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Purified chickpea or lentil proteins impair VLDL metabolism and lipoprotein lipase activity in epididymal fat, but not in muscle, compared to casein, in growing rats

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Abstract *Background* It is well known that the legume proteins have a lowering effect on plasma cholesterol and triacylglycerols (TG) concentrations compared to animal proteins. The protein itself, as well as non-protein constituents, naturally present in legumes may be implicated. *Aim of the study* The effects of various dietary purified legumes proteins compared to casein, were determined on plasma TG level, VLDL concentration and composition. Moreover, lipoprotein lipase (LPL) activity in epididymal fat, gastrocnemius and heart was investigated to evaluate in these tissues their capacity to release free fatty acids from their TG substrate and the liver capacity to stock the TG. *Methods* Weaning male Wistar rats were fed ad libitum one of the following diets: 200 g/kg diet of purified proteins of lentil (L), or chickpea (CP) or casein (CAS). At day 28, VLDL were isolated from plasma sample by a single ultracentrifugation flotation. Hepatic lipase and LPL activity in epididymal fat, gastrocnemius and heart were measured by using glycerol tri [9–10(n)-³H] oleate emulsion as substrate. *Results* Compared with CAS diet, the CP and L protein diets exhibited similar

cholesterolemia, but lower triglyceridemia (1.9-fold and 2.5-fold) and VLDL particle number, as measured by their reduced contents of TG and apolipoproteins. CP and L protein diets reduced liver TG and cholesterol by 31 and 45%, respectively compared to CAS diet. Furthermore, LPL activity in adipose tissue of rats fed CP or L was 1.6-fold lower than that of rats fed CAS. There was no significant difference in heart and gastrocnemius LPL activities with the three proteins. In contrast, hepatic lipase activity was higher in rats fed CP and L diets. *Conclusion* The low food efficiency ratio of purified CP and L proteins related to CAS is associated with decreased plasma VLDL and adipose tissue LPL activity. The low liver TG concomitant with reduced TG and apolipoproteins contents of VLDL confirm that hypotriglyceridemia is essentially due to impaired synthesis, exportation and transport of TG by VLDL which prevent lipid storage in adipose tissue.

Key words chickpea – lentil – lipoprotein-lipase – hepatic-lipase – VLDL metabolism – rat

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Introduction

It is well known that plant proteins, particularly soybean protein have a lowering effect on plasma cholesterol and triacylglycerols concentration compared to animal proteins [11, 26]. The protein itself and its amino acid composition, as well as nonprotein constituents, naturally present in soybean isolate such as isoflavones, may be implicated [41]. These effects are somewhat variable, but generally are greater in the hypercholesterolemic than in normocholesterolemic situations [36].

Although most of the studies carried out using soybean, other legume seeds species such as lentils [10, 19], chickpeas [33, 42, 45, 46] or faba bean [23], have also shown hypocholesterolemic and hypotriglyceridemic properties in experimental animals and human [31, 43].

However, the effect of these purified legumes seeds proteins on triglyceridemia and VLDL metabolism is less documented. We have previously demonstrated that highly purified soybean protein free-cholesterol diet did not change plasma total cholesterol, but involved a lower triacylglycerol (TG) level and very low density lipoprotein (VLDL) particles number as measured by diminished VLDL-TG and -apo B concentrations [25]. Conversely, Demonty et al. [12] have reported that using soybean-protein isolate in hypercholesterolemic rats lowered both of plasma cholesterol and triacylglycerol concentrations.

The mechanisms responsible for the decrease in plasma TG concentrations associated with consumption of legumes proteins involve a reduction in hepatic TG and VLDL synthesis and secretion, and increased VLDL uptake by liver. Indeed, Sirtori et al. [35] and Madani et al. [25] have shown in rats with cholesterol-free diet that VLDL binding to membranes is markedly enhanced by a cholesterol enriched soybean protein compared with a casein diet. Moreover, the activity of lipoprotein lipase (LPL) and hepatic lipase (HL) which involve the catabolism of triglycerides-rich lipoprotein may have also a potential mechanism of action on lowering plasma TG concentrations. Nevertheless the very few studies that have examined this issue showed that soy protein had little or no effect on postheparin-plasma LPL activities [12, 15].

Indeed, LPL which expressed in a variety of extrahepatic tissues plays an important role in lipoprotein metabolism through an efficient transfer of energy in the form of lipids from the sites of synthesis (gut and liver) to those of storage or use. This enzyme which requires, for maximal activity apo C-II, catalyzes the hydrolysis of TG contained in VLDL and chylomicrons leading to the release of free fatty acids and their subsequent uptake by muscles for energy

production or adipocytes for reesterification and storage [37]. Therefore the LPL activity is an important determinant in the tissue ability to uptake the plasma free fatty acids from TG.

Furthermore, HL which is a key enzyme involved in lipoprotein metabolism is almost exclusive to hepatic sinusoids; its catalytic activity contributes to hydrolyze phospholipids and TG of VLDL remnants, low density lipoprotein (LDL) and high density lipoprotein (HDL) [44].

It has been reported that LPL regulation is often opposite in adipose tissue and muscle in response to the same nutritional treatment. Indeed, starvation is associated with a decrease in LPL activity in adipose tissue while it increases or remains unchanged in oxidative skeletal muscles [6].

The effects of the legumes protein diets on LPL and HL activities compared to casein diet are less documented. Demonty et al. [11, 12] showed that soy protein and casein fed by rats do not affect LPL, but decrease hepatic lipase. Although these studies clearly demonstrate that legumes protein compared with casein decrease plasma TG; no comparative study has been conducted on the importance of extrahepatic LPL activities related to the VLDL concentrations and composition when rats were fed chickpea or lentil protein versus casein without cholesterol. Recently, Yang et al. [43] reported that epididymal adipose tissue LPL and hepatic lipase activity of rats increase with high fat diet compared to normal fat diet rats dietary chickpeas completely normalized these enzymatic activities.

The aim of the present study was to determine the effect of two dietary legumes proteins compared to casein, on plasma TG level, VLDL concentrations and composition. Moreover, LPL activity in epididymal fat, gastrocnemius and heart was investigated to determine in these tissues their capacity to release free fatty acids from TG and the liver capacity to stock the TG.

In the present study, two types of dietary proteins (purified chickpea and lentil proteins) were tested and compared with casein as control. We have chosen chickpea and lentil proteins because these legume seeds are commonly consumed in the Mediterranean area and in numerous developing countries, particularly in Algeria.

Materials and methods

■ Preparation of purified chickpea and lentil proteins

Protein extraction process was performed according to Swanson [39]. Lentil (*Lens culinaris*) or chickpea (*Cicer arietinum*) flours were diluted in distilled water

containing sodium sulphite (10 mM/l) in a ratio of 1:10 (w:v), after homogenization, the pH was adjusted to 10 with 1 M-NaOH. After sedimentation for 12 h at 4°C, the supernatant was obtained and brought to pH 4.5 with 5 M-H₂SO₄. Proteins were removed after centrifugation (3,000×g for 20 min), then rinsed with distilled water. The purified proteins were dried at 37°C in ventilated oven (Memmert, France) during 2 days. CP and L protein concentrations were estimated by their nitrogen contents, using mineralization followed by coloration with Nessler's reactive agent and ammonium nitrate as a standard. Then the values were multiplied by 6.25 to obtain proteins concentrations which represented 92% in CP and 90% in L. The protein content of casein (Prolabo, France) was 96%.

■ Animals and diets

Male Wistar rats at weaning ($n = 18$, Iffa Credo l'Arbresle, Lyon, France) weighing 65 ± 5 g at the beginning of the experiment were allowed for free access to a commercial pellet diet (A03 UAR, Villemoisson/Orge, France) for 5 days. After this adaptation period, when their weight was 80 ± 5 g, they were randomly divided into three groups and fed during 28 days 200 g protein/kg diet from different sources (purified lentil protein (L) or chickpea protein (CP). The control group was fed 200 g casein /kg diet (CAS).

The detailed composition of these isoenergetic diets was shown in Table 1. Each rat was allocated in a single wire bottom cages with controlled temperature (24°C), humidity (60%) and lighting (12 h-cycles). They had ad libitum access to fresh food and tap water. Food intake and body weight were measured daily. The general guidelines of the Council of European Communities [8] for the care and use of laboratory animals are followed.

■ Sampling procedure

At day 28, rats were food deprived for 12 h and anaesthetised between 09.00 and 10.00 h by intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight). The blood was drawn from abdominal aorta into tubes containing Na₂-EDTA (1 g/l blood) and plasma was then recovered by centrifugation at 600×g for 20 min. Organs were removed, washed with cold saline (150 mM-NaCl), quickly excised, blotted and weighed.

■ Lipids extraction

One gram of liver was extracted with chloroform:methanol (2:1, v:v) according to the method of

Table 1 Composition of experimental diets (g/kg diet)^a

Protein	Casein (CAS)	Chickpea (CP)	Lentil (L)
Casein ^b	200	–	–
Chickpea protein ^c	–	200	–
Lentilprotein ^c	–	–	200
Corn starch ^d	550	550	550
Sucrose	40	40	40
Olive oil ^e	100	100	100
Cellulose powder ^f	50	50	50
Mineral mix ^g	40	40	40
Vitamin mix ^h	20	20	20

^aDiets were semipurified, isoenergetic (16.97 MJ/kg of diet) and given in powdered form

^bProlabo, Paris France: provided the following amino acids as g/kg of protein: Arg: 36; His: 34; Ile: 42; Leu: 92; Lys: 74; Val: 57; Thr: 46; Phe: 49; Tyr: 53; Met: 23; Cys: 2; Ala: 30; Asp: 66; Glu: 207; Gly: 18; Pro: 116; Ser: 55

^cProteins purified from dry seeds in our laboratory. Chickpea protein provided the following amino acids as g/kg of protein [16]: Arg: 84; His: 30; Ile: 48; Leu: 87; Lys: 72; Val: 46; Try: 9; Thr: 31; Phe: 55; Tyr: 28; Met: 11; Cys: 6; Ala: 49; Asp: 110; Glu: 173; Gly: 37; Pro: 38; Ser: 37. Lentil protein: Arg: 78; His: 22; Ile: 41; Leu: 78; Lys: 70; Val: 50; Try: 7; Thr: 35; Phe: 50; Tyr: 32; Met: 8; Cys: 9; Ala: 42; Asp: 118; Glu: 215; Gly: 36; Pro: 35; Ser: 52

^dUAR (Villemoisson, 91360 Epinay sur Orge, France)

^eCommercial product, olive oil provided the following fatty acids (g/100 g of total fatty acids) saturated: 15; monounsaturated: 76; polyunsaturated ($n - 6$): 8.3; polyunsaturated fatty acids ($n - 3$): 0.7

^fProlabo, Paris, France

^gUAR 205B, (Villemoisson, 91360 Epinay sur Orge, France), the salt mixture provided the following amounts (mg/kg diet): CaHPO₄, 17,200; KCl, 4,000; NaCl, 400; MgO, 420; MgSO₄, 2,000; FeO₃, 120; FeSO₄·7H₂O, 200; trace elements, 400; MnSO₄·H₂O, 98; CuSO₄·5H₂O, 20; ZnSO₄, 80; CoSO₄·7H₂O, 0.16; KI, 0.32

^hUAR 200, (Villemoisson, 91360 Epinay sur Orge, France), vitamin mixture provided the following amounts (mg/kg diet): retinol, 12; cholecalciferol, 0.125; thiamine, 40; riboflavin, 30; panthotenic acid, 140; pyridoxine, 20; inositol, 300; cyanocobalamin, 0.1; ascorbic acid, 1,600; di- α -tocopherol, 340; menadione, 80; nicotinic acid, 200; para-aminobenzoic acid, 100; folic acid, 10; biotin, 0.6

Folch et al. [14]. After solvent evaporation under nitrogen stream, lipid extract was dissolved in isopropyl alcohol and immediately measured.

■ VLDL isolation

The technique used has been detailed in a previous paper [27]. Plasma samples (2 ml) were overlaid with 4 ml 0.15 M-NaCl and 1 mM-Na₂EDTA, pH 7.4 (density 1.006 kg l⁻¹). Floating VLDL were isolated by ultracentrifugation at 15°C for 17 h at 108,000×g in a Beckman L8-55 ultracentrifuge equipped with a 50 Ti rotor (Beckman Instrument, Palo Alto, CA). VLDL fractions were subjected to partial lyophilization, followed by a rapid delipidation with cold diethyl ether in order to avoid the precipitation of high molecular mass apolipoproteins. Apo-B100 was estimated by electrophoresis (SDS-PAGE), with 2.5–20% acrylamide according to the method of Irwin et al. [17]. Electrophoresis was performed in a LKB 2001.001 vertical electrophoresis unit (LKB produkte, Bromme, Sweden) at 4°C for 18 h with 20 mA/gel

slab. Gels were then stained with Coomassie Brilliant blue G250. The stained gels were scanned at 600 nm with a densitometer (Model Profil 26, Sebia, Issy les Moulineaux, France). To estimate the concentration of apo-B100, the percentage of the area was multiplied by total apolipoprotein concentration of each plasma sample.

Chemical analysis

Liver and plasma VLDL phospholipids were estimated by phosphorus contents according to the method of Bartlett [2]. Plasma, VLDL and liver TG, free and total cholesterol concentrations were assayed by enzymatic methods (kits Boehringer Mannheim, Meylan, France) by using respectively glycerol and cholesterol as standard. Protein contents of VLDL were measured by the method of Lowry et al. [22] using bovine serum albumin (Sigma Chemical, St Louis, MO) as standard.

Substrate preparation for lipoprotein lipase activities

The substrate emulsion was prepared according to Nelsson-Ehle & Eckman method [29]. A total of 7 mg unlabeled triolein (Sigma Chemical, St Louis, MO) were mixed with 5.4 μ Ci glycerol tri [9-10(n)-³H] oleate (NEN, Boston, MA) and 0.3 mg lysophosphatidylcholine (Sigma Chemical, St Louis, MO). After solvent removal under N₂ stream, the mixture was sonicated with 2.4 ml of 0.2 M-Tris buffer pH 8.2. After sonication, 0.3 ml of 4% bovine serum albumin fraction V and 0.3 ml of heat inactivated serum (providing apo C-II, an activator of LPL) were added to the emulsion.

Lipolytic activity determination

LPL (EC 3.1.1.34) and HL (EC 3.1.1.32) activities were measured according to the Bengtsson-Olivecrona & Olivecrona method [4] as following: 100 mg of epididymal fat pads, heart, gastrocnemius or liver were homogenized in ultraturax (Basic T25 Ika Werke) with 0.9 ml ice-cold buffer containing 0.025 M-NH₃, antiproteases (10 μ g/ml leupeptin, 1 μ g/ml pepstatin, 25 IU/ml aprotinin), 5 mM-EDTA, 5 IU/ml heparin, 100 mg/ml CHAPS and 0.08% (w/v) sodium dodecyl sulfate (SDS) (for HL extraction, SDS was substituted by 0.4% (w/v) triton X100) and adjusted at pH 8.2. The homogenates of each tissue were centrifuged at 1,200×g, at 4°C for 20 min. A total of 10 μ l of the fraction between the upper fat layer and the bottom sediment was collected for lipoprotein lipase assay. The incubation was performed in duplicate for 1 h at

28°C with 100 μ l of substrate prepared according to the method of Nelsson-Ehle & Eckman [29]. For HL determination, supernatant was adjusted to 1 M-NaCl to inhibit LPL activity and heat inactivated serum was omitted in the substrate preparation. At the end of the incubation period, released free fatty acids were extracted according to the Belfrage & Vaughan method [3] with a 2-phase solvent system: 3.5 ml CH₃OH:CH₃Cl:heptane (1.41:1.25:1, v/v/v) and 1.05 ml 0.1 M-tetraborate-carbonate pH 10.5.

[³H] radioactivity in 1.5 ml aliquots of the methanol/water upper phase was measured in 10 ml of scintillant (Ultima gold XR; Perkin Elmer, Boston, MA) in a 7500 LS scintillation counter (Beckman, Palo Alto, CA). Enzyme expressed as mU/mg proteins (one milliunit of enzyme activity corresponded to 1 nmol of fatty acid released per min at 28°C). Proteins were estimated according to the Lowry et al. method [22].

Statistical analysis

Data analysis was conducted by using STATISTICA (version 4.1, Statsoft, Tulsa, OK). Values are means \pm SEM. Data were subjected to ANOVA and Duncan's multiple range test [13]. A difference of $P < 0.05$ was considered significant.

Results

Body weight and energy intake

At day 28 of the experimental period, body weight and food efficiency ratio of rats fed CP and L diets were similar and lower than those of CAS group ($P < 0.05$). However, food and absolute energy intakes were similar in the three groups (Table 2).

Relative weights of organs

The relative weight expressed in g/kg BW of gastrocnemius, heart and liver were similar. In contrast,

Table 2 Body weight, food and energy intakes of rats fed diets containing 200 g/kg diet of purified protein of chickpea or lentil compared with casein

	Casein (CAS)	Chickpea (CP)	Lentil (L)
Body weight (g)	229.4 \pm 29.3 ^a	189.8 \pm 7.6 ^b	175.4 \pm 9.2 ^b
Food efficiency ratio	0.31 \pm 0.05 ^a	0.21 \pm 0.05 ^b	0.21 \pm 0.05 ^b
Weight gain (g/d/rat)	5.62 \pm 1.40 ^a	3.80 \pm 0.90 ^b	3.30 \pm 1.01 ^b
Food intake (g/d/rat)	17.53 \pm 1.50 ^a	17.00 \pm 1.22 ^a	15.13 \pm 1.96 ^a
Energy intake (kJ/d/rat)	315 \pm 27 ^a	305 \pm 22 ^a	272 \pm 36 ^a

Values are means \pm SEM of 6 rats per group. ^{a,b} means within a row with unlike superscript letters were significantly different (one-way ANOVA and Duncan's multiple range test, $P < 0.05$)

the relative weight of epididymal fat pads of CP and L groups were significantly lower than those of CAS group ($P < 0.05$) (Table 3).

■ Plasma and liver lipid concentrations

Plasma triacylglycerol concentrations in CP and L groups were respectively 1.9- and 2.4-fold lower than those of CAS group, whereas plasma total cholesterol (TC) and phospholipids (PL) levels were similar in the three groups. Compared with CAS group, liver TG concentrations were respectively 1.5- and 2-fold lower, whereas PL contents were significantly 1.2-fold higher in L and CP groups. Hepatic TC values of PC and L rats were 1.5- and 1.8-fold lower than those of CAS rats. However liver protein content was similar in the three groups (Table 4).

■ VLDL lipid concentrations and composition

Plasma VLDL mass calculated by adding their apolipoprotein and lipid components (TG, PL, unesterified cholesterol (UC) and cholesteryl esters (CE)), represented in CP and L groups, respectively 53 and 32% of the CAS group values. These lowered levels with CP

Table 3 Relative weights of epididymal fat, gastrocnemius, heart and liver of rats fed diets containing 200 g/kg diet of purified protein of chickpea or lentil compared with casein

Relative weight (g/kg BW)	Casein (CAS)	Chickpea (CP)	Lentil (L)
Epididymal fat	20.19 ± 2.70 ^a	16.70 ± 0.37 ^b	16.40 ± 0.29 ^b
Gastrocnemius	5.18 ± 0.20 ^a	5.41 ± 0.34 ^a	5.06 ± 0.28 ^a
Heart	3.00 ± 0.14 ^a	2.96 ± 0.16 ^a	2.90 ± 0.18 ^a
Liver	33.70 ± 1.60 ^a	33.50 ± 4.00 ^a	36.00 ± 3.50 ^a

Values are means ± SEM of 6 rats per group. ^{a,b} means within a row with unlike superscript letters were significantly different (one-way ANOVA and Duncan's multiple range test, $P < 0.05$)

Table 4 Plasma and liver lipid concentrations of rats fed diets containing 200 g/kg diet of purified protein of chickpea (CP), lentil (L) compared with casein (CAS)

	Casein (CAS)	Chickpea (CP)	Lentil (L)
Plasma (mmoles/l)			
Cholesterol	2.55 ± 0.33 ^a	2.52 ± 0.49 ^a	2.88 ± 0.56 ^a
Triglycerides	0.99 ± 0.23 ^a	0.53 ± 0.13 ^b	0.42 ± 0.19 ^b
Phospholipids	3.89 ± 0.75 ^a	4.21 ± 0.76 ^a	3.99 ± 1.12 ^a
Liver (μmoles/g)			
Cholesterol	19.31 ± 2.27 ^a	13.25 ± 1.95 ^b	10.96 ± 2.41 ^b
Triglyceride	18.41 ± 1.68 ^a	12.67 ± 3.41 ^b	9.20 ± 2.55 ^b
Phospholipids	20.18 ± 2.44 ^b	28.81 ± 3.17 ^a	27.63 ± 1.81 ^a
Protein content (mg/g)	154.60 ± 36.50 ^a	159.60 ± 40.60 ^a	132.20 ± 35.20 ^a

Values are means ± SEM of 6 rats per group. ^{a,b} means within a row with unlike superscript letters were significantly different (one-way ANOVA and Duncan's multiple range test, $P < 0.05$)

Table 5 VLDL composition of rats fed diets containing 200 g/kg diet of purified protein of chickpea or lentil compared with casein

	Casein (CAS)	Chickpeas (CP)	Lentils (L)
VLDL mass (g/l of plasma)	1.14 ± 0.32 ^a	0.61 ± 0.08 ^b	0.37 ± 0.1 ^c
VLDL apo (g/l of plasma)	0.21 ± 0.0 ^a	0.14 ± 0.02 ^b	0.14 ± 0.05 ^b
VLDL CE (mmol/l of plasma)	0.21 ± 0.04 ^a	0.14 ± 0.02 ^b	0.08 ± 0.04 ^b
VLDL UC (mmol/l of plasma)	0.05 ± 0.01 ^b	0.08 ± 0.03 ^a	0.06 ± 0.01 ^{ab}
VLDL TG (mmol/l of plasma)	1.20 ± 0.34 ^a	0.44 ± 0.05 ^b	0.19 ± 0.09 ^c
VLDL PL (mmol/l of plasma)	0.57 ± 0.04 ^a	0.32 ± 0.02 ^b	0.35 ± 0.09 ^b
Apo-B100 (AU)	125.0 ± 10.00 ^a	55.1 ± 16.00 ^b	30.00 ± 6.00 ^b

Values are means ± SEM of 6 rats per group. VLDL-mass was the sum of their apolipoproteins (apo), triacylglycerols (TG), cholesteryl esters (CE), unesterified cholesterol (UC) and phospholipids (PL) contents. ^{a,b,c} means within a row with unlike superscript letters were significantly different (one-way ANOVA and Duncan's multiple range test, $P < 0.05$)

and L diets respectively compared to CAS diet, were concomitant with their reduced composition in TG (−63 and −80%), apo (−33%), PL (−40%) and CE (−32 and −62%). VLDL-UC concentration was 1.6-fold higher in CP rats compared to CAS rats. Moreover, VLDL apo-B100 (the major VLDL apolipoprotein) was respectively 2.3- and 4-fold lower in CP and L groups (Table 5).

■ Tissue lipoprotein lipase activity

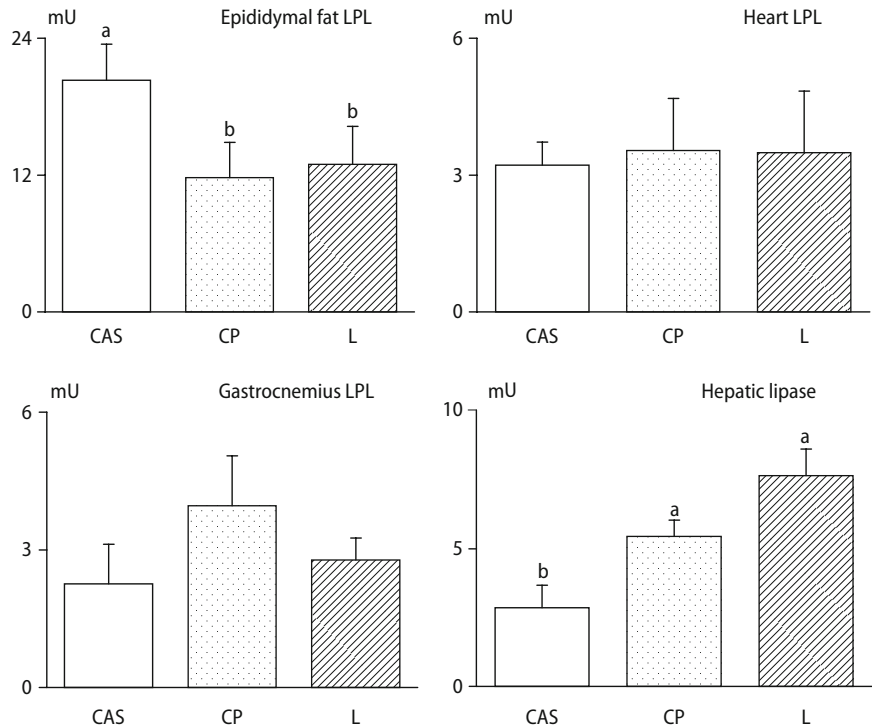
Compared to CAS group, epididymal fat LPL activity was respectively 1.7- and 1.5-fold lower in CP and L groups, whereas those of heart and gastrocnemius were similar in CP and L groups. In contrast, hepatic lipase activity was respectively 1.9- and 2.6-fold higher with CP and L diets compared with CAS diet (Fig. 1).

Discussion

The present investigation was conducted to determine whether VLDL levels and composition and tissue LPL activities were modified by purified chickpea and lentil proteins compared to casein.

In our experimental conditions, although we have not chose to use pair-fed controls for CP and L rats, the both groups ate the same quantity. Therefore, in spite of a similar energy intake (Table 2) in the three groups, rats fed CP and L diets showed a lower body weight gain than those of rats fed CAS diet. The reduced weight gain with CP and L diets could be attributable to the limited amino acids compared to casein, especially sulfur amino acids, associated with a lower protein efficiency ratio. Moreover, another explanation might be related to differences in the digestion-absorption process. Indeed, Rubio & Seiquer [34], Porres et al. [32] and Cuadrado et al. [9]

Fig. 1 Hepatic lipase and lipoprotein lipase activity of epididymal fat, gastrocnemius and heart of rats fed diets containing 200 g/kg of purified protein of chickpea (CP), lentil (L) compared with casein (CAS). Values are means \pm SEM of 6 rats per group. ^{a,b}Mean with different superscript letters were significantly different (one-way ANOVA and Duncan's multiple range test, $P < 0.05$)



have reported that the lower efficiency of protein utilization was due to a reduced net nitrogen-absorption from small intestine. In return, the two specific symptoms of protein malnutrition, i.e. liver steatosis and oedema, were not observed with these legumes proteins.

In the present study, compared to CAS rats, CP and L rats involved a significant decrease in plasma TG which reflected a low TG contents in VLDL. Plasma VLDL mass and their apo-B-100 concentrations were decreased in CP and L groups indicating a lower number of VLDL particles, as noted previously with highly purified soybean protein [24, 25]. The effects of these legume seed proteins on TG and VLDL cannot be attributed to energy intake which was similar among the three groups. The low plasma VLDL particle number and TG concentrations with purified chickpea and lentil proteins might reflect an increased uptake of VLDL and their remnants by liver, as reported previously with the same proteins [7].

The effects of these purified proteins could result from their limited essential amino acids composition. Indeed, it has been reported that essential amino acids (present in animal protein as casein) are relatively more effective in releasing insulin, whereas non-essential amino acids (present in plant protein as soybean) particularly arginine release preferentially glucagon [20]. Moreover, the higher arginine level noted in CP (8.4/100 g protein) and L (7.8/100 g protein) [21] compared with CAS (3.6/100 g protein)

increases VLDL uptake by liver as shown previously [7]. This lead to limited number of LDL particles available for the transport of cholesterol in the plasma. These results are in agreement with those of Macarulla et al. [23] using faba bean-protein isolate in hypercholesterolemic rats and with those of Lavigne et al. [21] using soybean-protein isolate. The low plasma VLDL-TG could also result from decreased TG synthesis by the liver and their transport by VLDL. The low content of some essential amino acids in PC and L diets could involve impaired apo-B-100 synthesis. Moreover, the low methionine level in the both diets has important effects on lipid metabolism. Indeed, the reduced VLDL concentration in rats fed chickpea and lentil proteins could be due to their low methionine content. This decrease was probably caused by the labile methyl group of methionine, this aminoacid being required as a substrate in conversion of phosphatidylethanolamine to phosphatidylcholine which is the main phospholipid required for assembly and secretion of apo B-containing lipoproteins [40].

Furthermore, TG-lowering effect of PC and L proteins compared with CAS might be caused by an alteration of mRNA transcript levels of the hepatic genes involved in the de novo synthesis of fatty acids as well as genes associated with TG hydrolysis. Indeed, Spielmann et al. [38] and Bettzieche et al. [5] showed recently that lupin protein lowered hepatic fatty acid synthesis compared with casein by inhibiting the sterol regulatory element-binding protein

(SREBP)-1c-mediated gene expression of lipogenic enzymes.

Whereas, the surprising higher HL activity obtained with purified lentil or chickpea proteins, could be explained by the lower liver cholesterol content. Indeed, it has been reported by Perret et al. [30] that an inverse relationship was found between the cultured hepatocyte cholesterol content and the levels of mRNA and activity of HL. This suggested that SREBP was involved in this regulatory process. Recently, Bettzieche et al. [5] have shown that lupin proteins reduce TG *via* down regulation of fatty acid synthesis (fatty acid synthase) genes and up regulation (but not statistically significant) of genes involved in TG hydrolysis (hepatic lipase). It was probably due to the moderate content of lupin proteins (5%) used by these authors compared with 20% of CP or L protein in our diets.

Moreover, the higher HL activity involved by CP and L could probably reduce TG- and PL- VLDL contents and increase their uptake via LDL receptor related protein (LRP). Indeed, Jansen et al. [18] reported that HL acts as a ligand in the binding of VLDL to LRP.

In the present study, chickpea and lentil protein diets compared to CAS did not affect heart and gastrocnemius LPL activities, but decreased LPL activity in epididymal fat.

The low LPL activity observed in epididymal fat of rats fed lentil or chickpea protein diets could probably diverted fatty acids availability from the lower VLDL-TG toward gastrocnemius and heart. The reduced LPL activity in this tissue was probably modulated in part, by a low level of its substrate (VLDL-TG) and in other part, by a low insulin level as shown by Baltzel et al. [1] who demonstrated that insulin was more specific for adipose tissue LPL. This could also

explain partly the responsiveness of LPL activities of gastrocnemius and heart of rats fed CP and L diets. Mulloy et al. [28] demonstrated that a diet containing 1% arginine, which is in close agreement with what is found in the chickpea protein (8.4/100 g protein) and the lentil protein (7.8/100 g protein) diets (Table 1) could probably induce lower plasma insulin concentration than a diet containing 0.5% arginine.

These data suggested that insulin could probably influence both the rate of hepatic TG synthesis and subsequent VLDL-TG secretion in the circulation and the rate of TG disappearance from the blood stream through its action on LPL and HL activities. Nevertheless, this hormonal hypothesis (insulin, glucagon) remains to confirm with these purified proteins.

Conclusion

Purified CP and L proteins compared to CAS have a low nutritional performance characterized by low food efficiency ratio and body weight but involve decreased plasma VLDL and adipose tissue LPL activity. The low liver TG and reduced -apolipoproteins and -TG contents of VLDL confirm that hypotriglyceridemia is essentially due to impaired synthesis, exportation and transport of TG by VLDL, preventing then a lipid storage in adipose tissue. Moreover, the enhanced HL activity can emphasize the hypotriglyceridemic effect of these purified proteins by facilitating the uptake of VLDL and their remnant via LDL receptor-related protein.

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