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Research Article

Effect the fasting and a ketogenic diet on some hematological and hormonal features of the reproductive system in male mice

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Abstract: Diet and lifestyle are the most important factors controlling maintaining the health of animals. Fasting is a type of physical stress that causes oxidative stress, which can increase reactive oxygen species (ROS). The ketogenic diet is low in carbohydrates, high in fat and sufficient protein. This system mimics fasting, causing ROS which affects the reproductive system, inflammation and apoptosis, thus affecting the secretion of hormones. This study aimed to compare some hematological and hormonal features of the reproductive system in male mice under fasting and ketogenic diets. For this purpose, 120 male mice were used and divided into three group's control, fasting, and ketogenic diet each consisting of 40 males. The control group was fed regular food, while the fasting group fasted for 24 hours, and the ketogenic diet group were fed a keto diet. The experiment lasted eight weeks and 10 male's mice from each group were killed at the end of every two weeks to obtain blood to measure CBC and hormones (LH, FSH and testosterone). Following fasting and a ketogenic diet in mice, significant changes in blood parameters were observed. Fasting did not affect the level of LH, while the ketogenic diet did reduce the level of LH. Fasting and the ketogenic diet equally reduced FSH, and testosterone was decreased more in the ketogenic diet group than in the fasting group.

Keywords: Fasting, Ketogenic diet, CBC, LH, FSH, Testosterone.
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Introduction

Food plays a major role in achieving a healthy lifestyle (Soleimani et al. 2016). Fasting is a self-disciplined procedure taken for a specified period of time without eating or drinking. It is a form of caloric restriction (CR). Fasting was used as one of the oldest treatments in medicine. Fasting causes a variety of changes such as metabolic, mineral, hormonal, immune and respiratory (Derakhshan & Derakhshan 2015). Fasting is one of the types of physical stress that can cause oxidative stress. Moreover, oxidative stress will increase the concentration of reactive oxygen species (ROS) and the increase in the proportion of ROS contributes to injury with infertility and reduce the weight of the testicles (Hafaz 2017). Studies have also shown that fasting for 12 hours/day and 24 hours/day for 65 days caused a decrease in concentration LH, FSH and testosterone in male albino rats (Omalaso et al. 2012).

The ketogenic diet consists of high fat, low carbohydrate, and moderate protein (Vidalia et al. 2015). Ketogenic diets are characterized by low carbohydrates (usually less than 50g/day) and relatively high levels of protein and fat (Paoli et al. 2013). This dietary intervention mimics fasting by ensuring a marked reduction in carbohydrates (the daily intake is usually less than 30g/day) which leads to ketone synthesis. These ketones are used as fuel for many tissues, including the central nervous system, skeletal muscle, and the heart (Mongioi et al. 2020). The goal of the ketogenic diet is to enter a state of nutritional ketosis and obtain energy from burning fat in the form of ketones (Mohorko et al. 2019).

The blood profile usually gives vital information about the body's reaction to damage, lesion, deprivation, and hematological stress (Rahman et al. 2001). In clinical pathology, important recommended hematological tests include WBC, RBC, HGB, HCT, PLT, MCH, MCV, and MCHC. These are the most important indicators for detecting anemia in most animals (Akpamu et al. 2011). Anemia has been associated with low testosterone levels. The researchers also hypothesized that men with hypogonadism had a physiologically related decrease in hematocrit (Ellegala et al. 2003).

Normal levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are important indicators of health (Mersane et al. 2017). LH is among the major pituitary hormones. It should be level LH naturally; testosterone is produced by Leydig cells and it plays a major role in steroid formation (Jamil et al. 2016). FSH is a glycoprotein hormone gonadotropin which is secreted through the anterior pituitary gland and regulates the reproductive system in the human body and plays a pivotal role in the formation of the ovarian follicle and the formation of sperm, so the natural concentration of this hormone is necessary for fertility in men and women (Kim et al. 2017). Testosterone is produced in Leydig cells in the testicle and is essential in maintaining the quality of semen by playing essential roles in sperm formation, sperm maturation, and sperm release (Mirsane et al. 2017)..Based on the above-mentioned background, this study aimed to compare some hematological and hormonal features of the reproductive system in male mice under fasting and ketogenic diets.

Materials and methods

Male mice were obtained from the Drug Control Department, the Iraqi Center for Cancer and Medical Genetics Research, and transferred to the animal house of the Biology Department, College of Science, Misan University. These mice were free of pathogens with an age of 8-12 weeks and had an average weight of 28g. The male mice were left for two weeks to adapt before the start of the experiment, and they were placed in plastic cages covered with metal mesh and furnished with sawdust, and the cages were cleaned twice a week. The study included 120 male mice that were divided into three groups, a control group of 40 mice, a fasting group of 40 mice, and a third group of mice that was placed on a ketogenic diet with a light/dark cycle of 12/12 hours. Animals are handled according to institutional guidelines approved by the local animal ethics committee for all experimental procedures. 10 male mice are killed at the end of every two weeks (2nd, 4th, 6^{th}, and 8th) to take blood.

Blood sample collection: Male mice are euthanized by placing the mice in a closed cage and placing chloroform on cotton inside the cage (Blackshaw et al. 1988). Blood is taken from the heart (1mL) (Parasuraman et al. 2010). Blood samples are taken every two weeks preferably from the ventricle and slowly so that the heart does not collapse, using a syringe (3ml) then the blood is divided into two groups; the first group was placed in tubes containing an anticoagulant. Blood parameters are measured using an automated blood analyzer, and this measurement includes white blood cells (WBC), neutrophils (GRAN), lymphocytes (LYME), Red blood cells (RBC), Platelets (PLT) hemoglobin concentration (HGB), platelet distribution (PDW), average pellet size (MCV), average platelet volume (MPV), hematocrit (HCT), average muscle hemoglobin (MCH), platelets (PCT), the average concentration of hemoglobin in the body (MCHC) red blood cell distribution (RDW) (Akpamu et al. 2011). The second part of the blood was placed in tubes that help to clot to obtain serum for measuring the hormones, including LH, FSH, and Testosterone.

Statistical analysis: The mean and standard deviation of the data were analyzed by software (SPSS) using One-way ANOVA (analyses of variance) followed by test LSD for statistical differences and at the level of significance ($P<0.05$) (Griffith 2007).

Results and discussion

The results showed in the second week no significant differences (P>0.05) in WBC and LYM in the fasting group and a significant increase (P<0.05) in the ketogenic diet group compared with the control group. In mono, a significant increase was observed in the fasting and ketogenic diet groups, and in Gran, HCT no significant differences were observed in the fasting group, while there was a significant decrease in the ketogenic diet group. In RBC and HGB, there was a significant increase in the fasting group and a significant decrease in the ketogenic group. In the MCH feature, there was a significant decrease in the fasting group equal to the significant increase in the ketogenic diet group, and in PDW, there were no significant differences in the fasting and ketogenic diet groups. Regarding, MCHC, MCV, PLT, and PCT, there was a significant decrease in the fasting group and a significant increase in the ketogenic diet group. There was a significant increase in MPV in the fasting and ketogenic diet groups (Table 1).

In the fourth week, the results showed a significant (P<0.05) increase in the level of WBC in the fasting and ketogenic diet groups. The fasting group did not show any significant difference in LYM and MCH, while there was a significant increase in the ketogenic diet group. There was a significant increase in Mono and Gran in the fasting group and no significant differences in the ketogenic diet group. In RBC and HCT, there was a significant increase in the fasting group and a significant decrease in the ketogenic diet group. The results showed a significant increase of PDW and MCV in the fasting and ketogenic diet groups. There is an equally significant decrease of MCHC in the fasting and ketogenic diet groups and MPV did not show any significant differences in the fasting and ketogenic diet groups. The results did not show any significant difference in HGB in the fasting group and showed a significant decrease in the ketogenic diet group. In PLT, a significant decrease in the fasting group and a significant increase in the ketogenic diet group were observed. The results showed a significant decrease in PCT in the fasting and ketogenic diet groups.

In the sixth week, the results showed a significant (P<0.05) decrease in the level of WBC and LYM in the fasting group and a significant increase in the ketogenic diet group. Regarding Mono and Gran, no significant differences were found in the fasting and ketogenic diet groups. The results showed a significant increase in RBC and PLT in the fasting group and a significant decrease in the ketogenic diet group. In MCH, a significant increase in the fasting group and no significant differences in the ketogenic diet group were recorded. The results showed no significant differences of PDW and HGB in the fasting group and a significant decrease in a significant decrease in the ketogenic diet group. MCHC, PCT, and HCT, significant decreases were found in the fasting and ketogenic diet groups. The results showed a significant increase and in MPV and MCV in the fasting and ketogenic diet groups.

In the eighth week, the results showed a significant increase (P<0.05) in the level of WBC, LYM, and MCV in the fasting and ketogenic diet groups. In the mono, no significant differences were observed in the fasting and ketogenic diet groups. In Gran, no significant difference was also observed in the fasting group and a significant decrease in the ketogenic diet group. The results of RBC, PDW, HGB, and HCT showed a significant decrease in the fasting and ketogenic diet groups, and the results of MCH, MCHC, PLT, and PCT showed a significant increase in the fasting group and a significant decrease in the ketogenic diet group. In MPV, a significant increase in the fasting group and no significant differences in the ketogenic diet group (Table 1). Reduces the secretion of LH and FSH from the pituitary gland, followed by a decrease in the level of testosterone (Knoll 1991; Al-Ghazali 2021). The results of the current study showed an equally significant decrease (P<0.05) in FSH levels in the fasting and ketogenic diet groups during the eight weeks (Table 3). These results are in agreement with the finding of Omolaso et al. (2012) who indicated a decrease in FSH in fasting white male rats.

Table 1. Shows the changes in hematological parameters in the three groups (control, fasting, and ketogenic diet) for the eight weeks.

CBC	2 Week			4 Week			6 Week			8 Week		
	C	F	K	C	F	K	C	F	K	C	F	K
WBC (x10^9/L)	4.3±0.558[a]	4.4±0.158[a]	9.6±0.158[b]	4.4±0.015[a]	10.2±0.688[b]	10.5±0.176[c]	4.4±0.189[a]	4±0.07[a]	10.3±0.295[b]	4.8±0.097[a]	10.3±0.58[b]	10.6±0.224[c]
LYM (x10^9/L)	2.6 ± 0.080[a]	2.7±0.141[a]	8±0.158[b]	2.5±0.013[a]	2.2±0.324[a]	8.5±0.13[b]	2.8±0.031[a]	2.1±0.1[b]	8.5±0.141[c]	2.9±0.07[b]	8.1±0.381[b]	9±0.217[b]
Mono (x10^9/L)	0.5 ±0.031[a]	0.8± 0.1[b]	0.8±0.071[b]	0.8±0.019[b]	1.6 ±0.161[b]	1.2±0.1[a]	0.6±0.018[a]	0.8±0.071[a]	0.7±0.13[a]	0.7±0.021[a]	0.6 ±0.1[a]	0.9±0.071[a]
Gran (x10^9/L)	1.2±0.077[a]	0.9±0.07[a]	0.8±0.130[b]	1.1±0.01[a]	6.4±0.22[b]	0.8±0.152[a]	1.1±0.02[a]	1.1±0.07[a]	1.1±0.1[a]	1.2±0.01[a]	1.6±0.17[a]	0.7±0.07[b]
RBC (x10^{12}/L)	6±0.158 [a]	8.7±0.054[c]	3.9±0.014[b]	6.1 ±0.01[a]	7.4 ±0.026[b]	3.3 ±0.02[c]	6.2±0.01[a]	6.9±0.106[b]	3±0.01[c]	6.1±0.012[b]	5.6±0.045[b]	5.2±0.021[c]
MCH (pg)	16.2±0.1[a]	14.3±0.13[b]	18.2±0.152[b]	15.8±0.205[a]	15.8±0.205[a]	18.2±0.1[b]	15.5±0.123[a]	17.5±0.071[b]	15.6±0.123[a]	15±0.1879[a]	16.9±0.187[c]	15.2±0.158[b]
PDW (%)	16±0.07[a]	15±0.07[a]	16.3±0.152[b]	15±0.15[a]	15.5±0.13[b]	16± 0.07[c]	16±0.07[a]	16.1±0.114[a]	15.5±0.13[b]	16.9±0.212[a]	15.9±0.07[b]	15.7±0.07[c]
MCHC (g/dL)	35.5±0.123[a]	33.9±0.245[a]	36.3±0.123b	36.5±0.176[a]	34±0.07[b]	34±0.07 [b]	38.2±0.1[a]	36.7±0.07[b]	34.3±0.141[c]	34.9±0.158[a]	36.5±0.141[b]	33.2±0.13[c]
MPV (fL)	5.6±0.141[a]	6.8±0.071[c]	6.5±0.158[b]	6.5±0.158[a]	6.2 ±0.1[a]	6.6±0.123[a]	5.1±0.071[a]	6.2 ±0.1 [c]	5.7±0.071[b]	5.7 ±0.071[a]	6.5±0.158 [b]	5.8 ± 0.1[a]
MCV (fL)	45.2±0.158[a]	42.3±0.071[b]	49.8±0.2[b]	40.5±0.205[a]	46.6±0.2[b]	53.5±0.164[c]	40± 0.354[a]	47.7±0.141[c]	45.5±0.152[b]	42.5±0.184[a]	46.2±0.192[c]	45.7±0.071[b]
HGB (g/dL)	10.9± 0.17[a]	12±0.187[b]	9.1±0.298[c]	11.2±0.243[a]	11.6±0.184[a]	9 ±0.21[b]	10.5±0.228[a]	10.1±0.182[a]	8.8±0.251[b]	11.8±0.278[a]	9.5±0.243 [b]	8±0.212 [c]
PLT (x10^9/L)	230±2[a]	220±1.517[b]	283±0.707[c]	243±1.14[c]	146±1[c]	253±0.707[b]	241±1.225[a]	310±1.817[b]	117±2.34[a]	262±1.643[a]	331±0.707[b]	183±1[c]
PCT (mL/L)	1.7±0.023[a]	1.5±0.007[c]	1.8±0.01[c]	1.8± 0.02[a]	0.9± 0.007[c]	1.7±0.223[b]	2.5± 0.31[b]	1.9±0.01[b]	0.8±0.01[c]	1.7±0.023[a]	2.8±0.007[c]	1.1±0.007[b]
HCT (%)	33±2[a]	36.6±0.339[a]	19.6±0.561[b]	27.2±0.281[a]	34.3±0.278[a]	17.6±0.176[c]	32.5±0.382[a]	14±0.316[b]	13.9±0.1[c]	34.7±0.381[a]	26±0.376[b]	23.9±0.13[c]

The values in the table are found by (mean±S.E of Mean). 2. Different small letters represent a significant difference in (*P*<0.05) between groups. Similar small letters represent no significant difference.

Table 2. Changes in hormone levels LH for the three groups (control, fasting and ketogenic diet) for the eight weeks.

Weeks Groups	LH (mlU/mL)		
	Control	Fasting	Ketogenic diet
2W	0.0100±0.0038 [a]	0.0052±0.0003 [a]	0.0123±0.0002 [a]
4W	0.0100±0.0007 [a]	0.0097±.0005 [a]	0.0074±0.0003 [b]
6W	0.0100±0.0036 [a]	0.0058±0.0001 [a]	0.0017±0.0002 [b]
8W	0.0100±0.0035 [a]	0.0063±0.0012 [a]	0.0065±0.0001 [a]

*The values in the table are found by (means±S.E of Mean). Different small letters represent a significant difference in (P<0.05) between groups. Similar small letters represent no significant difference.

Table 3. Changes in hormone levels FSH for the three groups (control, fasting and ketogenic diet) for the eight weeks.

Weeks Groups	FSH (mlU/mL)		
	Control	Fasting	Ketogenic diet
2W	0.01±0.0038 [a]	0.0001±0.0000 [b]	0.0001±0.0000 [b]
4W	0.01±0.0007 [a]	0.0001±0.0000 [b]	0.0001±0.0000 [b]
6W	0.001±0.0003 [a]	0.0001±0.0000 [b]	0.0001±0.0000 [b]
8W	0.01±0.0038 [a]	0.0001±0.0000 [b]	0.0001±0.0000 [b]

*The values in the table are found by (means±S.E of Mean). Different small letters represent a significant in (P<0.05) between groups. Similar small letters represent no significant difference.

Table 4. Changes in the level of testosterone for the three groups (control, fasting and ketogenic diet) for the eight weeks.

Weeks Groups	ng/ml) (Testosterone		
	Control	Fasting	Ketogenic diet
2W	10.900±0.158 [a]	0.025±0.0007 [b]	0.025±0.001 [b]
4W	9.180±0.007 [a]	0.040±0.003 [b]	0.025±0.001 [c]
6W	9.110±0.017 [a]	0.025±0.001 [b]	0.025±0.003 [b]
8W	10.010±0.316 [a]	0.038±0.002 [b]	0.025±0.002 [c]

*The values in the table are found by (means±S.E of Mean). Different small letters represent a significant difference in (P<0.05) between groups. Similar small letters represent no significant difference.

These results disagree with the results of Al-Chalabi (2013) and Mirsan et al. (2017) who showed the survival of FSH unchanged during fasting. Kayode et al. (2021) indicated a significant increase in FSH in the group that underwent the ketogenic diet.

The results of the current study showed a significant decrease (P<0.05) in testosterone levels in the fasting and ketogenic diet groups for the eight weeks compared to the control group (Table 4). These results are in agreement with the findings of Samuel et al. (2015) who indicated that the level of testosterone is negatively affected by fasting. However, Mongioi et al. (2020) showed an increase in testosterone in the ketogenic diet group and Liu et al. (2022) indicated that the ketogenic diet led to an increase in the level of testosterone. Kenuedy et al. (2007) also indicated that feeding animals a ketogenic diet maintained normal testosterone levels. The decreased concentration of testosterone may be due to the expression of aromatase in adipose tissue, which converts testosterone into estradiol (Fui et al. 2014). Low testosterone may also be caused by the breakdown of Leydig cells due to oxidative stress.

Conclusion

The study showed that both fasting and the ketogenic diet have affected their blood parameters and that fasting does not affect on LH hormone, but showed a decrease in the FSH and testosterone. While the ketogenic diet showed decreases in LH, FSH, and testosterone.

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