as same Steps mentioned in the kit [22].

Indices calculation

LI was calculated according to this equation [23]:
LI = Cube root of body weight (g) / nose-to-anus length (cm)

TyGI was calculated by using this equation [24]:
TyGI = Ln [fasting triglycerides (mg/dL) × fasting plasma glucose (mg/dL)/2]

AIP was calculated by using this equation [25]:
AIP = Log (fasting triglyceride/HDL-C)

Statistical analysis

Data of the two groups were presented as mean ± standard deviation (SD). Paired t-test were used to compare parameters between 0 and 8 weeks, while unpaired t-test were used to compare parameters between two types of feeding. A P value less than 0.05 was considered as significant, while a p-value <0.001 was considered as highly significant. Statistical package for social sciences (SPSS) version 26 and Microsoft Office Excel 2024 were the software used for analysis.

Results

The ordinary diet group LI at week 0 (0.37±0.03) (P value = 0.053) was not significantly different than that of the modified diet group (0.37±0.05) (P value = 0.372). In both groups, the LI was increased in the next eight weeks of the study. However, this LI increment was not significantly different in week 0 and week 8 (P value = 0.783, 0.927). The increase in LI during 8 weeks was less in those with a modified diet rather (102.58%) than in those with an ordinary diet (102.85%) (Table 1).

As shown in table (2), TyGI showed a highly significant increment in both groups (P value <0.001) after 8 weeks of feeding, however, the difference in TyGI was more obvious in a group with a modified diet than in a group with an ordinary diet (106.28% vs 104.56%). Yet, no significant difference was noticed when compared TyGI at week 0 and week 8 between the both study groups (P value = 0.930, 0.064) respectively.

Table 1. Comparison of Lee index at week 0 and week 8 among normal and modified diet group groups

Group	Lee index Week 0 Mean±SD	Week 8 Mean±SD	P value*	% of difference after 8 weeks
Ordinary diet	0.37±0.03	0.38±0.03	0.053	102.85
Modified diet	0.37±0.05	0.38±0.03	0.372	102.58
P value**	0.783	0.927		

* P value by paired t-test, ** P value by unpaired t-test

Table 2. Comparison of triglyceride glucose index at week 0 and week 8 among normal and modified diet group groups

Group	Triglyceride glucose index Week 0 Mean±SD	Week 8 Mean±SD	P value*	% of difference after 8 weeks
Ordinary diet	3.66±0.09	3.82±0.09	<0.001	104.56
Modified diet	3.66±0.11	3.89±0.12	<0.001	106.28
P value**	0.930	0.064		

* P value by paired t-test, ** P value by unpaired t-test

There was a highly significant increase in AIP after 8 weeks of feeding (P value <0.001) in both groups and more obvious in a group with a modified diet than in a group with an ordinary diet (127.71% vs 121.08%). But no significant difference in AIP was shown at two times of measurement (0 and 8 weeks) (P value 0.645, 0.138) respectively (Table 3).

Table 3. Comparison of atherogenic index of plasma at week 0 and week 8 among normal and modified diet group groups

Group	Atherogenic index of plasma Week 0 Mean±SD	Week 8 Mean±SD	P value*	% of difference after 8 weeks
Ordinary diet	0.79±0.04	0.96±0.07	<0.001	121.08
Modified diet	0.78±0.08	0.99±0.07	<0.001	127.71
P value**	0.645	0.138		

* P value by paired t-test, ** P value by unpaired t-test

Discussion

The present study investigated the impact of two distinct feeding regimens over an eight-week period on the LI, TyGI, and AIP. These indices were selected based on their ability to provide complementary insights into obesity, insulin resistance, and cardiovascular risk using relatively simple, non-invasive, and reproducible measurements.

Still, the question is what the main effect on the occurrence of obesity or dyslipidemia is with the types of food. This study applied to rats with comparable age and weight initially, aiming to identify the effects of type of food on indices.

Two study groups experienced an insignificant increase in the LI at week 8, with the same rate of percentage of increment. This may be explained as 8 weeks of feeding whether ordinary or modified is not enough to show significant change in LI. The mild increment in the LI in the two groups may be due to parallel changes in weight and height as they are in the stage of growing up and continuous feeding with limited activity for each rat as they were kept in a limited space within a cage whatever the type of feeding. The LI, which is a measure of obesity, was found to be significantly increased in rats fed a high-fat diet (26) compared the effects of different diets on obesity in rats and consistently found that high-fat diets led to increased weight gain, adiposity, and fat depot weights, as indicated by higher LI values (27,28). The specific composition of the high-fat diets varied between the studies, with some using a butter-based high-fat diet and others using diets with different percentages of energy from lipids (29).

In the present study, both ordinary and modified diet groups exhibited significant increases in the TyGI after 8 weeks, indicating that metabolic stress can arise even under standard dieting conditions. Importantly, however, the modified diet group demonstrated a greater percentage increase (106.28% vs. 104.56%), reflecting a trend toward more pronounced metabolic disturbance, even though the between-group difference did not reach statistical significance at this time point. These results align with previous work showing that high-fat diets induce adverse metabolic and endocrine changes in adipocytes and promote systemic inflammation when exposure is prolonged. Moreno-Fernández et al. (30) further demonstrated that high-fat/high-glucose feeding accelerates the development of metabolic syndrome in rats, reinforcing the notion that longer durations are required to capture the full extent of diet-induced impairment. In contrast, the rise in TyGI in the ordinary diet group may reflect the sensitivity of this index to understated metabolic shifts, as validated by Guerrero-Romero et al. (31) and Navarro-González et al., (32) who established TyGI as a reliable marker of insulin resistance and diabetes risk even in populations with normal fasting glucose. Thus, while both groups showed early increases, the trajectory of change suggests that high-fat diets may exacerbate metabolic risk over time. So, current findings verify the link between dietary fat intake and adverse metabolic outcomes observed in both animal models and human studies.

The AIP was more obvious in a group with a modified diet than in a group with an ordinary diet. A higher AI indicates a greater risk of developing atherosclerosis, the plaque buildup in arteries leading to CVD (33). Modified diet content is known to promote dyslipidemia, characterized by increased levels of atherogenic lipoproteins such as low-density lipoprotein cholesterol (LDL-C) and decreased levels of anti-atherogenic lipoproteins such as HDL-C. These alterations contribute to an unfavorable lipid profile and elevate the AIP, reflecting an increased risk of atherosclerosis and cardiovascular diseases (34-36). High-fat diets have established models for inducing atherogenic changes in rats, they typically lead to elevated LDL-C and triglycerides, along with a decreased HDL-C, resulting in a higher AIP (37). The type of fat plays a role; saturated fats tend to increase the LDL-C and AIP, while unsaturated fats, particularly polyunsaturated fats, can have a lowering effect (38). In this study, eight weeks may not be enough time for significant changes in body composition or established metabolic pathways and factors like weight gain or lipid profile might take longer to develop with a study needs more than 12 weeks (39).

In conclusion, both TyGI and AIP showed marked increases with no change in LI in both types of feeding for 8 weeks, which indicates a real change in lipid profile and blood glucose despite unchanging weight and height expressed by LI. Additionally, the type of food showed no difference on any studied index that suggests an insufficient interval of 8 weeks to make marked differences.

Future studies extending beyond 8 weeks, with additional markers of inflammation and insulin sensitivity, will be essential to determine whether the modified diet produces sustained and clinically relevant metabolic disturbances compared to ordinary diet.

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Author contribution
Oudah: Data collection, experimental work, writing the manuscript draft. Dr. Ahmed: Study design, statistical analysis, and final revision of the manuscript.

Conflict of interest
The authors declare that there was no conflict of interest.

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