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EFFECT OF RS4 RESISTANT STARCH IN HIGH-FAT DIETS ON MAGNESIUM AND IRON APPARENT ABSORPTION IN WISTAR RATS – A SHORT REPORT

Dagmara Orzeł, Monika Bronkowska, Marzena Styczyńska

Chair f Human Nutrition, Department of Food Storage and Technology, Wrocław University of Environmental and Life Science, Wrocław, Poland

Key words: resistant starch, Mg and Fe absorption, Wistar rats

The study was undertaken to investigate the effect of RS4 resistant starch in high-fat diets on the apparent absorption of magnesium (Mg) and iron (Fe) in Wistar rats. The animals (4 groups of males, n=32) were fed 4 types of diets: a control diet with 15% addition of soybean oil (K_1) and a control diet with 15% addition of lard and cholesterol (K_2) as well as 2 respective experimental groups with 10% addition of RS4 resistant starch (K_{1S} and K_{2S}). After a 3-week adaptive period, the animals were transferred into metabolic cages. Feed intake was measured and samples of faeces were collected within the 3 subsequent days. Diets and faeces were determined for contents of Mg and Fe with the method of atomic absorption spectrometry. The absorption of Mg and Fe in rats was calculated based on a difference between their contents in ingested diets and excreted faeces. An increase was observed in the apparent absorption of magnesium and iron by *ca.* 8% and 17.5%, respectively, in the group of rats fed the high-fat diet (lard + cholesterol) and resistant starch (K_{2S}) as compared to the control group (K_2).

INTRODUCTION

There is evidence showing that the amount of magnesium and iron in diets is often insufficient to individual needs. Because Mg and Fe play an essential role in a wide range of fundamental reactions, it is not surprising that their deficiency in the organism may lead to biochemical changes. Resistant starch (RS) as a functional ingredient in foods could bring beneficial health effect, enhancing Mg and Fe absorptions from the large intestine. RS shows promising physiological benefits in humans, which may result in disease prevention [Nugent, 2005; Topping & Clifton, 2001; Sajilata *et al*., 2006].

RS is subdivided into four fractions: RS1, RS2, RS3 and RS4. RS1 is the physical form of starch found in whole or partly milled grains and seeds. RS2 represents starch that is in a certain granular form and resistant to enzyme digestion (in raw potatoes, green bananas, some legumes and high amylase corn). RS3 is mainly retrograded amylose formed during cooling of gelatinized starch (in cooked and cooled potatoes, bread, cornflakes, food products with repeated moist heat treatment). RS4 is chemically-modified starch due to cross-linking with chemical reagents like starch phosphate, acetylated starch, hydroxypropylstarch, and oxidized starch [Leszczyński, 2004; Soral-Śmietana *et al*. 2004; Nugent, 2005; Sajilata *et al*., 2006].

Chemically-modified starches as food additives are used as a thickening agent, stabilizer, or an emulsifier in a range of baked and extruded products. They have high gelatinization temperature, good extrusion, film-forming qualities and lower water-holding properties, rheological properties than traditional fiber products. RS improves texture and increases coating crispness of products [Tharanthan *et al*., 2001; Sajilata *et al*., 2006].

Physiological effects of RS have been proved to be beneficial for health. Due to health-promoting properties, in recent years resistant starch (RS) has become a subject of extensive investigations [Bird *et al.,* 2000; Haralampu, 2000; Nugent, 2005]. Studies have shown that different classes of resistant starch are digested and fermented differently and thus must be considered individually. Resistant starches contribute specific health benefits. RS feeding has been reported to lower cholesterol and triglycerides concentration relative to digestible starch feeding [Hashimoto *et al.,* 2006;Figurska-Ciura *et al.,* 2007]. Diet containing resistant starch is low glycemic and low insulinemic [Severijnen *et al.,* 2007]. Resistant starch increases availability of mineral compounds, *e.g.* calcium, phosphorus, magnesium, iron, copper, zinc, in the colon. This may constitute a significant difference as compared to dietary fiber whose increased intake diminishes the absorption of minerals [Greger, 1999; Lopez *et al*., 2000; Coudray *et al*., 2003, Orzeł *et al*., 2007].

The reported study was aimed at evaluating the effect of a resistant starch RS4 preparation in high-fat diets on the absorption of magnesium and iron in organisms of Wistar rats. In the experiments, high-fat and high-energy diets that reflect irrational eating habits of populations from economically-developed countries contributing to the development of civilization diseases were adopted as model diets.

Author's address for correspondence: dr inż. Dagmara Orzeł, Department of Food Storage and Technology, Wrocław University of Environmental and Life Science, ul. Norwida 25, 50-375 Wrocław, Poland; e-mail: Dagmara.Orzel@wnoz.up.wroc.pl

MATERIAL AND METHODS

Experiments were carried out on 32 Wistar male rats with an initial body weight of 276 g, originating from the Animal Laboratory, Chair of Pathological Anatomy, Medical Academy of Wrocław. The study was approved by the Local Ethics Commission for Animal Experiments at the Wrocław University of Environmental and Life Sciences (No. 103/07). The animals were bred in cages adapted for that purpose, with constant access to feed and water. The rats were divided at random into four groups taking into account the type of diet ingested (8 rats each). The quantitative composition of each diet was presented in Table 1. The animals were fed a synthetic diet for laboratory rodents AIN-93 M [Reeves *et al.*, 1993], in appropriate modification. In all diets the content of fat was increased to 15%. Groups K_1 and K_{1S} received in diets vegetable oil – soybean oil, whereas groups K_2 and K_{2S} – animal fat – lard and 1% addition of cholesterol.

The rats from groups K_{1S} and K_{2S} were fed diets with 10% addition of resistant starch RS4 produced at the Department of Food Storage and Technology, Wrocław University of Environmental and Life Sciences. In analyses, use was made of starch monophosphate prepared from soluble potato starch. Based on the saccharification degree of that preparation, under the influence of glucoamylase applied at 60°C for 120 min, its resistance level was determined and preparation addition to diets calculated.

The rats received experimental diets for 4 weeks. Rats were habituated to new meals feeding for a period of 20–21 days, next the animals were transferred into metabolic cages. After a few days of adaptive period in metabolic cages, diet intake was measured and faeces were monitored for 3 consecutive days. Feed and water were provided *ad libitum*. This study also was aimed at determining the effect of resistant starch on lipid metabolism (hematocrit, hemoglobin, glucose, total cholesterol, HDL-cholesterol, and triglycerides) and activity of hepatic enzymes (alanine aminotransferase (Alat) and aspartate aminotransferase (Aspat)), in rats.

To determine contents of metals, 1 g samples were weighed and mineralized with 5 mL of 65% nitric acid and 1 mL of 30% H_2O_2 in a microwave furnace Mars 5. Determinations of magnesium and iron contents were carried out in diets and feces with the method of atomic absorption spectrometry using an AA 240FS atomic absorption spectrometer by Varian. Apparent absorption of Mg and Fe (%) was calculated in rats based on the difference between their contents in ingested diets and excreted feces. The contents of Mg and Fe in water drunk and excreted urine were also taken into account, however these were statistically insignificant values as compared to concentrations of those minerals in diets uptaken and faeces excreted. In order to verify results obtained apart from a calibration curve, use was also made of the standard addition technique. The average recovery of Mg reached about 91%, whereas that of Fe – 92%.

A statistical analysis of the results obtained was carried out with the use of Statistica 6.0 PL software. The effect of RS4 preparation in diets on the absorption of magnesium and iron in experimental rats was evaluated with the method of one-way analysis of variance (ANOVA). Differences between mean values were compared with the Duncan's test at a significance level of $p<0.05$. In Tables 2 and 3 the same letters denote statistically homogenous groups.

RESULTS AND DISCUSSION

Table 2 provides mean contents of magnesium and iron in diets administered to rats and their faeces. In the diets ex-

TABLE 2. Mean contents of magnesium and iron in diets and excreted feces of the rats examined ($\bar{X} \pm SD$).

Diet	Content of Mg in diets (mg/100 g)	Content of Fe in diets (mg/100 g)	Content of Mg in feces (mg/100 g)	Content of Fe in feces (mg/100 g)
K_1	144.2 ± 3.1^a	18.9 ± 0.8^a	85.2 ± 9.6^a	15.4 ± 1.9^a
K_{1S}	147.9 ± 4.8^a	22.0 ± 1.1^a	82.1 ± 13.3^a	19.3 ± 0.9^b
K_2	158.8 ± 3.9^a	22.0 ± 1.3^a	74.1 ± 14.4^b	15.2 ± 2.0^a
K_{2S}	161.2 ± 3.5^a	22.3 ± 2.3^a	71.6 ± 9.3^b	17.1 ± 2.3^b

One-way Anova, differences statistically significant at $p<0.05$; the same letters denote statistically homogenous groups.

TABLE 1. Quantitative composition of experimental diets.

Dietary component	Diet			
	K_1 (g/kg of diet)	K_2 (g/kg of diet)	K_{1S} (g/kg of diet)	K_{2S} (g/kg of diet)
Cellulose	50.00	50.00	50.00	50.00
Cholesterol	–	1.00	–	1.00
Choline	2.50	2.50	2.50	2.50
Cysteine	1.80	1.80	1.80	1.80
Casein	140.00	140.00	140.00	140.00
Vegetable fat – soybean fat	150.00	–	150.00	–
Saccharose	100.00	100.00	100.00	100.00
Mineral Mix AIN-93M-MX	35.00	35.00	35.00	35.00
Preparation of resistant starch	–	–	227.20	227.20
Wheat starch	510.7	509.7	283.5	282.5
Animal fat – lard	–	150.00	–	150.00
Vitamin Mix AIN-93-VX	10.00	10.00	10.00	10.00

amined, the mean concentrations of these minerals were statistically homogenous. Contents of magnesium in excreted faeces of rats fed diets with RS4 did not demonstrate any significant differences, when compared to their control groups. Mean concentrations of magnesium in faeces of rats fed diets with vegetables oil (K_1 and K_{1S}) were about 15% higher than those reported in groups of rats fed animal fat (K_2 and K_{2S}). Mean concentrations of iron in faeces of rats from groups K_{1S} and K_{2S} were higher than those reported in the respective control groups (K_1 and K_2).

Table 3 contains data on the apparent absorption of magnesium and iron in the examined rats depending on the type of diet applied. No significant differences were shown in the absorption of magnesium and iron between rats fed diets with vegetable fat (K_1) and those receiving vegetable fat and resistant starch (K_{1S}). Animals fed a diet with the addition of animal fat and cholesterol (K_2) were characterised by statistically significantly lower mean values of magnesium and iron absorption as compared to the respective values reported in the other groups of rats. The addition of RS4 resistant starch to a diet with animal fat and cholesterol (K_{2S}) evoked an increase in the mean apparent absorption of magnesium and iron by 8% and 17.5%, respectively, when compared to the control group (K_2).

Investigations of Schulz *et al.*, [1993] demonstrated an 8% increase in the apparent absorption of magnesium (calculated as the difference between mineral intake and fecal excretion) in female Wistar rats (aged ~3 weeks) fed a diet supplemented with RS2 resistant starch for two weeks in respect of the control diet. Beneficial effect of RS3 resistant starch on magnesium absorption were not confirmed in the study.

A research by Lopez *et al.* [2000] showed a positive impact of 20% addition of RS1 resistant starch (crude potato starch) to diets for male Wistar rats (weighing 150–160 g) on the apparent absorption of minerals. Absorption of magnesium and iron in the group of animals, receiving resistant starch for 20 days period, increased by 15% and 12%, respectively, as compared to the control group. Apparent absorption of magnesium and iron in the group of animals receiving resistant starch and wheat bran increased by 10% and 7%, respectively, as compared to the control group. Diet supplementation with wheat bran, as a source of dietary fiber, was found not to exert any positive effect on the absorption of minerals in the rats examined.

Enhanced apparent absorption of magnesium and iron (by *ca.* 9-10%) was observed in male Wistar rats (weighing ~150 g) fed diets with the addition of potato and maize RS2 for 3 weeks when compared to animals from control groups [Lopez *et al.,* 2001].

Investigations of Younes *et al.* [2001] demonstrated a 17% increase in the apparent absorption of magnesium in adult male Wistar rats administered a diet with 15% addition of RS1 resistant starch (crude potato starch) for 21 days in respect of the control group. A significant increase was shown in the absorption of that element (by 29%) after ingestion of a diet with the addition of resistant starch and inulin. It was observed that resistant starch could improve mineral absorption in animal studies conducted on different types of RS. RS1 or RS2 have confirmed their beneficial effect on mineral absorption in different animal studies. They have been thought to impair absorption of minerals such as calcium, magnesium, iron, phosphorus or zinc [Ohta *et al*., 1995; Lopez *et al.,* 1998, 2001].

The effect of resistant starch RS4 on apparent absorption of magnesium and iron was studied in Wistar rats by the authors earlier [Orzeł *et al*., 2007]. The 24 rats (divided on 4 groups) were fed diets: control with wheat starch (K) and 3 diets with 3 preparations of modified resistant starches. After an adaptation period (14 d), rats were transferred to metabolic cages. Dietary intake and feces were monitored for 3 days. Mg and Fe levels were assessed in diets and feces by atomic absorption spectrometry. Apparent absorption of minerals was calculated as mineral intake minus fecal excretion and expressed as percentage of intake. Our results confirmed that Mg apparent absorption in female rats fed the diet with one of resistant starch preparation (starch monophosphate) was significantly increased (+37%) compared with the control group.

Experiments of most authors showed that RS, together with an enlargement of the cecum and enhanced SCFA production, significantly increased Mg, Ca, Fe, P absorptions [Schulz & Beynen, 1993; Younes *et al.,* 1996; Topping & Clifton, 2001; Sajilata *et al.,* 2006]. Mineral absorption values were significantly higher in the RS groups than in controls, in parallel with an enlarged cecum and greater short chain fatty acid (SCFA) production than in control rats. This was accompanied by a significant acidification of cecal pH and a greater degree of cecal mineral solubilization.

Maybe high-fat diet (with lard) enrichment with resistant starch and saturated fatty acids indicated easier formation

TABLE 3. Effect of RS4 resistant starch on the apparent absorption of magnesium and iron in rats ($\bar{X} \pm SD$).

Animal group	Mg			Fe		
	intake with diet (mg/day)	excreted with feces (mg/day)	apparent absorption (% of intake)	intake with diet (mg/day)	excreted with feces (mg/day)	apparent absorption (% of intake)
K_1 (n=8)	25.7±2.1[a]	3.1±0.5[a]	87.9±5.4[a]	3.4±0.3[a]	0.6±0.1[a]	81.5±5.1[a]
K_{1S} (n=8)	22.8±2.4[a]	2.3±0.7[a]	90.0±6.3[a]	3.4±0.2[a]	0.5±0.1 [a]	85.5±4.2[a]
K_2 (n=8)	18.9±1.9[b]	3.3±0.6[b]	82.5±5.8[b]	2.6±0.2[b]	0.7±0.1 [b]	68.1±3.1[b]
K_{2S} (n=8)	25.4±2.1[a]	2.5±0.4[a]	90.2±4.9[a]	3.5±0.5[a]	0.6±0.2[a]	85.5±4.1[a]

One-way Anova, differences statistically significant at $p<0.05$; the same letters denote statistically homogenous groups.

of amylose-lipid complexes in rats than high-fat diet (with vegetable oil) enrichment with resistant starch and unsaturated fatty acids. Thus these amylose-lipids complexes were additionally a form of highly resistant RS and increased apparent absorption of minerals.

CONCLUSIONS

An increase of the apparent absorption of magnesium and iron by *ca.* 8% and 17.5%, respectively, was observed in the group of animals fed the diet (lard + cholesterol) with 10% addition of RS4 resistant starch (K_{2S}) as compared to the control group (K_2). The 10% addition of RS4 resistant starch in the diet with vegetable fat was found not to affect the absorption of magnesium and iron in the rats examined. These feeding study with rats indicates that RS4, as a starch monophosphate, had different effects on the absorption of magnesium and iron and depended on diet components like type of fat.

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