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ScienceDirect
Nutrition Research 27 (2007) 498 – 504
Nutrition Research
www.elsevier.com/locate/nutres

Soy protein without isoflavones reduces aortic total and cholesterol ester concentrations greater than soy protein with isoflavones compared with casein in hypercholesterolemic hamsters☆

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Received 11 September 2006; revised 23 April 2007; accepted 7 May 2007

Abstract

The present study was designed to investigate the effects of replacing casein with soy protein either containing isoflavones or not on plasma lipids and lipoprotein cholesterol concentrations and early aortic atherosclerosis (measured as aortic cholesterol concentration) in hypercholesterolemic hamsters. Thirty, 8-week-old, male F_1B Golden Syrian hamsters were individually housed and fed a semipurified hypercholesterolemic diet (HCD) containing 22% casein, 20% coconut oil, 2% corn oil, and 0.12% cholesterol (wt/wt) for a period of 2 weeks. The hamsters were then divided into 3 groups of 10 based on similar mean fasting plasma cholesterol concentrations and placed on experimental diets for 6 weeks. Group 1 continued on the HCD (casein). Group 2 was fed the HCD with 22% water-washed soybean protein isolate (soy + I) (1.76 mg of isoflavones per gram of protein) in place of casein. Group 3 was fed the HCD with 22% alcohol-washed soybean protein isolate (soy − I) (0.08 mg isoflavones per gram of protein) in place of casein. Compared with hamsters fed casein, hamsters fed both soy diets had significant decreases in their plasma total and non–high-density lipoprotein (very low and low-density lipoprotein)–cholesterol concentrations by week 2, which continued across weeks 4 and 6. However, the hamsters fed the soy − I diet accumulated significantly less aortic total cholesterol compared with the hamsters fed casein and the soy + I diet. In addition, the hamsters fed the soy − I diet accumulated significantly less aortic cholesterol ester compared with the hamsters fed casein only. In conclusion, dietary replacement of casein with soy protein either containing isoflavones or not in hypercholesterolemic hamsters lowers plasma total and non–high-density lipoprotein–cholesterol concentrations; however, only the soy protein without isoflavones reduced aortic cholesterol accumulation.

Keywords: Soy protein; Casein; Plasma cholesterol; Aortic cholesterol; Hamster

1. Introduction

There have been studies in human beings [1,2] and various animal models [3-10] that have shown that substitution of soybean protein for casein lowers plasma cholesterol concentrations. However, the extent to which plasma cholesterol is reduced when casein is replaced by soybean protein is highly variable and may depend on at least 3 factors: the intake of dietary cholesterol, the degree of

☆ This work was supported by Archer Daniels Midland Company, Randall Research Center, Decatur, Ill (RJN).

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doi:10.1016/j.nutres.2007.05.005

hypercholesterolemia, and the level of protein fed. Previous studies have shown that the differential cholesterolemic effect of dietary casein and soybean protein becomes evident only when high-cholesterol diets are fed [7]. In human beings, substitution of soybean protein for casein in the diet resulted in a decrease in plasma cholesterol levels when the diets contained cholesterol [1] or when fed to hypercholesterolemic patients [2]. The type of protein had little effect when low-cholesterol diets were used or when fed to normolipidemic subjects [11,12]. Similar results have been found in experimental animals. The feeding of cholesterol-containing diets to rats [4], pigs [5], monkeys [6,10], and hamsters [8,9] has shown a clear differential cholesterolemic effect of dietary casein and soybean protein. In contrast, the feeding of a cholesterol-free diet did not show a differential response to the type of dietary protein [13,14].

Most of these dietary protein studies including our previous communications on plasma lipids and lipoproteins in hamsters [8] and monkeys [6,10] used soybean protein isolate as the source of soy protein. Despite these compositional differences, in more recent publications [15,16] we demonstrated that, relative to casein, both soybean protein isolate and soybean protein concentrate fed at the 20% or 40% level significantly reduced plasma total cholesterol (TC) and low-density lipoprotein–cholesterol (LDL-C).

The cholesterol-lowering mechanism of soy protein is still far from clear. This effect on blood cholesterol concentrations is thought to be due largely to the amino acid composition of soy protein. However, studies in which amino acids patterned after soy protein or casein were fed to experimental animals have suggested that other nonprotein components present in soy may be partially responsible for the hypocholesterolemic effect [17-19]. There are a number of non–amino acid effects found in soy products that have been associated with lowering blood lipids [20]. These include soybean oil [21], soy lecithin [21], saponins [22], protein digestibility [23], protein phosphorylation [24], and isoflavones/phytoestrogens [25-27].

Although most studies have shown that the replacement of casein with soy protein lowers blood cholesterol levels, there still exists some controversy over whether soy protein containing isoflavones lowers it better than soy protein not containing isoflavones and whether there is a difference in early atherosclerosis development on these diets. Therefore, the present study was designed to investigate the effects of replacing casein as the sole protein source with water-washed soy protein, which contains isoflavones, vs alcohol-washed soy protein, which does not contain isoflavones, on the accumulation of cholesterol in the aortic arch of hypercholesterolemic hamsters.

2. Methods and materials

2.1. Experimental animals and diets

Thirty, 8-week-old, male F_1B Golden Syrian hamsters (*Mesocricetus auratus*) (BioBreeders, Inc, Watertown, Mass) were individually housed in hanging stainless steel wire-bottomed cages in an environmentally controlled room with a 12-hour light/dark cycle and were fed a nonpurified standard diet (Purina Rodent Chow, Purina, St Louis, Mo) ad libitum for 1 week to get acclimated to the facility. The hamsters were then fed a control semipurified hypercholesterolemic diet (HCD) (Research Diets, New Brunswick, NJ) containing 22% casein, 20% coconut oil, 2% corn oil, and 0.12% cholesterol, by weight, for a period of 2 weeks. After an overnight fast (16 hours), the hamsters were bled and plasma cholesterol concentrations were measured (week 0). The hamsters were then divided into 3 groups of 10 based on similar mean plasma cholesterol concentrations and placed on the different diets for an additional 6 weeks. Group 1 continued on the control HCD (casein). Group 2 was fed the HCD with 22% water-washed soybean protein isolate with isoflavones (1.76 mg of isoflavones per gram of protein) in place of casein as the sole protein source in the diet (soy + I). Group 3 was fed the HCD with 22% alcohol-washed soybean protein isolate without isoflavones (0.08 mg isoflavones per gram of protein) in place of casein as the sole protein source in the diet (soy − I). The complete composition of the diets is shown in Table 1. Food disappearance and body weights were monitored on a weekly basis throughout the study. Hamsters were maintained in accordance with the guidelines of the Committee on Animals of the University of Massachusetts Lowell Research Foundation and the guidelines prepared by the Committee on

Table 1
Composition of diets fed to hamsters for 10 weeks[a]

Ingredient (g/kg diet)	Casein	Soy + I	Soy − I
Casein	217.0	–	–
Water-washed soy protein[b]	–	217.0	–
Alcohol-washed soy protein[c]	–	–	217.0
L-Methionine	3.0	3.0	3.0
Lodex 10	125.0	125.0	125.0
Corn starch	225.0	225.0	225.0
Cellulose	151.8	151.8	151.8
Coconut oil (hydrogenated)	200.0	200.0	200.0
Corn oil	20.0	20.0	20.0
Mineral mix[d]	10.0	10.0	10.0
Calcium phosphate, dibasic	13.0	13.0	13.0
Calcium carbonate	5.5	5.5	5.5
Potassium citrate	16.5	16.5	16.5
Vitamin mix[e]	10.0	10.0	10.0
Choline bitartrate	2.0	2.0	2.0
Cholesterol	1.2	1.2	1.2
Protein (% energy)	18.0	18.0	18.0
Carbohydrate (% energy)	34.0	34.0	34.0
Fat (% energy)	47.0	47.0	47.0

[a] Semipurified diets were supplied by Research Diets, Inc.
[b] Soy protein supplied by Archer Daniels Midland Company and contains 1.04 mg genistein and 0.71 mg of daidzein per gram of soy protein.
[c] Soy protein supplied by Archer Daniels Midland Company and contains 0.03 mg genistein and 0.01 mg of daidzein per gram of soy protein.
[d] S10026 = mineral mix (rd-96 salt mix without calcium, phosphorus, and potassium).
[e] V10001 = vitamin mix for AIN-76A rodent diet.