

Changes In Some Haematological And Biochemical Parameters of Albino Rats In Response to Low-energy Diet

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ABSTRACT

The effect of low-energy diet (Low protein or low fat-diet) was studied using male albino rats. The experiment was expended to 45 days (30 days treatment and 15 days recovery period). Body weight gain or loss as well as blood samples for blood picture (R.B.C_S, W.B.C_S count, Hb. concentration, Hct value and erythrocyte indices such as MCV, MCH and MCHC) well recorded Biochemical examination for glucose concentration, total protein, albumin, globulin and A/g ratio. Triglyceride level,

total cholesterol and LDL-cholesterol, and HDL-cholesterol level, in addition to some hormones such as insulin, Testosterone and Leptin levels were examined at the end of the treatment period and the recovery period. A significant decrease in body weight gain percentage was observed in group fed low

protein or Low fat-diet and the body weight improved during and after the recovery period. A significant decrease in R.B.C_S, W.B.C_S, count and Hb. concentration, and Hct value in treatment period and improved during and after recovery period. Total proteins, albumin and globulin were significantly affected by low protein or low-fat diet treatment in comparison with the control group. These effects were significantly counteracted by balanced diet. A significant decrease in triglyceride, total cholesterol, LDL-cholesterol and HDL-cholesterol were observed in groups received diet containing low-protein or low fat as compared with the control group. The results of the present study showed a significant reduction in (Leptin, insulin and testosterone) level after treatment for 30 days and recovery period for 15 days.

INTRODUCTION

There is a widespread interest in dietary protein or fat effect on various aspects of protein and lipid metabolism and problems relating to hepatic and cardiovascular disease. Some problems can develop at the extremes either low fat or low protein intakes. Excess fat causes elevation of serum lipids and thus an increased risk of heart disease

and arteriosclerosis, but the low fat diet causes reduction of serum total lipids. Also there is a widespread agreement that consumption of food low in protein or low in fat resulted in substantial weight loss. The elegant model of Flatt (1987) predicts that low protein or low fat diet should induce weight loss. Long term trials of low-fat or low protein

Changes In Some Haematological And Biochemical Parameters

diets have resulted in decreases in body weight as compared to control group fed balanced diet Lee-Han (1988) and Retzl half (1991).

Low-protein diets are still major pediatric problems in many parts of the world and result in an overall reduction in the growth and development of all major tissues of the body. (Tover *et al.*, 1998). Anaemia is a very common manifestation in low-protein or low fat diet (Fondu *et al.*, 1978). Therefore, the present study aimed to provide additional information on the relationship between the role of low protein or low fat in diet on the haematological parameters and some association (Soheir *et al.*, 1996).

Impaired erythropoietin production was considered one of the reasons for anaemia as mentioned by Tazaki *et al.* (1994); Zaahkouk and Helal (1999) recorded a significant decrease in red blood cells count (R.B.C_s), or erythrocyte haemoglobin (Hb) concentrations and white blood cells count (W.B.C_s) or leucocyte in rats fed on low-energy diet. Caro *et al.* (1983) reported that erythropoietin (EPO) is produced not only by the kidneys but also by other organs, such as liver.

The haematological alterations produced after low-energy diet period have been improved greatly after recovery period when the rats fed on balanced diet. The improvements may be attributed to increased protein synthesis after the inclusion of amino acid in the diet and induce synthesis of red blood cells via stimulation of erythropoiesis. Also improvement in the immune system by improving the percentages of each kind of leucocytes that meet sustaining body activities (Jacobh *et al.*, 1996).

There was a significant decrease in blood glucose level in response to low-protein and fat diets Davalose *et al.*, (1996) and Zaahkouk and Helal,

(1991) postulated that the decrease in serum glucose of low-energy diet may be due to decrease gluconeogenesis, depletion of liver glycogen and increased utilization of glucose by tissue. The decrease in total protein, albumin and globulin in low-protein diet might be due to the depletion of daily requirements of amino acids and consequently the disturbance in protein synthesis. The same results were recorded by Zaahkouk and Helal (1999).

Hypotriglyceridemia was evident in low-fat diet due to decrease in the rate of synthesis of very low density lipoprotein (VLDL) the main triacylglycerol carrier, and its secretion from the liver to the circulation and correlated to the rate of lipid biosynthesis in the hepatic tissue particularly triacylglycerol (Dietschy *et al.*, 1978). Hypocholesterolemia was evident in all treated rats with low protein or low fat diet. To reduce plasma cholesterol concentration and the risk of coronary heart disease, recent recommendations have suggested that fat man intake of saturated fats should be reduced to below 10% of dietary energy (Men Sink, 1992). Sally *et al.* (1997) showed that low-fat diet enriched with olive oil provides advantages over a very low-fat diet in the control of serum lipoproteins among persons with hypercholesterolemia.

Katan (1997) concluded that there are some indications that high carbohydrates, high fibres and low-fat diets may help to produce ischemic heart disease in diabetes and obesity. Hypercholesterolemia has been

identified as a major risk factor of coronary artery disease (Neaton, 1992). Thus, reduction in concentration of total serum cholesterol, and low-density lipoprotein (LDL-cholesterol) have

been shown to decrease the risk for future coronary events.

Because serum cholesterol concentration can be lowered by altering intakes (Grundy *et al.*, 1990) and Dwyer (1995). It has been recommended generally that dietary fibre intake be increased but variable emphasis has been given to fibres-Lipids lowering effects (Mekky 1997). One reason low-fat diets have such appeal is that they are thought to prevent weight gain.

Adult population groups, that subsist on low-fat diets are often lean, and this may cancel out the effect of such diet on high density lipoprotein-cholesterol (HDL-cholesterol). To maintain weight loss, it may be physiologically necessary for successful weight loss maintainers to consume a lower-energy and lower-fat diet than persons who have been obese as recorded by Siao Mei, (1998).

Low-fat diet reduces the risk of cardiovascular disease, generally and is accepted to be a consequence of a decrease in circulating levels of LDL and HDL-cholesterol. Concentrations (William *et al.*, 1999).

Chronic low-energy diet is one of the most important causes of several metabolic dysfunctions such as hypoinsulinemia, hypoleptinemia and hypotestosteronemia. A positive correlation exists between leptin and insulin as well as between leptin and testosterone (Baranowska *et al.*, 1999). Leptin, the protein product hormone of the ob/gene (Zhang *et al.*, 1994) is produced in adipose tissue and synthesised in ovary, transported in the oocyte, and made by both fetus and placenta (Hima, 1999).

Also leptin occurs in the lung, kidney, heart, brain, spleen, liver, and

muscle (Daniel *et al.*, 1998). Leptin is thought to be an inhibitor to appetite. In addition leptin may have a role in erythropoiesis (Young *et al.*, 1997). Thus Bennett *et al.* (1996) reported a near significant positive correlation between serum leptin and haemoglobin level. Serum leptin is related significantly to body fat content, patients with congenital deficiency of leptin have severe morbid obesity starting in early childhood (Montague *et al.*, 1997).

Hyperinsulinemia have shown an effect on leptin regulation and is related to acute change in nutritional status. (Dugogo *et al.*, 1996). In support long term insulin infusion, via a direct trophic effect of insulin on the adipocyte, increase leptin secretion (Kolaczynski *et al.*, 1996). The present study was planned to investigate the effect of low-protein or low-fat diet on blood picture and some biochemical parameters of male albino rats.

MATERIAL AND METHODS

Thirty male albino rats of similar age and weight, weighing 150 ± 10 gm at the beginning of the experiment were used. All animals were provided free access to tap water throughout the study and were maintained in a temperature-controlled environment (22-25°C) with a 12h/12h-light/dark cycle. All rats were randomly divided into 3 main groups as follows.

- 1- First group (n=10) was fed the control diet (CD) consisting of 22% protein (casein), corn starch 60%, cellulose, 5% soybean oil 7%, ineral mixture 3% and vitamin mixture 3%.
- 2- Second group was fed low protein diet (LPD) consisting of 4% protein (casein), 78% corn starch, 5% cellulose, 7% soybean oil, 3%

Changes In Some Haematological And Biochemical Parameters

mineral mixture, and 3% vitamin mixture.

3- Third group was fed low fat diet (LFD) consisting of 4% fat (corn oil), 78% corn starch, 5% cellulose, 7% sucrose, 3% mineral mixture and 3% vitamin mixture.

The treatment diets nearly isocalori, thus the only dietary variable altered was protein and fat. Food consumption and spillage were measured to the nearest 0.1g. Each 5 rats were housed in a separated cage so that food consumption and body weight could be measured daily. Body weight was measured in the nearest gram using triple beam balance. Daily weighing ensured that there were no health problems occurring in rats fed the low protein or low fat diet and provided data for subsequent analysis.

After 30 days of treatment, five animals of each group were decapitated, while the other half of each group was kept for 15 days for recovery period and fed control or balanced diet. Blood samples were collected in two centrifuge tubes, one containing anticoagulant for haematological analysis and the second tube devoid any anticoagulant and centrifuged for 20 minutes at 4000 rpm. Serum was collected and transferred into another clean tube for biochemical analysis.

Haematological parameters include: red blood cell count (R.B.Cs) and white blood cells count (W.B.Cs) according to the method of Dacie and Lewis (1991), Haemoglobin (Hb) concentration was measured according to the method of Drabkin and Austin (1932). Haematocrit (Hct) value was determined by the method of Rodak (1995) using heparinized capillary tubes and erythrocyte indices according to the method of Rodack (1995).

Biochemical parameters include: glucose, concentration was determined in serum by using the method of Trinder

(1969), total protein level was measured colorimetrically using commercial available kit according to Doumas (1975) method, Albumin concentration was estimated by using the method of Doumas et al (1971) using commercial available kit.

Globulin level in serum was calculated by subtracting albumin from total protein, triglycerides concentration in serum was colorimetrically measured by using the method of Wahlefeld (1974), using Boehringer Mannheim (GmbH Diagnostica Kit). Total cholesterol was determined according to the method of Allain et al. (1974). Low density lipoprotein-cholesterol (LDL-cholesterol) was colorimetrically estimated by using the method of Friedwald et al. (1972). Also high density lipoprotein-cholesterol was measured according to the Benzie (1979) method.

$$\text{LDL-Cholesterol, } = \text{Total cholesterol} - \text{HDL-cholesterol} \frac{\text{Triglycide}}{5}$$

Insulin concentration in serum was measured by radio-immunoassay kit obtained from D.P.C. Los Angeles, according to the method of Reeves (1983). Leptin level in serum was measured according to MaZognium et al. (1996). Serum testosterone concentration was carried out using the method of Dolacardo et al. (1973). Statistical analysis: The statistical analysis of the obtained data was done according to Armitage (1974).

RESULTS

It is clear that the percentage change of body weight gain or loss as indicated in table (1) and figure (1) was 25% of change in group received diet containing low-protein diet and 50% of change in group received low fat diet

after 30 days of treatment as compared to balanced diet or control group. After 15 days of recovery period by balanced or control diet the percentage of change 20 and 36 in group received diet containing low-protein or low-fat diet respectively.

As for haematological parameters, R.B.C_s and W.B.C_s count, Hb% concentration, Hct value and erythrocyte indices (MCV, MCH and MCHC) results are presented in table (2) and figure (2). Remarkable changes were observed. R.B.C_s count and Hb concentration were lowered in case of the group receiving low-protein or low-fat diet during the treatment period and insignificant change after recovery period. Also W.B.C_s significantly decreased only in the group receiving low-protein diet as observed in table (2) and figure (2).

Investigation of other haematological indices revealed that the two low-energy diets caused significant reduction in the mean corpuscular volume (MCV). The mean corpuscular haemoglobin (MCH) showed no significant change, while the mean corpuscular haemoglobin concentration (MCHC) was significantly decreased as compared to the control group.

Table (3) and figure (2) represent serum glucose concentration in the group of rats receiving low-

protein or low-fat diet for 30 days treatment. Results showed a significant ($P < 0.01$) reduction as compared to the group fed on control or balanced diet. As shown in table (2) and figure (2) low-protein diet revealed a significant ($P < 0.05$) decrease in total protein after 30 days of treatment and this decrease was improved after recovery period. Both serum albumin and serum globulin showed a significant

decrease ($P < 0.05$) in the group fed low-protein diet.

Hypolipidemia was evident after 30 days of treatment by receiving diet containing low-protein or low-fat diet, therefore a significant ($P < 0.05$) decrease was shown in circulating triacylglycerolemia whereas circulating concentrations of total cholesterol were significantly ($P < 0.01$) decreased as shown in table (2) and figure (2). The increase in circulating cholesterol was evident in HDL-cholesterol, the main cholesterol carrier lipoprotein in rat after treatment period 30 days. However LDL-cholesterol levels were significantly below the control level after 30 days of treatment period.

The present data concerning hormones, insulin, leptin and testosterone as observed in table (4) and figure (3) showed a significant decrease ($P < 0.05$) in insulin level in the group which received low-protein or low-fat diet for 30 days and improved after recovery period 15 days by balanced diet. Hypoleptinemia was observed in the group receiving low-fat diet. Also there is a significant ($P < 0.0$) reduction in testosterone hormone in the group receiving low-protein or low-fat diet. A positive correlation between insulin and leptin hormone as well as a correlation was found between leptin and testosterone concentrations and low-fat diet or type of diet.

DISCUSSION

From the present data, all animals that received low-protein or low-fat diet were observed to have lesser body weight than control or the group fed balanced diet. Low-energy diet leads to decrease in body weight that may be attributed to or in part by the decrease in insulin-like growth factor-1 (IGF-1) levels as has been

Changes In Some Haematological And Biochemical Parameters

reported by keteslegers *et al.* (1995). The most consistent predictors of greater weight loss were low-intake of dietary fat, higher initial waist circumference, and higher levels of moderate intensity physical activity Besbas *et al.* (1998).

The lower the achieved fat intake, the greater the initial and sustained weight loss. Hill *et al.* (1993) postulated that the magnitude of the energy deficit rather, than the cause of the deficit is the most important factor in initiating weight loss. In contrast, a specific reduction in the dietary fat intake is likely, to be most important in achieving sustained weight loss because it counteracts the decline in fat oxidation after weight loss (Hill *et al.*, 1993). These data suggest that the successful weight loss maintainers consume less energy and a lower percent of dietary energy from fat (Siao Mei *et al.*, 1998).

Alterations of various haematological parameters in blood of the present work were also investigated. When rats were fed low-protein or low-fat, diet for 30 days exhibited significant decrease in both red blood cells (R.B.C_S) and white blood cells (W.B.C_S) count and haemoglobin concentration. These findings were in agreement with Fondu *et al.* (1978) and Soheir *et al.* (1996). The early response to chronic changes in blood volume in haemodilution. These help to maintain

the circulating blood volume but cannot replace the R.B.C_S. So drop in erythrocyte count will occur. These results were in agreement with Zaakhouk et al (1999) who reported that the low-fat diet cause anaemia and decrease in haematocrit value (Hct) which may be due to increased plasma volume and decrease in blood corpuscle relatively. The present results also demonstrated significant reduction in

Hct, MCV, and MCHC while MCH revealed no significant change as compared to control group. This may lead to anaemia as a result of iron and globin deficiency.

Low-energy diet decreases blood glucose level due to decreased gluconeogenesis, depletion of liver glycogen and increased utilization of glucose by tissues. Traianedes *et al.*, (1992) found that fasting glucose level in diabetic rats fed high fat diet was markedly higher relative to those fed low fat diet.

The present investigation indicated that concentration of serum total protein, albumin and globulin in groups that received low-fat or low protein diet significantly ($P < 0.05$) decreases. These data were in agreement with that of Zaakhouk *et al.*, (1999), who recorded that the marked decrease observed is probably due to disruption of hepatic cells, a decline in serum protein and level was noticed as a physiological response to malabsorption or low-energy diet.

In the current study, low-protein or low fat diet exhibited marked serum hypotriglyceridemia characterized by decreased serum triglycerides due to the low rate of synthesis of VLDL, the main triglyceride carrier and its secretion from the liver to the circulation and correlated to the low rate of lipid biosynthesis in the hepatic tissue particularly triacylglycerol (Dietschy *et al.*, 1978).

Hypocholesterolemia was evident in the group fed low-fat diet and the group that received diet containing low protein diet. Hypercholesterolemia has been identified as a major risk factor of coronary artery disease (Neaton 1992). Thus, reduction in total serum cholesterol and LDL-cholesterol which accounts for most of the cholesterol in the blood. It carries cholesterol to the tissue of the body

including the arteries. For this reason a high level of LDL-cholesterol increases the risk of dangerous build up and blockage in the arteries (Assmann, 1982)

Low-fat diet reduces the risk of cardiovascular disease, chronic low energy diet is one of the most important causes of several metabolic dysfunction, HDL-cholesterol is sometimes called the good cholesterol because it contains a relatively small amount of cholesterol itself and carries away harmful fatty deposits from cells and tissues to the liver for excretion from the body. This helps to prevent the build up of cholesterol in the wall of arteries (Assmann N.G., 1982).

Human insulin is a polypeptide hormone originating in the beta cells of the pancreas and serving as a principal regulator for the storage and production of carbohydrates. Its secretion is normally stimulated by increases in the

amount of glucose in circulation. This leads to higher insulin levels and more rapid tissue assimilation of glucose followed by a decline in the insulin level as the glucose level subsides. Low-fat diet decreases insulin secretion as recorded by Plagmann *et al.* (1999). These results were obtained by Nagatani *et al.* (1998). Low-fat diet led to Hypoleptinemia. Hypothalamic neuropeptide Y (NPY) neurons are influenced by circulating level of insulin and leptin and are thought to be involved in mediating hunger following undernutrition (Pick Kavanci 1998). The decrease in the testosterone level is expected to be effect of the biological activity of Sertoli cells or may be attributed to Leydig cells exhaustion (Dyme 1983) which is affected by low-energy diet or the interstitial tissues decreased capacity to synthesize testosterone (Yogev *et al.*, 1985).

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Changes In Some Haematological And Biochemical Parameters

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Table (1): Mean values of body weight changes of male albino rats fed on low-energy diet for 30 days treatment and 15 days recovery period.

	Treatment period 30 days			Recovery period 15 days		
	Control	L.P.D.	L.F.D.	Control	L.P.D.	L.F.D.
Percentage of change	180	155 25	130 50	185	165 20	149 36

Changes In Some Haematological And Biochemical Parameters

Table (2) : Mean values of haematological parameters of male albino rats fed on low-energy diet for 30 days treatment and 15 days recovery.

Parameters		Treatment period 30 days			Recovery period 15 days		
		Control	L.P.D.	L.F.D.	Control	L.P.D.	L.F.D.
R.B.C _S x 10 ⁶ Cell/mm ³	Mean ± S.E propability	6.5 0.12	4.5 0.2	5.1 0.3	6.4 0.15	5.5 0.18	6.1 0.2 insig
Hb.g%	Mean ± S.E propability	16.1 1.1	12.5 1.3	13.5 1.2	15.9 1.9	14.5 2.0	15.0 2.1 insig
W.B.C _S x 10 ³ Cell/mm ³	Mean ± S.E propability	9.5 1.1	7.2 1.0	7.5 1.0	9.6 1.5	8.5 1.2	8.0 1.0 insig
Hct %	Mean ± S.E propability	42.0 0.9	36.1 0.9	38.2 1.2	41.5 1.3	38.1 0.9	40.0 1.5 insig
MCV μ^3	Mean ± S.E propability	64.6 2.5	80.2 2.6	74.9 2.6	64.7 2.0	69.3 2.1	65.6 2.0 insig
MCH Pg	Mean ± S.E propability	24.7 1.2	27.7 0.9	26.4 0.7	24.8 1.2	26.4 1.0	24.6 1.1 insig
MCHC %	Mean ± S.E propability	38.1 1.0	34.6 1.2	35.3 0.9	38.3 1.7	39.1 1.2	37.5 1.3 insig

L.P.D = Low-protein diet

L.F.D = Low-fat diet

Table (3): Mean values of biochemical parameters of male albino rats fed on low-energy diet for 30 days treatment and 15 days recovery.

Parameters	Treatment period 30 days			Recovery period 15 days		
	Control	L.P.D.	L.F.D.	Control	L.P.D.	L.F.D.
Glucose mg/dL	Mean + S.E propability	100 1.6 P< 0.01	80 1.5 P< 0.01	75 1.7 P< 0.01	95 2.0 P< 0.01	89 1.5 P< 0.01
Total protein g/dL	Mean + S.E propability	6.5 0.5 P< 0.05	4.5 0.6 insig	5.9 0.9 insig	5.8 1.0 insig	6.0 0.8 insig
Albumin g/dL	Mean + S.E propability	3.8 0.1 P< 0.05	3.0 0.2 P< 0.05	3.0 0.1 P< 0.05	3.6 0.1 P< 0.05	3.5 0.2 insig
Globulin g/dL	Mean + S.E propability	2.7 0.1 P< 0.01	1.5 0.07 insig	2.9 0.03 insig	2.9 0.01 insig	2.5 0.02 insig
A/G ratio	Mean + S.E propability	1.4 0.01 P< 0.05	1.3 0.03 P< 0.05	1.0 0.01 P< 0.05	1.3 0.04 P< 0.05	1.2 0.01 P< 0.05
Triglyceride mg/dL	Mean + S.E propability	115.0 1.8 P< 0.05	105.0 2.1 P< 0.05	80 2.3 P< 0.05	108 2.0 P< 0.05	100 1.6 P< 0.05
Total cholesterol mg/dL	Mean + S.E propability	106.1 1.5 P< 0.01	90.4 1.2 P< 0.01	80 1.3 P< 0.01	104 1.4 P< 0.05	96 1.2 P< 0.05
LDL.cholesterol mg/dL	Mean + S.E propability	106.1 1.5 P< 0.01	90.4 1.2 P< 0.01	60 1.3 P< 0.01	104 1.4 P< 0.01	90 1.2 P< 0.01
HDL.cholesterol mg/dL	Mean + S.E propability	53.5 1.6 P< 0.05	60.4 1.2 P< 0.01	76 1.5 P< 0.01	54.0 2.5 insig	60.0 2.0 insig

Changes In Some Haematological And Biochemical Parameters

Table (4): Mean values of some hormones level in male albino rats fed low-energy diet for 30 days treatment and 15 days recovery period.

Parameters	Treatment period 30 days			Recovery period 15 days		
	Control	L.P.D.	L.F.D.	Control	L.P.D.	L.F.D.
Insulin μi-u/ml	Mean ± S.E propability	60.1 0.9 P< 0.01	55 0.7 P< 0.05	56.0 0.6 P< 0.05	59.0 0.7 P< 0.01	65.2 0.6 insig
Leptin ng/ml	Mean ± S.E propability	3.5 0.18 insig	3.4 0.20 insig	2.5 0.1 P< 0.01	3.5 0.2 insig	3.2 0.3 insig
Testosterone ng/ml	Mean ± S.E propability	0.5 0.01 P< 0.01	0.4 0.02 P< 0.01	0.25 0.01 P< 0.01	0.45 0.03 insig	0.40 0.02 P< 0.05

Changes In Some Haematological And Biochemical Parameters

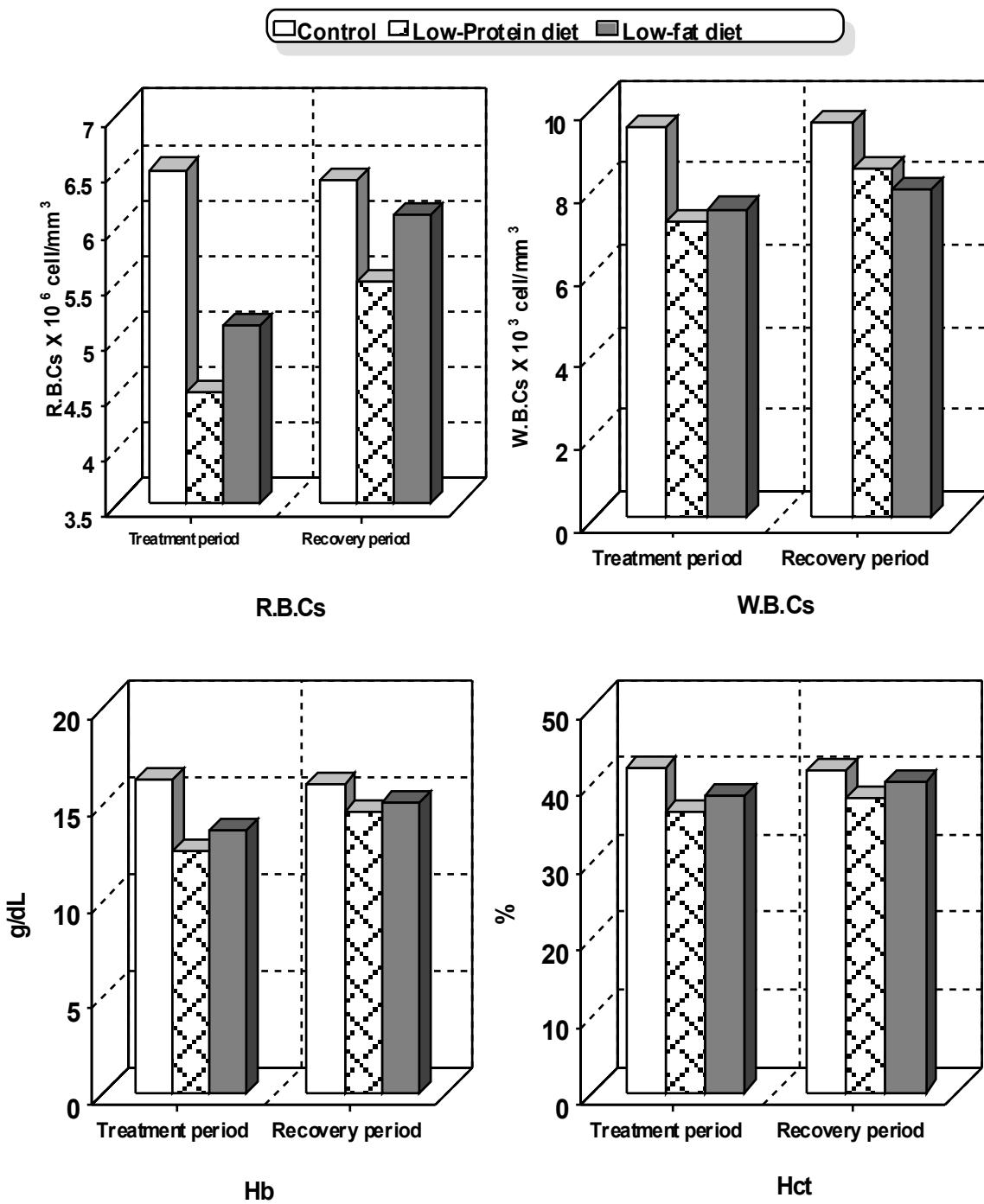


Fig.(1) Diagrammatic representations of some hormonesparameters of male albino rats fed on Low-protein and Low-fat diet for 30 days treatment and 15 days recovery periods.

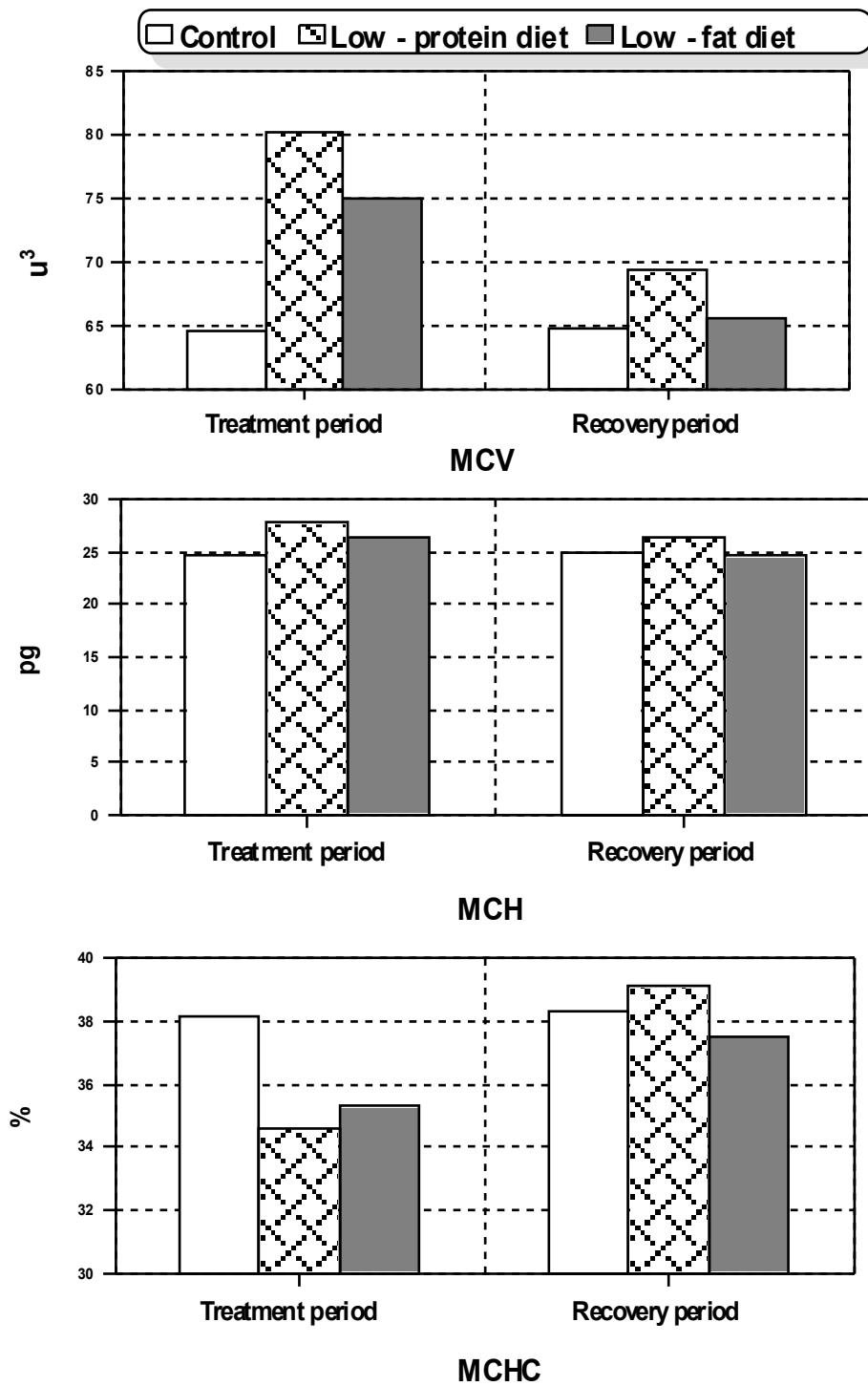


Fig.(2) Diagrammatic representations of some erythrocyte indices of male albino rats fed on Low-protein and Low-fat diet for 30 days treatment and 15 days recovery periods.

Changes In Some Haematological And Biochemical Parameters

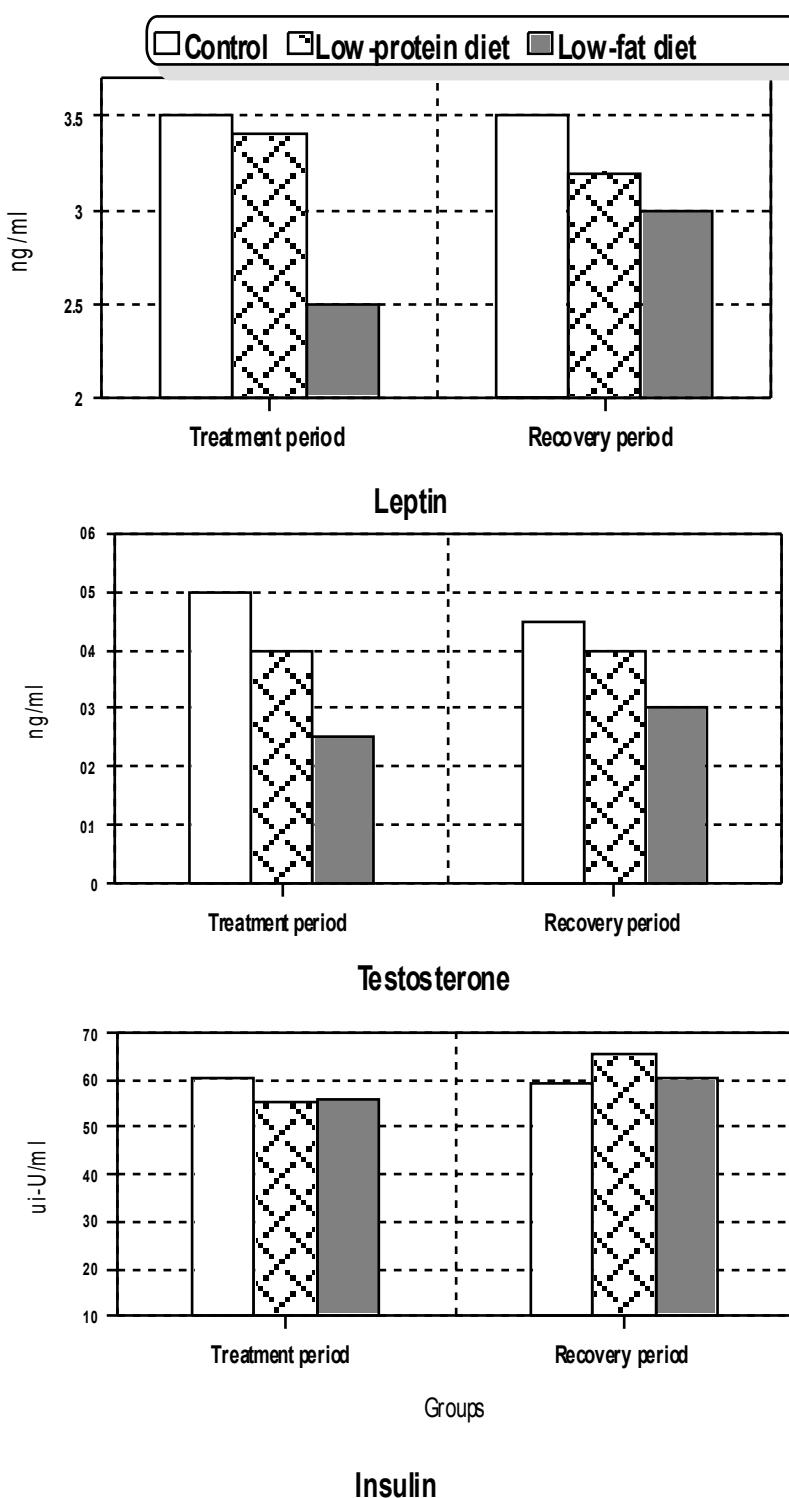


Fig.(3) diagrammatic representations of some hormones of male albino rats fed on Low-protein diet and Low - fat diet for 30days treatment and 15 days recovery periods

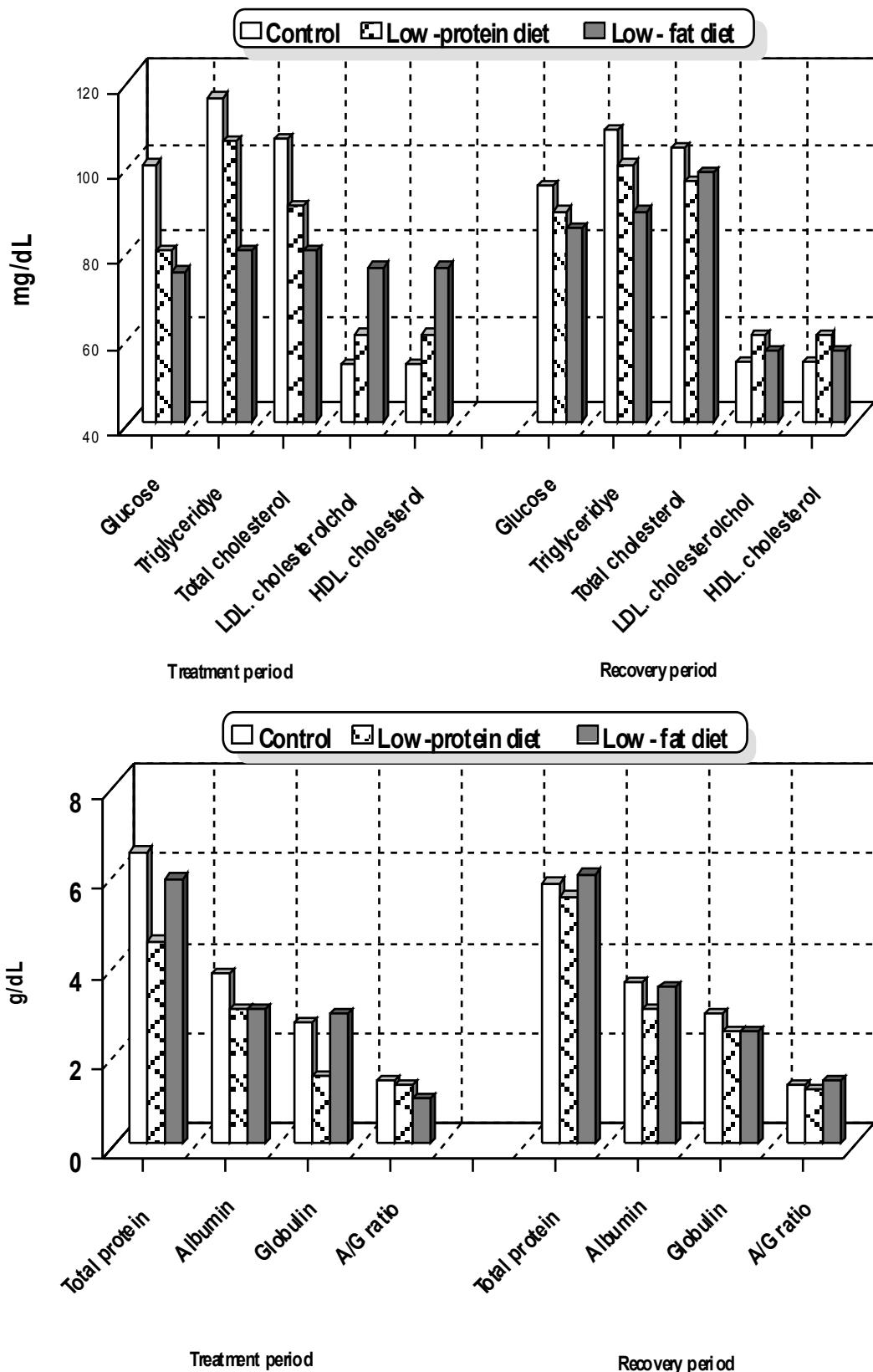


Fig.(4) diagrammatic representations of some biochemical parameters of male albino rats fed on Low - protein diet and Low - fat diet for 30days treatment and 15 days recovery periods

التغيرات فى بعض المعايير الدموية والقياسات البيوكيميائية
فى الفئران البيضاء إستجابة لوجبة منخفضة الطاقة

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