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The Influence of Different Levels of Acute Calorie Restriction on Several Hematological and Biochemical Parameters in Wistar Rats

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Abstract. Calorie Restriction (CR) is a diet in which calorie intake is reduced, also defined as under-nutrition without malnutrition. In this experiment we used two levels of calorie restriction: 25% and 40% of calorie intake reduction than normal. We used 24 male Wistar rats, divided in 4 groups: *ad libitum* fed, 100% of normal calorie intake, 75% and 60% of normal calorie intake, for a period of 30 days. After harvesting the blood samples and hematological and biochemical analisys of the blood, we statistically interpreted them by calculating the mean, standard deviation, standard eror of the mean and ``t`` test (Student). After the statistical interpretation of the result, we concluded that acute mild and moderate CR did not have any negative effects on hematological and biochemical parameters comprised in this study. Moreover, it suggests beneficial effects regarding the fat and glucose metabolisms.

Keywords. acute calorie restriction, Wistar rats, hematological parameters, biochemical parameters.

INTRODUCTION

Calorie Restriction (CR) is a diet in which calorie intake is reduced. CR is also sometimes called dietary restriction and, in simple terms is defined as under-nutrition without malnutrition. Typically, the experimental animal is kept on a diet which is 30% to 70% less compared to the amount of food taken when there are no restrictions. The quality of vitamins, minerals, protein, carbohydrate, lipids and other factors in the diet is not compromised, rather it is the amount of overall calories that is reduced. After a period of time on this diet, several biomarkers of aging return to normal levels and the animal looks and is healthy (Witte *et al.*, 2009). Researches performed by various researchers shows that many of the beneficial effects of CR are seen not only in mice or rats but also in primates and even humans. However, many scientists want to see more research into the human effects of CR, before they accept its clinical benefits beyond any doubt (Everitt *et al.*, 2006).

Evidence that mammalian longevity could be increased emerged in 1935 in a rodent study showing that CR extended average and maximum life span and delayed the onset of age-associated pathologies. It was not until the 1990s that CR became widely viewed as a scientific model that could provide insights into the retardation of the aging process and thereby identify underlying mechanisms of aging (Colman *et al.*, 2005).

Specifically in rats, concerning the length of CR period, it has been described 3 different stages of CR: 1) acute CR, if the diet spans over 30 days period (the individuals subjected to CR are lethargic, it is noticed a decrease in the digestive system activity and a

decrease in the skeletal muscle); 2) sub acute CR, if the diet spans over 60 days period (the individuals subjected to CR are less lethargic and unresponsive as during the first CR month as their organisms are getting habituated to the reduced energy level); 3) chronic CR, if the CR diet spans at least 90 days period (the individuals subjected to CR are fully habituated with their current energy level, marked by a constant hypoglycemia and hipocholesterolemia) (Teske and Kotz, 2009).

Through this experiment we intend to prove that acute CR influences in a statistically significative manor the hematological and biochemical parameters in Wistar rats.

MATERIALS AND METHODS

The animals. For this experiment we have used 24 male Wistar rats at 3 months age at the beginning of the experiment. The rats were obtained by breeding of our own Wistar line at the Faculty of Veterinary Medicine Cluj-Napoca Biobase, and were kept at a constant day-night cicle of 12/12 hours and at a constant temperature interval of 21 ± 2^0C. In the day 0 of the experiment, the rats were weighted and divided in 4 equal groups based on their initial weight. Each group was housed in 40/60/25 cm Plexiglas cage.

The chow was provided by Cantacuzino Institute, Bucharest, Romania, and consisted in combined granulated pellets, with 290 kcal/100 g, and recommended daily allowance of 20 g/rat. The animals had free access to drinking water. As bedding was used beech saw dust, which was changed once at every week during the experiment.

Group 1 was considered as control and had unrestricted access to food. Group 2 received 20 g/rat/day while groups 3 and 4 received 15 g/rat/day (mild CR) and 12 g/rat/day (moderate CR). The chow was provided daily between 8 and 10 o'clock in the morning. The experiment spanned over a 30 days.

Methods for hematological and biochemical evaluation. In the end, the blood samples were harvested from retro orbitary sinus under deep ether narcosis. Hematological analyses were done by Abacus Junior Vet, automatic analyzer Diatron Messtechnik, Budapest, Hungary, and the biochemical analysis by STAT FAX semiautomatic analyzer.

Statistics. The data were expressed as the mean and standard error of the mean (S.E.M.). ``T`` Student multiple range test from Excel Windows Software was used to assess the differences between groups. Differences at $0.01<p<0.05$ and $0.001<p<0.01$ were considered significant and respectively significantly distinct statistically, and $p<0.001$ was considered highly significantly statistically distinct.

RESULTS AND DISCUSSIONS

White blood cells. The controlled diet was responsible for significant decrease in WBC count in all group as compared to control. The groups subjected to CR were the most affected. Notably, all the WBC were within the normal range. The WBC count reduction was due to decreased levels of granulocytes and lymphocytes in all 3 experimental groups while middle cells remained almost unchanged (Fig.1).

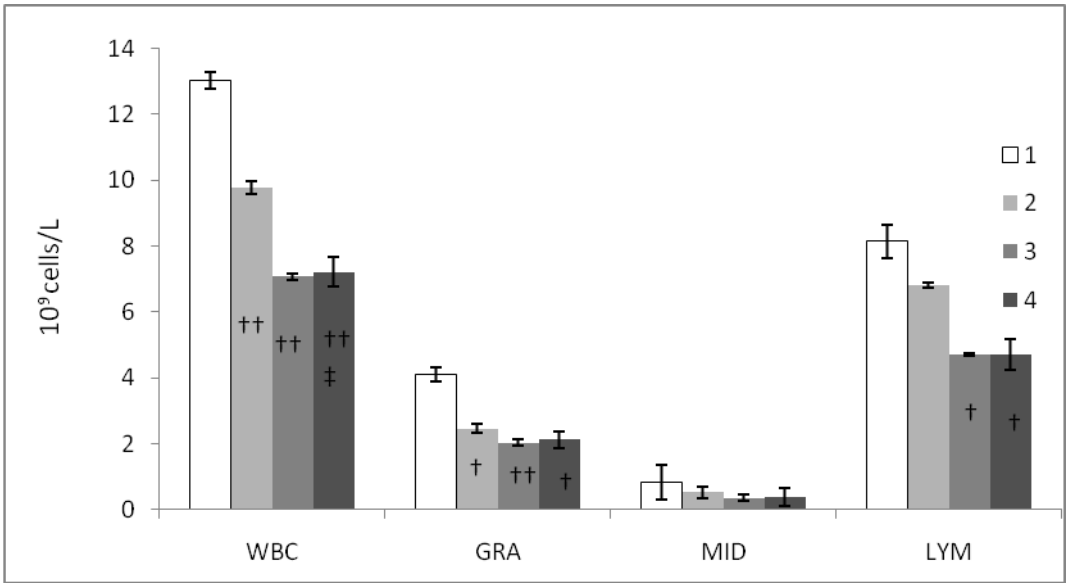

Fig. 1. The effect of acute CR over white blood cells count and differential (mean ± S.E.M.)
†= statistically significant at 0.01<p<0.05 as compared to Control group
††= significantly statistically distinct at 0.001<p<0.01 as compared to Control group
‡= statistically significant at 0.01<p<0.05 as compared to 2nd group
WBC-white blood cell count, LYM-lymphocytes, MID-middle cells, GRA-granulocytes
Normal values: WBC= 8-11.8x10^9cells/L, GRA= 1.98-2.92x10^9cells/L, MID=0.01-0.04 x10^9cells/L, LYM= 6.03-8.90 x10^9cells/L (Marcus, 2004).

Red blood cells. The red blood cells were slightly increased in experimental groups as compared to control, but significant differences were found in group 3 only. Hemoglobin and hematocrit followed the same trend but significant differences were also found in the 3rd group (Table 1).

Tab. 1.

The effect of acute CR on RBC parameters (mean ± S.E.M.)

Group/ Parameter	**RBC (10^{12}cells/L)**	**HGB (g/L)**	**HCT (%)**
1	7.67±0.18	141.25±2.62	39.98±0.7
2	8.03±0.1	145.67±1.54	41.8±0.45
3	8.68±0.13†††‡	151.33±2.59†‡	45.13±0.25†††‡‡‡
4	8.24±0.26	141.5±4.49	42.43±1.1

†= statistically significant at 0.01<p<0.05 as compared to Control group
††= significantly statistically distinct at 0.001<p<0.01 as compared to Control group
‡= statistically significant at 0.01<p<0.05 as compared to 2nd group
‡‡= significantly statistically distinct at 0.001<p<0.01 as compared to 2nd group
‡‡‡= highly significantly statistically distinct at p<0.001 as compared to 2nd group
RBC- red blood cells, HGB- hemoglobin, HCT-hematocrit
Normal values: RBC= 8.15-9.75 10^{12}cells/L, HGB=13.4-15.8 g/L, HCT= 44.4-50.6% (Marcus, 2004).

Red blood cells indices revealed a tendency of hipocromia in the 4th group, MCH and MCHC were in significant lower levels than control (Tab. 2). Despite those facts, all values

were within normal range, and this suggests that CR had no negative influence on red blood cells.

Tab. 2.

The effect of acute CR on RBC indices (mean ± S.E.M.)

Group/ Parameter	**MCV (fl)**	**MCH (p/g)**	**MCHC (g/L)**	**RDWcv (%)**
1	52.5±0.45	18.4±0.4	352.75±5.55	15.35±0.4
2	52.17±0.31	18.15±0.11	348±0.93	15.08±0.19
3	51.67±0.76	17.43±0.41	334.5±6.32†	15.13±0.22
4	51.83±0.4	17.15±0.26†‡‡	332.83±4.76†‡	14.68±0.18

†= statistically significant at $0.01<p<0.05$ as compared to Control group
‡= statistically significant at $0.01<p<0.05$ as compared to 2nd group
‡‡= significantly statistically distinct at $0.001<p<0.01$ as compared to 2nd group
MCV-mean corpuscular volume, MCH-mean corpuscular hemoglobin, MCHC-mean corpuscular hemoglobin concentration, RDW-red blood cells distribution width
Normal values: MCV= 53.6-56 fl, MCH= 48.1-50 pg, MCHC= 31.3-33.2 g/dl (Marcus, 2004)

Plasma biochemistry. The protein profile remains unchanged in groups subjected to CR, the single significant difference was found in the 3rd group that showed an increased level in total proteins due to high globulin level (Tab. 3). The significance of the present changes remained unclear, but in our point of view, this fact was not a direct consequence of CR. Glycemia was decreased in both group subjected to CR, but significant difference was found in severe CR only. The cholesterol remained virtually unmodified probably because of the internal synthesis. The triglycerides were significantly decreased in both experimental groups as compared to control, but also to normal fed group (Tab. 4). γ glutamyltransferase remained unchanged among all groups. The markers for the kidney function (urea and creatinine) didn`t change in a significant manor, some groups in study revealed a slightly decreased levels but no connection to CR diet could be found. The values fell well in the physiological parameters, there for the present study cannot prove any pathological changes in the hematological and biochemical profile. Moreover, mild and moderate CR proved to have beneficial effects regarding triglycerides and glicemia that were at the lower physiological range.

Tab. 3.

The effect of acute CR on protein measurements (mean ± S.E.M.)

Group/ Parameter	Total proteins (g/L)	Albumine (g/L)	Globulins (g/L)
1	74.24±2.99	36.44±1.25	37.8±2.85
2	71.27±3.55	39.17±1.07	32.1±3.03
3	79.43±3.14‡	34.83±1.46‡	44.6±2.48‡
4	73.23±6.08	37.33±2.17	35.9±7.68

‡‡= significantly statistically distinct at $0.001<p<0.01$ as compared to 2nd group
Normal values: Total proteins=45-85 g/L, Albumine=29-59 g/L, Globulins=16-26 g/L (Sharp and La Regina, 1998).

Tab. 4.

The effect of acute CR over serum glucose, cholesterol and triglycerides (mean ± S.E.M.)

Group/ Parameter	Glucose (mg/dL)	Cholesterol (mg/dL)	Triglycerides (mg/dL)
1	140.58±10,18	90.72±3.05	141.2±18.75
2	141.53± 12.21	98.9±7.06	94.37±11.63
3	112.82±3.37	92.37±3.38	49.13±2.61††‡‡
4	115.7±9.82‡	90.92±6.03	51.17±14.2††

††= significantly statistically distinct at 0.001<p<0.01 as compared to Control group
‡‡= significantly statistically distinct at 0.001<p<0.01 as compared to 2nd group
Normal values: Glucose=80-300 mg/dL, Cholesterol=40-130 mg/dL, Triglycerides=26-145 mg/dL (Sharp and La Regina, 1998).

Tab. 5.

The effect of acute CR over serum GGt, urea and creatinine (mean ± S.E.M.)

Group/ Parameter	GGt (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
1	5.02±0.9	30.6±0.8	0.56±0.04
2	5.72±1.51	26.52±1.42†	0.62±0.05
3	4.22±1.38	24.52±2.37	0.7±0.04†
4	4.73±1.43	25.8±2	0.67±0.08

†= statistically significant at 0.01<p<0.05 as compared to Control group
GGt- γ glutamyltransferase
Normal values: Urea=11-23 mg/dL, Creatinine=0,4-1,4 mg/dL (Sharp and La Regina, 1998; Marcus, 2004).

CONCLUSIONS

This study strongly suggests that acute mild and moderate CR did not have any negative effects on hematological and biochemical parameters comprised in present study. Moreover, it suggests beneficial effects regarding the fat and glucose metabolisms. Despite of these promising effects, further studies are required in order to asses long term effects of different levels of CR.

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