

SHORT REPORT

The effect of selection for body weight on the activity of lysosomal enzymes in the liver and kidney of mice

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The study was conducted on 20 Swiss line male mice divided into two groups – control (n=10) comprised of unselected animals (group C), and experimental (n=10) comprised of mice selected over 24 generations for high live body weight gain (group S). Beginning from day 42 of life mice of both groups received over a period of 14 days a standard pelleted feed containing 16% protein. After 14 days the animals were killed, a lysosomal fraction of liver and kidney cells was obtained and analysed for the activity of acid phosphatase, lysosomal esterase, lysosomal lipase, β -glucuronidase, β -galactosidase, β -glucosidase, β -N-acetylo-hexosaminidase, leucine aminopeptidase, alanine aminopeptidase and cathepsins D and L. In S mice the activities of a majority of the lysosomal hydrolases examined were found to be higher than in C animals.

KEY WORDS: adaptation / mice / lysosomal enzymes / selection

Selection is one of the most powerful factors deciding, among much else, about progress in the breeding of domestic animals. However it cannot be effectively conducted if animal nutrition is incorrect, even if other conditions are at optimum [LeBlanc and Thibault 2003, Ortmann *et al.* 2003, Hegarty *et al.* 2007].

Studies on selection demonstrated that, compared to animals mated randomly, selected animals may respond differently to factors disrupting their internal environment [Kołątaj 1993, Wagener *et al.* 2006]. Selection conducted over a long period may lead to results not always favourable, both for the organism of the animal and for the breeder [Berryman *et al.* 2006]. Selected animals may prove to be less resistant to stimuli from the external environment and their reactions may be deeper and of a greater amplitude when compared to unselected controls. Moreover, they have more difficulties with adaptation to unfavourable environmental conditions [Cowley *et al.* 2004, Guderley *et al.* 2006].

In light of these data the present work aimed at determining whether a prolonged selection for increased body weight has a modifying effect on the activity of lysosomal enzymes in the liver and kidneys of experimental mice.

Material and methods

The study was conducted on male Swiss mice at the age of 8-10 weeks. The control group (C, n=10) comprised unselected animals (*i.e.* obtained from randomly mated parents), with a mean body weight of 24.0 ± 1.8 g. The experimental group (S, n=10) consisted of mice selected over 24 generations for high weight gain, having a mean body weight of 38.4 ± 1.9 g. Until day 42 of life the animals of both groups were maintained in cages together with their dams. After weaning they received for 14 days a pelleted feed containing 16% crude protein, had free access to water and were under veterinary control. Animals of both groups were maintained under standard conditions of mice farm in the Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, in a ventilated room at a temperature of 21-22°C, relative humidity of 50-60%, and a natural light cycle.

On day 56 of life the mice were decapitated and tissue samples were immediately taken from the liver and kidney, homogenized and centrifuged according to Beaufay [1972]. In lysosomal supernatants obtained from liver and kidneys the activity of 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 the method by Barrett and Heath [1972];
- 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 the method by Barrett [1972];
- leucine aminopeptidase (LeuAP, EC 3.4.11.1) and alanine aminopeptidase (AlaAP, EC 3.4.11.2) according to the method by McDonald and Barrett [1986] and cathepsins D and L (Cath. D and L, EC 3.4.23.5) according to Langner and Wiederanders [1984].

The activity of the enzymes examined was expressed in nmol/mg protein/hour. The results were subjected to a statistical evaluation according to the Student t-test.

The experiment was conducted at the Biology Institute, Department of Animal Physiology, Świętokrzyska Academy, Kielce.

Results and discussion

As shown in Table 1, in the liver of S mice an increased (compared to group C) activity of β -GlcUr, β -Gal, Hex, El, Cath. D and L, and LeuAP was observed as well as a decreased activity of β -Glu and AcP. In the kidney (Tab. 2) an increase was observed in the activity of all the enzymes tested (compared to group C), though for β -Glu and AcP it was not confirmed statistically.

Table 1. Activity of lysosomal enzymes (mmol/mg protein/h) in the liver of unselected vs selected mice

Enzyme	Unselected (C)		Selected (S)		S/C (%)
	mean	SD	mean	SD	
β -GlcUr	5.00	0.945	6.57**	1.01	131
β -Gal	2.77	0.488	5.08***	1.03	183
β -Glu	2.66	0.483	1.18***	0.16	44
Hex	1.95	0.243	13.27***	2.92	680
AcP	25.06	4.49	20.80*	3.17	83
EL	16.47	3.86	31.30***	6.20	190
LL	8.92	1.26	9.18 ns	1.42	103
Cath.D and L	0.260	0.036	0.36**	0.07	138
LeuAP	2.29	0.307	8.52***	1.52	372
AlaAP	7.35	0.915	6.52 ns	1.16	89

Differences between groups significant at: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
ns – difference not significant.

It is known that as result of selection one may obtain highly productive animals, but simultaneously, compared to unselected animals, such individuals as a rule have more difficulty in adapting to unfavourable environmental conditions [Kadokawa and Martin 2006; Rezende *et al.* 2006].

In the liver and kidney of S animals an increased activity was observed of β -GlcUr, β -Gal and Hex, *i.e.* enzymes which render possible the release of glycoprotein chains of carbohydrates in the form of glucose and galactose, which increases the glucose concentration in blood [Garcia-Pascual *et al.* 1992, Borelli *et al.* 1994]. The increased activity of β -GlcUr in the liver and kidney caused by selection may be related to the fact that β -GlcUr, as a precursor form, is subjected to numerous post-translative modifications [Weyel *et al.* 2000]. It may also indicate an increased rate of its biosynthesis. Enzyme β -Gal catalyses the hydrolysis of terminal β -glycoside bindings of lactose, glycoproteins and glycosaminoglycanes, and its increased activity

Table 2. Activity of lysosomal enzymes (mmol/mg protein/h) in the kidney of unselected vs selected mice

Enzyme	Unselected (C)		Selected (S)		S/C (%)
	mean	SD	mean	SD	
β -GlcUr	1.28	0.143	3.62***	0.642	283
β -Gal	5.40	1.09	8.25***	1.18	153
β -Glu	3.31	0.620	3.60 ns	0.540	109
Hex	4.51	0.771	14.61***	2.16	324
AcP	26.50	4.81	29.20 ns	5.17	110
EL	6.90	1.29	12.60***	2.69	183
LL	5.11	0.919	11.25***	1.26	220
Cath.D and L	0.24	0.036	0.770***	0.121	321
LeuAP	12.50	2.10	29.13***	5.87	233
AlaAP	22.60	4.82	68.60***	11.60	304

Differences between groups significant at: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
ns – difference not significant.

in the liver and kidney of S mice may be connected with the more intensive rate of its biosynthesis in the cells [Tulsiani *et al.* 1995].

β -Glu is an exo-glucosidase determining the rapid breakdown of low-molecular glycosides. Its increased activity was observed in the heart of fasted mice [Wildenthal 1976].

The increased activity of enzyme Hex in the liver and kidney of S mice was probably related to the increasing requirements of the cells for an intensified rate of degradation of the material stored in autophagous vacuoles [Lorini *et al.* 1995, Popko *et al.* 2005, Lee *et al.* 2006]. In turn, the decreased activity of AcP in the liver of S mice may indicate slower rate of phosphorylation processes, related to the increased protein absorption [Sharma *et al.* 2005]. The symptomatic increased EL activity in the liver and kidney and LL in the kidney of S mice was probably related to the accelerated degradation of certain glycerol esters. It was demonstrated that the activity of enzyme LL may also be significantly affected by the concentration of lipids and cholesterol in the blood plasma [Miszczuk-Jamska *et al.* 1993] as well as by the level of glucose [Maciejewski *et al.* 2001].

The increased activity of enzymes Cath. D and L in the liver and kidney of mice shows that animals subjected to selection, *i.e.* responding more readily, have an accelerated rate of protein metabolism. Herrero *et al.* [1997] demonstrated that in kidney of mice selected for increased body weight an accelerated binding of alanine, glutamic acid, glycine and phenylalanine occur.

The increased activity of LeuAP observed in the liver and kidney, and of AlaAP in the kidney of S mice was related to the intensified lysosomal proteolysis in those organs, compared to control (C) animals. Similar results were reported by Jóźwik *et al.* [2003] and Witek [2004].

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Wpływ selekcji w kierunku wzrostu masy ciała na aktywność enzymów lizosomowych w wątrobie wątrobie nerce myszy

Streszczenie

Badania przeprowadzono na 20 samcach myszy linii Swiss. Podzielono je na dwie grupy – kontrolną (n=10) obejmującą osobniki nieselekcjonowane oraz grupę doświadczalną (n=10), do której należały osobniki selekcjonowane w kierunku wysokich przyrostów masy ciała. Myszy obu grup przez okres 14 dni żywiono paszą o standardowej, tj. 16% zawartości białka. Po upływie 14 dni zwierzęta uśmiercano i w otrzymanej frakcji lizosomowej komórek wątroby i nerek oznaczano aktywność fosfatazy kwaśnej, esterazy lizosomowej, lipazy lizosomowej, b-glukuronidazy, b-galaktozydazy, b-glukozydazy, b-N-acetylohexozaminidazy, aminopeptydazy leucynowej, aminopeptydazy alaninowej oraz katepsyn D i L. Uzyskane wyniki wskazują, że zastosowana selekcja istotnie zwiększała aktywność większości badanych hydrolaz lizosomowych.

