

Research Article

Hematological Profile in Food Deprivation Induced Food Hoarding in Mice

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Abstract

Background: The hoarding is one of the adaptive behaviors that formed during deficit of vital demands. This behavior and its disorder have several unknown biological aspects. This study was aimed to describe the blood profile in food deprivation induced food hoarding in mice.

Material and Methods: Sixteen female adult mice were allocated in two groups, high hoarder (n=8) and low hoarder (n=8) in order to evaluate blood profile and Fasting Blood Sugar (FBS).

Results: Positive correlations were found between relative abdominal fat pad and FBS ($r=0.709$, $p=0.002$), between White Blood Cells (WBC) and amount of hoarding ($r=0.454$, $p=0.008$), and between Mean Corpuscular Volume (MCV) and amount of hoarding ($r=0.538$, $p=0.038$). The FBS, WBC, Red Cell Distribution Width (RDW), neutrophil, eosinophil and basophile number tended to be higher in high hoarder mice than low hoarder mice ($p>0.05$). However, the MCV, Mean Corpuscular Hemoglobin Concentration (MCHC), Hemoglobin (Hb), Pematocrit (HCT), lymphocyte, plateletcrit (PCT), Platelet Distribution Width (PDW) were tended to be higher in low hoarder mice ($p>0.05$). The platelet number was higher in low-hoarder, while monocyte and basophile numbers were higher in high hoarder than low hoarder mice ($p<0.05$).

Conclusions: The results showed inflammatory and stress blood profile ($p<0.05$) and increased FBS ($p>0.05$) in high hoarder than low hoarder mice although different response to food deficit in this group compared to low hoarder mice is still an open question.

Keywords: Hoarding; Food Deprivation; Blood; Leukogram; Inflammation; Mice

Introduction

Food hoarding, the food storage for later consumption, is an adaptive strategy to increase animal's survival and reproduction in response to food deprivation [1]. Food hoarding is more noticed in animals while humans over hoard following restricted access to food [2]. The etiology of hoarding has not been yet determined precisely, however the involving of genetic factors, biological molecules such as enzyme, neurotransmitter and hormones in other psychiatric disorders besides environmental factors such as different types of deficits and deprivations have been investigated [3-6]. The different brain regions are involved in hoarding behavior depends on types of hoarding and involving various mechanisms such as decision-making, information processing, assessment of value, emotional attachment, reward-punishment system, appetite and ingestive-related processes and finally memory [1,6-9].

It should be mentioned that despite present behavioral characteristics in the hoarder animals, they are often encountered with one kind of chronic stress. The definition of stress term is not as simple as it seems. In other words, everyone knows stress while there is not a consensus on its definition [10]. The stress is a body response to stimuli that disturb its normal physiological conditions. In this regard, stress causes imbalances between body demands and body

ability to cope it [10]. According to duration, stress divides into acute and chronic one. While the first one is in short time, the latter lasts for months or even years. However, each one has its own effects on various body systems especially immune and hematopoietic systems. The stress can alter the immune molecules and cells especially T helper cells through activation of Hypothalamic Pituitary Adrenal (HPA) axis [10-14]. Moreover, stress modulates glucocorticoids and other metabolic hormones in which can affect blood parameters and cells, as well [11,13].

It has been reported that the stress effects on White Blood Cells (WBC), Tumor Necrosis Factor Alpha (TNF α), Natural Killer cells (NK cells), inflammatory responses, lymphocyte sensitivity to glucocorticoids, apoptosis, cellular deoxyribonucleic acid (DNA)-repair mechanisms, Interleukins (IL) and Fasting Blood Sugar (FBS) levels [10-15]. Meanwhile, there has no report about the evaluation of blood and immune cells in the presence of hoarding behavior. Hence, this study was aimed to describe the blood profile in food deprivation induced food hoarding in mice.

Materials and Methods

This study was reviewed and approved by the Laboratory Animal Care Committee of Razi University. The adult female NMRI mice with ad libitum food and water access were fed chow diet

and maintained under 12/12h artificial light cycles. The reference intervals of hoarding amounts were measured in the mice colony *via* using hoarder selecting protocol by specific apparatus as reported previously [16]. The chambers (13.0×32.0×6.5 cm³) with a whole (internal diameter 4cm) in their front side, chalky boxes and water bottles were designed in this apparatus. Hoarding wire mesh tubes (length 45cm, external diameter 4cm) were connected to the holes which were equipped with a 10cm plastic tube. The one end of these holes was sealed with a plastic plug.

The food restriction was applied from 8 am to 6 pm and then the access to food was allowed from 6 pm to 8 am next morning. The amount of hoarded food was weighted next day at 8 to 8:30 am. In this way, two groups high hoarder (n=8) and low hoarder (n=8) were selected. According to obtained results of initial screening, the hoarder with >20g and <5g were categorized into high-hoarder and low-hoarder group, respectively. The mice that hoarded in 5<g<20 range were considered as normal phenotype and excluded from the study.

After 12 hours fasting, selected mice received intraperitoneal injection of anesthetic cocktail of ketamine (80mg/kg; Alfasan, Netherland) and diazepam (0.5mg/kg; Chemidarou Co. Tehran, Iran) and the blood samples were taken *via* cardiac puncture into test tubes containing Ethylene Diamine Tetraacetic Acid (EDTA) for evaluation of morphological and selected hematological parameters besides measuring FBS. The abdominal fat pad also was weighted. The FBS measurement was performed by Elite glucometer while the hematology parameters were determined by autohematology analyzer (Mindray, China, #B30-280). The selected blood parameters included Red Blood Cell (RBC) counts, Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), Red Cell Distribution Width (RDW), Mean Platelet Volume (MPV), PLT (platelets), Plateletcrit (PCT), Platelet Distribution Width (PDW), WBC counts were measured as reported elsewhere. The Giemsa staining was used to determine differential leukocyte count.

Statistical analysis

The data were analyzed with SPSS, ver. 20.0, (Inc., Chicago, IL, USA). After the data were checked for their normality, the significant differences between each two defined groups were checked by t-test. Moreover, the correlations between hoarding and selected parameters were determined by the Pearson's correlation coefficient. The data were expressed as mean ± Standard Errors of the Mean (SEM) and the significant level was considered at p<0.05.

Results

The selected metabolic parameters are shown in Table 1, while the hematological results are presented in (Table 2,3) respectively. The FBS levels tended to be higher in high hoarder compared to low hoarder mice, but it was not statistically significant p<0.05 (Table 1).

The parameters that showed significant differences between high-hoarder and low-hoarder mice were platelet (p=0.045), monocyte (p=0.045) and basophile (p=0.047) counts (Table 2,3). In this context, platelet number was higher in low-hoarder, while monocyte and basophile numbers were higher in high-hoarder than low-hoarder mice (Table 2,3).

Table 1: The metabolic profile in high hoarder versus low hoarder mice.

Variable	Low-hoarder (n=8)	High-hoarder (n=8)	%Trend
Body weight (g)	31.93±1.15	28.62±2.75	-11.56
Relative abdominal fat weight (%)	1.45±0.41	1.37±0.27	-5.84
FBS (mg/dl)	105.00±4.50	128.12±21.88	18.04
Amount of hoarding (g)	3.12±1.18 ^b	51.65±4.7 ^a	93.96 ^c

Results are shown as mean ± SEM. Results with different superscript were significantly different in rows; Relative abdominal fat weight = (abdominal fat pad/bodyweight) ×100; FBS=Fasting blood sugar.

Table 2: The hematology values in high hoarder versus low hoarder mice.

Variable	Low-hoarder (n=8)	High-hoarder (n=8)	%Trend
RBC (×10 ⁹ /μL)	7.91±0.19	7.55±0.17	-4.77
Hb (g/dL)	11.40±0.35	10.67±0.20	-6.84
HCT (%)	34.23±0.90	32.62±0.62	-4.93
MCV (fL)	44.20±0.71	43.28±0.65	-2.12
MCH (pg)	14.36±0.22	14.08±0.31	-1.99
MCHC (g/dL)	33.21±0.24	32.67±0.31	-1.65
RDW/CV (%)	18.76±0.69	43.28±0.65	56.65
RDW/SD (fL)	29.04±1.46	14.08±0.13	-106.25
PLT (×10 ³ /μL)	625.50±42.04 ^b	32.67±0.31 ^a	-1814.6 ^c
MPV (fL)	10.36±1.44	20.08±0.89	48.81
PDW	14.27±0.22	29.68±1.29	51.29
PCT (%)	0.57±0.03 ^b	493.43±41.52 ^a	99.88

Results are shown as mean ± SEM. Results with different superscript were significantly different in rows.

RBC: Red Blood Cell; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean Corpuscular Volume; MCHC: Mean Corpuscular Hemoglobin Concentration; MCH: Mean Corpuscular Hemoglobin; RDW: Red Cell Distribution Width; MPV: Mean Platelet Volume; PLT: Platelets; PCT: Plateletcrit; PDW: Platelet Distribution Width; SD: Standard Deviation; CV: Coefficient Variance

The FBS, WBC, RDW, neutrophil, eosinophil, and basophile number were higher in high-hoarder mice than low-hoarder mice, although there were not statistically different (p>0.05, Table 2,3). However, the MCV, MCHC, HCT, Hb, lymphocyte, PCT, and PDW were tended to be higher in low-hoarder mice but they were not statistically significant, as well p>0.05, (Table 2,3).

The other point was positive correlations between abdominal fat and FBS (r=0.709, p=0.002), between WBC and amount of hoarding (r=0.454, p=0.008), and between MCV and amount of hoarding (r=0.538, p=0.038).

Discussion

The whole observed changes in high-hoarder mice revealed an inflammatory leukogram and blood stress profile. In other words, monocytosis, basophilia, thrombocytopenia and RBC/platelet anisocytosis revealed a general inflammatory condition. It should be mentioned that monocytosis is recognized as an indicator for blood stress profile, inflammation and body response to corticosteroids. This condition relates to pus, necrosis, neoplasia, hemorrhage and immune failure [17]. The basophilia is another indicator of blood stress profile that besides allergic conditions, it associates with inflammatory and myeloproliferative disorders, as well [17]. Finally, it should be mentioned that thrombocytopenia is associated with

Table 3: The leukogram in high-hoarder versus low-hoarder mice.

Variable	Low-hoarder (n=8)	High-hoarder (n=8)	%Trend
WBC ($\times 10^3/\mu\text{L}$)	5.72 \pm 1.16	6.24 \pm 0.56	8.33
Lymphocyte	70.37 \pm 4.03	61.37 \pm 2.72	-14.66
Monocyte	0.25 \pm 0.25 ^b	1.87 \pm 0.74 ^a	86.63 ^c
Neutrophil	25.37 \pm 3.77	31.62 \pm 2.72	19.76
Band neutrophil	3.87 \pm 0.89	3.75 \pm 0.75	-3.2
Eosinophil	0.12 \pm 0.12	0.50 \pm 0.27	76
Basophil	0.00 \pm 0.00 ^b	0.75 \pm 0.31 ^a	100 ^c
Neutrophil/Lymphocyte	0.39 \pm 0.09	0.53 \pm 0.07	26.41

Results are shown as mean \pm SEM. Results with different superscript were significantly different in rows.

neoplasia, aplastic anemia, immunity dysfunction, and poisoning [17].

The stress is one of the main features in the expression of hoarding behavior. Of course, the food deprivation can trigger the stress response in mice. There are different types of stress that psychological stress and physiological stress can be named based on its origin [10-12]. The body strategies to deal with these stresses are variable and include two general groups: Psychological (fear, anger, anxiety, uneasiness or instability) and physical (hypercortisolemia, neurological system derangement, hormonal and immunological alternations and negative effect on blood coagulation) [18,19]. Indeed, the response to perception of the disturbing stimulator is modulated *via* HPA and Renin Angiotensin System (RAS) through limbic regulation [11]. The stress can affect different systems, the immune and blood are two important examples. The stress especially chronic one can decrease NK cell activity, inhibit IL2 production from Antigen Presenting Cells (APCs), stimulate production of IL 4,10,13, up-regulate IL6 and cause imbalance of Th1/Th2 cell populations. The immune dysregulation is due to production of proinflammatory cytokines, increment of circulating inflammatory leukocytes through direct stimulation of hematopoietic stem cells and it is besides lymphocyte decrement [10-14,20]. In summary, stress leads to immunosuppression, cellular damaged DNA and affecting mesenchymal stem cells [11-13,20].

In regard to presence of stress in high-hoarder mice, it is rational to expect the role of corticosteroids and other metabolic hormones in the expression of hoarding behavior [13,14]. In this line, hyperglycemia in high-hoarder mice may be explained through high cortisol level. This pathway may cause acute immune and blood disorders and all these mentioned factors will prepare the underlying causes besides more susceptibility to chronic inflammatory diseases.

Although there was no comparable study focused on blood alternations in hoarding behavior, however there are reports addressed the effect of food restriction on decreasing lymphocytes, HCT, RBC besides increasing FBS and granulocytes in mink [21]. In this study, the level of PCV, monocyte, RBC, neutrophil and lymphocyte were lower in high-restricted feed intake group than low restricted feed intake one [21]. However, the plasma concentration of total protein, glucose, non-esterified fatty acids, alanine transaminase, urea, and cholesterol were similar in both groups [21]. Moreover, several blood alternations have been observed in behavioral disorders in human subjects, therefore there is a relationship between behavior and blood

alternations [20].

The other striking feature of our results was lower abdominal fat and body weight in high-hoarder in comparison to low-hoarder mice. Cautiously, corticotropin-releasing factor as a stress hormone may be one of the explanation for this finding since it causes negative energy balances and consequently decreases weight of body, fat and fat deposit [22]. Moreover, there is an inverse relation between corticotropin-releasing factor and hoarding incidence [22].

Conclusion

The common root in different types of hoarding is a deficit that may bring long-term stress for hoarders. This stress influences on corticosteroids and other inflammatory mediators and variable blood cell lines by itself. Therefore, the more susceptibility of hoarders to various immune-failure related diseases is understandable. This study evaluated the blood parameters in hoarder mice for the first time. The results revealed the occurrence of inflammatory condition and inflammatory blood profile in high-hoarder compared to low-hoarder mice.

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