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Research

Shrimp Protein Improves Oral Glucose Tolerance in High-Fat Diet-Induced Obese Mice

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Abstract

The present study was conducted to determine the effect of shrimp protein concentrate on insulin resistance in a diet-induced obese mouse model. Male C57BL/6J mice fed a commercial high-fat diet (60 kcal% from fat) for 12 weeks were divided into three groups and then switched to a high-fat diet prepared in the lab. One group was used as the high-fat diet control and the other two were fed the high-fat diet with 35% or 70% of casein replaced by the same amount of protein from shrimp protein concentrate for 9 weeks. A group of age and sex matched C57BL/6J mice fed a low-fat diet (10% kcal from fat) all the time were used as the normal or low-fat diet control. Weekly body weight, daily food intake, weekly 4-hr fasting blood glucose, oral glucose tolerance and overnight fasting blood glucose, insulin and lipids were measured. It was observed that replacing dietary casein with shrimp protein significantly improved oral glucose tolerance and 4-hr fasting blood glucose levels while having no effect on the fasting blood glucose and insulin levels. There was a trend of increasing body weight and food intake, particularly at 35% replacement, which also increased blood total cholesterol levels while having no effect on triacylglycerol levels. There were mixed results of shrimp protein concentrate on metabolic phenotypes while a dramatic improvement in oral glucose tolerance was seen. Additional studies are required to verify the observed benefits and further look into the underlying mechanisms.

Key Words: Diet-Induced Obese Mice; Shrimp Protein Concentrate; Glucose Tolerance; Glucose; Insulin; lipids

Introduction

The marine environment represents a large source of bioactive compounds which have recently been identified as having diverse biological activities and health benefits [1]. Thus, there is an increasing interest to explore marine bioactives for the discovery and development of drugs and natural health products. However, the majority of drug discovery and product development activities have been focused on small molecules.

Proteins and peptides are the largest portion of biomass of the marine environment and have been reported to possess various health benefits compared to other animal proteins. Peptides isolated from fish protein hydrolysates showed antihypertensive, antithrombotic [2,3,4], anticoagulant [5], immune modulatory and anti-oxidant [1,5] activities. Fish proteins are also reported to improve metabolic syndrome and contribute to the prevention of type II diabetes mellitus (T2DM) [6,7]. A clinical study demonstrated that cod proteins, compared to beef, pork, veal, egg and milk proteins, significantly improved insulin sensitivity and β-cell function in subjects with insulin-resistance [7]. Similar results were observed in another study, which showed that cod proteins improved glucose tolerance and insulin function on glucose disposal [6]. Oral administration of fish collagen hydrolysate in Chinese patients with T2DM decreased the fasting serum glucose, insulin, leptin, resistin, total cholesterol, low-density lipoprotein cholesterol and triacylglycerol levels while increasing adiponectin concentrations [8]. Dietary supplementation of the same product induced a sharp decrease in the serum levels of high-sensitivity C-reactive protein, nonesterified fatty acids (NEFA) and cytochrome P450, which all are associated with the development of insulin resistance and T2DM [9,10]. Furthermore, the serum levels of prostacyclin, a prostaglandin member of lipid molecules known as eicosanoids implicated in the development of diabetes [11] decreased after the prolonged administration of this fish hydrolysate. In rats fed a high-fructose diet, sardine proteins induced a marked improvement in insulin resistance and glucose tolerance, reduced blood leptin and NEFA levels, which was accompanied by a reduction of adiposity [12]. Although limited, available information has demonstrated the

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benefits of marine fish proteins, protein hydrolysates, or peptides derived from the protein hydrolysates on β-cell function, insulin action and glucose metabolism.

Shrimp processing produces a large amount of waste stream that contains predominantly proteins. There are no prior scientific reports available for the effect of shrimp proteins on insulin resistance and diabetes. The present study demonstrates for the first time that shrimp proteins are beneficial to blood glucose homeostasis and insulin sensitivity in a diet-induced obese and insulin resistant mouse model.

Materials and Methods

Preparation of Shrimp Protein Concentrate

Northern pink shrimp (*Pandalus borealis)* was processed at Island Fishermen Cooperative Association Ltd. (Lameque, NB, Canada). The wastes along with processing water were treated with the pending patent processing technology [13], which involves the addition of flocculating agent and dissolved air floatation system. The aggregates formed from suspended and dissolved solids were collected from the surface. The remaining suspension was then subjected to horizontal centrifugation to further separate the solids from liquids. The liquids were pumped into a 3-phase vertical centrifuge to separate oil, water and solids. The solid obtained at different stage was named as shrimp protein concentrate and transported from the processing plant to the laboratory under frozen condition and stored at -20°C until further processing. The material was thawed by leaving it at 4°C overnight, mixed manually, and dried in a Freezone 4.5 litter benchtop freeze dry system (Labconco, Kansas, MI, USA). The dried protein concentrate was ground using an electric rotary grinder and used in the animal study.

Analysis of Shrimp Protein Concentrate

Unless otherwise specified, the proximate analysis of shrimp protein concentrate was performed following the AOAC methods. Specifically, the ash content was analyzed by placing samples in a muffle furnace at 550°C for a minimum of 5 hr (AOAC 920.153); moisture was determined in an air-forced oven at 103-105°C for 18 hr (AOAC 930.15); protein was determined using the block digestion method (AOAC 981.10), and fiber was determined using the method of AOAC Ba 6a-05. Fat content was measured using the Folch method [14]. The content of carbohydrate was calculated as: 100% - (% moisture + % ash + % fat + % protein).

Animals and Diets

Sixty male C57BL/6J mice at 12 weeks of age were purchased from the Jackson Laboratory (Bar Harbor, ME). Twelve mice were fed a low-fat (10 kcal% fat; D12450J) and 48 were fed a high-fat (60 kcal% fat, D12492) diet (Research Diets, New Brunswick, NJ), starting at the age of 5 weeks in the Jackson Laboratory. After arrival, mice were housed individually in cages with a 12-hour light-dark cycle. They were fed the same diets for another 5 weeks to allow further development of insulin resistance. Then, the high-fat diet-fed mice were divided into three groups based on semi-fasting blood glucose and body weight, and switched to a high-fat diet prepared in the laboratory. One group was used as a high-fat diet control (HFC) and the other two groups were treated with shrimp protein concentrate by replacing 35% (T35) or 70% (T70) of casein with the same amount of proteins from the shrimp protein concentrate. The high-fat diet was a casein-cornstarch-sucrose-based AIN-93G diet modified to contain 60 kcal% from fat in a form of lard and sunflower oil mix (96:4, wt/wt). The mice on the low-fat diet were switched to the standard AIN-93G diet (LFC; 11 kcal% from fat) prepared in the laboratory. The composition of diets is presented in Table 1. The body weight was obtained weekly and food intake was recorded every day throughout the study period. After 9 weeks of treatment, the animals were fasted overnight and anesthetized with inhalation of isoflurane (Pharmaceutical Partners of Canada Inc.). Blood was collected by cardiac puncture into serum tubes, kept at room temperature for approximately 2 hr, and then placed on ice. After centrifugation, serum was collected and stored at -80°C. The study protocol and procedures performed in this study were reviewed and approved by the Joint Animal Care and Research Ethics Committee of the National Research Council Canada-Aquatic and Crop Resource Development Portfolio and the University of Prince Edward Island. The study was conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Measurement of Semi-Fasting Blood Glucose

Throughout the 9-week treatment period, 4-hr semi-fasting blood

Table 1: Composition of experimental diet (g)

Ingredient[#]	LFC[&]	HFC	T35	T70
Casein	200	200	130	60
Shrimp protein concentrate			134.6	269.2
Corn starch[$]	549	125	118.3	111.5
Sucrose	100			
Fructose		68.8	68.8	68.8
Lard[*]	48	245.0	225.4	205.7
Sunflower Oil	2	25	25	25
Cellulose	50	50	50	50
DL-methionine	3	3	3	3
AIN-93 G Mineral mix	35	35	35	35
AIN-93G Vitamin mix	10	10	10	10
Choline bitartrate	2	2	2	2
BHT	0.2	0.2	0.2	0.2
Cholesterol		15	15	15
Cholic Acid		1.9	1.9	1.9
Total	999	780.9	838.8	896.7

[#]All ingredients were purchased from Dyets Inc. (Bethlehem, PA, USA).

[$]Carbohydrate contributed by shrimp protein concentrate was substituted for the same amount of corn starch.

[*]Oil contributed by shrimp protein concentrate was substituted for the same amount of lard.

[&]LFC, low-fat diet control; HFC, high-fat diet control; T35, high-fat diet with 35% of casein being replaced with the same amount of protein from shrimp protein concentrate; T70, high-fat diet with 70% of casein being replaced with the same amount of protein from shrimp protein concentrate.

glucose was measured weekly from the lateral tail vein using an ACCU-Check glucometer (Roche Diagnostics, Ontario, Canada).

Oral Glucose Tolerance Test

The oral glucose tolerance test (OGTT) was performed after 7 weeks of treatment. Briefly, following 4-hr fasting, mice were orally gavaged with 2 g/kg body weight of glucose (D-(+)-glucose (99.5%; Sigma-Aldrich, Markham, ON, Canada) dissolved in water (40%, w/v). Blood glucose levels were measured with the glucometer from the tail vein at 0, 15, 30, 60, and 120 min, respectively.

Analysis of Fasting Blood Glucose

Fasting serum glucose was measured in duplicate in the laboratory using an enzymatic method, with the reagents purchased from Sekisui Diagnostics Inc. (Charlottetown, PE, Canada).

Analysis of Serum Insulin

Fasting serum insulin was determined in duplicate using mouse ELISA kits (Crystal Chem Inc. IL, USA) following the kit instructions. Standards at a series of concentrations were run in parallel with the samples. The concentrations were calculated in reference to the standard curve.

Analysis of Serum Lipids

Serum total cholesterol (T-C) and triacylglycerols (TAG) were measured in duplicate on a Pointe-180 chemistry analyzer using the reagents purchased from the manufacturer (Pointe Scientific Inc., Canton, MI).

Statistical Analysis

Data were analyzed using SAS 9.1 (SAS Institute, Cary, NC). The difference between the LFC and HFC groups was determined using the Student's *t*-test. The treatment effects were determined using one-way ANOVA. Each method with repeated measures was used for the parameters that were measured multiple times. When a significant treatment effect was detected, differences among the HFC and treatment groups were determined using the pair-wise comparison of the least squares means test. The significance level was set as $p < 0.05$. The data are presented as means ± S.E.M.

Results

Composition of Shrimp Protein Concentrate

The shrimp protein concentrate contained 52% protein as estimated based on the total nitrogen content, 24.8% ash, 14.6% fat, and 5% carbohydrate (Table 2). The macronutrients were adjusted in the treatment diets to ensure that the treatment and HFC diets had similar content of total protein, fat, carbohydrate and energy density. The ash content was not adjusted due to the compositional differences between ash and the mineral mix, meaning that T35 and T70 diets had slightly higher ash content than the HFC diet.

Food Intake and Body Weight

The food intake of mice in the HFC group was consistently lower than that in the LFC group, in accordance with the energy density of the diets (Table 3). Compared to the HFC group, treatments T35 and T70 increased food intake in weeks 4 and 7 while had no significant effect in other weeks. The HFC group was heavier than the LFC group at the beginning of treatment. Surprisingly, after the first week of treatment the difference disappeared (Table 4). This effect remained throughout the rest of the study period. In order to incorporate shrimp protein concentrate in diet, a high-fat control diet (AIN-93G modified to have 60 kcal% fat) was prepared in the laboratory and the treatment diets were prepared by replacing 35% and 70% of casein with the same amount of protein from the shrimp protein concentrate, respectively. The composition of the high-fat and low-fat diets was slightly different from the respective commercial ones from Research Diets Inc. The T35 group tended to increase the body weights throughout the treatment period and was significantly heavier than the HFC group at the end of weeks 6 and 7. However, the T70 group showed similar weekly body weights with the HFC group.

Effect of Shrimp Protein on Semi-Fasting Blood Glucose Levels

Before the treatment, the HFC group showed higher ($p < 0.05$) semi-fasting blood glucose levels than the LFC group (Table 5). After one week on the experimental diets, the difference between the LFC and HFC groups disappeared, consistently with the body weight changes, but started to show up again in the later of treatment. After 7 weeks, the HFC was higher than the LFC group at a marginal significance level ($p = 0.09$), but was reversed in the

Table 2: Composition of shrimp protein concentrate

	% of fresh weight
Moisture	5.2
Crude protein	52.0
Lipids	14.6
Carbohydrates	5.0
Ash	24.8

Table 3: Effect of shrimp protein concentrate on the food intake of high-fat diet-fed C57BL/6J mice

Treatment	Time post treatment (week)								
	1	2	3	4	5	6	7	8	9
LFC	3.90 ± 0.07	4.57 ± 0.11	4.17 ± 0.15	3.90 ± 0.12	3.98 ± 0.23	4.22 ± 0.09	4.02 ± 0.15	4.41 ± 0.17	3.57 ± 0.11
HFC	2.43 ± 0.28*	2.74 ± 0.16*	2.91 ± 0.13*	2.60 ± 0.11*	2.64 ± 0.11*	2.98 ± 0.15*	2.80 ± 0.11*	2.72 ± 0.14*	2.51 ± 0.07*
T35	1.84 ± 0.25	2.95 ± 0.15	3.24 ± 0.16	3.04 ± 0.10[a]	2.71 ± 0.12	3.20 ± 0.09	3.15 ± 0.07[b]	2.78 ± 0.09	2.75 ± 0.04
T70	2.53± 0.22	3.14 ± 0.12	3.04 ± 0.06	3.00 ± 0.08[b]	2.70 ± 0.09	3.21 ± 0.12	3.12 ± 0.10[b]	2.79 ± 0.10	2.90 ± 0.10

LFC, low-fat diet control; HFC, high-fat diet control; T35, high-fat diet with 35% of casein being replaced with the same amount of protein from shrimp protein concentrate; T70, high-fat diet with 70% of casein being replaced with the same amount of protein from shrimp protein concentrate. Data are presented as means ± SEM (g/d; n = 9-12). *Different from LFC group, $p < 0.0001$; [a]different from HFC, $p < 0.01$; [b]different from HFC, $p < 0.05$.

Table 4: Effect of shrimp protein concentrate on the body weight of high-fat diet-fed C57BL/6J mice

Treatment	Time post treatment (week)							
	0	1	2	3	4	6	7	9
LFC	30.2 ± 0.5	32.4 ± 0.5	34.3 ± 0.6	35.6 ± 0.6	36.7± 0.6	37.4 ± 0.7	38.8 ± 0.7	37.9 ± 0.7
HFC	40.1 ± 1.6*	35.6 ± 1.4	35.2 ± 1.6	35.6 ± 1.7	35.9 ± 1.6	36.5 ± 1.7	37.5 ± 1.8	37.3 ± 2.1
T35	41.6 ± 0.8	37.3 ± 1.1	37.8 ± 1.0	38.9 ± 1.0	40.3 ± 1.0	41.2 ± 1.0[b]	43.3 ± 1.0[b]	41.9 ± 1.2
T70	39.8 ± 1.3	35.9 ± 1.4	35.8 ± 1.2	36.2 ± 1.1	36.9 ± 1.2	36.7 ± 1.4	38.1 ± 1.5	37.5 ± 1.8

LFC, low-fat diet control; HFC, high-fat diet control; T35, high-fat diet with 35% of casein being replaced with the same amount of protein from shrimp protein concentrate; T70, high-fat diet with 70% of casein being replaced with the same amount of protein from shrimp protein concentrate. Data are presented as means ± SEM (g; n = 9-12). *Different from LFC group, $p < 0.0001$; [b]different from HFC, $p < 0.05$.

T70 group ($p < 0.05$).

Effect of Shrimp Protein on Oral Glucose Tolerance

Following oral glucose loading, the blood glucose levels quickly increased and reached the peak levels at 30 min in every group and then started to decline and returned nearly to the basal levels after 120 min (Figure 1). Mice in the HFC group showed higher ($p < 0.05$) levels of blood glucose at 30, 60, 90, and 120 min, respectively, than those in the LFC group, indicating that impaired glucose tolerance was developed in mice fed the high-fat diet. The T35 group showed similar blood glucose levels at each time point with the HFC group. However, the T70 group showed lower glucose levels than the HFC group at each time point and significantly lower ($p < 0.01$) at 60 min and 90 min, respectively. Consistently, the HFC group showed

Table 5: Effect of shrimp protein concentrate on semi-fasting (4-hr) blood glucose levels in high-fat diet-fed C57BL/6J mice

Treatment	Time post treatment (week)							
	0	1	2	3	4	6	7	8
LFC	10.1 ± 0.5	11.4 ± 0.7	11.7 ± 0.6	12.9 ± 0.6	12.9± 0.7	12.4 ± 0.7	12.8 ± 0.7	12.3±0.9
HFC	13.2 ± 0.6*	12.1 ± 0.5	11.8 ± 0.5	13.0 ± 0.6	13.2 ± 0.7	13.2 ± 0.6	14.8 ± 0.9	14.4±1.1
T35	12.9 ± 0.6	13.0 ± 0.6	13.4 ± 0.7	14.1 ± 0.5	14.0 ± 0.6	12.8± 0.5	13.7± 0.8	13.7±1.4
T70	13.2 ± 0.6	12.2 ± 0.8	11.6 ± 0.4	12.5 ± 0.6	12.6 ± 0.9	11.7 ± 0.6	11.9 ± 0.9[b]	12.0±1.2

LFC, low-fat diet control; HFC, high-fat diet control; T35, high-fat diet with 35% of casein being replaced with the same amount of protein from shrimp protein concentrate; T70, high-fat diet with 70% of casein being replaced with the same amount of protein from shrimp protein concentrate. Data are presented as means ± SEM (g; n = 9-12). *Different from LFC group, $p < 0.0001$; [b]different from HFC, $p < 0.05$.

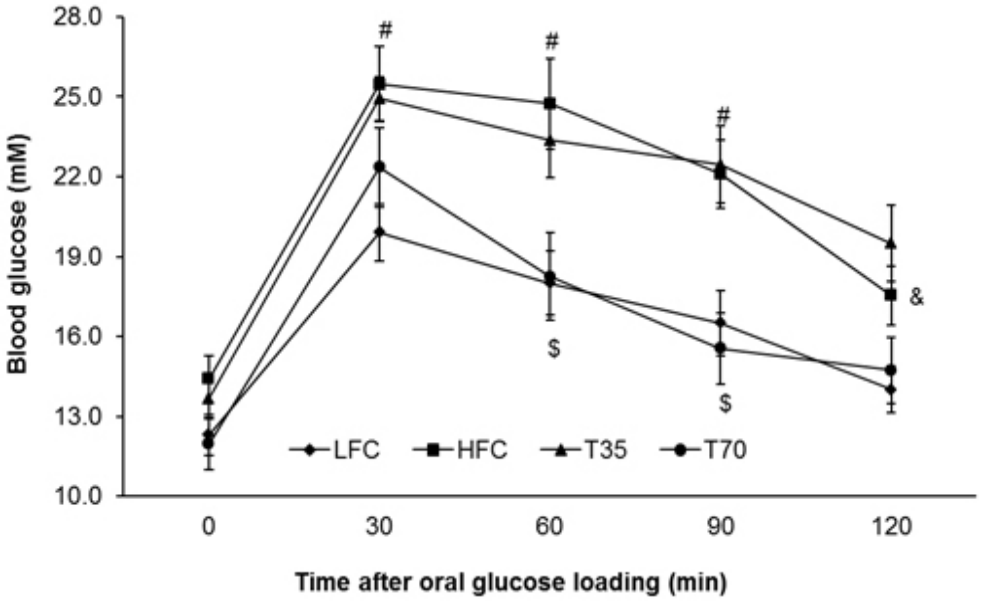

Figure 1: Effect of shrimp protein concentrate on oral glucose tolerance in mice. Values are means ± SEM, n=9-12. [$]different from HFC, $p < 0.01$; [#]different from LFC, $p < 0.01$; [&]different from LFC, $p < 0.05$. LFC, low-fat diet control; HFC, high-fat diet control; T35, high-fat diet with 35% of casein being replaced with the same amount of protein from shrimp protein concentrate; T70, high-fat diet with 70% of casein being replaced with the same amount of protein from shrimp protein concentrate.

higher ($p < 0.05$) AUC of the oral glucose tolerance than the LFC group but was reversed ($p < 0.05$) in the T70 group (Figure 2). The results demonstrated the beneficial effect of 70% replacement of casein with shrimp protein on insulin sensitivity.

Effect of Shrimp Protein on Fasting Serum Glucose and Insulin Levels

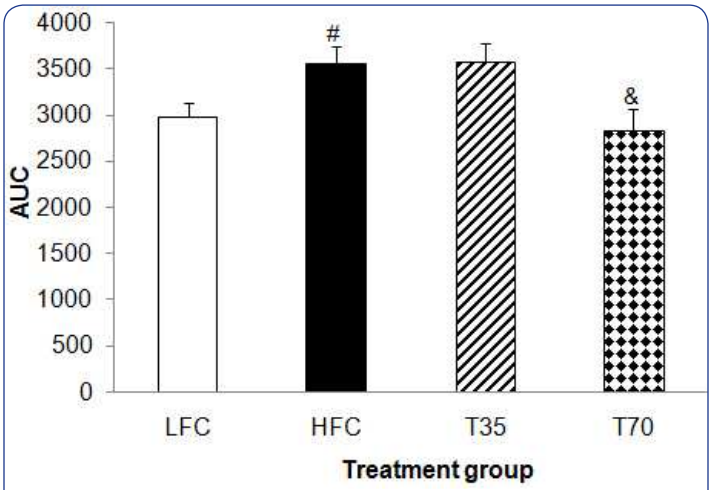

Figure 2: Effect of shrimp protein concentrate on the area under the curve (AUC) of oral glucose tolerance in mice. Values are means ± SEM, n=9-12. [&]different from HFC, $p < 0.05$; [#]different from LFC, $p < 0.05$. LFC, low-fat diet control; HFC, high-fat diet control; T35, high-fat diet with 35% of casein being replaced with the same amount of protein from shrimp protein concentrate; T70, high-fat diet with 70% of casein being replaced with the same amount of protein from shrimp protein concentrate.

The fasting serum insulin and glucose levels were similar between the HFC and LFC groups (Table 6). The replacement of dietary casein with shrimp protein concentrate had no significant effect on both fasting blood glucose and insulin levels compared to the HFC group.

Serum Lipids

The Serum total cholesterol levels were not altered in the HFC group compared to the LFC group (Table 6). Surprisingly, T35 increased ($p < 0.05$) blood cholesterol levels while T70 had no effect. The HFC lowered ($p < 0.05$) TAG levels as compared with the LFC group, and no effect of shrimp protein concentrate was observed.

Table 6: Effect of shrimp protein concentrate on fasting (12-hr) blood glucose and insulin levels and lipids in high-fat diet-fed C57BL/6J mice

Treatment	FBG (mmol/L)	Insulin (ng/mL)	T-C (mg/dL)	TAG (mg/dL)
LFC	11.2±0.6	0.25±0.03	163.1±11.4	58.5±4.7
HFC	10.2±0.4	0.24±0.01	181.5±10.9	37.8±2.6[#]
T35	10.4±0.4	0.33±0.04	232.1±12.2[&]	41.6±3.0
T70	10.2±0.4	0.45±0.11	200.7±12.7	33.0±2.7

FBG, fasting blood glucose; HFC, high-fat diet control; LFC, low-fat diet control; TAG, triacylglycerol; T-C, total cholesterol; T35, high-fat diet with 35% of casein being replaced with the same amount of protein from shrimp protein concentrate; T70, high-fat diet with 70% of casein being replaced with the same amount of protein from shrimp protein concentrate. Data are presented as means ± SEM (g; n = 9-12). [#]Different from LFC; [&]different from HFC, $p < 0.05$.

Discussion

Excessive caloric intake is a major driving force behind escalating obesity and type 2 diabetes epidemics worldwide [15]. The diet composition and dietary ingredients play an important role in the prevention of type 2 diabetes. Consumption of proteins such as soy protein and fish protein is associated with decrease of plasma glucose and insulin concentrations and improvement of glucose tolerance [7,16]. However, there was no information about the impact of shrimp protein on glucose homeostasis and insulin sensitivity. The present study demonstrated for the first time the antidiabetic property of a shrimp protein concentrate in mice fed a high-fat diet.

High-fat diet-induced obesity in C57BL/6 mice share human obesity phenotypes such as impaired glucose tolerance and type 2 diabetes [17]. Therefore, this model has been increasingly used to determine preclinically the *in vivo* anti-diabetic effects of pharmaceutical drugs, diets and naturally-occurring compounds. This model is especially useful for the evaluation of diets and natural health products wherein they are mainly used for the prevention rather than treatment due to their relatively lower efficacies but better safety profiles. Therefore, the findings of the current study are more applicable for the prevention of type-2 diabetes and insulin resistance. It may also be used as a co-treatment with a diabetic drug so as to lower the drug dosage and reduce the side effects associated with drugs.

The observations of the present study were largely consistent with the finding of a previous study in C57BL/6 mice, showing that a high-fat diet induced hyperglycemic and glucose intolerance [18]. The increase of insulin secretion is a compensatory response to insulin resistance [19]. Yet the fasting blood insulin was not elevated significantly in the HFC mice, which is not in line with the reports of previous studies [19,18]. This discrepancy might be a result of the mild insulin resistance developed in mice due to the weight loss in the first week of treatment and did not increase substantially throughout the treatment period. The mice lost weight in the first week after the switch from the commercial to the laboratory-made high-fat diet although they had the same energy density, protein concentration and similar fat content. The commercial high-fat diet contained 12.5% maltodextrin and 6.9% sucrose, while the high-fat diet prepared in the laboratory contained 12.5% starch and 10% fructose. In addition, the high-fat diet prepared in the laboratory contained 2.5% of sunflower oil instead of soybean oil and additional 0.19% cholic acid and 1.5% of cholesterol. It is not possible to conclude if these differences in the amount and composition of specific carbohydrate ingredients and other compounds led to the weight loss and partial reverse of hyperglycemia at the beginning of the treatment, which is worth further investigation. Although fasting blood glucose and insulin levels were not significantly different among the treatment groups and the HFC, the oral glucose tolerance test demonstrated

that the high-fat diet resulted in a significant impairment of oral glucose tolerance, which was substantially improved or reversed by replacing dietary casein with the same amount of protein from the shrimp protein concentrate.

The mechanism by which shrimp protein concentrate exerts its beneficial effects on glucose tolerance could be attributed to its specific amino acid composition. It is reported that leucine and isoleucine modify glucose homeostasis and insulin secretion [20,21]. Some dipeptides characterized by the presence of branched-chain amino acids stimulated glucose uptake and glycogen synthesis rate after oral administration [22] and these peptides could be released during *in vivo* enzymatic hydrolysis of marine proteins following their ingestion. These peptides may stimulate glucose uptake in skeletal-muscle cells and isolated muscles via the phosphoinositide 3-kinase and atypical protein kinase C pathways, which are different from the insulin signaling pathway. Another possible mechanism that has been postulated is the inhibitory action on the angiotensin-converting enzyme. It is reported that angiotensin converting enzyme inhibition by protein hydrolysates increases glucose metabolism by improving insulin secretion and sensitivity [23,24]. In the present study, although not significant the T35 and T70 consistently resulted in higher serum insulin levels than the HFC group. The angiotensin converting enzyme inhibitory peptides identified in some fish hydrolysates have been found to promote the serum levels of bradykinin and increase glucose transport [25,23, 26,24] and the expression of adiponectin, hence improving insulin sensitivity [8]. A similar relationship between angiotensin-converting enzyme inhibitory peptides of a sardine hydrolysate and suppression of rising blood-glucose levels after glucose loading was observed in an animal study [27]. A study in Chinese patients with T2DM demonstrated that the supplementation of marine collagen peptides from fish hydrolysate at a daily feeding rate of 13 g for 3 months significantly reduced the fasting blood glucose, glycated hemoglobin A1c, fasting blood insulin, TAG, total cholesterol, low-density lipoprotein cholesterol, and free fatty acid levels but increased high-density lipoprotein cholesterol levels and insulin sensitivity index after 1.5 or 3 months [8]. Significant decreases in high-sensitivity C-reactive protein and nitric oxide but increases in bradykinin, prostaglandin I_2, and adiponectin were also detected after consumption of marine collagen peptides from fish hydrolysates [8].

In addition to proteins, shrimp protein concentrate contained 14% lipids, contributing to approximately 1.3% and 2.6% lipids in the T35 and T70 diets. Shrimp oil has a better fatty acid profile than lard, in terms of unsaturated fatty acids, especially polyunsaturated omega-3 EPA and DHA. Many studies have demonstrated the beneficial effects of omega-3 fatty acids on insulin sensitivity [28,29]. The lipids in the shrimp protein concentrate also contained a significant amount of astaxanthin, vitamins E and A, which are strong anti-oxidants and have been reported to positively affect insulin function and glucose metabolism [30,31].

In summary, the present study demonstrated that replacing dietary casein with the same amount of protein from the shrimp protein concentrate improved the impaired glucose tolerance and semi-fasting blood glucose in high-fat diet-induce obese mice. The beneficial action of shrimp protein concentrate on insulin sensitivity occurred without reductions in body weight or adiposity, strongly suggesting that shrimp protein concentrate may protect against obesity-related insulin resistance by increasing insulin sensitivity and thus glucose disposal in the peripheral tissues. The anti-insulin resistant benefit of shrimp protein concentrate might be attributed to its specific composition of amino acids, fatty acids and antioxidants. Shrimp protein concentrate could be a novel functional ingredient for preventing insulin resistance and diabetes or improving insulin resistance in diabetic patients as a co-treatment product with diabetic drugs. Further investigations are needed to verify the observed benefits and further look into the underlying mechanisms.

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