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SHORT REPORT

The effect of feeding diets differing in protein content on activity of lysosomal enzymes in the liver and kidneys of mice

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The investigations were conducted on 20 Swiss male mice, fed a feed containing 16% (control group, n=10) or 10% protein (experimental group, n=10). After 14 days of feeding, sections of liver and kidneys were obtained from all animals and homogenized in order to obtain the lysosomal fraction, in which the activity of the following enzymes was determined: acid phosphatase, lysosomal esterase, lysosomal lipase, β-glucuronidase, β-galactosidase, β-glucosidase, β-N-acetyl-hexosaminidase, leucine aminopeptidase, alanine aminopeptidase and cathepsins D and L. Feed with the protein level reduced to 10% had, compared to the control, a significant and differentiated effect on the activity of enzymes examined, depending on the enzyme type and organ.

KEY WORDS: dietary protein / kidney / liver / lysosomal hydrolases / mice

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Feed is considered a factor affecting both the intensity of protein biosynthesis and its degradation and thus also the activity of corresponding enzymes, related at the given moment to the rate of metabolism [Bozhkov 2001, Jóźwik 2001, Mosoni and Mirand 2003]. Proteins are considered the most important structural elements of cells despite the fact, that the animal organism does not synthesize numerous amino acids that compose proteins. For this reason the requirement for protein is in fact a requirement for exogenous amino acids contained in the dietary protein. The proteins of live cells are continuously subjected to degradation, creating a pool of free amino acids, circulating in the organism and used for the synthesis of new proteins, depending on the current requirements of the organism [Timmerman and Volpi 2008].

Facing the above, studies have been undertaken in order to analyse the model of the lysosome fraction degradation enzymes in liver and kidney cells of mice when offered feeds containing 16 (standard) or 10% protein. The aim was to determine whether a reduction in protein content of a diet to 10% alters the activity of certain enzymes in comparison with a standard, control diet, containing 16% protein.

Material and methods

The experiment was conducted on 20 male, non-selected mice of the Swiss line, aged 8-10 weeks and with a mean live body weight of 25.0 (± 1.3) g. The animals were maintained in standard conditions at the Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, at a temperature of 21-22°C, relative humidity of 50-60% and a 12 hour lightening cycle. Throughout the experiment the animals remained under professional veterinary care.

On day 42 of life the animals were weaned and divided into two groups of ten – control, in which a standard pelleted feed containing 16% protein was fed (group C), and experimental in which the feed offered contained 10% protein (group E). The feeds were almost iso-caloric.

Both feeds were prepared at the Łomna Las Farm. Their composition and nutritive value according to the Jan Kielanowski Institute of Animal Physiology and Nutrition of the Polish Academy of Sciences are given in Table 1. A 100 g of the C group diet contained 4.7g lysine and 8.9 g leucine while a 100 g of the E group diet – 3.9 g lysine and 11.6 g leucine.

After 14 days of feeding the mice were decapitated and immediately fragments of liver and kidneys were taken, homogenized and centrifuged according to Beaufay [1972].

In the lysosomal supernatants the activity of the following enzymes was determined: acid phosphatase [AcP, EC 3.1.3.2], lysosomal esterase [EL, EC 3.1.1.2] and lysosomal lipase [LL, EC 3.1.1.13] according to Barrett and Heath [1977], β-glucuronidase [β-GlcUr, EC 3.2.1.31], β-galactosidase [β-Gal, EC 3.2.1.23], β-glucosidase [β-Glu, EC 3.2.1.21] and β-N-acetylo-hexosaminidase [Hex, EC 3.2.1.52] according to Barrett [1972], leucine aminopeptidase [LeuAP, EC 3.4.11.1] and alanine aminopeptidase

Table 1. Ingredients and protein and energy content of the pelleted feeds[1]

Item	Feed	
	group C (16% protein)	group E (10% protein)
Premix LSM	1.01	1.02
Salt	0.29	0.29
Chale	1.64	1.65
Phosphates	0.97	0.97
Powder milk	5.80	4.83
Soya meal	10.14	1.94
Germinated corn	15.44	-
Maize meal	64.71	89.30
Protein (%)	15.63	10.38
Gross energy (cal/g)	4122	3982
Gross energy (MJ/kg)	~14.04	~13.47

[1]Determined by the Jan Kielanowski Institute of Animal Physiology an Nutrition of the Polish Academy of Sciences , Jabłonna.

[AlaAP, EC 3.4.11.2] according to Mc Donald and Barrett [1986] as well as the activity of cathepsins D and L [Cath. D and L, EC 3.4.23.5, EC, 3.4.22.15] according to Langner *et al*. [1973]. In lysosomal supernatants of liver and kidneys total protein was also determined according to Kirschke and Wiederanders [1984]**.** The activity of the lysosomal enzymes examined was expressed in nmol/mg protein/hour.

The results obtained were subjected to a statistical evaluation using the Student t test. The experiment was conducted on the basis of certificate issued by the Local Commission for Ethics in Animal Experimentation, Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec.

Results and discussion

As shown in Tables 2-3, in the liver was observed a higher activity of Hex, Cath. D and L as well as LeuAP, while that of β-GlcUr, EL and LL appeared lower. In the kidneys a significant increase was observed in the activity of GlcUr, β-Gal, Hex, AcP, Cath. D and L, LeuAP and AlaAP.

Lysosomal enzymes are the subject of considerable interest of scientists [Cuervo and Dice 1998, Lloyd 2000, Jóźwik *et a*l. 2003, Lettau *et a*l. 2007, Paris *et a*l. 2007; Salminen-Mankonen *et al*. 2007].

Protein deficiency in the diet significantly changes the rate of protein degradation in the liver and other glands, and also in muscles. Moreover, it extends the duration of their biosynthesis. Prolonged feeding with low-protein diet may lead to the use by the organism of amino acids obtained from the degradation of its own proteins [Jóźwik, 2001]. Protein hydrolysis, taking place particularly during starvation, may protect proteins of a special metabolic importance and simultaneously supply substrates for non-protein metabolism [Bozhkov 2001].

Table 2. Effect of protein content of feed on enzymes activity (nmol/mg protein/h) in the liver of mice (n=10)

Enzyme		Protein content of feed		Per cent difference (control = 100)
		16% (control)	10%	
β-GlcUr	mean	5.00	1.50	30^{***}
	SD	0.945	0.113	
β-Gal	mean	2.77	3.07	111^{ns}
	SD	0.448	0.535	
β-Glu	mean	2.66	2.17	82^{ns}
	SD	0.483	0.318	
He*	mean	1.95	14.00	718^{***}
	SD	0.243	2.11	
AcP	mean	25.00	24.80	99^{ns}
	SD	4.49	3.71	
EL	mean	16.47	2.13	13^{***}
	SD	3.86	0.321	
LL	mean	8.92	1.14	13^{***}
	SD	1.26	0.184	
Cath.D and L	mean	0.260	0.770	296^{***}
	SD	0.036	0.119	
LeuAP	mean	2.29	5.11	223^{***}
	SD	0.307	0.835	
AlaAP	mean	7.35	6.07	83^{ns}
	SD	0.915	1.19	

$^{***}P \leq 0.001$; ns – not significant.

Reducing the protein content of a diet to 10% revealed a considerable reactivity of the lysosome enzymes examined in the liver and kidneys of mice. The increase observed in the activity of lysosome enzymes Hex, Cath. D and L as well as LeuAP in the liver and of β-GlcUr, β-Gal, Hex, AcP, Cath. D and L, LeuAP andi AlaAP in the kidneys may be linked – among much else – with the increased production of glucose obtained from the degradation of own intra-cell protein, what is a response of the cells of both glands to the low-protein diet. Jóźwik [2001] demonstrated that non-standard diets, including low-protein diets [10%], compared to a standard diet [16%], led to an activation of some lysosomal hydrolases in the liver and kidneys of mice.

The lower activity of β-GlcUr, EL and LL observed in the liver, and of β-Glu, EL and LL observed in the kidneys of mice fed a low-protein diet (group E) was most probably related to the decreased biosynthesis rate of those enzymes [Weir 2007].

In this report the demonstrated lower activity of β-GlcUr in the liver, and its simultaneously increased activity in the kidney, may indicate that in those organs a threat of protein deficiency leads to a different metabolism rate. The increased activity of β-Gal renders possible a rapid degradation of glycoside compounds occurring in the liver and kidney cells [Sorensen *et al*. 2004] and is associated with the increased

Table 3. Effect of protein content of feed on enzymes activity (nmol/mg protein/h) in the kidney of mice (n=10)

Enzyme		Protein content of feed		Per cent difference (control = 100)
		16% (control)	10%	
β-GlcUr	mean	1.28	3.21	250***
	SD	0.143	0.597	
β-Gal	mean	5.40	6.40	118*
	SD	1.09	1.07	
β-Glu	mean	3.31	1.08	33***
	SD	0.620	0.117	
He*	mean	4.51	11.13	247***
	SD	0.771	1.75	
AcP	mean	26.50	114.0	430***
	SD	4.81	20.40	
EL	mean	6.90	0.944	14***
	SD	1.29	0.102	
LL	mean	5.11	1.33	26***
	SD	0.919	0.113	
Cath.D and L	mean	0.240	0.280	116*
	SD	0.036	0.082	
LeuAP	mean	12.50	98.20	785***
	SD	2.10	10.43	
AlaAP	mean	22.60	140.0	619***
	SD	4.82	18.20	

***P≤0.001; *P≤0.05; ns – not significant.

production of glucose from glycoproteides, what is an expression of the organism's adaptation to the low protein level of the diet [Wray-Cahen *et al*.1997].

Markedly higher activity of AcP in kidneys of mice maintained on a low-protein (group E) diet, may indicate an increased turnover of phosphates and thus also the dephosphorylation rate of the products of proteolytic and lipolytic metabolism [Rüdiger *et al*. 1998].

A lower activity of EL and LL was observed both in the liver and kidneys of mice from group E than from group C. EL is an enzyme participating in the degradation of fatty acid esters in the cytoplasm as well as in the β-oxidation of fatty acids in the mitochondrial membrane [Kerner and Hoppel 2000, Weinberg 2006]. Lower activity of those enzymes as related to a reduced protein level in the diet, may be caused by a more efficient use of fats under protein deficiency [Jóźwik 2001, Jóźwik *et al*. 2003].

The increased activity of cathepsins D and L observed in the liver and kidneys, as well as of AlaAP in the kidneys of mice, may indicate a higher protein turnover resulting from the necessity of degradation of endogenous proteins [Yamamoto *et al*. 1998].

The results reported here indicate that enzymes of the lysosomal fraction, examined in the liver and kidneys of mice, respond dynamically to a reduced protein level in the diet, what reveals considerable possibilities of adaptation under protein deficiency.

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Wpływ żywienia dietami o zróżnicowanym poziomie białka na aktywność enzymów lizosomowych w wątrobie i nerkach myszy

S t r e s z c z e n i e

Badania przeprowadzono na 20 samcach myszy linii Swiss, utrzymywanych na paszy zawierającej 16% i 10% białka. Po upływie 14 dni żywienia od wszystkich zwierząt pobrano fragmenty wątroby i nerek, które poddano homogenizacji dla uzyskania frakcji lizosomowej, w której oznaczono aktywność fosfatazy kwaśnej, esterazy lizosomowej, lipazy lizosomowej, β-glukuronidazy, β-galaktozydazy, β-glukozydazy, β-N-acetylo-hexozaminidazy, aminopeptydazy leucynowej, aminopeptydazy alaninowej oraz katepsyn D i L. Uzyskane wyniki wskazują, że pasza o zmniejszonej do 10% zawartości białka wywierała w porównaniu z paszą kontrolną [16% białka] istotny, różnokierunkowy wpływ na aktywność badanych enzymów, zależny od rodzaju enzymu i rodzaju narządu.