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Basic nutritional investigation

Caffeoylquinic acid-rich extract from chicory seeds improves glycemia, atherogenic index, and antioxidant status in rats

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ABSTRACT

Objective: Comparison of the effects of a high-fructose diet supplemented with rutin, a phenolic compound with well-recognized bioavailability and bioactivity, and a chicory (Cichorium intybus L.) seed extract rich in caffeoylquinic acids (CQA) on gut physiology and the development of disorders related to metabolic syndrome.
Methods: A 28-d experiment was conducted on 32 young male Wistar rats. In comparison with control rats fed a standard corn starch diet (group C), the experimental group (group E) was fed a diet with an increased content of cholesterol and fructose (to 1% and 66% of the diet, respectively), as well as with oxidized soybean oil. Rats from the other two experimental groups were administered the same diet as group E during the first 2 wk of feeding, whereas at the beginning of the last 2 wk, the diet was enriched with rutin (group ER) or the CQA-rich ethanol extract from chicory seeds (9.6% of CQA, group EC), so the amount of added phenolics was equal in both dietary groups (0.15%).
Results: The diet administered in group E caused hyperglycemia and increased blood serum atherogenicity in rats, but did not induce other manifestations of the metabolic syndrome, i.e., dyslipidemia and oxidative stress. Additionally, it affected gut physiology through increasing mucosal sucrase activity and disturbing fermentative processes in the cecum, such as the production of short-chain fatty acids and the activity of microbial enzymes. Similarly to rutin, the dietary addition of the chicory seed extract improved glycemia, which was comparable to that determined in group C. In addition, the extract was found to decrease the atherogenic index to the level observed in group C and to increase blood antioxidant status. Both dietary supplements reduced the content of thiobarbituric acid-reactive substances in kidney and heart tissue when compared with group E.
Conclusion: The potential efficacy of the CQA-rich extract from chicory seeds in improving diet-induced metabolic disturbances proved to be better than that of rutin; thus, the extract might be considered as a dietary supplement for carrying out clinical trials.
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Introduction

Metabolic syndrome (MS) is a widespread diet-related disorder, defined as a cluster of interrelated risk factors for cardiovascular disease and type 2 diabetes. According to the new standardized criteria for clinical diagnosis of MS, these factors include central obesity, raised blood pressure, low concentration of high-density lipoprotein (HDL) cholesterol, as well as elevated glycemia and triglyceridemia [1]. Moreover, there is convincing evidence that oxidative stress is also involved in the development and progression of MS [2]. Most of the aforementioned manifestations can be induced experimentally in rats, in a relatively short time, by a high-fructose diet. However, the range of metabolic disturbances in response to excess fructose ingestion seems to be complex and may depend on many factors, such as age of the animals, as well as amounts of fructose and other components in the diet [3–5].

Prevention and treatment of MS are crucial for public health. In recent years, much research has been focused on dietary flavonoids, the antioxidants that can potentially improve

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disorders clustered in MS. In this context, one of the most investigated and relatively well-recognized flavonoids is quercetin and its glycosides, in particular, rutin (quercetin 3-O-glucorhamnoside) [6]. In experiments on rats, rutin administered orally or intraperitoneally improved glycemia, lipidemia, and antioxidant status [7–9], as well as ameliorated obesity induced by a high-fat diet [10]. Interestingly, the bioavailability of rutin after oral administration is low and it is mainly metabolized by large intestinal bacteria, among others to the aglycone form and phenolic acids [11,12]. Apart from flavonoids, there are also many other plant phenols exhibiting a vast array of biological activities. These include esters of caffeic and quinic acids, whose activity against disorders of MS has not been studied extensively thus far, to our knowledge, considering especially caffeoylquinic acids (CQA) with more than one caffeic acid residue. The most known and recognized CQA is a monocaffeoyl derivative chlorogenic acid (5-CQA), which occurs in many plants, fruits, and vegetables, such as coffee beans, apples, tomatoes, etc. [13]. For instance, chlorogenic acid has been shown to exhibit antiobesity properties and improve lipid metabolism in mice [14]. Moreover, some authors have observed delayed glucose absorption, suggesting its shift to more distal parts of the intestine, after the ingestion of chlorogenic acid-rich decaffeinated coffee [15]. However, similarly to rutin, CQA are also low-absorbable in the upper part of the gastrointestinal tract and most probably reach the large intestine in quantities [13,16].

In our laboratory, CQA have been recently isolated from different parts of chicory (*Cichorium intybus* L.) [17], which is a biennial plant with many applications in the food industry, for instance, it is used as an additive to coffee (roasted roots) and salads (young leaves) or as a source of prebiotic fructans (roots).

The aim of this study was to compare the effects of a high-fructose diet supplemented with rutin, a phenolic compound with relatively well-recognized bioavailability and bioactivity, and a CQA-rich extract from chicory seeds on gut physiology and the development of disorders in rats related to MS. To enhance the metabolic disturbances, a high-fructose diet was additionally enriched with oxidized fat and cholesterol. We speculated that, like rutin, the multiple ethanol extract from chicory seeds can reduce adverse effects of such a diet.

Materials and methods

Dietary supplements

Seeds of chicory (*C. intybus* L.) were provided by Cykoria Co. (Wierzchosławice, Poland), a company specializing in the cultivation and processing of the plant. After drying at <70°C, the seeds were ground and then extracted four times with 75% ethanol (1:3 sample-to-solvent ratio). The extraction procedure was performed in a hermetic container without access to light. Afterwards, the ethanol was removed by vacuum distillation and the extract was then freeze-dried for 24 h beginning at −30°C, followed by an additional drying at 40°C for 2 h. Determination of the antioxidant activity of the extract was performed using 2,2-diphenyl-1-picrylhydrazyl radical according to the modified method of Brand-Wiliams et al. [18], and the results were expressed as nanomoles of Trolox equivalent per gram of sample. Dry matter, ash, protein, and fat contents in the extract were assayed according to the Association of Analytical Communities (AOAC) official methods, whereas the determination of saccharides and phenolic compounds was conducted with high-performance liquid chromatography methods. Dicaffeoylquinic acid (diCQA) standard was isolated by semipreparative high-performance liquid chromatography and confirmed by mass spectrometry. CQA were quantified as grams of chlorogenic acid (5-caffeoylquinic acid; Sigma, St. Louis, MO, USA) equivalents per 100 g fresh mass. The procedure of extraction, as well as the analysis of the antioxidant activity and chemical composition of the extract tested, with detailed description of the methods applied, was described previously [17]. Table 1 presents chemical composition and antioxidant activity of the extract.

Rutin (quercetin 3-O-glucorhamnoside, trihydrate, purity 95%) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 1
Chemical composition and antioxidant activity of the ethanol extract from chicory seeds

	g/100 g fresh mass
Dry matter	95.0 ± 0.20
Ash	8.4 ± 0.05
Protein	10.7 ± 0.3
Fat	14.4 ± 0.2
Mono- and disaccharides	34.9 ± 0.3
Glucose	12.1 ± 0.1
Fructose	21.4 ± 0.2
Sucrose	1.4 ± 0.02
Inulin	1.7 ± 0.04
Phenolic compounds	9.6 ± 0.03
MonoCQA	2.8 ± 0.01
DiCQA	6.8 ± 0.02
Antioxidant activity (nmol/g)	505.1 ± 6.02

CQA, caffeoylquinic acids
Data are expressed as mean values ± SD ($n = 3$)

Animals and diets

The animal protocol used in this study was approved by the Local Animal Care and Use Committee (permission no. 23/2008). The experiment was conducted on 32 young male Wistar rats, distributed into four groups of eight animals each. All animals were housed individually in standard conditions with free access to water and semipurified casein diets (Table 2). Control rats (group C) received for 28 d a diet containing among others cholesterol (0.5%), soybean oil (8%), fructose (5%), and corn starch (66.5%). In comparison with group C, rats from the experimental group (group E) were fed for 28 d a diet with increased amounts of cholesterol and fructose (to 1% and 66% of the diet, respectively), added at the expense of cornstarch, as well as with oxidized soybean oil added instead of fresh. Rats from the other two experimental groups (ER and EC) were administered the same diet as in group E for the first 2 wk of feeding, whereas at the beginning of the last 2 wk the diet was enriched with rutin (group ER) or a chicory seed extract (group EC), added at the expense of cornstarch. In all experimental groups, the diets had comparable sums of mono- and disaccharides, whereas in the ER and EC groups the diets had additionally the same amount of phenolic compounds.

Oxidized soybean oil was obtained by intensive aeration while heating at 70-80°C for 72 h. The degree of oxidation was monitored daily by determining the peroxide value according to the AOAC official method. The final degree of oxidation amounted to 120 miliequivalents of O_2 per kg of soybean oil.

Sample collection and analysis

On termination of the experiment, rats were anaesthetized with sodium pentobarbital according to the recommendations for euthanasia of experimental animals. After laparotomy, blood samples were taken from caudal vein; then gut

Table 2
Composition of the diets fed to rats*

Ingredient (g/100 g diet)	Group			
	C	E	ER	EC
Casein	14.6	14.6	14.6	14.6
DL-methionine	0.2	0.2	0.2	0.2
Soybean oil	8	—	—	—
Oxidized soybean oil†	—	8	8	8
Cholesterol	0.5	1	1	1
Mineral mix‡	3.5	3.5	3.5	3.5
Vitamin mix‡	1	1	1	1
Choline chloride	0.2	0.2	0.2	0.2
Rutin	—	—	0.15	—
Chicory seed extract	—	—	—	1.57
Sucrose	0.5	0.5	0.5	—
Fructose	5.0	66.0	66.0	66.0
Corn starch	66.50	5.00	4.85	3.93

* Rats from the ER and EC groups have the same diet during first 2 wk of feeding as in group E, whereas the preparation of rutin or chicory seed extract was supplemented at the beginning of the last 2 wk. In all experimental groups, the diets have comparable sum of mono- and disaccharides, whereas in the ER and EC groups the diets have additionally equal amount of phenolic compounds.
† Degree of oxidation = 120 meq O_2/kg.
‡ Recommended for American Institute of Nutrition-93G diet.

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