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The Influence of Maca (*Lepidium meyenii*) on Antioxidant Status, Lipid and Glucose Metabolism in Rat

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Abstract This work focused on the effect of maca on lipid, anti-oxidative, and glucose parameters in hereditary hypertriglyceridemic (HHTg) rat. Maca (1%) was administred to rats as a part of a high-sucrose diet (HSD) for 2 weeks. Rosiglitazone (0.02%) was used as a positive control. Maca significantly decreased the levels of VLDL (very low density lipoproteins), LDL (low density lipoproteins), and total cholesterol, and also the level of TAG (triacylglycerols) in the plasma, VLDL, and liver. Maca, as well as rosiglitazone, significantly improved glucose tolerance, as the decrease of AUC (area under the curve) of glucose showed, and lowered levels of glucose in blood. The activity of SOD (superoxide dismutase) in the liver, the GPX (glutathione peroxidase) in the blood, and the level of GSH (glutathione) in liver increased in all cases significantly. Results demonstrate that maca seems to be promising for a positive influence on chronic human diseases (characterized by atherogenous lipoprotein profile, aggravated antioxidative status, and impaired glucose tolerance), and their prevention.

Key words: Cholesterol, Dietary supplement, Lipoproteins, Maca, Triacylglycerols

Introduction

Oxidative stress, increased lipid levels, and disturbances in glucose metabolism are important risk factors for diabetes, cardiovascular, oncologic and many other diseases. Diet undoubtedly plays a key role and optimalizing the diet in both quality and quantity, has a preventative function. Fruit and vegetables are an invaluable source of many biologically active substances, including antioxidants. For this reason a diet rich in fruit and vegetables has a positive effect on reducing the incidence of these serious lifestyle diseases [1].

Maca (*Lepidium meyenii*, Walp., Brassicaceae family; sometimes considered synonymous with *L. Peruvianum* Chacón) is widely dispersed on high plateaus (altitudes between 4000 and 4500 m above the sea level) of the Andean mountain range in Peru. The underground part of the plant, a pear-shaped tuberous root, is the main product of maca and evidence for its cultivation has been found dating back as far as 1600 B.C. Lepidium is a genus of a plant which includes about 175 species found worldwide, but cultivated *Lepidium meyenii* is the only species in the entire genus that produces a tuberous root [2]. These roots are used for production of salads, jams, bread, coffee substitutes, and even beer [3]. The use of maca is recommended for malabsorption syndrome, ethylism, as a laxative [4], and during reconvalescence [5], owing to its excellent nutritional characteristics [6]. Maca is also known for its supportive effect on fertility and increase in libido [7]. It contains a range of substances, such as fatty acids, amino acids, many microelements, tannins, saponins, alkaloids (macaines), etc. It reduces plasma glucose levels and free fatty acids [8], and it also has anti-oxidative activity (most likely due to aromatic isothiocyanates) [3, 9].

This study focused on the effect of maca on lipid, glucose, and anti-oxidative parameters in the laboratory rat. The study model was rats with hereditary hypertriglyceridemia (HHTg), which suffer from the metabolic syndrome (high levels of plasmatic triacylglycerols, increased oxidative stress, insulin resistance, hyperinsulinemia, hypertension). The symptoms are intensified by feeding them a high-sucrose diet [10]. These rats are an appropriate model [10] for studies of the hypolipidemic, anti-oxidative, and hypoglycemic effects of maca.

Material and Methods

Plant Material and Chemicals

For the preparation of the experimental diets we used a commercial dehydrated powdered Maca andina naturalia (Quimica Suiza, Peru), rosiglitazone (SmithKline Beecham, UK), Bio-La-Test Cholesterol 250 E and Bio-La-Test Triacylglycerol T 500 (Kits for lipids analysis, PLIVA-Lachema, Czech Republic), and Standard laboratory feed (KrmiMo Mohelsky, Czech Republic). Other chemicals were of analytical grade.

Animals and Diets

HHTg rats were bred from Wistar rats on the basis of a high-sucrose diet-induced increase in plasma triacylglycerols,

at the Institute for Clinical and Experimental Medicine (Prague, Czech Republic). Rats (males, body weight 340–360 g) were kept in standard laboratory conditions with free access to water and feed. They were fed for 2 weeks *ad libitum* on the following diets: 1) HSD – high-sucrose diet (standard laboratory diet, 70 cal% sucrose = 70 % of energy as sucrose), 2) HSD enhanced with 1% (w/w) of maca, or 3) HSD enhanced with 0.02% (w/w) of rosiglitazone (non-sulfonamid peroral antidiabetic drug, insulin sensitizer). Other dietary components were the same (casein, dry milk, dry yeast powder, mineral and vitamin mix), proteins 20 cal%, fat 10 cal%, and alpha-tocopherol 10 mg% (w/w).

After two weeks of feeding, the non-fasted rats were anaesthetised by intramuscular administration of fentanyl (40 μg kg^{-1} of body weight) in combination with medetomidin (200 μg kg^{-1} of body weight), followed by an intramuscular administration of diazepam (5 mg kg^{-1} of body weight). Blood was taken from the aortic bifurcation and divided into two aliquots. The first aliquot of blood was taken into Na_2EDTA (1 mg ml^{-1}) and NaN_3 (0.1 mg ml^{-1}); plasma was separated by centrifugation at 2500 $\times$ *g* for 20 min (10 °C) and used for isolation of plasma lipoproteins (VLDL, LDL and HDL). An aliquot of plasma was frozen for determination of cholesterol and triacylglycerols (TAG). The second aliquot of the blood (300 μl) was hemolysed by ice-cold purified water (2.7 ml). The liver was removed and rinsed in ice-cold saline, weighed and divided into two portions. One piece of liver was frozen for analysis of lipid content, the other liver sample was immediately homogenised (10% w/v) with ice-cold Tris-HCl buffer (25 mmol l^{-1}). Homogenates were then centrifuged (4000 $\times$ *g*, 10 min, 4 °C). The homogenous supernatants and blood hemolysates were stored at − 20 °C for determination of enzyme activities (superoxide dismutase – SOD, glutathione peroxidase – GPX, catalase – CAT), levels of glutathione (GSH), thiobarbituric acid reactive substances (TBARS), and conjugated dienes (CD). One piece of the gluteal muscle was removed and rinsed in ice-cold saline, weighed, and frozen for analysis of lipid content (cholesterol and TAG). After 12 h of fasting, glucose (3 g kg^{-1} of body weight) was intragastrically administered for the oral glucose tolerance test (oGTT). Blood samples were taken from the tail vein at time intervals 0 (immediately) 30, 60, and 120 min after glucose application). The area under the curve ($AUC_{0\rightarrow\infty}$) of glucose was calculated using the linear trapezoidal method.

All procedures with animals were approved by the Ethics Committee, Ministry of Education, Czech Republic. Each experimental group of rats consisted of 6 animals. Rats were fed their respective diets *ad libitum* and the quantity consumed was weighed daily per cage of two rats.

The Analytical Methods

Plasma lipoproteins (VLDL, LDL and HDL) were isolated by sequential density gradient ultracentrifugation [11]. Liver lipids were extracted according to Haugh & Hostmark [12]. Cholesterol and TAG were measured enzymatically using Bio-La-Tests. The activity of SOD was determined spectrophotometrically using *p*-tetrazolin nitro-blue [13]. The activity of catalase was measured spectrophotometrically by ammonium molybdate reaction with H_2O_2 [14]. The activity of GPX [15] and content of GSH [16] were analysed spectrophotometrically using Ellman's reagent. The level of CD was determined spectrophotometrically after extraction into *n*-heptane [17]. Thiobarbituric acid reactive substances (TBARS) were measured according to Naito et al. [18].

Statistical Analysis

Results are given as means ± S.E., $n = 6$. Intergroup differences were evaluated using ANOVA and Student's *t*-test (Microsoft Office Excel 2003).

Results and Discussion

HHTg rats are characterized by hypertriglyceridemia, peripheral insulin resistance, hyperinsulinemia, hypertension and oxidative stress [10]. This condition—so called "metabolic syndrome"—is an important risk factor for type II diabetes mellitus and other cardiovascular complications [19]. When these rats are fed a high-sucrose diet, the metabolic defects are strongly aggravated, including the appearence of hypertriglyceridemia and signs of oxidative stress [10]. Two weeks of feeding with maca as a dietary supplement (1% of HSD) led to a significant decrease in plasma cholesterol in the lipid spectrum parameters, and to a positive effect on the lipoprotein profile (significant decrease of VLDL and LDL cholesterol), as demonstrated in Table 1. Maca significantly reduced the level of total plasma TAG and VLDL-TAG, and the content of TAG in the liver. The TAG level in muscle tissue was not significantly influenced by maca (Table 1). Decrease in $AUC_{0\rightarrow\infty}$ (Area under the curve) of glucose demonstrates that maca significantly improved the glucose tolerance after 3 g of glucose load per kg of body weight; maca also significantly decreased blood glucose levels (Table 1). The oral antidiabetic drug rosiglitazone, which reduces insulin resistance, had a similar effect (Table 1). This drug has also a positive effect on TAG and cholesterol levels in plasma and in the liver [20], apart from its antidiabetic properties. In our study, rosiglitazone significantly decreased VLDL cholesterol and slightly increased HDL cholesterol, as shown in Table 1. Triacylglycerols in plasma, VLDL, and also in the

Table 1. Effect of high-sucrose diet supplemented with maca on the cholesterol, triacylglycerols in various organs and basic glycemia

	HSD	HSD + Maca (1%)	HSD + Rosiglitazone (0.02%)
Cholesterol			
Plasma [mmol l^{-1}]	1.371 ± 0.056	1.07 ± 0.060***	1.21 ± 0.042
VLDL [mmol l^{-1}]	0.502 ± 0.035	0.373 ± 0.034*	0.247 ± 0.016†
LDL [mmol l^{-1}]	0.119 ± 0.008	0.091 ± 0.007*	0.118 ± 0.007
HDL [mmol l^{-1}]	0.453 ± 0.032	0.426 ± 0.030	0.551 ± 0.029
Triacylglycerols			
Plasma [mmol l^{-1}]	5.443 ± 0.313	3.655 ± 0.362***	2.62 ± 0.151†
VLDL [mmol l^{-1}]	4.259 ± 0.326	2.588 ± 0.179†	1.744 ± 0.132†
Liver [μmol g^{-1}]	18.643 ± 0.741	16.299 ± 0.662*	12.759 ± 0.663†
Muscle [μmol g^{-1}]	3.62 ± 0.263	3.353 ± 0.269	4.258 ± 0.314
Glucose parameters:			
Basal glycemia [mmol l^{-1}]	4.782 ± 0.427	3.436 ± 0.177**	3.076 ± 0.153***
$AUC_{0\to\infty}$ [mmol l^{-1} min]	662 ± 21	554 ± 27**	585 ± 11**

Note. HSD: High-sucrose diet, AUC: Area under the curve.
Values are means ± S.E., $n = 6$; $^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{\dagger}p < 0.001$ *vs* HSD.

liver decreased significantly after rosiglitazone administration (Table 1).

Markers of lipid peroxidation (TBARS and CD) in the blood and liver were not significantly affected by maca administration (Table 2). The glutathione system responded by a significant increase in GPX acivity in blood and by a higher level of reduced GSH in the liver after maca administration (Table 2). The SOD activity in the liver was significantly higher after the maca diet (Table 2). These results suggest that maca may improve the efficiency of superoxide radical conversion to hydrogen peroxide due to the increased SOD activity, and following deactivation of hydrogen peroxide by the glutathione system [21]. The activity of catalase was not changed either in blood or in the liver (Table 2). Rosiglitazone also exhibits antioxidant properties by stimulating catalase activity [22], reducing H_2O_2 production [23] and LDL cholesterol oxidation [24]. Rosiglitazone administration led to an increase in TBARS and CD levels in the liver (Table 2), but on the other hand the SOD activity and the level of reduced GSH increased

Table 2. Effect of high-sucrose diet supplemented with maca on the TBARS and conjugated dienes and some key enzymes in the blood and liver

	HSD	HSD + Maca (1%)	HSD + Rosiglitazone (0.02%)
TBARS [μmol mg^{-1} of protein]			
Blood	1.731 ± 0.117	1.808 ± 0.062	1.639 ± 0.054
Liver	2.353 ± 0.104	2.624 ± 0.165	2.955 ± 0.216*
CD [nmol mg^{-1} of protein]			
Blood	72.24 ± 4.93	77.30 ± 3.96	63.72 ± 2.73
Liver	71.22 ± 5.92	82.89 ± 7.99	99.48 ± 4.96***
GPx [μmol(GSH) min^{-1} mg^{-1} of protein]			
Blood	258 ± 22.22	343 ± 15.21***	461 ± 30.38†
Liver	576 ± 35.50	657 ± 57.59	467 ± 44.37
GSH [μmol mg^{-1} of protein]			
Blood	8.92 ± 0.753	9.96 ± 0.317	13.33 ± 0.738***
Liver	10.74 ± 0.528	19.13 ± 1.292†	20.06 ± 0.911†
SOD [U I mg^{-1} of protein]			
Liver	0.354 ± 0.013	0.461 ± 0.023***	0.558 ± 0.046***
CAT [μmol(H_2O_2) min^{-1} mg^{-1} of protein]			
Blood	400 ± 34.83	442 ± 21.97	606 ± 41.42***
Liver	531 ± 32.25	536 ± 31.11	627 ± 42.77

Note. TBARS: Thiobarbituric acid reactive substances, CD: conjugated dienes, GPx: Glutathione peroxidase, GSH: Glutathione, SOD: Superoxide dismutase, CAT: Catalase.
Values are means ± S.E., $n = 6$; $^{*}p < 0.05$, $^{**}p < 0.02$, $^{***}p < 0.01$, $^{\dagger}p < 0.001$ *vs* HSD.

significantly in this organ (Table 2). The activities of GPX, catalase and GSH level were increased significantly in blood after rosiglitazone administration (Table 2).

The results show a number of positive effects of maca on rats with hereditary induced disturbances in lipoprotein profile, antioxidant status, and glucose tolerance. The hypotriglyceridemic effects were accompanied by a decrease in TAG content in the liver, reduced plasma cholesterol, and changes in distribution of cholesterol in lipoproteins (significant decrease in VLDL cholesterol and TAG, reduced atherogenous LDL cholesterol). This positive effect of maca on atherogenous lipoprotein profile is especially promising in the light of preventing lifestyle diseases of the cardiovascular system [25]. Maca lowered blood glucose levels and also improved glucose tolerance. Its antioxidative effects were demonstrated by means of antioxidant status markers—reduced GSH, GPX and SOD. However, we cannot say which compounds are responsible for the abovementioned effects, including its cytoprotective [26] and other effects. There are a number of substances contained in the tuberous roots, such as fatty acids (linoleic, palmitic and oleic acid mainly) [27], amino acids (lysine and arginine), many microelements (Cu, Sn, Mn, Al, etc.), and also tanins and saponins. An important component of maca is a mixture of alkaloids known as macaines 1, 2, 3 and 4 [5]. Apparently, this group of structurally undefined substances represents the main component of *Lepidium meyenii* [7]. Other substances should be also considered, such as alkamides (macamides), including alcamide 1 to 5, which have been discovered recently [28]. Some authors suggest that active substances are not just prostaglandins and sterols, but also aromatic isothiocyanates, such as benzyl-isothiocynate or p-methoxy-benzyl-isothiocyanate [3]. The antioxidative activity of maca is linked to these substances [9]. There are a number of other components - macaridine [29], lepidiline A, lepidiline B [30], etc., which are important for the way maca influences the living organism, and which still remain unknown.

The results suggest that maca could be used, as a dietary supplement, in the treatment of chronic deseases characterized by atherogenous lipoprotein profile, liver steatosis, aggravated antioxidant status and impaired glucose tolerance and also in their prevention.

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