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Research Article

The Effect of Starvation on Acute Phase Proteins and Adiponectin Levels in Rats

Şenay Akça[1], Pınar Alkım Ulutaş[2]

[1] Aydın Adnan Menderes University, Health Sciences Institute, Department of Biochemistry, Aydın, Turkey

[2] Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Department of Biochemistry, Aydın, Turkey

ABSTRACT

In this study, it was aimed to investigate the effect of starvation constituted in rats, on C-reactive protein (CRP), haptoglobin (Hp), albumin, ceruloplasmin and cytokine (adiponectin) synthesized from the adipose tissue.

Twenty adults, 250-300 g of weight, non-pregnant, Wistar type of female rats were used in this study. The rats were randomly divided into two groups, as control and experimental groups. The rats in the experimental group were given only water, while the rats in the control group were fed regularly according to their routine feeding habits for 48 hours. Blood samples were collected intracardiac under the anaesthesia. Serum CRP, Hp, albumin, ceruloplasmin, adiponectin, cholesterol, triglyceride, total protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), glucose and urea levels were studied. Albumin, total protein, triglyceride, glucose and ALT levels were significantly lower, whereas urea and AST levels were higher in the starving/fasting group compared to the control group. Regarding the acute phase proteins (APP), there was a statistically insignificant decrease in the CRP and Hp levels, and increase in ceruloplasmin levels in the starving/fasting group. Adiponectin and cholesterol levels were higher; GGT level was a lover in the starving group compared to the control group and both of the differences were statistically insignificant. Statistically significant increases in urea and AST levels were observed.

As a result, the levels of positive APP's didn't change significantly with acute starving whereas a negative APP, albumin, decreased significantly. The tendency in the increase of adiponectin levels is another important data that we obtained in this study. Starving of human beings may occur as a result of various psychological, social and organic reasons, and we speculate that the physiological and biochemical effects of starving stated in our study could be a reference for further studies related to this subject.

Key Words: Rat, starvation, acute phase proteins, adiponectin

Ratlarda Açlığın Akut Faz Proteinleri ve Adiponektin Düzeyleri Üzerine Etkileri

ÖZET

Bu çalışmada ratlarda açlığın C-reaktif protein (CRP), haptoglobin (Hp), albümin, seruloplazmin ve yağ dokusundan sentezlenen sitokin (adiponektin) üzerindeki etkisinin araştırılması amaçlanmıştır.

Bu çalışmada 250-300 g ağırlığında , gebe olmayan, 20 yetişkin Wistar tipi dişi rat kullanıldı. Ratlar, rastgele, kontrol ve deney grubu olarak iki gruba ayrıldı. Deney grubundaki ratlara sadece su verilirken, kontrol grubundaki ratlar 48 saat rutin beslenme programlarına göre beslendi. Kan örnekleri anestezi altında intrakardiyak olarak toplandı. Serum CRP, Hp, albümin, seruloplazmin, adiponektin, kolesterol, trigliserit, total protein, aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), gama glutamiltransferaz (GGT), glukoz ve üre analizleri yapıldı. Kontrol grubuna göre açlık grubunda albümin, total protein, trigliserit, glukoz ve ALT düzeyleri anlamlı olarak düşük, üre ve AST düzeyleri yüksek olarak belirlendi. Açlık grubunda CRP ve Hp seviyelerinde istatistiksel olarak önemsiz bir azalma, seruloplazmin seviyelerinde artış görüldü. Açlık grubunda kontrol grubuna göre adiponektin ve kolesterol seviyeleri yüksek; GGT düzeyi ise düşük bulundu. Her iki fark istatistiksel olarak anlamsızdı. Üre ve AST düzeylerinde istatistiksel olarak önemli artışlar belirlendi.

Sonuç olarak, pozitif akut faz proteinleri (AFP) seviyeleri akut açlıkla değişmezken, negatif AFP olan albümin önemli ölçüde azaldı. Çalışmadan elde edilen diğer önemli bir veri adiponektin düzetindeki artma eğilimiydi. Bu çalışmanın sonuçlarının, insanlarda çeşitli psikolojik, sosyal ve organik nedenlerle ortaya çıkabilen açlığın fizyolojik ve biyokimyasal etkilerinin inceleneceği çalışmalarda referans olabileceği kanısına varıldı.

Anahtar Kelimeler: Rat, açlık, akut faz proteinleri, adiponektin

Introduction

Starvation is a metabolic process that is a result of inappropriate balance or absence of all or a portion of the basic constituents required for the survival of an organism (Lee et al., 2015a). Insufficient nutrition and starvation lead to the deficiency of essential nutrients which results in the catastrophic destruction of the body and even death (Pointer et al., 2013). Short and long term starvation causes some changes in the carbohydrate, fat and protein metabolisms (Lee et al., 2015a). The findings related to these metabolic changes caused by starvation are not clearly stated.

Acute phase reaction (APR) is a local or systemic nonspecific immune reaction, that may develop as a result of trauma, infection, neoplastic growth, tissue damage, inflammation or immunologic defects of a metabolism (Eckersall and Bell, 2010). The aim of the development of APR is the removal of infectious agents, harmful molecules and wastes that cause damage to the organism; so that the tissue or organ can be protected from further damage and the repair process can be initiated so that physiological homeostasis can be provided. APP's are proteins synthesized by the liver that are generally a reaction to inflammation and serum concentrations of APP's change during APR (Gruys et al., 2006). The levels of APP's are correlated with the magnitude and severity of the tissue damage. Therefore the determination of APP levels provides diagnostic and prognostic information (Eckersall and Bell, 2010). Adiponectin is a glycoprotein adipocytokine synthesized from the fat tissue (Frankenberg et al., 2017). Studies related to the physiologic importance and efficiency of adiponectin have increased in recent years. The most important effect of adiponectin besides its antiatherogenic and anti-inflammatory effects is its regulatory effect on insulin hormone activity (Sun et al., 2009).

There are not many studies related to the effect of starving on APP's and adiponectin in the literature. We consider that the results of this study regarding the effect of starvation on adiponectin, albumin, APP's like Hp, CRP, ceruloplasmin will be beneficial for further studies.

Material and Methods

Animals: The experimental protocols were approved by the ethics committee of Adnan Menderes University Animal Experiments Local Ethics Committee (64583101/2016/155).

Adult, healthy, female Wistar rats weighing approximately 250-300 g were used. During the study, the rats were kept in 425 x 265 x 180 mm sized transparent polycarbonate cages, covered with stainless steel in the controlled rooms of Adnan Menderes University, Veterinary Faculty, Experimental Animals Unit; which were regulated to have an optimal temperature (22°C), 40-60% relative humidity, 12 hours light and 12 hours dark media. The animals were put into the experiment rooms 1 week before the experiment for their adaptation to the environment. Food and water were given *ad libitum*. The groups were randomly selected as two groups; each consisting of 10 animals. Two rats were put into each cage, making a total of 10 cages.

Before the onset of the study, each rat was weighed and the result was recorded. The rats were put into two groups as experimental and control groups, each consisting of 10 rats. As the study started, the study group was given only water for two days, while the control group was fed as their routine feeding habit. At the end of the second day, each rat was weighed and the results were recorded. After the weighing procedure, intracardiac blood samples were taken, after being anaesthetized by ether. Blood samples were centrifuged and divided into three parts; and kept at -20ºC temperature until the analysis was performed.

Biochemical Analysis: Serum albumin, total protein, cholesterol, triglyceride, urea, glucose, ALT, AST and GGT levels were measured with commercial test kits (Archem Diagnostic, Turkey) used in the biochemistry autoanalyzer (Rayto Chemray 120). CRP levels were measured with a quantitative competitive immunoassay technique kit, on the ELISA device (AssayPro, Assay Max Rat CRP, USA). Serum haptoglobin levels were measured with commercial test kits (ELISA commercial kit Tridelta LTD, Ireland) on the ELISA device. Serum ceruloplasmin levels were determined with the spectrophotometric technique that Sunderman and Numato (1970) presented (Schimatzu UV 1601, Japan). Rat adiponectin ELISA Kit (Boster Biological Technology, USA) based on standard enzyme-linked immunosorbent experiment technology was used for the measurement of serum adiponectin levels.

Statistical Analysis: The statistical analysis of the data obtained at the end of the study was performed using SPSS (for Windows Release Standart Version 15) program. Independent sample test (Mann-Whitney U) was used for the identification of the difference between the groups. The results were given as mean ± standard deviation. The interpretation of statistical results was determined as NS: Not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Results

Albumin levels were 4,04±0,14 g/dL in the control group, and

Table1. Control and starvation groups mean serum albumin (ALB), Total Protein (TP), cholesterol, triglyceride, urea and glucose results
*****:p<0,001 **:p<0,01 *:p<0,05**

	ALB (g/dl) X±SD	TP (g/dl) X±SD	Cholesterol (mg/dl) X±SD	Trygliceride (mg/dl) X±SD	Urea (mg/dl) X±SD	Glucose (mg/dl) X±SD
Control (n=10)	4,04±0,14	6,57±0,25	75,33±17,25	64,15±11,41	38,70±5,28	130,05±24,81
Starvation (n=10)	3,97±0,63	6,12±0,38	90,00±26,64	50,90±11,03	47,48±6,94	106,20±26,12
t	0,346	3,107	-0,254	2,639	-3,185	2,093
p	0,043 *	0,007 **	0,529	0,023 *	0,011 *	0,019 *

Table 2. Control and starvation groups mean serum AST, ALT and GGT results
*****:p<0,001 **:p<0,01 *:p<0,05**

	ALT (U/L) X±SD	AST (U/L) X±SD	GGT (U/L) X±SD
Control (n=10)	55,05±10,93	135,64±26,73	2,63±0,67
Starvation (n=10)	38,25±6,30	214,88±103,20	2,44±0,83
t	4,211	-3,017	0,573
p	0,001 **	0,004 **	0,247

3,97±0,63 g/dL in the study group. Albumin levels were lower in the study group compared to the control group, statistically p<0,05. Total protein levels were 6,57±0,25 g/dL in the control group, whereas 6,12±0,38 g/dL in the study group. Regarding the serum total protein levels, the statistical difference between the two groups is p<0,01. Cholesterol level was 75,33±17,25 mg/dL in the control group, and 90,00±26,64 mg/dL in the study group. The increase in serum cholesterol levels in the starving group was statistically insignificant. Triglyceride levels were 64,15±11,41 mg/dL in the control group and 50,90±11,03 mg/dL in the study group. The decrease in the study group was statistically significant, p<0,05. Urea levels were 38,70±5,28 mg/dL in the control group, and 47,48±6,94 mg/dL in the study group; the increase was statistically significant p<0,05. Blood glucose levels were 130,05±24,81 mg/dL in the control group, and 106,20±26,12 mg/dL in the study group; the decrease in the study group was statistically significant p<0,05 (Table 1).

ALT levels were 55,05±10,93 U/L in the control group and 38,25±6,30 U/L in the study group. The relative decrease of serum ALT levels in the study group was statistically significant, p<0,01. AST levels were 135,64±26,73 U/L in the control group and 214,88±103,20 U/L in the study group. The relative increase of serum AST levels in the study group was statistically significant, p<0,01. The difference between the GGT levels between the control and study groups was statistically insignificant, 2,63±0,67 U/L and 2,44±0,83 U/L respectively (Table 2).

CRP levels were measured as 27,46±13,77 µg/mL in the control group and 17,11±12,65 µg/mL in the study group. The decrease in the study group was statistically insignificant. The difference of serum Hp levels were also statistically insignificant, 0,96±0,84 g/L in the control group and 0,69±0,33 g/L in the study group. Ceruloplasmin levels were 50,08±5,36 mg/dL in the control group and 55,17±12,40 mg/dL in the study group; the difference was also statistically insignificant. The difference between adiponectin levels of the control and study groups was statistically insignificant; 5,85±1,54 ng/mL and 6,41±1,52 ng/mL respectively (Table 3).

The mean weight of the rats in the control group was 323,7±18,80 g in the beginning of the study and increased to 326,8±22,84 g at the end; whereas the weight of rats decreased from 325,4±39,00 g to 292,1±36,34 g. The 48 hours starvation led to a 10% decrease in the body weights of the rats (Table 4).

Discussion

Starvation is a biological process in which the access of an organism to nutritional sources is restricted as a result of various causes. It is known that the restriction or cessation of food intake initiates a process that can result in death in all of the living organisms. The restriction of food intake causes in some changes of systemic activities of the organism (Karataş, 2014). We aimed to study the effect of starvation on blood levels of biochemical parameters, APP's and adiponectin levels in rats.

In our study, the total protein and albumin levels in starved rats were lower than the control group; and the difference was p<0,01 for total protein and p<0,05 for albumin, both of which were statistically significant. Similarly, Lee et al. (2015b) reported a decrease in total protein levels in the rats that were starving. The decrease in total protein was interpreted to be a reflection of inadequate food intake or a sign of the deterioration of the liver function in starvation. In our study, the blood AST levels were significantly higher in the study group compared to the control group; which was concluded to be the result of the effect of starvation on the liver function impairment. Albumin is the most important negative APP (Cray et al., 2009). The most important factors affecting albumin levels are nutrition and inflammation. Protein loss of the body causes a decrease in albumin levels. A decrease in protein intake results in reduced albumin synthesis and finally albumin concentration decreases (Burl et al., 2004). Factors like liver function impairment, starvation affect serum albumin levels (Hirvonen and Pyörälä, 1998; Petersen et al., 2004). Lee et al. (2015b) stated that long-term starvation leads to a severe decrease in serum albumin levels. Albumin synthesis of the organism is partly related to its nutrition, and especially to the protein intake. But studies have shown that inadequate nutrition itself -excluding severe starvation statuses- does not cause a significant decrease in albumin levels (Kaysen and Don, 2003).

As soon as the body faces malnutrition or starvation, it begins to provide the supplementation of some nutrients, like lipids, from organs and tissues like the muscles and the liver. This causes a decrease in blood and tissue triglyceride levels. In our study, serum triglyceride levels were significantly lower in the starvation group, which is compatible with the literature. Similarly, Lee et al. (2005) reported a decrease in serum triglyceride levels in long-term starved rat models. Zauner et al. (2017) stated a significant decrease in serum triglyceride levels in the second day of their study, in which 1, 2, 3, 4 days of starvation was performed on rats. The results of our study are compatible with the results of other experimental starvation animal model studies and we concluded the decrease in the triglyceride levels was a result of the usage of lipids by the metabolism to fulfil the energy need. In our study, blood cholesterol levels were not statistically different between the study and control groups. We concluded that 48 hours of starvation didn't have any effect on total cholesterol levels. Lee et al. (2015b) performed a prolonged (two weeks) starvation study on rats and stated that cholesterol and HDL levels don't change, but LDL cholesterol levels significantly increase with starvation. In another study, Zauner et al. (2017) reported that short term starvation didn't

Table 3. Control and starvation groups mean serum CRP, Hp, ceruloplasmin and adiponectin results
*****:p<0,001 **:p<0,01 *:p<0,05**

	CRP (µg/ml)	Haptoglobin (g/L)	Ceruloplasmin (mg/dl)	Adiponectin (ng/ml)
Control (n=10)	27,46±13,77	0,96±0,84	50,08±5,36	5,85±1,54
Starvation (n=10)	17,11±12,65	0,69±0,33	55,17±12,40	6,41±1,52
t	1,751	0,956	-0,454	0,814
p	0,089	0,796	0,143	0,353

cause a significant change in total cholesterol levels.

The decrease in blood glucose levels is directly related to the reduction of nutritional intake. In this study, blood glucose levels were significantly lower in the starved rats compared to the control group. The decrease in blood glucose levels in both short- and long-term starvation is reported in various studies (Karataş, 2014; Ryu et al., 2005; Zauner et al., 2017). The drop in the glucose with hunger is related to the decreased intake of glucose which is the major energy source for the metabolism and the consumption of hepatic glycogen.

The body develops various adaptation mechanisms during starvation, like decreasing the weight to decrease the energy need. This adaptation is correlated with the obesity status of the organism before starvation. The energy is obtained from the stored lipid in the individuals who have high-fat tissue; whereas it is obtained from the catabolism of proteins in individuals who have not. The usage of proteins as an energy source may lead to an increase in urea levels. Lee et al. (2015b) established two weeks starved rat model study, and reported a significant increase in the blood urea nitrogen levels and concluded this finding to be a result of the renal dysfunction and failure. In our study, the blood urea levels of starvation constituted rats were significantly higher compared with the control group, which we considered to be the related with the catabolism of proteins in starvation states of the organism. Similarly, Zauner et al. (2017) reported an increase in the blood urea nitrogen levels in all periods of short-term starvation, where the rats were kept at 1, 2, 3 and 4 days of starved periods. In our study, there was a decrease in the serum ALT levels. It is considered to be the result of decreased functional parenchymal tissue loss during starvation. The GGT levels didn't vary among the two groups.

Non-statistically significant decrease was observed in the levels of CRP, haptoglobin and ceruloplasmin in the fasting group compared to the control group in this study. It was thought that this decrease might be related to the decrease in protein synthesis in the liver and the bodyweight lost as a result of 48 hours starvation. Positive APPs are important parameters in monitoring and evaluating the disease in inflammatory processes. However, positive APPs may not give sufficient and correct answers in nutritional disorders and chronic infections. In such cases, looking at negative APPs helps to make a more accurate diagnosis (Gruys et al., 2005).

Recent studies have shown that adipose tissue is not only an energy store, but also an organ that has effects on energy metabolism and insulin sensitivity, and secretes hormones and various metabolites. The most important of these are adiponectin and leptin. Plasma adiponectin levels decrease in obesity and type 2 diabetes, unlike other adipocytokines. This condition is associated with insulin resistance and sensitivity. In a screening study conducted in India, it was observed that the rate of type 2 diabetes was low in individuals with high blood adiponectin levels and high in those with low blood adiponectin levels (Lindsay et al., 2002). Koca et al. (2005) reported that adiponectin levels decreased in their study on obese individuals. Arita et al. (1999) reported that there is a negative correlation between the decrease in adiponectin level and body mass index in obese patients. They stated that it could be associated with increased insulin resistance and TNF-α level with arteriosclerotic lesion and / or increase in adipose tissue. Pannaacciulli et al. (2003) reported in a study on 11 women with anorexia nervosa that blood adiponectin levels were higher than healthy samples in the control group. Besides, in studies conducted both in obese rats (Milan et al., 2002) and in humans (Yang et al., 2001), the decrease in body weight caused an increase in adiponectin level despite the decrease in adipose tissue, which is the only organ that synthesizes itself. Similarly, in our study, adiponectin levels in rats with starvation were found to be higher than the control group, but no statistical significance could be determined between the groups. It was concluded that the reason for this was related to the small number of samples in the experimental and control groups and the duration of fasting. Adiponectin is an adipocytokine that has an anti-inflammatory effect and suppresses TNF-α and IL-1 levels (Yalnız et al., 2012). APPs are proteins released by the stimulation of these cytokines in inflammatory reactions. Therefore, decreased adipose tissue and increased adipokine level due to starvation decreased the synthesis of proinflammatory cytokines. APP levels do not increase as a result of decreased protein synthesis from the liver with decreased protein intake; it is obviously clear that APP's levels have a decreasing trend. Expected decreases in total protein, albumin, triglyceride and glucose levels and increases in urea and AST levels were observed in this study. Impairment of liver function due to starvation resulted in a decrease in the levels of proteins synthesized from the liver. It is thought that the results obtained from this study will provide information about APPs occurring in starvation, some biochemical values and adiponectin concentrations and maybe a reference for future studies.

Conflict of interest

The authors declare that they have no competing interests.

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