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Effects of hypercholesterolemic diet enriched with onion as functional ingredient on fatty acid metabolism in Wistar rats

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ABSTRACT

The complex biochemical composition of onions has been studied as a source of biological components with health-related properties. The evolution of hypercholesterolemia is associated with a large range of alterations considered as strong risk factors for many cardiovascular events. The objective of this study was to investigate the effects of onion as functional ingredient on plasma, erythrocyte, liver and adipose tissue fatty acid composition in hypercholesterolemic male Wistar rats. Rats (n = 24) were randomly divided into three groups: control (C), high-cholesterol (HC), and high-cholesterol enriched with onion (HCO) groups. At the end of 7 weeks, animals were anesthetized and euthanized by extracting blood by cardiac puncture. Plasma, erythrocytes, liver and adipose tissue were collected and immediately stored at −80 °C. Fatty acid methyl esters were identified and quantified by GC/MS. Total fatty acid concentration decreased in liver and adipose tissue both in HC and HCO groups. SFA content was significantly higher in plasma, erythrocytes and liver in the C group compared to HC and HCO groups. In contrast, SFAs increased in adipose tissue both in HC and HCO groups compared to the C group. A significant increase in MUFA content in plasma was found in HC and HCO groups compared to the C group; in erythrocytes and liver the increase was lower. In plasma, PUFA content was significantly lower in HC and HCO groups compared to the C group. Interestingly, in liver and adipose tissue, PUFAs increased in HC and HCO groups compared to the C group. Results showed noticeable effects on individual fatty acid composition when assaying high-cholesterol diets in rats, in some cases enhanced by onion enrichment. Further research is needed to deeper understand the involved mechanisms and pathways.

1. Introduction

The evolution of hypercholesterolemia is associated with a large range of alterations considered as strong risk factors for many cardiovascular events. Cardiovascular disease is the main cause of mortality in the United States, Europe and most parts of Asia (Harris, 2008). Polyunsaturated and monosaturated fatty acids are important for normal growth, development and are suggested to play an important role in modulation of cardiovascular inflammatory diseases and cancer (Makni et al., 2008). However, high-cholesterol diets can also modify fatty acid metabolism. Thus, stearoyl-CoA desaturase has an important role in lipid metabolism by catalyzing the synthesis of monounsaturated fatty acids (MUFAs) from saturated fatty acids (SFAs) (Soliman, 2012). Dietary cholesterol intensifies MUFA synthesis while inhibiting polyunsaturated fatty acid (PUFA) pathways (Pita et al., 2002). Due to these changes, fatty acid composition of cellular membranes would be affected, modifying the normal function of receptors, transporters and enzymes.

Nowadays, investigation has focused on identifying ways to prevent atherosclerosis and other diseases through changes in diet. Many epidemiological studies have firmly proposed a correlation between the intake of food rich in polyphenols and low mortality due to coronary heart disease (Arts & Hollman, 2005; Heim, Tagliaferro, & Bobilya, 2002; Middleton, Kandaswami, & Theoharides, 2000; Nijveldt et al., 2001). High fruit and vegetable content diet can help to prevent the development of cancer and cardiovascular diseases (Ezz El-Arab, 2009; Goulet, Lamarche, Nadeau, & Lemieux, 2003). The healthful effects of these foods have been related to the presence of polyphenols and other nutrients like fiber, fatty acids and phytosterols. It has been shown that *Allium* species may help to prevent tumor promotion, cardiovascular disease and aging (Lee, Jung, & Kim, 2012; Nakayama, Tsuge, Sawada, & Higashi, 2013; Roldán-Marín et al., 2010, 2009). The onion (*Allium cepa* L.) forms part of the basic diet of many consumers. The complex biochemical composition of onions has been studied as a source of flavonoids, sulfur compounds, fructooligosaccharides and dietary fiber (Benítez et al., 2011; Colina-Coca, de Ancos, & Sánchez-Moreno, 2014; González-Peña et al., 2013; Griffiths, Trueman, Crowther, Thomas, & Smith, 2002) with antioxidant, anti-inflammatory

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and antimicrobial properties (González-Peña et al., 2013; Griffiths et al., 2002). Flavonoids have been proven to be successful in attenuating hypercholesterolemia (Bok et al., 1999; Kumar, Sudhahar, & Varalakshmi, 2005; Peluso, 2006; Rehrah et al., 2007). The most abundant flavonols in onions are derivatives of quercetin, mainly quercetin 4′-*O*-glucoside and quercetin 3,4′-*O*-diglucoside (González-Peña et al., 2013). Quercetin has been associated to reduction of lipids in hepatic tissue and hyperlipidaemia in obese mice induced by an atherogenic diet (Park, Ahn, Kim, & Ha, 2008) through oxidation of fatty acids (Czeczot, 2000) and also showing hepatoprotective effects (Cipak, Novotny, Cipakova, & Rauko, 2003; Le Marchand, Murphy, Hankin, Wilkens, & Kolonel, 2000; Pavanato et al., 2003; Peres et al., 2000).

Despite these positive effects on lipid metabolism, very little information is available on the role of *Allium* spices in fatty acid composition and lipid metabolism. The aim of this study was to investigate the effects of onion as functional ingredient on plasma, erythrocyte, liver and adipose tissue fatty acid composition in hypercholesterolemic male Wistar rats.

2. Materials and methods

2.1. Chemicals

Methanol, *N*,*N*-dimethylformamide (DMF), and hexane were purchased from LabScan (Dublin, Ireland). Sodium bicarbonate and sulfuric acid (98%) were purchased from Panreac (Barcelona, Spain) and glyceryl tritridecanoate from Sigma (St. Louis, Missouri, USA). All reagents were HPLC grade. Reference butter fat BCR 164 (EU Commissions; Brussels, Belgium) was purchased from Fedelco Inc. (Madrid Spain).

2.2. Onion powder preparation

Raw onions (*Allium cepa* L. var *cepa*, 'Recas') were supplied by Cebacat (Lleida, Spain). Onions were harvested in April 2012 in Spain and their bulbs were free of external damages and stored at 4 °C until processing (5 days later). The onions were hand-peeled, cut into 10 mm pieces, in bags with very low gas permeability (Doypack®, Polyskin XL, Amcor Flexibles Hispania, S.L., Granollers, Barcelona, Spain) and treated with high-pressure (400 MPa, 5 min, 25 °C) (High Pressure Iso-Lab System, model FPG7100:9/2C, Stansted Fluid Power Ltd., Essex, UK). High-pressure treatment was applied in order to ensure onion microbiological and nutritional quality. After high-pressure treatment, the onion was immediately frozen with liquid nitrogen, freeze-dried in a lyophilizer (model Lyoalfa, Telstar, S.A., Barcelona, Spain), pulverized with a ultra centrifugal mill ZM 200 (Retsch GmbH, Haan, Germany) obtaining a fine powder (final size particle ≤ 250 μm), and stored at -20 ± 0.5 °C until use.

2.3. Animal experimental design and diet preparation

A total of twenty four male Wistar rats with a body weight of approximately of 250 g at the outset were obtained from Harlan Laboratories Models (Harlan, SL, Barcelona, Spain). The animals were housed individually in metabolic cages in a temperature-controlled room (22.5 ± 0.5 °C) with a 12 h light–12 h dark cycle. The present study was approved by the Spanish Ministry of Science and Innovation Advisory Committee [project AGL2010-15910 (subprogram ALI)] and by an Ethics Committee of the Complutense University of Madrid (Spain). All experiments were performed in compliance with the Directive 2010/63/UE regarding the protection of animals used for scientific purposes. The rats were fed commercial rat pellets (Panlab, SLU, Barcelona, Spain) during 3 d for adaptation to environmental conditions and then distributed into three groups of eight animals each, according to average body weight and fed the control diet for 4 d for the adaptation to the metabolic cages. The diet compositions, based on the AIN-93M semi-purified rodent diet (Reeves, 1997) are shown in Table 1. The following three experimental semi-synthetic diets were prepared: (1) the control diet (C) was composed of a homogeneous mixture of 100% rodent diet; (2) the high-cholesterol diet (HC) was the control diet with 2% cholesterol and 0.5% cholic acid, substituting an equal amount of maize starch; and (3) the high-cholesterol diet enriched with onion (HCO) was identical to the high-cholesterol diet, but with 10% onion powder, balancing the dietary fiber with cellulose powder. Water and food were provided *ad libitum* over the 7-week experimental period. Body weight and food consumption were recorded weekly and daily, respectively.

2.4. Plasma, erythrocyte, liver and adipose tissue sampling

At the end of the experiment, in order to avoid inter-assay variations that could affect the comparison of data from the different groups, animals in fasting conditions were anesthetized and euthanized by extracting blood by cardiac puncture with a syringe, taking one animal at a time, of each one of three groups. Blood was collected from the heart and taken into tubes with EDTA as anticoagulant. Erythrocytes were separated from EDTA-blood by centrifugation at $1000 \times g$ for 10 min (4 °C) and then hemolyzed in the tris–HCl buffer (pH 7.6, 10 mmol/L). Plasma was recovered after centrifugation ($1500 \times g$, 15 min) at 4 °C and immediately stored at −80 °C until analysis. The whole liver and mesenteric, retroperitoneal and perirenal adipose tissue were removed, frozen in liquid nitrogen and stored at −80 °C until analysis.

2.5. Nutritional, phytochemical, and antioxidant activity analyses in onion powder

Analysis of protein, lipids, total fructans, total dietary fiber and ash was performed using standard laboratory procedures (AOAC, 1995).

Table 1
Composition of the experimental diets.[c]

Ingredient (g/kg)	Control	HC	HCO
Onion powder	–	–	100
Casein	200	200	200
Sucrose	100	100	100
Maize starch	470.49	445.49	368.69
Soya oil	50	50	50
Maize oil	80	80	80
Mineral mixture[a]	35	35	35
Vitamin mixture[b]	10	10	10
Cellulose powder	50	50	26.8
Choline bitartrate	2.5	2.5	2.5
tert-Butylhydroquinone	0.010	0.010	0.010
L-Cysteine	2	2	2
Cholesterol	–	20	20
Cholic acid	–	5	5

HC, high-cholesterol diet; HCO, high-cholesterol enriched with onion diet.

[a] Mineral mix for the AIN-93M diet, g/kg: calcium carbonate anhydrous, 357.00; potassium phosphate monobasic, 250.00; potassium citrate, tripotassium monohydrate, 28.00; sodium chloride, 74.00; potassium sulfate, 46.00; magnesium oxide, 24.00; ferric citrate, 6.06; zinc carbonate, 1.65; sodium meta-silicate $9H_2O$, 1.45; manganous carbonate, 0.63; cupric carbonate, 0.30; chromium potassium sulfate $12H_2O$, 0.275; boric acid, 0.0815; sodium fluoride, 0.0635; nickel carbonate, 0.0318; lithium chloride, 0.0174; sodium selenate anhydrous, 0.01025; potassium iodate, 0.0100; ammonium paramolybdate $4H_2O$, 0.00795; ammonium vanadate, 0.0066; powdered sucrose, 209.806.

[b] AIN-93-VX vitamin mix for the AIN-93M diet, g/kg: niacin, 3.000; calcium pantothenate, 1.600; pyridoxine-HCl, 0.700; thiamin-HCl, 0.600; riboflavin, 0.600; folic acid, 0.200; biotin, 0.200; vitamin B12 (0.1%), 2.500; vitamin E (all-*rac*-α-tocopheryl acetate, 500 IU/g), 15.000; vitamin A (all-*trans*-retinyl palmitate, 500,000 IU/g), 0.800; vitamin D3 (400,000 IU/g), 0.250; vitamin K1, 0.075; powdered sucrose, 974.655.

[c] Diet energy content was calculated using the factors 16.73 kJ/g (4 kcal/g) for protein, 15.69 kJ/g (3.75 kcal/g) for monosaccharides, 16.53 kJ/g (3.95 kcal/g) for disaccharides, 17.49 kJ/g (4.18 kcal/g) for starch, 8.37 kJ/g (2 kcal/g) for dietary fiber, and 37.65 kJ/g for fat. Control diet, 18540.9 kJ/kg (4431.4 kcal/kg); HC diet, 18856.6 kJ/kg (4506.8 kcal/kg); HCO diet, 18642.4 kJ/kg (4455.6 kcal/kg).