Physiological and Biochemical Alterations in Sprague-Dawley Female Rats Subjected to High Fat Diet and Intermittent Fasting

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Abstract: Physiologically, it is known that diets rich in fat are generally associated with complicated metabolic disorders and health problems. Hyperlipidemia, atherosclerosis and obesity are one of the most interesting topics worldwide. Looking for safe solutions and beneficial strategies towards overcoming of dietary fat problems still need more scientific efforts. Fasting has been identified as the mechanism that allows one to improve one's body. Fasting has been recommended as therapy for various conditions by physicians of most cultures. The purpose of the present study was to characterize the effect of intermittent fasting on Sprague-Dawley female rats fed with high fat (HF) diet. The experimental rats were divided into 4 groups. Group 1 was fed with normal diet. Group 2 was fed with normal diet and subjected to fasting. Rats of group 3 were fed with HF diet. Animals of group 4 were fed with HF diet and subjected to fasting. Body weight gain was more increased after 2 and 4 weeks in rats fed with HF Diet. Furthermore, the values of serum cholesterol, triglycerides, low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), atherogenic indices, glucose and lactate dehydrogenase (LDH) were significantly increased, while the levels of high density lipoprotein cholesterol (HDL-C) and HDL-C ratio were statistically decreased. Fasting reduced the highly alterations of body weight gain and serum biochemical parameters in rats fed with HF diet. Also, fasting induced several changes in rats received the normal diet. According to these findings, fasting has no inappropriate effect and it is nutritionally safe and could be used as an effective strategy for prevention and control of hyperlipidemia, atherosclerosis, obesity and metabolic disturbances.

Key words: High fat diet, fasting, body weight, hematobiochemical analyses, female rats.

INTRODUCTION

Diet is an important factor controlling serum lipids and, consequently, the occurrence of coronary heart disease[1]. Nutrients may be classified as beneficial or harmful if they decrease or increase the serum level of lipids involved in cardiovascular disease development. Cardiovascular diseases are the leading causes of death worldwide, accounting for an estimated 14 million death in 1990 and projected to cause 25 million death in 2020[2]. Dyslipidemia is associated with hypertension, diabetes mellitus and obesity, and is one of the major risk factors for the development of cardiovascular disease[2-3]. Obesity is a chronic disease of increasing prevalence in most countries, which leads to substantial increase in morbidity, and mortality in association with many important complications such as insulin resistance, diabetes, hyperlipidemia, hypertension, coronary heart disease, sleep apnea and pulmonary dysfunction, stroke, diseases of the gallbladder, liver and the musculoskeletal system, reproductive dysfunction, venous insufficiency, deep vein thrombosis, and poor wound healing[8-11]. Obesity is a severe metabolic disorder, characterized with increases in energy intake and a decrease in energy output concerning body weight and glucose metabolism[12]. Many factors have been attributed to an epidemic of obesity including sedentary lifestyle, high-fat (HF) diets, and consumption of large amount of modern fast foods[13]. Obesity is an increasing problem in modern societies, due to the adoption of rapid lifestyle which results in high dietary intake of carbohydrates and fat accompanied by reduced energy consumption[9]. More than half of adult Americans are overweight or obese, like populations of many other countries[9]. Ramos et al.[14] thought that obesity should be a low-grade inflammatory disease because most of the obese patients had shown increased levels of interleukin-6 and tumor necrosis factor-alpha, both markers of inflammation. Obesity may be the underlying reason of cancers of the breast, endometrium, colon and prostate. Fat not only increases the palatability of food but also

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Ramadan fasting and starvation are not synonymous. Many physiological and psychological changes take place during Ramadan, most probably due to the changes in eating patterns, eating frequency and sleep patterns\[40\]. Additionally, several studies have reported on the physiological effects of Ramadan fasting, especially concerning changes in blood pressure, lipids profile, apoproteins, fasting blood sugar (FBS), hormones and other hematological and biochemical parameters\[11-39\]. However, any fasting by individuals suffering from a particular illness of medical condition should involve the supervision of a doctor and or fasting therapist. Generally, fasting can take on three essential forms: (1) Dry fasting involves abstaining from all manner of food and liquid. (2) Juice fasting involves abstinence from all food and drink except water and pure vegetable and fruit juices. (3) Modified fasting includes small amounts of food, usually raw fruits and steamed vegetables or the use of herbal teas or broths. Scientifically, fasting, especially the dry form, is characterized by a coordinated set of metabolic changes designed to spare carbohydrate and increase reliance on fat as a substrate for energy supply. As well as sparing the limited endogenous carbohydrate, and increased rate of gluconeogenesis from amino acids, glycerol and ketone bodies help maintain the supply of carbohydrate\[39\]. Till today there is no any investigation concerning the influence of fasting on hyperlipidemia, biochemical and physiological disturbances induced by HF diet in experimental female animals. Therefore, the current study was aimed to determine the physiological and biochemical effects of intermittent fasting on Sprague-Dawley female rats fed with HF diet.

**MATERIALS AND METHODS**

**Animals and Experimental Protocol:** Twenty four Sprague-Dawley strain female albino rats weighing 195-205 g were taken for the present experiment. The principles of laboratory animal care were followed through out the duration of experiment and instruction given by King Abdul Aziz University ethical committee was followed regarding experimental treatments. The rats were distributed into four groups (six rats per group) and were housed in standard cages at an ambient temperature of 20±1°C with 12 h light: 12 h dark cycle. The experimental rats had free access to standard diet and water for one week. After one week, Rats of group 1 were only fed with standard diet and served as control group. Group 2 was fed with standard (normal) diet and daily fasted for 10 h (7 am - 5 pm), five times weekly for 4 weeks. Animals of group 3 were fed with HF diet (15% mutton tallow). The experimental animals of group 4 were fed with same

is converted into body fat far more efficiently than carbohydrates\[19\]. The mechanism of HF diet-induced obesity is still unclear, but long-term exposure to a HF diet can increase body weight and adiposity in human and animals\[18,17\]. Another characteristic of HF diet-induced obese rodents is that they have a low sympathetic activity, which in turn results in decreased energy expenditure\[18\]. However, it is not known whether reduced sympathetic activity is due to a defect in the central nervous system (CNS) during the development of diet-induced obesity. The role of dietary fat in energy balance has been a topic of interest for researchers and public health investigators because many epidemiological studies and animal models have characterized response to HF feeding in humans and rodents\[19,20\]. HF diet produces a consistent and significant increase in body fat content that is dependent on both the amount of fat in the diet and the duration of feeding. Hyperphagia may be one important mechanism by which HF diets promote obesity because fat is less satiating than carbohydrate\[21\]; thus, it has been suggested that fat may lead to passive diet overconsumption\[22\]. HF diet to rodents has proved to be a useful model of the putative effects of dietary fat in humans\[21,24\]. There is a large body of evidence that rats fed HF chronically produce obesity and insulin resistance\[15,12\]. Medical studies show that about 70% of adults over 50 years old suffer atherosclerosis. Hyperlipidemia is a well known risk factor for atherosclerosis\[24\]. To prevent this disease a number of hypolipidemic drugs have been administrated in clinic. However, they all have obvious or potential side effects to some extent\[27\]. The search is in continuity for safe, dependable, and effective drugs.

Fasting has been used in religious and health traditions around the world to improve health and to aid in the achievement of enlightenment and to help strengthen self-control for thousands of years. From the Bible to the Holy Quran to the medical texts of ancient Greece, abstinence from food and drink has played a central role in the treatment of illness and disease. Today, many practitioners turn to fasting for a variety of conditions, but does this practice actually work?. Is there any hard scientific evidence backing up this practice, or is the idea of fasting little more than self-induced starvation?. Ramadan (Islamic) fasting, involving one month of abstention from food and fluid intake during daylight hours, is practised by a large part of the world population. This period involves a shift in the pattern of intake from daytime to the hours of darkness\[24\]. As a result, the Ramadan fast provides an excellent opportunity to study the effects of various diets on the human body and can serve as an excellent research model for metabolic and behavioral studies\[29\].
diet given to group 3 and fasted at the same treatment durations of group 2. All experimental rats' body weight were recorded after 2 and 4 weeks. At the end of experimental duration, all rats were fasted for 6 h and blood samples were taken from orbital venous plexus under total anesthesia using diethyl ether. Obtained blood sample were transferred to test tubes and the sera were separated using the standard centrifugation process. Serum samples were kept in freezer for biochemical measurements. Serum triglycerides, cholesterol, high density lipoprotein cholesterol (HDL-C), glucose, total protein, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were estimated using Automated Clinical Chemistry Analysis System, Dimension® type RXL Max (Dade Behring Delaware, DE 19714, U.S.A.). Serum low density lipoprotein cholesterol (LDL-C) was calculated using the equation of Friedewald et al.[40]. Serum very low density lipoprotein cholesterol (VLDL-C) was evaluated using the following equation:

VLDL Cholesterol = Triglycerides/2.175

The methods of Martirosyan et al.[41], Frohlich and Dobiasova[42], Brehm et al.[43] and Dobiasova[44] were used to calculate the atherogenic indices as follows:

Cardiac Risk Ratio (CRR) = Total Cholesterol / HDL Cholesterol
Atherogenic Coefficient (AC) = (Total Cholesterol – HDL Cholesterol) / HDL Cholesterol
Atherogenic Index of Serum (AIS) = Log (Triglycerides / HDL Cholesterol)
HDL-Cholesterol (HDL-C) Ratio was calculated using the following equation:

HDL Cholesterol Ratio = HDL Cholesterol X 100 / Total Serum Cholesterol – HDL Cholesterol.

Statistical Analysis: The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) for Windows, version 12.0. Quantitative data were reported as mean ± standard deviation (SD) and compared using the paired two-tailed student’s t-test. A probability level of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Results: Effects of fasting, HF diet and HF diet plus fasting on rats' body weight and their percentage changes are shown in Figures 1 and 2. In control group, the percentage changes of body weight gain are 13.7 % after 2 weeks and 17.2 % after 4 weeks. The percentage changes of body weight gain in rats fed with normal diet and subjected to fasting (group 2) are 9.8 % after 2 weeks and 12.2 % after 4 weeks, while the percentage changes were more increased in rats exposed to HF diet (group 3) after 2 weeks (14.3%) and 4 weeks (22.1%). Fasting also had pronounced effects on the percentage changes of body weight gain in rats fed with HF diet (group 4). In rats of group 4, the percentage changes of body weight gain are 13.5 % after 2 weeks and 17.3 % after 4 weeks. Serum cholesterol (-12.8%) and LDL-C (-35.6%), cardiac risk ratio (CRR) (-13.3%) and atherogenic coefficient (AC) (-23.3%) were significantly decreased in rats fed with normal diet and exposed to fasting (group 2), while the level of HDL-C ratio (+35.7%) was statistically increased compared with control rats (Table 1). In group 2, insignificant alterations of serum triglycerides, HDL-C, VLDL-C, atherogenic index of serum (AIS), glucose, total protein, creatinine, AST, ALT and LDH levels were noted. In rats fed with HF diet (group 3), the values of cholesterol (+28.1%), triglycerides (+42.1%), LDL-C (+79.7), VLDL-C (+42.9%), CRR (+63.4%), AC (+111.3%), AIS (+440.0%), glucose (+6.1%) and LDH (+15.4%) were statistically evoked, while the levels of HDL-C (-21.1%) and HDL-C ratio (-50.1%) were notably declined. Furthermore, the levels serum total protein, creatinine, AST and ALT were statistically unchanged in rats of group 3 compared with control and other treated groups. In comparison with control group, the levels of all serum biochemical parameters and atherogenic indices were insignificantly altered in rats fed with HF diet combined with fasting process (group 4), but the levels of LDL-C, CRR, AC and AIS were markedly enhanced, and the value of HDL-C ratio was statistically declined compared with group 2 (Table 1).

Discussion: Hyperlipidemia is one of the established major risk factors of coronary heart disease and cerebrovascular disease. Diet modification is the cornerstone of therapy for mild to moderate hyperlipidemia and is also recommended along with pharmacological treatment in people at higher risk of coronary heart disease as a part of other interventions like exercise, smoking cessation, cessation of excessive alcohol, and weight control. Diet therapy has shown modest reductions in lipid levels in the population[44-45]. The present study indicates that fasting reduced the percentage changes of the body weight gain in rats fed with normal or HF diet. These results are in line with the reports of several researchers who observed a significant decrease in body weight during Ramadan fasting[46-49]. The decrease in body weight was attributed to efficient utilization of body fat during fasting[50]. It has also been reported that overweight persons lose more weight than normal or under-weight subjects.
**Fig. 1**: Body weights (mean ± SD) at 0, 2 and 4 weeks of control, fasting, HF diet, HF diet plus fasting treated rats (n=6).

**Fig. 2**: Body weight's percentage changes at 2 and 4 weeks of control, fasting, HF diet, HF diet plus fasting treated rats.

**Table 1**: Serum cholesterol, triglycerides, HDL-C, VLDL-C, atherogenic indices, HDL-C ratio, glucose, total protein, creatinine, AST, ALT and LDH values (mean ± SD) of control, fasting, HF diet, HF diet plus fasting treated rats (n=6). Percentage changes are included in parentheses.

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<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
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<td></td>
<td>Control</td>
<td>Fasting</td>
<td>HF diet</td>
<td>HF diet+ fasting</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>1.64±0.11*</td>
<td>1.43±0.15 (-12.8%)</td>
<td>2.10±0.23(+28.1%)</td>
<td>1.68±0.25(+2.4%)</td>
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<td>Triglycerides (mmol/L)</td>
<td>0.76±0.05*</td>
<td>0.73±0.05 (-4.0%)</td>
<td>1.08±0.20(+42.1%)</td>
<td>0.84±0.13(+10.5%)</td>
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<td>HDL-C (mmol/L)</td>
<td>0.71±0.66*</td>
<td>0.71±0.60 (0.0%)</td>
<td>0.56±0.07(-21.1%)</td>
<td>0.67±0.04(-5.6%)</td>
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<tr>
<td>LDL-C (mmol/L)</td>
<td>0.59±0.09*</td>
<td>0.38±0.15(-35.6%)</td>
<td>1.06±0.21(+79.7%)</td>
<td>0.63±0.20(+6.8%)</td>
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<td>VLDL-C (mmol/L)</td>
<td>0.35±0.02*</td>
<td>0.34±0.02 (-2.9%)</td>
<td>0.50±0.09(+42.9%)</td>
<td>0.39±0.06(+11.4%)</td>
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<tr>
<td>Cardiac Risk Ratio</td>
<td>2.33±0.19*</td>
<td>2.02±0.26(-13.3%)</td>
<td>3.81±0.71(+63.4%)</td>
<td>2.51±0.27(+7.7%)</td>
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<tr>
<td>Atherogenic Coefficient</td>
<td>1.33±0.19*</td>
<td>1.02±0.26(-23.3%)</td>
<td>2.81±0.71(+111.3%)</td>
<td>1.51±0.27(+13.5%)</td>
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<tr>
<td>Atherogenic Index of Serum</td>
<td>0.05±0.02*</td>
<td>0.04±0.02 (-20.0%)</td>
<td>0.27±0.10(+440.0%)</td>
<td>0.10±0.06(+100.0%)</td>
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<tr>
<td>HDL-C Ratio</td>
<td>76.74±13.44*</td>
<td>104.16±26.95 (+35.7%)</td>
<td>38.27±7.70(-50.1%)</td>
<td>67.49±11.07 (-12.1%)</td>
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<tr>
<td>Glucose (mmol/L)</td>
<td>7.43±0.24*</td>
<td>7.45±0.33(+0.3%)</td>
<td>7.88±0.34(+6.1%)</td>
<td>7.57±0.47(+1.9%)</td>
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<tr>
<td>Total protein(g/L)</td>
<td>65.00±4.29*</td>
<td>63.67±4.95(-1.9%)</td>
<td>60.33±6.74(-7.2%)</td>
<td>62.17±5.49(-4.4%)</td>
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<tr>
<td>Creatinine(μmol/L)</td>
<td>36.50±3.27*</td>
<td>35.33±2.86(-3.2%)</td>
<td>35.67±4.4 (+2.3%)</td>
<td>36.67±8.22(+3.2%)</td>
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<tr>
<td>AST(U/L)</td>
<td>80.83±3.71*</td>
<td>77.17±2.99(-4.5%)</td>
<td>76.50±4.64(-5.4%)</td>
<td>79.00±5.05 (-2.3%)</td>
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<td>ALT(U/L)</td>
<td>49.33±4.13*</td>
<td>48.33±5.96(-2.0%)</td>
<td>47.60±2.04(-4.7%)</td>
<td>48.33±3.19(-2.0%)</td>
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<tr>
<td>LDH(U/L)</td>
<td>205.17±12.12*</td>
<td>204.00±7.62(-0.6%)</td>
<td>236.67±15.33(+15.4%)</td>
<td>208.67±9.29(+1.7%)</td>
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The values within the same line that do not share the same superscript letter are significantly different (P <0.05).
during Ramadan fasting. Maislos et al. suggested that the decrease in body weight through Ramadan fasting may be related to reduction in fluid intake, a decrease in water stores that related to glycogen-bound, short reduction of body tissue with a moderate degree of hypohydration, and extracellular volume contraction. Obesity has been recognized as a serious risk factor for mortality and morbidity of cardiovascular diseases in general population. Kissebah and Krakower and Wajchenberg have demonstrated that cardiovascular disease-related morbidity and mortality might be affected not only by the total amount of fat but also by the regional distribution of body fat. Subsequent epidemiological reports have established that abdominal fat accumulation increases the incidence of cardiovascular disease and death. A balanced energy-restricted diet is the most reasonable method for weight reduction in human. Ideally, the diet should be nutritionally adequate except for energy. The number of calories must be decreased to the point where the body mass mobilize fat stores to meet daily energy needs. The energy restriction should be maintained at level where weight loss is 0.5-2 Lbs (226.8-907.2 g) per week. In such type of regimen, there will be no increase in ketone bodies in blood and thus, the side effects will be minimum. The caloric value of body fat is approximately 3500 Kcal/Lb (453.6 g). Thus, if a person reduces daily caloric intake by 500 Kcal the weekly deficit will be 3500 Kcal or 1 Lb. of weight loss. Moreover, fasting could be a safe nutritional approach for prevention and treatment of obesity. The Fast imposes a temporary ban on food intake resulting into a less energy intake. Hence, the observed reduction of rats' body weight after 2 and 4 weeks of fasting in rats fed with normal and HF diets may translate into a significant health benefit.

The data obtained in the present study revealed that rats received normal diet with fasting showed significant decreases in the level of serum cholesterol and LDL-C, and the values of CRR and AC, while the level of HDL-C ratio was statistically increased. These results indicate that fasting improves the lipid profile. The values of cholesterol, triglycerides, LDL-C, VLDL-C, CRR, AC, AIS, glucose, LDH were statistically increased, while the levels of HDL-C and HDL-C ratio were notably decreased in rats fed with HF diet. Several studies showed similar observations in experimental animals treated with HF diet. Fasting does have an implication on the blood levels of several biochemical parameters. Blood glucose level is decreased due to dietary restriction and its continuous metabolism by the body. Cholesterol synthesis is reported to decrease during fasting. Moreover, formation of triacylglycerol is also reduced. Carbohydrate and lipid metabolism is influenced by fasting resulting in changes in blood chemistry. There is no caloric intake during fasting and the continual use of glucose in the body for various vital functions lead to lowering of blood glucose level. The depletion of glycogen stores after prolonged fasting further decreases its level. The most common risk factors associated with increased risk of atherosclerotic heart disease or stroke are abnormalities of lipids, hypertension, smoking and some coagulation and hemostatic factors. Diets supplemented with either saturated fats or cholesterol lead to both hypercholesterolemia and hypertriglyceridemia concurrent with hepatic steatosis. Dyslipidemia, characterized by abnormally elevated plasma triacylglycerol and cholesterol concentrations, is an established risk factor for the development of coronary heart disease. Excessive intake of HF diet leads to changes in lipid metabolism. It is well established that the liver regulates plasma levels of cholesterol and triglyceride by secretion and transport of these lipids in the lipoproteins. Hypercholesterolemia and hypertriglyceridemia, is a major risk factor for the development of cardiovascular diseases. The abnormal metabolism and elevation of plasma cholesterol and lipoproteins are well documented risk factors for the development of atherosclerosis. Evidence from clinical trials indicates that reducing plasma cholesterol by dietary and/or pharmacological means leads to reductions in the incidence of death from cardiovascular disease. Hyperlipidemia in experimental rats was evidenced by a significant enhancement in the level of triglyceride and phospholipid in blood and liver. Cholesterol feeding has been often used to elevate serum or tissue cholesterol levels to assess hypercholesterolemia-related metabolic disturbances in different animal models. The increased blood cholesterol up to 25%, seems to be the result of liver lipid deposition, which provides acetyl coenzyme A to liver cells for cholesterol synthesis. The excessive liver lipid deposition leads to steatosis which represents an imbalance between triglyceride synthesis in the liver and its secretion. The present insignificant alterations in the levels of serum total protein, creatinine, AST and ALT in all experimental groups indicate an absence of kidney and liver dysfunctions. LDH is an intracellular enzyme capable of reversible formation of pyruvate and lactate in all eukaryotic and prokaryotic cells. LDH is found particularly in the kidney, heart, liver, lungs and skeletal muscle. In a cell metabolic level of pyruvate is normally utilized through one of three pathways, such as (1) conversion of pyruvate to lactate by LDH, (2) generation of glucose through gluconeogenesis and (3) formation of acetyl coA and its reutilization in the tricarboxylic acid (TCA) cycle. In contrast, lactate is released into the blood stream by red blood cells (RBC) and skeletal muscle cells, for converting it to glucose. Hence, modulations in the cellular status of
pyruvate or lactate will affect metabolic pathways involving pyruvate in a coordinated manner. The measurement of serum LDH has therefore been used as a diagnostic tool for the clinical elevation of subjects\(^{[76,79]}\). Increased serum level of LDH is usually found in cellular death and/or leakage from cells or in some cases it is a useful marker of myocardial or pulmonary infarction\(^{[74]}\). Amin and Nagy\(^{[80]}\) and Suvarunrsawat et al.\(^{[81]}\) reported that HF and high cholesterol diets-induced high serum lipid profile as well as serum LDH and other biochemical parameters in experimental rats. However, the present high activity of serum LDH demonstrated that the cellular membranes integrity of myocardial tissues may be disturbed. Also, the increase of LDH level in serum suggests an increased leakage of this enzyme from mitochondria as a result of HF effect. As observed in the present study and comparison with control data, the levels of all serum biochemical parameters and atherogenic indices were insignificantly altered in rats fed with HF diet combined with fasting process. These results confirmed the usefulness of fasting application against HF feeding. Further investigations are required in human to ascertain the beneficial influence of fasting, especially Islamic and dry forms, as a promising therapeutic agent for hyperlipidemia, atherosclerosis, obesity and metabolic disorders.

REFERENCES


