

The effect of calorie restricted diet on anthropometric and biochemical parameters in Sprague Dawley rats

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Received 22 January 2021
Revised 1 March 2021
Accepted 12 March 2021

Abstract

Purpose – Calorie restricted diets are known to improve health and promote healthy aging. This happens because of controlled inflammation and metabolism in the body. This study aims to evaluate the differences in anthropometric and biochemical parameters in Sprague Dawley (SD) rats because of consumption of a calorie restricted diet.

Design/methodology/approach – The study divided SD rats ($n = 15$) into 3 groups consuming high calorie intake (HCI), medium calorie intake and low calorie intake (LCI). Anthropometric parameters were determined through measurement of abdominal circumference (AC), thoracic circumference (TC), body length and body weight (BW). Biochemical parameters analyzed in this study were fasting blood glucose level and full blood lipid profile. Nutritional status was obtained based on food consumption, energy intake (EI) and food efficiency rate. Measurements were taken for a period of four weeks.

Findings – Analysis on anthropometric parameters indicates a significant difference in mean BW between HCI (230.44 ± 1.47 g) and LCI (188.54 ± 1.50 g). There is a significant difference in abdominal TC ratio ($p < 0.001$; $F = 13.599$) in the LCI group (1.01 ± 0.00714) compared to the HCI group (1.04 ± 0.00858). Post hoc for nutrition parameters indicates a significant difference in mean EI between HCI (9.71 ± 0.006 kJ) and LCI (3.21 ± 0.001 kJ). There is a significant effect ($p < 0.0001$; $F = 3042872.02$) of EI on rats in all three groups. HDL levels were significantly higher ($p < 0.0001$; $F = 1536.89$) in the LCI group (68.60 ± 0.55 mg/dL) compared to the HCI group (49.40 ± 0.55 mg/dL). The Pearson's correlation results show a strong positive correlation in EI with BW ($p < 0.01$; $r = 0.988$), AC ($p < 0.01$; $r = 0.970$) and body mass index ($p < 0.01$; $r = 0.972$).



Originality/value – Low calorie diet has been proven to affect anthropometric development and has shown improvements in biochemical parameters of the rats. This may result in healthy aging which could prevent later-life diseases.

Keywords Nutrition, Obesity, Anthropometric, Biochemical, Calorie restricted diet

Paper type Research paper

Introduction

Obesity is a major public health concern worldwide because of its high prevalence within the past decade. According to WHO (2016) worldwide data, approximately more than 1.9 billion people of age >18 years are known to be overweight. Out of these, more than 650 million individuals were found to be obese (WHO, 2016). Obese or overweight is a serious condition which can lead to severe non-communicable diseases such as a cardiovascular disease or a kidney disease (Lambrinou *et al.*, 2019). Moreover, obesity can give rise to age-related health complications such as type 2 diabetes mellitus (T2DM), dyslipidemia, cancers and non-alcoholic fatty liver disease (Brahe *et al.*, 2016). It is known to be one of the main risk factors contributing to motility and morbidity rate in chronic diseases (Szewczyk-Golec *et al.*, 2017). Increased calorie intake remains to be one of the main causes giving rise to the obesity epidemic. For this reason, there is a growing demand for effective weight loss methods and diet strategies when it comes to treatment and prevention of obesity. Specifically, there is no effective long-term weight loss strategy proven safe to be used by overweight individuals. Energy restricted diets are currently the most popular and commonly used treatment in obesity (Dayan *et al.*, 2019). However, this particular method of treatment greatly requires further studies to confirm their effects on biochemical and anthropometric parameters.

Previous studies have shown that a calorie intake reduction up to 20–50% without malnutrition can prolong lifespan up to 50% in some strains of rats and mice (Most *et al.*, 2017). A chronic reduction of dietary energy intake (EI) by approximately 30% without incurrence of malnutrition has been found to be a broadly effective dietary intervention. It significantly decreases the adiposity, inflammation and is known to improve metabolic profiles among non-obese humans and rodents (O'Flanagan *et al.*, 2017). Metabolic changes in response to lower caloric intake has proven to encourage certain safety characteristics such as improved insulin sensitivity and reduced blood glucose levels (Templeman *et al.*, 2017). Moreover, it has shown to decrease chances of age-related complications such as cancer, diabetes, cardiovascular and neurodegenerative diseases in experimental models (O'Flanagan *et al.*, 2017).

Restriction of calories without starvation is the most studied experimental technique among experimental models. Studies conducted in multiple animal models have shown that calorie restriction can improve health duration along with lifespan. Over the past two decades, the physiology of aging research community has held a close watch on calorie restriction studies on non-primates. In addition to these findings, there is plenty of continuous research ongoing to study the impact of calorie constrains on metabolic and molecular adaptations in humans (Most *et al.*, 2017). Systematic reviews conducted in obese/overweight women have showed that the amount of visceral adipose mass and inflammatory cytokine levels to have improved along with the adiponectin/leptin ratio upon consuming energy restricted diets (Razmpoosh *et al.*, 2020). Furthermore, calorie restriction also remains to be the most successful intervention to date which has proven to delay the progression of aging and age-related chronic diseases.

The study aims to determine the effect of calorie restricted diets on the anthropometric and biochemical parameters in Sprague Dawley (SD) rats. Thus, the effect of changes in body weight (BW), body length (BL), abdominal circumference (AC) and thoracic circumference (TC) between high caloric intake (HCI), medium caloric intake (MCI) and low caloric intake (LCI) at different intervals were monitored. In addition to this, changes in biochemical parameters such as fasting blood glucose level (FGL), LDL, HDL and triglycerides (TG) cholesterol levels were also studied within the experimental groups. The study attempts to observe the consumption of food and EI between experimental intervals by all experimental animals.

Nutrition research and experimental studies often use animal models such as rodents as subjects instead of humans. Rodent animals such as rats and mice provide researchers with opportunities to study how factors such as genetics affect them. This can be achieved by using transgenic and knockout strains of mice (Vaughan *et al.*, 2017). Moreover, animals are crucial for both *ex vivo* and *in vivo* nutrition studies as non-nutrient and nutrient elements present in food can alter with metabolic pathways by means of various unknown mechanisms. Hence, it is recommended to use animal models for nutrition-based studies before validating and translating it to humans (Chalvon-Demersay *et al.*, 2017).

Some of the previous dietary intervention studies conducted in animals consisted of either monitoring their effects in relation to a certain drug or a specific disease. Up until now, most of the studies concerning anthropometry and biochemical parameters were conducted alongside physical activity. Therefore, current study was strictly focused on monitoring the effects of dietary intervention on anthropometric and biochemical parameters without inclusion of physical activity or any drug. Thus, this paper aims to determine the difference in anthropometric and biochemical parameters as an effect of consuming a calorie restricted diet by SD rats between experimental groups HCI, MCI and LCI. The results of this study showed important relationships between variables of anthropometric, biochemistry and nutritional parameters with regard to calorie restriction. Variations in EI per day have played a role in displaying differences between experimental groups.

Methodology

Research design

The experiment in this study used healthy SD rats obtained from Animal Laboratories Department Health of Science, Management and Sciences University (Shah Alam, Malaysia). This study used SD rats ($n = 15$) weighing 61.51 ± 5.62 g (3 weeks old). They were each kept individually in standard animal housing at a controlled room temperature (22.24°C) with 12-h day/night cycle with free access to standard pellets, Gold Coin pellets (Port Klang, Malaysia) and tap water, ad libitum (Fattepur *et al.*, 2018). The acclimatization period for the rats took 10 days after which all rats were weighed and divided into 3 experimental groups, namely, HCI ($n = 5$), MCI ($n = 5$) and LCI ($n = 5$). This study used the “resource equation” method for sample size calculation because of the lack of availability of effect size and standard deviation estimates from previous studies (Charan and Kantharia, 2013).

This study prepared three different diets. Calories for the diets were calculated in the form of energy per gram (kJ/g) before being fed to the respective experimental groups. For the purpose of growth requirements, the rats consumed 8–25 g amount of food intake gram per day (g/day) (Ritskes-Hoitinga and Chwalibog, 2002). This study implemented a standardized procedure for the groups. They were standardized by providing food consumption (FC) of 5 g/100g per BW of each rat (Atiqah *et al.*, 2015).

The study fed the LCI group with only standard pellets and fed the HCI group with a homemade semi-solid and semi purified fat foods along with standard pellets (Hintze *et al.*, 2018; Sheng *et al.*, 2017). The study fed the MCI group 60% of the daily food intake of HCI diet (Hussain *et al.*, 2016; Park *et al.*, 2012). MCI group is considered as the control group in this study as their nutrient composition amounts are at a balance between HCI and LCI diet composition. LCI group is provided with a standard rat chow diet where in the fat component amounts up to 3% while the other nutrient components are restricted in comparison to HCI and MCI nutrient components. Hence is why, the LCI group is not considered as a control group in this study despite being fed a standard rat chow diet. The study used diet concepts prepared using diet dilution methods which are highlighted under diet preparation (Delorme *et al.*, 1981; Nk and Naa, 2013; Samadi *et al.*, 2017). For four weeks, the study monitored FC and the BW for careful characterization of energy ingestion and weight gain. In addition, the study assessed the anthropometric parameters and biochemical parameters on a weekly basis in all groups for four weeks.

Ethical consideration

This experiment was performed at the Animal House Laboratory, Management and Science University, Shah Alam, Malaysia. The Ethics Committee at Management and Science University approved all experimental protocols with respect to ethics code: MSU_RMC-02/FR01/02/L3/020. All efforts were made to follow the ethical principles of animal handling and to minimize animal suffering.

Diet preparation

The HCI diet approximately contains 5.24 kcal/g with 8.4% protein, 19.5% carbohydrates, 61.2% fats and 10.9% others (Atiqah *et al.*, 2015; Konopelnjuk *et al.*, 2015). Ingredients used for the diet preparation were standard pellet (40 g) and pure duck yolk fats (60 g) (Echeverria *et al.*, 2018; Getz and Reardon, 2006; Hintze *et al.*, 2018; Karaçor *et al.*, 2014). Diet preparations were carried out under sterile conditions on a weekly basis. The same ingredients were used in the preparation of MCI diet with 64 g of the standard pellet and 36 g of duck yolk fats. The LCI diet entirely consists of standard pellet. Every 100 g of a standard pellet diet contains 48.8% carbohydrates, 3% fats, 21% proteins and 27.2% others (Beheshti *et al.*, 2017). Diet compositions and ingredient dilution followed for each diet were detailed in Tables 1 and 2, respectively.

	HCI	MCI	LCI
Nutrients	%/100g	%/100g	%/100g
Carbohydrates	19.5	31.2	48.8
Protein	8.4	13.4	21
Fat	61.2	37.9	3
Others	10.9	17.4	27.2
Total energy	5.24 kcal/g	4.366 kcal/g	3.062 kcal/g

Table 1.
Diet composition for
100 g preparation

Notes: Other nutrients are made up of calcium, phosphorous, fiber, moisture and ash. A total of 60% of the 61.2% fats in HCI diet is from duck yolk fats. A total of 36% of 37.9% fats in MCI diet is from duck yolk fats

Anthropometrical parameters

The study used a measuring tape to measure the AC (immediately anterior to the forefoot), TC (immediately behind the foreleg) and BL (nose–anus length) of the rats (Konopelnyuk *et al.*, 2015; Novelli *et al.*, 2007). An electrical balance (A&D GF3000) was used to monitor the BW. All the parameters were measured and the average for all parameters was calculated on a weekly basis for each group. BW and BL measurements were used to calculate the body mass index (BMI) and specific rate of body mass gain (SRBMG) of the rats as formulated below. The normal BMI for male rats is known to be between $0.45 \pm 0.02 \text{ g/cm}^2$ and $0.68 \pm 0.05 \text{ g/cm}^2$ (Atiqah *et al.*, 2015):

- $\text{BMI} = \text{BW (g)}/\text{length}^2 \text{ (cm}^2\text{)}$,
- $\text{SRBMG (g/kg)} = dM/M \text{ dt}$,

where dM is the gain of BW during $dt = t2-t1$ and M is the rat BW at $t1$.

Nutritional parameters

Uneaten food was weighed everyday using an electrical balance. The uneaten food weight was then subtracted from the total amount of food provided to calculate the daily food intake. The food intake amount is considered to be the amount of food (g) consumed by each rat within 24 h (Patel *et al.*, 2017). The food intake amounts were used to calculate EI (kJ/day) and food efficiency rate (FER) (Novelli *et al.*, 2007). The formulas used for the calculation are:

- $\text{EI (kJ/day)} = \text{mean of FC} \times \text{dietary metabolized energy}$.
- $\text{Food efficiency (FE, g gained/kcal)} = (\text{mean of BW gain} \times 100)/\text{total EI (kcal)}$.

Biochemical parameters

SD rats were fasted for 12 h or overnight for all weekly assessments of the biochemical parameters. The study used a 26 G needle and a syringe to withdraw up to 0.2 ml of blood from the marginal tail of the rats. The blood samples were collected during the fasting state

HCI		MCI		LCI	
Ingredients	Gram	Ingredients	Gram	Ingredients	Gram
Standard pellet	40	Standard pellet	64	Standard pellet	100
Duck yolk fat	60	Duck yolk fat	36	Duck yolk fat	0

Notes: *For 100 g of diet preparation, HCI diet consists of 40 g standard pellet with 60 g duck yolk fat. MCI diet is made up of 64 g standard pellet with 36 g of duck yolk fat. LCI is entirely 100 g standard pellet with no added extra ingredient

Table 2.
Diet dilution for
100 g preparation

EI (kJ/g)	Week 1	Week 2	Week 3	Week 4
HCI	3.29 ± 0.01^a	6.02 ± 0.01^a	7.95 ± 0.01^a	9.71 ± 0.01^a
MCI	2.18 ± 0.01^b	3.99 ± 0.00^b	5.28 ± 0.01^b	6.45 ± 0.00^b
LCI	1.08 ± 0.00^c	1.98 ± 0.00^c	2.62 ± 0.00^c	3.20 ± 0.00^c

Note: *EI between groups have increased in all 3 groups within the span of 4 weeks (^a, ^b and ^c denote significant difference at $p < 0.05$ between groups)

Table 3.
EI between groups
for four weeks

(Saxena *et al.*, 2017). Fasting blood samples taken were used to monitor blood glucose levels as well as blood lipid profile. An Accu-check Oncall-plus glucose meter and test strips were used to determine FGL (Ghanbari *et al.*, 2016). Readings were obtained in mg/dL. The blood lipid profiles of the rats were analyzed using a handheld 3-in-1 Combo Test Device for a complete lipid panel (Mission cholesterol meter). Blood samples collected for blood lipid profiling were allowed to stand for 15 min to clot. They were then centrifuged at 6,000 rev/min for 5 min. Up to 35 μ l of serum was collected with Pasteur pipette and introduced into cholesterol test strips. The results were displayed within 60s after the insertion of the MEMO clip (Banda *et al.*, 2018). HDL, TG and total cholesterol values obtained from the meter were used to calculate the LDL levels using the Fredwald's equation shown below (Francis *et al.*, 2019).

$$\text{LDL cholesterol} = \text{total cholesterol} - (\text{HDL cholesterol}) - (\text{TG}/5).$$

Statistical analysis

All data were analyzed using SPSS for windows version 23. The paper uses data reported as mean \pm standard deviation. Continuous variables with parametric distributions were analyzed using the analysis of variance (ANOVA) and if the results were significant, a post-hoc test was performed. The differences between groups were defined to be statistically significant if p -value was less than 0.05 (Konopelnyuk *et al.*, 2015). Differences of parameters between baseline and week four within groups were analyzed using repeated measures ANOVA.

Results

Food intake

All SD rats from week 1 to 4 had a FC of 8.48 ± 0.24 g to 25.02 ± 0.15 g of food. Based on the post-hoc results of week four, there was a significant difference in EI by rats at $p < 0.05$ in three diet groups ($F = 3042872.02$, $p < 0.0001$). There was a significance difference in the FER of LCI at $p < 0.05$ with MCI and HCI ($F = 59.55$, $p < 0.0001$). As the FC and EI significantly increased up to four weeks, the rate of food efficiency significantly decreased among the rats as shown in Figure 1. Mean EI (kJ/g) for each experimental interval between groups are demonstrated in Table 1. According to Table 1, EI within groups have increased

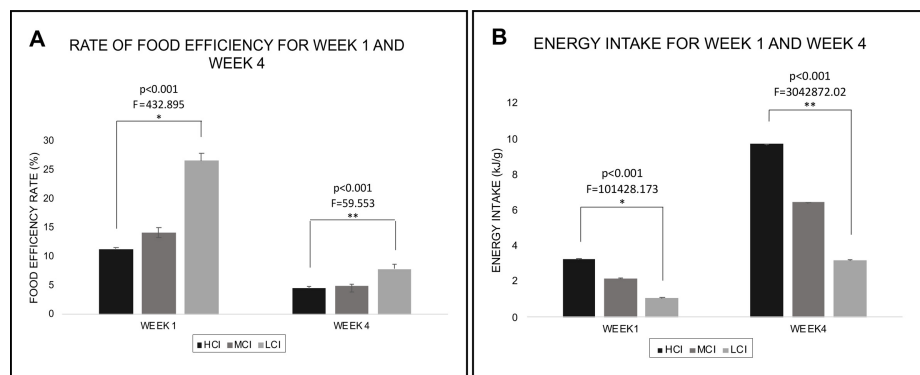


Figure 1. Changes in nutritional parameters between groups

Notes: FER (%) between groups at week one and week four (A). EI (kJ/g) between groups at week one and week four (B)

significantly during the course of four weeks. EI for HCI, MCI and LCI increased from 3.29 ± 0.01 kJ/g to 9.71 ± 0.01 kJ/g, 2.18 ± 0.01 kJ/g to 6.45 ± 0.01 kJ/g and 1.08 ± 0.01 kJ/g to 3.20 ± 0.01 kJ/g, respectively, at $p < 0.0001$. Increase is observed to be highest in HCI group followed by MCI and LCI groups.

Analysis of anthropometric parameters. BW significantly increased in all SD rats by week four between HCI (mean, MCI and LCI experimental groups). The mean score for BW significantly increased in HCI (M = 230.44, SD = 1.47), MCI (M = 204.30, SD = 0.72) and LCI (M = 188.53, SD = 1.50) at $p < 0.05$. Significant increase was observed in average BL at week 4 in HCI (M = 19.12, SD = 0.08), MCI (M = 18.84, SD = 0.05) and LCI (M = 18.54, SD = 0.05). The average weekly increase of BW and BL for all experimental groups is shown in [Table 4](#). The weekly mean BMI and abdominal thoracic circumference (ACTC) ratio between groups are displayed in [Table 5](#). [Figure 2A](#) shows the weekly increase of BMI. A significant effect was observed in BMI of the rats at $p < 0.05$ in all three groups (F = 316.27, $p < 0.0001$). The SRBMG gain was significantly decreased with the increase of the age of the SD rats used ([Figure 2B](#)). AC ([Figure 2C](#)) and TC ([Figure 2D](#)) have increased throughout the experiment.

There was a significant difference in TC at $p < 0.05$ in LCI (M = 10.38, SD = 0.12) with both MCI (M = 10.50, SD = 0.12) and HCI (M = 10.72, SD = 0.08). Significant increase was recorded in the ACTC ratio ([Figure 2E](#)) between LCI (M = 1.02, SD = 0.01) and HCI (M = 1.04, SD = 0.01) at $p = 0.002$. Based on the Pearson correlation results, a strong positive correlation was observed between the final BW and the final BL of the SD rats at week 4, $r = 0.957$, $n = 15$ and $p < 0.0001$.

Analysis of biochemical parameters. According to FGL demonstrated in the [Figure 3A](#), levels remained the same for HCI group at week one and week four. A decrease is observed in FGL at week four among the LCI group in comparison to week one levels. However, there was no significant difference in FGL at $p < 0.05$ in LCI (M = 87.93, SD = 1.50) with both MCI (M = 88.65, SD = 1.50) and HCI (M = 90.09, SD = 1.27) at week four. Repeated measured ANOVA conducted between HCI and LCI group at week one and week four showed no significant difference in FGL ($p = 0.585$ and $p = 0.091$, respectively).

[Figure 3B](#) shows that LDL levels remained constant between groups at week 1. LDL levels for HCI group and MCI group have increased from week one to week four while the levels remained the same for the LCI group. Increase appears to be highest among the HCI group in comparison to MCI group. There is a significant difference in LDL cholesterol level observed at week four for HCI group (M = 18.44, SD = 1.61 mg/dL) than with the MCI group (M = 13.80, SD = 0.37 mg/dL) and LCI group (M = 11.28, SD = 0.30 mg/dL) at $p < 0.05$. Repeated measured ANOVA conducted to compare LDL levels between HCI and LCI group at week one and week four showed a significant difference at week four ($p < 0.001$).

[Figure 3D](#) shows the HDL levels among experimental groups at week one and week four. Week one of the experiment demonstrates a constant HDL level between the HCI (M = 68.40, SD = 1.14), MCI (M = 69.40, SD = 0.54) and LCI (M = 69.20, SD = 0.83) groups. A decrease in HDL levels is observed in both HCI and MCI groups at week four while the HDL level remained at a constant for LCI. There is a significant difference noticed in week-four HDL levels between HCI (M = 49.40, SD = 0.54 mg/dL) with MCI (M = 59.40, SD = 0.54 mg/dL) and LCI (M = 68.60, SD = 0.54 mg/dL) at $p < 0.0001$.

TG levels between groups for week one and week four are shown in the [Figure 3C](#). TG levels at week four for HCI, MCI and LCI were noted to be (M = 142.80, SD = 1.92 mg/dL), (M = 106.20, SD = 1.64 mg/dL) and (M = 94.40, SD = 0.54 mg/dL), respectively. No significant increase is observed for LCI group from week one (M = 92.40, SD = 1.67) to week four (M = 94.40, SD = 0.54). An increase in TG levels is observed for both HCI and MCI group from week

Table 4.
 BMI (g/cm²),
 SRBMG (g/kg), AC
 (cm), TC (cm) and
 ACTC ratio at week
 one and week four

Groups Parameters/week	HCI		MCI		LCI	
	1	4	1	4	1	4
BMI	0.30 ± 0.01 ^a	0.63 ± 0.01 ^b	0.26 ± 0.01 ^a	0.58 ± 0.00 ^b	0.25 ± 0.01 ^a	0.55 ± 0.01 ^b
SRBMG	0.01 ± 0.00 ^a	0.01 ± 0.00 ^b	0.02 ± 0.00 ^a	0.02 ± 0.00 ^b	0.02 ± 0.00 ^a	0.01 ± 0.00 ^b
Abdominal (cm)	10.58 ± 0.13 ^a	11.16 ± 0.05 ^b	10.36 ± 0.11 ^a	10.7 ± 0.07 ^a	10.10 ± 0.10 ^a	10.38 ± 0.11 ^a
Thoracic (cm)	10.52 ± 0.11 ^a	10.72 ± 0.08 ^a	10.36 ± 0.11 ^a	10.50 ± 0.12 ^a	10.10 ± 0.10 ^a	10.18 ± 0.13 ^a
ACTC ratio	1.00 ± 0.01 ^a	1.04 ± 0.01 ^b	1.00 ± 0.01 ^a	1.02 ± 0.01 ^b	1.00 ± 0.00 ^a	1.02 ± 0.01 ^b

Notes: *BMI, SRBMG, AC, TC and ACTC ratio have increased from week one to week four in all groups. ^a, ^b and ^c denote significant difference at $p < 0.05$ within groups at week one and week four

one to week four. According to the post-hoc results of week four, there is a significant increase of TG levels for both HCI group and MCI group at $p < 0.0001$.

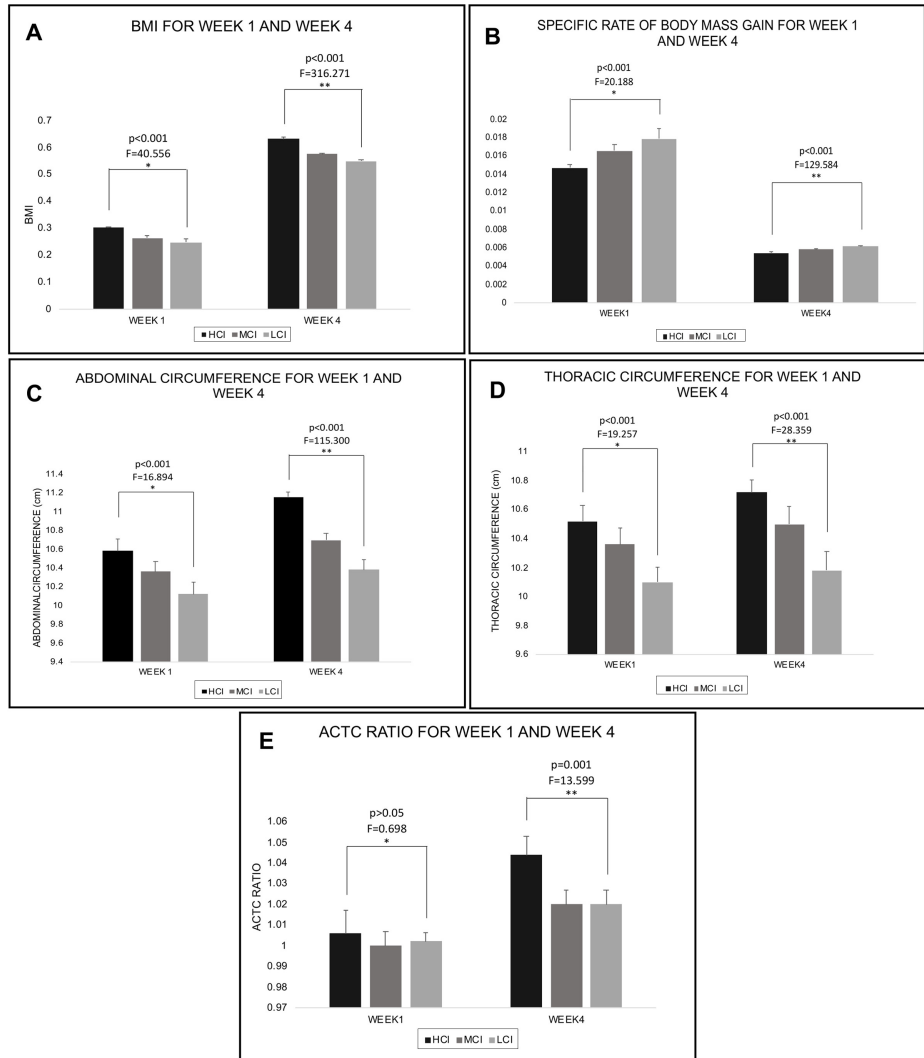
Discussion

The study used repeated measures ANOVA to study the difference in anthropometric and biochemical parameters within groups during the beginning (baseline) and end (week four) of the experiment. It helped to understand and assess the changes taking place within the groups over time. Present study shows a significant increase in BW, BMI and ATCT ratio between groups at week four. The HCI group was found to show a greater increase in these parameters in comparison to MCI and LCI. This increase is because of increased EI via a high calorie diet. Amount of EI and FER among groups showed an increase from week one to week four (Figure 1). Although the LCI group rats did not show any decrease in these parameters, the rate of increase in these parameters was noticeably lesser than the other two experimental groups. According to Romieu *et al.* (2017), increased energy consumption is the major cause behind excessive weight gain leading to obesity (Romieu *et al.*, 2017). Obesity is a condition highly correlating to oxidative stress. The condition can cause free radicals in the body to increase while simultaneously reducing the number of antioxidants neutralizing them. This could lead to chronic inflammation and adverse effect into cell survival within the body. A study conducted by Sandra *et al.* (2019) demonstrated the suppression of high fat diet (HFD)-induced liver steatosis by co-administrating the antioxidant hydroxytyrosol (HT) and *n*-3 PUFA docosahexaenoic acid (DHA) in weaning male mice. The study used 14 animals. They were randomly divided into control diet group and HFD group. Each group was supplemented with either saline or HT or DHA or HT and DHA. The experiment results showed an increase in liver fat contents such as FFA, TGs and cholesterol for the HFD group. HFD group further showed a significant correlation with SREBP-1c mRNA levels. SREBP-1c upregulation in the liver can activate expression of other genes in the liver involved in FA synthesis such as PERK, ATF6 α , ACC, FAS and SCoAD-1. These changes were paralleled by a down regulation of PPAR α in HFD. PPAR α down regulation can cause oxidative stress enhancement in the liver. This was evidenced by the increased ROS and TBARS (Soto-Alarcón *et al.*, 2019). The oxidative stress effect is further supported by the study conducted by Takatsu *et al.* (2013). They used DahlS.Z-*Lepr^{fa}/Lepr^{fa}* (DS/obese) rats which belong to a cross breed between Zucker rats and Dahl-salt sensitive. The rats were divided into a normal group consuming a chow diet and a 65% calorie restricted diet. The experiment was done for a period of 13 weeks. Findings of the results showed a reduction in ameliorated left ventricular hypertrophy, body fat content, fibrosis and diastolic dysfunction among DS/obese rats present in calorie restricted group. Moreover, results further showed calorie restriction to have attenuated the inflammation in the rats as well as oxidative stress.

Week	BW (g)			BL (cm)		
	HCI	MCI	LCI	HCI	MCI	LCI
0	67.92 ± 1.62 ^a	60.52 ± 2.53 ^b	56.08 ± 3.45 ^b	18.50 ± 0.00 ^a	18.62 ± 0.04 ^b	18.52 ± 0.04 ^a
1	104.96 ± 1.24 ^a	91.59 ± 2.8 ^b	85.03 ± 4.72 ^c	18.66 ± 0.05 ^a	18.62 ± 0.04 ^a	18.52 ± 0.04 ^b
2	151.20 ± 0.73 ^a	130.73 ± 3.19 ^b	122.27 ± 5.85 ^c	18.76 ± 0.05 ^a	18.62 ± 0.04 ^b	18.52 ± 0.04 ^c
3	186.66 ± 3.88 ^a	172.59 ± 1.46 ^b	163.52 ± 1.18 ^c	18.82 ± 0.04 ^a	18.64 ± 0.05 ^b	18.62 ± 0.04 ^b
4	230.4 ± 1.47 ^a	204.30 ± 0.722 ^b	188.54 ± 1.50 ^c	19.12 ± 0.08 ^a	18.84 ± 0.05 ^b	18.54 ± 0.05 ^c

Notes: *Overall increase in BW and BL are observed in all groups for four weeks. ^a, ^b and ^c denote significant difference at $p < 0.05$ between groups

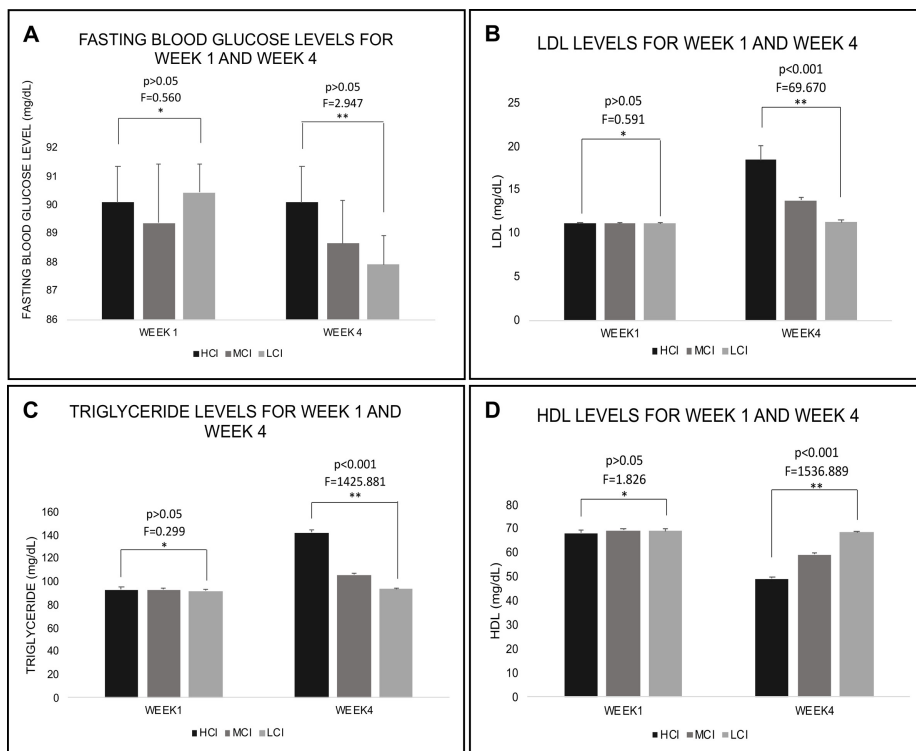
Table 5.
Weekly average BW
(g) and BL (cm)
between groups

**Figure 2.**

Anthropometric changes between groups

Notes: BMI between groups at week one and week four (A). SRBMG between groups at week one and week four (B). AC between groups at week one and week four (C). TC between groups at week one and week four (D). ACTC ratio between groups at week one and week four (E)

Apart from this, the expression of angiotensin II type 1A receptor genes and the angiotensin-converting enzyme present in the heart were found to be dysregulated (Takatsu *et al.*, 2013). Oxidative stress is a process known to be linked with age-related and aging diseases such as cancer, neurodegeneration, diabetes and cardiovascular diseases. Calorie restriction is a regimen known to prevent age-related diseases by reducing oxidative stress. Experimental studies have shown that calorie restriction can reduce aging by decreasing the



Notes: FGL (mg/dL) between groups at week zero and week four (A). LDL levels (mg/dL) between groups at week zero and week four (B). TG levels (mg/dL) between groups at week zero and week four (C). HDL levels (mg/dL) between groups at week zero and week four (D)

Figure 3.
Changes in
biochemical
parameters between
groups

amount of ROS production in mitochondria and increasing the antioxidant enzyme activity. Antioxidant enzymes such as the catalase, glutathione and superoxide dismutase are responsible for detoxifying ROS and other harmful radicals (Walsh *et al.*, 2014). A study conducted by Daniele La *et al.* (2020) found that calorie restriction can reverse aging in aged and obese rats. The study used young and aged rats where aged rats were divided into two groups (control group and calorie restriction group). Calorie restriction was given for a period of six months. The results showed that calorie restriction increased the plasma adiponectin levels and simultaneously reduced the levels of anti-inflammatory protein TSG-6 pro-inflammatory marker. It brought about improvements in redox balance along with inflammatory process in tissues and plasma levels. This was supported by decreased expression of GSTP-1 anti-oxidant. GSTP-1 is an anti-oxidant which possess proliferative activity (La Russa *et al.*, 2020). Based on these studies, we can safely agree that calorie restriction can promote healthy aging in rodents.

HCI group rats were recorded to have a significantly high amount of LDL cholesterol levels. Eating a high calorie diet can cause LDL levels to increase. This in turn increases TG levels in the blood and causes a reduction in HDL cholesterol levels. HCI rats were recorded to have low HDL levels and high TG levels in this study. Increased EI could lead to an

accumulation of fat in the body. The findings of this study are in line with a study conducted by [Gao *et al.* \(2017\)](#) to study the effect of dietary restriction (DR) in hyperlipidemic mice. The study was conducted for a period of five weeks. Mouse models were divided into three groups, namely, HFD, DR 30% group and DR 50% group. DR 30% group consumed 70% of HFD while DR 50% consumed 50% of HFD. The results of the study showed a significant decrease in serum lipids along with serum glucose levels, liver index, average BW and liver weight among both DR groups in oppose to HFD ([Gao *et al.*, 2017](#)). A different study done by [Salesi and Mehrtash \(2018\)](#) examined the effects of calorie restricted diet on lipid coat proteins of SD rats. Lipid coat proteins are proteins which can prevent lipolysis of adipose tissue. A total of 30 male adult SD rats were used in this study. After eight weeks of acclimatization, the rats were divided into two groups: calorie restricted diet group and HFD group. PCR was used to assess the expression levels of lipid coat proteins while HOMA-index was used to calculate the insulin resistance. Findings of this study reported to have a significant decrease of weight and insulin resistance in calorie restricted group. It also showed that expression levels of lipid coat protein perilipin-1 were higher in the skeletal muscle of the HFD group in comparison to calorie restricted group. Thus, this study demonstrates that calorie restriction can prevent insulin resistance along with the accumulation of fat ([Salesi and Mehrtash, 2018](#)). Additionally, our data is further supported by a study done by [Kim *et al.* \(2016\)](#) where they investigated the metabolic changes in obesity induced SD rats. The study used a Hyperpolarized ^{13}C magnetic resonance spectroscopy to measure the metabolic and serum enzyme levels. Ten SD rats of seven weeks old were randomly divided into two groups. Five rats were fed a HFD while remaining five rats were fed a normal diet. Serum biochemistry results showed HFD rats to have significantly increased cholesterol and serum ALT levels. Increased levels of metabolites such as alanine pyruvate ratio were observed in HFD rats along with increased BW and LDH levels. Macro vesicular steatosis and ballooning degeneration in hepatocytes were identified in histopathological examination of liver tissues of HFD rats ([Kim *et al.*, 2016](#)).

Several animal research studies have shown that calorie restriction boosts cardiovascular function by reducing cholesterol levels of LDL and TG ([Longo *et al.*, 2015](#)). Calorie restriction has been known to reverse the effects of obesity-related cardiovascular diseases. This is caused as a result of increased HDL levels. HDL concentration is a traditional marker used as risk stratification for atherosclerotic cardiovascular diseases. HDL possesses cardio protective properties because of its anti-oxidant and anti-diabetic properties. They have the potential to inhibit vascular inflammation and enhance endothelial function ([Barter and Genest, 2019](#)). Calorie restriction can ameliorate the two major risk factors of an ischemic heart disease known as hyperglycemia and hyperlipidemia ([Fontana and Klein, 2007](#)). Furthermore, it has been known to reduce the formation of atherosclerotic lesion along with the accumulation of visceral fat ([Guo *et al.*, 2002](#)). In the present experiment, LCI group rats were found to have reduced levels of LDL cholesterol and TG from week one to week four of the study ([Figure 3B and C](#)). It was also noticed that LCI rats had increased HDL cholesterol in week four when compared to week one ([Figure 3D](#)). These results are in line with the experiment conducted by [Melo *et al.* \(2016\)](#). Their study investigated the effects of calorie restriction on risk factors of cardiovascular diseases in adult Wistar rats. The results showed that calorie restricted Wistar rats had increased cardiac functions with reduced lipid profiles for LDL and TG ([Melo *et al.*, 2016](#)). Apart from the aforementioned study, an experiment conducted by [Ciobanu *et al.* \(2017\)](#) tested the hypothesis that calorie restriction can reduce the incidence of age-related metabolic syndrome and obesity. This strategy is thought to have potential for preventing consequences relating to an ischemic stroke in the future. SD rats used for this experiment were randomly

divided into 3 groups: 30 young aged control, 30 aged control and 30 aged calorie restricted group. The experiment was conducted for a period of eight weeks. The rats were fasted overnight and then anesthetized to carry out the experimental infarction. At the end of the study, it was found that there was a continuous reduction in BW among aged control rats. Whereas, no significant BW decrease was observed in those rats which consumed the calorie restricted diet. A significant post-stroke increase was seen in serum glucose, IGF-1 levels and insulin among calorie restricted group rats. This means that there is a possibility for enhancement in recovery when a rat already consuming a calorie restricted diet is subjected to stroke. At the same time, the study's findings showed that over nutrition can lead to slow recovery from stroke (Ciobanu *et al.*, 2017). Hence, with regard to this literature, care should be given to avoid over nutrition or over feeding to laboratory-based animals as it is considered unhealthy.

Apart from obesity, increased calorie intake can potentially cause increased adipose store. This could lead to other chronic disorders such as metabolic syndrome and T2DM (Nikhra, 2018). Metabolic syndrome consists of multiple combinations of metabolic disorders such as dyslipidemia, hyperlipidemia, obesity and impaired glucose tolerance. In the current study, LCI rats showed a decrease in FGL throughout the duration of this experiment while it remained more or less the same for HCI and MCI rats (Figure 3A). Increased blood glucose level can be caused because of T2DM as a result of insulin resistance. Intake of high calorie diet could result a spike in blood glucose levels. However, in case of LCI without malnutrition, the blood glucose amount can be decreased to an appropriable amount in blood thereby preventing the risk of T2DM. A study conducted by Wong *et al.* (2018) have shown that intaking a high carbohydrate high fat (HCHF) diet can result in significant changes in metabolic syndrome parameters. This experiment was conducted for 16 weeks. A total of 14 male Wistar rats were used in this experiment. At the beginning of the study, they were randomly divided into two groups: HCHF diet and normal standard chow diet group. Results showed a significant increase in systolic blood pressure, LDL cholesterol, dyslipidemia and glucose intolerance among HCHF rats in comparison to normal rats, thereby indicating that metabolic syndrome can be induced among rats consuming a HCHF diet (Wong *et al.*, 2018). In addition to this, Park *et al.* (2006) conducted a study by using 1.5 months and 18 months old rats to investigate the effect of calorie restriction in young age and middle age rats. The rats were subjected to 30% calorie restriction for a period of four to six weeks. Immunoblotting for protein abundance of phosphorylated insulin receptors was used for the analysis of quadriceps femoris muscle. They observed that calorie restriction greatly improved glucose tolerance and showed lower serum insulin response to glucose in both younger and middle age rats (Park *et al.*, 2006).

Limitations

The study was conducted for a period of one month. This is a considerably short duration of time to study the effects and changes of calorie restriction effectively. As a result of this, the current study was unable to report the long-term effects of consuming a calorie restricted diet. The sample size of the rats used in this experiment is less. Moreover, the rats used for this experiment are younger in terms of age and are not obese. This limits the extent to which accurate conclusions can be drawn when doing anti-aging and nutrition studies for obesity prevention. Zucker rats are known to be a good animal model for obesity studies. However, this experiment uses SD rats as the animal model. Therefore, additional correlation studies using obese Zucker rats are greatly needed to further confirm anti-aging effects of DR. Furthermore, only two biochemical parameters (blood glucose and blood lipid profile) were focused on this experiment, which is insufficient to confirm the long-term

effects of calorie restriction on biochemical aspects. This study further lacked histological evidences which are crucial to evaluate the extent of damage HCI can do to tissues.

Conclusion

In conclusion, our caloric restriction experiment showed significant changes in anthropometric measurements such as BMI, AC, TC and ACTC ratio and biochemical markers such as blood lipid profiles and blood glucose levels in SD rats. The nutrition parameters of EI and FER displayed how they could affect anthropometric and biochemical parameters based on the number of calories consumed by the diet. This study shows that efficiency of calorie intake and FC can affect blood cholesterol levels and BMI significantly. In future thought, more calorie restricted studies involving longer experimental duration is essential to support the findings of this study. In addition to this, other laboratory methods such as histological analysis and more molecular level analysis are required to gain a better understanding of the mechanism and morphological changes taking place inside the body as a result of consuming a calorie restricted diet. More experimental studies are needed to study the advanced effects of calorie restriction on parameters such as insulin level, growth hormones and organ weight before translating the results to humans. More repetition studies involving the aforementioned parameters could pave a way for an effective diet plan to be used in the treatment or prevention of obesity in the future.

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