SUPPLEMENT

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Effect of One Unit Blood Donation on Appetite-Related Biomarkers

Abstract

Purpose: It is commonly reported that blood donation (BD) leads to an increase in appetite. To investigate this claim, a questionnaire was offered to 306 people who had a history of BD at least once in their life. Following a positive outcome from the questionnaire, we further investigated the impact of BD on appetite.

Materials and Methods: The questionnaire study consists of a 5-question survey with VAS was performed on 399 volunteers. Later, 108 volunteers were enrolled in a laboratory study. Blood samples were withdrawn before and after BD. Leptin, ghrelin, neuropeptide-Y (NPY), and alpha-melanocyte stimulating hormone (α -MSH) levels were measured by ELISA kits. VAS assessment was also performed on all participants before and after BD.

Results: There was a significant decrease in the level of leptin but the levels of ghrelin, NPY and α -MSH were unchanged. A significant change in VAS value in terms of appetite was also observed.

Conclusion: The elevation of appetite after BD is predominantly psychological. Hormonal changes may contribute to the control of hypothalamic hunger and satiety centers.

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It is a common perception that blood donation (BD) causes an increase in appetite and that individuals who donate blood gain weight. This might deter some people from donating blood. National Blood Transfusion Service, Turkish Red Crescent, is the foundation exclusive authorized for blood collection in Turkey. In total, 1,860,255 units of blood were donated to the Turkish Red Crescent in 2014. The 2016 target of Turkish Red Crescent is 2,200,000 units of blood [1]. Unfortunately, we think that there is an obstacle to reach this target.

The most frequently-asked questions by individuals who are considering BD are “Will I gain weight?” and “Will I lose weight?” These questions are so prevalent that their answers are written on the formal website of Turkish Red Crescent: “There is no medically demonstrated effect of BD on weight gain or loss.” It is usually said that appetite increase after BD due to production of new blood cells, nevertheless this issue is an outcome of psychology. There is an idea that eating more after BD is like a substitute of the blood donated; thus, the person gains weight. It is also written in the website that the ratio of female volunteers who donate blood is lower than male volunteers. The reason to this may be due to the women being more afraid of gaining weight than the men.

The question we wanted to answer was whether appetite was increased after BD and if so, why. We designed a two stage study plan to determine the effects of BD (450 mL = 1 unit) on healthy volunteers. The first stage was a questionnaire study and the second stage was a laboratory study. Firstly, we aimed to conduct a 5-question survey with visual analogue scale (VAS) with 399 people. Secondly, we intended to apply the next (laboratory) stage based on the results obtained. The laboratory study consisted of both biochemical analysis of appetite regulator hormones including leptin, ghrelin, neuropeptide-Y (NPY), and alpha-melanocyte stimulating hormone (α -MSH) and VAS analysis. For this stage, we wanted to investigate the effect of donating one unit of blood on appetite regulation. Two vial of blood were collected from individuals who donated blood voluntarily: one vial just before donation and one vial 24 hours after donation. All participants will also be asked to mark the appetite degree they felt after the donating blood on VAS.

The originality of our study was that we investigated this important public health issue for the first time: based on our results, the Turkish Red Crescent, and the other similar National Blood Transfusion Services, would be able to more accurately answer the questions “Will I gain weight?” and “Will I lose weight?”

Materials and methods

Questionnaire Study

The questionnaire study consists of five questions survey with VAS:

1. Do you smoke? If answer is yes, how many pack of cigarette per day?
2. Have you ever donated blood before? If answer is yes, how many times? And when did you last donate?
3. Do you think that blood donation increases appetite? Five different choices from no idea increases a lot.
4. Before donating blood, please mark your appetite status (range is from 0 to 10).
5. After donating blood, please mark your appetite status (range is from 0 to 10).

VAS was originally designed to help the patients to define the degree of appetite they felt by plotting on a 10 cm long straight horizontal line. This questionnaire study was performed on 306 students from the Faculty of Engineering and Economics at the Turgut Ozal University, who had donated blood at least once in their life, aged 18–25 years (mean age 22.24 ± 2.16 years).

Subjects and study design

TURKMISC association invited Turkish Red Crescent to carry out BD campaign and Faculty of Medicine, Turgut Ozal University hosted the event in January 2015. The personnel of Turkish Red Crescent asked the volunteers to fill the form and their blood was taken by finger stick to measure hemoglobin levels.

In addition to Turkish Red Crescent’s criteria, inclusion criteria of our study were to be a first time donor and a non-smoker. Our study population consisted of 88 healthy volunteers, 39 females and 49 males; aged 18–25 years (mean age 21.47 ± 0.74 years). The two groups, questionnaire and study, were matched for age and BMI. Venous blood samples were collected to measure appetite hormones just before blood donating and placed into an enclosed pink topped aprotinin tube. Second venous blood samples were collected 24 hours after BD and placed into a similar test tube. To eliminate the factors which might affect the levels of appetite hormones, we reminded the volunteers to be the same level of satiety before giving second blood samples. Plasma fraction was obtained by centrifugation ($2,000 \times g$, 10 min, and 4°C) approximately 2 minutes after being drawn and stored at -80°C until analysis.

All participants were also asked to mark the degree of appetite they felt before and 24 hours after blood donating on VAS. Verbal and written consent was obtained from all

participants, and authorization was given by the Ethics Committee of School of Medicine, Turgut Ozal University, Ankara, Turkey.

Biochemical analysis

Ghrelin, leptin, NPY and α -MSH concentrations of the samples were measured in plasma samples by double antibody sandwich enzyme-linked immunosorbent assay (ELISA) with human commercial kits (Shanghai Sunred Biological Technology, China) according to the manufacturer's instructions. Absorbance values were read at 450 nm in an automatic ELISA reader. The concentrations were calculated by converting the optical density readings against a standard curve. All samples were assayed in duplicate.

Plasma ghrelin levels were expressed as pg/L. Detection range was between 30 pg/mL and 900 pg/mL. The minimum detectable level of human ghrelin is 28.225 pg/mL. Plasma leptin levels were expressed as ng/L. Detection range was between 0.2 ng/mL and 60 ng/mL. The minimum detectable level of human leptin is 0.153 ng/mL. Plasma NPY levels were expressed as ng/L. Detection range was between 7 ng/mL and 2000 ng/mL. The minimum detectable level of human leptin is 5.115 ng/mL. Plasma α -MSH levels were expressed as ng/L. Detection range was between 15ng/mL and 4200 ng/mL. The

minimum detectable level of human α -MSH was 14.068 ng/mL.

Statistical analysis

Results are expressed as mean $\pm$ standard deviation. Distributions were evaluated by using One Sample Kolmogorov Smirnov test. A two-tailed paired t-test or Wilcoxon test was used to compare as appropriate. Pearson or Spearman Rho correlation test was used to indicate relationships between variables. Differences were considered statistically significant at $P < 0.05$. The SPSS statistical software package (SPSS, version 16.0 for windows; SPSS Inc., Chicago, Illinois, USA) was used to perform all statistical calculations.

Results

The results of questionnaire study are shown Table 1: 53% of participants thought blood increased their appetite. For 93 participants, appetite increased significantly, from 4.9 ± 1.2 to 7.5 ± 1.8 ($p = 0.018$). After this result we made a decision to undertake biochemical analysis of appetite hormones measurement. In total, 108 volunteers were recruited in the study; 13 volunteers were excluded for not coming to get blood drawn next day (after 24 hours), three volunteers were excluded because of taking pain killer medicine (after BD), four volunteers were excluded because of forgetting to mark

TABLE 1. The questionnaire study and results

Question	Allowed answer/Comments
Do you smoke?	No: 316
	Yes: 83 (mean=1 pack of cigarettes per day)
Have you ever donated blood before?	No: 93
	Yes: 306 (mean=once)
Do you think that blood donation increase appetite?	increases a lot: 59
	increases: 153
	does not increase: 88
	decreases: 12
	no idea: 48
Before donating blood, please mark your appetite status	Range: 0 – 10
	4.9 ± 1.2
After donating blood, please mark your appetite status	Range: 0 – 10
	7.5 ± 1.8

TABLE 2. Changes in appetite and appetite-related hormones before and 24 hours after blood donation

	Before blood donation	After blood donation	<i>P</i> value
Appetite; according to VAS	5.58±2.45	6.16±2.70	0.018
Ghrelin (pg/mL)	5497.27±634.13	8491.07±3023.48	0.297
Leptin (ng/mL)	23.94±3.67	18.77±3.10	0.001
Neuropeptide-Y (ng/mL)	1354.93±151.53	1392.34±154.78	0.262
Alpha-melanocyte-stimulating hormone (ng/mL)	2731±310.87	2954±344.83	0.331

All results are expressed as mean±standard deviation

NS: Non significant

P value: comparison with before and after blood donation

their appetite status on VAS. Thus, a total 88 volunteers completed the study. There were no significant difference in basic characteristics (age and BMI) between the questionnaire group and study group. Biochemical results of the study groups are shown in Table 2. Mean hemoglobin level of volunteers was 14.61±1.4 (range: 12.5-18.0). There was a significant increase between the appetite degrees as assessed by using VAS ($p=0.018$). Appetite degrees of blood donors elevated from 5.58±2.45 to 6.16±2.70. There was a significant decrease in the level of leptin ($p=0.001$). The levels of ghrelin, NPY and α -MSH were unchanged after BD.

Discussion

Medical emergencies, illnesses and injuries take place constantly, and according to the American Red Cross, one of the largest organizations to collect BDs, someone needs blood every two seconds in America. Although the number of blood donors increases daily, only 80% of the need can be filled [2]. In developed countries, the ratio of voluntary BD to population is 5%, but this ratio is lower (3.6%) in our country [1]. An important problem is inadequacy in the number of voluntary blood donors. One reason potential donors decline to donate blood is due to a concern over weight gain after BD. We performed this study to determine whether this phobia was valid.

The cardinal findings of this study are as follows: compared with before BD (i) the level of leptin decreased; (ii) the levels of ghrelin, NPY and α -MSH were unchanged; and, (iii) the appetite degrees assessed by using VAS were elevated. The mechanisms for controlling food intake involve mainly interplay between the gut, brain and adipose tissue [3]. The arcuate nucleus (ARC), located at the base of the hypothalamus plays a crucial role in the regulation of food

intake and energy homeostasis. The ARC contains two populations of neurons with opposing effects on food intake [4]: prexigenic neurons (i.e., those stimulating appetite) express NPY whereas anorexigenic neurons (i.e., those inhibiting appetite) express α -MSH [5]. The ARC acts as an integrative centre. The activities of these neuronal pathways are also influenced by numerous factors such as nutrients, fasting and disease to modify appetite and hence affect food intake and energy expenditure.

Ghrelin is a peptide secreted primarily from the stomach and, plasma levels decrease after a meal and potently stimulate food intake. The factors involved in the regulation of ghrelin secretion have not yet been identified. Blood glucose levels seems a probable candidate; thus, administration of glucose decrease plasma ghrelin levels, which exhibit a nocturnal increase and is low in obese people and high in lean people [6, 7]. Exogenous growth hormone decreases stomach ghrelin mRNA expression and plasma ghrelin levels, but does not affect stomach ghrelin stores. BD may be another factor that affects ghrelin secretion. Blood donors had higher level of ghrelin (5497.27±634.13 *vs.* 8491.07±3023.48 pg/mL) after donating but this elevation was not statistically significant ($p=0.06$). A potential reason for insignificant increase ghrelin may be the time of taking sample from donors in the same satiety state. If blood donating increases the appetite, increased ghrelin levels are expected. It is important because ghrelin increases cumulative food intake and decreases energy expenditure, resulting in body weight gain.

NPY is one of the hypothalamic orexigenic peptides and the most noticeable effect of it is the stimulation of feeding (8). This effect is regulated by inhibitory afferent signals such as leptin and insulin. In the present study, we revealed that the NPY levels were not changed after BD.

The ARC is also a target of leptin. It is known to suppress appetite and regulate energy expenditure and produced mainly (95%) in adipose tissue. Leptin directly inhibits the appetite-stimulating effects of NPY and agouti-related protein whereas hypothalamic ghrelin blocks leptin-induced reduction of feeding (9). Thus, ghrelin and leptin have a competitive interaction in the regulation of feeding. The production of leptin is being stimulated by insulin and blood glucose but inhibited by sympathetic activity, lipolytic catecholamines, and free fatty acids (3). Little is known about leptin response to BD. The other finding of this study was that there was a reduction in the levels of leptin after BD. The reduction of leptin levels may cause an increase food intake, a decrease in energy expenditure, enhanced satiating effect of cholecystokinin, and induce down-regulation of the hippocampal somatostatinergic system which may potentiate its anorexigenic effect.

These results are in agreement with those obtained by Sliwowski *et al.* [10]. They investigated the immediate effects of strenuous exercise in healthy volunteers under fasting or fed condition and before and one day after BD. They conclude that strenuous physical exercise following BD resulted in a marked reduction in plasma leptin levels.

Anorexigenic peptide α -MSH, product of pro-opiomelanocortin inhibits food intake by activating melanocortin (MC) 3 and MC4 receptors and inhibiting the release of NPY. The MC4R is a key molecule underlying appetite control and energy homeostasis [11]. In the present study, we found out that BD did not affect the level of α -MSH.

VAS, a valid and sensitive measure, has been used to assess appetite degree and pain intensity in numerous clinical investigations [12]. Results of both the questionnaire and laboratory studies indicated higher appetite VAS score after BD and donors feel more appetite.

BD is good for health. It reduces the amount of iron in the body and reduces the risk of heart diseases: blood donors have an 88% reduced risk of acute myocardial infarction. Zacharski *et al.* reported that BD was associated with lower cancer risk such as colon cancer and mortality due to iron reduction [13]. Metabolic syndrome (METS) includes obesity, hypertension, insulin resistance and glucose and lipid metabolic abnormalities. The main treatment for METS is weight loss but the findings suggest doctors might consider BD as a possible treatment for people with METS who have above-normal iron levels (a common situation). Houshyar *et al.* reported that donating blood may potentially help obese patients with METS, through a reduction in blood pressure,

along with other changes that are linked with a reduced risk of heart disease, and overall heart health [14].

Although BD is generally safe, blood donors expose themselves to a variety of risks such as iron deficiency [15], restless legs syndrome [16], vasovagal reactions, citrate-related events, nerve injury [17], bruises and arterial puncture [18]. Fortunately, most of these risks are rare. With an ever-increasing demand on blood supplies worldwide, there are psychological factors that influence people's willingness to donate blood. This is the first scientific attempt to address the problem of whether BD will cause increased appetite and therefore weight gain. In conclusion, the results of this study showed that there was a statistically significant alteration in the level of leptin. This might provide a pathophysiologic link between excess appetite and BD. The other three neuropeptides that regulate appetite and feeding behavior remained unchanged.

Limitations

We investigated the effect of BD on appetite only for one day. The long-term effects of it also need to be studied. But it is very difficult to perform because it may be influenced by changes in life style such as eating habits during the study period. The other limitation was the lack of a control group. We do not think that people would agree to being stuck twice with needles, and perhaps having an increase in appetite and without even being able to donate blood. However, we do not suppose the act of piercing a vein with a needle can be responsible for these metabolic changes.

Acknowledgments

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