develop atheroma plaque within a few months, with the aim of evaluating the relationships between chronic fat intake, postprandial lipemia and atheroma plaque deposition.

Thirteen NZW male rabbits, 12–13 weeks old, weighing 2.20–2.40 kg were fed two different diets: a low fat (3% lipids), no cholesterol (LF, $n = 6$) or a high fat diet (HF, $n = 7$) with 0.17% cholesterol and 5.20% lard. On days 0 and 42 for LF rabbits and on days 14, 28, 42, 63, 84 for HF rabbits, the animals received a test meal (25 g bolus) containing 6 g peanut oil and 200 mg cholesterol with $^{3}$H-cholesterol and $^{14}$C-triolein. Blood samples were collected after a 24 h fast and 10, 24, 34 and 48 h after the test meal intake. Lipoproteins (chylomicrons + very low density lipoproteins [VLDL] or triglyceride-rich particles, low density [LDL] and high density lipoproteins [HDL]) were isolated by ultracentrifugation. Triglycerides, free and esterified cholesterol and phospholipids were assayed. On day 105, lipoprotein lipase (LPL) and hepatic lipase (HL) activities were measured on postheparin plasma. On day 119, the rabbits were killed and the atheroma plaques were quantified in the aorta.

Fasting lipids and lipoproteins as well as postprandial lipemia after the test meal intake were not modified in the LF rabbits and the extent of atheroma plaque was 0%. The HF diet induced hypercholesterolemia and hyperphospholipemia after a few weeks and led to 30.87 ± 3.76% ($P < 0.05$) of the rabbit aorta being covered by atheroma plaque. In HF rabbits, postprandial plasma triglycerides peaked after 24–36 h and the 0–48 h AUCs triglycerides steadily increased as rabbits became more hypercholesterolemic and were significantly higher than in LFs. Plasma $^{14}$C-triolein and $^{3}$H-cholesterol concentrations were higher in HF rabbits than in LFs during the postprandial period and were proportional to fasting cholesterolemia. LPL and HL activities were two-fold higher in HF rabbits than in LFs on day 105.

The present study shows that i) as compared to a LF diet, ingestion of a HF cholesterol diet led to stepwise increases in fasting plasma cholesterol, postprandial lipemia and postprandial occurrence of dietary lipids in the circulation; ii) these changes are related to a dramatic increase of atheroma deposition; iii) these changes could originate from the inability of hepatocytes to remove intestinal lipoproteins even when the lipase activity levels are doubled.

**Sequential hydrolysis of bovine caseins in the preparation of a low-phenylalanine fraction for the treatment of phenylketonuria.** E Perrin, L Miclo, A Driou, JL Gaillard, G Linden *(Laboratory of Food Bio-Sciences associated with Inra, Faculty of Sciences, University H-Poincaré Nancy-I, BP 239, 54506 Vandœuvre-lès-Nancy cedex, France)*

Children with phenylketonuria – an autosomal recessive disorder, concerning about one birth per 15 000, caused by reduction or absence of activity of hepatic phenylalanine hydroxylase (EC 1.14.16.1) – are constrained to a strictly controlled phenylalanine diet to prevent irreversible damage to the nervous system [Følling (1934) Hoppe-Seylers *Z Physiol Chem* 227, 169-176].

Proteic intake is made of an equilibrated amino acid mixture without phenylalanine. This bad tasting mixture has to be taken in rather high quantities. The rest of the meal is composed of food with low protein content (mainly vegetables and fruits) in amounts corresponding to the minimal needs of phenylalanine for maintenance or growth.

This study evaluates a preparation of a low-phenylalanine hydrolysate made from bovine casein. Proteolysis was performed with two sets of proteases chosen for their ability to cleave proteins at the phenylala-

nine sites. A model study was performed by sequential hydrolysis of purified $\alpha_{s1}$-casein with immobilized pepsin (EC 3.4.23.1) and/or α-chymotrypsin (EC 3.4.21.1) (cleavage of Phe-X peptidic bonds) and immobilized thermolysin (EC 3.4.24.4) (cleavage of X-Phe peptidic bonds). Biochemical characterization of the hydrolysates was performed by analyzing: i) the molecular weight using size-exclusion chromatography; ii) the individual aromatic amino acid composition of the peptides using reversed-phase high-performance liquid chromatography and spectrophotometric analysis [Perrin et al (1995) *J Chromatogr* 664, 267-276]; iii) the global amino acid content.

Peptidic fractions showed a decreased phenylalanine content (reduction of phenylalanine concentration about three times). This could not, however, be directly used for phenylketonuria treatment due to the presence of free phenylalanine that had been released by proteolysis.

Further elimination of free phenylalanine was achieved by size-exclusion chromatography, hydrophobic affinity chromatography or electrodialysis.

We demonstrated that the residual phenylalanine level in the peptidic fraction was the consequence of the resistance of the hydrophobic fragment CN$\alpha_{s1}$-f(21-32) containing four phenylalanyl residues to the enzymes.

The hydrolysates have a more neutral taste and technofunctional properties than the mixture currently in use.

**Soy protein may have a hypercholesterolemic effect in rats and may potentiate lipoprotein susceptibility to peroxidation when its dietary level leads to methionine deficiency.** C Moundras, C Rémésy, MA Levrat, C Demigné *(Laboratoire des maladies métaboliques, Inra-Clermont-Ferrand/Theix, 63122 Saint-Genès-Champanelle, France)*

It is widely accepted that casein, compared to soy protein, is hypercholesterolemic. However, this effect depends on various factors, such as animal species, age, sex, dietary protein level and the presence of cholesterol in the diet. Casein and soy protein differ in their amino acid composition and the supply of methionine may be deficient with soy protein diets. Besides their effects on protein synthesis and growth, the sulfur amino acids are the precursors of important intracellular compounds such as taurine and glutathione (GSH). GSH is the major intracellular-SH molecule, and may be involved in a number of processes, including cellular protection against oxidative stresses.

The aim of the present study was to further investigate the effect of dietary protein on lipid metabolism (particularly cholesterol). In parallel, lipoprotein susceptibility to in vitro Cu-induced peroxidation was examined in rats adapted to moderate (13%) protein levels, with or without methionine supplementation (0.4%). This methionine level was selected to produce plasma and liver methionine concentrations similar to those present in casein fed rats: as a result, liver GSH concentrations were greatly elevated by methionine supplementation in soy protein fed rats (from 1.20 to 3.87 µmol/g liver).

In this experiment, it turned out that soy protein failed to lower plasma cholesterol and there was even a slight cholesterol-raising effect (+18%), despite a higher rate of bile acid excretion than in the rats adapted to the casein diet. In contrast, soy protein feeding resulted in a marked triglycerides-lowering effect. Methionine supplementation of the soy protein diet counteracted its hypercholesterolemic effect. It also elevated the plasma triglyceride levels. The elevation of plasma cholesterol in rats fed soy protein was characterized by higher cholesterol lev-