Biomedical Reviews 2022; 33: 77-88

ISSN 1314-1929 (online)

BMR Biomedical Reviews

Research Article

HEMATOLOGICAL PROFILE OF HIGH-FAT DIET-INDUCED MURINE MODEL OF METABOLIC SYNDROME

Fatemeh Ranjbar[1], Isaac Karimi[1,2*], and Nima Yakhchalian[2]

[1]*Laboratory of Molecular and Cellular Biology 1214, Department of Basic Veterinary Sciences, School of Veterinary Medicine, Razi University, Kermanshah, Iran*

[2]*Department of Biology, Faculty of Science, Razi University, Kermanshah, Iran*

The present study was conducted to address how high-fat diet (HFD)-induced metabolic syndrome (MetS) makes alterations to murine hematological profile. Fasting plasma glucose (FPG) of 80 male NMRI mice was measured and mice with FPG falling within the range of 80-160 were included as healthy or non-diabetic mice. Afterward, mice in the selected population were categorized into two separate main groups including normal control (NC, n = 32) and HFD -induced MetS (n = 32) having received a normal chow diet and a HFD, respectively, and 8 mice sacrificed for a biochemical and hematological profile at weeks 2, 4, 8, and 16. Lipid profiling, peripheral blood analysis, and bone marrow (BM) interpretation were considered endpoints. Results were analyzed in a separated time panel using a non-paired t-test at the significance level of $p<0.05$. Gained weight at week 16, increased accumulation of abdominal fat at week 8, raised FPG at weeks 2 and 8 and increased high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) at week 8 were observed in MetS compared to NC group confirming successful translation of a murine model of MetS. Hematologically, no change in serum ferritin, serum iron, hematocrit, platelet count, and differential leukocyte count (DLC) was observed in MetS compared to NC group. By contrast, MetS group showed raised absolute basophil count compared to NC group at week 8. Strikingly, MetS group showed a downward trend in hemoglobin concentration compared to NC group. However, this downward trend was only significant in the 2nd week. In addition, mean corpuscular volume and mean corpuscular hemoglobin in MetS group dropped at week 8 compared to NC group. Furthermore, MetS group showed decreased erythroid lineage cells including proerythroblast, polychromatophilic erythroblast, and orthochromatophilic erythroblast proposing the presence of anemia in the murine model of MetS. Histopathologic evaluation of BM showed decreased cellularity and increased infiltration of lipids in MetS compared to NC group. Additionally, MetS group showed the reduced number of basophils, eosinophils, and monocytes in BM during the first 8 weeks of the study. However, in the 16th week, lymphocytes were the only decreased cells, and the absolute count of neutrophils, basophils, and monocytes was raised possibly towards higher production of inflammatory cells in MetS group. It is worth noting that change in the number of plasma cells was shown to be highly variable throughout study. Following the successful development of a HFD-induced murine model of MetS, histological examination of BM in MetS mice showed reduced cellularity and enhanced accumulation of adipose tissue. Additionally, BM analysis indicated significantly decreased basophils, eosinophils, and monocytes at early weeks of receiving diet; however, increased neutrophils, basophils, and monocytes were observed at the end of the study which can primarily be considered reactive leukocytosis due to MetS-mediated inflammatory response. Furthermore, enumeration of erythroid lineage cells in BM demonstrated a significant decrease in proerythroblasts, polychromatophilic erythroblasts, and orthochromatophilic erythroblasts, highly suggestive of anemia. ***Biomed Rev* 2022; 33: 77-88**

Keywords: metabolic syndrome, blood, bone marrow, inflammation, ferritin, anemia

Received 15 April 2022, revised 3 May 2022, accepted 4 May 2022.

* Correspondence to: Isaac Karimi, E-mails: isaac_karimi2000@yahoo.com, karimiisaac@razi.ac.ir

INTRODUTION

Metabolic syndrome (MetS) plays a causal role in developing numerous (veterinary) clinical disturbances threatening the health and economic life of both society and animal husbandry in companion animals. Therefore, a plethora of seminal reviews have placed much emphasis on the etiopathology of MetS (1, 2)). In brief, overeating, decreased physical activity and sedentary lifestyle, depression, obesity, and high-calorie diets can cause MetS (2).

The implication of fat infiltration as an invasive tissue during the pathogenesis of MetS may affect the bone marrow (BM; *osteomyelosteatosis*) and finally leads to hematological disorders like anemia or inflammation (reviewed in 3). To dig deeper into the pathogenesis of hematological disorders that occurred or developed during diet-induced MetS and to prevent or treat them, handy non-genetic animal models are needed. Once we successfully developed a murine model of MetS not based on genetic modification, but through dietary intervention in our laboratory, we concentrated upon hematological study of our MetS model in order to introduce reference intervals for our high-fat diet (HFD)-induced mouse model of MetS.

MATERIALS AND METHODS

Animals and Sampling

The Animal Ethical Committee of our university reviewed and approved this study. Male Naval Medical Research Institute (NMRI) mice, *Mus musculus*, were maintained under relative humidity (45–55%), 12h/12h light/dark cycle, and temperature within the range of 25 ± 2°C. The animals (20–30 g) were divided into 2 groups ($n = 32$ for each) housed in colony cages. One group was fed with normal pelleted feed (Gharbdaneh Co., Iran) and was considered the control group (NC), and another received a high-fat induced-MetS regimen for 2, 4, 8, and 16 weeks as described previously(2) and was considered MetS group. The diets were formulated based on the daily requirement of 15 g / bodyweight according to the American Institute of Nutrition Rodent Diets (AIN93)(4).

The mice were sacrificed under deep anesthesia induced by intraperitoneal injection of ketamine (80 mg/kg) / Diazepam (0.5 mg/kg) cocktail and underwent blood collection at the end of the second, fourth, eighth, and sixteenth weeks of dietary intervention. The mice were examined for successful induction of MetS and pathobiological effects exerted by a high-fat diet on their serum lipid and lipoprotein, blood, and their BM profiles (*vide infra*) were analyzed through a necropsy study.

Biochemical Criteria Used to Define MetS

To determine the status of MetS in mice, a number of parameters were evaluated including fasting plasma glucose (FPG) level which was measured after 16 hours of food deprivation over the night using a glucometer. Blood samples were centrifuged at 2,500 rpm for 10 minutes at 20 °C and sera were isolated. In addition, triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), and total cholesterol (TC) were measured using commercial kits (Pars Azmoon Diagnostics, Iran). Moreover, concentration of very-low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein (LDL-C) coupled with atherogenic index (AI) were also calculated using corresponding formula(5, 6): VLDL = (TG) / 5; TC = LDL-C + HDL-C + VLDL-C; AI = LDL-C / HDL-C; and body mass index = body weight (g) / (nose length up to the anus (centimeter))^2 * 100. Total serum iron was measured (ELIThec Group Co., French) using Sinnowa D280 Biochemistry Auto Analyzer, China. In addition, serum ferritin level was also assessed using an ELISA kit (Padtan Alam Co., Iran).

Evaluation of Blood Profiles

Blood samples were collected into tubes containing anticoagulant (EDTA) and serum tubes, and immediately blood smear was prepared, dried, fixed with methanol, and stained with Giemsa stain. Once, a peripheral blood smear was finally prepared, differential leukocyte count, i.e. enumeration of lymphocytes, monocytes, neutrophils, eosinophils, band cells, and basophils was performed via counting 100 cells per blood spread. Afterward, 0.5 ml of each blood sample was stored in EDTA-containing tubes and analyzed using a hematology analyzer machine. The hematological assessment was accomplished on blood samples including quantitative analysis of cellular components of blood, hemoglobin (Hb) concentration, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH).

Bone Marrow Evaluation

Once the animals were sacrificed, removal of the sternum was immediately implemented and bones were placed in nitric acid 20% solution for 24 hours to get decalcified. Thereafter, the bones were cut into thin sections (5-6 μm) and stained with hematoxylin-eosin for further microscopic assessments. Major characteristics which were examined through bone marrow (BM) microscopy include cellularity, megakaryocytes count and maturity, and presence of pathologic features including any sign of malignancy, granuloma, abnormal cell, and fibrosis.

In addition, femur bone was also detached and kept over ice

and bone marrow was separated using the flushing method (7). In a nutshell, two heads of femur bone were separated under the hood by means of the sterile scapular method. Afterward, by using 1 ml of phosphate buffer solution (PBS), bone marrow was washed and harvested into EDTA-containing tubes. The procedure adopted to wash bone marrow is as follows: at first, an insulin syringe needle was inserted from one head and slowly rinsed PBS to drain the contents of bone into the tubes. This process was repeated several times on both heads of bone until the red color of the bone marrow changed in white color. At the second stage, the bone marrow cell count, myeloperoxidase index, and lobularity index were examined with Technicon H*1 Automated Hematology Analyzer (Technicon Instruments Corporation, Tarrytown, NY 10591). Following the current procedure, the samples were centrifuged at 1,500 rpm for 5 minutes and the remaining deposition in the tubes was stained with methanol and Wright-Giemsa colors. At the final stage, 500 cells per smear were counted and myeloid to the erythroid ratio (M/E ratio) was reported.

STATISTICAL ANALYSIS

All results are expressed as means ± standard error of the mean (SEM). Shapiro–Wilk test showed that all data fitted a Gaussian distribution. The parametric analysis of variance revealed a significant difference, and a *post hoc* Turkey's test was used to check the difference between groups. The significance level was set at $p < 0.05$ and all statistical analyses were carried out using SPSS package version 16.0 for Windows (Chicago, Illinois, USA).

RESULTS

Although the body weight of mice showed an upward trend in response to the intake of a HFD, body weight of the mice in MetS group on the 2nd, 4th, and 8th weeks was not significantly different compared to the body weight of mice in NC group. However, body weight of mice in MetS group showed a significant difference on 16th week compared to NC mice ($P = 0.04$; Table 1).

It is worth stating that abdominal fat weight was not measurable at the beginning of the study because of low fat mass in the studied mice. However, abdominal fat weight continued to show a rising trend toward the end of the study, and on the 8th week, this increase in abdominal fat weight was significant in the MetS group compared to the NC group ($p = 0.03$; Table 1). Although there was an increasing trend in body mass index (BMI) of the MetS group which was considered an indicator of obesity development in mice over the course of study, neither BMIs nor other somatic/physical indices showed any significant difference between MetS and NC mice (Table 1).

Biochemical Profiling

Although MetS groups showed increased fasting plasma glucose over the whole course of study, increased FPG values were only significant on the 2nd and the 8th week compared to NC groups ($p = 0.44$ and $p = 0.011$, respectively; Table 2). Regarding HDL-C, MetS group showed a significantly higher value on 8th week compared to NC group ($p = 0.015$; Table 2). In the current research, plasma concentration of LDL-C in all MetS groups was higher than that of the NC group, but this higher amount of LDL-C in MetS groups compared to NC mice was only significant on 8th week ($p = 0.001$; Table 2). Furthermore, atherogenic index, total cholesterol (TC), triglyceride (TG), VLDL-C, iron, and ferritin were higher in all MetS groups in comparison with those of NC group; however, the differences were not significant (Table 2).

Table 1. *Evaluation of somatic (physical) indices in murine model of metabolic syndrome and normal control mice*

Parameter	NC				MetS			
	W2	W4	W8	W16	W2	W4	W8	W16
Weight (g)	23.00.80±	26.11.67±	27.00.80±	29.80.53*±	26.14.36±	24.81.21±	28.61.20±	33.80.85±*
Length (cm)	8.90.30±	9.80.30±	8.90.30±	9.40.00±	10.00.18±	9.30.14±	9.30.33±	10.00.11±
BMI (g/cm^2 × 100)	71.87±	60.013±	43.03±	33.03.2±	59.56.6±	63.57±	39.04.3±	25.81.9±
Relative abdominal fat weight (G)	ND	ND	ND	ND	ND	ND	0.010.0 ±*	10.100.0±

Note: body mass index = body weight (g) / (nose length up to the anus (centimeter)2 * 100. In each row, parameters showing significant difference ($p < 0.05$) between MetS group and the same group of NC mice at each week are shown with a * sign.
ND: Uncertain due to low levels of fat in the abdomen.

Table 2. *Analysis of biochemical markers and atherogenic index in murine model of metabolic syndrome and normal control mice*

Parameter	NC				MetS			
	W2	W4	W8	W16	W2	W4	W8	W16
FBS (mg/d)	109.68.95±*	129.156.36±	152.49.00±*	137.612.00±	238.236.23±*	163.102.75±	274.253.47±*	273.015.00±
TC (mg/d)	191.012.8±	200.710.0±	183.77.7±	186.25.5±*	199.165.9±	293.011.0±	268.314.2 ±	281.3±32.3*
TG (mg/dl)	164.84.9±	196.012.8±	160.210.4±	144.04.4±	68.67.9±	121.212.6±	189.0±36	156.415.4±
HDL-C (mg/dl)	127.211.4±	148.614.8±	176.0217±*	144.44.4±	152.610.3±	192.613.6±	187.511.1±*	115.09.5±
VLDL-C (mg/dl)	32.90.9±	36.43.4±	32.02.0±	31.22.5±	13.71.5±	24.22.5±	45.09.3±	31.23.0±
LDL-C (mg/dl)	34.14.1±	25.50.00±	34.00.00±*	41.714.1±	30.19.99±	69.610.5±	35.86.5±*	185.627.8±
AI	0.30.1±	0.20.0±	0.140.09±	0.30.1±	0.20.0±	0.30.0±	0.190.03±	1.90.1±
Fe (µM/l)	167.810.4±	18917.2±	157.817.4±	166.824.0±	168.3±1.0	18417.70±	236.612.4±	25116.8±
Ferritin (µg/l)	37.313.9±	14.36.66±	14.63.6±	19.65.9±	24.33.0±	36.63.4±	27.03.2±	28.79.8±

Note: In each row, biomarkers showing a significant difference ($p < 0.05$) between MetS group mice and NC mice are indicated by * sign. LDL-C level was calculated by the formula: TC = LDL-C + HDL-C + VLDL-C. VLDL-C was calculated by the formula: VLDL-C = TG / 5. The rate of atherogenic index was calculated by the formula: AI = LDL-C / HDL-C. a: Up to 6 decimal places SEM has been zero.

Blood Profile and Erythrocyte Indices

As shown in Table 3, although decreased Hb concentration was observed among MetS groups, this reduced Hb level in MetS group was only significant on the 2nd week compared to NC mice ($p = 0.04$). Decreased HCT was also observed; however, it was not significant (Table 3). Additionally, MCV in MetS group showed a significant increase compared to NC group on the 4th week ($p = 0.019$; Table 3). Furthermore, MCH significantly rose in MetS group on the 4th week compared to NC group ($p = 0.031$; Table 3). No other parameter showed any significant changes.

Blood Profile and Counting Leukocytes and Thrombocyte

Among the leukocytic and thrombocytic indices analyzed, the only significantly increased value was basophil count in MetS group on 8th week compared to that of NC group ($p = 0.029$, Table 4). Although a consistent rising trend in platelet count was observed in MetS group, no significant difference was revealed between NC and MetS groups in terms of platelet count and in other indices (Table 4).

Differential Counting of Bone Marrow Cells

Among BM indices analyzed, a significant difference in the basophil count in was observed in MetS group on the 2nd week compared to NC group ($p = 0.045$; Table 5). In addition, eosinophil count in MetS group on the 4th week showed a significant decrease compared to NC group ($p = 0.05$; Table 5). Regarding monocyte count, MetS group demonstrated significantly decreased count on the 8th week compared to the

Table 3. *Blood profile and erythrocyte indices in the murine model of metabolic syndrome and normal control mice*

Parameter	NC				MetS			
	W2	W4	W8	W16	W2	W4	W8	W16
RBC (10^6/µl)	7.40.2±	7.10.1±	6.90.2±	7.0±0.2	6.40.6±	5.80.4±	6.30.4±	5.8±0.5
HB (g/dl)	11.60.2±	11.10.4±	9.50.8±	9.60.5±	10.70.6±*	9.90.7±	9.20.7±	8.80.8±
HTC (%)	34.71.9±	33.80.8±	32.21.6±	30.81.4±	34.41.5±	28.61.8±	31.61.7±	28.42.1±
MCV (fl)	46.80.8±	47.0±0.20*	46.20.8±	43.70.9±	52.15.7±	49.40.7±*	50.0±0.9	48.80.9±
MCH (pg/dl)	15.60.2±	42.526.6±*	13.70.9±	13.70.4±	15.1±4.7	17.0±0.5*	15.60.5±	14.90.3±
MCHC (g/dl)	33.50.7±	34.20.8±	29.72.1±	31.30.5±	29.0±3.7	34.40.8±	31.21.0±	30.61.0±

Note: In each row, the parameters showing a significant difference ($p < 0.05$) between MetS and NC group are indicated by * sign.
MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, MCH: mean corpuscular hemoglobin.

***Table 4**. Evaluation of blood profile and differential count of leukocyte and thrombocyte in murine model of metabolic syndrome and normal control mice*

Parameter	NC				MetS			
	W2	W4	W8	W16	W2	W4	W8	W16
WBC (*10^3/µl)	13.14.6±	16.62.9±	30.21.5±	40.4±4.3	36.5±8.5	6.0±0.5	16.42.8±	37.45.0±
Lymphocyte (%)	64.65.1±	63.86.5±	63.74.5±	48.65.6±	60.83.9±	47.19.0±	58.62.4±	45.79.0±
Neutrophil (%)	31.45.2±	32.67.4±	38.06.2±	48.85.8±	33.43.0±	43.59.9±	39.2±2.9	33.77.2±
Eosinophil (%)	0	0	0	0	0	0	0	0
Monocyte (%)	0. 6±0.4	0.2±0.2	0.4±0.4	0.4±0.4	4.7±1.0	3.1±1.2	0.4±0.2	0.6±0.6
Band Cell (%)	3.41.0±	3.4±1.1	4.21.1±	4.60.9±	2.5±0.0	6.1±1.1	3.80.7±	6.60.9±
Basophil (%)	0	0	0*	0	0	0	0.2±0.2*	0
Platelet (%)	532.657.6±	598.6±69.7	537.568.8±	610.041.8±	480.396.8±	397.560.6±	423.634.3±	428.855.9±

Note: In each row, the parameters showed a significant difference ($p < 0.05$) between MetS and NC group are indicated by * sign.

***Table 5.** The cell count of bone marrow in murine model of metabolic syndrome and normal control mice*

Parameter	NC				MetS			
	W2	W4	W8	W16	W2	W4	W8	W16
RBC (10^6/µl)	0	0	0.01±0a	0.02±0 a	0	0	0.01±0a	0.01±0a
WBC (10^3/µl)	17.61.6±	14.11.3±	14.30.9±	19.74 .2±	16.38.5±	8.9±1.3	12.41.0±	14.83.8±
Neutrophil (%)	8.00.6±	7.81.4±	12.3±5.7	6.6±0.3*	8.38.1±	6.50.6±	8.5±0.6	15.0±6.3*
Lymphocyte(%)	88.51.3±	25.2±4.87	95.1±5.88	68.7±3.80*	36.1±5.88	86.1±4.87	92.6±3.84	47.0±8.91*
Monocyte (%)	1.5±0.2	1.0±0.1	1.8±0.5*	0.8±0.0b	1.4±0.1	1.0±0.3*	1.4±0.1a	2.9±0.8b
Eosinophil (%)	0.6±0.1	0.80.1±*	1.0±0.5	0.40.1±	4.10.0±	0.20.01±*	0.4±0.1	0.80.1±
Basophil (%)	1.4±0.8*	0.40.1±	0.4±0.1	0.2±0.0*	5.0±0.0*	2.8±0.7	1.0±0.1	0.8±0.4*
MPOI	25.5±1.9	19.22.4±	21.3±.6	19.61.1±	13.28.0±	20.31.9±	16.41.0±	15.51.3±
LI	1.1±0.0	1.1±0.0	1.2±0.0	1.3±0.0	1.00.6±	1.40.0±	1.4±0.0	1.2±0.0

Note: In each row, the parameters showed a significant difference ($p < 0.05$) between MetS and NC group are indicated by * sign.
a: Up to 6 decimal places SEM has been zero.

NC group ($p = 0.10$; Table 5). On the 16th week, MetS group mice showed a significantly higher basophil count, significantly decreased neutrophil count, and a significantly increased lymphocyte count group compared to NC group ($p = 0.003$), ($p = 0.038$), ($p = 0.018$) and ($p = 0.00$) increased, increased, decreased and increased significantly (Table 5). No significant difference was recorded concerning other parameters (Table 5). Figures 1 and 2 illustrate cellular distribution analyzed by the H*1 automated hematology analyzer.

A significant difference was recorded among the cell lines studied in BM. On the 2nd week, orthochromatophilic erythroblasts and plasma cells in MetS group were compared with the NC group and both of them demonstrated a significant decrease in MetS group ($p = 0.043$) and ($p = 0.029$) respectively (Table 6). On the 8th week, myelocytes in MetS group and NC group, plasma cell the MetS group and the NC group, basophil the MetS group and the NC group, respectively ($p = 0.029$), ($p = 0.029$), ($p = 0.013$), there is a significant decrease (Table 6). On the 16th week, proerythroblasts significantly decreased ($p = 0.038$) compared to NC group. Additionally, polychromatophilic erythroblasts and plasma cells in MetS group showed significantly increased values ($p = 0.046$) and ($p = 0.00$) respectively (Table 6). As shown in Table 6, there was an upward trend myeloid index and myeloid/erythroid ratio

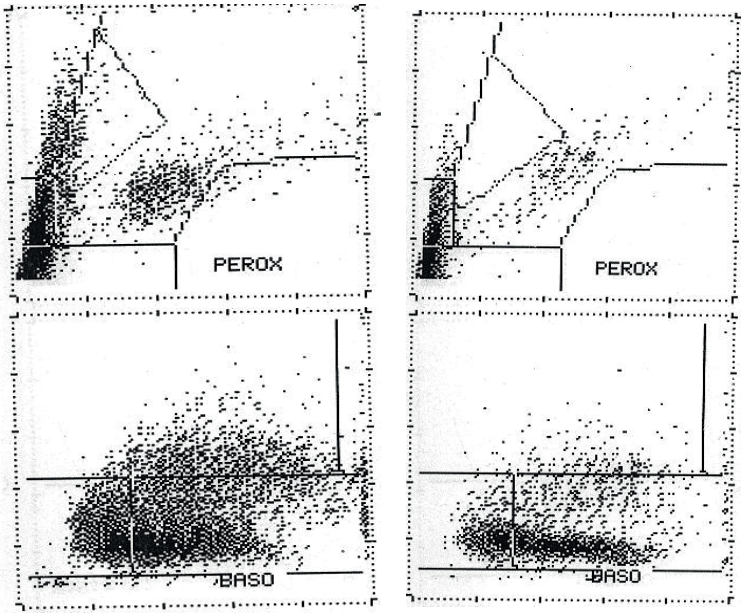

***Figure 1**. The ratio of the polymorphonuclear to mononuclear cells.*

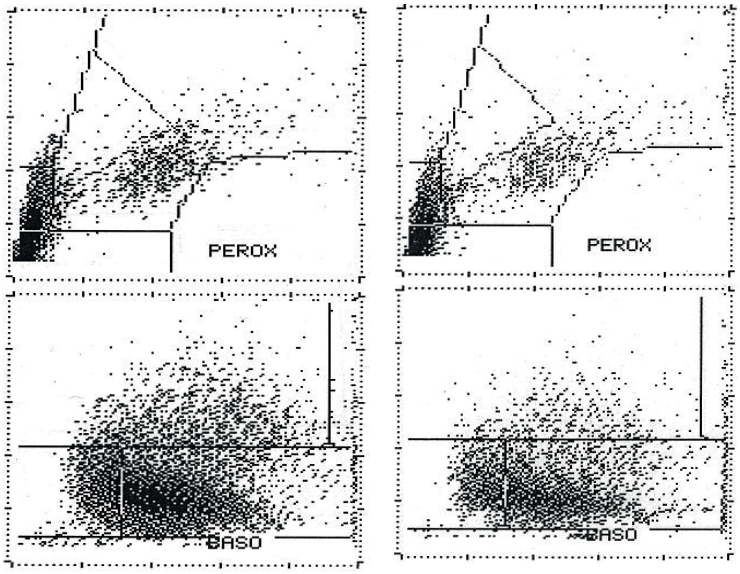

***Figure 2.** Evaluation of myeloperoxidase activity in bone marrow by differential counting of neutrophils and eosinophils cell lines.*

(M/E ratio) and a downward trend in erythroid index which is not significant. Pathological evaluation of BM in this study revealed decreased cellularity with a mild and non-significant trend (Table 6). Moreover, the number and maturity status of megakaryocytes were normal and no specific complications were observed (Figs 3-4 and 4 -4).

DISCUSSION

MetS is characterized as a set of adverse metabolic dysfunctions including obesity, insulin resistance, and dyslipidemia. It has recently been reported that obesity-mediated inflammatory response can play a fundamental role in the development of cardiovascular diseases (CVD) and cancers (8, 9). MetS

***Table 6**. Differential count in the bone marrow of murine model of metabolic syndrome and normal control mice*

Parameter	NC				MetS			
	W2	W4	W8	W16	W2	W4	W8	W16
Eosinophil	5.40.9±	8.4±0.5	5.6±1.5	3.4±0.6	6.0±2.8	9.0±0.5	6.2±0.8	3.0±1.3
Proerythroblast	2.40.5±	2.6±0.5	3.8±1.3	3.2±0.5	2.2±0.3	2.4±0.7	2.2±0.3	1.8±0.2a
Basophilic erythroblast	3.40.6±	2.4±0.5	13.0±0.8	7.8±1.1	3.2±0.5	3.2±1.0	3.2±0.5	4.6±0.2
Polychromatophilic erythroblast	18.2±0.8	13.6±1.4	17.6±1.2	11.0±1.1a	18.8±0.0	12.6±0.7	18.0±0.8	9.0±0.3a
Orthochromatophilic erythroblast	20.20.9±*	21.8±1.0	8.4±0.5	32.2±1.0	19.0±6.5a	20.2±0.9	19.6±0.5	19.6±1.2
Myeloblast	2.60.5±	3.4±0.6	3.4±0.6	1.2±0.2	4.6±1.0	4.6±1.3	1.6±0.4	1.2±0.2
Promyelocyte	5.6±0.5	2.4±0.5	2.6±0.5	2.4±0.3	2.4±0.0	2.0±0.3	2.0±0.4	3.0±0.3
Myelocyte	3.0±0.9	4.8±0.8	6.4±1.0a	8.6±0.2	4.4±0.0	4.4±1.2	4.0±0.4a	9.0±0.4
Metamyelocyte	7.0±0.5	5.2±0.5	7.2±1.1	7.4±0.8	7.0±4.8	5.4±0.7	7.2±0.8	8.4±0.6
Band	8.4±1.1	9±0.7	12.2±1.8	15.2±1.1	9.1±0.2	9.8±0.5	9.2±0.9	23.0±0.9
Neutrophil	10.01.3±	11.6±0.9	9.0±1.0	8.2±0.8	10.1±1.8	9.6±1.6	10.8±1.1	9.2±0.3
Plasma cell	1.20.2±a*	1.6±0.6	1.2±0.2b	1.0±0.0c	1.0±0.0a	1.2±0.2	1.0±0.0b	1.4±0.2c
Lymphocyte	9.61.5±	10.6±0.8	1.6±0.4	3.0±0.6	11.1±6.5	12.4±1.0	11.6±1.5	2.6±0.6
Monocyte	1.80.3±	1.4±0.4	1.4±0.2	2.8±0.4	8.2±1.0	1.4±0.2	1.8±0.2	2.6±0.6
Basophil	1.40.2±	1.2±0.2	6.6±2.2a	1.8±0.3	6.4±1.0	1.8±0.3	1.6±0.4a	1.6±0.4
Myeloid	36.0±0.7	36.4±1.3	40.2±2.7	43.0±1.3	35.8±0.0	35.8±1.5	35.0±0.8	53.8±0.9
Erythroid	44.21.1±	40.4±1.4	40.2±1.6	45.2±2.4	43.1±1.0	38.2±1.8	43.0±1.1	35.4±1.2
M.E ratio	0.820.0±	0.90.0±	04.0±0.1	1.010.1±a	0.810.0±	0.950.0±	0.810.0±a	1.50.06±
Cellularity1	682.0±	66±2.4	63±3.0	60±1.5	66.42.0±	642.4±	601.5±	532.5±

Note: In each row, the parameters showed a significant difference ($p < 0.05$) between MetS and NC group are indicated by * sign.
M / E: Myeloid/Erythroid ratio. A: Up to 6 decimal places of the SEM has been zero.

models developed *via* genetic modification, cannot holistically reflect the entire physiological conditions of the body; therefore, MetS models not developed based upon genetic modification must be put at the center of attention as these models can provide us with a vivid image of physiological conditions(10). The case in point is the diet-induced model of diabetes mellitus (DM) produced/developed by HFD-induced obesity for searching anti-diabetic drugs or remedies (11). This model is much more similar to type 2 DM which primarily develops as a consequence of obesity and overweight (12). Numerous advantages have led us to recall mouse model in the current study including its easy transportation, fully sequenced genome, low diversity, and short reproductive period. Although mice have a high blood total cholesterol, low LDL-C, and high HDL-C, they cannot be a reliable and fully translated model for developing MetS (13).

In this study, we bent all efforts to the task of developing a HFD-induced murine model of MetS. As clearly mentioned, MetS group showed increased body weight over the course of study. In harmony with what has been reported (4, 14, 15), a significant difference in body weight between MetS group and NC group was observed on the 2nd week, thus, it is recommended that HFD should be used for longer temporal periods in order to increase weight throughout the course of study and individual weight changes showed us that even in one colony, we can see obesity-prone and obesity-resistant mice that gives rise to large standard deviations.

In the current study, significantly enhanced accumulation

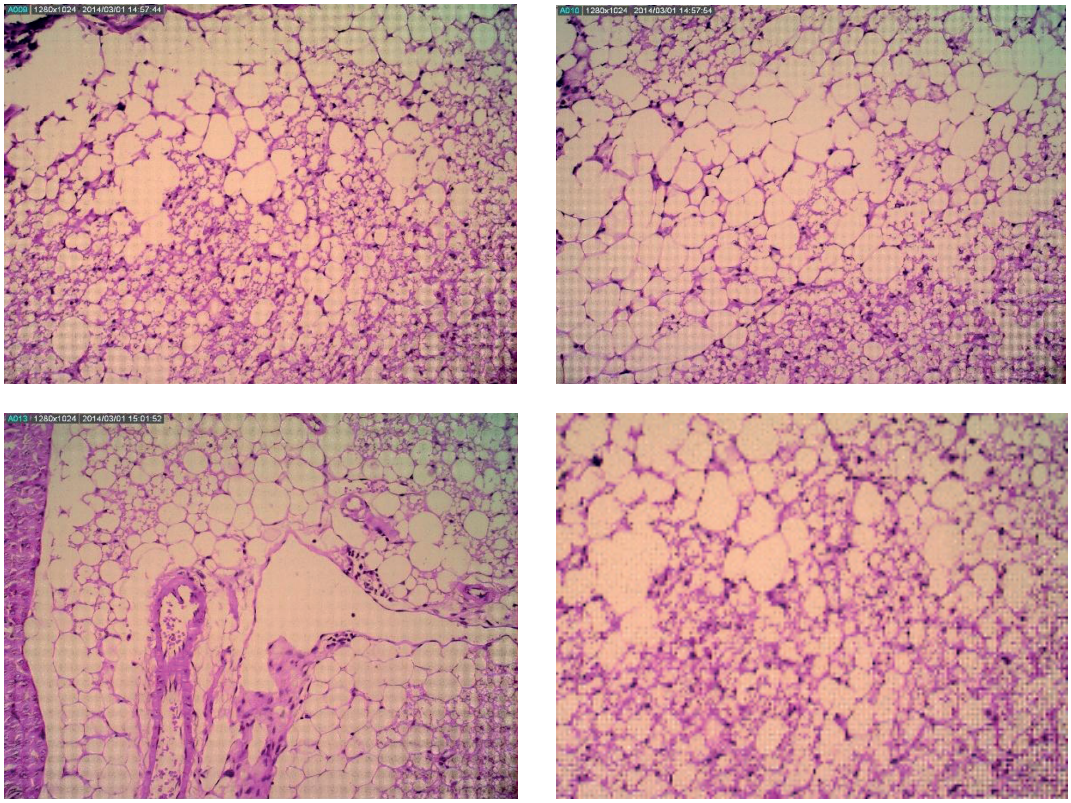

Figure 3. *Hematopoietic compartment with associated adipose tissue. × 100*

of abdominal adipose tissue was observed from the 8th week in MetS compared to NC group supported by confirmatory evidence such as the results obtained (4). Despite the fact that BMI in MetS group did not show any significant difference in comparison with that of NC, increased BMI in MetS group can suggest the occurrence/successful induction of obesity in the experimental mice.

Concerning FPG, significant enhancement was reported in MetS group on the 2nd and 8th weeks of study compared to NC group which is harmoniously supported by the research conducted (4). Although above-mentioned increasing trend continued until the end of the study, this enhancement was not significant on other weeks of experiment. It is suggested that along with increased fat intake in our experimental mice, carbohydrate and animal drinking water get increased (data not shown).

Regarding the characterization of lipid profile, MetS group showed a significantly higher level of LDL-C at all weeks of study in comparison with NC group. This finding is consistently endorsed by the results of previous studies (4, 15). According to our results, HDL-C concentration significantly increased in MetS group on week 8 compared to NC group which paradoxically contradicts the results obtained (15).

Our research enlightened striking features including the development of a murine MetS model, accumulation of abdominal adipose tissue, weight gain, increased blood lipid markers and FPG in MetS compared to NC group. Although no significant difference was reported regarding numerous biomarkers between MetS and NC groups, multiple signs of MetS such as enhanced BMI, accumulation of abdominal fat, increased FPG, and dyslipidemia emerged in response to HFD in MetS group. To recapitulate, it can be stated that murine MetS model was successfully developed in this study; however, there is still room for improvement of current research in the future using animal models showing a higher animal-to-human translatability, particularly using those animals with higher LDL-C level (13).

When it comes to the interpretation of blood profile in the current study, "inflammatory response" should be placed at the center of attention. To be more precise, inflammation is the part and parcel of MetS acting as an indispensable mediator towards increasing serum ferritin in patients with MetS. In this line, studies propose that the level of serum ferritin can contribute to MetS diagnosis since being a positive acute phase reactant in-

creasing in response to inflammatory conditions. Therefore, it can be said that MetS-mediated inflammation (or HFD-induced inflammation in MetS?) can give cause for increased level of serum ferritin in the body. The things which should be kept in mind is the fact that in order to confirm the presence of any inflammatory condition, quantitative analysis/measurement of other pro-inflammatory mediators such as cytokines and adipokines must be accomplished (16). Mechanistically, iron can contribute to the hydroxylation of radicals in the body and may exert adverse effects upon hepatic function. Therefore, enhanced serum ferritin can be suggestive of type 2 DM and MetS (17, 18). Hypothetically, hypoxia-induced erythropoiesis and suppressed hepcidin production can result in increased level of ferritin; however, some studies suggested increased expression of *HAMP* gene encoding hepcidin (16). The iron accumulation in tissues can adversely make alterations to pancreatic secretion and production of insulin and it can also interfere with roles of insulin in hepatic tissue ultimately leading to insufficiency of insulin secretion and decreased insulin level. In addition, muscular iron overload can cause pathological consequences for muscles and decreased glucose absorption in the muscles. Furthermore, iron also triggers rapid onset of atherosclerosis and vascular endothelial injury in laboratory model mice through production of free radicals such as H_2O_2 sticking to plasma membrane and other cellular components eventuating into deleterious damages to cells (19). Insulin can stimulate iron reabsorption (19). In the same context, insulin can also stimulate erythropoietin production and increase iron absorption by activating α-1 activated factor in hypoxic circumstances (20).

In a research carried out by Sun and colleagues, a strong positive correlation between ferritin level and the risk of type 2 DM as well as MetS was reported among Chinese men and women. This correlative relationship depends not only upon factors including lifestyle, level of education, family history, chronic diseases, and diet, but also upon obesity, inflammatory mediators, and adiponectin. There is a mountain of evidence highlighting the role of iron in the development of metabolic diseases in developing countries. In this study, a positive correlation between ferritin concentration and the level of inflammatory mediators such as IL-6 and CRP was found proposing that inflammation affects ferritin secretion. Therefore, enhanced ferritin can not only be an indicator of iron overload, but also it can reflect the presence of any systemic inflammatory response and neoplastic conditions (17, 21).

Shi and colleagues showed that women with enhanced serum ferritin are at higher risk of developing MetS and co-presence of anemia and MetS. As heavily supported by evidence, level of ferritin, as an acute phase reactant, is associated with inflammation being linked to numerous complications such as anemia and MetS in women. Therefore, preventing and treating both clinical entities, especially in women above 50 years old, is recommended. What must be considered is the alarming fact that higher incidence of MetS and anemia among the population may reflect the presence of an underlying inflammation, which requires further examination(18).

In the study carried out by Jehn *et al*., the association between excessive iron storage (confirmed by ferritin measurement) and the incidence rate of MetS was reported. They found a positive correlation between ferritin level and different manifestations/consequences of MetS, especially increased plasma TGs, plasma glucose, and insulin resistance (20). It has also been reported that raised CRP and ferritin in a contextual response to underlying inflammation may be indicative of MetS and insulin resistance; thus, put the patients at higher risk of developing CVD (19).

In contrast with results provided by the aforementioned studies, increased iron and ferritin in MetS mouse model were not significantly different from that of the control group; therefore, the current study did not confirm the enhancement of ferritin and iron in response to MetS-mediated inflammation. Needless to say, ferritin is not the only biomarker raised by the presence of inflammation and not always does it raise in the context of any inflammation; as a result, other inflammatory markers including serum acute phase reactants and pro-inflammatory mediators such as CRP, TNFα, IL-6 and erythrocyte sedimentation rate (ESR) are highly recommended to evaluate whether any active inflammatory response exists in the body or not.

Additionally, contrary to a vast number of studies having shown an association between the count of WBC and RBC with MetS (22-28), current study did not find any significant relationship between WBC and RBC count with MetS. Of course, there was a significant difference in basophil count on the 8th week of the experiment between MetS group mice and NC mice. Noticeably, some studies have reported hyperhemoglobinemia in MetS (29).

Mechanistically, diminished oxygenation of adipose tissue and consequent hypoxia can establish insulin resistance which inhibits phosphorylation of insulin receptors and reduce glucose transfer. Previous studies have shown that reduced oxygenation in adipose tissue upregulates erythropoietin

gene. According to evidence, hypoxia can trigger erythropoietin production and lead to elevated Hb. In current research, erythropoietin and Hb were significantly higher in mice in MetS group. Research executed by Shi *et al.* reported a high incidence of anemia and MetS in people over 20 years old and older. Their study also demonstrated that a small proportion of the population (about 2.9%) suffer from both complications simultaneously. Co-existence/ co-occurrence of these two complications appeared to be higher among women over 50 years of age. Obviously, social, economic, and nutritional factors are associated with these complications. In this study, anemia, MetS, and the co-presence of both complications were more prevalent among women (18). In another study, a higher incidence of anemia was reported in diabetic patients compared to non-diabetics. Additionally, it has evidently known that diabetics serve poorer renal function compared to non-diabetics, which can be attributed to the higher rate of anemia in these patients. Evidence shows that renal hypoxia secondary to lower Hb plays a vital role in developing diabetic kidney disease.

Diabetes-mediated anemia may be due to impaired erythropoietin production in the context of chronic kidney disease. Routinely, hematological parameters are not measured in diabetic patients; therefore, finding diabetics with anemia can be challenging. Early management of anemia in diabetics seems to be of great importance to the health of these people. As a result, in developing countries, complete blood count (CBC) should routinely be checked in these patients. It is worth noting that higher plasma glucose and glycosylated hemoglobin A1C (Hb A1C) are associated with developing CVD in diabetic patients. Considering that diabetes-induced CVD is considered among the top causes of death in diabetic patients, evaluation of plasma glucose level and Hb A1C is highly recommended and paves the way for effective management and prevention of cardiovascular complications in these patients (30). The results of current research are in harmony with the results obtained in a vast number of studies (18, 27, 30) and are in contrast to the results obtained (16). In our study, significantly decreased Hb on the 2nd week in MetS group compared to NC group confirms successful induction of anemia in MetS group. Lack of any change in MCV, MCH, and MCHC, may be because of the normocytic normochromic nature of anemia. However, significantly increased MCV and MCH in the MetS group were observed on the 4th week, but according to the lack of any changes in other biochemical and blood markers, this increase is not likely due to the HFD.

It is worth stating that both primary and secondary thrombocytosis were observed. Primary thrombocytosis is due to increased PLT production in BM, and secondary thrombocytosis is a response to inflammation and infection (31). Following the increase in platelet count, the probability of cerebrovascular accidents will increase as a result of thrombosis and formation of PLT clump in veins (32). In line with the results (28), there is no significant correlation between PLT count and other aspects of MetS; however, some studies such as the one conducted by (27), reported a significant relationship between PLT count and various aspects of MetS and has shown raised PLT count can be considered one of the risk factors for MetS, thrombosis, and CVD.

The relationship between obesity and bone metabolism can be found at both systemic and local levels. Usually in subjects with obesity, the systemic one is dominant which is a hormone-directed mechanism. Increased presence of adipose tissue in BM exerts adverse impacts on osteoblastic activity and activates osteoclasts (33). Numerous theories have been proposed to explain this phenomenon. As evidence suggest, since obesity eventuates into a chronic inflammation in the body and raised pro-inflammatory cytokines such as TNF-α and IL-6, osteoblastic activity will be inhibited as a result of such an inflammatory response. Additionally, regarding the fact that osteoblasts and adipocytes originate from multipotent stem cells, obesity can drive these stem cells to producing more adipocytes. Of course, enhanced plasma leptin due to obesity also causes suppression of bone formation (34).

CONCLUSION

For the first time in this study, BM activity was evaluated in a HFD-induced murine MetS model. Histological examination of BM revealed that mice in MetS group showed reduced cellularity and increased adipose tissue accumulation. Additionally, BM analysis indicated significantly decreased basophils, eosinophils, and monocytes at *ab initio* stages; however, increased neutrophils, basophils, and monocytes were observed at the end of the study which can primarily considered reactive leukocytosis due to inflammatory response. Furthermore, enumeration of erythroid lineage cells in BM demonstrated a significant decrease in proerythroblasts, polychromatophilic erythroblasts, and orthochromatophilic erythroblasts, highly suggestive of anemia. One of the limitations of current study was the size of the laboratory animal which limited the possibility of serial sampling of BM and peripheral blood. It is recommended that larger animal models should be used such

as guinea pigs or rabbits in order for the serial sampling of different tissues to be applicable.

ACKNOWLEDGMENTS

This paper emanates from the doctoral thesis of the first author in Veterinary Medicine at School of Veterinary Medicine, Razi University, Kermanshah, Iran. *In vivo* part of the current study was financially supported by a grant at Razi University.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Chan SMH, Selemidis S, Bozinovski S, Vlahos R. Pathobiological mechanisms underlying metabolic syndrome (MetS) in chronic obstructive pulmonary disease (COPD): clinical significance and therapeutic strategies. *Pharmacol Ther*. 2019;198:160-88. PMID: 30822464;PMCID: PMC7112632
2. Motamedi S, Karimi I, Jafari F. The interrelationship of metabolic syndrome and neurodegenerative diseases with focus on brain-derived neurotrophic factor (BDNF): Kill two birds with one stone. *Metab Brain Dis*. 2017;32(3):651-65. PMID: 28361262
3. Wang H, Leng Y, Gong Y. Bone Marrow Fat and Hematopoiesis. *Front Endocrinol* 2018;9. PMID: 30546345;PMCID: PMC6280186
4. Akagiri S, Naito Y, Ichikawa H, Mizushima K, Takagi T, Handa O, *et al*. A Mouse Model of Metabolic Syndrome; Increase in Visceral Adipose Tissue Precedes the Development of Fatty Liver and Insulin Resistance in High-Fat Diet-Fed Male KK/Ta Mice. *J Clin Biochem Nutr*. 2008;42(2):150-7. PMID: 18385833;PMCID: PMC2266061
5. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499-502. PMID: 4337382
6. Sagud M, Mihaljevic-Peles A, Pivac N, Jakovljevic M, Muck-Seler D. Lipid levels in female patients with affective disorders. *Psychiatry Res*. 2009;168(3):218-21. PMID: 19560828
7. Heib T, Gross C, Müller ML, Stegner D, Pleines I. Isolation of murine bone marrow by centrifugation or flushing for the analysis of hematopoietic cells - a comparative study. *Platelets*. 2021;32(5):601-7. PMID: 32726163
8. Bou Malhab LJ, Abdel-Rahman WM. Obesity and Inflammation: Colorectal Cancer Engines. *Curr Mol Pharmacol*. 2022;15(4):620-46. PMID: 34488607
9. Kachur S, Lavie CJ, de Schutter A, Milani RV, Ventura HO. Obesity and cardiovascular diseases. *Minerva Med*. 2017;108(3):212-28. PMID: 12361492
10. Wang Y, Zheng Y, Nishina PM, Naggert JK. A new mouse model of metabolic syndrome and associated complications. *J Endocrinol*. 2009;202(1):17-28. PMID: 19398498;PMCID: PMC2853727
11. Philouze C, Martin JC, Riva C, Marziou A, Defoort C, Couturier C, *et al*. Vitamin D3 Supplementation Alleviates Left Ventricular Dysfunction in a Mouse Model of Diet-Induced Type 2 Diabetes: Potential Involvement of Cardiac Lipotoxicity Modulation. *Cardiovasc Drugs Ther*. 2022;36(2):245-56. PMID: 33661433
12. Homayounfar R, Ehrampoush E, Koohpaye S, Meshkibaf M, Taghizade S, Almasi A, *et al.* Diet-induced metabolic syndrome model in rats. *J Fasa Univ Med Sci* 2013;2:288-96.
13. Karimi I. Animal Models as Tools for Translational Research: Focus on Atherosclerosis, Metabolic Syndrome and Type-II Diabetes Mellitus. 2012; DOI: 10.5772/47769
14. Gallou-Kabani C, Vigé A, Gross MS, Rabès JP, Boileau C, Larue-Achagiotis C, *et al.* C57BL/6J and A/J mice fed a high-fat diet delineate components of metabolic syndrome. *Obesity* (Silver Spring). 2007;15(8):1996-2005. PMID: 17712117
15. Hattori T, Murase T, Ohtake M, Inoue T, Tsukamoto H, Takatsu M, et al. Characterization of a new animal model of metabolic syndrome: the DahlS.Z-Leprfa/Leprfa rat. *Nutr Diab* 2011;1(1):e1-e. PMID: 23154293;PMCID: PMC3302131
16. Hämäläinen P, Saltevo J, Kautiainen H, Mäntyselkä P, Vanhala M. Erythropoietin, ferritin, haptoglobin, hemoglobin and transferrin receptor in metabolic syndrome: a case control study. *Cardiovasc Diabetol*. 2012;11:116. PMID: 23016887;PMCID: PMC3471017
17. Sun L, Franco OH, Hu FB, Cai L, Yu Z, Li H, et al. Ferritin concentrations, metabolic syndrome, and type 2 diabetes in middle-aged and elderly chinese. *J Clin Endocrinol Metab*. 2008;93(12):4690-6. PMID: 18796516
18. Shi Z, Hu X, Yuan B, Pan X, Meyer HE, Holmboe-Ottesen G. Association between serum ferritin, hemoglobin, iron intake, and diabetes in adults in Jiangsu, China. *Diabetes Care*. 2006;29(8):1878-83. PMID: 16873796

19. González AS, Guerrero DB, Soto MB, Díaz SP, Martinez-Olmos M, Vidal O. Metabolic syndrome, insulin resistance and the inflammation markers C-reactive protein and ferritin. *Eur J Clin Nutr*. 2006;60(6):802-9. PMID: 16493453
20. Jehn M, Clark J, Guallar E. Serum Ferritin and Risk of the Metabolic Syndrome in U.S. Adults. *Diabetes Care.* 2004;27:2422-8. PMID: 15451911
21. Bozzini C, Girelli D, Olivieri O, Martinelli N, Bassi A, De Matteis G, *et al.* Prevalence of body iron excess in the metabolic syndrome. *Diabetes Care*. 2005;28(8):2061-3. PMID: 16043762
22. Galan P, Noisette N, Estaquio C, Czernichow S, Mennen L, Renversez J-C, *et al*. Serum ferritin, cardiovascular risk factors and ischaemic heart diseases: a prospective analysis in the SU.VI.MAX (SUpplementation end VItamines et Minéraux AntioXydants) cohort. *Public Health Nutr* 2006;9(1):70-4. PMID: 16480536
23. Facchini F, Hollenbeck CB, Chen YN, Chen YD, Reaven GM. Demonstration of a relationship between white blood cell count, insulin resistance, and several risk factors for coronary heart disease in women. *J Intern Med*. 1992;232(3):267-72. PMID: 1402624
24. Jeppesen JL, Maheux P, Facchini FS. White blood cell count and insulin resistance in healthy nonsmoking men. *Am Heart J*. 1994;127(2):459-61. PMID: 8296722
25. Koenig W, Ernst E, Matrai A. Blood rheology associated with cardiovascular risk factors and chronic cardiovascular diseases: results of an epidemiologic cross-sectional study. *Angiology*. 1988;39(11):986-95. PMID: 3263066
26. Targher G, Seidell JC, Tonoli M, Muggeo M, De Sandre G, Cigolini M. The white blood cell count: its relationship to plasma insulin and other cardiovascular risk factors in healthy male individuals. *J Intern Med*. 1996;239(5):435-41. PMID: 8642236
27. Lohsoonthorn V, Jiamjarasrungsi W, Williams MA. Association of Hematological Parameters with Clustered Components of Metabolic Syndrome among Professional and Office Workers in Bangkok, Thailand. *Diab Metab Syndr*. 2007;1(3):143-9. PMID: 19543435 PMCID: PMC2699274
28. Wang Y-Y, Lin S-Y, Liu P-H, Cheung B, Lai W-A. Association between hematological parameters and metabolic syndrome components in a Chinese population. *J Diab Complic*. 2004;18:322-7. PMID: 15531181
29. Hämäläinen P, Saltevo J, Kautiainen H, Mäntyselkä P, Vanhala M. Erythropoietin, ferritin, haptoglobin, hemoglobin and transferrin receptor in metabolic syndrome: a case control study. *Cardiovasc Diabetol* 2012;11:116**.** PMID: 23016887; PMCID: PMC3471017
30. Ezenwaka CE, Jones-Lecointe A, Nwagbara E, Seales D, Okali F. Anaemia and kidney dysfunction in Caribbean type 2 diabetic patients. *Cardiovasc Diabetol* 2008;7:25. PMID: 18752687;PMCID: PMC2542986
31. Ghaemi M, Ramyar A, Zavareh SA. A case report of newborn with thrombocytosis born to an opioid-addict mother. *J Kerman Univ Med Sci* 2010;17:379-82.
32. Sasannejad P, Mellat Ardekani A, Velayati A, Shoeibi A, Saeidi M, Foroughipour M, *et al.* Cerebral vein thrombosis in women using short course oral contraceptive consumption. *Iran J Reprod Med* 2012;10(6):537-42. PMID: 25246923;PMCID: PMC4169846
33. Duque G. Bone and fat connection in aging bone. *Curr Opin Rheumatol* 2008;20:429-34. PMID: 18525356
34. Cao JJ. Effects of obesity on bone metabolism. *J Orthop Surg Res* 2011;6:30. PMID: 21676245; PMCID: PMC3141563