Acta Veterinaria (Beograd), Vol. 54. No. 1, 13-20, 2004.

UDK619:612.015.36

THE EFFECT OF CHRONIC FOOD RESTRICTION ON LIVER ACUTE PHASE PROTEIN RESPONSE IN FEMALE AND MALE WISTAR RATS

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(Received 15. July 2003)

The acute inflammatory response of the liver associated with chronic food restriction was examined in adult female and male Wistar rats. The changes in the levels of serum markers of liver injury, AST and ALT and the appearance of a serum marker of inflammation, the acute phase protein (APP) haptoglobin (Hp) were assessed following turpentine treatment of well-nourished (WN) controls and undernourished (UN) rats. Undernutrition was induced by food restriction during a six week period by offering chow equivalent to 50% of the normal food intake. In the female rats undernutrition significantly potentiated liver injury and increased their sensitivity to the toxic effects of turpentine, which was opposite to the results obtained for males. Differences in the basal levels of AST, ALT and Hp between females and males imply that the effects of chronic food restriction on protein synthesis in the liver are gender related.

Key words: undernutrition, liver inflammation, haptoglobin, AST, ALT, acute phase response, female and male rats.

INTRODUCTION

The sequence of events known as the inflammmatory reaction or acute-phase (AP) response is induced in response to stress stimuli such as inflammation, infection and tissue injury, and a number of plasma proteins called acute-phase proteins (APPs) are synthesized in the liver as part of the host defense mechanism (Mackiewicz, 1997). The main source of amino acids for APP synthesis comes from the breakdown of proteins in skeletal muscles (Fürst *et al.*, 1982; Rennie *et al*., 1985). In addition to an increased influx of amino acids, changes in activity of different enzymes, ion and metabolic transporters as well as activation of many metabolic pathways occur in the liver as a result of activation of the protein synthesis system during the AP response (Kuschner, 1982; Baumann and Gauldie, 1994; Mackiewicz, 1997). Since it includes an array of changes designed to enable organisms to survive and to regain normal function following both minor and major threats to their integrity, the AP response represents a phenomenon of

enormous biological importance.It is therefore significant to determine factors which can modulate it.

Some studies have indicated that the ability of an organism to respond to and recover from various stress stimuli depends on its age and/or nutritional status. It was found that obesity (Loffreda *et al*., 1998; Tschop and Heiman, 2001) and poor nutritional status affects reproductive, adipocyte, metabolic and neuroendocrine functions (Peralta *et al.*, 2002; Tappy *et al*., 2000). Based on these data, our objective was to study the liver inflammatory and AP response in relation to chronic food restriction. In the present study, AP response was induced by administration of turpentine oil to Wistar albino rats, because this sterile tissue injury model successfully reproduces changes occuring during inflammation (Baumann *et al*., 2000). We compared the sensitivity to food restriction and liver injury by turpentine of female to that of male rats by determining the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and haptoglobin (Hp). Serum AST and ALT concentrations reflect damage to liver cells and the level increase when these cells have been inflamed or undergone cell death. In our opinion, the concentration of Hp can be used as a sensitive marker of host inflammation status because the serum level of this prominent APP is elevated three to six fold during the inflammatory response (Baumann and Gauldie, 1994). Its functions are pleiotropic, and mostly related to the binding and clearance of hemoglobin, inhibition of superoxide production and stimulation of angiogenesis (Mackiewicz, 1997).

MATERIAL AND METHODS

Animals

Wistar albino female and male rats aged 1 and 2.5 months, bred at the Institute for Biological Research in Belgrade were used. The animals were maintained at constant temperature (22±2^{o}C) with a 12 h light/dark cycle and fed standard laboratory chow (D.D.Veterinarski Zavod, Subotica, Serbia & Montenegro). Tap water was available *ad libitum.*

Food restriction

One-month-old female and male rats were placed in individual cages and fed daily for 6 weeks with an amount of chow equivalent to 50% of the normal food intake until they reached and maintained a body weight about 50% of that of *ad libitum* fed age-mates. Undernourished (UN) animals were weighed weekly and the amount of food provided was adjusted individually to maintain the weight.

Turpentine treatment

Undernourished (UN) and *ad libitum* fed female and male rats aged 2.5 months, termed well-nourished (WN) controls, received a subcutanious injection of turpentine oil (250 μl) in the dorsal lumbar region (sterile tissue injury model). Each experimental series included 5 animals per treatment. Animals were sacrificed by cervical dislocation at various time points after injection and blood and liver samples were collected individually.

Determination of AST and ALT

The serum was obtained after blood clotting and centrifugation at 5000 x g for 15 min. The concentrations of AST and ALT were determined using an ILab-600 Analyzer (International Laboratory, USA).

Rocket immunoelectrophoresis

The serum level of Hp was measured by rocket immunoelectrophoresis according to Baumann (1988) using polyspecific antibodies to human Hp (Sigma-Aldrich Inc), which where cross-reactive with rat Hp. The relative concentration of Hp was established by quantification of the areas under the respective immunoprecipitation peaks and expressed as relative increase in relation to the initial control values, which were taken as 100%.

Statistical analysis

Statistical comparisons were performed by Student's *t* test.

RESULTS

Data on body and liver weights and serum levels of AST and ALT in 2.5 month old female and male Wistar rats fed *ad libitum* (WN) or exposed to food restriction for six weeks previously (UN) are summarized in Table 1. As expected, food restriction kept the body weight of UN rats at about 50% of WN females and 44% of WN males. Total liver weights of both female and male UN rats were significantly lower than those of their WN controls ($p<0.01$).

Table 1. Characteristics of the well-nourished (WN) and undernourished (UN) female (F) and male (M) rats before exposure to turpentine: the effect of food restriction on body weight, liver weight and serum levels of AST and ALT.

	WN		UN	
	F	M	F	M
Body weight, g	211±10	275±39	110±6	122±12
Liver weight, g	7.55±0.48	10.96±1.5	3.37±0.29	3.83±0.25
Liver weight, % body weight	3.58±0.35	3.98±0.31	3.06**±0.21	3.13**±0.34
AST, units/liter	51.3±7.09	124±7.8	142.6*±12.1	101.3±24.4
ALT, units/liter	18.3±3.21	32.3±3.51	41*±4.0	44.3±7.09

The values represent the mean ± SD (n = 14-16/group for body and liver weight; n = 5/group for AST and ALT)
* $p<0.05$; ** $p<0.01$ compared with lean WN control

The mean concentrations of AST and ALT were significantly higher in the sera of UN female rats than in the lean WN controls ($p<0.05$). In the case of UN male rats the serum level of AST decreased and that of ALT increased but not signifi-

cantly. These results suggest that food restriction may lead to liver injury especially in females.

Table 2. Effect of turpentine on serum activities of liver-associated enzymes. Changes in serum levels of AST and ALT in WN and UN female and male rats 24 h after turpentine treatment

	FEMALES			MALES		
	WN	WN + turpentine	UN + turpentine	WN	WN + turpentine	UN + turpentine
AST, units/l	51.3±7.09	143.3*±6.5	275***±20.6	124±7.8	207.2*±4.9	305**±50
ALT, units/l	18.3±3.21	47.6**±12.07	62.8**±6.8	32.3±3.51	26.75±1.78	109**±13.2

The values represent the mean ± SD (n = 5/group)
*p<0.05; **p<0.01; ***p<0.005 compared with WN control

Table 2 shows that turpentine treatment of WN controls resulted in significant increases of serum levels of AST and ALT 24 h after administration ($p<0.05$). In females, this increase was similar for AST and ALT (2.8 and 2.6 fold respectively), whereas in males AST increased 1.6 fold and ALT decreased but not significantly. In UN groups, administration of the same amount of turpentine as in WN animals significantly amplified the increase of serum AST and ALT in both female and male rats ($p<0.01$). This was especially marked in UN female rats, where AST increased 5.3 fold ($p<0.005$).

Figure 1 demonstrates that food restriction had a tendency to increase basal Hp level in the serum of female and decrease it in the serum of male rats. Following turpentine treatment, Hp level remained higher in UN females than in lean WN

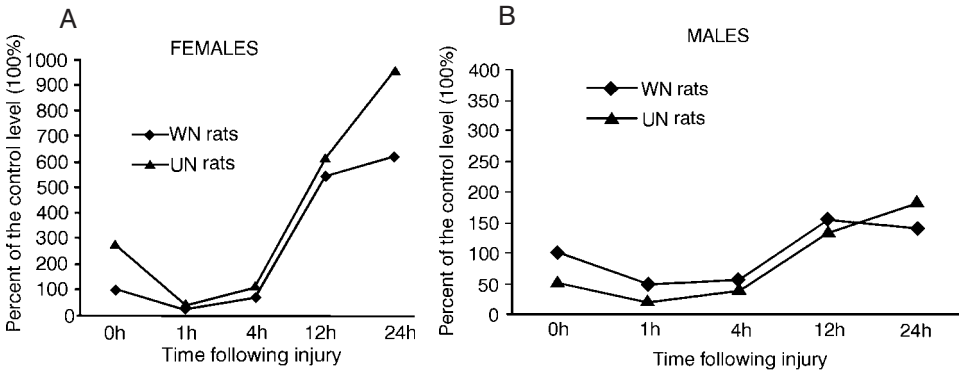

Figure 1. Changes in the relative Hp level in the serum of WN and UN female (A) and male (B) rats at different times after turpentine treatment. Data are expressed as percentages of initial control values.

controls at all time points after treatment. In contrast, the initial Hp level in UN males was almost 50% lower than in the WN group and it remained lower relative to the lean WN males treated with turpentine until 12 h after treatment. After this time point, Hp level decreased in WN males, but increased in UN rats.

These results imply that changes in the relative concentration of Hp in the serum of WN and UN rats injured with turpentine are time dependent. Besides initial differences in its relative concentration between WN and UN rats, the pattern of changes in Hp concentration at different times after turpentine treatment was similar. In both groups, a significant drop of serum Hp level was noted 1 and 4 h after the injection, followed by a dramatic increase up to 24 h in female and 12 or 24 h in male rats.

DISCUSSION

Chronic malnutrition is one of the most important causes of several metabolic, immune and neuroendocrine dysfunctions (Giovambatista *et al*., 2000). The results presented in this paper demonstrate that chronic food restriction affects the inflammatory status of female and male Wistar rats in different ways. Consistent with the significant increase in serum AST and ALT concentrations, as well as the level of the AP protein Hp, we conclude that in females chronic food restriction potentiates liver inflammation and increases its sensitivity to the toxic effects of turpentine.

Thus, our results revealed a significant increase in the basal level of Hp in the serum of UN female rats. Since the rat liver response to acute inflammation is characterized by massive production and secretion of several APPs including Hp (Mackiewicz, 1997), it seems that chronic food restriction is able to affect the hepatic synthesis/secretion of Hp. Undernutrition is well known to cause a reduction in plasma concentrations of some APPs, such as prealbumin, transferrin (Fleck *et al*., 1985) and 2-macroglobulin (Jennings *et al*., 1992). This reflects the diversion of amino acids from these proteins into other proteins that are more essential for survival. The study of Giovambatista *et al*. (2000) showed that basal hypoglycemia, hypotriglyceridemia, hypoleptinemia, hypercorticosteronemia and enhanced adrenal glucocorticoid content in female rats were induced by undernutrition. Stimulation of Hp synthesis is incorporated in to the complex interchange of cytokines, growth factors and glucocorticoid hormones resulting in a specific assembly of *trans*-acting proteins affecting the Hp gene regulatory elements (Marinković and Baumann, 1990; Ševaljević *et al*., 1995). Consequently, through modulation of immune and neuroendocrine functions (Giovambatista *et al*., 2000) food restriction could change the structure and/or function of certain liver-specific or liver-enriched regulatory proteins binding to the Hp gene regulatory elements and thus determine its expression. In liver–derived cells, glucocorticoids induce the expression of alpha and beta C/EBP isoforms at both the level of mRNA and protein (Crosson *et al*., 1997; Matsuno *et al*., 1996). Roesler (2001) reported that transactivating capacity of C/EBPs, a family of transcription factors with important roles in constitutive regulation of APPs gene transcription and energy metabolism, can be modulated by nutrients. Our previous studies have shown the involvement of sev-

eral C/EBPs in the regulation of Hp gene transcription (Grigorov *et al*., 1998; Milosavljević *et al*., 2003).

When the UN females were treated with turpentine, the relative level of serum Hp appeared to be higher than after the same treatment of WN controls. These data imply that food restriction sensitizes liver cells and makes them more susceptible to the action of a second inflammatory stressor. Therefore, the effects of turpentine treatment may be potentiated by undernutrition. However, the molecular mechanism involved in the interaction of these two "stressors" must be studied in each sex separately, because the results obtained for the acute inflammatory response of the liver in UN male Wistar rats were different. Contrary to UN females, UN male rats displayed a slight insignificant decrease in AST level and a slight increase in ALT level (Table 1). The mean level of serum Hp (Fig. 1) was significantly reduced suggesting that in male rats food restriction probably inhibited the Hp synthesis system in the liver. The difference in Hp response in UN female and male Wistar rats could result from different palettes of gender related types of inflammatory mediators, which would lead to different liver responses. As a result UN females might be more sensitive and prepared for inflammation than UN males, which is in an accordance with the data shown here.

In conclusion, together with the reports of other authors, our results support the involvement of nutritional status in the ability of an organism to respond to and recover from various stress stimuli. By recognizing the influence of food restriction on liver metabolism and transcription rates of genes involved in a wide variety of metabolic events, the actual role of nutritional status during acute and chronic inflammatory disease can be assessed.

ACKNOWLEDGEMENT

This work was entirely supported by the Research Science Fund of the Serbian Ministry of Science and Technology, Contract No. 1722.

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UTICAJ HRONIČNOG IZGLADNJIVANJA ŽENKI I MUŽJAKA WISTAR PACOVA NA ODGOVOR JETRINIH PROTEINA AKUTNE FAZE

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SADRŽAJ

Ispitivan je akutni inflamatorni odgovor jetre odraslih ženki i mužjaka Wistar pacova u uslovima hroničnog izgladnjivanja. Praćene su promene koncentracije AST-a i ALT-a kao serumskih markera povrede jetre, i prisustvo akutno faznog proteina haptoglobina - serumskog markera inflamacije, nakon tretiranja normalno hranjenih (WN) i neuhranjenih pacova (UN) terpentinom. Neuhranjenost je bila in-

dukovana restrikcijom hrane u periodu od šest nedelja količinom koja je za 50% bila manja od one koja se normalno uzima. Kod ženki pacova neuhranjenost je značajno potencirala inflamaciju jetre i povećava senzitivnost na toksične efekte terpentina što je suprotno od onog dobijenog za mužjake. Razlike u bazalnim nivoima AST-a, ALT-a i haptoglobina između ženki i mužjaka ukazuju da su efekti hroničnog izgladnjivanja na sistem za proteinsku sintezu u jetri specifični za pol.