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Impact of dietary protein on lipid metabolism in hamsters is source-dependent and associated with changes in hepatic gene expression

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This study tested the hypothesis that protein source is a factor determining the impact of the diet on lipid metabolism in hamsters. Twenty-eight hamsters of similar body weight were assigned for a period of 8 weeks to one of the following four diets (seven per group) containing either 20 % (w/w) casein (CAS), beef protein (BF), wheat gluten (WG) or soya protein (SOY). The fat composition of the diet was the same (15·5 % w/w) in all groups and provided SFA, MUFA and PUFA representative of the average Canadian diet. After an overnight fast, blood and liver were collected for the measurement of serum lipids, fatty acid composition of liver phospholipids and mRNA levels of selected genes involved in lipid metabolism. WG resulted in lower total cholesterol, HDL-cholesterol and non-HDL-cholesterol but, along with SOY, in higher mRNA levels of cholesterol 7 α-hydroxylase and LDL receptor. Furthermore, both WG and SOY resulted in lower 18 : 3*n*-3, 20 : 4*n*-6, total *n*-6 PUFA, 18 : 1*n*-9 and total MUFA, but higher 22 : 6*n*-3, total *n*-3 PUFA, 22 : 6*n*-3/18 : 3*n*-3 and 22 : 5*n*-3/18 : 3*n*-3 ratios in liver phospholipids, and higher hepatic Δ6-desaturase mRNA levels. These results show that the impact of dietary protein on lipid metabolism is source-dependent and associated with changes in mRNA abundances of key hepatic enzymes and receptors.

Lipid metabolism: Fatty acids: Cholesterol: Gene expression: Hamsters

CVD are the leading causes of death in Canada[1] and incur tremendous costs for the Canadian health care system[2]. Therefore, primary prevention is the ultimate strategy warranted to reduce the rate of mortality from CVD and alleviate the economic burden of its morbidities. Defective lipid metabolism is a cluster of modifiable risk factors for CVD, including hypercholesterolaemia (total cholesterol (TC) and LDL-cholesterol (LDL-C)), hypertriacylglycerolaemia and low circulating concentrations of HDL-cholesterol (HDL-C), that can be corrected by diet.

The impact of the three classes of macronutrients on lipid metabolism has been investigated to various degrees, with that of dietary fats and cholesterol receiving the greatest attention[3]. Recent years have witnessed a surge in the popularity of high-protein diets for the management of body weight in obesity[4]. These diets were shown to be effective not only in inducing weight loss[5], but also in improving lipid profile and reducing CVD risk[6]. However, these and other effects appear to be dependent on the source of protein[7,8]. It is generally agreed that vegetable protein decreases circulating cholesterol concentrations compared to animal protein[9,10].

The limitations of most of these studies are the comparison of the effects of only two sources of protein at a time on lipid indices, the use of an animal model whose lipid metabolism in response to diet differs from that of man, and large variations in the quantity and quality of dietary lipids. The majority of studies compared the effects of soya protein (SOY) and casein (CAS)[10,11]. However, wheat gluten (WG) was also found to be hypocholesterolaemic compared to CAS[12,13]. When more than two dietary groups were included, the limiting amino acid was added to the investigated protein in an attempt to provide a mechanistic explanation for the observed effects. For example, the limiting amino acids lysine + threonine and methionine were added to WG and SOY, respectively, to elucidate their role in cholesterol and long-chain PUFA metabolism[14,15].

In addition, it is imperative to use an animal model whose lipid metabolism in response to dietary nutrients closely mimics that of man. The hamster, in opposition to the rat, is a valid model for this purpose[16], especially in light of the high content of fat in human diets in comparison with commonly used rodent diets (see Materials and methods section and Table 1). Last but not least, because lipid metabolism is primarily determined by the amount and type of dietary fats, it is important to investigate the effect of protein source under conditions that reflect habitual fat intakes, in this case under fatty acid intakes that are representative of the Canadian diet[17] (see Materials and methods section and Table 2). However, dietary cholesterol was not added to the diets in the present study because it strongly impacts

Abbreviations: BF, beef protein; CAS, casein; CYP7A1, cholesterol 7 α-hydroxylase; HMG-CoAr, 3-hydroxy-3-methylglutaryl-CoA reductase; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; LDL-R, LDL receptor; PL, phospholipids; SOY, soya protein; SR-B1, scavenger receptor B1; TC, total cholesterol; WG, wheat gluten.

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Table 1. Composition of the experimental diets (g/kg)

Ingredient	CAS*	BF†	WG‡	SOY*
Protein§	222	222	264	222
Cornstarch‡	273	273	231	273
Sucrose‖	136	136	136	136
Fat blend¶	155	155	155	155
Cellulose*	100	100	100	100
Wheat bran*	50	50	50	50
Hayes Mineral Mix*	46	46	46	46
Hayes Cathcart Vitamin Mix*	12	12	12	12
Choline dihydrogen citrate*	6	6	6	6
Total	1000	1000	1000	1000
Metabolizable energy (kJ/g)**	16·49	16·49	16·49	16·74

BF, beef protein; CAS, casein; SOY, soya protein; WG, wheat gluten.
* Harlan Teklad (Madison, WI, USA): vitamin-free CAS and isolated SOY.
† Extra lean ground beef (local butcher, Gatineau, QC, Canada), defatted and freeze-dried.
‡ Dyets Inc. (Bethlehem, PA, USA).
§ Provided as 20 % (w/w) actual protein content, as per manufacturer's powder composition sheet for CAS, WG and SOY, and based on nitrogen content of BF by micro-Kjeldahl method.
‖ Lantic Ltd (Montreal, QC, Canada).
¶ Consisting of low-erucid acid rapeseed oil (50 %), coconut oil (28 %), lard (12 %), soyabean oil (5 %), butter (5 %) and flaxseed oil (1 %).
** Energy was calculated by adding the energy contributed from protein (CAS and BF = 15·07 kJ/g; WG = 15·36 kJ/g; SOY = 16·24 kJ/g), carbohydrate (starch and sucrose = 16·74 kJ/g), fat (fat blend = 37·68 kJ/g) and wheat bran (9·40 kJ/g).

hepatic cholesterol and LDL-C metabolism in a dose-dependent manner in the hamster[18], but not in man[19]; and because it modulates the expression of genes and/or activity of enzymes involved in lipid metabolism[20] to an extent that might mask the effect of dietary protein on such indices. Although humans consume protein from different sources and the impact of diet on lipid metabolism is modulated by several nutrients, elucidating the effects of individual nutritional factors, in this case protein, will help develop dietary strategies aiming at optimizing or improving cardiovascular health.

Therefore, the objective of the present study was to compare systematically the effect of four different un-supplemented proteins of both animal and vegetable sources on plasma lipids, long-chain fatty acid metabolism in the liver, and expression of genes involved in hepatic lipid metabolism in hamsters fed cholesterol-free diets.

Table 2. Fatty acid (FA) composition of the experimental diets (% of total FA)*

Fatty acid	Fat blend†	CAS	BF	WG	SOY
10 : 0	1·3	1·4	1·3	1·1	1·4
12 : 0	12·2	12·2	11·9	10·9	11·8
14 : 0	5·8	5·7	5·6	5·3	5·4
16 : 0	9·8	9·9	10·1	10·8	10·2
18 : 0	4·4	4·4	4·6	4·2	4·4
Total SFA	35·0	35·1	34·9	33·4	35·2
18 : 1*n*-9	37·6	37·0	36·8	34·9	35·8
Total MUFA	41·8	41·1	41·0	38·8	39·8
18 : 2*n*-6	16·1	16·7	16·7	20·8	18·0
20 : 4*n*-6	0·0	0·0	0·1	0·0	0·0
Total *n*-6 PUFA	16·3	16·9	17·2	21·0	18·1
18 : 3*n*-3	6·1	6·0	5·8	5·9	6·0
20 : 5*n*-3	0·0	0·0	0·0	0·0	0·0
22 : 6*n*-3	0·0	0·0	0·0	0·0	0·0
Total *n*-3 PUFA	6·1	6·1	6·1	6·2	6·2
18 : 2*n*-6/18 : 3*n*-3	2·7	2·8	2·9	3·5	3
Total *trans* FA	0·8	0·8	0·8	0·7	0·8

BF, beef protein; CAS, casein; SOY, soya protein; WG, wheat gluten.
* See Materials and methods section for details of lipid extraction and analysis.
† Fat blend consisted of low-erucid acid rapeseed oil (50 %), coconut oil (28 %), lard (12 %), soyabean oil (5 %), butter (5 %) and flaxseed oil (1 %).

Materials and methods

Animals and diets

The Health Canada Animal Care Committee approved the animal experimental protocol, and maintenance of the animals followed the guidelines of the Canadian Council for Animal Care. Male golden Syrian hamsters (*n* 28; age 30–33 d) were purchased from Charles River (St-Constant, QC, Canada). Upon arrival, they were housed individually in ventilated plastic transparent cages with bedding in a climate-controlled environment maintained at a temperature of 22 ± 1°C and 60 % relative humidity with a 12:12 h light–dark cycle. After a week of acclimation during which a commercial rodent feed (Purina chow # 5001) was provided, the hamsters (seven per group; average body weight 84 g) were given free access to one of the four experimental diets: CAS, beef protein (BF), WG and SOY (Table 1). The diets were formulated based on a modification of the Hayes Purified Diet for Hamsters[21]. Dietary protein accounted for 20 % (w/w) based on pure protein content (as per manufacturer's sheet composition for CAS, WG and SOY, and based on nitrogen content of BF by micro-Kjeldahl method). Extra lean ground beef was purchased from a local butcher (Gatineau, QC, Canada), defatted, freeze-dried and pulverized into a powdered form before mixing it with other ingredients in the diet.

Dietary fat accounted for 15·5 % (w/w) of the total diet (Table 1) and consisted of a blend of rapeseed oil (50 %), coconut oil (28 %), lard (12 %), soyabean oil (5 %), butter (5 %) and flaxseed oil (1 %). This fat blend was chosen to provide SFA, MUFA and PUFA at levels similar to those estimated for the average Canadian diet[17]. Lard and butter did not contribute significantly to the cholesterol content of the diet (approximately 0·003 %). Total nitrogen in proteins and diets was determined (in duplicate) by the micro-Kjeldahl method using a Kjeltec Auto 2400 Analyser (Höganäs, Sweden). Protein was calculated using a nitrogen-to-protein ratio factor of 6·25. Diets were hydrolysed (in duplicate) with 6 M-HCl at 110°C for 22 h for the determination of total amino acids except sulphur amino acids and tryptophan[22]. Hydrolysates for the determination of methionine as methionine sulphone and cyste(i)ne as cysteic acid were prepared by performic acid oxidation of protein followed by hydrolysis with 6 M-HCl[22]. All amino acids, except tryptophan, in hydrolysates were determined by liquid chromatography of precolumn phenylisothiocyanate derivatives[23] using Waters 600 Controller with Waters 717plus Autosampler and Waters 996 Photoiode Array Detector (254 nm; Waters, Milford, MA, USA) (Table 3). The fatty acid composition of the diet (Table 2) was determined as described later.

Procedures

Fresh food was provided and measurements of food intake and body weight were taken twice a week. On these same days, cages were changed and fresh bedding was provided, spiked with a small amount of the old bedding from the same cage to

Table 3. Amino acid composition of the experimental diets (g/100 g protein)*

Amino acid	CAS	BF	WG	SOY
Alanine	3·1	5·0	2·5	3·9
Arginine	4·1	6·0	3·7	7·3
Aspartic acid	6·6	7·8	2·9	9·9
Cystine	0·7	1·4	2·2	1·7
Glutamic acid	22·4	14·3	34·4	18·4
Glycine	2·2	4·3	3·4	4·2
Histidine	3·2	3·7	2·4	2·8
Isoleucine	5·8	4·6	4·1	4·8
Leucine	9·7	7·7	6·8	7·7
Lysine	7·6	7·8	2·0	5·9
Methionine	3·1	2·6	1·4	1·2
Phenylalanine	5·4	4·0	5·0	5·2
Proline	10·8	3·8	11·9	5·1
Serine	6·4	4·3	5·4	5·7
Threonine	4·6	4·4	2·8	3·9
Tyrosine	5·6	3·3	3·4	3·8
Valine	6·7	4·8	3·9	4·7

BF, beef protein; CAS, casein; SOY, soya protein; WG, wheat gluten.
* Amino acid composition of the diets was determined according to the method developed by Sarwar *et al.*[23].

minimize variation in the surroundings. Hamsters had unlimited access to food and water during the entire experimental phase. At the end of the eighth week, the animals were fasted for at least 16 h prior to killing by exsanguination through cardiac puncture under general anaesthesia with isoflurane. Blood was collected in serum separator tubes gel and clot activator tubes and centrifuged at 1000 ***g*** for 10 min at 4°C to obtain serum for the analysis of lipids. The liver was removed and immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Serum lipids

Serum TAG, TC and HDL-C were measured using a microplate enzymatic colorimetric method (Randox Laboratories, Mississauga, ON, Canada; catalogue numbers TR 201, CH 201 and CH 204, respectively) according to the manufacturer's instructions. Non-HDL-C was determined by subtracting HDL-C from TC.

Fatty acid composition of diets and liver phospholipids

Total lipids were extracted from diets and livers with chloroform–methanol–water (2:2:1·8, by vol.)[24]. Phospholipids (PL) from liver total lipids were separated and isolated by TLC with development in hexane–diethyl ether–acetic acid (85:15:1, by vol.). Lipids from the diets and liver PL were then transmethylated with 0·5 M-methanolic HCl. The fatty acid methyl esters were analysed by GC with a flame ionization decay detector and auto-sampler (Agilent Technologies 6890N) using a Supelcowax-10 flexible fused silica capillary column (30 m × 0·25 mm × 0·25 μm, Supelco Inc., Bellefonte, PA, USA). The fatty acids were identified by comparing their retention times with those of a standard mixture of fatty acid methyl esters (Supelco 37 FAME catalogue number 47 885-U; Supelco Inc.). The fatty acids were expressed as % of total fatty acids.

Quantitation of mRNA abundances of selected hepatic enzymes and receptors involved in lipid metabolism

Total RNA was isolated from hamster liver samples with TRIzol reagent (Invitrogen Canada Inc., Burlington, ON, Canada) according to the manufacturer's instructions. Total RNA (500 ng) was reverse transcribed with M-MLV RT (Invitrogen Canada Inc.) for cDNA synthesis. One-tenth of the synthesized cDNA was then amplified using Taq DNA polymerase (New England Biolabs, Beverly, MA, USA) with the primers listed in Table 4 for the following genes: LDL-R, scavenger receptor B1 (SR-B1), also known as HDL receptor, cholesterol 7 α-hydroxylase (CYP7A1), 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoAr), lecithin-cholesterol acyltransferase, Δ6-desaturase, Δ5-desaturase (Invitrogen Canada Inc.), and universal 18S rRNA primers and competimers (Ambion Inc., Austin, TX, USA). PCR conditions were 94°C for 5 min, 94°C for 30 s, annealing temperature (Table 4), 72°C for 30 s and 72°C for 10 min. The annealing temperatures, ratios of 18S rRNA primer–competimer and amplification cycle numbers for each gene were optimized to ensure that the amplifications were in the linear range. Samples were resolved on pre-cast 2 % agarose gels in the

Table 4. Oligonucleotide sequence for reverse transcription and RT–PCR

Genes	Species	Accession no.	Primer sequence*	Length (bp)	Temp. (°C)	Cycles	18S P:C ratio
LDL-R	*Cricetulus griseus*	M94387	Forward: 5′-TGGCTACGAGTGCTTGTGTC-3′ Reverse: 5′-ATCCCCACTGATGATGGTGT-3′	411	60	31	2:8
SR-B1	*Cricetulus griseus*	U11453	Forward: 5′-TGCAGACCCTGTGCTATCAG-3′ Reverse: 5′-TGAATGGCCTCCTTATCCTG-3′	416	60	30	2:8
CYP7A1	*Rattus rattus*	X17595	Forward: 5′-CTGCAAACTGATGGGAAAT-3′ Reverse: 5′-TGCATCATGGCTTCAGAAAG-3′	236	60	33	2:8
HMG-CoAr	*Rattus norvegicus*	X55286	Forward: 5′-CCTGCTGCCATAAACTGGAT-3′ Reverse: 5′-GCCATTACAGTGCCACACAC-3′	466	58	34	2:8
LCAT	*Rattus norvegicus*	U62803	Forward: 5′-CTGTTGCTGTTGGGGCTACT-3′ Reverse: 5′-TTGAAATCCAGCCAGATGGT-3′	232	60	30	2:8
Δ6D	*Rattus norvegicus*	AB021980	Forward: 5′-AAGGGAGGTAACCAGGGAGA-3′ Reverse: 5′-ATGATGTGGGACAGGAGGAG-3′	415	58	30	3:7
Δ5D	*Rattus norvegicus*	AF320509	Forward: 5′-GAGCAGTCCAGCTTTGAACC-3′ Reverse: 5′-TGGTTGTATGGCATGTGCTT-3′	496	60	28	4:6

CYP7A1, cholesterol 7 α-hydroxylase; Δ5D, Δ5-desaturase; Δ6D, Δ6-desaturase; HMG-CoAr, 3-hydroxy-3-methylglutaryl-CoA reductase; LCAT, lecithin-cholesterol acyltransferase; LDL-R, LDL receptor; P:C, primer:competimer; SR-B1, scavenger receptor B1; Temp., annealing temperature.
* All primers listed in the table were purchased from Invitrogen Canada Inc. (Burlington, ON, Canada).

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presence if ethidium bromide (Invitrogen Canada Inc.). Multiple images of each gel were taken with different exposure time using the BioDoc-It Imaging System (UVP Inc., Upland, CA, USA). Densities of the appropriate images were measured using the Scion Image software, and mRNA abundances of each gene were normalized against their respective 18S rRNA content, which was not affected by our treatments (data not shown).

Statistical analysis

Data were analysed by one-way ANOVA. If significance was observed ($P \leq 0{\cdot}05$), Duncan's multiple range test was used to identify differences in the effect of individual diets. This *post hoc* test was used instead of the more rigid Tukey's honest significant differences because of the relatively small sample size and the large variability within dietary groups. Statistica 7 for Windows 2000 (StatSoft, Tulsa, OK, USA) was used in all analyses. All data are expressed as means and their standard errors.

Results

Food intake and body weight

There were no significant differences in average food intake per day ($P=0{\cdot}14$), body weight at the end of the study ($P=0{\cdot}94$) and body weight gain ($P=0{\cdot}94$) of hamsters during the feeding phase among the four dietary protein groups (Table 5).

Serum lipids

Protein source had a significant effect on serum TC ($P=0{\cdot}0086$), HDL-C ($P=0{\cdot}027$) and non-HDL-C ($P=0{\cdot}049$), but not on TAG ($P=0{\cdot}065$) (Table 5). Hamsters fed WG had significantly lower serum TC and HDL-C than hamsters fed either BF or SOY. Hamsters fed CAS also had significantly lower HDL-C than those fed BF or SOY, but significantly lower TC than only BF. However, only hamsters fed WG had significantly lower non-HDL-C than those fed BF. A trend to lower TAG concentrations was observed in the animals fed WG and SOY compared to BF, but this was not significant ($P=0{\cdot}065$).

Fatty acid composition of liver phospholipids

Protein source had a significant effect on the relative level (% of total) of several fatty acids in liver PL, including 18 : 3*n*-3 ($P=0{\cdot}0011$), 20 : 5*n*-3 ($P=0{\cdot}0045$), 22 : 5*n*-3 ($P=0{\cdot}0001$), 22 : 6*n*-3 ($P=0{\cdot}0007$), total *n*-3 PUFA ($P=0{\cdot}0013$), 20 : 4*n*-6 ($P=0{\cdot}0000$), 18 : 3*n*-6 ($P=0{\cdot}04$), 20 : 3*n*-6 ($P=0{\cdot}0013$), total *n*-6 PUFA ($P=0{\cdot}0005$), 18 : 1*n*-9 ($P=0{\cdot}0002$), total MUFA ($P=0{\cdot}0001$), as well as 20 : 5*n*-3/18 : 3*n*-3 ($P=0{\cdot}0089$) and 22 : 6*n*-3/18 : 3*n*-3 ratios ($P=0{\cdot}0001$), but not on 18 : 2*n*-6 ($P=0{\cdot}56$), 16 : 0 ($P=0{\cdot}27$), 18 : 0 ($P=0{\cdot}17$) or total SFA ($P=0{\cdot}08$) (Table 6).

For the *n*-3 PUFA series, hamsters fed WG or SOY had significantly lower percentages of 18 : 3*n*-3 and 22 : 5*n*-3, but significantly higher percentages of 22 : 6*n*-3, total *n*-3 PUFA, as well as ratio of 20 : 5*n*-3/18 : 3*n*-3 and 22 : 6*n*-3/18 : 3*n*-3 than hamsters fed BF. However, only hamsters fed WG or BF had significantly lower percentages of 20 : 5*n*-3 than those fed CAS. In addition, hamsters fed CAS had percentage of 22 : 5*n*-3 significantly lower than those fed BF, but higher than those fed WG or SOY, and a significantly lower ratio of 20 : 5*n*-3/18 : 3*n*-3 than hamsters fed SOY.

For the *n*-6 PUFA series, hamsters fed WG, SOY or CAS had significantly lower percentages of 20 : 4*n*-6 and total *n*-6 PUFA than those fed BF. However, hamsters fed WG or SOY had significantly lower 20 : 3*n*-6 than those fed either BF or CAS. On the other hand, hamsters fed WG had significantly higher percentages of 18 : 3*n*-6 than those fed BF or CAS.

For the MUFA, hamsters fed WG or SOY had significantly lower percentages of 18 : 1*n*-9 and total MUFA than those fed BF or CAS.

mRNA abundances of selected hepatic enzymes and receptors involved in lipid metabolism

Protein source had a significant effect on the mRNA abundances of CYP7A1 ($P=0{\cdot}052$), LDL-R ($P<0{\cdot}0049$) and Δ6-desaturase ($P<0{\cdot}032$), but not those of HMG-CoAr ($P=0{\cdot}21$), lecithin-cholesterol acyltransferase ($P=0{\cdot}49$), SR-B1 ($P=0{\cdot}15$) or Δ5-desaturase ($P=0{\cdot}74$) (Fig. 1).

With respect to the selected enzymes and receptors involved in cholesterol and lipoprotein metabolism, hamsters fed WG or SOY had significantly higher mRNA abundances (ratio to

Table 5. Food intake, body weight and plasma lipid concentrations of hamsters fed one of the four different sources of protein in their diets for 8 weeks

(Mean values with their standard errors for seven hamsters per group)

	CAS		BF		WG		SOY		
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*
Food intake (g)*	6·1	0·17	6·7	0·27	6·3	0·11	6·2	0·22	0·14
Body weight (g)†	128·9	3·93	131·2	4·20	132·1	2·99	131·3	4·52	0·94
Body weight gain (g)	45·6	4·41	45·5	3·96	48·3	2·92	46·9	3·55	0·94
Serum lipids (mmol/l)									
TAG	1·0	0·13	1·3	0·18	0·9	0·09	0·8	0·08	0·065
TC	$2{\cdot}5^{bc}$	0·19	$3{\cdot}4^{a}$	0·36	$2{\cdot}3^{c}$	0·08	$3{\cdot}0^{ab}$	0·13	0·0086
HDL-C	$1{\cdot}9^{b}$	0·14	$2{\cdot}3^{a}$	0·16	$1{\cdot}8^{b}$	0·10	$2{\cdot}3^{a}$	0·14	0·027
Non-HDL-C	$0{\cdot}6^{ab}$	0·07	$1{\cdot}1^{a}$	0·25	$0{\cdot}5^{b}$	0·06	$0{\cdot}7^{ab}$	0·10	0·049

BF, beef protein; CAS, casein; HDL-C, HDL-cholesterol; SOY, soya protein; TC, total cholesterol; WG, wheat gluten.
[a,b,c] Mean values within a row with unlike superscript letters were significantly different ($P<0{\cdot}05$).
* Food intake is the average intake per day over 8 weeks.
† Body weight at the end of the 8-week feeding period.

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Table 6. Fatty acid (FA) profile of liver phospholipids of hamsters fed one of the four different sources of protein in their diets for 8 weeks

(Mean values with their standard errors for six hamsters per group)

	CAS		BF		WG		SOY		
FA	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	*P*
% of total FA									
16 : 0	17·5	0·20	17·3	0·42	17·1	0·42	18·1	0·48	0·27
18 : 0	19·8	0·54	20·2	0·36	21·3	0·51	20·9	0·57	0·17
Total SFA	38·9	0·64	38·9	0·28	39·9	0·31	40·6	0·67	0·08
18 : 1*n*-9	8·5^{a}	0·47	8·5^{a}	0·19	6·5^{b}	0·18	7·1^{b}	0·27	0·0002
Total MUFA	10·8^{a}	0·58	10·8^{a}	0·20	8·3^{b}	0·18	9·1^{b}	0·28	0·0001
18 : 2*n*-6	15·8	0·51	15·8	0·35	15·3	0·21	15·1	0·55	0·56
18 : 3*n*-6	0·49^{b}	0·02	0·47^{b}	0·04	0·61^{a}	0·05	0·59^{a}	0·04	0·04
20 : 3*n*-6	1·1^{a}	0·07	1·1^{a}	0·08	0·81^{b}	0·04	0·87^{b}	0·03	0·0013
20 : 4*n*-6	13·9^{b}	0·30	15·9^{a}	0·22	14·5^{b}	0·25	14·0^{b}	0·14	0·0000
Total *n*-6 PUFA	32·2^{b}	0·32	34·4^{a}	0·54	32·2^{b}	0·17	31·5^{b}	0·54	0·0005
18 : 3*n*-3	0·21^{a}	0·02	0·19^{a}	0·01	0·15^{b}	0·01	0·15^{b}	0·02	0·0011
20 : 5*n*-3	0·43^{a}	0·02	0·36^{a}	0·01	0·35^{a}	0·01	0·40^{ab}	0·01	0·0045
22 : 5*n*-3	0·56^{b}	0·02	0·64^{a}	0·02	0·49^{c}	0·02	0·47^{c}	0·02	0·0001
22 : 6*n*-3	16·2^{a}	0·62	14·0^{b}	0·53	18·0^{a}	0·31	17·1^{a}	0·78	0·0007
Total *n*-3 PUFA	18·0^{a}	0·61	15·8^{b}	0·51	19·5^{a}	0·31	18·8^{a}	0·79	0·0013
Ratio									
20 : 5*n*-3/18 : 3*n*-3	2·2^{bc}	0·20	1·9^{c}	0·09	2·4^{ab}	0·14	2·6^{a}	0·13	0·0089
22 : 6*n*-3/18 : 3*n*-3	80·8^{b}	6·99	73·7^{b}	4·41	124·6^{a}	8·59	114·5^{a}	6·66	0·0001
22 : 6*n*-3/20 : 5*n*-3	38·0^{b}	2·77	39·5^{b}	2·28	51·9^{a}	1·02	43·2^{b}	1·02	0·0003
20 : 4*n*-6/18 : 2*n*-6	0·88	0·04	1·0	0·02	0·94	0·03	0·93	0·04	0·11

CAS, casein; BF, beef protein; WG, wheat gluten; SOY, soya protein.
^{a,b,c} Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).

18S) of LDL-R (Fig. 1 (a)) and CYP7A1 (Fig. 1 (b)) than hamsters fed BF, whereas hamsters fed CAS had significantly higher mRNA abundances of only LDL-R compared to those fed BF.

With respect to key enzymes involved in desaturation of the essential fatty acids linoleic acid and α-linolenic acid, hamsters fed WG or SOY had significantly higher mRNA abundances (ratio to 18S) of Δ6-desaturase than those fed BF (Fig. 1 (c)).

Discussion

The results of the present study support the hypothesis that the protein source is a determinant of the effects of the diet on lipid metabolism in the hamster. In addition, these effects appear to be attributed, at least in part, to changes in the mRNA levels of enzymes and receptors involved in cholesterol, lipoprotein and long-chain fatty acid metabolism.

The observations that neither food intake, nor body weight, nor body weight gain were different among the four protein sources (Table 5) are of particular importance. First, although WG is deficient in lysine (Table 3), there is no evidence that this was limiting at the level provided in the experimental diets, supporting previous findings that the importance of protein quality to meet the hamster's requirements is reduced by virtue of its forestomach microbial activity that ferments dietary protein[25]. Second, and most importantly, the observed differences in lipid metabolism indices can be attributed solely to the protein source.

Although the protein source in the diet had an impact on serum lipid concentrations in the present study, the differences were observed mainly between WG and BF protein, whereas the effects of SOY and CAS were intermediate. The present observations are inconsistent with previous studies showing hypocholesterolaemic actions of either WG or SOY compared to CAS. For example, Endo *et al.* [13] reported that a 12 % WG diet fed to adult male Wistar rats resulted in significantly lower TC and HDL-C, concomitant with higher expression of HMG-CoAr, HMG-CoA synthase and CYP7A1 than one with 12 % CAS. Although we also observed higher expression of CYP7A1 and a trend towards higher expression of HMG-CoAr, the discrepancy in the effects on circulating cholesterol could be due to the fact that the basal rate of hepatic cholesterol synthesis is high in the rat, whereas it is very low in the male hamster[16]. Because the contribution of the liver to whole body cholesterol synthesis is higher in the rat than in the hamster, an up-regulation of the expression of genes involved in cholesterol metabolism would have a greater impact on circulating cholesterol levels in the former. To our knowledge, this is the first study to investigate the effect of WG on lipid metabolism in hamsters, and thus, direct comparisons cannot be made. It is important to note as well that the *n*-6 + *n*-3 PUFA content of the WG diet was up to 4 % higher than that of the other diets. Although the effect of PUFA on cholesterol metabolism is well established, we do not believe that they contributed to the observed effect as WG and CAS, which have the highest and lowest levels of PUFA, resulted in similar cholesterol concentrations (Table 5).

Unlike WG, the cholesterolaemic effects of SOY and CAS have been compared in male hamsters, but the results are conflicting. Some studies have reported reduction of total, VLDL + LDL-C and HDL-C[26–29], whereas others[30], like us, have not. Such differences might be explained by both the cholesterol content and fatty acid composition of the diet. In most of these studies, cholesterol at a minimum level of 0·01 % was added to the diet. Because dietary cholesterol substantially elevates TC and LDL-C in hamsters, an effect of protein can be more clearly observed when

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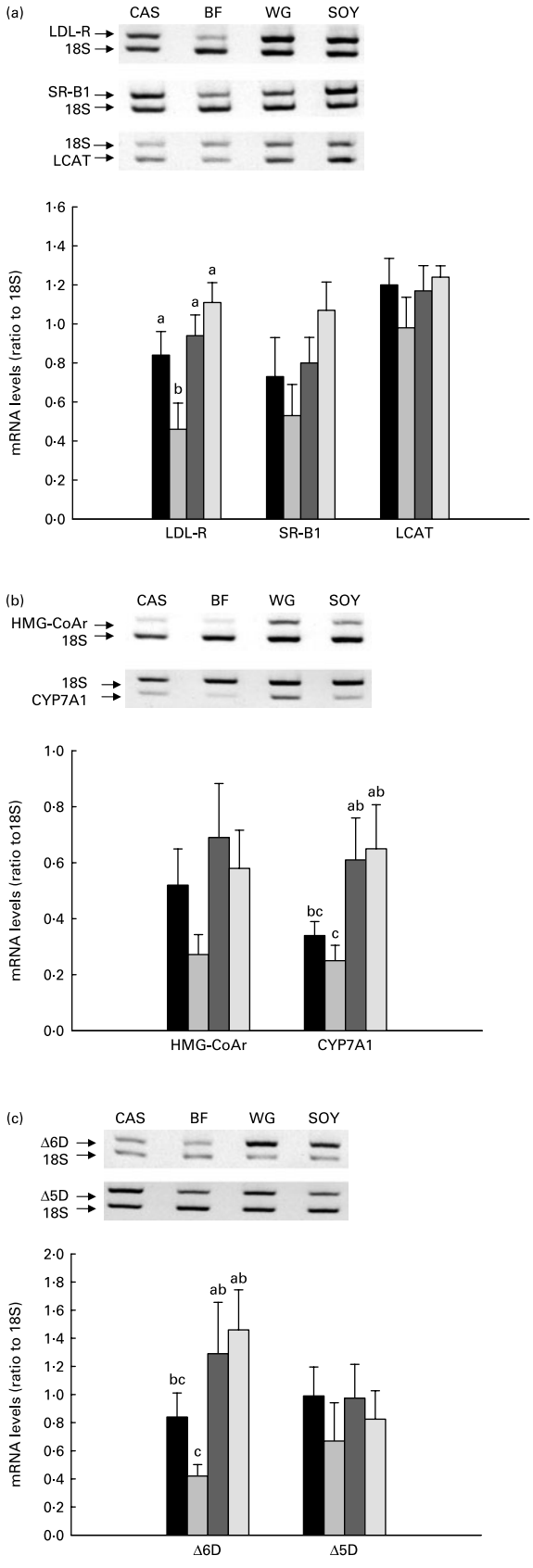

cholesterol is included in the diet. However, two groups also demonstrated a hypocholesterolaemic effect of SOY in hamsters fed cholesterol-free diet[27,31]. In these studies, the major source of fat was the SFA-rich coconut oil, which was shown to increase plasma LDL-C in hamsters in a diet essentially free of cholesterol, albeit the effect was less pronounced than in the presence of cholesterol[18]. Because the present diets were higher in unsaturated fatty acids than in SFA (ratio 1·7/1·0), it is possible that the hypocholesterolaemic effect of SOY (and WG) relative to CAS was obliterated in the absence of dietary cholesterol.

Of particular interest though was the observation that SOY resulted in higher HDL-C concentrations than either CAS or WG. Whereas it is not surprising that the hypocholesterolaemic effect of WG compared to SOY would affect HDL-C, the major carrier of plasma cholesterol in hamsters[32], the higher HDL-C levels suggest a better lipoprotein profile in SOY-fed hamsters than CAS-fed ones. However, the present results cannot be explained by changes in SR-B1 or lecithin-cholesterol acyltransferase mRNA levels, which were similar among all dietary groups, albeit there was a trend for higher transcript levels in the SOY groups (Fig. 1 (a)). The SR-B1, commonly referred to as HDL receptor, selectively transports cholesterol esters from HDL particles to the liver[33]. Although such function results in lower circulating HDL-C, overexpression of SR-B1 has been shown to protect against CHD, whereas its absence leads to the opposite[34]. The anti-atherogenic effect of enhanced SR-B1 expression and/or activity despite decreased plasma HDL-C levels is indicative of enhanced reverse cholesterol transport, possibly due to negative hepatic cholesterol balance. Therefore, the higher concentrations of HDL-C in hamsters fed the SOY and BF diets could be due to alterations in the protein levels of these genes, in the expression and/or activity of other genes involved in HDL metabolism, such as Apo-AI or cholesteryl ester transfer protein, or simply to the fact that hamsters from both these diets had the highest levels of TC.

Perhaps the most important observation with respect to cholesterol metabolism was that BF protein resulted in the highest non-HDL-C (VLDL-cholesterol + LDL-C) that is accompanied by the highest and lowest expression of LDL-R and CYP7A1, respectively. HMG-CoAr was also lowest in BF-fed hamsters, albeit not significantly. However, the absence of statistical significance should be interpreted with caution because fasting down-regulates the expression of sterol regulatory element binding proteins, which are transcription factors that control the transcription of a plethora of genes involved in lipid and lipoprotein metabolism, including HMG-CoAr and CYP7A1[35]. Consequently, the

Fig. 1. Expression of genes involved in lipoprotein metabolism (a), hepatic cholesterol metabolism (b) and desaturation of *n*-6 and *n*-3 PUFA (c) in hamsters fed one of the four different sources of protein in their diets for 8 weeks (■, CAS, casein; □, BF, beef protein; □, SOY, soya protein; ■, WG, wheat gluten). Values are means with their standard errors depicted by vertical bars (seven hamsters per group). [a,b,c] Mean values within a cluster of bars with unlike superscript letters were significantly different by one-way ANOVA followed by Duncan's multiple range test ($P<0·05$). CYP7A1, cholesterol 7 α-hydroxylase; Δ5D, Δ5-desaturase; Δ6D, Δ6-desaturase; HMG-CoAr, 3-hydroxy-3-methylglutaryl-CoA reductase; LCAT, lecithin-cholesterol acyltransferase; LDL-R, LDL receptor; SR-B1, scavenger receptor B1.

down-regulation of sterol regulatory element binding protein-1c by fasting would alleviate the inhibition of CYP7A1 gene expression. On the other hand, that of sterol regulatory element binding protein-2 would suppress the expression of HMG-CoAr, making it difficult to observe a significant effect of diet. Although it cannot be concluded that the changes in cholesterol metabolism are brought about by changes in the expression of the selected genes, the present results strongly suggest that protein source exerts its cholesterol-modulating effect at the level of the liver.

In addition to cholesterol, the present study provides further evidence that protein source affects long-chain PUFA metabolism. Specifically, WG, SOY and CAS resulted in higher relative concentrations of 22 : 6*n*-3 and long-chain *n*-3 PUFA and lower concentrations of 20 : 4*n*-6, long-chain *n*-6 PUFA, 18 : 1*n*-9 and total MUFA than BF in liver PL. However, only WG and SOY led to lower 18 : 3*n*-3 levels and a higher 22 : 6*n*-3/α-linolenic acid ratio than BF, supporting previous findings that protein source impacts long-chain *n*-6 and *n*-3 PUFA metabolism[36,37]. Consistent with the present observations, WG- and SOY-fed hamsters had higher relative mRNA levels of Δ6-desaturase than those fed BF and CAS, although the difference was not significant with the latter. On the other hand, the relative abundance of Δ5-desaturase was similar in all groups despite lower levels of 20 : 5*n*-3 in WG-fed hamsters. This observation can be explained by enhanced elongation and desaturation of 20 : 5*n*-3, as shown by higher ratios of 22 : 6*n*-3/20 : 5*n*-3 and 22 : 6*n*-3/18 : 3*n*-3.

The metabolic conversion of α-linolenic acid to 20 : 5*n*-3 and 22 : 6*n*-3 has been the centre of much debate. Based on stable isotope tracer studies, it is estimated that only 8 % of the 18 : 3*n*-3 pool enters the desaturation/elongation pathway in man, and that its overall efficiency of conversion to 20 : 5*n*-3 and 22 : 6*n*-3 is 0·2 and 0·05 %, respectively[38]. It is generally agreed that the Δ6 desaturation of 18 : 3*n*-3 to 18 : 4*n*-3 is the rate-limiting reaction within the long-chain *n*-3 PUFA cascade[39]. Because 18 : 2*n*-6 is also a substrate for Δ6-desaturase, the desaturation and elongation of 18 : 3*n*-3 to 20 : 5*n*-3 might be compromised at high intake ratios of 18 : 2*n*-6/18 : 3*n*-3. The ratio of 18 : 2*n*-6/18 : 3*n*-3 in our diets varied between 2·7 and 3·5, considered optimal for conversion of 18 : 3*n*-3 to 20 : 5*n*-3 and 22 : 6*n*-3[40]. Although we did not directly measure conversion, the 20 : 5*n*-3 and 22 : 6*n*-3 levels in liver PL of our hamsters mostly reflect desaturation and elongation of 18 : 3*n*-3 because the test and adaptation period diets were devoid of both these fatty acids, and the tissue levels of 20 : 4*n*-6 and long-chain *n*-6 PUFA were lower in hamsters fed WG, SOY and CAS diets. Therefore, it is possible that hamsters have higher expression and activity of Δ6-desaturase than man and that the fatty acid profile of the diet enhanced the effect of protein on 22 : 6*n*-3 levels in liver PL.

The finding that protein source impacts PUFA metabolism has important implications for cardiovascular health. Higher heart or erythocyte levels of *n*-3 PUFA have been associated with a lower risk for CVD[41]. This is partly due to the fact that they have anti-arrhythmic properties[42] and are precursors of anti-inflammatory and anti-aggregatory eicosanoids[43]. Although we measured fatty acid profile only in liver PL, it has been reported that entry into this compartment was a limiting factor for the conversion of 18 : 3*n*-3 to 22 : 6*n*-3, and that the hepatic conversion is a predictor of plasma PL composition[44,45].

The exact mechanisms by which dietary protein affects lipid metabolism are not fully understood. The proteins provided in the diet are reported to have a high digestibility : 94 % (WG and BF), 96 % (SOY) and 99 % (CAS)[46], and it is unlikely that the small differences in digestibility account significantly to the observed results. Individual and/or groups of amino acids have been proposed to modulate cholesterol and fatty acid metabolism[47]. For example, dietary methionine content strongly and positively correlated with serum cholesterol levels[48] and methionine supplementation abolished the hypocholesterolaemic effects of SOY in rats fed a cholesterol-free diet[49]. In addition, methionine increased Δ6-desaturase activity in rats[14], and hepatic Δ6-desaturase activity appears to be regulated by Δ6-desaturase mRNA levels[50]. However, it is unlikely that the methionine content of our proteins explained the present results because CAS, which contains the highest amount of methionine (Table 3), had significantly lower concentrations of TC than BF, but similar ones to WG and SOY (Table 5), which contain the lowest amount (Table 3). Moreover, WG and/or SOY had the highest percentage of 22 : 6*n*-3 in liver PL and hepatic Δ6-desaturase mRNA levels. In addition to methionine, cystine, lysine, glycine and arginine have been proposed to modulate cholesterol and PUFA metabolism, but the evidence is inconclusive and none of them appear to explain the present results.

The influence of bioactive peptides derived from protein digestion on lipid metabolism offers an alternative hypothesis. It has been increasingly acknowledged that the nutritional role of protein extends beyond the provision of amino acids for the synthesis of proteins. Bioactive peptides released during protein digestion can act locally (in the gut) or, if absorbed, in target organs to modulate a variety of physiological functions[51]. Bioactive peptides have been identified in many dietary proteins, including CAS, whey, BF, SOY and WG[50]. Whether bioactive peptides play a role in modulating cholesterol and fatty acid metabolism, as we observed in the present study, is currently unknown. Some studies have shown that the hypocholesterolaemic effect of SOY is partly explained by the binding of undigested peptide fraction to cholesterol and bile acids and their subsequent excretion in faeces[52,53]. However, SOY was not hypocholesterolaemic compared to other proteins and whether such a mechanism accounts for the hypocholesterolaemic effect of WG remains to be investigated.

In conclusion, we have provided evidence that the source of dietary protein modulates cholesterol and fatty acid metabolism in hamsters, an animal model suitable to study the impact of diet on lipid metabolism, in the absence of dietary cholesterol and at fat intakes representative of the average Canadian diet. The present findings call for further investigation on the mechanisms of action of dietary protein with respect to lipid metabolism and suggest that dietary strategies aiming at preventing and/or managing CVD might need to take the source of the protein into consideration.

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