Interaction Between Dietary Proteins and Lipids in the Regulation of Serum and Liver Lipids in the Rabbit. Effect of Fish Protein

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Purified diets varying in dietary protein, namely casein (CA), soy protein (SP), fish protein (FP), and lipid origin (corn oil (CN), coconut oil (CO)) were fed to rabbits to evaluate the effects of protein and fat source, as well as protein-lipid interactions, on serum total, lipoprotein and hepatic lipid levels. Dietary proteins and lipids exerted a separate effect on serum total cholesterol (C), very low-density lipoprotein cholesterol (VLDL-C), and low-density lipoprotein cholesterol to high density lipoprotein cholesterol (LDL-C/HDL-C) ratio. Hence, CA increased serum cholesterol compared to SP, while coconut oil enhanced serum and VLDL-C, and decreased LDL-C/HDL-C compared to corn oil. Dietary proteins interacted with dietary lipids to modulate HDL-C levels. Thus, FP maintained a high level of HDL-C regardless of lipid origin, compared to CA and SP whose HDL-C levels were decreased by corn oil, compared to coconut oil. A dietary protein-lipid interaction was also observed in the regulation of liver cholesterol levels. Coconut oil, compared to corn oil, decreased liver cholesterol in rabbits fed FP, whereas hepatic cholesterol concentration was unaltered by dietary lipid source in CA- and SP-fed rabbits. These results demonstrate that dietary proteins act synergistically with dietary lipids to regulate cholesterol metabolism in the rabbit.


The hypercholesterolemic effect of casein compared to soy protein, irrespective of the high saturated or polyunsaturated nature of the fats included in the purified diets of rabbits, is well established (1,2). Nevertheless, the origin of dietary lipids has been shown to affect the magnitude of the cholesterolemic response of rabbits to dietary proteins (3). Butter, a fat rich in saturated fatty acids, has been reported to intensify casein-induced hypercholesterolemia compared to corn oil, a fat rich in polyunsaturated fatty acids (2).

The effect of fish protein as well as the modulatory role exerted by saturated and unsaturated dietary lipids included in a fish protein diet on cholesterol metabolism are more variable and still poorly understood. While fish protein exerts a cholesterolemic action similar to that of casein when fed to the rabbit as part of a low-fat (1%) corn oil diet (2), fish protein is hypocholesterolemic compared to casein, when included in a high-fat (14%) coconut oil diet (4). In a corn oil-coconut oil diet with an intermediate lipid content (5%), fish protein exerts a cholesterolemic action intermediary to that of casein and soy protein (5).

Meanwhile, regardless of the lipid content of the purified diet, fish protein, compared to casein (4,5) and soy protein (5), has consistently been shown to increase high-density lipoprotein cholesterol (HDL-C) levels in the rabbit. The variation in serum cholesterol levels induced by fish protein when included in purified diets which differ in their lipid content and origin suggests that dietary proteins and lipids may act synergistically to regulate cholesterol metabolism in the rabbit.

Numerous studies have been conducted to determine the individual effects of various dietary proteins (1,2) or lipids (6) on serum and lipoprotein cholesterol levels. However, studies that have been statistically designed to isolate the dietary protein effects from the dietary lipid effects in rabbits (3,7) and other animal models (8–10) are scarce. It is therefore not possible at present to identify with certainty which specific nutrients are responsible for serum total and lipoprotein cholesterol changes subsequent to feeding purified diets. Inasmuch as dietary proteins and lipids are normally consumed as part of a mixed diet and are thus liable to exert an overall effect on cholesterol metabolism, the present study was designed by means of a 3 X 2 factorial model, to isolate the dietary protein effect from the dietary lipid effect, and to detect the interaction between dietary proteins and lipids simultaneously present in purified diets on cholesterol metabolism in the rabbit. For this purpose, rabbits were fed casein, soy protein and fish protein in purified diets differing in their lipid sources. The latter consisted of either corn oil, rich in n-6 polyunsaturated linoleic acid (18:2n-6), or coconut oil, rich in saturated lauric acid (12:0), two dietary lipid sources known to have contrasting effects on lipid and lipoprotein metabolism (6).

MATERIALS AND METHODS

Experimental animals. Upon arrival, 53 male New Zealand white rabbits were housed individually in stainless steel cages with mesh floors, suspended over sawdust in a room maintained under constant conditions of temperature (20–24°C), lighting (12-hr cycle) and humidity (45–55%). Following a 5-day adaptation to the animal quarters where animals were maintained on a non-purified diet (NPD) (Performance blend no. 5301, Ralston Purina, Québec, Canada), rabbits were divided into 6 experimental groups balanced for initial body weight (1400 g) and gradually adapted to their experimental rations by feeding mixtures of NPD and purified diets over a 5-day period. For the duration of the 30-day study, fresh food and water were provided once daily on an ad libitum basis. Food
intake was measured daily while growth of animals was monitored twice a week.

**Purified diets.** In the present study high fat (11%), cholesterol-free, powdered purified diets, assigned to rabbits in a 3 × 2 factorial arrangement, consisted of either casein-corn oil (CA-CN), casein-coconut oil (CA-CO); soy protein-corn oil (SP-CN), soy protein-coconut oil (SP-CO); fish protein-corn oil (FP-CN) and fish protein-coconut oil (FP-CO). The composition of the corn oil and coconut oil based purified diets is detailed in Table 1. The coconut oil diets were supplemented with 1% corn oil in order to meet the essential fatty acid requirements of the rabbit (11). The nutrient content of the vitamin and mineral mix has been described in a previous study (5). Casein and soy protein were purchased from ICN Biomedicals (Cleveland, OH). Fish protein, prepared in our laboratory, was obtained by lyophilization of frozen cod fillets which were then delipidated in an industrial Soxhlet-type apparatus during 12-hr cycles, using a mixture of ethyl acetate/hexane (1:1, v/v) in order to extract both neutral and polar lipids. The delipidation procedure was done with the collaboration of the Food Research and Development Centre (St-Hyacinthe, Quebec, Canada). Residual lipid content of casein (0.02%), soy protein (0.4%) and fish protein (0.2%) was verified with a Goldfish Lipid Extractor (model 35001, Labconco Corporation, Kansas City, MO). The protein content (N × 6.25) of casein (88.5%), soy protein (67.4%) and fish protein (92.0%) was assayed by the Kjeldahl method using a Kjell Foss auto analyzer (Model 16210, Foss Co., Hillerod, Denmark). The level of protein in the purified diets was then adjusted to 20% on an isonitrogenous basis, at the expense of cornstarch. As confirmed by bomb calorimetry, the CA-CN (4.71 kcal/g), CA-CO (4.64 kcal/g), SP-CN (4.65 kcal/g), SP-CO (4.56 kcal/g), FP-CN (4.59 kcal/g) and FP-CO (4.43 kcal/g) purified diets were isocaloric providing an average of 4.60 kcal/g of feed. The American Institute of Nutrition (AIN) mineral mix, corn starch, and cellulose (Alphacel) were purchased from ICN Biomedicals. Coconut oil was obtained from Sigma Chemical Company (St. Louis, MO) and the vitamin mix was purchased from Teklad test diets (Madison, WI). Corn oil was bought from a local food market.

**Serum, lipoprotein and hepatic lipid analyses.** At the end of the 30-day experimental period, 18-hr fasted rabbits were submitted to a light ketamine-xylazine anesthesia (0.4 mL/kg body weight) before extracting blood by cardiac puncture. Blood samples were allowed to clot at room temperature, followed by low-speed centrifugation to isolate serum. Livers were also removed, weighed, frozen in liquid nitrogen, and stored at −70°C until subsequent chloroform/methanol (2:1, v/v) extraction of hepatic lipids by the method of Folch et al. (12). All 4-mL aliquots of serum were stained with Sudan black dye (13) to later visualize the lipoprotein bands, and were submitted to a 25-hr discontinuous gradient ultracentrifugation (14) using a SW-41 swinging bucket rotor in a Beckman ultracentrifuge, model L8 M70 (Palo Alto, CA). Very low-density lipoproteins (VLDL; d < 1.006 g/mL), low-density lipoproteins (LDL; 1.006 < d < 1.063 g/mL) and high-density lipoproteins (HDL; 1.063 < d < 1.21 g/mL) thus separated were collected by aspiration and frozen at −70°C until determination of their cholesterol and triglyceride (TG) contents.

Serum, liver and lipoprotein cholesterol and TG concentrations were determined enzymatically using the CHOD-PAP and triglycerides without free glycerol kits supplied by Boehringer Mannheim (Laval, Quebec, Canada) according to the methods described by Siedel et al. (15) and Kohlmeier (16), respectively.

**Statistical analyses.** Data were subjected to an analysis of variance (ANOVA) according to a 3 × 2 factorial arrangement (17) in a randomized block design using the statistical analysis system (SAS) general linear model (GLM) procedure allowing for missing cells in factorial models (18), in order to determine the main protein and lipid effects, and to identify any interaction among dietary protein and lipid sources (P × L) at a probability level inferior to 0.05. Overall dietary protein and lipid effects, as well as protein-lipid interactions detected by ANOVA, were then submitted to least square mean (LSmean) multiple comparisons (18) to identify the statistical differences (P < 0.05) among the groups. All data were log transformed (18) prior to statistical analysis in order to make comparisons among homogeneous means. Values in tables and figures are however expressed as the original means ± SEM.

**RESULTS**

Food intake, weight gain and gross weight gain efficiency (GWGE) [g of body weight gained/g of food ingested daily] are presented in Table 2. Although the CA-CO rabbits had a significantly (P < 0.05) lower food intake than the animals of the other groups, weight gain and GWGE were similar in all dietary groups. These results indicate that all experimental rations supported normal and linear growth throughout the study period and were thus nutritionally adequate and equivalent.

Mean serum total cholesterol, VLDL-C, LDL-C and LDL-C/HDL-C are listed in Table 3. The overall analyses of variance performed on these data are reported in the top half of Table 4, while the multiple comparisons are shown on the bottom half of this table. As indicated by the P values lower than 0.05, both dietary proteins and lipids separately and significantly affected serum total cholesterol, VLDL-C, and the LDL-C/HDL-C ratio. Hence feeding FP, as well as CA, significantly (P < 0.05) increased serum total cholesterol compared to SP. By comparison, VLDL-C concentrations were significantly (P < 0.05) decreased by FP, in contrast to the CA and SP groups

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**TABLE 1**

**Composition of the Purified Diets**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Corn oil diets</th>
<th>Coconut oil diets</th>
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<tbody>
<tr>
<td>Protein</td>
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</tr>
<tr>
<td>Mineral mix</td>
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<td>5</td>
</tr>
</tbody>
</table>

*Composition of the vitamin and mineral mix has been described previously (5).*