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Brief report

Effects of whey protein and leucine supplementation on insulin resistance in non-obese insulin-resistant model rats

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ABSTRACT

Objective: Whey protein (WP) has been reported to reduce body weight gain and improve glucose metabolism in obese individuals. This study aims to assess and compare the effects of WP and its hydrolysate-leucine (Leu) supplementation in non-obese, insulin-resistant (IR) rat models, particularly the effects on insulin sensitivity, lipid profile, and antioxidant activity.
Methods: Wistar rats were fed a diet consisting of 38.5% fat for 12 wk and 51.3% fat for an additional 4 wk to establish non-obese IR rats. The IR rats were then switched to regular AIN-93 diet containing 0% WP, 5% WP, 15% WP or 1.6% Leu for 8 wk. The Leu content was the same in the 15% WP and 1.6% Leu groups based on high-performance liquid chromatography. The IR rats' body weight, fasting blood glucose, fasting insulin, and homeostasis model assessment-insulin resistance were measured before and after supplementation. An oral glucose tolerance test was performed after supplementation. Body composition, plasma concentrations of the lipids profile, and antioxidant index also were analyzed.
Results: No significant difference was observed in body weight, energy intake, and fasting blood glucose in the non-obese IR rats at the end of the experiment. Compared with the 0% WP group, the fasting insulin and homeostasis model assessment-insulin resistance significantly decreased in the 15% WP and 1.6% Leu groups. Furthermore, the blood glucose area under the curve of the oral glucose tolerance test was significantly less in the 15% WP and 1.6% Leu groups. There were no differences in the lipids profile, except for the increase in the high-density lipoprotein cholesterol in the 15% WP and 1.6% Leu groups. For the antioxidant index, the 15% WP group had significantly increased plasma levels for total antioxidation capacity, superoxide dismutase, and glutathione, and a decreased malondialdehyde concentration. The 1.6% Leu group was shown to have the same effect as the 15% WP group, except for the glutathione.
Conclusion: Our findings demonstrate that the supplementation of WP and Leu may improve IR and antioxidant stress without resulting in changes in body weight and energy intake in non-obese IR rats.

Introduction

Milk is a naturally nutrient-rich food. A previous meta-analysis indicated that milk intake might have the potential to protect against type 2 diabetes mellitus [1]. Milk contains two high-quality proteins, namely, casein and whey protein (WP). WP is usually a by-product of cheese manufactured from cow milk, and accounts for 20% of the total protein in bovine milk. An increasing number of studies have demonstrated the beneficial effect of WP intake on obese individuals, patients with type 2 diabetes mellitus, and insulin-resistant (IR) rats, as indexed by weight loss, increased satiety, and the regulation of insulin sensitivity [2–5]. WP is rich in branched-chain amino acids, such as leucine (Leu), which can stimulate muscle protein synthesis, regulate body weight, and improve the glucose metabolism in obese animals [6–8]. Thus, Leu was considered as one of the

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active components in WP that may have beneficial effects for IR patients. In this study, an IR model of rats was induced by a two-stage high-fat diet (HFD) to explore the potential effects of WP and Leu on the improvement of insulin sensitivity.

Materials and methods

Determination of the amino acids profile in WP

WP concentrate powder (81.4% protein) was purchased from Warmambool Cheese and Butter Factory Co., Ltd (SUNPRO, Australia). For WP hydrolysis, 2 mg of WP was dissolved in 1 mL of Tris-HCl (pH 8.0). Then, 3.5 μL of trypsin, 10 μL of peptidase, and 100 μL of chymotrypsin (all from Sigma, St. Louis, MO, USA) were added, and the mixture was incubated at 37°C for 24 h. The amino acid profile was determined by high-performance liquid chromatography (Waters Corporation, Milford, MA, USA), as previously described [9] but with minor modifications. Norleucine was used as an internal standard. The recovery rate was analyzed by adding 50, 200 and 1000 μmol/L Leu to a sample in the same batch, and the recovery rates were 94.1%, 92%, and 103.4%, respectively. The amino acids profile in the WP was determined before the animal experiment to determine the Leu amount in this study. Using the internal standard method, we found that the percentage for Leu in WP used was 14.4%.

Induction of the IR model in rats

Sixty 6-wk-old male Wistar rats were obtained from the Shanghai Laboratory Animal Center (Shanghai, China) and housed individually in stainless-steel cages in an air-conditioned room (23°C ± 2°C) with a 12-h light/dark cycle. The experimental protocols were approved by the Animal Experimentation Committee of Soochow University, China. After 1 wk of acclimatization with an AIN-93-based diet, 50 rats in the HFD group were fed a moderate HFD (41.7: 38.5: 19.8 energy profile of carbohydrate: fat: protein, respectively) for 12 wk, followed by a higher HFD (33.0: 51.3: 15.7) for another 4 wk. The other 10 rats were maintained on an AIN-93-based diet. The body weight was measured weekly. After the induction period, blood was obtained from the tail vein after overnight food deprivation. The fasting blood glucose (FBG) was immediately determined using a Roche blood glucose meter (F. Hoffmann-La Roche Ltd, Basel, Switzerland). The remaining blood was immediately centrifuged, and the plasma was stored at −80°C for fasting insulin (FINS) determination using a rat/mouse insulin enzyme-linked immunosorbent assay kit (Millipore, Billerica, MA, USA). The IR was estimated by homeostasis model assessment-insulin resistance (HOMA-IR) using the following formula: HOMA-IR = FBG (mmol/L) × FINS (pmol/L)/22.5. The HFD-fed rats in the upper 80% (40 of 50) of the HOMA-IR were selected as IR rats for the following intervention study.

Animal intervention and index measurement

After HFD feeding, 40 IR rats were randomly assigned to four groups. The IR rats in each group were then fed with a regular AIN-93 diet containing 0% WP, 5% WP, 15% WP and 1.6% Leu, respectively. The Leu amount in the 1.6% Leu group was the same as that in the 15% WP group, which was calculated based on the Leu composition (14.4%) of WP used in this study. The diet was mixed completely and dispensed in a powdered diet feeding jar (Natsume Seisakusho Co., Ltd, Tokyo, Japan). Before supplementation, blood was obtained from the tail vein after overnight food deprivation. The FBG and FINS were measured, and the HOMA-IR was calculated.

All animals were weighed weekly, and the food intake was measured once every 2 wk. After 8 wk of supplementation, an oral glucose tolerance test (OGTT) was performed. The rats were given an oral glucose bolus of 2 g/kg, and the blood glucose levels were determined at 0, 30, 60, and 120 min. The trapezoidal rule was used to determine the area under the curve (AUC).

After 2 d of the washout of an OGTT, the rats were deprived of food for 12 h and sacrificed. The abdominal cavity was opened, and blood was drawn from the inferior vena cava. The plasma was used to determine the lipids profile and antioxidant indexes using commercial kits obtained from the Shanghai Kehua Bio-engineering Co., Ltd. and the Jiancheng Bio-engineering Institute, respectively. The lipid profile included triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The antioxidant indexes included total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione (GSH), and malondialdehyde

Table 1
Changes in body weight and insulin resistance of rats fed with different diets

	N	Body weight (g)	FBG (mmol/L)	FINS (IU/mL)	HOMA-IR
AIN-93-based diet	10	419.8 ± 20.2	5.2 ± 0.3	23.4 ± 3.6	5.4 ± 0.6
High-fat diet	40	469.2 ± 30.3*	5.3 ± 0.3	34.1 ± 6.8*	8.1 ± 1.8*

FBG, fasting blood glucose; FINS, fasting insulin; HOMA-IR, homeostasis model assessment-insulin resistance

* $P < 0.01$, significantly different from the rats fed with AIN-93-based diet.

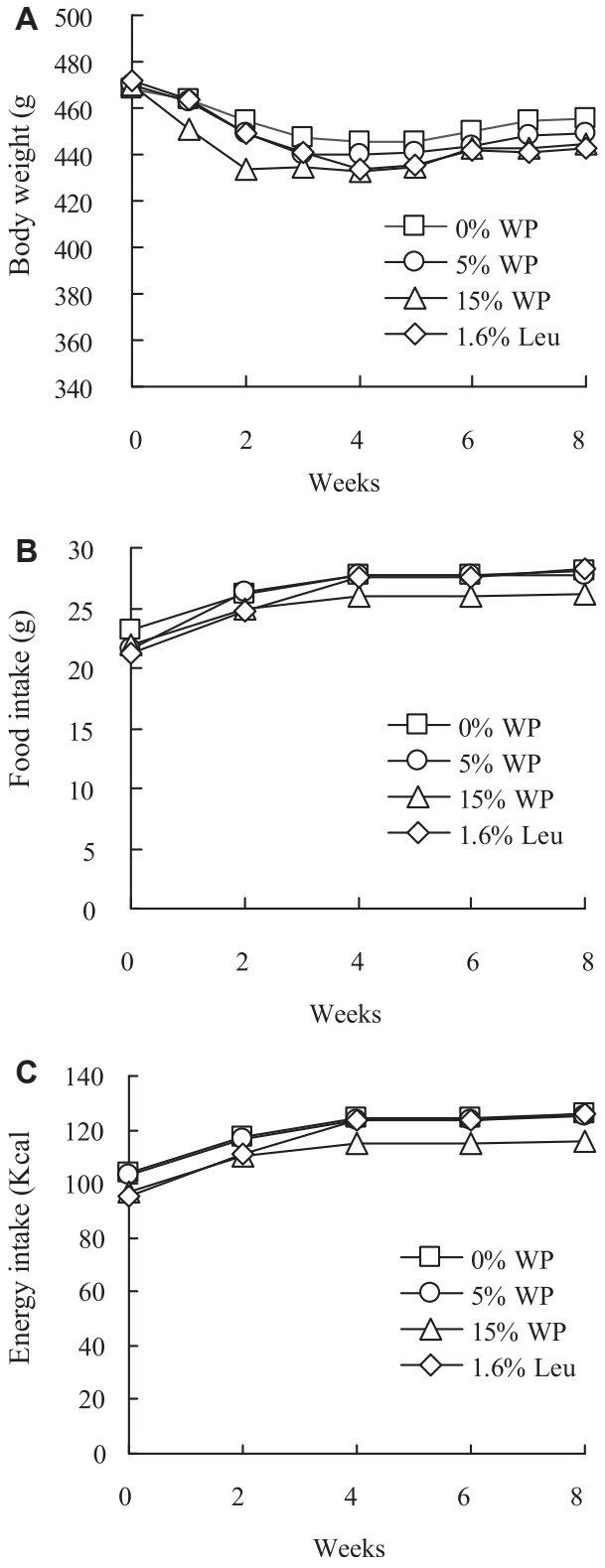

Fig. 1. Changes in body weight, and food and energy intake during the experiment. Leu, leucine; WP, whey protein.