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Effect of Starvation and Refeeding on Spermatological Parameters and Oxidative Stress in Rats

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Abstract

In this study, we set out to determine the effects of starvation and refeeding on rats. The main objectives were observing the changes in spermatological variables and oxidative stress. The test subjects consisted of 24 Wistar Albino rats, all healthy-males. Dividing them into four equivalent groups, we determined the first group to be the control group. We did not perform any starvation or refeeding on them. The second, third and fourth groups were starved for 5 days. As for refeeding, the third group was refed for 5 days, and the fourth group was refed for 7 days. We measured the weights of the rats' bodies and reproductive organs. Certain parameters were also measured, such as sperm motility and concentration, changes in blood glutathione, malondialdehyde levels and the antioxidant enzyme activities. The results showed that starving the rats caused a decrease in body weights and the weights of reproductive organs and concentration of sperm. Glutathione and malondialdehyde levels increased, while antioxidant enzyme activities decreased. The refed groups, the third and the fourth, exhibited production of amelioration and signs of biochemical. We infer that starving the rats has caused an increase in lipid peroxidation and damage in the reproductive cells and organs.

Keywords: antioxidant, oxidative stress, spermatological parameters, starvation.

1. Introduction

Balanced nutrition is necessary for any organism to maintain its healthy vitality. However, it should be debated whether or not food is taken in as and when needed. In particular, it seems that there is no strict control mechanism regulating the amount that can be consumed in living beings, particularly in the supply of food. Rapidly increasing obesity rates in developed societies and the fact that specially-raised animals are heavier than their untamed relatives in the wild are the most concrete indicators of this (Masoro, 1985; Habib et al. 1990).Caloric restriction has been shown to prevent weight gain, to delay aging and to prevention various pathologies associated with aging. To explain these effects, it was necessary to determine the effects of starvation on oxidative stress in rats. It has been shown that calorie restriction is an effective means of protection from many diseases (atherosclerosis, hypertension, diabetes mellitus, etc.). It also delays aging and leads to a prolonged life span in living organisms (Gong et al., 1997; Caglayan et al., 2001; Gultekin et al., 2004; Pannacciulli et al., 2006; Mannarino et al., 2008). Although the mechanism underlying these positive effects of calorie restriction is not clear, many hypotheses have been raised in this regard. One of these has been the positive effects of caloric restriction on reducing the damage caused by free radicals, but this is not certain (Craft et al., 2005).

However, findings on the metabolic effects of starvation, a very advanced level of calorie restriction, are not very clear. In particular, the effects of starvation on oxidant stress and antioxidant capacity in organs have been investigated and various results have been obtained, both positive and negative (Wohaieb and Godin, 1987; Vreugdenhil et al., 1993; Langkamp et al., 1995).

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Fasting may also have some effects on the gonadotropic hormone level (Gonzales, 2004) and the testosterone level in fertile males, either via the hypothalamus pituitary-testicular axis or by directly effecting the testis, and could therefore affect spermatogenesis. The quality of spermatozoa is a significant determinant of male fertility. It is therefore important to determine how fasting effects the general quality of the semen (Segal and Nelson, 1959).Studies in the literature have already showed that food is a crucial environmental parameter for animals. Reproductive activities are greatly influenced by starvation (Rodjmark, 1987; Cameron and Nosbisch, 1991).

In studies on starvation, it is first necessary to keep the duration of the experiment very short. The resistance of the organism to starvation is proportional to its triglyceride stores, which are the source of fatty acid (Goodman et al., 1980). Absolute absence of nutrients will change the experimental conditions after a certain period of time. Increased oxidation of fatty acids as an energy source increases hydrogene peroxide (H_2O_2) production (Crescimanno et al., 1989). On the other hand, starvation definitely reduces intracellular ATP stores (DeVivo et al., 1975; Kaur and Kaur, 1990).

Oxidative stress occurs in cells or tissues when the concentration of reactive oxygen species (ROS) generated exceeds the antioxidant capability of that cell. Studies show an increase in the occurrence of tissue disorders and oxidative stress due to changing lifestyles, leading to serious problems in the area of public health. Oxidative damage in the tissue occurs when the concentration of ROS (superoxide radical ($O^{\cdot}_2$), H_2O_2, and hydroxyl radical ($\cdot OH$)) generated exceeds the antioxidant capacity of the cell, or when the antioxidant capacity of the cell decreases (Trush and Kensler, 1991)

The oxidant stress-reducing effect of calorie restriction has been demonstrated by several investigators (Rao et al., 1990; Cadenas et al., 1994). However, as mentioned earlier, starvation is more advanced than calorie restriction. In this context, the effects of starvation on oxidative stress may be different than the effects caused by calorie restriction.It has been shown that short-term starvation and calorie restriction have protective effects against many conditions. However, this can change when the starvation period is longer (Li et al., 2006). Since the available data are both limited and contradictory, the aim of our study was to find out if five days of starvation followed by refeeding significantly changes the level of free radicals and other parameters of oxidative status and sperm quality.

2.Materials and Methods

2.1. Animals and Experimental Design

Twenty four healthy male Wistar-Albino rats (250-400 g body weight, 3 months old) were used in this study. The protocol for the use of animals was approved by the Firat University Animal Experiments Local Ethics Committee (04.04.2013/No:57). We fed the rats with standard rat pellet diet and gave them tap water as needed. Housing conditions were those of a standard laboratory, at a temperature of 25±2°C and light and dark cycles of twelve hours each. The animals were randomly divided into four experimental groups, including six rats in each. These groups were arranged as follows:Group 1 served as the control and received food during study. Group 2 was starved for five days, group 3 was starved for five days and refed for five days, group 4 was starved for five days and refed for seven days.

2.2. Sample Collection, Spermatological and Biochemical Analysis

At the end of the experiment, under ether anesthesia, blood samples were withdrawn by injector from the heart of the animals and collected into tubes containing EDTA for biochemical analysis. Then, the rats in the control and experimental groups were sacrificed by decapitation under ether anesthesia. Reproductive organs (testis, epididymides, seminal vesicles, and ventral prostate) were removed, cleaned of the adhering connective tissue, and weighed for spermatological analysis. All spermatological analyses were made using the methods reported in the study by Turk et al. (2008). Right epididymis tissue was trimmed in 1ml saline. Then, the sperm concentration in the right cauda epididymal tissue was determined using a hemocytometer. Freshly isolated left cauda epididymal tissue was used for the sperm motility analysis. The percentage of sperm motility was evaluated using a light microscope (37 ˚C) with a heated stage.

Blood samples containing EDTA were centrifuged at 1,500 g for 15 min at +4°C to obtain plasma, which was used to measure the malondialdehyde (MDA) level as a marker of lipid peroxidation (LPO). The MDA and reduced glutathione (GSH) levels, catalase (CAT), glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities were analyzed in blood. The MDA level was measured according to the method developed by Placer et al. (1966). This method was based on the reaction of thiobarbituric acid (TBA) and MDA, one of the aldehyde products of LPO.

GSH level was determined using the method developed by Ellman et al. (1961). This method was a spectrophotometric method based on the formation of highly stable yellow colored sulfhydryl groups when 5,5'dithiobis-2-nitrobenzoic acid (DTNB) was added. CAT activity was carried out using the Aebi method (1984). CAT activity was determined by measuring the resolution of H_2O_2 at 240 nm. GSH-Px activity was measured using the Beutler method (1984). GSH-Px catalyzes the oxidation of GSH to oxide glutathion (GSSG) using H_2O_2. The rate of formation of GSSG was measured according to the glutathion reductase (GR) reaction. SOD activity was measured by using xanthine and xanthine oxidases to generate $O^{\cdot}_2$ reacting with nitroblue tetrazolium (Sun et al., 1988). Hemoglobin concentrations were determined according to the method developed by Drabkin (1970). In addition, hemoglobin levels were measured for each sample and spesific enzyme activities were calculated using hemoglobin levels.

2.3. Statistical Analysis

Expression of the results was as mean ± standard error (S.E.). We used ANOVA to observe significant differences among the groups. To compare the groups to each other, we implemented the post hoc test and used the Tukey test for multiple comparison, with a level of significance at $p<0.05$. We used the Statistical Package for Social Sciences (SPSS)/PC soft ware program (Version 22.0; SPSS, Chicago, Illinois, USA).

3. Results

3.1. Body and Reproductive Organ Weights

Initial and final body weight means are presented in Table 1. Starvation caused a significant ($p<0.05$) decrease in the final body weight when compared with the control group. A significant increase was observed in final body weight in the refeeding group in comparison with the starvation group (group 2).Absolute and relative reproductive organ weights are presented in Table 2. Significant decreases ($p<0.05$) were observed in the absolute weights of testis and prostate in group 2 when compared with the control group. Significant increases were observed in absolute weights of testis in the refeeding groups (in groups 3 and 4) when compared with group 2. A significant increase was observed the in absolute weight of testis in the refeeding group 4 when compared with group 2. Significant increases ($p<0.05$) were observed in the relative weights of testis in group 2 when compared with the control group. No significant decreases were observed in the relative weights of testis in the refeeding groups (in groups 3 and 4), when compared with group 2.No statistically significant changes were found in the absolute and relative weights of whole epididymis, right cauda epididymis and seminal vesicles. Additionally, no statistically significant change in the relative weight of the prostate was observed.

Table 1. The effect of starvation and refeeding on initial and final body weight means (g)

Parameters	Group 1	Group 2	Group 3	Group 4
Mean Initial Body Weight (g)	337.17±25.34	364.83±15.87	358.50±18.95	374.92±29.49
Mean Final Body Weight (g)	325.75±55.82^{a}	276.66±13.28^{b}	343.33±51.89^{a}	326.17±30.58^{a}

The data are expressed in mean±S.E. for six animals per group. Within rows, means with different letters (a and b) are significantly different. Group 1 served as the control, received food during study. Group 2 were starved 5 days, group 3 were starved 5 days and refeed 5 days, group 4 were starved 5 days and refeed 7 days.

Table 2: The effect of starvation and refeeding on absolute and relative organ weights (g)

Parameters	Group 1		Group 2		Group 3		Group 4	
	Absolute	**Relative**	**Absolute**	**Relative**	**Absolute**	**Relative**	**Absolute**	**Relative**
Testis (Right+left/2)	1.70±0.06^{ab}	0.503±0.01^{a}	1.61±0.04^{b}	0.586±0.02^{b}	1.80±0.04^{a}	0.555±0.02^{ab}	1.79±0.02^{a}	0.526±0.01^{ab}
Whole epid.(Right+left/2)	0.53±0.02	0.160±0.01	0.49±0.01	0.172±0.01	0.53±0.02	0.168±0.01	0.54±0.01	0.160±0.01
Right cauda epididymis	0.21±0.01	0.061±0.01	0.19±0.01	0.070±0.01	0.21±0.01	0.069±0.01	0.22±0.01	0.065±0.01
Seminal vesicles	1.07±0.09	0.319±0.03	0.82±0.08	0.288±0.02	1.10±0.10	0.358±0.04	1.00±0.07	0.292±0.02
Ventral prostate	0.35±0.04^{a}	0.101±0.01	0.20±0.02^{b}	0.076±0.01	0.29±0.03^{ab}	0.096±0.01	0.42±0.06^{a}	0.110±0.01

The data are expressed in mean±S.E. for six animals per group. Within rows, means with different letters (a and b) are significantly different. Group 1 served as the control, received food during study. Group 2 were starved 5 days, group 3 were starved 5 days and refeed 5 days, group 4 were starved 5 days and refeed 7 days.

3.2. Sperm Parameters

Epididymal sperm concentration and sperm motility in all groups are presented in Table 3. A significant decrease ($p<0.05$) was determined in sperm concentration in group 2 when compared with the control group. Significant increases were observed in sperm concentration in the refeeding groups when compared with group 2. No statistically significant change was found in sperm motility between the groups.

Table 3: The effect of starvation and refeeding on sperm motility (%) and concentration (million/right cauda epididymis)

Parameters	Group 1	Group 2	Group 3	Group 4
Motility	67.33±6.03	63.00±4.09	68.00±6.24	74.00±2.32
Concentration	66.66±4.58^{a}	46.33±5.12^{b}	71.66±5.20^{a}	76.3±9.97^{a}

The data are expressed in mean±S.E. for six animals per group. Within rows, means with different letters (a and b) are significantly different. Group 1 served as the control, received food during study. Group 2 were starved 5 days, group 3 were starved 5 days and refeed 5 days, group 4 were starved 5 days and refeed 7 days.

3.3. MDA, GSH Levels and Antioxidant Enzymes Activities

Table 4 shows the MDA, GSH levels and the activities of antioxidant enzymes such as CAT, GSH-Px and SOD in the blood in the control and experimental groups. The data indicated that group 2 had significantly higher MDA and GSH levels in plasma than the control group. Improvement in MDA and GSH levels was observed in the refeeding groups (in group 3 and 4) when compared with group 2.

A significant reduction was found in CAT, GSH-Px and SOD activities in group 2 when compared with the control group. Generally, while significant increases were observed in antioxidant enzyme activities in the refeeding groups when compared with group 2, these increases in GSH-Px activities were numerical but not statistically significant in comparison with group 2.

Table 4: The effect of starvation and refeeding on plasma MDA level and erythrocyte GSH level, CAT, GSH-Px, SOD activities

Parameters	Group 1	Group 2	Group 3	Group 4
MDA (nmol/ml)	8.22±0.40^{a}	10.22±0.24^{c}	9.18±0.11^{b}	8.90±0.17^{ab}
GSH (µmol/ml)	61.17±2.62^{a}	105.94±10.47^{b}	65.35±6.39^{a}	82.81±2.13^{a}
CAT (k/g Hb)	46.09±1.39^{a}	40.92±1.71^{b}	43.65±0.93^{ab}	45.95±1.81^{a}
GSH-Px (U/g Hb)	142.48±2.92^{a}	133.31±1.88^{b}	135.03±3,22^{ab}	137.78±1.82^{ab}
SOD (U/g Hb)	64.27±0.90^{a}	60.17±0.97^{b}	62.88±0.85^{a}	63.73±0.53^{a}

The data are expressed in mean±S.E. for seven animals per group. Within rows, means with different letters (a, b and c) are significantly different. Group 1 served as the control, received food during study. Group 2 were starved 5 days, group 3 were starved 5 days and refeed 5 days, group 4 were starved 5 days and refeed 7 days.

4.Discussion

Starvation is a metabolic process that can occur as a result of being deprived of all or some of the basic building blocks necessary for organisms to survive, or taken in an inappropriate balance. This metabolic process can be concomitant with physiological, biochemical, and histological equilibrium and defensive mechanisms depending on the duration and weight of the starvation, or can lead to various consequences ranging from malnutrition to death. Starvation, such as that produced by dietary restriction, may result in severe metabolic impairment and small-intestine hypofunction in animals and humans (Cahill et al., 1970).

Different results can occur as the duration of the starvation lengthens. In the light of this information, the aim of our study was to find out if five days of starvation followed by refeeding significantly changes the level of free radicals and the other parameters of oxidative status as well as sperm quality.

Prolonged starvation may result in decreased body weight. In the mechanism of fasting, there are phases 3. The duration of phases 1 (carbonhidrate destruction), and 2 (fat destruction) is approximately 3-4 days. After the onset of starvation (Bertile and Raclot. 2006; Li et al., 2006). Phase 3 (proteine destruction) began on day 3-4 in rats. It is stated that weight loss will be rapid due to protein destruction at the 3rd stage. (Chediack et al., 2012). In our study, starvation caused a significant ($p<0.05$) decrease in the final body weight when compared with the control group. Previous studies reported a decrease in body weight during starvation (Athar et al,1984; Hussein et al., 1987; Omolasa et al., 2012). Results show that there may be a significant weight loss in the fasted group possibly resulting from hypoglycemia. In contrast, one study reported weight gain (Frost and Pirani, 1997). The relative weight of the testis increased in the group 2 in the current study. The reason for this is that the decrease in testicular weights is less than the rate of body weight decrease.

The sperm motility of rats in the group 2 was decreased but not statistically significant. The inability of starvation to decrease sperm motility is explained by glucose levels remaining within normal range throughout the five days of fasting used in this study.A significant decreased ($p<0.05$) was determined in sperm concentration in group 2 when compared with the control group. Significant increases were observed in sperm concentration in the refeeding groups when compared with group 2. The reduction in the sperm concentration explains the decrease in testicular organ weight, which is an indication that fasting reduced or inhibited spermatogenesis.

Activities of certain antioxidative enzymes, metabolites from LPO and MDA levels, all of which had been defined as pro-oxidant activity biomarkers in mammals, have been measured to determine what effects starvation had on the antioxidant defenses of the rats (Robinson et al., 1997; Gomi and Matsuo, 1998; Domenicali et al., 2001). There are a number of changes in the cell in oxidative stress. MDA, one of the most important products emerging as the result of LPO, is one of the parameters showing LPO. In many experimental studies, it has been reported that long-term starvation causes LPO, characterized by elevated levels of MDA in tissue damage (Neu, 1997; Morales et al., 2004). In this study, the data indicated that the starvation group (in group 2) had a significantly higher MDA level in plasma than the control group. It demonstrates that starvation causes the formation of high levels of free radicals that cannot be tolerated by the cellular antioxidant defense system. We observed that the refed rats showed an decreased in MDA levels, reaching those of the control group. We conclude from this that refeeding rats can help suppress free radicals, protect membrane lipids from oxidative damage and limit LPO.

One of the major detoxification and free-radical scavenging systems, GSH and GSH-related enzymes may play a role in controlling diseases (Balal et al., 2004). GSH can act via the direct interaction of -SH group with ROS, as a cofactor or coenzyme. It helps to protect biological membranes, which are susceptible to peroxidation (Naaz et al., 2014). In this study, the starved rats had significantly higher levels of GSH than the control rats. Refeeding led to a normalization in GSH concentrations. We suggest that the increased GSH concentrations may reflect oxidative stress in starved rats. However, increased GSH concentration may be an adaptation to maintain adequate redox milieu in the red blood cells. The effects of refeeding on cellular GSH may be directly due to antioxidant effects, enhanced biosynthesis of GSH (Wohaieb et al., 1987).

Oxidative damage in a cell or tissue occurs when the concentration of ROS ($O^{\cdot-}_2$, H_2O_2, and $\cdot OH$) generated exceeds the antioxidant capability of the cell (Sies, 1991). Free radical scavenging enzymes like CAT and SOD protect the biological systems from oxidative stress. The CAT further detoxifies H_2O_2 into H_2O and O_2 (Murray et al., 2003). The SOD dismutates $O^{\cdot-}_2$ into H_2O_2 and O_2 (Fridovich, 1986). GSH-Px also functions in detoxifying H_2O_2 similar to CAT. Thus, SOD, CAT and GSH-Px act mutually and constitute the enzymic antioxidative defense mechanism against ROS (Bhattacharjee and Sil, 2006).

Regarding the antioxidant state of the animal in this study, results indicate that the changes in LPO were also accompanied by a concomitant decrease in the activities of the enzymes involved in the disposal of $O^{\cdot-}_2$ anions and peroxides, namely SOD and CAT. From these findings, it appears that the initial changes induced by long-term starvation are due to the formation of LPO, and that toxicity is mediated through antioxidant enzymes as well as by GSH metabolism.

As H_2O_2 is created in the cells in the period of starving, the component decays at high levels and it is followed by consumption. This, along with the starving process limiting the antioxidant enzymes, might be the reasons behind the inactivity of these enzymes. The decreased activity of CAT, GSH-Px and SOD would suggest an

impairment of tissue defense mechanisms against oxidative stress. Indeed, in the present study we found an increase in MDA levels, a marker of lipid peroxidation, and a decrease in antioxidant enzyme activities in the blood of the starved rats.Changes in antioxidant enzyme activities in various tissues of starvation experimental animals were also observed. However, these changes were not always found in the same direction. Baker et al. (1990), there was a decrease in SOD, CAT and GSH cycles in the rat small intestine after 72 h fasting. CAT and GSH-Px mRNA did not change when SOD mRNA was decreased. However, Langkamp et al. (1995) found that the small intestines of starved rats were more resistant to ischemia-reperfusion injury. Wohaieb et al. (1987) found that the effects of starvation on antioxidant enzymes in rats differed among tissues. According to this, CAT, heart, and pancreas increase while the liver decreases. SOD decreases in the heart, while increasing in the kidney and pancreas. Smith et al. (1990) did not observe significant change in the SOD and CAT activities in the lungs of hungry mice. In their study on sheep, Gaal et al. (1993) reported that hunger increased antioxidant enzyme levels in the erythrocytes of sheep.

In addition to these different enzyme activity changes, several studies have been published that demonstrate the antioxidant properties of starvation. Yegen et al. (1990) reported a slight decrease in the plasma lipid peroxide level of starvation. MDA production due to H_2O_2 in rat erythrocytes after 72 h fasting was lower in the control group (Wohaieb et al., 1987). In conclusion, starvation causes damage in male reproductive system by increasing lipid peroxidation, and refeeding normalizes it by balancing oxidative status.

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