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Short communications

Transgenic flax overexpressing polyphenols as a potential anti-inflammatory dietary agent ☆

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ABSTRACT

The influence of chronic administration of seed cake of genetically modified flax W92/72, which overexpressed polyphenols, on diet-evoked inflammation in the liver and other physiological disturbances was examined. High-fat-diet-induced rat obesity models were administered *ad libitum* the experimental diets (standard, high-fat with lard, and standard and high-fat diets enriched with 30% seed cake of non-transgenic or transgenic flax) for 14 weeks. The beneficial effects of transgenic seed cake were related to carbohydrate metabolism, serum total antioxidant capacity and lipid peroxidation. The levels of the liver pro-inflammatory cytokines tumor necrosis factor-α and interleukin-6 were decreased in the transgenic and non-transgenic seed cake groups. Transgenic seed cake consumption elevated the anti-inflammatory cytokines interleukin-4 and -10. The flaxseed cake groups had improved liver ultra-structure. Diet supplementation with genetically modified flax W92/72 seed cake may contribute to solving health problems resulting from high-energy diet consumption.

1. Introduction

Food fortification is the process of the addition of essential nutrients and health-promoting compounds (Espin, Garcia-Conesa, & Tomás-Barberán, 2007). Biofortification involves crop fortification at the source to synthesize and accumulate these compounds (Gómez-Galera et al., 2010). Plants are able to be biofortified by modulating endogenous metabolic pathways, which increase the production of flavonoids (Wang, Chen, & Yu, 2011).

An interesting subject of biofortification using genetic engineering methods is flax (*Linum usitatissimum*). It has resulted in the overexpression of polyphenolic antioxidants:

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flavonoids, anthocyanins, phenolic acids and lignans in seeds which increased the protection against oxidation of the unsaturated fatty acids (UFA) (Lorenc-Kukuła et al., 2005; Żuk et al., 2011). Even after cold extraction of oil polyphenols remain associated with the flaxseed cake.

Numerous studies have demonstrated that polyphenols are protective against pathologies caused by oxidative stress, such as metabolic disorders. Flavonoids and phenolic acids have demonstrated antioxidant and anti-inflammatory activities (Coppin et al., 2013; González et al., 2011; Martin et al., 2014; Moon, Yang, & Park, 2006; Soobrattee, Neergheen, Luximon-Ramma, Aruoma, & Bahorun, 2005; Wang et al., 2014). Flaxseeds are the richest dietary source of lignans; they contain secoisolariciresinol and trace quantities of matairesinol (Adlercreutz, 2007) which, during consumption, are converted to enterodiol and enterolactone. They have antioxidative and estrogen-like activities, which may lower chronic disease risks, including the risk for obesity (Adlercreutz, 2007; Prasad, 2000).

A potentially useful target for polyphenol treatment is obesity, energy imbalance, a state of low-grade, chronic inflammation that promotes the development of insulin resistance and diabetes (Johnson, Milner, & Makowski, 2012). Inflammation is a common cause of liver disease (Berasain et al., 2009; Park et al., 2010). Innate immune system liver cells initiate and maintain liver inflammation through cytokine production (Liaskou, Wilson, & Oo, 2012).

The objective of this study was to investigate the influence of chronic administration of seed cake of genetically modified (GM) flax with overexpressed polyphenols on the disturbances caused by the consumption of a high-fat diet, i.e., alterations in redox homeostasis, glucose and insulin concentrations, inflammatory state and the liver ultra-structure. To obtain the seed cake, oil was cold-pressed from flaxseeds (Żuk et al., 2011). The experimental dose of flaxseed cake was determined to not cause nutritional imbalances or metabolic disturbances in the experimental animals – rats (EFSA (Panel on Genetically Modified Organisms, GMO), 2011; EFSA (Scientific Committee), 2011).

2. Materials and methods

2.1. Plant material and transgenesis

Non-GM flax (*Linum usitatissimum* L. cv. Linola) seeds were obtained from the Flax and Hemp Collection of the Institute of Natural Fibers and Medicinal Plants (Poznan, Poland). The GM W92/72 line was received from the Department of Genetic Biochemistry of the University of Wroclaw (Wroclaw, Poland). The GM flax simultaneously overexpresses *Petunia hybrida* major enzymes of the flavonoid biosynthesis pathway: chalcone synthase (CHS), chalcone isomerase (CHI) and dihydroflavonol reductase (DFR). The expression of these enzymes improved antioxidant capacity of the seed extract which resulted in compositional changes for fatty acids and an increase in the production of quercetin and kaempferol derivatives, anthocyanins, phenolic acids (caffeic, ferulic, *p*-coumaric) and secoisolariciresinol diglucoside (SDG), the most important lignan component. The antioxidant capacity and bioactive compound concentrations were also increased in the transgenic flaxseed cake extracts. The W92/72 flax production procedures and compositional details have previously been described (Lorenc-Kukuła et al., 2005; Żuk et al., 2011). The GM and non-GM plants were grown in a field in the vicinity of Wroclaw (trial No. 26, AM-13; Polish Environment Ministry No. 36/2011 decision, dated September 29th, 2011).

2.2. Nutritional experiment and animal material preparation

An experimental model of obesity was used to determine the effect of a transgenic flaxseed cake diet on the development of diet-induced liver inflammation. Male Wistar–Crl:WI(Han) rats (The Center for Experimental Medicine, The Medical University of Bialystok, Poland) were fed a high-fat diet that was rich in pork lard (701 kcal GE/100 g, 456 kcal GE from crude fat/100 g) for 14 weeks, from the age of 5 weeks. The rats were maintained in individual growth cages (21 °C, 12 h/12 h, 40% humidity). For 14-week nutritional research the animals were divided into six groups (n = 6) with standardized body weights (bw) and were administered *ad libitum*: (1) standard diet (SD), (2) high-fat diet rich in lard (HFD), standard diet supplemented with 30% seed cake of either (3) the non-transgenic flax Linola (S Linola) or (4) the transgenic flax W92/72 (S W92), and high-fat diet supplemented with the same amount of seed cake of either (5) the non-transgenic flax (HF Linola) and (6) the transgenic flax W92/72 (HF W92) (Table 1). The diets were fabricated in the 'Morawski' Feed Production Plant (Kcynia, Poland). The standard diets met animal nutritional requirements; the high-fat diets substantially exceeded these requirements (NRC, 1996). The diet compositions and their nutritional values were determined according to the Association of Official Analytical Chemists (AOAC, 1996) procedures. The rats had free access to water. The body weight of animals and food intake were monitored weekly. After 12 hours of fasting, the rats were euthanized using an isoflurane (Aerrane, Baxter, Deerfield, IL, USA) overdose. Blood samples were collected from the heart in serum separating tubes or tubes containing lithium heparin for plasma separation. After blood clotting, the samples were centrifuged (10 min., 1006 ×*g*) and the serum was aliquoted and stored at –25 °C until further use. The erythrocytes were divided from the plasma using centrifugation (10 min., 1006 ×*g*, 4 °C) and were washed four times with 0.9% NaCl solution and stored at –25 °C. Liver tissue samples (right lobe) were stored at –80 °C, put into 4% formalin for histological analysis or into 2.5% glutaraldehyde in phosphate buffer pH 7.2 for transmission electron microscopic (TEM) observations.

The experimental procedures were approved by the local ethics committee [Resolution No. 65/2010 of the III Local ethics committee on animal experiments in Warsaw, dated October 27th, 2010].

2.3. Serum glucose and insulin and a homeostatic model assessment of insulin resistance

Serum glucose was determined using a photometric method on a Miura One analyzer (I.S.E. S.r.l., Rome, Italy). The serum insulin was determined using an enzyme-linked immunosorbent assay (ELISA, Wuhan EiAab Science Co., Ltd,