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# Altered lipid metabolism in rat offspring of dams fed a low-protein diet containing soy protein isolate

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ABSTRACT

*Aims:* Substantial studies have reported that maternal protein restriction may induce later development of cardiovascular disease in offspring by impairing antioxidant system and lipid metabolism. Because a unique amino acid composition of soy protein isolate has been shown to provide health benefits, including hypolipidemic effects, we investigated effects of maternal low-protein diet composed of low-isoflavone soy protein isolate (SPI) on oxidative stress and lipid metabolism in offspring.
*Main methods:* Sprague-Dawley dams were fed 20% or 10% SPI diet throughout pregnancy and lactation. On postnatal day 21, male offspring and their dams were studied.
*Key findings:* Maternal consumption of low-protein diet composed of SPI did not induce hepatic oxidative stress in offspring. Although serum triacylglycerol and cholesterol levels in dams were not different between groups, serum triacylglycerol levels were lower in offspring of dams fed a 10% SPI diet (10% SPI group) compared to offspring of dams fed a 20% SPI diet (20% SPI group). Maternal protein restriction also reduced serum HDL/total cholesterol levels. The mRNA levels of apolipoprotein A1, which is required for HDL formation, were lower in 10% SPI group compared to 20% SPI group and were positively correlated with serum HDL-cholesterol levels.
*Significance:* Although maternal consumption of low-protein diet containing SPI did not induce oxidative stress and hypertriglyceridemia, the present study indicates that it may disturb cholesterol metabolism of rat offspring on postnatal day 21. Further studies are warranted to investigate the effect of maternal diet composed of soy protein isolate on later development of cardiovascular disease in offspring.


## 1. Introduction

Because various organs are developed and organized during the fetal and perinatal periods, environmental factors in early life have lifetime significance in health and disease development [1,2]. Among several nutrition models of the early life origins of chronic disease, the low-protein model is one of the well-established animal models of early growth restriction, which may possibly be relevant to the challenges faced in groups of people. It is noteworthy that maternal low-protein diet consumption even only during the preimplantation period is able to induce the altered development in multiple organ systems, including liver and kidney [3].

Most protein restriction studies in animals have employed about a 50% restriction in maternal dietary protein during pregnancy and/or lactation to induce growth retardation [4,5]. In addition to an inadequate supply of nutrients, maternal protein restriction has been shown to cause poor fetal growth by impairing the placental nutrient transfer [6] and increased expression of genes involved in negative regulation of cell growth in the placenta [7]. Several studies demonstrated that low growth rate and altered organ structure of offspring born to protein-restricted dams are linked to high blood pressure and cardiac dysfunction, which result in an increased risk for cardiovascular disease in later life [5,8–11]. Furthermore, maternal low-protein diet decreased mitochondrial respiration capacity and antioxidant defense system, which led to an increased susceptibility to oxidative injury in offspring [12,13]. When casein was used as the sole protein source in maternal diet, altered lipid metabolism, especially hypercholesterolemia, was also observed in offspring of dams fed a low-protein diet [14].

Previous studies have reported that protein source in maternal diet affects lipid metabolism in offspring. Especially, soy protein isolate compared to casein lowered serum and/or hepatic lipid parameters in offspring [15–17]. Various bioactive compounds in soybean, including isoflavone and soyasaponins, have been shown to alleviate dyslipidemia and oxidative stress [18,19]. Furthermore, several studies have reported that amino acid patterns of soy protein isolate may influence cholesterol

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levels [20,21]. Soy protein isolate contains higher amounts of several amino acids, including cysteine, arginine, and glycine, than casein. Low ratio of methionine-to-cysteine [22], and low ratios of methionine-to-glycine and lysine-to-arginine [23] have been suggested to contribute to hypocholesterolemic effect of soy protein. Previous studies have reported that maternal consumption of a low-protein diet with lower methionine content did not result in a reduced maternal weight gain during pregnancy and did not induce hypertension in their offspring [24], suggesting that protein source may play an important role in programming of adult disease development. Similarly, glycine supplementation to a maternal low-protein diet reversed the development of hypertension in offspring [25,26].

Because most previous studies have reported the effect of maternal protein inadequacy in offspring using a casein as the sole protein source; in the present study, we investigated the effect of maternal restricted protein diet formulated with low-isoflavone soy protein isolate (SPI) on oxidative stress and lipid metabolism in male offspring on postnatal day 21.

## 2. Materials and methods

### 2.1. Diets

The composition of diets is shown in Table 1. A control diet (20% SPI diet) and an isoenergetic low-protein diet (10% SPI diet) were prepared according to AIN-93G [27] with corn oil substituting for soybean oil and contained SPI (PRO-FAM® 974; Archer Daniels Midland Company, Chicago, IL, USA) as a sole protein source. In a 10% SPI diet, half of protein was substituted with equivalent amounts of mixture containing cornstarch, dextrinized cornstarch, and sucrose (3:1:1). SPI contains 3.73 mg genistein, 14.71 mg genistin, 1.37 mg daidzein, and 6.40 mg daidzin/100 g protein as determined by HPLC. Amino acid content was provided by the supplier of protein source.

### 2.2. Animals

Seven-week-old virgin female Sprague-Dawley rats were obtained from the local animal facility (Orient Bio Inc., Seongnam, Korea) and were maintained in a temperature (22 ± 3 °C) and humidity (50 ± 10%)-controlled room with a 12 h-dark–light cycle. The experimental procedures used in the present study were approved by Seoul National University Institutional Animal Care and Use Committee (#SNU-081006-4). After two weeks of acclimation period, rats were mated and vaginal plugs were identified (day 0 of gestation). As shown in Fig. 1, the plug-positive rats were transferred to individual cages and were fed a diet containing 20% SPI or 10% SPI throughout pregnancy and lactation (3 dams per group). Diets and water were provided ad libitum. After delivery, litter size was recorded at birth and adjusted to 8 pups to standardize food supply at 7 days of age. Dams and their male offspring (3 male offspring per litter) at 3 weeks of age, were sacrificed after an overnight fast. Blood samples were rapidly obtained by cardiac puncture and serum was obtained after centrifugation. Tissues were removed and were snap-frozen immediately in liquid nitrogen. Serum and tissues were stored at −80 °C until analyzed.

**Table 1**
Composition of experimental diet.

| Composition (g/kg diet) | Diet | |
|---|---|---|
| | 20% SPI | 10% SPI |
| SPI[a] | 200 | 100 |
| Cornstarch | 397.5 | 459 |
| Dextrinized cornstarch | 132 | 152 |
| Sucrose | 100 | 120 |
| Corn oil | 70 | 70 |
| Fiber | 50 | 50 |
| Mineral mix[b] | 35 | 35 |
| Vitamin mix[c] | 10 | 10 |
| L-Cystine | 3.0 | 1.5 |
| Choline bitartrate | 2.5 | 2.5 |
| *t*-Butylhydroquinone | 0.014 | 0.014 |

[a] SPI, low-isoflavone soy protein isolate: alanine 4.3, arginine 7.5, aspartic acid 11.5, cystine 1.2, glutamic acid 19.2, glycine 4.1, histidine 2.7, isoleucine 4.8, leucine 8.0, lysine 6.3, methionine 1.4, phenylalanine 5.2, proline 5.2, serine 5.5, threonine 3.7, tryptophan 1.1, tyrosine 3.8, and valine 4.8 g/100 g protein.
[b] Mineral mix: AIN-93G-MX (Dyets Inc., Bethlehem, PA, USA).
[c] Vitamin mix: AIN-93-VX (Dyets Inc., Bethlehem, PA, USA).

### 2.3. Serum biochemical analysis

Serum glucose, triacylglycerol, total cholesterol, HDL-cholesterol, and albumin levels were detected with a colorimetric method, using commercial kits (Asan Pharmaceutical Co., Seoul, Korea). Serum free fatty acid levels were also measured with a commercially available kit (Shinyang Diagnostics, Seoul, Korea) and serum insulin levels were measured using a rat/mouse insulin ELISA kit (Millipore, Temecula, CA, USA). Serum total protein levels were measured with a Protein Assay Reagent (Bio-Rad, Hercules, CA, USA). The activities of serum aspartate transaminase (AST) and alanine transaminase (ALT) were measured by using the commercial kit (Asan Pharmaceutical Co., Seoul, Korea).

### 2.4. Serum free amino acid analysis

Serum samples were mixed with 2 mmol/L norvaline as an internal standard and 20% sulphosalicylic acid to precipitate protein and were kept for 60 min on ice. After the centrifugation at 12,000 ×*g* for 5 min at 4 °C, the supernatant was filtered through a 0.2 μm filter. Filtered samples and free amino acid standards (Agilent Technologies, Santa Clara, CA, USA) were analyzed by the Agilent 1200 HPLC (Agilent Technologies, Santa Clara, CA, USA) equipped with an Inno C18 column (4.6 mm × 150 mm, 5 μm, Young Jin Biochrom Co., Seongnam, Korea) and the fluorescence detector. The derivatization reagents of amino acids were *o*-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC). The analytes were eluted with a gradient of eluent A (20 mM phosphate buffer, pH 7.8) and eluent B (acetonitrile:methanol:water, 45:45:10, v/v/v). The flow rate was 1.5 mL/min, and the column and sample were maintained at 40 °C and 20 °C, respectively. The fluorescence detector was set at excitation 340 nm and emission 450 nm for OPA, and excitation 266 nm and emission 305 nm for FMOC.

### 2.5. Hepatic biochemical analysis

Total lipids were extracted according to the method of Folch et al. [28]. Briefly, 5% (w/v) liver homogenates were prepared in PBS, and 300 μL of homogenates were incubated in 1.2 mL of methanol-chloroform (1:2, v/v) at 4 °C for 3 h. After incubating, 240 μL of 0.88% KCl was added for aggregation of non-lipid contents and centrifuged at 1000 ×*g* for 15 min at 4 °C. The bottom layer was concentrated by nitrogen gas and lipid pellets were resuspended in isopropanol. Hepatic triacylglycerol and cholesterol concentrations were determined by enzymatic colorimetric methods using commercial kits (Asan Pharmaceutical Co., Seoul, Korea). To determine hepatic total glutathione and lipid peroxides, liver tissues were homogenized in 10 vol (w/v) of homogenizing buffer containing 154 mmol/L KCl, 50 mmol/L Tris-HCl, and 1 mmol/L EDTA (pH 7.4) and the supernatant was used after centrifugation at 600 ×*g* for 10 min at 4 °C. Total glutathione levels were analyzed by the method of Griffith [29]. The level of lipid peroxides was analyzed by measurement of thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa et al. [30].