



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)
Clinical Nutrition Experimental
 journal homepage: [http://
 www.clinicalnutritionexperimental.com](http://www.clinicalnutritionexperimental.com)



Original Article

Biochemical changes associated with low and very low calorie diets on lipid metabolism, iron profile and kidney function in obese rats

H.A. Abdel Maksoud ^a, Mohamed G. Elharrif ^{b,*}, Raafat R. Mohammed ^a,
 M.A. Omnia ^a, Nihal E. El Sayed ^a

^a Department of Biochemistry, Benha University, Egypt

^b Department of Basic Medical Sciences, Shaqra University, Saudi Arabia

ARTICLE INFO

Article history:

Received 16 May 2020

Accepted 30 July 2020

Available online 8 August 2020

Keywords:

Low calorie

Lipid metabolism

Iron profile

Kidney function

SUMMARY

Obesity is the most prevalent nutritional disorder. Healthy diets helps people maintain and improve their general health. This study aimed to assessment of low and very low calorie diets through determination of its effect on lipid metabolism, iron profile and kidney function in obese male rats. To achieve our aim forty five male albino rats were used and divided into 4 groups: control group, obese group with 75 calories diet, obese group with 60 calories diet and obese group with 45 calories diet. Our result showed significant disturbance of lipid, iron and kidney profile on supplementation of 75 calories diet to obese group of male rats and significant improvement in lipid, iron and kidney profile on supplementation of 60 calories diet or 45 calories diet to obese group of male rats. The results confirmed that, high fat diet able to produce significant disturbance in lipid metabolism, iron profile and kidney function while low calorie diets able to improving the disturbance in such parameter.

© 2020 The Author(s). Published by Elsevier Ltd on behalf of European Society for Clinical Nutrition and Metabolism. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

* Corresponding author.

E-mail address: al_harrif@yahoo.com (M.G. Elharrif).

1. Introduction

Obesity is a worldwide disease related to genetic, environmental, and behavioral factors and it is associated with high rates of morbidity and mortality [1]. It defined as an excess of body fat relative to lean body mass [2]. Clinically, obesity is defined on the basis of body mass index (BMI). Individuals with BMI between 25 and 29.9 kg/m² is termed overweight and those with BMI 30 kg/m² or more is classified as obese. [3].

Obesity is associated with significant health problems including high blood pressure, heart diseases, diabetes, stroke, osteoarthritis, sleep apnea, premature death and decreased life quality [4].

A healthy diet helps maintain or improve overall health and provides the body with essential nutrition: fluid, macronutrients, micronutrients, and adequate calories [5].

Energy intake (calories) should be in balance with energy expenditure. Evidence indicates that total fat should not exceed 30% of total energy intake to avoid excessive weight gain. Saturated fats should be less than 10% of total energy intake and trans-fats to less than 1% of total energy intake, with a shift in fat consumption away from saturated fats and trans-fats to unsaturated fats, and towards the elimination of industrial trans fats [6].

Restricted consumption of free sugars to less than 10% of total energy intake is part of a healthy diet but further reduction to less than 5% of total energy intake is suggested for additional health benefits. Weight loss promoting diets can be categorized as: low-fat, low-carbohydrate, low-calorie, very low calorie and more recently flexible dieting [7].

The current research was designed to evaluate biochemical changes associated with low and very low calorie diets on lipid metabolism, iron profile and kidney function in obese male rats through determination of: plasma AST (Aspartate Aminotransferase), total protein, albumin, cholesterol, plasma triglycerides, VLDL (Very Low Density Lipoprotein), LDL (Low Density Lipoprotein), HDL (High Density Lipoprotein), plasma iron, ferritin, plasma transferrin, TIBC (Total Iron Binding Capacity), serum urea and serum creatinine.

2. Materials and methods

Forty five male albino rats, 6–8 weeks old, with average body weight 150–180 gm used in the experimental investigation of this study and obtained from “The Laboratory Animals Research Center” Benha University. Rats were housed in separate wire mesh cages, exposed to good ventilation, humidity and to a 12-hr light, dark cycle and provided with a constant supply of standard pellet diet and plenty of fresh, clean drinking water ad-libitum.

Animals were kept at constant environmental and nutritional conditions throughout the period of the experiment and left for 15 days adaptation period prior to the inception of experiment.

2.1. Experimental design

Rats were randomly assigned into four groups, placed in individual cages and classified as following:

2.1.1. Group 1 (Control normal group)

Comprised 15 rats, provided only with a basal ration throughout the course of the experiment and served as control.

2.1.2. Group II (Obese group with 75 calories diet)

Included 10 rats, received a high fat diet according to [8] for 4 week period then each rat was fed daily with 75 calories diet according to [9] for 3 months.

25 gm mash 21% protein. 20 gm crushed sorghum.

21 gm crushed beans 22 gm wheat.

2.1.3. *Group III (Obese group with 60 calories diet)*

Included 10 rats, was received a high fat diet according to [8] for 4 week period then each rat was fed daily with 60 calories diet according to [10] for 3 months.

20 gm mash 21% protein. 16 gm crushed sorghum.
13 gm crushed beans 17 gm wheat.

2.1.4. *Group IV (Obese group with 45 calories diet)*

Included 10 rats, was received a high fat diet according to [8] for 4 week period then each rat was fed daily with 45 calories diet according to [10].

15 gm mash 21% protein. 12 gm crushed sorghum.
10 gm crushed beans 13 gm wheat.

2.2. *Blood samples*

At the end of the experiment, rats of each group were sacrificed by cervical decapitation. Blood samples were collected in dry, clean, screw capped serum separating tubes then plasma were separated by centrifugation at 2500 r.p.m for 15 min and kept in deep freeze at 20° C until used for subsequent biochemical analysis: plasma AST, total protein, albumin, cholesterol, plasma triacylglycerol, VLDL, LDL, HDL, plasma iron, ferritin, plasma transferrin, TIBC (Total Iron Binding Capacity), serum urea and serum creatinine. (see Tables 1–4)

3. Discussion

Increased consumption of nutrient poor foods with high levels of sugar and saturated fats have led to three folds rise in obesity rates. The rising epidemic reflects the profound changes in society and in behavioral pattern of communities over recent decades [11].

The obtained results showed that supplementation of 75 calories diet to obese group of male rats daily for 3 months, exhibited a significant increase in serum AST activity, total protein and serum albumin concentration when compared with control normal group while supplementation of 60 calories diet to obese group of male rats daily for 3 months, exhibited a significant improvement in serum AST, total protein and serum albumin concentration activity when compared with obese group. Moreover, additional improvement in serum AST in the obese rats administrated 45 calories diet daily for 3 months.

Our results came in accordance with [12] who found that, the mean values of serum AST, ALT and glucose were significantly decreased after weight loss phase. High-fat diet (HFD) and high-fructose beverages (HF) or both (HFHF) increased body weight, leptin, Homeostatic Model Assessment Indexes (HOMA2-IR) associated to steatosis, oxidative stress in plasma and tissues. HFHF induced fasting hyperglycemia and persistent hyperinsulinaemia and fasting hyperglycemia with complicated steatosis. High fat and high carbohydrate induced type 2 diabetes with widespread tissues effects. The oxidative stress has passive role toward pathogenesis and hepatic and vascular complications [13].

In addition [14], demonstrated that, overweight and obesity are major risk factors for diseases such as type 2 diabetes, coronary heart disease, sleep apnea, cancer, and liver disease such as non alcoholic

Table 1
High fat diet according to [8].

Composition	High fat diet (g/kg)	Composition	High fat diet (g/kg)
Casein	200	Dextrin	100
Saturated fat (Lard)	200	Cellulose	50
Corn oil	150	Vitamins mix	10
Cholesterol	3	Minerals mix	10
Coconut oil	25	Amino acids	5
Corn starch	75	Cholic acid	2
Sucrose	170	Total	1000 g

Table 2

The effect of 75, 60 and 45 calories diet supplementation in obese male rats on different biochemical parameters.

Parameter	Groups	Durations of treatment	
		After 2 months	After 3 months
AST activity (U/l)	Control normal group	74.63 ± 3.12 ^d	79.84 ± 3.45 ^c
	Obese group + 75 calories diet	90.54 ± 3.03 ^b	99.95 ± 3.24 ^b
	Obese group + 60 calories diet	81.19 ± 2.99 ^c	75.16 ± 2.63 ^c
	Obese group + 45 calories diet	143.67 ± 4.58 ^a	116.17 ± 6.22 ^a
Total protein concentration (g/dL)	Control normal group	6.58 ± 0.22 ^b	6.77 ± 0.26 ^b
	Obese group + 75 calories diet	7.32 ± 0.13 ^a	7.90 ± 0.22 ^a
	Obese group + 60 calories diet	6.95 ± 0.16 ^b	6.61 ± 0.18 ^b
	Obese group + 45 calories diet	5.52 ± 0.19 ^c	5.33 ± 0.18 ^c
Albumin concentration (g/dL)	Control normal Group	2.97 ± 0.12 ^c	3.08 ± 0.10 ^c
	Obese group + 75 calories diet	3.49 ± 0.11 ^a	3.89 ± 0.10 ^a
	Obese group + 60 calories diet	3.35 ± 0.12 ^b	3.10 ± 0.10 ^b
	Obese group + 45 calories diet	2.99 ± 0.08 ^c	2.89 ± 0.06 ^c
Urea concentration (mg/dL)	Control normal group	24.82 ± 1.32 ^c	26.61 ± 1.39 ^c
	Obese group + 75 calories diet	29.76 ± 1.06 ^b	35.22 ± 1.35 ^b
	Obese group + 60 calories diet	25.39 ± 1.04 ^c	23.33 ± 1.29 ^c
	Obese group + 45 calories diet	58.09 ± 2.94 ^a	60.83 ± 3.19 ^a
Creatinine concentration (mg/dL)	Control normal group	0.54 ± 0.03 ^c	0.59 ± 0.05 ^c
	Obese group + 75 calories diet	0.88 ± 0.04 ^b	0.98 ± 0.06 ^b
	Obese group + 60 calories diet	0.43 ± 0.03 ^d	0.37 ± 0.05 ^d
	Obese group + 45 calories diet	1.96 ± 0.11 ^a	2.09 ± 0.12 ^a

Data are presented as (Mean ± S.E), S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at (P < 0.05).

Table 3

The effect of 75, 60 and 45 calories diet supplementation in obese male rats on different biochemical parameters.

Parameter	Groups	Durations of treatment	
		After 2 months	After 3 months
Total cholesterol concentration (mg/dL)	Control normal group	71.62 ± 2.92 ^b	75.09 ± 2.36 ^b
	Obese group + 75 calories diet	126.97 ± 3.28 ^a	142.36 ± 3.79 ^a
	Obese group + 60 calories diet	56.67 ± 2.73 ^c	52.62 ± 2.14 ^c
	Obese group + 45 calories diet	47.40 ± 1.76 ^d	41.38 ± 1.86 ^d
triacylglycerols concentration (mg/dL)	Control normal group	66.43 ± 3.12 ^b	71.84 ± 3.45 ^b
	Obese group + 75 calories diet	176.53 ± 2.73 ^a	185.65 ± 2.69 ^a
	Obese group + 60 calories diet	62.45 ± 1.97 ^c	54.54 ± 2.92 ^c
	Obese group + 45 calories diet	50.77 ± 2.37 ^d	42.91 ± 2.02 ^d
VLDL concentration (mg/dL)	Control normal group	14.55 ± 0.42 ^b	16.33 ± 0.64 ^b
	Obese group + 75 calories diet	22.37 ± 3.03 ^a	26.01 ± 0.38 ^a
	Obese group + 60 calories diet	12.83 ± 0.28 ^c	11.05 ± 0.53 ^c
	Obese group + 45 calories diet	10.30 ± 0.32 ^c	9.17 ± 0.43 ^c
LDL concentration (mg/dL)	Control normal group	22.19 ± 1.12 ^b	23.94 ± 1.65 ^b
	Obese group + 75 calories diet	32.92 ± 2.38 ^a	36.65 ± 2.48 ^a
	Obese group + 60 calories diet	24.33 ± 1.76 ^b	20.69 ± 1.82 ^b
	Obese group + 45 calories diet	18.89 ± 1.37 ^c	16.21 ± 1.10 ^c
HDL concentration (mg/dL)	Control normal group	25.91 ± 1.75 ^a	25.04 ± 1.51 ^b
	Obese group + 75 calories diet	13.72 ± 1.18 ^d	9.55 ± 1.43 ^d
	Obese group + 60 calories diet	21.86 ± 0.91 ^c	24.92 ± 1.02 ^c
	Obese group + 45 calories diet	23.38 ± 1.17 ^b	28.94 ± 1.35 ^a
Average body weight	Control normal group	162.35 ± 2.78 ^c	178.47 ± 2.83 ^b
	Obese group + 75 calories diet	258.66 ± 3.12 ^a	244.09 ± 3.36 ^a
	Obese group + 60 calories diet	198.34 ± 3.28 ^b	173.84 ± 2.77 ^c
	Obese group + 45 calories diet	147.12 ± 3.19 ^d	124.25 ± 3.57 ^d

Data are presented as (Mean ± S.E), S.E = Standard error.

Mean values with different superscript letters in the same column are significantly different at (P < 0.05).

Table 4

The effect of 75, 60 and 45 calories diet supplementation in obese male rats on different biochemical parameters.

Parameter	Groups	Durations of treatment	
		After 2 months	After 3 months
serum iron concentration ($\mu\text{g/mL}$)	Control normal Group	0.73 ± 0.07^c	0.85 ± 0.08^c
	Obese group + 75 calories diet	2.17 ± 0.21^a	2.41 ± 0.45^a
	Obese group + 60 calories diet	1.82 ± 0.14^b	1.75 ± 0.13^b
	Obese group + 45 calories diet	1.61 ± 0.17^b	1.31 ± 0.18^b
serum ferritin concentration ($\mu\text{g/mL}$)	Control normal group	17.62 ± 0.61^c	18.99 ± 0.51^c
	Obese group + 75 calories diet	45.71 ± 1.52^a	50.79 ± 1.77^a
	Obese group + 60 calories diet	23.42 ± 0.51^b	21.87 ± 0.48^b
	Obese group + 45 calories diet	12.52 ± 0.62^d	11.31 ± 0.39^d
serum transferrin concentration (mg/dL)	Control normal group	256.12 ± 3.65^a	249.12 ± 3.65^a
	Obese group + 75 calories diet	219.08 ± 2.58^d	207.42 ± 2.91^d
	Obese group + 60 calories diet	237.62 ± 2.57^c	245.55 ± 3.62^c
	Obese group + 45 calories diet	242.08 ± 6.22^b	247.20 ± 3.45^b
Serum TIBC concentration ($\mu\text{g/dL}$)	Control normal group	453.96 ± 5.02^b	462.32 ± 3.25^b
	Obese group + 75 calories diet	411.35 ± 4.21^d	426.12 ± 4.82^d
	Obese group + 60 calories diet	434.25 ± 6.61^c	445.60 ± 5.81^c
	Obese group + 45 calories diet	516.49 ± 9.61^a	492.68 ± 8.57^a

Data are presented as (Mean \pm S.E), S.E = Standard error.Mean values with different superscript letters in the same column are significantly different at ($P < 0.05$).

fatty liver and non alcoholic steatohepatitis which recognized as being predominantly associated with insulin resistance, which is commonly found in obese subjects, especially those with a high accumulation of visceral fat.

Our results came in accordance with [13] who reported that, high caloric diet give rise to oxidative stress causing inflammation and elevated stress protein level. This result may be justified by [15] who noticed that, albumin is involved in many bioactive functions such as regulation of plasma osmotic pressure, binding and transport of various endogenous or exogenous compounds, and finally extra-cellular antioxidant defenses. It may lower the availability of pro-oxidants and be preferentially oxidized to protect other macromolecules. Overall improvement in liver outcome measurements in obesity associated with weight loss diet [16].

The obtained results showed that supplementation of 75 calories diet to obese group of male rats daily for 3 months, exhibited a significant increase in serum urea and creatinine concentration when compared with control normal group while supplementation of 60 calories diet to obese group of male rats daily for 3 months, exhibited a significant improvement in serum urea and creatinine concentration when compared with obese group. Meanwhile the serum urea and creatinine concentration exhibit significant increase in the obese rats administrated 45 calories diet daily for 3 months.

This result is agreed with [17] who reported that obesity causes a decline or loss of renal function and elevation of serum urea, creatinine and uric acid levels. Uremia resulted from high protein diet cause by increased production of urea in the liver and increased protein catabolism by oxidative stress [18].

In addition [19], demonstrated that, obesity is known as an independent risk factor for renal injury. Sympathetic nerve activation may have an important role of the pathogenesis of obesity and hypertension may underpin the development of cardiovascular events.

Our results came in accordance with [12] who reported that, the mean values of uric acid, creatinine, and blood urea nitrogen in were significantly decreased after weight-loss phase. Weight loss diet improved renal function (as evident from measures of creatinine and creatinine clearance) in overweight individuals. Total body fat mass could be predictor for improvement in renal function associated with weight loss. Suppression of sympathetic nervous activation associated with weight loss may have a role in the ameliorative effects on renal function. The unexpected increase of urea and creatinine levels in obese rats administrated 45 calories diet may be due to disturbance in protein metabolism and glomerular filtration rate.

1	Carbohydrates	58 %	2	Protein	20.5 %
3	Lipid	3.4 %	4	Cellulose	3.1 %
5	Vitamins	2.3%	6	Minerals	2.7 %
7	Moisture	10%	Total		100

The obtained results showed that supplementation of 75 calories diet to obese group of male rats daily for 3 months, exhibited a significant changes in lipid profile concentration when compared with control normal group while supplementation of 60 calories diet to obese group of male rats daily for 3 months, exhibited a significant improvement in lipid profile when compared with obese group. Moreover additional improvement in lipid profile in the obese rats administrated 45 calories diet daily for 3 months.

This results is corroborated with [20] who reported, serum levels of triglyceride, phospholipid, total cholesterol, and FFA were significantly elevated in the high fat hyper caloric diet. These results indicate that feeding of high fat hyper caloric diet makes normal wistar male adult rat obese associated with hyperlipidemia, hyperinsulinemia and glucose intolerance.

Our results is in the same line with [21] who revealed that obesity is commonly associated with elevated triacylglycerols, low HDL, and normal low-density lipoproteins. HDL has a beneficial role in the reverse cholesterol transport process where cholesterol in peripheral tissues is transported to the liver for reuse or bile acid synthesis, preventing the accumulation of cholesterol in the arteries. HDL is also thought to be cardio protective due to its antioxidant activity [22].

Low HDL observed in obesity is associated with an enhanced risk of atherogenesis possibly due to increased degradation and/or decreased production of HDL particles. Adipose cells have been shown to bind to HDL; hence increased body fat may lead to an increased uptake of HDL particles from circulation resulting in a reduction in plasma HDL levels [23].

Our results is agreed with [12] who reported that, after weight-loss phase, the mean serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) in the treated group were decreased significantly at the end of weight-loss phase, whereas serum high-density lipoprotein (HDL) was increased when compared with the obese group.

The obtained results showed that supplementation of 75 calories diet to obese group of male rats daily for 3 months, exhibited a significant changes in iron profile, when compared with control normal group while supplementation of 60 calories diet or 45 calories diet to obese group of male rats daily for 3 months, exhibited a significant improvement in iron profile when compared with obese group.

High fat diet HFD showed iron accumulation in the spleen, but not in the heart or liver. Increased percentages of the splenic red pulp and macrophages were observed in HFD-fed mice and iron accumulation in the spleen was found mainly in the splenic red pulp. The HFD also showed decreased iron content in the duodenum and increase mRNA expression of divalent metal transporter-1 (DMT-1), an iron absorption-related gene [24].

It is well known that there were negative correlation between ferritin and transferrin as demonstrated with [25], and this justified in elevation serum level of iron and ferritin and reduction in serum level of transferrin obese rats administrated high calorie diet, although the reduction in serum level of iron and ferritin and the elevation in serum level of transferrin obese rats administrated low calorie diet. Also, the elevated serum ferritin levels are associated with increased insulin resistance and risk of diabetes in obese. Higher serum ferritin levels were correlated with a greater risk of hyperglyceridemia in obese [26].

This results in the same line with [27] who reported that total iron-binding capacity (TIBC) indicates the maximum amount of iron needed to saturate plasma or serum transferrin thus there were a positive correlation between total iron-binding capacity (TIBC) and transferrin. The significant increase of (TIBC) in obese rats administrated 45 calories diet may be as a result of dropped in iron concentration showed in same group.

4. Conclusion

In conclusion, we suggest that, high fat diet able to produce significant disturbance in lipid metabolism, iron profile and kidney function while low calorie diets able to improving the disturbance in such parameter.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- [1] De Freitas Junior WR, Ilias EJ, Kassab P, Cordts R, Porto PG, Rodrigues FCM, et al. Assessment of the body composition and the loss of fat-free mass through bioelectric impedance analysis in patients who underwent open gastric bypass. *Sci World J* 2014;2014:843253.
- [2] Li Z, Bowerman S, Heber D. Health ramifications of the obesity epidemic. *Surgical Clinics* 2005;85(4):681–701.
- [3] Falaschetti E, Malbut K, Primatesta P. The general health of older people and their use of health services. 2002. Norwich, the stationary office.
- [4] Aggarwal T, Bhatia RC, Singh D, Sobti PC. Prevalence of obesity and overweight in affluent adolescents from Ludhiana, Punjab. *Indian Pediatr* 2008;45(6):500.
- [5] Mitchell D, Haroun L. Introduction to health care. 3 ed. Delmar Cengage; 2012. p. 279.
- [6] Hooper L, Abdelhamid A, Moore HJ, Douthwaite W, Skeaff CM, Summerbell CD. Effect of reducing total fat intake on body weight: systematic review and meta-analysis of randomised controlled trials and cohort studies. *Brith Med J* 2012;345: e7666.
- [7] Strychar I. Diet in the management of weight loss. *Can Med Assoc J* 2006;174(1):56–63.
- [8] Surapaneni KM, Jainu M. Comparative effect of pioglitazone, quercetin and hydroxy citric acid on the status of lipid peroxidation and antioxidants in experimental non-alcoholic steatohepatitis. *J Physiol Pharmacol* 2014;65(1):67–74.
- [9] Yamaguchi T, Miyashita Y, Saiki A, Watanabe F, Watanabe H, Shirai K. Formula diet is effective for the reduction and differentiation of visceral adipose tissue in Zucker fatty rats. *J Atherosclerosis Thromb* 2012;19(2):127–36.
- [10] Chen JH, Ouyang C, Ding Q, Song J, Cao W, Mao L. A moderate low-carbohydrate low-calorie diet improves lipid profile, insulin sensitivity and adiponectin expression in rats. *Nutrients* 2015;7(6):4724–38.
- [11] Popkin BM. The nutrition transition and obesity in the developing world. *J Nutr* 2001;131(3):871S–3S.
- [12] Nassar IOM. Effect of 3-month treatment of obesity by low-calorie diet on anthropometric, health, and nutritional status for obese female individuals. *Menoufia Medical J* 2014;27:115–21.
- [13] Lozano I, Van der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, et al. High-fructose and high-fat diet-induced disorders in rats: impact on diabetes risk, hepatic and vascular complications. *Nutr Metabol* 2016;13(1):15.
- [14] Neuschwander-Tetri BA. Nonalcoholic steatohepatitis and the metabolic syndrome. *Am J Med Sci* 2005;330(6):326–35.
- [15] Sitar ME, Aydin S, Sakatay U. Human serum albumin and its relation with oxidative stress. *Clin Lab* 2013;59(9–10): 945–52.
- [16] Wang RT, Koretz RL, Yee HF. Is weight reduction an effective therapy for on alcoholic fatty liver? A systematic review. *Am J Med* 2003;115:554–9. 2003.
- [17] Khan HN, Pergulwar A, Siddiqui AM, Shinde AR. Estimation of serum urea, creatinine and uric acid in obese subjects. *Int J Innov Res Med Sci* 2017;2(8):1201.
- [18] Meyer TW, Hostetter TH. Uremia *N Engl J Med* 2007;357(13):1316–25. 2007.
- [19] Masuo K, Rakugi H, Ogihara T, Esler MD, Lambert GW. Effects of weight loss on renal function in overweight Japanese men. *Hypertens Res* 2011;34(8):915.
- [20] Akiyama T, Tachibana I, Shirohara H, Watanabe N, Otsuki M. High-fat hypercaloric diet induces obesity, glucose intolerance and hyperlipidemia in normal adult male wistar rat. *Diabetes Res Clin Pract* 1996;31(1–3):27–35.
- [21] Mooradian AD, Haas MJ, Wehmeier KR, Wong NC. Obesity-related changes in high-density lipoprotein metabolism. *Obesity* 2008;16(6):1152–60.
- [22] Tall AR. Plasma high density lipoproteins. Metabolism and relationship to atherogenesis. *J Clin Invest* 1990;86(2):379–84.
- [23] Shoji TY, Nishizawa H, Koyama H, Hagiwara S, Aratani H, Azumotani-sasao K, et al. Lipoprotein metabolism in normolipidemic obese women during very low calorie diet: changes in high density lipoprotein. *J Nutr Sci Vitaminol* 1991;37: S57–64.
- [24] Yamano N, Ikeda Y, Sakama M, Izawa-Ishizawa Y, Kihira Y, Ishizawa K, et al. A long-term high-fat diet changes iron distribution in the body, increasing iron accumulation specifically in the mouse spleen. *J Nutr Sci Vitaminol* 2015;61(1):20–7.
- [25] Majoni SW, Lawton PD, Barzi F, Cass A, Hughes JT. Assessing the association between serum ferritin, transferrin saturation, and C-reactive protein in northern territory indigenous Australian patients with high serum ferritin on maintenance haemodialysis. *Int J Nephrol* 2017;5490963.
- [26] Ko PC, Huang SY, Hsieh CH, Hsu MI, Hsu CS. Serum ferritin levels and polycystic ovary syndrome in obese and nonobese women. *Taiwan J Obstet Gynecol* 2015;54(4):403–7.
- [27] Yamanishi H, Iyama S, Yamaguchi Y, Kanakura Y, Iwatani Y. Total iron-binding capacity calculated from serum transferrin concentration or serum iron concentration and unsaturated iron-binding capacity. *Clin Chem* 2003;49(1):175–8.