

# Transgenic potato tubers with overexpression of 14-3-3 protein in growing rat diets. 1. Selected hormone activities and liver function status\*

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## ABSTRACT

In a 4-week experiment, growing rats were fed diets containing 30% transgenic potatoes with overexpression of P14-3-3 protein, non-transgenic dehydrated potato tubers of the same variety (Desiree), or a control diet. The activity of dehydroepiandrosterone (DHEA) as well as increases in the concentrations of NO<sub>3</sub><sup>-</sup> ions, Fe and Zn in the liver of rats fed diets containing transgenic potatoes were observed. The reaction might be related to oxidative stress linked to the altered chemical composition of potato tubers resulting from transgenesis, changed concentrations of some minerals or biologically active substances.

KEY WORDS: transgenic potato, rats, selected hormone activities, liver

## INTRODUCTION

Assessment using transgenic plants in human and animal diets requires many nutritional experiments, including evaluation of potential cytotoxic risk and mutagenic or immunosuppressive interactions. Modification of 14-3-3 protein, which is a regulator of numerous processes in plant cells modulating protein metabolism and the activity of key enzymes connected with N and C metabolism in plants, influences not only the content of nutrients in potato tubers of transgenic plants but can also change the content of biologically active substances (Wilczyński et al., 1998). Earlier experiments did not find any significant effects

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of adding potatoes with modified 14-3-3 protein to the diet on the growth of rats (Kosieradzka et al., 2004). The aim of the present study was the examination of the influence of genetically modified potatoes with overexpression of 14-3-3 protein on the level of selected hormones and indicators of liver functional status.

## MATERIAL AND METHODS

The experimental material consisted of potato tubers of *Solanum tuberosum* L. cv. Desiree with increased synthesis of protein 14-3-3 (derived from *Cucurbita pepo* cDNA) developed at the Department of Genetic Biochemistry of University of Wrocław. The transgenic potato variety contained more crude protein in DM of tubers than conventional varieties (16.2 vs 10.7 %), fat (0.6 vs 0.2%), Fe (130 vs 58 ppm) and less Zn (29 vs 34 ppm). Male rats from the outbred IF<sub>z</sub>Jaz herd with a mean body weight of 150 g were allotted to 3 groups, and were kept in individual cages. During the four-week experiment the rats received an isoproteinous diet containing 30% DM of steamed and dried (50°C) tubers of transgenic and non-transgenic cv. Desiree potatoes. The control group was fed the standard casein diet. All diets were supplemented with 3.5% mineral mixtures (AIN-93, ICN). At the end of the experiment the rats were fasted for 12 h and euthanized by ketamine overdose, blood was sampled from hearts, and livers were removed and frozen (-70°C). In liver tissue, Fe and Zn were determined by FAAS, NO<sub>3</sub><sup>-</sup> and NO<sub>2</sub><sup>-</sup> by HPLC (Waters). In blood serum, glucose, creatinine, cholesterol, HDL, triglycerides, urea N, total protein, asparagine transferase (AST) and alanine transferase (ALT) were determined using Vitros DT II kits (Johnson and Johnson, USA). Serum steroid hormone dehydroepiandrosterone (DHEA) was measured by RIA kits (DSL-9000 Active DHEA, Diagnostic Systems Laboratories, Inc., Webster, TX). Concentrations of plasma triiodothyronine, (T<sub>3</sub>), thyroxine (T<sub>4</sub>) were assayed by a chemico-luminescent method using DPC-Immulete kits (Immulete 2000 automated enzyme immunoassay analysers). The results were subjected to statistical analysis by single-factor analysis of variance ANOVA and Duncan's range test, using Statgraphic 4.1 Plus software package.

## RESULTS AND DISCUSSION

Oxidative stress caused by diet composition may result in increased thyroid hormone activity and lead to intensification of catabolism, thermogenesis and increase in tissue oxygen requirements. Giving diets containing 30% transgenic or non-transgenic potatoes caused a statistically significant increase in thyroxine (T<sub>4</sub>) levels. This thyroid hormone can induce liver oxidative stress leading to enhancement in lipid and protein oxidation and to nitration through peroxynitrite formation (Radi et al., 1995). The increase of T<sub>4</sub> activity was not accompanied by changes in the values

of biochemical parameters connected with lipid and protein metabolism. However, in the livers of rats fed diets with potatoes, a statistically significant increase in  $\text{NO}_3^-$  ions in comparison with the control group was found. Feeding transgenic potatoes in comparison with the non-transgenic ones increased  $\text{NO}_3^-$  ion levels in the liver (Table 1). The biological significance of the oxidative modification of proteins in liver induced by thyroid-hormone oxidative stress could be connected with loss of protein function and increased protein degradation (Tapia et al., 1999).

Table 1. Relative liver weight, concentration of nitrite and nitrate ions and Fe, Zn in liver tissue (fresh matter) biochemical parameters, thyroid hormones ( $T_3$ ,  $T_4$ ) and dehydroepiandrosteron (DHEA) in blood serum

	Control	Potato		SEM	p
		non-transgenic	transgenic		
<i>Liver</i>					
liver weight mg/100g BW	4.97	4.96	4.94	0.1528	0.0064
$\text{NO}_2$ mg/kg	< 0.07	<0.07	< 0.07	-	-
$\text{NO}_3$ mg/kg	1.79 <sup>A</sup>	2.40 <sup>B</sup>	3.44 <sup>C</sup>	0.0272	0.0001
Fe mg/kg	36.0 <sup>A</sup>	68.7 <sup>B</sup>	84.2 <sup>C</sup>	0.1164	0.0000
Zn mg/kg	27.4 <sup>A</sup>	27.0 <sup>A</sup>	28.0 <sup>B</sup>	0.0759	0.0000
<i>Serum</i>					
asparagine transferase U/L	192.8	138.0	147.0	34.7939	0.5076
alanine transferase U/L	44.5	44.5	43.4	4.2988	0.2975
cholesterol, mmol/L	1.82	1.79	1.69	0.0712	0.4540
HDL, mmol/L	1.07	0.96	0.97	0.0438	0.2171
triglycerides, mmol/L	1.72	1.82	1.25	0.2294	0.2100
urea N mmol/L	3.36	3.58	3.92	0.1802	0.1280
creatinine, $\mu\text{mol/L}$	30.94	33.15	31.82	1.9601	0.7307
$T_3$ , $\mu\text{g/dL}$	149.5	159.25	149.25	6.7464	0.5152
$T_4$ , $\mu\text{g/mL}$	58.18 <sup>A</sup>	73.95 <sup>B</sup>	75.75 <sup>B</sup>	4.4691	0.0404
DHEA, $\mu\text{g/mL}$	1.40 <sup>A</sup>	1.30 <sup>A</sup>	1.68 <sup>C</sup>	0.0276	0.0000

<sup>A,B</sup> means with different superscripts are significantly different ( $P < 0.01$ )

There were no statistically significant differences in asparagine and alanine transferase activities in the blood serum of rats fed the experimental and control diets. Increased intensity of lipid degradation in liver cells was accompanied by increased concentrations of Fe and Zn (Table 1). The increased accumulation of essential metals (Fe, Zn) and nitrate ions in liver tissue may be associated with liver tissue damage (Chen et al., 1998). Iron may act as a catalyst for the Fenton reaction and play a role in initiating the lipid peroxidation of this organ. In the blood of rats fed diets with transgenic potatoes, the concentration of DHEA was higher than in other groups, which could be a symptom of earlier onset of puberty of the males in this group (Table 1). Precise evaluation of the reasons for degradation of liver tissue and hormone activity requires further study of

the concentration of biologically active substances in tubers of both transgenic and non-transgenic potato forms (glycoalkaloids, polyphenolics, etc.) and also carrying out long-term experiments on the use of pure substances isolated from the tubers in synthetic diets.

## CONCLUSIONS

Giving growing rats diets containing 30% dehydrated potatoes with overexpression of protein 14-3-3 caused a change in the activity of DHEA, increased the concentration of  $\text{NO}_3^-$  ions and Fe and Zn in liver in comparison with the tissues of rats fed diets with non-transgenic potatoes. The reaction might be related to changed concentrations of some minerals in the diets and oxidative stress linked to the altered chemical composition of potato tubers resulting from transgenesis.

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## STRESZCZENIE

### **Transgeniczne ziemniaki z nadekspresją białka 14-3-3 w diecie dla rosnących szczurów. 1. Aktywność wybranych hormonów i stan funkcjonalny wątroby**

W 4-tygodniowym doświadczeniu rosnącym szczurom podawano izobiałkowe diety z 30% udziałem suszonych bulw ziemniaków transgenicznych z nadekspresją białka 14-3-3 lub nietransgenicznych odmiany Desiree oraz standardową dietę kontrolną. Stwierdzono zmianę aktywności dehydroepiandrosteronu (DHEA), zwiększenie koncentracji jonów  $\text{NO}_3^-$ , oraz Fe i Zn w tkance wątroby szczurów otrzymujących diety z ziemniakami transgenicznymi. Reakcja organizmu może mieć związek ze stresem oksydacyjnym wywołanym zmienionym, w wyniku transgenezy, składem ziemniaków, zróżnicowaną zawartością niektórych składników mineralnych lub substancji biologicznie czynnych.