Institute of Agricultural and Nutritional Sciences
(Director: Prof. Dr. M. Rodehutscord)
of the
Faculty of Natural sciences III
(Dean: Prof. Dr. P. Wycisk)
of the
Martin-Luther-University
Halle-Wittenberg, Germany

"Investigations on influence of different dietary proteins on lipid metabolism in rats fed a hyperlipidemic diet"

Dissertation

to achieve the degree of
Doctor of Nutritional Sciences (Dr. troph.)

presented by

Anjali Shukla (Master of Science) born on 1. Juli 1977 in Sitapur (India)

Referee: Prof. Dr. Habil. Klaus Eder
Prof. Dr. Habil. Gabriele I. Stangl
Prof. Dr. Habil. Gerald Rimbach

Defence on: 12.02.2007

Halle/Saale 2007

urn:nbn:de:gbv:3-000011438
[http://nbn-resolving.de/urn/resolver.pl?urn=nbn%3Ade%3Agbv%3A3-000011438]

I. Contents

List of Abbreviations

ACAT	Acyl coenzyme A **:** cholesterol acyl transferase
ACO	Acyl coenzyme A oxidase
APO-B	Apolipoprotein-B
bp	Base pair
cDNA	Copy de-oxyribonucleic acid
CE	Cholesterol ester
CHD	Coronary heart disease
CPT	Carnitine palmitoyl transferase
CYP4A1	Cytochrome 450 4A1
CYP7A1	Cholesterol 7 α1 hydroxylase
dATP	De-oxy adenosine triphosphate
DEPC	Diethylpyrocarbonate
dH_2O	Distilled water
dNTP	De-oxy nucleotide triphosphate
ER	Endoplasmic reticulum
Exp	Experiment
FAS	Fatty acid synthase
FC	Free cholesterol
G6PDH	Glucose 6 phosphate dehydrogenase
GAPDH	Glyceraldehyde-3-phosphate-dehydrogenase
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
HDL	High density lipoprotein
HMG CoA	3-hydroxy-3-methylglutaryl-CoA
IDL	Intermediate density lipoprotein
Insig	Insulin induced gene
LDL	Low density lipoprotein
MCAD	Medium chain acyl coenzyme A dehydrogenase
$MgCl_2$	Magnesium chloride
mRNA	Messenger ribonucleic acid
MTP	Microsomal triglyceride transfer protein
NaCl	Sodium chloride
NBT	p-Nitro-blue-tetrazolium chloride
PAGE	Poly-Acrylamide Gel Electrophoresis
PCR	Polymeric Chain Reaction
PL	Phospholipids
PPAR-α	Peroxisome proliferator activated receptor-alpha
rpm	Rounds per minute
RT	Room temperature
SDS	Sodium dodecyl sulphate
SREBP	Sterol regulatory element binding protein
SR-BI	Scavenger receptor class B type I
TC	Total cholesterol

TEMED	N,N,N,N-Tetramethylene diamine
UV	Ultra violet
VLDL	Very low density lipoprotein

1 Introduction

Cholesterol, fatty acids, phospholipids and triglycerides are important lipids in the body (Luskey 1988, Semenkovich 1997). Lipids are a major source of energy (triglycerides) and have many other functions such as the structure of membrane (phospholipids) and hormone synthesis (prostaglandins and steroid hormones). However, imbalances of lipid metabolism can lead to some of the major clinical problems such as atherosclerosis and obesity (Semenkovich 1997, Stamler et al. 1986). Atherosclerosis is the main cause of coronary heart disease (CHD), which is the leading cause of mortality in the western countries (Glass and Witztum 2001, Grundy 1994). Atherosclerotic vascular disease develops from complex multifactorial processes that contribute to the deposition and accumulation of cholesterol in focal areas of the arterial wall (see Figure 1.1). Elevated or modified blood levels of total cholesterol, particularly low density lipoprotein (LDL) cholesterol, free radicals caused by cigarette smoking, hypertension, elevated plasma homocysteine level, infectious microorganisms, and combinations of the above are risk factors for CHD (Grundy 1996). There is substantial evidence that lowering total and LDL-cholesterol levels reduce the incidence of CHD and coronary death (Grundy 1996). Many primary and secondary prevention trials have proven the benefits of cholesterol-lowering (Grundy et al. 1999). Aside from cholesterol, elevated levels of plasma triglyceride have been associated with an increased risk of cardiovascular disease (Austin 1991). A meta analysis revealed that increased triglyceride level is a risk factor for cardiovascular disease independent of high density lipoprotein (HDL) cholesterol level (Austin et al. 1998). Studies in both human and animal models have shown that triglyceride-rich lipoproteins such as intermediate density lipoprotein (IDL) and very low density lipoprotein (VLDL) are related to the extent and severity of atherosclerosis (Krauss 1998). Lowering triglyceride levels may reduce the risk of CHD (Ericsson et al. 1996).

One of the strategies to lower blood lipids is lifestyle intervention such as diet modifications and controlling body weight (Kris-Etherton et al. 1997). In October 1999, the FDA approved a claim stating that 25 grams of soy protein a day, as a part of diet low in saturated fat and cholesterol may reduce the risk of heart disease. Soy protein or lupin has been shown to reduce plasma lipid levels in studies with human and animal models (Bakhit et al. 1994, Ni et al. 1998, Anderson et al. 1995, Wang et al. 1998, Potter et al. 1998, Sirtori et al. 2004). Recent research shows that the effects of soy protein include decreases in plasma total cholesterol and LDL-cholesterol concentrations with an increase in HDL-cholesterol

levels in different model animals and hyperlipidemic patients when compared to an animal protein diet mainly casein (Anderson et al. 1995, Wang et al. 1998, Sirtori and Lovati 2001, Madani et al. 1998, Potter 1996). The hypocholesterolemic effect is directly correlated to the patient's cholesterolemia.

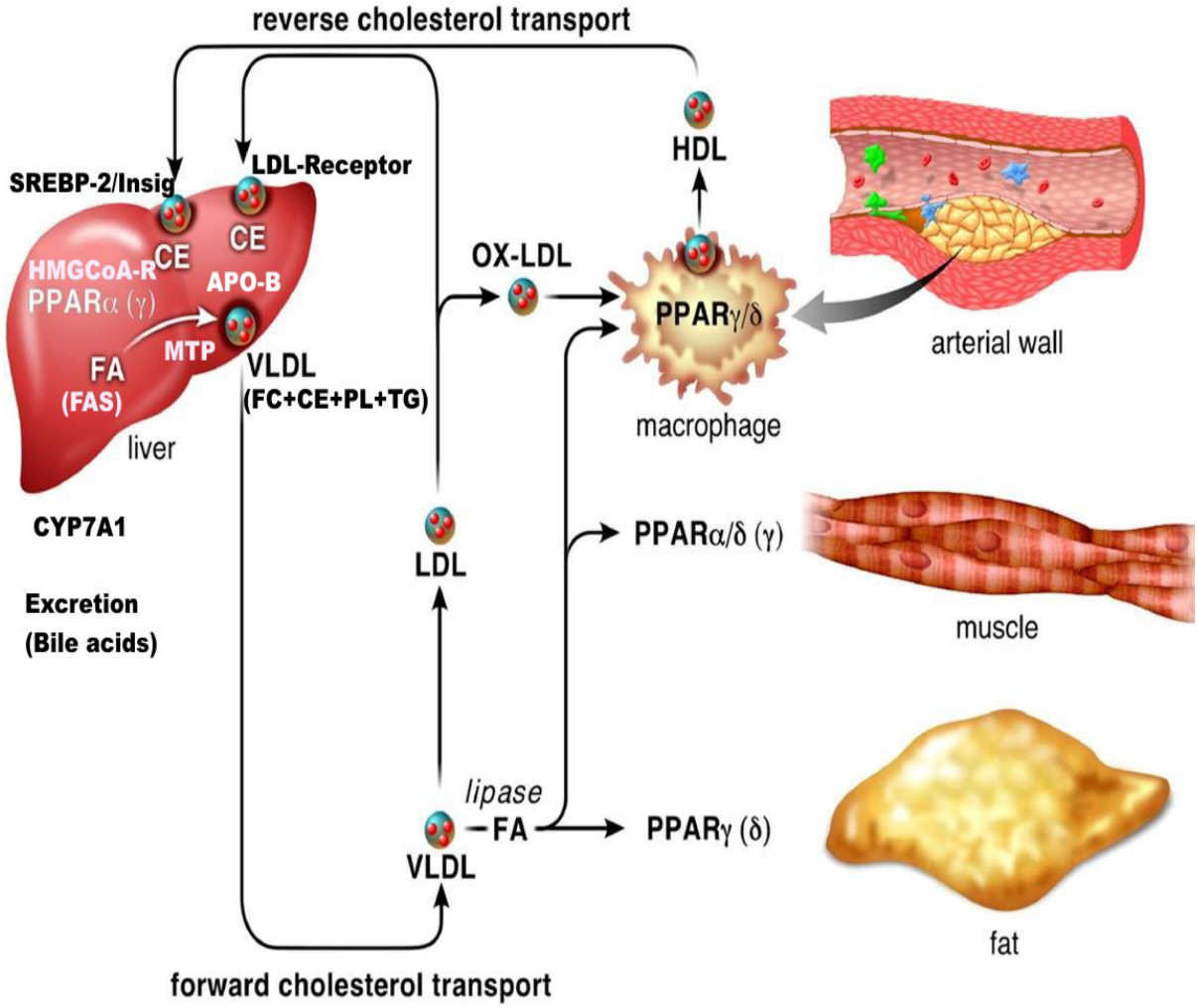

Figure 1.1: Cholesterol transport cycle. The liver secretes cholesterol in the form of VLDL. VLDL can subsequently be converted into LDL. In humans, LDL particles contain most of the cholesterol and the oxidized form of LDL can deposit in the artery wall (www.Google.com). VLDL, very low density lipoprotein; LDL, low density lipoprotein; ox-LDL, oxidized LDL; HDL, high density lipoprotein Apo-B, apolipoprotein-B; HMG CoA R, 3-hydroxy 3-methylglutaryl coenzyme A reductase; PPAR α (γ), peroxisome proliferators activated receptor alpha (gamma); FAS, fatty acid synthase; MTP, microsomal triglyceride transfer protein; Cyp7A1, cholesterol 7 alpha hydroxylase; SREBP, sterol regulatory element binding protein; Insig, insulin induced gene; FA, fatty acids; PL, phospholipids; TG, triglycerides; FC free cholesterol; CE, cholesterol ester.

There are several components of soybeans that may contribute to the lipid lowering properties of soy. Components such as soy protein, amino acids, peptides, isoflavones, saponins, phytic acid, fibers and protease inhibitors have been implicated in the hypolipidemic effect of soy protein preparations (Anderson et al. 1995, Wang et al. 1998, Potter et al. 1998). The major isoflavones (phytoestrogen) in the soybeans are genistein and daidzein. The hypocholesterolemic effect of isoflavones was noted in mice (Kirk et al. 1998), hamsters (Balmir et al. 1996), non human primates (Anthony et al. 1997) and humans (Wang et al. 1998, Carroll and Kurowska 1995, Gardner et al. 2001, Damasceno et al. 2001). Lichtenstein et al. (2002) reported that ingestion of ethanol extract rich in isoflavones increases the abundance of hepatic mRNA for cholesterol 7α-hydroxylase (CYP7A1) and LDL receptors in rats, which play important roles in cholesterol catabolism. However, other studies demonstrated that soy protein rather than its isoflavones contribute to the lipid lowering properties of soy (Claudia et al. 1998, Adams et al. 2002, Jenkins et al. 2002, Lichtenstein et al. 2002). Moreover, soy protein enhances the expression of the LDL receptors in hypercholesterolemic type II diabetic patients (Lichtenstein 1998, Wang et al. 1998, Potter 1995), animals (Iritani et al. 1996, Iritani et al. 1986, Wright & Salter 1998) and cultured human hepatoma cells. But these effects of soy protein were strongly related with the subject's initial serum cholesterol concentrations. It is also shown that the methionine content of the dietary protein is positively related with serum total cholesterol concentration in rats and atherosclerosis like alterations in the aorta of rats (Sautier et al. 1983, Antony et al. 1998). It is also reported that dietary supplementation of L-arginine prevents intimal thickening in the coronary arteries of hypercholesterolemic rabbits (Forsythe 1995). In vitro studies have clearly indicated that specific protein fractions of soybean, in particular the alpha' subunit of the 7S globulin and thereof peptides can directly activate LDL receptors in liver cells (Lovati et al. 2000). All these studies showed partly different effects and the underlying mechanism of this lipid lowering effect is not fully understood yet.

Therefore one aim of our first experiment was to investigate the mechanism of soy protein isolate on lipid metabolism, to minimize interference of isoflavones the soy protein isolate used for this study was additionally ethanol-washed. The results indicate that ethanol extracted soy protein also has positive effects on lipid metabolism.

In general, animal proteins are considered to be hypercholesterolemic when compared with plant proteins. But the number of proteins examined in this connection, is so far very limited. Most of the studies are based on casein as representative of animal proteins and soy

protein as representative of plant proteins (Ni et al. 1998, Damasceno et al. 2001). Besides casein, animal proteins such as those from beef, pork, poultry, or fish protein play an important role in human nutrition worldwide. Therefore, we planned a second experiment to investigate the effects of meat proteins isolated from pork, beef, and turkey, and fish protein isolated from Alaska pollack fillets and compared their effects on the lipid metabolism with casein and ethanol washed soy protein isolate. Casein served as reference protein of animal origin, and soy protein isolate, which is known to have hypocholesterolemic action when compared with casein, served as reference protein of plant origin. To study whether dietary proteins isolated from beef, pork, turkey and fish influence the metabolism of cholesterol or triacylglycerols, we determined the concentrations of cholesterol and triacylglycerols in plasma, lipoproteins and liver. In the liver, moreover, we determined the ratio between esterified and free cholesterol to investigate whether esterification of the free cholesterol could be influenced by these animal proteins.

In a third experiment, we decided to investigate alternative legume proteins isolated from peas and sweet lupin seeds. Moreover, we intended to verify the results obtained after feeding fish protein in the second experiment because until now there is little knowledge available about the effects of fish protein on lipid metabolism. Bergeron and Jacques (1989) demonstrated that the serum cholesterol level of rabbits fed fish protein was intermediate and not different from that of rabbits fed casein or soy protein. Iritani et al. (1985) reported that feeding fish protein to rats had a hypocholesterolemic effect equivalent to that of soy protein rather than casein. Thus fish protein has been shown to induce variable effects on serum cholesterol level compared with casein and soy protein (Jacques et al. 1995).

As it has been suggested that protein induced alterations of the lipid metabolism could be mediated by certain amino acids such as methionine, lysine or arginine (Sugiyama et al. 1996, Sugiyama et al. 1997, Gudbrandsen et al. 2005), we measured the concentrations of amino acid in the diets and in plasma. The synthesis of cholesterol and fatty acids begins with a common precursor, acetyl CoA, which originates from the catabolism of carbohydrates, proteins, and lipids. Acetyl CoA is converted to cholesterol in a pathway involving at least 23 enzymes. Acetyl CoA is also polymerized to form fatty acids in a pathway involving up to 12 enzymatic steps. Therefore, we planned to analyze lipid metabolism at the level of activities of enzymes like glucose 6-phosphate dehydrogenase (G6PDH), fatty acid synthase (FAS), and microsomal triglyceride transfer protein (MTP). Moreover, we intended to analyze the mRNA concentrations of various proteins involved in lipid metabolism like 3-hydroxy-3-

methylglutaryl-CoA reductase (HMG CoA R), apolipoprotein-B (APO-B), LDL receptor, delta 6-desaturase, FAS, CYP7A1, acyl CoA oxidase (ACO), and cytochrome 450 4A1 (CYP4A1). Since the effects of fish protein are less investigated we intended to apply the cDNA macro arrays which allow insight in the expression of more than 1000 proteins/genes at once. We decided to evaluate the gene expression profile of the liver because this tissue is the major site of lipid metabolism.

In the regulation of lipid metabolism several transcription factors like peroxisome proliferator-activated receptor α (PPAR-α) and sterol regulatory element binding proteins (SREBPs) are involved. Therefore we analyzed the expression of PPAR-α and SREBPs.

PPARs are nuclear proteins that belong to the super family of nuclear hormone receptors. They mediate the effects of small lipophilic compounds such as long chain fatty acids and their derivatives on transcription of genes commonly called PPAR target genes. Of the three PPAR types (PPAR-α, PPAR-β and PPAR-γ) known to date, PPAR-α has been best characterized. PPAR-α is involved in the regulation of peroxisomal β-oxidation (ACO, Thiolase B), mitochondrial ω-oxidation (CYP4A1, 4A6-Z), mitochondrial β-oxidation (CPT-I, CPT-II, MCAD). Thus, PPAR-α serves as a master regulator of fatty acid catabolism (Mandard et al. 2004, Dreyer et al. 1992).

SREBPs are a family of three proteins (SREBP-1a, SREBP-1c and SREBP-2) which control lipid metabolism in the liver and other organs (Horton et al. 2002). SREBPs are intrinsic membrane proteins of the endoplasmic reticulum (ER). SREBP-1a and SREBP-1c both activate genes involved in the synthesis of fatty acids and their incorporation into triglycerides and phospholipids. SREBP-2 preferentially activates genes for cholesterol synthesis and the LDL receptor (Horton et al. 1998). In liver SREBP-1c and SREBP-2 are the predominant isoforms. SREBP-1a is present in lesser amounts (Shimomura et al. 1997).

We also analyzed Insulin Induced Gene (Insig) proteins, which are recently identified ER proteins (Insig-1 and Insig-2). The gated movement of SREBPs from ER to the Golgi complex is the central event in lipid homeostasis in animal cells, which is controlled by Insig proteins (Horton et al. 2002). The combined actions of Insig-1 and Insig-2 permit feedback regulation of cholesterol synthesis over a wide range of sterol concentrations (Yang et al. 2002). The SREBP precursor protein is anchored in the membranes of the ER and nuclear enveloped by two membrane-spanning helices (Figure 1.2) (Hua et al. 1995).

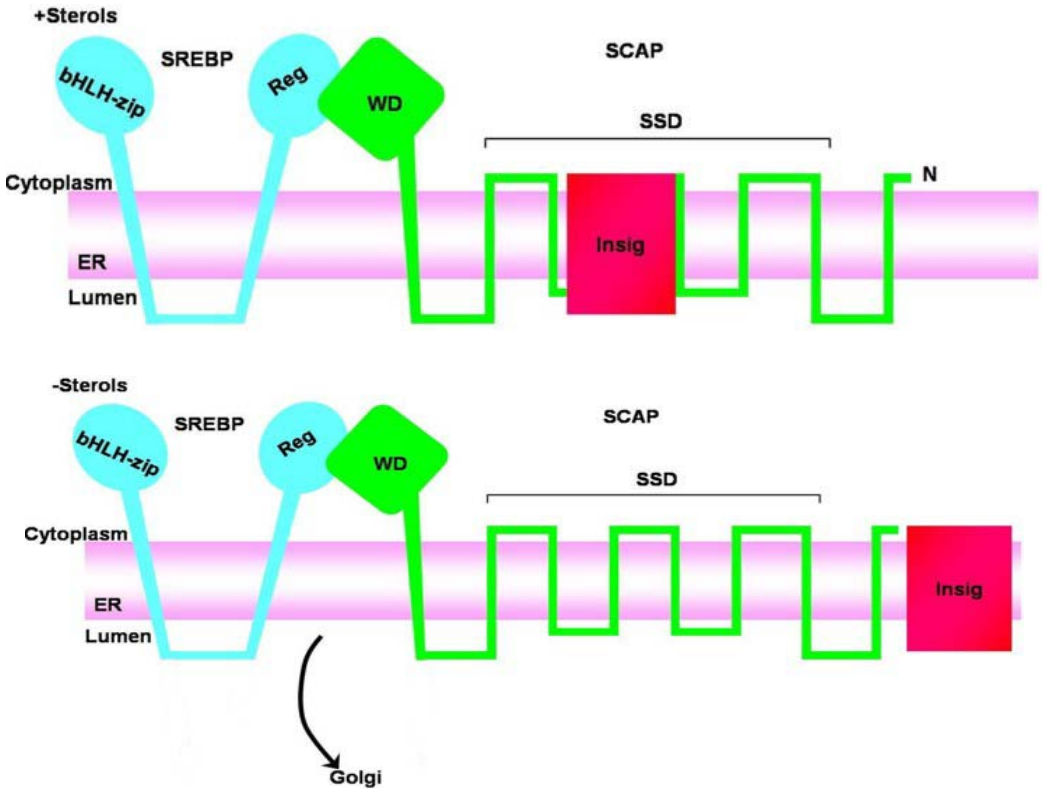

Figure 1.2: Sterol-regulated interaction between Insig and SCAP. In the presence of sterols, the SREBP-SCAP complex remains in the endoplasmic reticulum (ER). Retention in the ER requires interaction between the sterol sensing domain (SSD) of SCAP and Insig-1 or 2. In the absence of sterols, SCAP and the Insig proteins do not interact. The SREBP-SCAP complex is then free to travel to the Golgi apparatus to be processed (Rawson 2003). bHLH-zip, basic helix-loop-helix leucine zipper; Reg, regulatory; WD, aspartate-tryptophan motif; SCAP , SREBP cleavage activating protein; Insig, Insulin induced gene; N, amino terminal.

As shown in Figure 1.2, when cellular cholesterol levels are high, Insig proteins bind and trap proteolytic activation in the Golgi complex and therefore the level of SREBPs in the nucleus drops resulting lower expression of genes involved in lipid metabolism. When the cellular demand for the cholesterol rises, the SREBP-SCAP (SREBP-cleavage-activating protein) complex leaves the ER and travels to the Golgi apparatus where the SREBP-SCAP complex cleavage occurs by two proteases, site 1 protease and site two protease. This releases the transcriptionally active SREBPs, which can then travel to the nucleus where it can activate the transcription of target genes (Rawson 2003).

2 Materials and Methods

2.1 Animal Studies

Three experiments were carried out with growing male Sprague Dawley rats supplied by Charles River GmbH (Sulzfeld, Germany). The animals were housed individually in Macrolon cages, in a room maintained at a temperature of 22±2°C and 50-60% relative humidity with lighting from 06.00 to 18.00 hours. All described experimental procedures were according to the established guidelines for the care and handling of laboratory animals, and were approved by the council of Saxony-Anhalt, Germany. To standardize the feed intake, the diets were fed daily in restricted amounts in between 8 and 9 O'clock. The diets varied in the protein source. Water was provided ad libitum from nipple drinkers.

In experiment 1, 20 rats with an initial body weight (± SD) of 70.2 ± 4.3 g were randomly assigned to two groups of 10 rats each and fed semi-synthetic diet for 21 days with casein or ethanol washed soy protein isolate as protein source.

In experiment 2, 72 rats with an initial body weight (± SD) of 72.3 ± 5.6 g were randomly assigned to one of the six groups (n = 12/group) and fed semi-synthetic diet for 20 days with protein isolated from pork, beef, fish, turkey, casein or ethanol washed soy protein isolate.

In experiment 3, 60 rats with an initial body weight (± SD) of 76.5 ± 5.3 g were randomly assigned to one of 5 groups (n = 12/group) and fed semisynthetic diet for 22 days with fish protein, casein, ethanol washed soy protein isolate, pea or lupin protein.

The rats within one experiment were offered equal rations daily, and the amount fed was adjusted to meet the expected consumption based on the feed intake of the previous day. The body weight of the experimental animals was recorded weekly.

2.2 Characterisation of the Diet

The compositions of the experimental diets used in experiment 1, 2 and 3 are shown in Table 2.1. All diets contained 200 g protein/kg diet. The protein source varied according to the experimental design. All diets contained 100 g lard/kg (Laru, Langensiepen, Ruckebier, Bottrop, Germany) as a proatherogenic fat. The diet contained sufficient amounts of minerals (Table 2.2) and

vitamins (Table 2.3) based on the recommendations by the American Institute of Nutrition (AIN) for rodents (Reeves et al. 1993).

The diets were freshly prepared by mixing 900 g dry substance with 100 g fat and water and subsequent freeze drying (CHRIST, BETA 1-8, freeze dryer, Germany). The residual water content of the diet was <5g/100 g. The diets were stored at 4°C up to the consumption. In all experiments, diets were administered in restricted amounts to standardize the diet intake.

Table 2.1: Diet Components (Experiment 1, 2 and 3)

Components	Amount [g/kg Diet]
Protein[1]	200
Fat (lard)	100
Sugar	200
Cellulose (fibre)	50
Corn starch	389.5
Minerals + Vitamins	60
Cholesterol	0.5

[1]Casein and ethanol washed soy protein isolate in experiment 1.

Pork, beef, fish, turkey protein isolated from meat, casein, and ethanol washed soy protein isolate were used in experiment 2.

Pea, lupin, ethanol washed soy protein isolate, fish protein and casein were used in experiment 3.

In order to meet the AIN recommended amounts in experiment 3, all plant protein diets were supplemented with DL-methionine (Pea diet: 3.6 g/kg; Lupin diet: 4.4 g/kg; Soy protein diet: 3 g/kg). Lupin protein diet was also supplemented with lysine (6.4 g/kg). These amino acids were supplemented on the expense of cellulose.

Table 2.2: Mineral Composition of the diets, used in Experiment 1, 2 and 3

Elements	Components	Amount [mg/kg Diet]
Calcium	Calcium carbonate	5000
Calcium, Phosphorus	Dicalcium phosphate	1561
Magnesium	Magnesium oxide	507
Potassium	Potassium sulphate	3600
Sodium, Chlorine	Sodium chloride	1019, 1571
Iron	Ferrous sulphate	35
Zinc	Zinc oxide	30
Manganese	Manganese oxide	10
Copper	Copper sulphate	6
Selenium	Sodium silinite	0.15
Iodine	Calcium iodate	0.2

Table 2.3: Vitamin Composition of the diets, used in Experiment 1, 2 and 3

Components	Amount [/kg Diet]
Vitamin A	5000 IU
Vitamin D3	1000 IU
Vitamin K	0.75 mg
Thiamin B1	5 mg
Riboflavin B2	6 mg
Pyridoxine B6	6 mg
Nicotinic acid	30 mg
Folic acid	2 mg
Calcium pentathenate	15 mg
Biotin	0.2 mg
Choline chloride	1000 mg
Vitamin E-Acetate	75 IU

2.3 Analysis of the Experimental Diets

For analysis of the fatty acid composition of the complete diet (experiment 2 & 3), total lipids were extracted according to method of Hara and Radin (1978) modified by Eder and Kirchgessner (1994). To extract total lipids, 7.0 g ground diet was dissolved in 30 ml Hexane/Isopropanol (3:2, v/v) solvent. The samples were shaken (Incubator Shaker G 25, Brunswick Scientific, Edison, New Jersey, USA), overnight at ambient temperature, and afterwards centrifuged at 2000 rpm, for 10 min. The clear supernatant was used for the fatty acid analysis. Fatty acids concentrations of the diets were analysed by gas chromatography (Eder and Brandsch 2002).

Table 2.4.a: Fatty Acid Concentrations of the Diets used in Experiment 2

Fatty Acids	Pork diet	Beef diet	Fish diet	Turkey diet	Casein diet	Soy diet
	[g Fatty acid/kg dry matter]					
16:0	25.9	26.1	25.5	25.7	25.7	25.6
16:1 n-7 + n-9	2.5	2.5	2.6	2.5	2.5	2.4
18:0	16.8	16.8	16.0	16.0	16.2	16.2
18:1 n-9 + n-11	37.2	37.3	37.3	37.3	37.6	37.4
18:2 n-6	10.6	10.6	10.7	10.6	10.8	11.5
18:3 n-3	0.9	0.9	0.9	0.9	0.9	1.0
20:1 n-9	0.8	0.8	0.8	0.9	0.9	0.8
20:2 n-6	0.5	0.5	0.5	0.5	0.5	0.5
20:3 n-6	0.1	0.1	0.1	0.1	0.1	0.1
20:4 n-6	0.1	0.1	0.1	0.1	0.1	0.1
20:5 n-3	0.1	0.1	0.1	0.2	0.1	0.1
22:3 n-9	0.2	0.1	0.1	0.1	0.0	0.0
22:5 n-3	0.1	0.1	0.1	0.1	0.1	0.1

22:6 n-3	0.0	0.0	0.0	0.2	0.0	0.0

Conditions:

Column: FUSED FATTY ACID PHASE silica rectifying column
30 m/0.53 mm inside diameter (Macherey and Nagen, Düren)

Feed Gas: Helium, 8.4 ml/min

Quantity: 1µl

Detector: Flame ionization detector

Table 2.4.b: **Fatty Acid Concentrations of the Diets used in Experiment 3**

Fatty Acids	**Fish diet**	**Casein diet**	**Soy diet**	**Lupin diet**	**Pea diet**
	[g Fatty acid/kg dry matter]				
16:0	25.4	25.3	25.4	25.3	25.1
16:1 n-7 + n-9	2.27	2.27	2.27	2.27	2.22
18:0	15.0	15.1	15.1	15.1	14.8
18:1 n-9 + n-11	39.6	39.8	39.9	39.9	39.4
18:2 n-6	10.4	10.4	10.5	10.4	11.3
18:3 n-3	0.87	0.88	0.89	0.88	1.12
20:1 n-9	0.89	0.89	0.89	0.89	0.88
20:4 n-6	0.12	0.12	0.12	0.12	0.12
20:3 n-6	0.03	0.02	0.02	0.03	0.02
20:5 n-3	0.08	0.06	0.05	0.06	0.05

Table 2.4.a shows the fatty acid concentrations of different diets used in experiment 2 and Table 2.4.b shows the fatty acid concentrations of different diets used in experiment 3. Within one experiment, fatty acid concentrations of the different diets were very similar. Only in experiment 3, the pea diet showed a bit higher concentration of linoleic acid (18:2, n-6); and α-linoleic acid (18:3, n-3) than the other diets.

2.4 Processing of the Dietary Proteins

Soy Protein Isolate: Pre-treatment

To minimize possible effects of soy protein associated isoflavones that may partly be responsible for cholesterol reduction by soy protein. The soy protein isolate (90% protein: Protein Technologies International, Ieper, Belgium) used for this study was additionally ethanol washed, a process which has been shown to be an efficient way to remove remaining isoflavones (Fukui et al. 2004). The soy protein isolate was washed twice with 10 volumes of 60% ethanol by stirring for 2 hours at RT. Protein was filtered, air dried and ground to fine powder.

Casein: (Nähr Kasein, Meggle, Wasserburg/Inn, Germany), was used without further processing.

Meat Proteins: Pork, beef, and turkey proteins were isolated from lean meat, and fish protein was isolated from Alaska pollack fillets (Shukla et al. 2006a), which were purchased in a local supermarket. The frozen fish fillets and all kinds of meat were chopped into small pieces, all visible fat and connective tissues were removed. Chopped meat were boiled for 30 minutes and then chilled over night at 4°C. Meat mash was homogenized in a grinder (Ika Universalmühle M20, Staufen, Germany). The crude protein was obtained after freeze drying (CHRIST, BETA 1-8, Germany) and rehomogenization. To remove protein associated lipids, the crude protein powder was treated twice with 10 volumes of acetone and once with 10 volumes of acetone/ethanol (1:1, v/v) solution. The protein was filtered air dried and ground to fine powder.

Pea protein: (PISANE, Cosucra, Belgium), was used without further processing.

Lupin protein: (Lupin Protein Isolate Type E, Fraunhofer Institüt für Verfahenstechnik und Verpackung, Freising, Germany), was used without further processing.

2.5 Analysis of the Dietary Proteins

Dry substances and amounts of raw protein, raw fat and raw ashes of all dietary proteins used are shown in table 2.5.

Dry Matter Analysis

Percent dry matter of each protein was determined by the method of Naumann and Basler (1993).

The glass wares with lid were dried in an oven (105°C, 1h), cooled down in a desssicator and weighed (i.e. weight A). 1.0 g fine homogenized protein sample was added (i.e. weight B), and dried for 3 h at 105°C. The probes were cooled down in desiccators and weighed (i.e. weight C). After an additional drying period of 1 h, process was repeated until constant weight achieved.

$$\%\ \text{dry matter} = \frac{(C - A) \times 100}{(B - A)}$$

Table 2.5: Dry matter and raw nutrients of the dietary Proteins used in Experiment 1, 2 and 3

Dietary proteins		Dry Matter	Protein	Fat	Ashes
		[g/100 g Protein]			
Exp 1	Casein	90.9	91.8	0.88	4.74
	Soy	84.7	93.0	1.24	5.03
Exp 2	Pork	91.6	94.1	1.97	3.19
	Beef	91.1	94.0	1.81	2.94
	Fish	91.6	92.6	0.76	4.21
	Turkey	91.3	95.0	0.66	2.96
	Casein	90.9	91.8	0.88	4.74
	Soy	84.7	93.0	1.24	5.03
Exp 3	Fish	93.3	91.8	0.86	4.69
	Casein	90.9	91.8	0.88	4.74
	Soy	91.8	98.0	0.65	3.65
	Pea	93.7	85.7	8.07	4.81
	Lupin	93.9	96.0	1.06	4.02

Protein Analysis

Protein was determined by the method of Kjeldahl (1883). The samples were digested with a strong acid so that it releases nitrogen which can be determined by the titration technique. Since all the proteins contain about the same amount of nitrogen (16%), the protein concentration was calculated by multiplying the nitrogen content with 6.25.

The samples were weighed and digested by heating in the presence of sulphuric acid, and anhydrous sodium sulphate. Digestion converts nitrogen in the food into ammonia, and other organic matter to CO_2 and H_2O. The ammonia gas liberated from the solution moves into the receiving flask which contains excess of boric acid. The low pH of the solution converts ammonia gas into ammonium ion, and simultaneously converts the boric acid to the borate ion. The nitrogen content was estimated by titration of the ammonium borate formed with standard sulphuric or hydrochloric acid. The concentration of the hydrogen ions (in moles) required to reach the end point is equivalent to the concentration of nitrogen that was in the original sample. Once the nitrogen content has been determined it is converted to the protein content using the appropriate conversion factor:

$$\% \text{ Protein} = 6.25 \times \% \text{ N}$$

Fat Analysis

Extraction of the crude fats was performed with the Soxtec HT 1043 extraction unit (Foss GmbH, Hamburg, Germany) on the basis of return-back principle with the solvent petroleum ether and followed by evaporation of the solvent and collection of the crude fat through weighing extracted material. Before the extraction an acidic hydrolysis was carried out to extract all fat substances which are not extractable by solvents. 1 g of the sample was hydrolysed with 120 ml 0.3 mole/L HCl for 15 min.

Ashes Analysis

The ash fraction contains all the mineral elements jumbled together. It allows calculation of nitrogen free extract (by difference) from dry matter and provides an estimate of contamination.

This method consists of oxidizing all organic matter in a weighed sample of the material by incineration and determining the weight of the ash remaining.

Homogenized samples were weighed (5.0 g) into dried (ignited and tarred) crucibles and placed in a drying oven at 100°C for 24 hours, followed by transferred to a cool muffle furnace and temperature was increased stepwise to 550°C ± 5°C. Temperature was maintained, until a white ash appeared (~8 hours).

$$\text{Ash (\%)} = \frac{(\text{crucible + ash weight}) - (\text{crucible weight}) \times 100}{(\text{crucible + sample weight}) - (\text{crucible weight})}$$

Amino Acid Analysis of the Dietary Proteins

Determination of the amino acid composition was performed with ion exchange chromatography using an amino acid analyser (Biotronic L C 3000**,** Eppendorf, Hamburg, Germany).

In order to determine methionine and cysteine the probes were oxidised: 250 mg diet was mixed with 5 ml of oxidation mixture (0.5 ml 30% H_2O_2, 4.5 ml phenol containing 98% formic acid and 25 mg phenol) and incubated for 24 h at 0°C. The oxidation was stopped by adding 0.9 g sodium disulfide. Afterwards, oxidised samples were hydrolysed with 50 ml of a hydrolysis mixture (492 ml HCl, 1 g phenol, and water to 1 L) for 24 h at 110°C (Bassler and Buchholz 1993). After cooling the probes, NaOH was added until the pH-value reached 2.2. The solution volume was adjusted and an internal standard (20 µmol Norleucin/ml citrate buffer pH 2.2) was added to the probe. An aliquot of this hydrolysate was applied to the amino acid analyzer. The separation of the amino acids was carried out by elution with buffer solutions of different pH-values. Elute was mixed continuously with ninhydrin-reagent and the extinction was read at 440 nm for proline and at 570 nm for all other amino acids. For calculation the peak volume of the amino acids and internal standard were used.

Amino Acid Concentrations of the Diets:

The amino acid concentrations of the diets used in experiment 2 are presented in table 2.6.a. Soy protein had higher concentrations of cysteine, aspartic acid, glycine, alanine, arginine and lower concentrations of serine, glutamic acid, proline, tyrosine, valine methionine and lysine. The ratios of lysine/arginine and mthionine/glycine were also lower in soy protein.

Table 2.6.a: Amino Acid Concentrations of the Diets used in Experiment 1

Amino acid	Soy	Casein
	[g/kg diet]	
Cysteine	1.8	0.8
Aspartamic acid	19.5	13.2
Serine	8.6	10.8
Glutamic acid	29.0	42.2
Proline	7.5	20.2
Glycine	6.9	3.4
Alanine	7.6	5.4
Tyrosine	5.5	8.6
Arginine	11.6	6.4
Methionine	2.5	5.2
Threonine	6.2	7.8
Valine	8.1	12.1
Isoleucine	7.6	8.8
Leucine	14.8	17.6
Phenylalanine	8.6	9.6
Histidine	5.7	6.4
Lysine	10.1	14.6
Lysine/Arginine	0.9	2.3
Methionine/Glycine	0.4	1.5

The amino acid concentrations of the diets used in experiment 2 are presented in table 2.6.b. Soy protein and proteins isolated from meat, pork, beef, turkey and fish protein had higher concentrations of cysteine, aspartic acid, glycine, alanine, arginine and lower concentrations of

serine, glutamic acid, proline, tyrosine, valine, compared to casein. Soy protein had lower concentrations of methionine and lysine compared to casein. Meat proteins and fish protein had similar amino acids concentration only turkey protein had higher concentration of histidine. Ratios of lysine/arginine, and methionine/glycine were highest in the casein.

Table 2.6.b: Amino Acid Concentrations of the Diets used in Experiment 2

Amino acid	Fish	Pork	Beef	Turkey	Soy	Casein
	[g/kg diet]					
Cysteine	1.7	1.7	1.5	1.7	1.8	0.8
Aspartamic acid	20.0	18.5	18.3	18.4	19.5	13.2
Serine	7.8	7.5	7.3	7.0	8.6	10.8
Glutamic acid	30.6	30.4	31.6	29.2	29.0	42.2
Proline	5.6	6.9	6.5	6.0	7.5	20.2
Glycine	7.9	8.9	8.1	8.0	6.9	3.4
Alanine	11.4	11.7	11.6	11.8	7.6	5.4
Tyrosine	5.1	5.0	5.4	4.8	5.5	8.6
Arginine	10.7	11.5	11.5	11.0	11.6	6.4
Methionine	5.7	4.7	4.8	5.1	2.5	5.2
Threonine	8.1	8.5	8.6	8.2	6.2	7.8
Valine	9.3	11.7	8.8	9.4	8.1	12.1
Isoleucine	7.9	7.9	8.1	8.6	7.6	8.8
Leucine	15.4	15.6	15.8	15.6	14.8	17.6
Phenylalanine	6.6	6.5	6.7	6.6	8.6	9.6
Histidine	6.0	7.4	7.0	10.4	5.7	6.4
Lysine	17.4	17.3	17.5	17.3	10.1	14.6
Lysine/Arginine	1.6	1.5	1.5	1.6	0.9	2.3
Methionine/Glycine	0.72	0.5	0.6	0.6	0.4	1.5

Table 2.6.c: Amino Acid Concentration of the Diets used in Experiment 3

Amino Acids	Pea	Lupin	Soy	Fish	Casein
	[g/kg diet]				
Cysteine	1.4	2.0	2.1	1.6	0.8
Aspartamic acid	18.7	19.6	20.9	17.4	13.2
Serine	8.3	9.5	9.3	7.4	10.8
Glutamic acid	29.2	42.9	37.3	27.5	42.2
Proline	7.2	7.4	9.8	5.7	20.2
Glycine	6.1	6.0	7.1	7.5	3.4
Alanine	6.4	5.1	7.2	10.0	5.4
Tyrosine	5.2	8.2	5.9	5.1	8.6
Arginine	14.1	18.3	14.1	11.0	6.4
Methionine	1.4	0.6	2.0	5.0	5.2
Threonine	5.9	5.8	6.5	7.7	7.8
Valine	8.0	6.7	8.5	8.7	12
Isoleucine	7.6	8.8	8.4	7.7	8.8
Leucine	13.9	14.4	14.6	14.2	17.6
Phenylalanine	8.6	7.3	9.6	7.1	9.6
Histidine	4.7	4.2	5.8	5.7	6.4
Lysine	11.4	6.7	10.8	15.7	14.6
Lysine/Arginine	0.8	0.4	0.8	1.4	2.3
Methionine/Glycine	0.2	0.1	0.3	0.7	1.5

In order to meet the recommended amounts in experiment 3, all plant protein diets were supplemented with DL-methionine (Pea diet: 3.6 g/kg; Lupin diet: 4.4 g/kg; Soy protein diet: 3 g/kg). Lupin protein diet was also supplemented with lysine (6.4 g/kg).

The amino acid concentrations of the diets used in experiment 3 are presented in table 2.6.c. The fish protein and casein had higher levels of lysine and methionine compared to plant proteins

(pea, lupin and soy protein). Soy protein had higher concentration of cysteine, aspartic acid, glycine, alanine, arginine compered to casein. Lupin and casein had higher concentrations of glutamic acid and tyrosine. Casein had higher concentrations of proline and valine compared to fish and plant proteins. Ratios of lysine/arginine, and methionine/glycine were lower in fish and plant proteins diets compared to casein.

Genistein Quantification in the Ethanol Washed Soy Protein Isolate

Quantitative determination of genistein, the major isoflavone in soy protein, was used to proof the efficacy of the ethanol extraction process. Genistein was determined by a modification of the method of Bilia et al. (2001).

In brief, soy protein isolate was hydrolyzed with HCl (1mol/L) in 50 % ethanol for 1 h at room temperature. After neutralization with NaOH, isoflavones were extracted three times with 20 ml ethyl acetate. After evaporation of ethyl acetate, isoflavones were dissolved in ethanol and used for a quantitative HPLC analysis of genistein using an ELITE LaChrom HPLC system (Hitachi, Manheim, Germany). The calibration was performed with Genistein.

Conditions:
Eluent A: Phosphoric acid, pH: 3
Eluent B: Acetonitrile
Flow rate: 1 ml/min
Detection wavelength: 329 nm

Sample	Concentration (mg/g)
Soy protein isolate before washing (Exp. 1 & 2)	0.42
Ethanol washed soy protein isolate (Exp. 1 & 2)	0.03
Soy protein isolate before washing (Exp. 3)	0.50
Ethanol washed soy protein isolate (Exp. 3)	0.08

2.6 Sample Preparation

After completions of the feeding period, rats were starved overnight (Experiment 1 and 2) to ensure that all rats had nearly the same interval of starvation until killing and to avoid the contamination of plasma with chylomicrones. While in Experiment 3, rats were killed in non-fasted state. Rats were anesthetized lightly with diethyl-ether and killed by decapitation. Whole blood was collected in heparinized monovettes. The liver was excised, rinsed in ice cold NaCl solution and immediately shock frozen in liquid nitrogen. Small portion of liver was stored at -80°C, for RNA isolation. The remaining organ was stored at -20°C. Faeces were collected from the last week of feeding, freeze dried (CHRIST; BETA 1-8, Germany), weighed and stored at -20°C up to the analysis.

2.6.1 Blood, Plasma and Lipoproteins

For the preparation of plasma, blood was centrifuged (3000 rpm, 4°C, 10 min). Plasma was stored at -20°C up to the analysis. Plasma lipoprotein fractions were separated by step-wise ultracentrifugation (RC-M150, SORVALL 1997), appropriate density cuts commonly used for the measure of rat lipoproteins (Sparks et al. 1998, Giudetti et al. 2003, Sirtori et al. 2004).

Reagents**:**

Solution A**:** 0.195 M Sodium chloride solution

Solution B**:** 0.195 M Sodium chloride solution and 2.44 M Sodium bromide solution

The plasma densities were adjusted with solution A and centrifuged at 150,000 rpm, at 4°C, for 1.5 h (RC-M150, SORVALL 1997). The lipoproteins of very low density (VLDL) were removed as upper phase ($\delta < 1.006$ g/ml). After the separation of VLDL the plasma densities were again adjusted with solution B and centrifugation process was repeated in the same way. Lipoprotein of low density (LDL) deposited as upper phase ($1.006 < \delta < 1.063$ g/ml). The remaining portion contained high density lipoprotein (HDL) fraction ($\delta > 1.063$ g/ml). All the lipoprotein samples were stored at -20°C.

2.6.2 Preparation of Homogenate, Cytosol and Microsomes

Liver homogenate and liver cytosol were prepared by the method of Garg et al. (1998) and Christiansen et al. (1991).

Liver Homogenate: 1.5 g frozen liver was chopped into small pieces, 4 ml of homogenizing buffer (0.1 M phosphate buffer pH 7.4 with 0.25 M sacccharose) was added and homogenized at 4°C in a homogenizer (Potter-S, Brown, Biotech International, Melsungen). The cell debris were separated by centrifugation (3000 rpm, 4°C, and 10 min). The homogenate in the supernatant was stored at -20°C.

Liver Cytosol: Liver homogenate was centrifuged at 26,000 rpm, at 4°C, for 15 min. The supernatant was centrifuged again at 105,000 rpm, at 4°C, for 1 h. The resulting clear cytosol in the supernatant was stored at -20°C.

Liver Microsomes: For preparation of liver microsomes, liver homogenates were centrifuged at 15,000 rpm for 20 min at 4°C. The microsomal pellet was obtained by centrifugation of the 15,000 rpm supernatant at 105,000 rpm for 1 h at 4°C.

2.6.3 Extraction of total Lipids from the Liver and Faeces

The extraction of the total lipid was performed by the method of Hara and Radin (1978) modified by Eder and Kirchgessner (1994). The total lipids from the homogenized sample materials (Liver 400 mg, Faeces 500 mg) were extracted with Hexane/Isopropanol solvent (3/2, v/v), by shaking (Incubator Shaker G 25, Brunswick Scientific, Edison, New Jersey, USA) for 18 h at ambient temperature. The supernatant was removed and used for further analysis.

2.7 Analytical Methods

2.7.1 Cholesterol and Triglyceride Determination

Cholesterol and triglyceride concentration in liver, plasma and lipoproteins (VLDL, LDL, and HDL) were determined using enzymatic reagent kits obtained from Merck (Ecoline 25, MERCK Eurolab, Germany).

Cholesterol and its esters are released from lipoproteins by detergents. Cholesterol esterase hydrolyses the esters. In the subsequent enzymatic oxidation by cholesterol oxidase, H_2O_2 is formed. This is converted into a coloured quinonimine in a reaction with 4-aminoantipyrine and salicylic alcohol catalyzed by peroxidase. The absorbance was measured at 500 nm. Determination of triglycerides was performed after enzymatic splitting with lipoprotein lipase. Indicator was quiononeimine which is generated from 4-aminoantipyrine and 4-chlorophenol by hydrogen peroxide under the catalytic action of peroxidase. The absorbance was measured at 550 nm. The

cholesterol and triglyceride concentrations in the plasma and lipoproteins were measured by directly mixing with reaction solution. For measuring the cholesterol and triglyceride concentration in liver and faeces total lipid extracts (2.6.3) were used. The aliquots were dried in a vacuum centrifuge (RC10.22 JOUAN, France). Lipids were dissolved using Triton X-100 (De Hoff et al. 1978).

The quantification of cholesterol and triglyceride was measured by adequate standards.

$$C_s = \frac{A_s \times C_{st}}{A_{st}}$$

C is the Concentration of the cholesterol or triglyceride in the sample, A_s and A_{st} are the absorbance of the sample and standard respectively, and C_{st} is the concentration of the standard.

2.7.2 Estimation of Apolipoproteins

Separated plasma lipoproteins (VLDL + chylomicrones, LDL, HDL) were subjected to analytical sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and apolipoproteins were estimated after densitometric scanning of Coomassie Blue-stained bands according to a modified method of Karpe and Hamsten (1994). Briefly, polyacrylamide gels were cast by a gradient from 4 to 15% acryl amide. The 4% acryl amide solution contained acryl amide (38.96 g/l), bisacryl amide (1.04 g/l), Tris (0.375 M), SDS (0.1%), TEMED (0.875 µl/ml) and ammonium persulphate (0.05%), whereas the 15% acryl amide solution contained acryl amide (146.1 g/l), bisacryl amide (3.9 g/l), Tris (0.375 M), SDS (0.1%), TEMED (0.875 µl/ml) and ammonium persulphate (0.05%).

Total protein content of lipoproteins was determined by the BCA assay with bovine serum albumin as standard. Lipoproteins (250 µg/ml) were dissolved (1:1) in buffer containing 0.22 M Tris, 41% glycerol, 0.7% SDS, 5% mercaptoethanol and 0.002% bromphenol blue and subsequently denaturated at 95°C for 5 min. After chilling 20 µl of denaturated probes containing 2.5 µg proteins were applied to the gels. Electrophoresis was run at 210 V for 1 h. Gels were stained in 0.25% Coomassie, 40% methanol, 10% acetic acid for 1 h and distained in 12% methanol, 7% acetic acid over night. Gels were scanned with a computer connected gel documentation system (Gel-Pro Analyzer, Intas, Göttingen, Germany). Bands of apo B-100, B-48, E and AI were identified by comparing the R_f values with that of high molecular weight standards (Serva, Heidelberg, Germany).

2.7.3 Amino Acids Analysis in Plasma

Protein precipitation was performed by adding 50 µl of 10% sulfosalicylic acid (4°C) to 200 µl plasma, followed by 30 min incubation at 4°C. After centrifugation (15,000 rpm, 10 min at 4°C), 20 µl of the supernatant was mixed with 20 µl internal standard (Norvalin, SERVA) and 80 µl deionized water. Concentrations of free amino acids in the plasma of rats were measured as isoindole derivatives by high performance liquid chromatography (Hewlett Packard, Waldbronn, Germany) according to Schuster 1988**,** after pre-column derivatization. Isoindole derivatives were detected at an excitation wavelength of 337 nm and emission wavelength of 454 nm.

2.7.4 Protein Determination

The protein concentration was determined by the Bradford assay (1976). The assay is based on the observation that the absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 shifts from 465 nm to 595 nm when binding to the protein occurs. Both hydrophobic and ionic interactions stabilize the anionic form of the dye, causing a visible colour change.

To 50 µl of the sample 200 µl of the Bradford reagent was added , incubated for 10 minutes at room temperature and absorbance was measured at 595 nm by spectrophotometer (Spectro flour plus, TECAN, Germany), A standard curve with known protein (bovine serum albumin) concentrations was prepared in parallel with the samples. By that standard curve the concentrations of the samples were determined.

2.7.5 Phospholipid Analysis in Plasma

Phospholipids in the plasma were determined enzymatically by a test kit (Phospholipids enzymatiques PAP 150, bioMerieux, France). Phospholipids (lecithins, lysolecithins and sphingomylin) are hydrolysed by phospholipase D and the liberated choline is measured by the TRINDER reaction.

The phospholipids concentration in the plasma was measured by directly mixing with working solution and incubating at 37°C, for 10 min. The optical density (OD) was measured at the wavelength of 505 nm against the reagent blank (ULTRASPEC 2000). The quantification was done by a standard.

$$C\text{sample} = \frac{OD \text{ sample}}{OD \text{ standard}} \times C\text{standard}$$

The phospholipid concentrations were measured in the plasma of rats fed casein or soy protein in experiment 1. No difference was observed between the rats fed casein or soy protein (1.5 mmol/L vs. 1.6 mmol/L), respectively.

2.7.6 Bile Acid Analysis

Bile acid concentrations in plasma and faeces were analyzed enzymatically (MERCK) by a modified method of Marlett & Fisher (2002). 3-α-hydroxy-bile acids are specifically converted to the corresponding 3-keto derivatives in the presence of NAD^+ and with the aid of 3-α-hydroxysteroid dehydrogenase. The NADH formed reacts with Nitrotetrazolium Blue under the catalytic influence of diaphorase to give a blue formazan derivative, which was measured at 500 nm.

Preparation of Bile Acid Extract

About 0.4 g freeze dried, fine ground faeces were dissolved in 4 ml Toluol/Methanol solvent (1:1, v/v), and shaken (Incubator Shaker, G 25) for 18 hour at ambient temperature, vortexed and centrifuged at 2000 rpm, for 10 min. Supernatant was transferred and resuspended in toluol/methanol solvent (1:1, v/v). Supernatant was pipetted for each probe and corresponding blank and vacuum centrifuged. Afterwards, 20µl triton/chloroform (1:1, v/v) solvent was added to each probe and corresponding blank, vortexed and vacuum centrifuged.

The bile acid concentrations in plasma and faeces were measured by incubation of the sample and their corresponding blanks with sample reaction solution and blank reaction solution. The reaction was stopped by the addition of stop reagent. The absorbance was read at 500 nm and used for quantification. The calculation of bile acid concentration in the samples was performed by means of standard after subtracting the corresponding sample blanks.

2.7.7 Thin layer chromatography

The fractions of free and esterified cholesterol in the liver were separated by thin layer chromatography and were determined densitometrically (Hojnacki et al. 1976).

Running solvent**:** n-Hexane: Diethyl-ether: 100 % acetic acid = 80: 20: 3

Staining solution: 20 % Ammonium sulphate (Isocommerz)

Probe: Liver extracts (2.6.3)

Standard**:** Cholesterol /Cholesterol ester**:** (3 mg/3mg)/1 ml Hexane /Isopropanol (3:2, v/v) solvent

Cholesterol and Cholesterol ester (cholesterol oleate, 98%) obtained from Sigma were mixed in the ratio (1**:**1, w/w), that makes the concentration of each (C/CE) 1.5 mg/ml. Several concentrations (1.5; 3.0; 7 µg/µl) of the above standard were used.

All the samples and standards were spotted on a Thin Layer Chromatography (TLC)-aluminum foil (Kieselgel 60, 20X20 cm, MERCK) with the device LINOMAT 5 (CAMAG, Switzerland). The foil was inserted in the running chamber and the solvent was run on the plate up to ½ cm left from upper side. The foil was air dried, and stained in the staining chamber (30 sec). Brown colour bands appeared after drying in an oven at 200°C for 5 min. The foil was photographed and evaluated by the associated device (Syngene Gene Genious Synopsis, USA). Calculation was performed with a standard curve.

2.7.8 Activity of Lipogenic Enzymes

The activities of the enzymes Glucose 6-phosphate dehydrogenase (G6PDH) and fatty acid synthase (FAS) were determined in liver cytosol (2.6.2). The results were related to the protein concentration.

2.7.8.1 Glucose 6-Phosphate Dehydrogenase

Activity of G6PDH (E.C.1.1.1.49) was measured by the method of Deutsch (1995). Glucose 6-phosphate and $NADP^+$ react in the presence of enzyme G6PDH and form 6-phosphogluconolactone and $NADPH/H^+$. The rate of increase in absorbance at 339 nm corresponds to the increase of $NADPH/H^+$ and therefore to the G6PDH activity.

Reagents:

Phosphate buffer: 0.1 M K_2HPO_4/KH_2PO_4 (pH 7.4)

Test medium**:** 500 mM Tris-(hydroxymethyl)-amino methane
3.8 mM NADP (Na-salt)
63 mM $MgCl_2$
33 mM glucose 6 phosphate

5 mM Malenimide

Liver cytosol was diluted 1:10 by the phosphate buffer and incubated at 25°C. Test medium was also maintained at 25°C and added to the cytosol. After an incubation of 2 minutes the increase in absorption was measured at 339 nm for 1 minute (ULTRASPEC 2000). One blank was also measured simultaneously and the sample reading was corrected by the blank reading.

$$Activity = \frac{V \times \Delta E}{\varepsilon \times d \times v}\left[\frac{nmol\ NAPDH / H^{+}}{mg\ \ \mathrm{Pr}otein \times \min}\right]$$

V→Total volume (1000 µl)

ΔE→Change in the absorption at 339 nm

∈→Molar extinction coefficient of NADPH (6.22 *10^{-3}[mL* $nmol^{-1}$* cm^{-1}])

d→Thickness [cm]

v→ Volume of the sample (50µl)

2.7.8.2 Fatty Acid Synthase

The activity of fatty acid synthase (EC.2.3.1.85) was measured by the method of Nepokroeff et al. (1975). In the presence of FAS malonyl CoA and acetyl CoA oxidize NAPDH into $NADP^{+}$ and n-carbonic acid is formed. The activity of FAS is proportional to the NADPH required.

Reagents:

Preincubation medium:	500 mM K_2HPO_4/KH_2PO_4 (pH 7.0), 5.0 mM Dithiothreitol
Medium 1:	700 mM K_2HPO_4/KH_2PO_4 (pH 7.0), 0.14 mM $NADPH/H^{+}$
	1.4 mM EDTA, 1.4 mM Dithiothreitol
Medium 2:	0.33 mM Acetyl CoA
Medium 3:	1.0 mM Malonyl CoA

Test Medium = 700 µl Medium 1 + 100 µl Medium 2 + 100 µl Medium 3

Liver cytosol diluted with preincubation medium (1:5, v/v) was incubated at 37°C for half an hour. Test mediums were incubated at 25°C and were mixed with cytosol probe in a cuvette, the absorption was measured by spectrophotometer (ULTRASPEC 2000) at 340 nm for 1 minute. One

blank without malonyl CoA was measured; from blank, oxidation of NADPH by FAS was corrected.

$$Activity = \frac{V \times \Delta E}{\varepsilon \times d \times v}\left[\frac{nmol}{mg\ \ \mathrm{Pr}otein \times \min}\right]$$

V→ Total volume (1000 µl)

ΔE→ Change in absorption at 340 nm

∈→ Molar extinction coefficient of NADPH (6.22 *10^{-3}[mL* $nmol^{-1}$* cm^{-1}])

d→ Thickness [cm]

v→ Volume of the sample (20µl)

2.7.9 MTP activity

The microsomal triglyceride transfer protein (MTP) activity was measured in the liver homogenate by a commercially available Fluorescence assay (Chylos, Inc., Woodburg, NY, USA) kit. MTP activity was measured by incubating liver homogenates with donor vesicles containing fluorescence-labeled lipids, and measuring the fluorescence transferred to acceptor vesicles.

Triplicate vesicles were pipetted in a fluorescence microtiter plate. Water and samples were added to the vesicles. Ingredients were allowed to reach room temperature. Reaction was started by adding homogenate to control and test, and isopropanol to the totals. Microtiter plate was incubated at RT for 30 min. Fluorescence units (FU) were measured using excitation wavelengths of 470 nm and emission wavelengths of 550 nm.

% Transfer in controls (C): (Control $_{FU}$ – Blank $_{FU}$) / (Total $_{FU}$ – Blank $_{FU}$) X 100

% Transfer in test samples (D): (Test $_{FU}$ – Blank $_{FU}$) / (Total $_{FU}$ – Blank $_{FU}$) X 100

2.7.10 Expression of Enzymes in the Liver

All expression experiments were based on the principle of reverse transcriptase and polymerase chain reaction (PCR).

2.7.10.1 Isolation of total RNA

Isolation of total RNA was performed according to Guanidinium Thiocyanate method following Chirgwin et al. (1979) with TRIZOL Reagent (Invirogen, Life technologies). About 10 mg frozen liver was homogenized in 1 ml of Trizol in an ice cooled hand homogenizer followed by incubation at 25°C, for 5 min. By adding chloroform and following centrifugation, probes were separated into an aqueous phase and an organic phase. RNA remains in aqueous phase. The RNA in the aqueous upper phase was transferred in a new eppendorf tube. The RNA was recovered by precipitation with isopropyl alcohol. The resulting RNA pellet was washed twice with 75% ethanol, vortexed, centrifuged (9700 rpm, 4°C, 5 min), and air dried. Finally the RNA pellet was dissolved in 50 µl of RNAse free water with slow vibration (Thermal Mixer Comfort, Eppendorf, Hamburg) at 60°C for 5 min. The RNA samples were stored at -80°C.

2.7.10.2 Quality and Concentration of RNA

To determine quality and concentration of RNA, the reaction tubes and solutions to be used were RNAse free, either in suitable form of commercial manufacturers referred or 0.1 % diethylpyrocarbonate (DEPC) treated and autoclaved. All the reagents/buffers needed were prepared with 0.1 % DEPC treated high purity water. The RNA concentration was measured spectrophotmetrically (ULTRASPEC 2000). The RNA was diluted to 1**:**100 and the absorption was measured against water at 260 nm and 280 nm in quartz cuvettes.

$$RNA\left[\mu g/ml\right] = A_{260} \times 40 \times 100$$

A_{260} is the absorption at 260 nm, 40 is the RNA [µg/ml] concentration at A_{260} and 100 is the dilution factor.

The purity estimation of RNA was done by the quotient absorption at 260 nm and absorption at 280 nm. All samples pointed a quotient of 1.6 or up which indicates good quality of RNA.

The quality of RNA was also analzyed by gel electrophoresis.

For the RNA gel electrophoresis following reagents were used:

Gel buffer (pH 7.0)	Running buffer	Loading buffer
200 mM Mops	100 ml gel buffer	4 ml 10 × gel buffer
50 mM Sodium acetate	20 ml Formaldehyde	20 mg Bromophenol blue
10 mM EDTA	880 ml water	80 µl 500 mM EDTA (pH 8.0)
		720 µl Formaldehyde (37%)
		2 ml Glycerol (100%)
		3 ml Formamide

MOPs: 3-[N-Morpholino] propanesulphonic acid; EDTA: Ethylene Diamine Tetra Acetic acid;

The gel was prepared from 0.36 g Agarose (SERVA), 3 ml gel buffer and 26.5 ml DEPC-water. The gel was heated to clear solution (5 min), and cooled down to 65°C. Afterwards, 540 µl formaldehyde and 14 µl ethidium bromide (0.5 µg/ml) were added to the gel. The gel was dried and then equilibriated in the running buffer for 30 min. 10 µl RNA (4 µg) with 4 µl loading buffer was poured in the slots and electrophoresis was ran (1 h, 5-7 V/cm). The gel was photographed under UV light and bands were evaluated with the associated device.

2.7.10.3 cDNA Synthesis

The RNA was copied into cDNA. **(**Omniscript transferase and buffer were purchased from Quigen, Hilden, Germany and dNTPs were purchased from Carl Roth GmbH, Karlsruhe, Germany).

Mater mix was added to the RNA and cDNA was synthesized for 59 minutes at 37°C (Mastercycler, Personal, Cologne, Germany). Reaction was stopped by denaturing at 93°C for 5 min.

Reagents	1× Sample (Master mix)
RNAse free water	10.5µl
10×buffer	2.0µl
Di-oxynucleotide triphosphate (10 mM)	2.0 µl
Oligonucleotides (T 18)	2.0 µl
Omniscript transferase	1.0 µl
RNA (0.4 µg/µl)	5.8 µl

2.7.10.4 Semiquantitative PCR

The gene expression for the enzymes HMG-CoA reductase, FAS, MTP, ACAT-2, SREBP-1c, SREBP-2, APO-B, CYP7A1 and LDL-receptor were determined by semi-quantitative PCR method and compared with the household gene GAPDH which was run in parallel. Tag-polymerase, buffer and $MgCl_2$ were purchased from Promega (MADISON, WI, USA).

The reagents used for the PCR and their ratio:

Reagents	1× Sample (Master mix)
DEPC-Water	10.2 µl
25 mM $MgCl_2$	1.2 µl
10×Buffer	2.0 µl
10mM dNTPs	0.4 µl
Forward primer (5 pmol/µl)	2.0 µl
Reverse primer (5 pmol/µl)	2.0 µl
Tag-polymerase	0.2 µl
cDNA	2.0 µl

Master mix was added to the cDNA and PCR was run, for corresponding gene annealing temperature and cycle numbers (Mastercycler, Personal, Cologne, Germany).

PCR Program:

Reaction cycle	Temperature (°C)	Time
Denaturing	93°C	30 seconds
Annealing	Depending on the gene	30 seconds
Extension	72°C	1 minute

Primer sequences: All the Primers and dNTPs were purchased from Carl Roth GmbH, (Karlsruhe, Germany).

Primer	Sequences (Forward and Reverse)	Product length	Annealing temp	Cycle numbers
HMG CoA Reductase	Forward 5'-AAG GGG CGT GCA AAG ACA ATC -3' Reverse 5'- ATA CGC CAC GGA AAG AAC CAT AGT- 3'	404 bp	56.7°C	30
FAS	Forward 5'-CCT CCC CTG GTG GCT GCT ACA A-3' Reverse 5'-CCT GGG GTG GGC GGT CTT T-3'	224 bp	60°C	30
MTP	Forward 5'-CGC GAG TCT AAA ACC CGA GTG-3' Reverse 5'-CCC TGC CTG TAG ATA GCC TTT CAT-3'	241 bp	57°C	35
SREBP-1c	Forward 5'-GGA-GCC-ATG-GAT-TGC-ACA-TT-3' Reverse 5'-AGG-AAG-GCT-TCC-AGA-GAG-GA-3'	191bp	60°C	35
SREBP-2	Forward 5'-CCG GTA ATG ATG GGC CAA GAG AAA G-3' Reverse 5'-AGG CCG GGG GAGACA TCA GAA G-3'	404 bp	60°C	35
APO-B	Forward 5'-A AAG GGG AGG GAA AAG GTT-3' Reverse 5'-A GGT AGG GGC TCA CAT TAT TGG-3'	286 bp	56°C	24
CYP7A1	Forward 5'-CAA GAC GCA CCT CGC TAT CC-3' Reverse 5'-CCG GCA GGT CAT TCA GTT G-3'	206 bp	60°C	38

LDL Receptor	Forward 5'-ACG GGC TGG CGG TAG ACT GGA-3' Reverse 5'-TGA GGC GGT TGG CAC TGA AAA-3'	474 bp	59°C	35
GAPDH	Forward 5'-A TGG CCT TCC GTC TTC C-3' Reverse 5'-GGG TGG TCC AGG GTT TCT TAC TC-3'	337 bp	60°C	24
PPAR α	Forward 5'-CCCTCTCTCCAGCTTCCAGCCC-3' Reverse 5'-CCACAAGCGTCTTCTCAGCCATG-3'	555 bp	65°C	29
Glutathione-S-Transferase	Forward 5'-AGG GGG AGA ATG CCA AGA AGT TC 3' Reverse 5'-GGG TAG AGC CTG TGG ATG GTA GTC-3'	466 bp	56°C	50
ACO	Forward 5'-CTTTCTTGCTTGCCTTCCTTCTCC-3' Reverse 5' GCCGTTTCACCGCCTCGTA 3'	415 bp	60°C	27
CYP4A1	Forward 5'-CAG AAT GGA GAA TGG GGA CAG C-3' Reverse 5'-TGA GAA GGG CAG GAA TGA GTG G-3'	459 bp	65°C	29
INSIG-1	Forward 5'-ATT TGG CGT GGT CCT GGC TCT GG-3' Reverse 5'-GCG TGG CTA GGA AGG CGA TGG TG-3'	389 bp	62°C	25
INSIG-2	Forward 5'-AAG CGT GGC CCC TAC ATT TCC TC-3' Reverse 5'-GGC CAC GCA GCG CAT AAC AC-3'	303 bp	59°C	28
Δ 6-Desaturase	Forward 5'-TCC CCA CTA TGC CAA GAC ACA ACT T-3' Reverse 5'-CAA AAG CCC TCC CCT CCC TCT G-3'	246 bp	60°C	30

Gel Electrophoresis: The PCR products were evaluated by the Agarose gel electophoresis. The separation of the PCR product developed was performed through Electrophoresis. 2% Agarose (SERVA) gel was prepared in 0.5×TBE buffer, boiled to clear solution and cooled down to 55°C and ethidium bromide was added to the gel. After drying, gel was equilibriated in the running buffer (0.5 ×TBE) for 30 min. 10 µl PCR products (stained with loading buffer) were poured in the wells and electrophoresis was run for 45 min at 80 V. The gels were photographed under the UV light and evaluated with the associated device (SYNGENE).

Regents used for Gel electrophoresis:

Gel buffer	5×Running buffer(TBE)	6×Loading buffer
0.7 g agarose (SERVA)	54 g Tris base	300 µl Glycerol (30%,v/v)
35 ml 0.5×TBE	27.5 g Boric acid	2.5 mg Bromophenol blue (0.25%)
14 µl Ethidium bromide	3.72 g $Na_2EDTA.2H_2O$	700 µl water
(0.5 µg/ml)	1 litre with water	

2.7.10.5 cDNA Array Analysis

For the expression analysis cDNA Macro array ATLAS™ Nylon 1.2 Array (Clontech, Heidelberg, Germany) were used. These Rat 1.2 Nylon membranes contain 1176 protein/genes involved in lipid metabolism and metabolism of carbohydrate, amino acid, xenobiotics, vitamins as well as genes, different receptors, hormones, protein and factors (Translation, Transcription, and Growth) according to the manufacturer's protocol.

For the cDNA arrays, total RNA isolated from the liver (2.7.10.1) was used. The analysis was performed with the RNA of the third experiment rats (Soy, Fish, and Casein group). Equal amounts of the RNA of three rats within the same dietary treatment group were mixed into one pool of 50 µg. Therefore, 4 RNA pools per treatment group were used for a separate hybridization.

Method: 50 µg total RNA (total volume 45 µl) per pool was prepared as described in ATLAS Pure Total RNA Labelling System (Clontech).

The substantial work procedures are described in the following:

Enrichment of the RNA: Total RNA (50 µg) was enriched with Biotinylated Oligo (Deoxy-Thymine) (dT). Streptavidin magnetic beads were added and the magnetic RNA was concentrated on a magnetic particle separator (Promega, Mannheim, Germany).

cDNA synthesis and Radioactive labelling: The cDNA was synthesized as described for ATLAS NYLON 1.2 ARRAYS (Clontech). The non bound fragments were separated by column chromatography. The cDNA was labeled with radioactive Alpha-^{33}Phosphorus [α-^{33}P] Deoxyadenosine triphosphate (dATP) (Perkin Elmer, Boston, USA).

Hybridization: The membranes were placed in hybridizing bottles. Before hybridization the quality of probes were checked by hybridizing them to a blank nylon membrane (supplied). After the addition of radioactivity, labeled samples were hybridized for 18 h at 68°C.

Exposition: Membranes were washed accordingly, afterwards mounted on whatman paper (3 MM) and wrapped in a plastic wrap. The array membranes were exposed to the Phosphorimager screen for 24 h. The array images were visualized using the Bio-Image analyzer Fujifilm BAS-1500 and TINA 2.0, software (Raytest).

Evaluation of the Data: Gene specific signal intensities on the arrays were quantified using ATLAS IMAGE 2.01 software (Clontech) and corrected for background. The allocation of the signals to the genes of the ATLAS rat 1.2 Arrays took place via the adaptation of the appropriate mask to the respective array. The background was the mean signal intensity of the unspotted fields on the array. Genes whose signal intensities were at least 1.5-fold the background level, were considered.

Normalization and comparison of the arrays: The level of the signal intensities of the particular arrays varied due to the quality and quantity of the assigned RNA. In order to be able to compare, the array data were normalized. Normalization of the signal intensities was done by relating them to the mean signal intensities of all genes. In our group prestudies (Sülzle et al. 2004, Ringseis and Eder 2005), this method of normalization yielded a higher reproducibility than normalization by various housekeeping genes. For the comparison of the twelve arrays, the four arrays of the control group (casein) were taken as one control array. But the four arrays of the control group were individually normalized; their relative signal intensities were multiplied by a coefficient. All the 8 treatment arrays were compared from one control array. For the Evaluation of data EXEL 2000 (Microsoft, USA) was used. The gene expression was taken as a relation ship between the signal intensities from treatment to the control array.

2.7.11 Western Blotting

For western blotting, liver microsomal fractions (2.6.2) of the rats fed soy protein or casein containing diets in experiment 3 were used. Samples were dissolved (1**:**4) in a buffer containing 0.22 M Tris, 41% glycerol, 0.7% SDS, 5% mercaptoethanol and 0.002% bromphenol blue and subsequently denaturated at 95°C for 5 min. After chilling 20 µl of denaturated probes containing 40 µg proteins were applied to the gels. For gel electrophoresis, 5% stacking gel and 10% running gel gradients were used. Electrophoresis was run at 210 V for 1 h.

Running gel (10%) Reagents	Volume
Acrylamide/Bisacrylamide (30%)	5 ml
Running Buffer (1.125 M, pH 8.8)	5 ml
SDS (10%)	0.15 ml
DEPC Water	4.85 ml
TEMED	12.5 µl
Ammonium per sulphate (10%)	75 µl

Stacking gel (5%) Reagents	Volume
Acrylamide/Bisacrylamide (30%)	0.72 ml
Stacking Buffer (0.5 M, pH 6.8)	2.576 ml
SDS (10%)	6 µl
DEPC Water	0.98 ml
TEMED	11.4 µl
Ammonium per sulphate (10%)	56.6 µl

Proteins resolved by electrophoresis were transferred from gel to nitrocellulose membrane electrophoretically (95 mA, 90 min). The nitrocellulose membrane which contained the resolved proteins was first incubated with milk protein (5%) for 10 min, to block nonspecific protein binding sites. Then the membrane was incubated with the primary antibody anti HMG CoA R (rabbit polyclonal IgG, Catalog 07-457, UPSTATE, Lake Placid, New York) (diluted 1**:**1000), for 2 h at RT. The unbound primary antibody was washed away with TBST-buffer and the membrane was incubated with the secondary antibody (anti rabbit conjugate, Sigma) (diluted 1**:**5000) for 1 h at RT. Afterwards, the membrane was washed three times with TBST buffer and once with alkaline phosphate buffer. The membrane was stained with p-Nitro-Blue-Tetrazolium chloride (NBT) and 5-Bromo-4-chloro-3-indolylphosphate (BCIP), dissolved in alkaline phosphate buffer, till the brown colour bands develop.

Running buffer for SDS-Page	Blot buffer for SDS-PAGE	5x TBST (pH 7.5)	Alkaline phosphate buffer (pH 9.8)
3.0 g Tris 14.4 g Glycine 1.0 g SDS 1 litre with water	3 g Tris 14.4 g Glycine 0.5 g SDS 200 ml Methanol 1 litre with water	50 mM Tris 750 mM NaCl 0.5 % TWEEN 20	25 ml 1 M Tris/HCl pH 9.8 15 ml 5 M NaCl 2.5 ml 1 M MgCl2 500 ml with water

Evaluation: The membranes were photographed with a computer connected system (Gel-Pro Analyzer, Intas, Göttingen, Germany). Bands of HMG CoA R were identified by comparing with the high molecular weight standards (Serva, Heidelberg, Germany).

2.8 Statistical Analysis

In all the three experiments, treatment effects were analyzed using the MINITAB statistical software with one-factorial analysis of variance (Minitab, State College, PA, USA). For statistical significant F-values, individual mean values were compared by the Fisher's multiple range tests. Treatment effects were considered statistically significant for $p<0.05$. All results are mean ± SD.

3 Results

3.1 Body Weight, Body Weight Gain and Liver Weight of Rats

Table 3.1: Body Weight, Body Weight Gain and Relative Liver Weight of Rats used in Experiment 1, 2 and 3

Groups		Body Weight at the start [g]	Body Weight at the end [g]	Body Weight gain [g]	Relative liver weight [g/100 g BW]
Exp 1	Casein	70 ± 4	205 ± 4^{a}	135 ± 4^{a}	3.96 ± 0.21^{a}
	Soy	70 ± 4	186 ± 5^{b}	116 ± 3^{b}	3.29 ± 0.20^{b}
Exp 2	Pork	72 ± 5	197 ± 6^{a}	125 ± 8^{ab}	3.63 ± 0.41^{ab}
	Beef	73 ± 6	194 ± 7^{a}	122 ± 11^{ab}	3.57 ± 0.51^{ab}
	Fish	72 ± 6	195 ± 7^{a}	122 ± 13^{ab}	3.78 ± 0.61^{a}
	Turkey	72 ± 6	202 ± 7^{a}	130 ± 9^{a}	3.32 ± 0.27^{b}
	Casein	72 ± 6	196 ± 7^{a}	123 ± 7^{ab}	3.54 ± 0.36^{ab}
	Soy	72 ± 6	177 ± 4^{b}	105 ± 8^{b}	3.23 ± 0.37^{b}
Exp 3	Fish	76 ± 5	202 ± 6^{a}	125 ± 6^{a}	4.08 ± 0.37^{ab}
	Casein	77 ± 5	191 ± 6^{ab}	115 ± 5^{ab}	4.51 ± 0.37^{a}
	Soy	77 ± 7	193 ± 6^{ab}	116 ± 6^{ab}	4.13 ± 0.28^{ab}
	Pea	77 ± 5	186 ± 6^{b}	109 ± 3^{b}	3.59 ± 0.25^{b}
	Lupin	76 ± 5	153 ± 10^{c}	77 ± 10^{c}	3.85 ± 0.75^{b}

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. P< 0.05 (Fisher's multiple range test). BW = Body weight, Experiment 1 (n = 10), Experiment 2 and 3 (n = 12). Rats were food deprived for 12 h in experiment 1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In table 3.1, the body weight, body weight gain and relative liver weights are summarized. All experimental rats completed their feeding period.

In Experiment 1, the average diet intake per rat was 13.5 g/day and the average body weight at the starting was 70.2 ± 4.3 g (n = 20). The amount of the diet was increased starting from 6 g/day to 14 g/day. The diet was fed for 21 days. The body weight gain and the relative liver weight of the rats fed diet containing soy protein were significantly lower (-10%, -17%, respectively) than that of the rats fed diet containing casein as protein source.

In Experiment 2, the average diet intake per rat was 13.5 g/day. The average body weight at the starting was 72.3 ± 5.6 g (n = 72). The amount of diet was increased starting from 8 g/day to 14 g/day. The diet was fed for 20 days. Rats fed diet containing soy protein had significantly lower body weight gain than rats fed diet containing turkey protein. Rats fed diets containing pork, beef, fish or casein had body weight gain values in between. The relative liver weight was significantly higher of the rats fed diet with fish protein compared to the rats fed diet with turkey or soy protein. Rats fed pork, beef, or casein diets had relative liver weight values in between.

In Experiment 3, average diet intake per rat was 10.3 g/day. The average body weight at the start was 76.5 ± 5.3 g (n = 60). The amount of the diet was increased starting from 6 g/day to 14 g/day. The diet was fed for 22 days. In this experiment the amount of diet provided to the rats was a bit lower because some rats from the lupin group did not ingest the entire amount of diet and to standardize the diet intake of all the groups, we provided a bit lesser diet to each group. Rats fed fish protein had significantly higher body weight gain compared to rats fed pea or lupin protein. Rats fed casein and soy protein had body weight gain values in between. Rats fed lupin protein had the lowest body weight gain. The relative liver weight was significantly higher of rats fed casein compared to rats fed pea protein. Rats fed fish or soy protein had values in between.

3.2 Cholesterol Concentrations in Plasma and Lipoproteins

In the table 3.2, the cholesterol concentrations in plasma, lipoproteins and the quotient from LDL-/HDL-cholesterol are specified. In Experiment 1, no difference was found in the concentrations of plasma cholesterol, LDL-cholesterol as well as in HDL-cholesterol, only VLDL-cholesterol concentration was significantly lower in rats fed soy protein (-53%) compared to rats fed casein. The quotient of LDL-/HDL-cholesterol was significantly lower in the rats fed soy protein (-12%) compared to rats fed casein.

Table 3.2 Cholesterol Concentrations in Plasma and Lipoproteins of the Rats used in Experiment 1, 2 and 3

Groups		Plasma	VLDL	LDL	HDL	LDL/HDL
		[mmol/L]				
Exp 1	Casein	2.17 ± 0.39	0.15 ± 0.04^{a}	0.66 ± 0.09	1.00 ± 0.17	0.66 ± 0.09^{a}
	Soy	2.08 ± 0.18	0.07 ± 0.03^{b}	0.60 ± 0.06	1.04 ± 0.10	0.58 ± 0.07^{b}
Exp 2	Pork	1.84 ± 0.28^{a}	0.29 ± 0.09^{a}	0.61 ± 0.16	1.08 ± 0.16^{a}	0.56 ± 0.13^{ab}
	Beef	1.92 ± 0.33^{a}	0.31 ± 0.14^{a}	0.61 ± 0.15	1.12 ± 0.24^{a}	0.55 ± 0.10^{ab}
	Fish	1.56 ± 0.23^{b}	0.35 ± 0.16^{a}	0.54 ± 0.14	0.80 ± 0.16^{b}	0.69 ± 0.17^{a}
	Turkey	1.71 ± 0.31^{ab}	0.33 ± 0.09^{a}	0.55 ± 0.12	1.04 ± 0.22^{a}	0.53 ± 0.10^{ab}
	Casein	1.80 ± 0.21^{a}	0.30 ± 0.13^{a}	0.58 ± 0.17	1.05 ± 0.14^{a}	0.54 ± 0.11^{ab}
	Soy	1.74 ± 0.32^{ab}	0.08 ± 0.06^{b}	0.58 ± 0.12	1.10± 0.16^{a}	0.46 ± 0.23^{b}
Exp 3	Fish	2.46 ± 0.25	0.53 ± 0.24^{a}	0.58 ± 0.12^{b}	1.11 ± 0.17^{b}	0.54 ± 0.10^{a}
	Casein	2.75 ± 0.63	0.47 ± 0.12^{ab}	0.44 ± 0.09^{c}	1.43 ± 0.31^{ab}	0.31 ± 0.05^{b}
	Soy	2.39 ± 0.33	0.23 ± 0.09^{b}	0.49 ± 0.09^{c}	1.48 ± 0.24^{ab}	0.33 ± 0.06^{b}
	Pea	2.70 ± 0.54	0.14 ± 0.05^{c}	0.78 ± 0.12^{a}	1.64 ± 0.22^{a}	0.48 ±0.06^{ab}
	Lupin	2.60 ± 0.26	0.06 ± 0.03^{d}	0.73 ± 0.11^{a}	1.77 ± 0.14^{a}	0.41 ± 0.08^{ab}

Values are mean values ± SD. VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, High density lipoproteins; Different superscript letters within one column denote significant difference within one experiment. $P < 0.05$ (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 and 3 (n = 12). Rats were food deprived for 12 h in exp.1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In Experiment 2, the concentration of plasma cholesterol was significantly lower in rats fed fish protein than in rats fed beef, casein and pork proteins. Rats fed turkey or soy protein had values in between. VLDL-cholesterol concentration was significantly lower in rats fed soy protein compared to rats fed casein, pork, beef, turkey or fish protein, which had similar concentrations. No difference was observed in the LDL-cholesterol concentration among the groups. HDL-cholesterol concentration was significantly lower in rats fed fish protein than rats fed any other experimental protein. The quotient of LDL-/HDL-cholesterol

was significantly lower in the soy protein fed rats compared to the rats fed fish protein, rats fed pork, beef, turkey or casein had values in between.

In Experiment 3, no significant difference was found in plasma cholesterol concentration among the groups. VLDL-cholesterol concentration was significantly lower in rats fed plant proteins (soy, pea and lupin proteins) than in rats fed animal proteins (fish and casein proteins). LDL-cholesterol concentration was highest in rats fed pea or lupin proteins. Casein and soy protein fed rats had lowest LDL-cholesterol concentrations and in the same range. Fish protein fed rats had values in between. HDL-cholesterol concentration was significantly lower in rats fed fish protein than in rats fed pea or lupin protein. Rats fed casein and soy protein had similar concentration of HDL-cholesterol and in the mean range. The quotient of LDL-/HDL-cholesterol was significantly higher in the fish protein fed rats compared to the casein and soy protein fed rats. The quotient of LDL-/HDL-cholesterol was similar in the soy protein fed rats and in the casein protein fed rats. The quotients of LDL-/HDL-cholesterol were similar in the pea protein fed rats and lupin protein fed rats, and the values were in between of the rats fed fish protein and soy protein.

3.3 Triglyceride Concentrations in Plasma and Lipoproteins

In table 3.3, concentrations of triglycerides in plasma and lipoproteins are presented

In Experiment 1, no significant difference was found in the concentrations of triglycerides in plasma, VLDL, LDL or HDL.

In Experiment 2, triglyceride concentration in plasma was lowest in rats fed soy protein. Rats fed pork and fish protein had significantly lower triglyceride concentration than rats fed casein. Rats fed beef and turkey proteins had values in between. Triglyceride concentration in the VLDL was lowest in rats fed soy protein. Rats fed pork, beef, or fish protein had significantly lower triglyceride concentration in the VLDL than rats fed casein and significantly higher concentration than rats fed soy protein. Rats fed turkey protein had significantly higher triglyceride concentration in the VLDL than rats fed soy protein, but did not differ from the other groups. Triglyceride concentration in the LDL was significantly lower in rats fed pork, beef or soy protein compared to rats fed casein. Rats fed turkey or fish proteins had triglyceride concentrations in between. Triglyceride concentration in HDL was

significantly lower in rats fed fish protein compared to rats fed casein, rats of the other groups had concentrations in between these two groups.

Table 3.3 Triglyceride concentrations in plasma and Lipoproteins of rats used in experiment 1, 2 and 3

Groups		Plasma	VLDL	LDL	HDL
		[mmol/L]			
Exp1	Casein	0.66 ± 0.17	0.38 ± 0.11	0.09 ± 0.02	0.10 ± 0.01
	Soy	0.63 ± 0.18	0.31 ± 0.11	0.08 ± 0.02	0.11 ± 0.02
Exp 2	Pork	1.10 ± 0.34^{b}	0.85 ± 0.33^{b}	0.21 ± 0.08^{b}	0.13 ± 0.04^{ab}
	Beef	1.25 ± 0.44^{ab}	0.89± 0.32^{b}	0.21 ± 0.06^{b}	0.15 ± 0.05^{ab}
	Fish	1.11 ± 0.57^{b}	0.86 ± 0.45^{b}	0.23 ± 0.10^{ab}	0.12 ± 0.03^{b}
	Turkey	1.30 ± 0.59^{ab}	1.07 ± 0.52^{ab}	0.23 ± 0.08^{ab}	0.14 ± 0.03^{ab}
	Casein	1.52± 0.21^{a}	1.24 ± 0.51^{a}	0.29 ± 0.09^{a}	0.17 ± 0.04^{a}
	Soy	0.75 ± 0.11^{c}	0.43 ± 0.10^{c}	0.18 ± 0.06^{b}	0.14 ± 0.01^{ab}
Exp 3	Fish	1.31 ± 0.67^{b}	1.23 ± 0.38^{b}	0.20 ± 0.06^{b}	0.08 ± 0.02^{b}
	Casein	2.34 ± 0.85^{a}	1.54 ± 0.44^{a}	0.32 ± 0.08^{a}	0.12 ± 0.05^{a}
	Soy	1.20 ± 0.79^{b}	1.02 ± 0.34^{b}	0.22 ± 0.07^{b}	0.08 ± 0.03^{b}
	Pea	0.80 ± 0.46^{bc}	0.64 ± 0.26^{bc}	0.21 ± 0.08^{b}	0.08 ± 0.03^{b}
	Lupin	0.58 ± 0.26^{c}	0.32 ± 0.26^{c}	0.19 ± 0.05^{b}	0.08 ± 0.03^{b}

Values are mean values ± SD. VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, High density lipoproteins; Different superscript letters within one column denote significant difference within one experiment. $P < 0.05$ (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 and 3 (n = 12). Rats were food deprived for 12 h in exp.1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In Experiment 3, triglyceride concentration in plasma was lowest in rats fed lupin protein. Rats fed fish, soy or pea protein had lower concentrations than rats fed casein. Triglyceride concentration in the VLDL was significantly lower in rats fed fish, soy or pea

protein compared to rats fed casein. All the groups had significantly higher VLDL triglyceride concentrations than rats fed lupin protein. Concentrations of triglycerides in the LDL and HDL were significantly higher in rats fed casein compared to rats of all other groups, which had similar concentrations.

3.4 Lipid Concentrations in the VLDL

Results of the lipid analysis of VLDL from rats fed casein or soy protein in experiment 3 analyzed by thin layer chromatography are shown in figure 3.1. Rats fed diet containing soy protein had significantly lower triglyceride (-30%) in the VLDL than rats fed diet containing casein. Rats fed diet containing soy protein tended to have lower phospholipid concentrations compared to rats fed diet containing casein ($P<0.10$). No significant difference was observed in the cholesterol concentrations between the groups. Rats fed diet containing soy protein had significantly lower cholesterol ester values (-54%) than rats fed diet containing casein. The core surface ratio (TG + CE)/ (FC + PL) which provides an indication of the relative differences in the average size of the VLDL, was not different between rats fed casein (2.66 ± 0.72) and soy protein (2.50 ± 1.10).

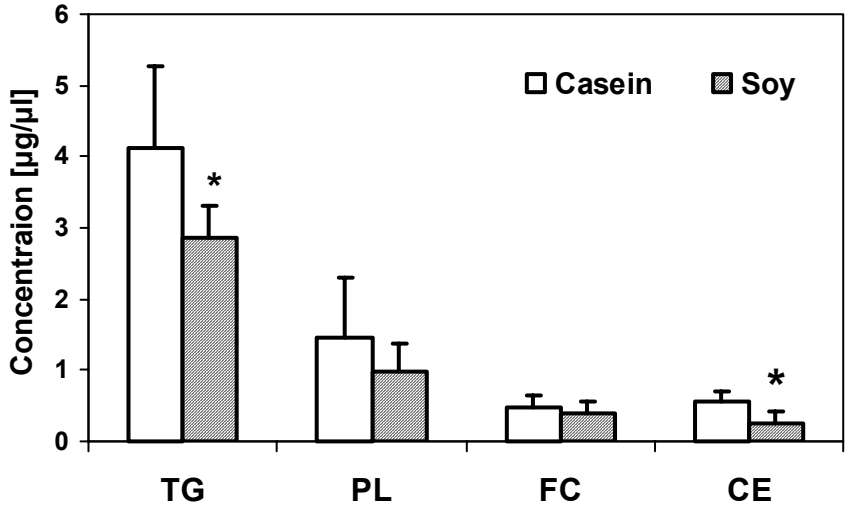

Figure 3.1: Concentrations of different lipids in the Very Low Density Lipoprotein fraction of rats fed either casein or soy protein determined by thin layer chromatography (Experiment 3)

Values are mean values ± SD. * significant difference between casein and soy group. $P<0.05$ (t-test). (n = 12). TG; triglycerides, PL; phospholipids, FC; free cholesterol, CE; cholesterol esters. Rats were killed without prior food deprivation.

3.5 Protein Concentrations in Lipoproteins and Plasma

Table 3.4 Protein Concentrations in Lipoproteins and Plasma of Rats used in Experiment 1, 2 and 3

Groups		VLDL protein [mg/L]	LDL protein [mg/L]	HDL protein [mg/L]	Plasma protein [g/L]
Exp 1	Casein	65.6 ± 13.2	173 ± 60	n.d.	47.3 ± 9.1
	Soy	57.6 ± 18.4	164 ± 35	n.d	41.6 ± 4.2.
Exp 2	Pork	345 ± 86[a]	n.d.	n.d.	n.d.
	Beef	348 ± 78[a]	n.d.	n.d.	n.d.
	Fish	357 ± 98[a]	n.d.	n.d	n.d.
	Turkey	372 ± 109[a]	n.d.	n.d.	n.d.
	Casein	394 ± 112[a]	n.d.	n.d.	n.d.
	Soy	206 ± 26[b]	n.d.	n.d.	n.d.
Exp 3	Fish	283 ± 111[b]	256 ± 101[b]	1338 ± 467	n.d.
	Casein	414 ± 125[a]	234 ± 46[b]	1671 ± 420	n.d.
	Soy	269 ± 147[b]	260 ± 43[b]	1706 ± 425	n.d.
	Pea	140 ± 43[bc]	382 ± 67[a]	1756 ± 386	n.d.
	Lupin	76 ± 33[c]	354 ± 59[a]	1802 ± 254	n.d.

Values are mean values ± SD. VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, High density lipoproteins; Different superscript letters within one column denote significant difference within one experiment. $P < 0.05$ (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 and 3 (n = 12). (n.d., not determined). Rats were food deprived for 12 h in exp.1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In the table 3.4, concentrations of proteins in the lipoproteins are presented. In Experiment 1, no significant difference was observed in VLDL, LDL and plasma protein concentrations between the groups.

In Experiment 2, rats fed soy protein diet had significantly lower protein concentration in the VLDL than rats fed diets containing pork, beef, fish, turkey or casein, which had similar concentrations.

In Experiment 3, rats fed lupin protein had lowest protein concentration and rats fed casein had highest protein concentration in the VLDL. Rats fed fish protein and soy protein had similar protein concentrations in the VLDL. Rats fed pea protein had VLDL protein concentration in between soy and lupin group. Rats fed lupin protein and rats fed pea protein had significantly higher protein concentration in the LDL than rats fed fish, casein and soy protein. No significant difference was observed in HDL protein concentration.

3.6 Apolipoprotein concentrations in the Lipoproteins

Relative amounts of selected apolipoproteins in different lipoprotein fractions are shown in figures 3.2 a and 3.2 b. Apo-B 100 and Apo-B 48 concentrations were significantly lower (-66% and -54%) in the rats fed diet containing soy protein compared to the rats fed diet containing casein ($P<0.05$). No significant difference were observed in the Apo-E concentrations between the two groups (Figure 3.2 a). No significant difference was observed in the concentrations of Apo-B 100 in LDL and Apo-AI in HDL fraction between the two groups (figure 3.2 b).

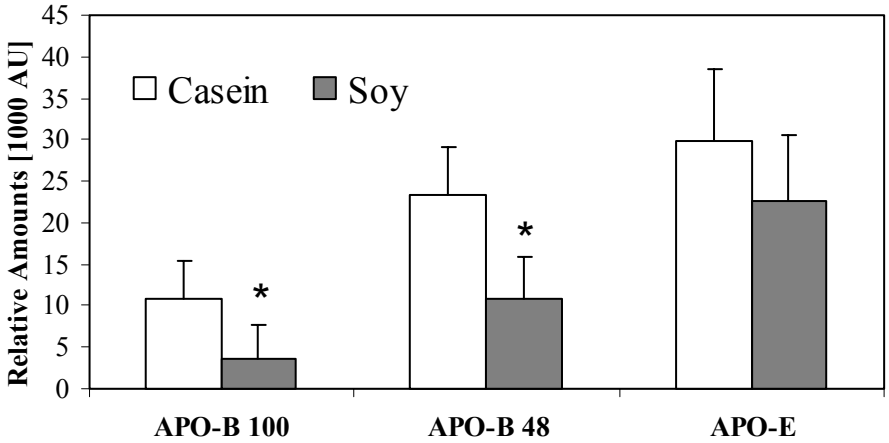

Figure 3.2 a: Apolipoprotein concentrations in the triglyceride rich lipoprotein (VLDL + Chylomicrones) of rats fed diets containing either casein or soy protein (Experiment 3)

Values are mean values ± SD. AU, Absorbance units. APO, Apolipoprotein. * Significant difference between casein and soy group ($P< 0.05$) (t-test). n = 12. Rats were killed without prior food deprivation.

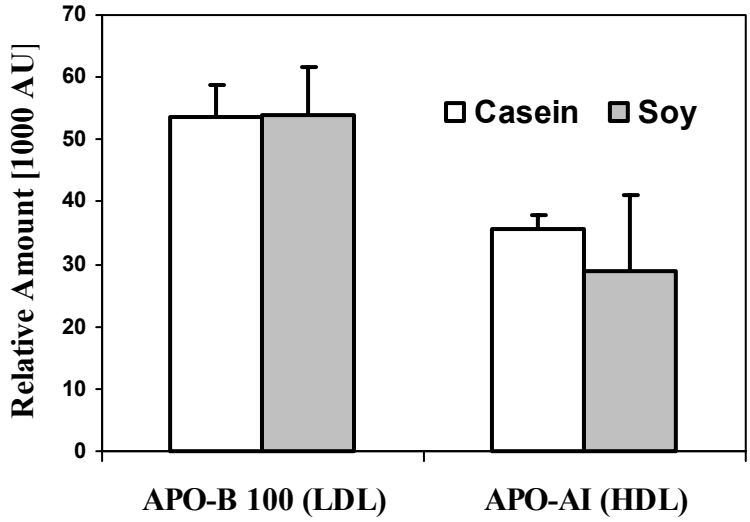

Figure 3.2 b: Apolipoprotein concentration in the LDL and HDL of rats fed diets containing either casein or soy protein (Experiment 3)

Values are mean values ± SD. AU, Absorbance units. Apo, Apolipoprotein. n = 12. Rats were killed without prior food deprivation.

3.7 Amino acid concentrations in Plasma

In table 3.5 a, the amino acid concentrations in plasma of rats used in experiment 1 are presented.

Plasma amino acid concentrations of isoleucine, leucine, lysine, methionine, threonine, valine, alanine, and phenylalanine were significantly higher in casein fed rats compared to soy protein fed rats. But no differences were found in the concentrations of arginine, histidine, tryptophan, asparagine, glutamine, glutamic acid, glycine, serine, tyrosine and taurine.

Table 3.5 a Amino Acid Concentrations in Plasma of Rats used in Experiment 1

Amino acids	Casein	Soy
	[µM]	
Arginine	125 ± 20	138 ± 20
Histidine	87 ± 9	96 ± 11
Isoleucine	58 ± 8[a]	49 ± 6[b]
Leucine	143 ± 25[a]	115 ± 17[b]
Lysine	432 ± 62[a]	370 ± 64[b]
Methionine	46 ± 4[a]	33.6 ± 5[b]
Threonine	619 ± 132[a]	429 ± 55[b]
Tryptophan	69 ± 9	59 ± 15
Valine	198 ± 29[a]	166 ± 25 [b]
Alanine	471 ± 56[a]	377 ± 41[b]
Asparagine	62 ± 9	53 ± 10
Glutamine	928 ± 82	852 ± 85
Glutamic acid	194 ± 24	181 ± 30
Glycine	329 ± 44	342 ± 40
Serine	424 ± 43	469 ± 46
Tyrosine	110 ± 20	80 ± 41
Phenyl alanine	78 ± 12[a]	61 ± 12[b]
Taurine	79 ± 45	78 ± 26

Values are mean values ± SD. Different superscript letters denote significant difference between casein and soy group. $P < 0.05$ (Fisher's multiple range test). n = 10. Rats were food deprived for 12 h.

In table 3.5 b, the amino acid concentrations in plasma of the rats used in experiment 2 are presented.

Table 3.5.b Amino Acid Concentrations in the Plasma of Rats used in Experiment 2

Amino acids	Pork	Beef	Fish	Turkey	Casein	Soy
	[µM]					
Arginine	128 ± 15^{ab}	131 ± 20^{ab}	146 ± 22^{a}	125 ± 18^{ab}	112 ± 18^{b}	117 ± 17^{b}
Histidine	51 ± 9^{ab}	43 ± 7^{b}	41 ± 6^{b}	41 ± 6^{b}	51 ± 8^{ab}	57 ± 9^{a}
Isoleucine	63 ± 11^{ab}	63 ± 8^{ab}	68 ± 12.4^{a}	61 ± 8^{ab}	67 ± 11^{a}	53 ± 10^{c}
Leucine	95 ± 17^{ab}	92 ± 13^{ab}	94 ± 17^{ab}	93 ±13^{ab}	105± 17^{a}	85± 14^{b}
Lysine	441 ± 124^{ab}	472 ± 79^{ab}	518 ± 85^{a}	402 ± 81^{b}	476 ± 81^{ab}	338 ± 63^{c}
Methionine	35 ± 5^{ab}	36 ±5^{ab}	42 ± 10^{a}	34 ± 3^{ab}	41 ± 5^{a}	28 ± 5^{b}
Threonine	434 ± 86^{ab}	430 ± 99^{ab}	313 ± 50^{b}	337 ± 90^{b}	527 ± 117^{a}	313 ± 49^{b}
Tryptophan	56 ± 19^{ab}	59 ± 10^{a}	61 ± 11^{a}	52 ± 7^{b}	56^{ab} ± 9	44^{c} ± 8
Valine	122 ± 16^{ab}	114 ± 12^{b}	107 ± 16^{b}	108 ± 17^{b}	130 ± 21^{a}	105 ± 20^{b}
Alanine	352 ± 76^{ab}	337 ± 53^{ab}	464 ± 198^{a}	321 ± 54^{b}	351 ± 67^{ab}	298± 52^{b}
Asparagine	51 ± 5^{b}	50 ± 4^{b}	54 ± 4^{ab}	49 ± 5^{b}	61 ± 10^{a}	48 ± 3^{b}
Glutamine	665 ± 62^{ab}	616 ± 43^{b}	614 ± 82^{b}	625 ± 57^{b}	689 ± 66^{a}	687 ± 39^{a}
Glycine	352 ± 64^{a}	315 ± 52^{ab}	354 ± 54^{a}	303 ± 50^{ab}	246 ± 25^{b}	274 ± 51^{b}
Serine	290 ± 29^{b}	263 ± 35^{b}	276 ± 26^{b}	265 ± 42^{b}	331 ± 33^{ab}	379 ± 36^{a}
Tyrosine	70 ± 11^{ab}	71 ± 10^{ab}	65 ± 10^{ab}	67 ± 13^{ab}	94 ± 21^{a}	58 ± 16^{b}
Phenyl alanine	46 ± 4^{a}	46 ± 4^{a}	46 ± 9^{a}	46 ± 5^{a}	49 ± 9^{a}	39 ± 6^{b}
Cysteine	159± 25^{a}	157 ± 25^{a}	147 ± 27^{b}	165 ± 34^{a}	156 ± 23^{a}	117 ± 23^{c}
Taurine	129 ± 29^{b}	113 ± 32^{b}	171 ± 30^{a}	97± 31^{b}	50 ± 10^{c}	55^{c} ± 8

Values are mean values ± SD. Different superscript letters within one row denote significant difference. $P<0.05$ (Fisher's multiple range test). n = 12. Rats were food deprived for 12 h.

Plasma concentration of arginine was higher in rats fed fish protein compared to rats fed casein or soy protein, rats fed pork, beef or turkey protein had values in between. Rats fed fish, turkey or beef protein had lower histidine concentration compared to rats fed soy protein. Rats fed pork and casein had values in between. Isoleucine concentration was significantly higher in rats fed fish protein and casein compared to rats fed soy protein, rats fed pork, beef and turkey protein had isoleucine concentrations in between. Leucine was present in significantly higher concentration in rats fed casein compared to rats fed soy protein, rats fed pork, beef, turkey or fish protein had concentrations in between. Lysine concentration was highest in the rats fed fish protein and lowest in the rats fed soy protein. Rats fed turkey protein had lower concentration then rats fed fish protein, rats fed pork, beef or casein had values in between. Methionine concentration was significantly higher in rats fed fish protein and casein compared to rats fed soy protein rats fed, pork, beef or turkey protein had concentrations in between these groups. Threonine concentration was significantly higher in rats fed casein compared to rats fed fish, turkey or soy protein. Rats fed pork and beef proteins had concentrations in between rats fed casein and soy protein. Tryptophan was present in significantly higher concentration in rats fed beef or fish protein compared to rats fed turkey or soy protein. Rats fed pork protein or casein had tryptophan concentrations significantly higher compared to rats fed soy protein. Valine concentration was significantly higher in rats fed casein compared to rats fed beef, fish, turkey or soy protein. Rats fed pork protein had concentration in between. Plasma concentration of alanine was significantly higher in rats fed fish protein compared to rats fed soy protein. Rats fed pork, beef, turkey or casein had plasma concentration of alanine in between. Asparagine concentration was significantly higher in rats fed casein compared to rats fed soy, pork, beef or turkey protein. Rats fed fish protein had concentrations in between. Rats fed casein and soy protein had similar glutamine concentrations and significantly higher than rats fed beef, fish or turkey protein. Rats fed pork protein had concentrations in between. Glycine concentration was significantly higher in rats fed pork or fish protein compared to rats fed casein or soy protein. Rats fed beef or turkey protein had concentrations in between. Serine was present in significantly lower concentrations in rats fed pork, beef, fish or turkey protein compared to rats fed soy protein. Rats fed casein had concentrations in between. Tyrosine and phenylalanine was present in significantly higher concentrations in plasma of rats fed casein compared to rats fed soy protein. Rats fed pork, beef, turkey or fish protein had tyrosine and phenylalanine concentrations in between.

Table 3.5.c Amino Acid Concentrations in the Plasma of Rats used in Experiment 3

Amino acids	Fish	Casein	Soy	Pea	Lupin
	[µM]				
Arginine	145 ± 22^{b}	85 ± 20^{c}	134 ± 23^{b}	177^{ab} ± 25	209 ± 59^{a}
Histidine	30 ± 9^{b}	43 ± 12^{a}	36 ± 9^{b}	47± 14^{a}	46 ± 14^{a}
Isoleucine	69 ± 11	81 ± 20	72 ± 13	71 ± 10	65 ± 21
Leucine	86 ± 17^{b}	123 ± 31^{a}	94 ± 17^{b}	96 ± 14^{b}	87 ± 29^{b}
Lysine	644 ± 124^{a}	487 ± 113^{b}	317 ± 192^{c}	444 ± 91^{b}	406 ± 193^{b}
Methionine	54 ± 4.7	55 ± 5.0	61 ± 8	64 ± 9	50 ± 18
Threonine	422 ± 81^{b}	526 ± 135^{a}	190 ± 32^{c}	252 ± 46^{bc}	315 ± 120^{bc}
Tryptophan	68 ± 14^{a}	66 ± 11^{a}	69 ± 8^{a}	29± 9^{b}	16 ± 6^{c}
Valine	107 ± 18^{b}	189 ± 54^{a}	103 ± 18^{b}	110^{b} ± 17	84 ± 26^{b}
Alanine	548 ± 133^{a}	539 ± 62^{a}	540 ± 78^{a}	510 ± 80^{a}	349 ± 80^{b}
Asparagine	337 ± 52^{c}	456 ± 101^{b}	569 ± 76^{a}	601 ± 112^{a}	450 ± 156^{b}
Glutamine	531 ± 77^{b}	663 ± 80^{a}	602 ± 49^{a}	621 ± 152^{a}	563 ± 86^{b}
Glycine	337 ± 66^{a}	152 ± 30^{c}	288 ± 47^{b}	238 ± 29^{b}	141± 19^{c}
Serine	250 ± 34	261 ± 38	236 ± 29	276 ± 36	252 ± 56
Tyrosine	55 ± 10^{b}	92 ± 21^{a}	63 ± 11^{b}	79 ± 22^{ab}	91 ± 45^{a}
Phenyl alanine	37 ± 8	43 ± 7	44 ± 7	46 ± 6	42 ± 16
Taurine	158 ± 26^{a}	62 ± 18^{c}	122 ± 38^{b}	103 ± 21^{b}	170 ± 70^{a}
Glutamic acid	93 ± 18^{a}	93 ± 11^{a}	98 ± 16^{a}	83 ± 20^{b}	78 ± 13^{b}

Values are mean values ± SD. Different superscript letters within one row denote significant difference. $P<0.05$ (Fisher's multiple range test). n = 12. Rats were killed without prior food deprivation.

Cysteine was present in significantly higher concentrations in rats fed pork, beef turkey or casein compared to rats fed soy protein. Rats fed fish protein had significantly higher concentration compared to rats fed soy protein, and lower compared to rest of the groups. Taurine concentration was significantly higher in rats fed fish protein compared to rats fed pork, beef, turkey, casein or soy protein. Rats fed pork, beef or turkey protein had

significantly lower taurine concentration than rats fed fish protein but significantly higher than rats fed casein or soy protein.

Amino acid concentrations in the plasma of rats used in experiment 3 are specified in table 3.5 c. Plasma concentration of arginine was significantly higher in rats fed lupin protein compared to rats fed casein, soy or fish protein. Rats fed pea protein had significantly higher plasma arginine concentration than rats fed casein. Rats fed fish and soy protein had significantly lower histidine concentration in plasma compared to rats fed casein, pea or lupin protein. Plasma isoleucine and methionine concentration were not different among the groups. Leucine concentration was significantly higher in rats fed casein compared to rats fed fish, soy, pea or lupin protein. Plasma lysine concentration was significantly higher in rats fed fish protein compared to rats fed casein, soy, pea or lupin protein. Rats fed soy protein had significantly lower lysine concentration compared to rats fed casein. Threonine concentration was significantly higher in rats fed casein or fish protein compared to rats fed soy, pea or lupin protein. Tryptophan concentration was similar among rats fed fish, casein or soy protein, but rats fed pea or lupin protein had significantly lower concentration compared to rats fed soy protein or casein. Plasma valine concentration was significantly higher in rats fed casein compared to rats fed fish, soy, pea or lupin protein. Alanine concentration was not different among rats fed fish, casein, soy or pea protein, only rats fed lupin protein had significantly lower concentration. Rats fed pea and soy protein had significantly higher asparagine concentration compared to rats fed fish, casein or lupin protein. Rats fed fish protein had significantly lower asparagine concentration compared to rats fed casein. Glutamine concentration was similar among rats fed casein, soy or pea protein, but rats fed fish and lupin protein had significantly lower concentration compared to rats fed casein or soy protein. Glycine concentration was significantly higher in rats fed fish protein compared to rats fed casein, soy, pea or lupin protein. Rats fed soy or pea protein had higher glycine concentrations compared to rats fed casein or lupin protein. No differences were observed in the serine and phenylalanine concentrations among the groups. Rats fed fish or soy protein had significantly lower plasma tyrosine concentration compared to rats fed casein or lupin protein, rats fed pea protein had plasma tyrosine concentration in between. Plasma taurine concentration was significantly higher in the rats fed fish or lupin protein compared to rats fed casein, soy or pea protein. Rats fed casein had plasma taurine concentration significantly lower compared to rats fed soy or pea protein. Plasma glutamic acid concentration was significantly higher in rats fed fish, casein or soy protein compared to rats fed pea or lupin protein.

3.8 Homocysteine concentrations in plasma

In figure 3.3, homocysteine concentrations in plasma of rats used in experiment 2 are shown. Homocysteine concentration was not different between rats fed casein and soy protein. Rats fed beef, fish or turkey protein had significantly lower homocysteine concentrations compared to casein and soy protein fed rats. Pork protein fed rats had homocysteine concentrations in between.

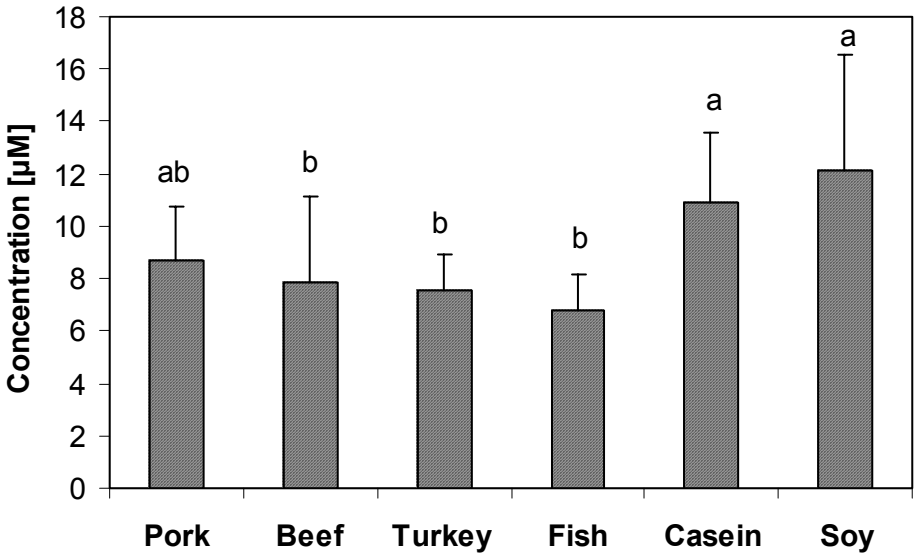

Figure 3.3 Homocysteine concentrations in plasma of rats used in experiment 2

Values are mean values ± SD. Different superscript letters denote significant difference. $P<0.05$ (Fisher's multiple range test). n = 12. Rats were food deprived for 12 h.

3.9 Cholesterol and Triglyceride in Faeces

In the table 3.6, cholesterol and triglyceride concentration in faeces and excretion values of rats used in experiment 1 and 2 are presented. In Experiment 1, no significant difference was found in the cholesterol concentration as well as in excretion between rats of the two groups. The triglyceride concentration as well as excretion was significantly higher in rats fed soy protein compared to rats fed casein (+192%, +256%), respectively.

In Experiment 2, cholesterol concentration in faeces was significantly higher in the rats fed casein compared to rats fed pork, beef, fish, turkey or soy protein, which had similar values. Cholesterol excretion was significantly higher in the rats fed casein or soy protein

compared to rats fed pork, beef, fish or turkey protein, which had similar values. Faecal triglyceride concentration as well as excretion was significantly higher in the rats fed soy protein compared to rats fed pork, beef, turkey, fish or casein protein.

Table 3.6 Cholesterol and triglyceride concentration in faeces and excretion of rats used in Experiment 1 and 2

Groups		Cholesterol		Triglyceride	
		Concentration [µmol/g]	Excretion [µmol/d]	Concentration [µmol/g]	Excretion [µmol/d]
Exp 1	Casein	6.8 ± 1.6	8.2 ± 2.0	0.4 ± 0.1^{b}	0.5 ± 0.1^{b}
	Soy	5.6 ± 1.0	8.4 ± 1.4	1.3 ± 0.5^{a}	1.9 ± 0.6^{a}
Exp 2	Pork	6.1 ± 1.7^{b}	7.2 ± 2.0^{b}	0.4 ± 0.2^{b}	0.5 ± 0.3^{b}
	Beef	6.2 ± 1.2^{b}	6.6 ± 2.7^{b}	0.5 ± 0.4^{b}	0.4 ± 0.3^{b}
	Fish	5.4 ± 0.7^{b}	6.5 ± 1.4^{b}	0.5 ± 0.3^{b}	0.6 ± 0.4^{b}
	Turkey	5.9 ± 1.5^{b}	6.5 ± 2.1^{b}	0.6 ± 0.4^{b}	0.4 ± 0.2^{b}
	Casein	10.7 ± 1.5^{a}	13.7 ± 3.1^{a}	0.2 ± 0.1^{b}	0.2 ± 0.1^{b}
	Soy	7.2 ± 2.7^{b}	10.5 ± 4.2^{a}	1.4 ± 0.8^{a}	1.9 ± 1.1^{a}

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. $P<0.05$ (Fisher's multiple range test). Experiment 1, (n = 10), Experiment 2 (n = 12). Rats were food deprived for 12 h.

3.10 Bile Acids in Plasma and Faeces

In the table 3.7, bile acid concentration in plasma and bile acid concentration and excretion values in the faeces are presented.

In Experiment 1, bile acid concentration in the plasma was not different between the rats fed soy protein and rats fed casein. But bile acid concentration and excretion was

significantly higher (+120% and +170%) in rats fed soy protein compared to rats fed casein, respectively.

In Experiment 2, bile acid concentration was significantly higher in rats fed fish protein than in rats fed casein. Rats fed pork, beef turkey or soy protein had values in between. The bile acid excretion was significantly higher in rats fed fish or soy protein than in rats fed pork, beef, turkey or casein.

Table 3.7 Bile Acid Concentrations in Plasma and Faeces and Excretion in Rats used in Experiment 1 and 2

Groups		Bile acid in Plasma	Bile acid in Faeces	
		Concentration (µmol/g)	Concentration (µmol/g)	Excretion (µmol/d)
Exp 1	Casein	16.9 ± 7.0	0.9 ± 0.2^{b}	1.1 ± 0.3^{b}
	Soy	15.2 ± 8.3	2.0 ± 0.7^{a}	2.9 ± 0.8^{a}
Exp 2	Pork	n.d.	2.2 ± 0.4^{b}	2.6 ± 0.4^{b}
	Beef	n.d	2.1 ± 0.5^{b}	2.1 ± 0.6^{b}
	Fish	n.d.	2.9 ± 0.7^{a}	3.5 ± 0.9^{a}
	Turkey	n.d.	2.4 ± 0.4^{b}	2.7 ± 0.6^{b}
	Casein	n.d.	2.0 ± 0.3^{b}	2.6 ± 0.5^{b}
	Soy	n.d.	2.3 ± 0.2^{b}	3.4 ± 0.4^{a}

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. $P<0.05$ (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 (n = 12). Rats were food deprived for 12 h.

3.11 Cholesterol and Triglyceride Concentrations in the Liver

In the table 3.8, cholesterol and triglyceride concentration in the liver of rats used in experiment 1, 2 and 3 are specified.

In Experiment 1, cholesterol concentration was significantly lower (-30%) in rats fed soy protein compared to the rats fed casein and the triglyceride concentration in the liver was 24% lower in rats fed soy protein compared to rats fed casein.

In Experiment 2, rats fed fish protein had significantly higher cholesterol concentration in the liver compared to rats fed casein or soy protein. Rats fed soy protein had significantly lower cholesterol concentration compared to rats fed casein. Rats fed pork, beef and turkey protein had significantly higher values compared to rats fed soy protein. Rats fed pork protein or casein had similar cholesterol concentrations. The triglyceride concentration in liver was significantly higher in rats fed fish protein and casein compared to rats fed pork protein. Rats fed beef, turkey and soy protein had values in between.

Table 3.8 Cholesterol and Triglyceride Concentrations in the Liver of Rats used in Experiment 1, 2 and 3

Groups		Liver cholesterol	Liver triglyceride
		[µmol/g]	
Exp 1	Casein	8.7 ± 2.1^{a}	8.5 ± 3.5
	Soy	6.1 ± 1.0^{b}	6.5 ± 1.7
Exp 2	Pork	12.7 ± 4.3^{b}	7.5 ± 2.1^{b}
	Beef	14.3 ± 3.5^{b}	9.2 ± 2.5^{ab}
	Fish	20.9 ± 6.5^{a}	13.9 ± 4.8^{a}
	Turkey	16.8 ± 3.5^{ab}	11.1 ± 4.4^{ab}
	Casein	13.2 ± 1.8^{b}	13.1 ± 3.2^{a}
	Soy	9.4 ± 2.2^{c}	10.6 ± 1.7^{ab}
Exp 3	Fish	10.1 ± 2.1^{a}	8.3 ± 2.5^{b}
	Casein	8.4 ± 1.6^{ab}	12.1 ± 5.2^{a}
	Soy	4.3 ± 1.5^{b}	7.7 ± 2.4^{b}
	Pea	3.2 ± 1.2^{b}	7.0 ± 1.4^{b}
	Lupin	2.8 ± 1.0^{b}	7.0 ± 2.1^{b}

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. P<0.05 (Fisher's multiple range test). Experiment 1, (n = 10), Experiment 2 and 3, (n = 12). Rats were food deprived for 12 h in exp.1 & 2, while in experiment 3 rats were killed without prior food deprivation.

In Experiment 3, rats fed fish protein had significantly higher liver cholesterol concentration and rats fed plant proteins had significantly lower cholesterol concentration, rats fed casein had cholesterol concentration in between. The triglyceride concentration in the liver was significantly higher in the rats fed casein compared to rats fed fish, soy, pea or lupin proteins which had similar triglyceride concentrations.

3.12 Free and Esterified Cholesterol in the Liver

Table 3.9 Concentrations of Free and Esterified Cholesterol in the Liver of Rats used in Experiment 1 and 2

Groups		Free Cholesterol (FC)	Cholesterol ester (CE)	CE/FC
		[µmol/g]		
Exp 1	Casein	4.3 ± 1.1[a]	25.6 ± 10.1[a]	6.4 ± 2.9
	Soy	1.9 ± 0.6[b]	11.9 ± 6.1[b]	6.7 ± 3.6
Exp 2	Pork	7.8 ± 3.4	25.1 ± 7.3[b]	3.5 ± 1.9[b]
	Beef	7.6 ± 3.5	23.0 ± 11.3[b]	3.5 ± 1.9[b]
	Fish	7.9 ± 2.9	40.8 ± 15.5[a]	6.4 ± 3.8[a]
	Turkey	8.5 ± 2.0	35.6 ± 14.3[a]	4.4 ± 1.9[b]
	Casein	6.8 ± 2.2	22.9 ± 12.6[b]	3.6 ± 1.5[b]
	Soy	6.0 ± 3.8	14.3 ± 9.4[c]	2.1 ± 1.2[c]

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. P<0.05 (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 (n = 12). Rats were food deprived for 12 h.

In table 3.9, concentrations of free and esterified cholesterol in the liver of rats used in experiment 1 and 2 are specified. In Experiment 1, free cholesterol as well as cholesterol ester

concentration in the liver was significantly lower (-56%, -54%)) in the rats fed soy protein compared to the rats fed casein. The esterified to free cholesterol ratio was not different between rats fed casein or soy protein.

In Experiment 2, no significant difference was found in the free cholesterol concentration among the groups. Esterified cholesterol concentration in the liver was significantly higher in the rats fed fish and turkey protein compared to rats fed pork, beef, casein or soy protein. Rats fed soy protein had significantly lower esterified cholesterol than rats fed casein. The esterified to free cholesterol ratio was significantly higher in rats fed fish protein compared to rats fed pork, beef, turkey, casein or soy protein. Rats fed soy protein had significantly lower esterified to free cholesterol ratio compared to rats fed casein.

3.13 HMG CoA Reductase in the Liver

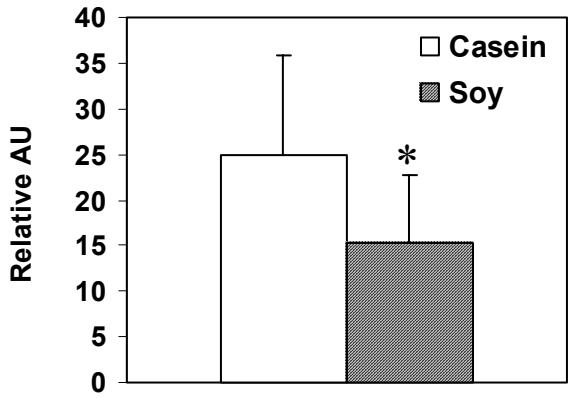

Figure 3.4: HMG CoA Reductase protein concentration in microsomal fraction of rats fed either casein or soy protein (Experiment 3)

Values are mean values ± SD. AU, Absorbance units, * Significant difference between casein and soy group. P<0.05 (t-test). Rats were killed without prior food deprivation, n= 12.

Rats fed soy protein diet had significantly lower (-38%) HMG CoA reductase protein concentrations in the microsomal fraction of the liver compared to rats fed casein protein diet (figure 3.4).

3.14 Enzyme activity in the Liver

In table 3.10, the activities of enzymes determined in experiment 1 and 2 are specified.

In experiment 1, rats fed soy protein had significantly lower MTP activity (-35%) in the liver compared to the rats fed casein. In experiment 2, rats fed turkey protein had significantly higher FAS activity in the liver compared to rats fed pork or soy protein. Rats fed beef protein, fish protein and casein had values in between. The activity of G6PDH was significantly lower in the rats fed pork and soy proteins compared to rats fed casein.

Table 3.10 Activities of Lipogenic Enzymes in the Liver Cytosol of Rats used in Experiment 1 and 2

Groups		MTP [ΔF/µg protein]	FAS	G6PDH
			[nmol/mg protein*min]	
Exp 1	Casein	43 ± 14[a]	n.d.	n.d.
	Soy	28 ± 7[b]	n.d.	n.d
Exp 2	Pork	n.d.	0.60 ± 0.23[b]	19.1 ± 4.4[b]
	Beef	n.d.	0.75 ± 0.35[ab]	n.d.
	Fish	n.d.	0.65 ± 0.24[ab]	n.d.
	Turkey	n.d.	0.99 ± 0.58[a]	n.d.
	Casein	n.d.	0.70 ± 0.45[ab]	31.5 ± 9.3[a]
	Soy	n.d.	0.52 ± 0.35[b]	16.4 ± 6.4[b]

Values are mean values ± SD. Different superscript letters within one column denote significant difference within one experiment. P< 0.05 (Fisher's multiple range test). Experiment 1 (n = 10), Experiment 2 (n = 12), n.d.: not determined. Rats were food deprived for 12 h. MTP, microsomal triglyceride transfer protein; FAS, fatty acid synthase; G6PDH, glucose 6 phosphate dehydrogenase.

3.15 Relative mRNA Concentrations in the Liver

Relative mRNA concentrations in the liver of rats fed diets containing casein or soy protein in experiment 1 are shown in figure 3.5. The mRNA concentrations of FAS and MTP were significantly lower (-30% and -27%) in rats fed soy protein than in rats fed casein (P<0.05). The mRNA concentrations of SREBP-1c and SREBP-2 tended to be lower (-30%

and -24%) in rats fed soy protein than in rats fed casein (P<0.10). No significant difference was found in the relative mRNA concentrations of HMG CoA reductase, LDL-receptor, and CYP7A1.

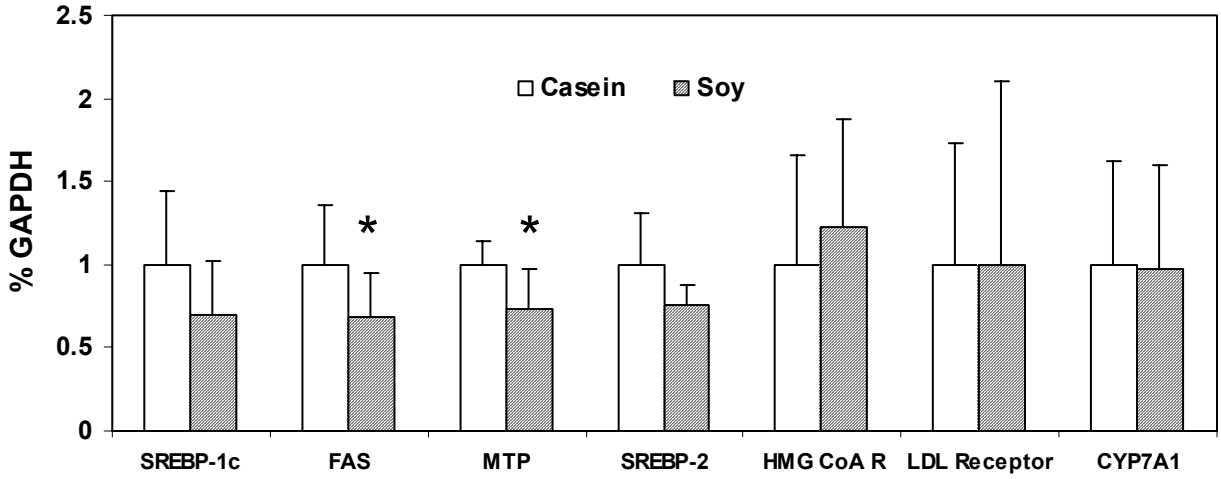

Figure 3.5: Relative mRNA concentrations in the liver of rats fed diets containing casein or soy protein in overnight food deprived state (Experiment 1)

Values are the mean values ± SD. n = 10. * denote significant difference between the two groups. P<0.05 (t-test). SREBP, sterol regulatory element binding protein; FAS, fatty acid synthase; MTP, microsomal triglyceride transfer protein; HMG CoA R, 3 hydroxy 3-methylglutaryl CoA reductase; LDL, low density lipoprotein; CYP7A1, cholesterol 7 α 1 hydroxylase.

Relative mRNA concentrations in the liver of rats fed diets containing casein or fish protein in experiment 2 are shown in figure 3.6. The mRNA expression of SREBP-2 was 65% higher in rats fed fish protein compared to rats fed casein (P<0.001) but however, we did not find significant difference in the mRNA concentration HMG Co A reductase. Although no significant difference was found in the mRNA concentrations of SREBP-1c, and G6PDH the mRNA concentrations of FAS and Delta 6-Desaturase were significantly higher 67% and 19% respectively, in the rats fed fish protein compared to rats fed casein.

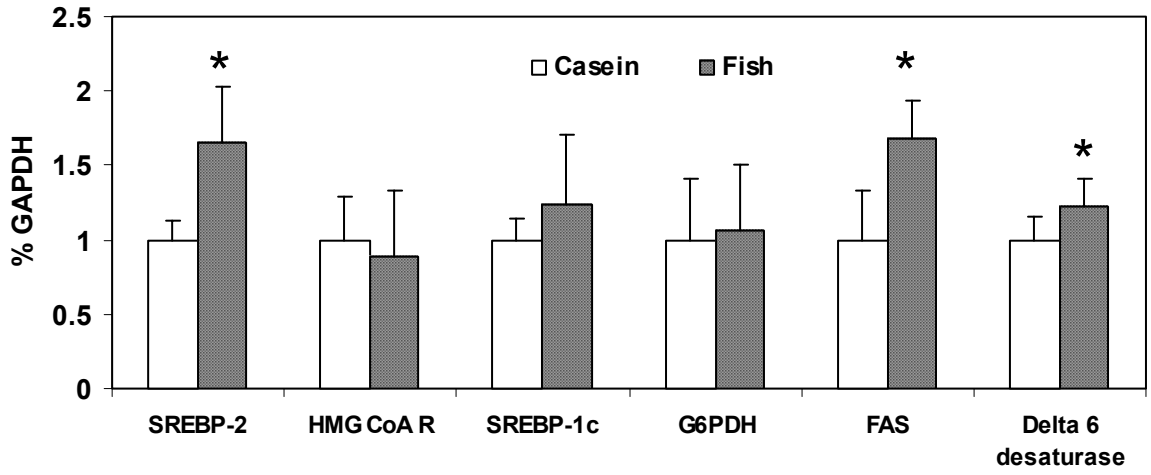

Figure 3.6: Relative mRNA concentrations in the liver of rats fed diets containing casein or fish protein in overnight food deprived state (Experiment 2)

Values are the mean values ± SD. n = 12. * denote significant difference between the two groups. P<0.05 (t-test). SREBP, sterol regulatory element binding proteins; HMG CoA R, 3 hydroxy 3 methyl glutaryl CoA reductase; G6PDH, glucose 6 phosphate dehydrogenase; FAS, fatty acid synthase.

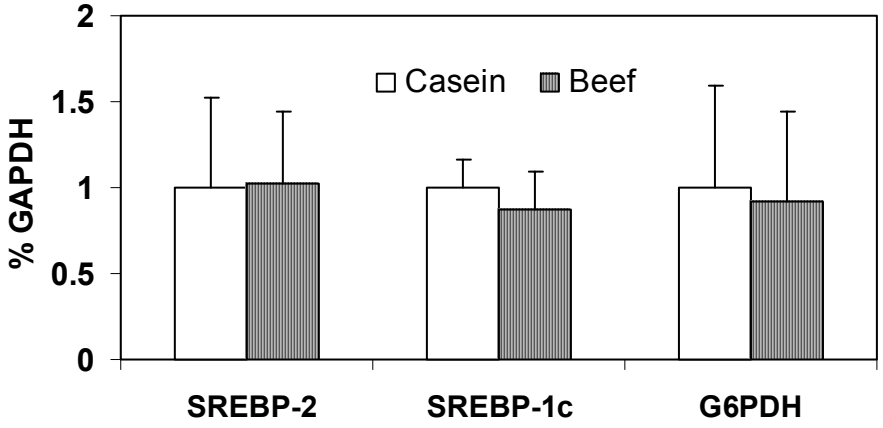

Figure 3.7: Relative mRNA concentrations in the liver of rats fed diets containing casein or beef protein in overnight food deprived state (Experiment 2)

Values are the mean values ± SD. n = 12, P<0.05 (t-test). SREBP, sterol regulatory element binding proteins; G6PDH, glucose 6-phosphate dehydrogenase.

There was no significant difference found in the relative mRNA expression of SREBP-1c, SREBP-2, or G6PDH in between the rats fed casein and beef protein (Figure 3.7).

3.16 cDNA Array data Soy Protein versus Casein:

Table 3.11: cDNA array data of selected proteins involved in lipid metabolism in the liver of rats fed diets containing soy protein compared with rats fed diet containing casein (Experiment 3)

GenBank Accession No.	Proteins/Genes	Ratio Soy/Casein group	Functions
D37920	Squalene epoxidase	0.8 ± 0.5	Cholesterol synthesis
M95591	Squalene synthetase	0.8 ± 0.4	Cholesterol synthesis
U12791	3-hydroxy-3-methylglutaryl-CoA synthase	0.9 ± 0.1	Cholesterol synthesis
X17595	Cholesterol 7-alpha-hydroxylase	0.8 ± 0.4	Bile acid metabolism
M38566	Sterol 27-hydroxylase	0.9 ± 0.1	Bile acid metabolism
M67465	3-beta hydroxy-5-ene steroid dehydrogenase type III (3beta-HSD III)	0.7 ± 0.2	Steroid metabolism
S63167	3-beta-hydroxysteroid dehydrogenase	0.8 ± 0.3	Steroid metabolism
P52233	11-beta-hydroxysteroid dehydrogenase 2	1.0 ± 0.5	Steroid metabolism
M00001	Apolipoprotein A-I precursor (APO-AI)	1.4 ± 0.4	HDL structural protein
U17697	Scavenger receptor class B type I	1.1 ± 0.5	Plasma HDL uptake
M00002	Apolipoprotein A-IV precursor (APO-AIV)	1.4 ± 1.0	Chylomicrones structural protein
U62803	Lecithin: cholesterol acyl transferase	0.9 ± 0.3	Extracellular cholesterol esterification
M35991	Fatty Acid-binding Protein	0.9 ± 0.2	Fatty acid binding, and transport
L46791	Liver Carboxylesterase 10 precursor	0.8 ± 0.3	Triglyceride degradation
D90109	Long chain acyl-CoA synthetase 2	0.9 ± 0.1	Fatty acid synthesis

J02791	Medium chain acyl-CoA dehydrogenase precursor	1.1 ± 0.4	Mitochondrial ß oxidation
U64451	Short chain acyl-CoA dehydrogenase precursor	1.6 ± 0.5	Mitochondrial ß-oxidation
M32801	3-ketoacyl-CoA thiolase A + B	1.6 ± 0.4	Paroxismal ß oxidation
J02752	Acyl-CoA Oxidase	0.7 ± 0.2	Paroxismal ß oxidation

Values are mean values ± SD. n = 4, For each probe RNA of 3 rats within same dietary treatment was pooled. Rats were killed without prior food deprivation. From 1176 gene/proteins spotted on the membrane about 575 gene/protein expressions were detectable. Only 127 were significantly different (at least 1.4 fold up or down regulated). HDL, high density lipoprotein.

In table 3.11, the cDNA array data of selected proteins involved in lipid metabolism in the liver of rats fed soy protein compared to rats fed casein are presented.

In the cDNA array analysis, soy protein fed rats showed a bit lower expressions of many proteins involved in cholesterol metabolism like squalene epoxidase, squalene synthetase and 3-hydroxy-3-methylglutaryl-CoA synthase, 3-beta-hydroxysteroid dehydrogenase III, 3-beta-hydroxysteroid dehydrogenase, cholesterol 7-alpha-hydroxylase, sterol 27-hydroxylase. apolipoprotein A-I precursor (APO-AI), apolipoprotein A-IV precursor (APO-AIV), and scavenger receptor class B type I were highly expressed in soy protein fed rats compared to casein fed rats. Medium chain acyl-CoA dehydrogenase precursor, short chain acyl-CoA dehydrogenase precursor, 3-ketoacyl-CoA thiolase A + B which are involved in fatty acid oxidation were highly expressed in rats fed soy protein.

Relative mRNA Concentrations Casein versus Soy protein in Experiment 3:

Relative mRNA expressions in the liver of rats fed diets containing casein or soy protein used in experiment 3 are shown in Figure 3.8. Relative mRNA expressions of APO-B (-37%), SREBP-2 (-35%), HMG CoA reductase (-38%), LDL receptor (-58%) and CYP7A1 (-38 %), were significantly lower in soy protein fed rats compared to casein fed rats. No difference was observed in the relative mRNA expressions of Insig-2. There was a tendency of higher Insig-1 (+ 49%) in rats fed soy protein compared to rats fed casein ($P<0.10$). The semiquantitative RT-PCR was performed to verify the results of cDNA arrays and these results prove the cDNA array findings (table 3.11).

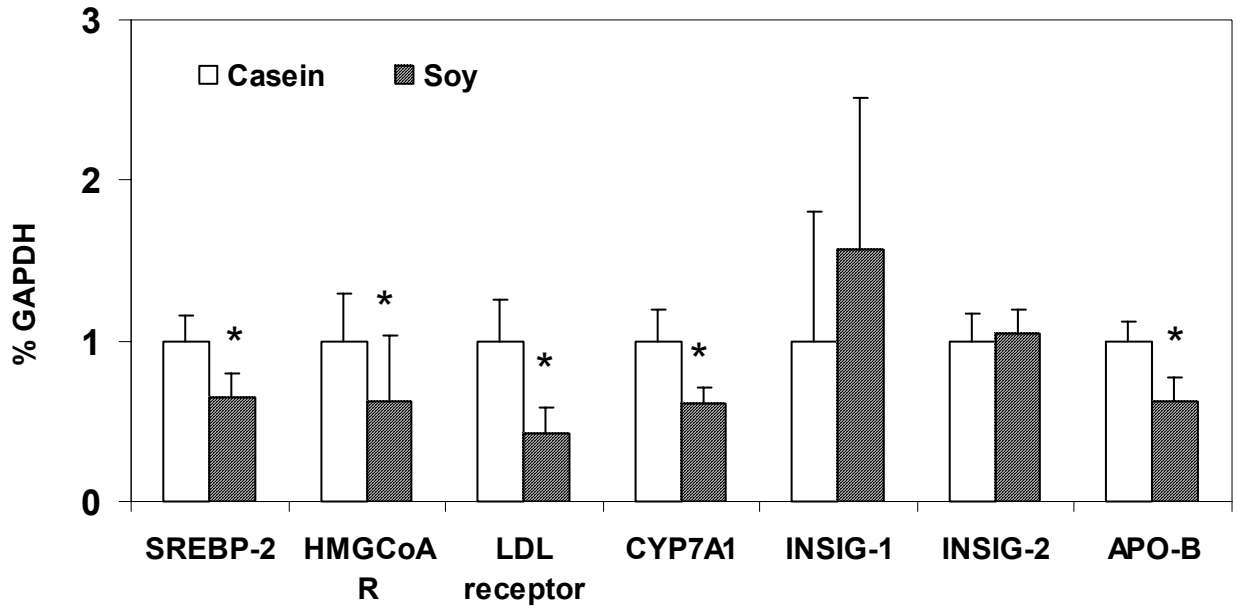

Figure 3.8: Relative mRNA concentrations in the liver of rats fed diets containing casein or soy protein in the postprandial state (Experiment 3)

Values are the mean values ± SD. * denote significant difference between the two groups. n = 12, P< 0.05 (t-test).). Rats were killed without prior food deprivation. SREBP, sterol regulatory element binding protein; HMG CoA R, 3 hydroxy 3-methylglutaryl CoA reductase; LDL, low density lipoprotein; CYP7A1, cholesterl 7 α 1 hydroxylase; INSIG, insulin induced gene; APO-B, apolipoprotein-B.

3.17 cDNA Array data Fish Protein versus Casein

In table 3.12, the cDNA array data of selected proteins involved in lipid metabolism in the liver of rats fed diets containing fish protein compared with rats fed diet containing casein are presented. The cDNA expression of genes involved in mitochondrial ß-oxidation of fatty acids like Carnitine palmitoyl transferase (CPT) I, CPT-II, short chain acyl-CoA dehydrogenase, medium chain acyl-CoA dehydrogenase, and long chain-specific acyl-CoA dehydrogenase were 1.1-2.0 times higher expressed in rats fed fish protein compared to rats fed casein. 3-ketoacyl-CoA thiolase A + B an enzyme involved in peroxisomal ß-oxidation of fatty acids was 1.6 times higher expressed in the liver of rats fed fish protein, CYP4A1, CYP4A3, CYP3A1, CYP4F6, which are involved in microsomal ω-hydroxylation of fatty acids were 1.3-2.2 times higher expressed in rats fed fish protein compared to rats fed casein.

Cholesterol 7-alpha-hydroxylase and sterol 27-hydroxylase which are involved in bile acid metabolism were 1.5 and 1.3 times higher expressed in rats fed fish protein. Acetyl-CoA carboxylase, a key enzyme of fatty acid synthesis was about twice expressed in rats fed fish protein compared to rats fed casein. The cDNA array expression of genes involved in HDL metabolism like Apo AI, and Lecithin: cholesterol acyl transferase were not different in between rats fed fish protein and casein, while SR-BI, otherwise known as HDL receptor was 1.5 times more expressed in rats fed fish protein compared to rats fed casein.

Table 3.12: Relative cDNA array expressions of proteins involved in lipid metabolism in the liver of rats fed diets containing fish protein compared with rats fed diet containing casein (Experiment 3)

GenBank Accession no.	Proteins/Genes	Ratio Fish/Casein group	Funcrions
L07736	Carnitine palmitoyl transferase I	2.0 ± 1.2	Mitochondrial ß oxidation
J05470	Carnitine palmitoyl transferase II	1.1 ± 0.16	Mitochondrial ß oxidation
U64451	Short chain acyl-CoA dehydrogenase	1.3 ± 0.5	Mitochondrial ß oxidation
J02791	Medium chain acyl-CoA dehydrogenase	1.5 ± 0.9	Mitochondrial ß oxidation
J05029	Long chain-specific acyl-CoA dehydrogenase	1.5 ± 0.9	Mitochondrial ß oxidation
J02752	Acyl-CoA oxidase	1.0 ± 0.8	Peroxisomal ß oxidation
M32801	3-ketoacyl-CoA thiolase A + B	1.6 ± 1.1	Peroxisomal ß oxidation
P00502	Glutathione S-transferase alpha	0.8 ± 0.3	Biotransformation
P08011	Glutathione S-transferase	0.8 ± 0.4	Biotransformation
K02422	Cytochrome P450 IA2	0.9 ± 0.2	Biotransformation
J02657	Cytochrome P450 2C11	0.9 ± 0.4	Biotransformation
M58041	Cytochrome P450 2C22	1.6 ± 1.3	Biotransformation
U39943	Cytochrome P450 2J3	1.2 ± 0.4	Biotransformation

X07259	Cytochrome P450 4A1	2.1 ± 1.7	Microsomal ω-hydroxylation
M33936	Cytochrome P450 4A3	2.2 ± 1.4	Microsomal ω-hydroxylation
M10161	Cytochrome P450 3A1	2.2 ± 1.6	Microsomal ω-hydroxylation
U39208	Cytochrome P450 4F6	1.3 ± 0.4	Microsomal ω-hydroxylation
X17595	Cholesterol 7-alpha-hydroxylase	1.5 ± 1.0	Bile acid metabolism
M38566	Sterol 27 Hydroxylase	1.3 ± 0.5	Bile acid metabolism
P19100	Steroid 17-alpha-hydroxylase	2.1 ± 0.5	Steroid metabolism
M55315	Acetyl-CoA carboxylase	1.9 ± 1.7	Fatty acid synthesis
M00001	Apolipoprotein A-I precursor	1.0 ± 1.1	HDL structural protein
M00002	Apolipoprotein A-IV precursor	1.4 ± 0.6	Chylomicrones structural protein
U17697	Scavenger receptor class B type I	1.5 ± 0.7	Plasma HDL uptake
P35952	Low density lipprotein (LDL) receptor	1.2 ± 0.6	LDL uptake
U62803	Lecithin: cholesterol acyl transferase	1.0 ± 0.3	Extracellular cholesterol esterification
U12791	3-hydroxy-3-methylglutaryl-CoA synthase	1.1 ± 0.1	Ketogenesis

Values are mean values ± SD. n = 4, HDL, High density lipoprotein; For each probe RNA of 3 rats within same dietary treatment group was pooled. Rats were killed without prior food deprivation. From 1176 gene/proteins spotted on the membrane about 590 gene/protein expressions were detectable. Only 154 were significantly different (at least 1.4 fold up or down regulated).

Relative mRNA Concentrations Casein versus Fish protein in experiment 3:

Relative mRNA concentrations in the liver of rats fed diets containing casein or soy protein used in experiment 3 are shown in Figure 3.9. The relative mRNA concentrations of PPARα regulated genes ACO and CYP4A1 were found significantly higher in rats fed fish protein compared to rats fed casein. The relative mRNA concentrations of glutathione-S-transferase α, and PPARα were not different between rats fed casein and fish protein. The cDNA array results and the RT-PCR performed to prove the array results suggest that fish

protein up regulated PPAR alpha activity because lots of genes up regulated by fish protein are PPAR alpha target genes.

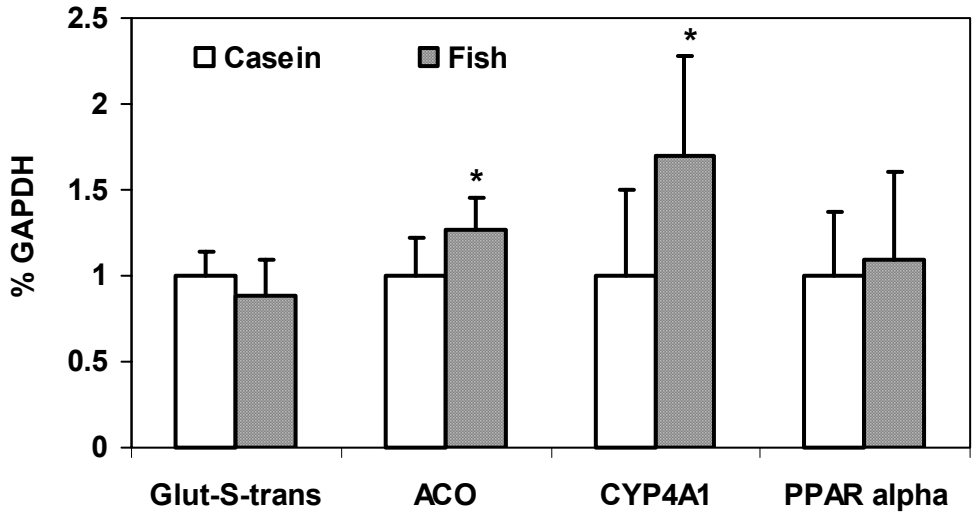

Figure 3.9: Relative mRNA concentrations of PPAR α down stream gene in the liver of rats fed diets containing casein or fish protein in the postprandial state (Experiment 3)

Values are the mean values ± SD. * denote significant difference between the two groups. $P<0.05$ (t-test). n = 12. Rats were killed without prior food deprivation. Glut-S-trans, Glutathione S transferase; ACO, Acyl CoA oxidase; CYP4A1, Cytochrome 450 4A1; PPAR, Peroxisome proliferator activated receptor.

4 Discussion

It has been established that dietary proteins influence lipid metabolism in men and animals (Anderson et al. 1995, Sirtori et al. 1998, Sirtori & Lovati 2001). Coronary heart disease (CHD) mortality and morbidity in Asian countries are substantially lower than in western countries (Robertson et al. 1997). Adlercreutz (1990) has suggested that this may be due to the considerably higher intake of soy protein in Asian countries. Previous studies, replacing dietary animal protein with intact soy protein, have shown significant improvements in CHD risk factors, particularly total cholesterol, LDL-cholesterol and triglycerides in humans and laboratory animals (Anderson et al. 1995, Carroll 1991, Carroll and Kurowska 1995). It has been reported many times that soy protein has hypocholesterolemic (Anderson et al. 1995, Iritani et al. 1996, Sugiyama et al. 1996) and hypotriglyceridemic action (Tovar et al. 2002, Ascencio et al. 2004, Tovar et al. 2005) in laboratory animals, and humans when compared to casein. However, the components of soy that may contribute to the lipid lowering properties have not been well characterized. Several studies have been made to elicit possible mechanisms by which soy protein isolate may act on lipid metabolism. Based on studies with cell cultures, animals and humans, suggested mechanisms of soy protein include increases of low-density lipoprotein (LDL) receptor expression and activity (Lovati et al. 1987, Sirtori et al. 1984, Lovati et al. 1991, Manzoni et al. 1998, Lovati et al. 2000), down regulation of sterol regulatory element-binding protein-1 (SREBP-1), a transcriptional factor that is primarily responsible for the regulation of genes involved in fatty acid biosynthesis (Tovar et al. 2002, Ascencio et al. 2004), and increases in the synthesis and faecal excretion of bile acids (Tachibana et al. 2005). Tachibana et al. (2005) have found that soy protein isolate significantly up-regulates the expression of genes related to steroid catabolism that may result in a reduction of the serum cholesterol level. Other experiments with cultured hepatocytes have indicated that certain peptide components of soy protein stimulate expression of LDL receptors (Lovati et al. 1992, 1996). However, the results of a study in LDL receptor null mice did not support widely suggested LDL receptor mediated mechanism (Adams et al. 2002), in which soy protein isolate lowers the LDL and VLDL cholesterol concentrations and inhibited atherosclerosis despite the absence of LDL receptor in the mice. The LDL receptor is one of a number of genes regulated by the transcription factor SREBP-2. SREBP-2 is critical for the regulation of intracellular sterol homeostasis and besides regulation of LDL receptor expression SREBP-2 is primarily responsible for the regulation of genes involved in cholesterol biosynthesis such as 3-hydroxy 3-methylglutaryl CoA (HMG CoA) reductase

(Hua et al. 1993, Vallett et al. 1996, Horton et al. 2002). Although, recent evidence exist that rats fed a soy protein diet have reduced SREBP-1 expression (Tovar et al. 2002, Ascencio et al. 2004), however, the expression of SREBP-2 and its sterol regulatory element-related genes that may also play a role in the hypolipidemic effects of soy protein isolate have not yet been investigated.

Therefore, one aim of our first experiment was to investigate the effects and mechanism of soy protein on the cholesterol metabolism. To minimize possible effects of soy protein-associated isoflavones that may partly be responsible for cholesterol reduction by soybean protein preparations, the soy protein isolate used in our studies was additionally ethanol-washed. To mimic western diets, which are commonly rich in saturated fats and cholesterol, we chose lard as type of dietary fat and supplemented the diet with cholesterol (0.5 g/kg). In spite of removal of most of isoflavones by ethanol extraction, the soy protein diet was able to show effects on cholesterol and triglyceride metabolism.

Besides casein, animal proteins such as those from beef, pork, poultry and fish play an important role in human nutrition worldwide. Yet less is known about their effects on the lipid metabolism. Therefore, we planned a second experiment to investigate the effects of proteins isolated from beef, pork, turkey meat and fish protein isolated from Alaska pollack fillets on lipid metabolism and compared their effects with casein and soy protein. Casein served as reference protein of animal origin; and soy protein served as reference protein of plant origin.

In a third experiment, we planned to investigate the effect of other easily available and commonly used plant proteins isolated from peas (used in infant formula food) and sweet lupin seeds. Moreover, we intended to affirm the results obtained after feeding fish protein in the second experiment because until now there is little knowledge available about the effects of fish protein on lipid metabolism.

As parameters of lipid metabolism, concentrations of cholesterol and triglycerides in plasma, lipoproteins, and liver, faecal excretion of bile acids, activities and concentrations of selected enzymes and hepatic expression of genes encoding proteins involved in lipid homeostasis were determined. In all three studies we used rats as model animal, a model which is used in many investigations of the dietary proteins (Iritani et al. 1996, Ascenio et al. 2004, Gudbrandsen et al. 2005). Growing rats were used, as we expected that the effects of the dietary treatments would be greater than in adults. We evaluated the gene expression profile and enzyme activity of the liver because this tissue is the major site of lipid metabolism. To avoid the interference of chylomicrons and postprondial rise of lipids, we

sacrificed the animals of the first two studies after overnight fast. However, under these conditions a lot of regulating proteins were probably down regulated (Hortan et al. 1998, Shimano et al. 1999) and the effects of proteins are indistinguishable. Therefore, in the third study we sacrificed animals in the postprandial state. The differences in the observations of the three experiments can be explained by different amount of food ingested during different experiments.

We used a relatively short experimental period as recent studies have shown that effects of dietary proteins on the lipid metabolism of rats become manifest within a short period, usually within two weeks or even earlier (Sugiyama et al. 1996, Sugiyama et al. 1997, Iritani et al. 1986, Ascencio et al. 2004). Most of the effects of soy protein on lipid metabolism observed in this study are in accord with those reported by other investigators (Madani et al. 1998, Madani et al. 2003, Tover et al. 2002, Ascencio et al. 2004). The fact that rats fed the soy protein diet exerted markedly alterations of their lipid metabolism confirms that the feeding period was long enough to adequately study effects of dietary proteins on the lipid metabolism.

4.1 Body and Liver Weight

Although the rats were fed a restrictive diet in order to exclude secondary effects which might result from different feed intakes, in the first two studies casein fed rats grew at the faster rate, whereas rats fed soy protein grew at slower rate. These results are in agreement with Tovar et al. (2002), Iritani et al. (1996), and Zhang and Beynen (1993). Tovar et al. (2002) observed that in spite of similar food intake the casein fed rats grew at the faster rate than soy protein fed rats. The growth retardation by the soy protein diet may be due to the lower concentration of lysine in the soy protein compared to casein, because lysine has been reported to be an important factor for growth. In the third experiment, there was no difference observed in the body weight gain between rats fed casein and soy protein, but in the third experiment additional DL-methionine was supplied to the soy protein diet. Therefore, the lower methionine concentration in the soy protein might be at least in part responsible for the slower growth rate of rats fed this protein.

In third experiment, rats fed lupin protein grew at the slower rate, compared to rats fed casein or soy protein. Few of the rats fed lupin protein containing diet showed health problems from the second week of feeding (skin hair fall etc.) and this group rats did not

consume all the food provided daily, which may explain the lower body weight gain. The health problems observed by feeding lupin protein diet might be because of higher content of plant alkaloid or antinutritional facors (like tannins and protease inhibitors etc.) found in the plant proteins, although the protein was recommended for nutritional use by the supplier.

In first two experiments, the relative liver weight of rats fed soy protein was significantly lower compared to rats fed casein. In third experiment, the relative liver weight was lower of rats fed pea or lupin than rats fed casein. These results are in agreement with many previous studies. Sugiyama et al. (1996) reported significantly lower relative liver weight with soy protein than with casein and fish protein diet. Iritani et al. (1986) also reported that the liver weight relative to body weight was lower in rats fed gluten or soybean protein than in those given casein or fish protein . Terpstra et al. (1983) and Peluso et al. (2000) also reported substitution of soy protein for casein reduced the liver weight. Zhang and Beynen (1993) reported soybean protein induced significantly lower liver weight than did either casein or cod meal.

4.2 Cholesterol Concentration

In all three experiments, rats fed soy protein and in experiment 3 rats fed pea and lupin protein had much lower cholesterol concentration in liver and VLDL compared to rats fed casein. In the first two experiments the ratio of LDL-/HDL-cholesterol was significantly lower in rats fed soy protein compared to rats fed casein. These results are in agreement with Damasceno et al. 2001, who reported that soy protein isolate in comparison with casein, promoted a decrease of cholesterol and triglyceride content of atherogenic lipoproteins. The lower cholesterol concentrations by soy protein diet were accompanied by a lower gene expression of SREBP-2. SREBPs are important transcription factors which belong to the basic helix-loop-helix-leucine zipper family and bind in its activated form to the sterol regulatory element in the promoter or enhancer regions of genes involved in cholesterol or fatty acid synthesis (Hua et al. 1993, Shimano et al. 1999). SREBP transcription factors are synthesized as inactive precursors bound to the endoplasmic reticulum membranes. Activation of these membrane-bound transcription factors involve a two-step proteolytic cascade through which the SREBP molecule is released from the membrane and obtains its mature form as a transcription factor which enters in the nucleus. Although, we did not measure the mature fraction of SREBP in nucleus because there was not enough sample material available from each animal, several additional parameters indicate an actually lower activity level of SREBP

in rats fed soy protein compared to rats fed casein. Parameters indicating a diminished activity level of SREBP-2 were a lower gene expression of HMG-CoA reductase and of LDL-receptor, both are downstream genes of SERBP-2, as well as diminished concentrations of cholesterol in liver.

Plasma cholesterol concentration did not differ between rats fed casein and soy protein. These observations are in agreement with Tovar et al. (2002), Madani et al. (2003), Sugiyama et al. (1996), and Ni et al. (1998), who reported no effect on serum total cholesterol concentration in soy protein isolate fed rats and mice, compared with those fed casein. Lucas et al. (2001) reported that ethanol extracted soy protein isolate does not modulate serum cholesterol concentration in ovarian hormone-deficient Golden Syrian hamsters. Van Raaij et al. (1981, 1982) assessed the effect of casein, soy protein isolate and soy protein concentrates on serum cholesterol levels in young and middle aged healthy humans. They reported that neither soy protein preparation had a significant effect on blood cholesterol levels compared with casein. But these observations are in contrast to a number of animal (Potter 1996, Carroll and Kurowska 1995) and human studies (Anderson et al. 1995) in which soy protein isolate or other soy bean products exerted hypocholesterolemic actions, compared to casein based diets. This effect is somewhat variable but is generally greater in hypercholesterolemic subjects than in normocholesterolemic subjects.

There was no effect of soy protein on the LDL cholesterol concentration in our studies, this is in contrast to a series of previously published results that show a distinct reduction of plasma and/or LDL cholesterol after administration of soy protein in man and animals (Bakhit et al. 1994, Carroll 1991, Carroll & Kurowska 1995). We suggest that lower mRNA concentrations of LDL receptor in rats fed soy protein isolate may explain the finding that plasma LDL concentration remained unchanged although liver cholesterol concentration was markedly decreased by dietary soy protein compared to casein. The LDL receptor is a major regulator of circulating LDL cholesterol (Meddings et al. 1986). Decreased removal of LDL from the circulation due to a diminished LDL receptor expression may increase plasma or LDL cholesterol concentration albeit down regulated cholesterol synthesis by soy protein isolate. LDL receptor expression lowering effect of the soy protein is in agreement with Ni et al. (1999), who reported that the isoflavone-intact soybean protein, but not the alcohol-extracted soybean protein enhanced hepatic LDL receptor mRNA in exogenously hypercholesterolemic rats. Additionally, in most of the studies cited in the literature the animals were fed soy protein, which was not characterized regarding its isoflavones.

Therefore, results from those experiments cannot actually be compared with the results from our experiments.

Our experiments are moreover the first showing that modulation of SREBP-2 by isoflavone-poor soy protein isolate was not due to an altered expression of Insig 1 and 2 (Shukla et al. 2006 b). Insigs are protein regulators that prevent movement of the SREBP/SCAP complex from the endoplasmic reticulum to the Golgi, thus blocking proteolytic cleavage and transcriptional activation of SREBP (Yang et al. 2002, Yabe et al. 2002). The reduced mRNA and protein concentrations of apo-B100 and apo-B48 could also be a consequence of altered intracellular cholesterol content. It has been shown that the secretion rate of lipoprotein cholesterol from the liver in rats fed a soybean protein diet is significantly lower than in rats fed a casein diet (Sugano et al. 1982, Pfeuffer and Barth 1986).

In our studies the main effects of the fish protein on cholesterol metabolism compared to casein were an increase of liver cholesterol concentration, a reduction of the HDL cholesterol concentrations, along with a stimulated gene expression of scavenger receptor class B type I (SR-B1), SREBP-2, and FAS. The higher relative mRNA concentrations of SREBP-2 in rats fed fish protein suggest that the cholesterol synthesis might possibly be stimulated by the fish protein which in turn could be responsible for the observed accumulation of cholesterol in the liver. This assumption is supported by the findings of Wergedahl et al. (2004) who observed a higher activity of HMG-CoA reductase in rats fed fish protein compared to those fed casein, although this effect was only observed in genetically hyperlipidemic obese Zucker rats but not in normal Wistar rats. In our experiments, besides a slight increase of HMG-CoA synthase expression, gene expression of LDL receptor was also slightly increased in rats fed fish protein. Both are target genes of SREBP-2 and indicate an activation of this transcription factor by fish protein feeding. Fish protein fed rats had significantly higher liver cholesterol and cholesteryl esters concentration compared to casein or soy protein fed rats. The increase of cholesteryl esters in the liver of rats fed fish protein support the fact that enlarged amounts of cellular cholesterol are normally associated with a higher concentration of esterified cholesterol (Field et al. 1987). Since the hepatic cholesterol homeostasis is achieved by a balance of biosynthesis, storage, catabolism, and export processes that influence cholesterol excretion could contribute to the observed cholesterol accumulation in livers of fish protein-fed rats. Abundant cholesterol is normally eliminated from liver mainly via bile acids and CYP7A1 is the key enzyme of synthesis of bile acids from cholesterol (Vlahecevic et al. 1999). However, since expressin of CYP7A1 in

the liver and the amounts of bile acids excreted via faeces, was higher in the fish protein fed rats compared with casein fed rats, it is suggested that the cholesterol accumulation in livers of fish protein-fed rats was not due to the reduced excretion of the cholesterol via bile acids.

Despite the observed cholesterol accumulation in livers of rats fed fish protein the concentration of cholesterol in plasma was not increased compared to rats fed casein. By measuring the different density lipoprotein fractions in plasma from rats of both experiments we found a lower cholesterol concentration in the HDL fraction in those fed fish protein compared to casein. The cut off chosen for lipoprotein was typical for humans, but also matched that used in previous rat studies (Sparks et al. 1998, Giudetti et al. 2003, Sirtori et al. 2004). As rat lipoproteins have a different density compared to human lipoproteins. This means the 1.006 < δ> 1.063 lipoprotein fraction contained besides LDL also intermediate density lipoproteins (IDL) and some HDL lipoproteins and this is possibly the reason for the inconsistent effects of fish protein on the lipoprotein fraction in both experiments. A major regulator of circulating LDL cholesterol is the LDL receptor (Meddings et al. 1986). The observed higher expression of LDL receptor in livers of rats fed fish protein could have possibly contributed to an increased removal of LDL from the circulation, thereby preventing a distinct LDL accumulation in plasma albeit an increased expression of SREBP-2.

The HDL-cholesterol lowering effect observed with fish protein feeding is in agreement with the recent findings in hyperlipidemic obese zucker rats and in hamsters that were fed fish protein from salmon (Wergedahl et al. 2004, Tsai and Huang 1999), and in humans fed fish diet (Li et al. 2004). HDL is the principle vehicle for removal of surplus cholesterol from the peripheral tissues for disposal in the liver. Several genes are involved in HDL metabolism like Apo-AI, a structural component of HDL, whereas the esterification of cholesterol by lecithin-cholesterol acyl transferase (LCAT) is critical for optimal cholesterol uptake and maturation of HDL (Genest et al. 1999) and SR-BI, otherwise known as HDL receptor, responsible for the selective uptake of HDL and unloading of HDL cholesterol in the liver (Acton et al. 1996). cDNA array results of Apo-AI and LCAT were not different between rats fed casein and fish protein. Although, the observed reduction in HDL-cholesterol concentration in rats fed fish protein could possibly be related to an increased expression of SR-BI. Further investigations will be necessary to clarify the mechanisms.

This study demonstrates that proteins from beef, pork and turkey did not alter the concentrations of cholesterol in plasma, lipoproteins, and liver and the ratio of esterified to free cholesterol in the liver compared with casein. This is consistent with previous work

showing that proteins from beef and chicken meat and also egg albumin and ovalbumin had no influence on cholesterol concentration in liver and plasma compared with casein (Lapre et al. 1989). Scott et al. (1994) showed that a diet with lean beef or chicken and fish had similar effects on serum lipoproteins of men with borderline hypercholesterolemia.

Since dietary proteins had to be treated in this way for experimental reasons, we cannot exclude the possibility that they were denaturized by the isolation procedure and that functional peptides in the proteins were changed. Therefore, we cannot absolutely exclude the possibility that the same proteins could have induced different effects on the lipid metabolism if they would have been prepared in another way such as under cooking condition.

4.3 Triglyceride Concentration

Soy protein lowered Triglyceride concentrations in liver, plasma, VLDL, LDL and HDL compared to casein. These results are in agreement with number of previously reported studies. Horigome and Cho (1992) have shown that soybean protein compared with casein results in lower plasma triglyceride levels. Sugiyama et al. (1996) and Terpstra et al. (1983) reported that the hepatic triglyceride concentration of soybean fed rats was significantly lower than that of casein and fish protein fed rats. Lucas et al. (2001) reported that ethanol extracted soy protein isolate lowered serum triglyceride concentration in Golden Syrian hamsters. In our study, SREBP-1c and FAS expressions were down regulated by soy protein compared to casein in experiment 1. Lower activities of FAS and G6DPH observed in experiment 2 show that soy protein lowers triglyceride synthesis. These results confirm the findings from previous studies that have found a decreased expression of SREBP-1, and FAS in rats fed soy protein compared to rats fed casein (Ascencio et al. 2004, Tovar et al. 2002). Ascencio et al. (2004) suggested that soy protein regulates SREBP-1 expression by modulating serum insulin concentration. Iritani et al. (1986) reported that the activity of lipogenic enzymes (G6PDH, malic enzyme, acyl CoA carboxylase, and FAS) in the whole liver of rats fed gluten or soybean protein were reduced to half the levels found in the rats fish protein or casein. It is well established that plasma triglycerides are supplied by the triglyceride-rich lipoproteins VLDL, in the fasted state, and by VLDL and chylomicrons, in the non-fasted state (Tso et al. 1984). Rat used in the first two experiments were food deprived for 12 h but the rats used in third experiment were non-fasted before killing because observations from our laboratory and a study of Shimano et al. (1999) have shown that food deprivation a few hours before killing led to a significant down regulation of genes involved in lipid metabolism. The VLDL

fractions obtained from plasma of our third experiment rats were contaminated with chylomicrons from intestine. In plasma, the concentrations of both apolipoproteins B-100 and the B-48 were reduced in rats fed soy protein compared to rats fed casein. This demonstrates that the soy protein lowered VLDL particle number. Similar findings were reported in rats and mice (Madani et al. 2003, and Nagata et al. 1981) and in soy-treated postmenausal women (Vigna et al. 2000). This might, in part result from diminished VLDL production and in part result from enhanced VLDL uptake. Madani et al. (2003) have shown that lower plasma VLDL particle number and plasma triglyceride concentrations in rats fed soybean protein compared with casein resulted from an enhanced VLDL uptake in the liver. In our study, the lower MTP activity as well as mRNA concentration measured in experiment 1, and lower apo-B protein concentration and mRNA expression measured in experiment 3, indicate for a lower production of VLDL particles. Apo-B is essential for the transport of lipids packaged into VLDL and chylomicrones. MTP function is required for the assembly of Apo-B containing plasma lipoproteins. MTP transports lipid molecules from the endoplasmic reticulum membrane where they are synthesized, to developing lipoprotein particles in the endoplasmic reticulum lumen. The assembly of VLDL particles in the liver is believed to occur in two steps; first lipid is transferred by the MTP to apo-B during translation, and second, the apo-B containing precursor particles fuse with triacylglycerol droplets to form mature VLDL (Shelness and Sellers 2001). Hepatic levels of components such as cholesteryl ester and triacylglycerol also play a role in modulating VLDL formation (Mason 1998). Therefore, we suggest that the effect of soy protein on triglyceride concentration is mediated by a reduced endogen triglyceride synthesis and a diminished apo-B synthesis resulting in a reduced VLDL assembly and secretion.

In experiment 3, triglyceride concentration in the liver, plasma, VLDL, LDL and HDL was significantly lower in the rats fed soy protein isolate, pea protein or lupin protein compared to rats fed casein. These observations are in agreement with Iritani et al. (1985, 1986, 1996), who reported that the hepatic and plasma triglyceride content of the plant protein fed rats were significantly lower compared to animal protein fed rats. They also reported, that in the groups fed gluten and soybean protein compared with the groups fed casein or fish protein, the triglyceride contents were more markedly affected than the lipogenic enzyme activities. Another finding of our study was triacylglycerol-lowering effects of pea and lupin proteins like soy protein. Lupin protein lowered triacylglycerol concentration in plasma and VLDL more than soy protein but the triacylglycerol concentration in the liver were similar.

We suggest that like soy protein pea and lupin protein also lower triglyceride synthesis. But further experiments are required to find out the mechanism.

In both experiments, rats fed fish protein had significantly lower Triglyceride concentrations in plasma, VLDL, LDL and HDL compared to rats fed casein. These results are in agreement with Tsai and Huang (1999) who reported similar serum and VLDL triglyceride concentration in hamsters fed ethanol washed soy protein isolate or fish protein based diets. Recent studies with rats (Murata et al. 2004, Ait Yahita et al. 2005) also found a reduction in plasma and VLDL triacylglycerol concentrations in groups fed fish protein compared with groups fed casein and soybean protein, respectively. The hypotriglyceridemia primarily resulted from reduced amounts of circulating triglyceride carrying lipoproteins ($\delta <$ 1.006kg/L) and confirms other reports on the effect of fish protein in spontaneously hypertensive rats (Ait Yahita et al. 2004), rabbits (Bergeron et al. 1992), and premenopausal women (Gascon et al. 1996). Hypotriglyceridemia could possibly be caused by a diminished synthesis of TG in liver, an increased catabolism of fatty acids or a diminished secretion of triglycerides from liver via VLDL. The measure of SREBP-1c mRNA concentration along with the mRNA concentrations of the SREBP-1c target genes such as FAS, G6PDH and the Delta 6-desaturase and the activity of FAS and cDNA array analysis of acyl CoA carboxylase were not indicative of an inhibition of lipogenesis. But feeding fish protein probably enhanced fatty acid catabolism. As cDNA array results have shown lots of genes involved in fatty acid catabolism were up regulated by fish protein. Carnitine palmitoyl transferase (CPT) I and II, short chain acyl CoA dehydrogenase, medium chain acyl CoA dehydrogenase, and long chain acyl CoA dehydrogenase, which are involved in mitochondrial β oxidation of fatty acid were higher expressed in rats fed fish protein. There results are in agreement with Wergedahl et al. (2004) who reported higher activity of CPT-I in wistar rats fed fish protein. Acyl CoA esters can not cross the mitochondrial membrane, and their entry to the mitochondrion is the major point for control and regulation of the β-oxidation flux (Eaton 2002). Transfer across the mitochondrial membrane is achieved by transference of the acyl group from CoA to carnitine. This is accomplished by CPT-I on the outer membrane and by CPT-II on the inner face of the inner membrane. Twice expression of CPT-I in the liver of rats fed fish protein explains higher fatty acid catabolism in rats fed fish protein. Mitochondrion is the major site of fatty acid oxidation. In spite of mitochondria fatty acids are also oxidized in peroxisomes and microsomes. Higher expression of CYP4A1, CYP4A3, and CYP4A6 indicates higher rate of microsomal ω-hydroxylation. Higher expression of acyl CoA oxidase and 3-ketoacyl thiolase a + b indicates higher peroxisomal β-oxidation of fatty acid. Therefore, we suggest that

hypotriglyceridaemia observed in the fish protein-fed rats could at least be partially the result of increased oxidation of fatty acids. A former study with rabbits has found a higher lipoprotein lipase activity in the animals fed fish protein compared to animals fed soybean protein (Bergeron et al. 1992). Therefore hypotriglyceridaemia observed in the fish protein-fed rats could also be caused by a stimulation of plasma triglyceride clearance.

Another important finding is that most of the genes up-regulated by fish protein feeding like CPT-I, CPT-II, MCAD, Thiolase B, CYP4A1, CYP4A6, CYP7A1 are PPAR alpha target genes. Because PPAR alpha mRNA was not affected by feeding fish protein, the increased expression of PPAR alpha down stream genes was not due to a higher PPAR alpha expression. Hence, the up-regulation of PPAR alpha down stream genes should be the result of the activation of PPAR alpha protein by its agonist. PPARs mediate the effects of small lipophilic compounds such as long chain fatty acids and their derivatives on transcription of target genes. Of the three PPAR types (PPAR-α, PPAR-β and PPAR-γ) known to date, PPAR-α has been best characterized. PPAR-α is mainly expressed in tissues exhibiting high rates of β-oxidation such as liver, heart, kidney and muscle (Desvergne and Wahli 1999). PPAR-α agonists increase the β-oxidation of fatty acids and therefore diminish the pool of fatty acids available for triglyceride synthesis and incorporation into VLDL. PPAR-α is involved in the regulation of peroxisomal β-oxidation (ACO, Thiolase B), mitochondrial ω-hydroxylation (CYP4A1, 4A6-Z), mitochondrial β-oxidation (CPT-I, CPT-II, MCAD), (Mandard et al. 2004, Dreyer et al. 1992).

In experiment 2, most remarkable differences by the animal proteins were observed in their effects on triglycerides. An interesting result of our study was the triglyceride-lowering effect of pork protein compared to casein. The extent of the reduction of the triglyceride concentration was 28% in plasma, 31% in the VLDL and 27% in LDL fraction and 46% in the liver. A previous experiment also found distinct differences between dietary proteins such as egg albumin, casein and wheat gluten on the concentration of triacylglycerols in liver of rats in which egg albumin has hypotriglyceridemic and wheat gluten hypertriglyceridemic action when compared to casein (Sugiyama et al. 1996). Although the authors did not explain these effects, their findings indicate a protein-mediated influence on the metabolism of triglycerides. Concentrations of triglycerides in liver and plasma are mainly dependent on the rate of hepatic lipogenesis (Foufelle et al. 1996). We therefore suggest that pork protein might have reduced the synthesis of triglycerides in the liver when compared to casein. Although we did not measure the mature fraction of SREBP-1c in nucleus or mRNA expression in the liver,

the reduced activities of FAS and G6PDH in the liver suggest diminished lipogenesis via SREBP-1c (Brandsch et al. 2006). This study therefore proposes that pork protein lowered fatty acid synthesis in the liver compared to casein via SREBP-1c mediated pathway.

4.4 Bile acid excretion

The liver centrally regulates whole body cholesterol excretion through the production and secretion of bile (Sautier et al. 1983). Several mechanisms may explain the hypocholesterolemic effect of soy proteins. Enhanced faecal steroid excretion which is the major route for cholesterol excretion may be one mechanism that explains the cholesterol lowering effect of soy protein (review by Potter 1996). In fact, a number of studies have shown that feeding soybean protein diets increases the faecal excretion of both neutral and acidic steroids in rats and rabbits (Potter 1996). Nagaoka et al. (1997) reported that lower serum cholesterol concentrations in rats as a result of soy feeding were associated with increased fecal excretion of total steroids. Wright and Salter (1998) reported an increase in bile acid excretion in hamsters fed intact soy protein compared to animals fed casein. They also reported a significant correlation between soy intake and bile acid excretion. In an earlier study, Nagata et al. (1982) also found increases in faecal steroid excretion in rats with dietary soy protein compared to casein. Sugano et al. 1982 reported an increase in faecal bile acid excretion and a reduction in hepatic cholesterol content with soy consumption compared to dietary casein.

Dietary soy protein enhanced bile acid excretion compared to the casein as we observed in our first two experiments, this may be partially responsible for the lower cholesterol concentration in the liver of rats fed soy protein. In first two experiments the expression of cholesterol 7 α hydroxylase was not different between the two groups. In our third experiment we did not measure the faecal bile acid excretion but the expression of cholesterol 7 α hydroxylase was significantly lower in rats fed soy protein compared to casein. This may be explained by the observation that in postprandial state both synthesis of cholesterol as well as bile acids were reduced by soy protein.

Fish protein also enhanced bile acid excretion compared to casein as we found in our second study. This higher excretion of bile acid was supported by higher expression of cholesterol 7 alpha hydroxylase, which is key enzyme involved in bile acid synthesis. In previous studies feeding fish protein, as compared with casein, was shown to increase faecal excretion of bile acids (Lapre et al. 1989, Iritani et al. 1985).

4.5 Amino Acids Concentrations in Plasma and Dietary Proteins

The difference in amino acid composition of dietary proteins can be reflected in the free amino acid pattern of plasma and some tissues either directly or indirectly. Based on this assumption, the relationship between the concentrations of plasma cholesterol and plasma free amino acids have been studied in humans (Sanchez et al. 1988), minipigs (Hagemeiser et al. 1990), rabbits (Kurowska and Carroll 1995), and rats (Horigome and Cho 1992). Barth et al. (1990) reported in pigs significant differences of postprandial plasma concentrations for 8 amino acids (cysteine, valine, methionine, leucine, tyrosine, lysine, tryptophan, and arginine) depending on whether they consumed a meal containing casein or isolated soy protein. They reported that the postprandial plasma amino acid pattern corresponds to the amino acid composition of the dietary proteins. We also observed that plasma amino acids concentrations of most of the amino acids were influenced by the type of dietary proteins and these observations were prominent in postprandial state (experiment 3). In experiment 3, plasma concentrations of cysteine, tyrosine, arginine, valine, leucine and lysine correspond to the amino acid composition of the dietary proteins.

Studies dealing with the effects of different dietary proteins from plant and animals sources on lipid metabolism suggest that specific amino acids could be responsible for the several effects observed (Kritchevsky et al. 1982, Sugiyama et al. 1986, Morita et al. 1997, Wergedahl et al. 2004). Sautier et al. (1983) reported that there was a significant correlation between the serum cholesterol concentration and the content of tyrosine, glutamic acid, cysteine, or alanine in dietary proteins in rats fed cholesterol-free diets containing one of the four types of proteins. They also reported that the serum cholesterol concentration could be significantly correlated with the content of proline, methionine, or alanine in dietary proteins when eight types of dietary proteins were used (Sautier et al. 1986). It has been suggested that the differences in plasma amino acid composition could affect cholesterol metabolism by altering hormone concentrations (Forsythe et al. 1986).

It has been suggested that amino acids such as methionine, glycine, arginine or lysine could be responsible for the different effects of dietary proteins on the lipid metabolism (Sugiyama et al. 1996, Sugiyama et al. 1997, Giroux et al. 1999, Gudbrandsen et al. 2005). Kurowska and Carroll (1995) reported that lysine and methionine contents of the diets seemed closely correlated with their hypocholesterolemic effect. Methionine was shown to elevate serum cholesterol concentration (Sugiyama et al. 1986). However, methionine

supplementation to a soy protein diet did not abolish the hypocholesterolemic effect of soy protein relative to casein (Kern et al. 2002, our third experiment, Shukla et al. 2006 b), suggesting that some factor other than methionine may be responsible at least in part for the cholesterol lowering effect of soy protein. Sugiyama et al. (1996) reported significantly positive correlation between the plasma cholesterol concentration and the plasma methionine or valine concentration. It was suggested that the higher ratio of methionine to glycine in casein may be responsible for the elevation in serum cholesterol (Morita et al. 1997), and glycine supplementation to a casein based diet lowered the serum cholesterol concentration in rats (Sugiyama et al. 1986).

In the present experiments the glycine and arginine contents of the soy protein diet were higher than that of the casein diet, and methionine and lysine content of the soy protein diet were lower that of the casein diet yielding lower methionine to glycine and lysine to arginine ratios in the soy protein diet. It was suggested that the increased serum cholesterol level that occurs with casein feeding was caused by the high ratio of lysine to arginine and methionine to glycine in casein (Morita et al. 1997, and Kritchevsky and Czarnecki 2000, Wergedahl et al. 2004). The dietary lysine to arginine ratios in the current study were 2.3 in the case of casein and 0.8 in the case of soy protein, and methionine to glycine ratios were 1.5 in the case of casein and 0.4 in the case of soy protein, favouring a cholesterol lowering effect by soy protein. Zhang and Beynen (1993) reported that the concentrations of cysteine and glycine in the diet were negatively associated with plasma and liver cholesterol concentrations. Similarily, significant negative correlations have been reported between serum cholesterol and dietary cysteine or alanine (Sautier et al. 1986, Sugiyama and Muramatsu 1990). In our study, cysteine and alanine concentration of soy protein were higher compared to casein. Thus, the amino acid composition of dietary proteins may be responsible in part for the effect of the protein source on liver cholesterol levels.

It was expected from previous reports that the higher proportion of methionine in casein would contribute to a higher plasma homocysteine, which is a risk factor for the development of atherosclerosis (Nehler et al. 1997, Toborek et al. 1995). Homocysteine is a sulphur-containing amino acid and is believed to act as an activator of SREBP-2 and thereby stimulates the gene expression of HMG-CoA reductase (Woo et al. 2005). Despite a significantly higher plasma concentration of methionine in rats fed casein compared to rats fed soy protein isolate, the homocysteine concentration in plasma did not differ between the two groups. Plasma homocysteine therefore provide no explanation for the observed activation of

SREBP-2. Ni et al. (1998) also observed no differences in serum homocysteine concentration in between soy protein isolate and casein fed apolipoprotein E-deficient mice.

However, it is more likely that soy protein peptides could be responsible for the observed effects. Alpha and alpha′ subunits from 7S soy globulin have been identified as peptides from soy protein that may regulate cholesterol homeostasis in HepG2 cells (Lovati et al. 2000). It seems not very probable that the remaining isoflavones in the ethanol-washed soy protein isolate may contribute to the observations made, because their concentration is extremely low compared to the concentrations normally used for induction of hypolipidemia (e.g. Mullen et al. 2004, Ali et al. 2004). However, it cannot be totally excluded that the remaining isoflavones may, at least in part contribute to the observations made.

When comparing the amino acids found in the dietary proteins extracted from meat with those of casein the greatest differences were observed for alanine, arginine, cysteine, glycine and aspartic acid (higher concentration in the meat proteins than in casein) and phenylalanine, glutamic acid proline, serine and tyrosine (lower concentration in the meat proteins than in casein). The ratios of methionine to glycine and lysine to arginine were also lower in proteins extracted from different meat compared to casein. The marked differences in the amino acid composition of the dietary proteins were not directly reflected in plasma. The most striking difference was observed for taurine, which was lowest in rats fed casein and highest in rats fed pork protein. Previous findings have shown that taurine supplementation reduced the concentration of triacylglycerols in liver and plasma of rats (Yan et al 1993, Park and Lee 1998). Since cysteine is a precursor of taurine, high intakes of cysteine might enhance taurine conjugation and, thus, have cholesterol lowering activity. Nanami et al. (1996) reported a hypocholesterolemic action of taurine supplemented diet in rats fed a high cholesterol diet. Another effect observed in rats fed diets containing proteins of beef, pork and turkey meat was reduction of the plasma homocysteine concentration compared with rats fed diets containing casein or soy protein. Plasma homocysteine concentrations are associated with the risk of cardiovascular and cerebrovascular disorders resulting from atherosclerosis (Clarke et al. 1991, Refsum et al. 1998, Welch et al. 1998). The reduction of plasma homocysteine concentrations by the animal proteins used in this study could therefore be of some physiologic relevance. The mechanisms underlying the homocysteine-lowering effects of these proteins are unknown, and require further investigation.

Fish protein when compared with casein had higher cysteine, aspartic acid, glycine, alanine, arginine content and lower serine, glutamic acid, proline, tyrosine, valine and phenyl

alanine contents. Fish protein had lower ratios of lysine to arginine and methionine to glycine. Fish protein lowered plasma homocysteine concentration. Plasma taurine concentration was significantly higher in rats fed fish protein compared to casein. Taurine is used for bile acid conjugation and may facilitate bile acids excretion in faeces. Moreover, taurine itself may enhance the biotransformation of cholesterol to bile acids, which may be partially responsible for the triacylglycerol lowering effect observed by fish protein feeding.

Pea and lupin proteins used in experiment 3, had higher cysteine, arginine and glycine, concentrations (about twice in the plant proteins than in casein). Valine, lysine and proline concentration of the plant proteins were lower compared to casein. The ratios of lysine to arginine and methionine to glycine were also lower in the plant proteins compared to casein. The plasma concentrations of arginine, asparagines and taurine were higher in the rats fed plant proteins and plasma concentrations of leucine, threonine, valine and tyrosine were lower compared to casein. Therefore, we suggest that dietary amino acids are at least partly involved in the cholesterol and triglyceride lowering effect observed by these proteins but we suggest that methionine might not be involved in the effects of these proteins on lipid metabolism observed in this study because we adjusted methionine concentrations of all plant proteins to a similar level as of casein in third study.

4.6 Final Thoughts:

When discussing the relevance of the effects observed in this rat study for human nutrition, several experimental points must be considered. Although rodents have been often used as an animal model to study the effects of dietary proteins on the metabolism of lipids (Terpstra et al 1982, Madani et al. 1998, Sugiyama et al. 1997, Ascencio et al. 2004, Gudbrandsen et al. 2005), it should be noted that they differ in their lipid metabolism in several regards from humans. For instance, they have generally lower concentrations of lipids (triglycerides, cholesterol) in plasma than humans and they carry most of their cholesterol in HDL which is also in contrast to humans (Jawie et al. 2004). In this study, we used a diet containing 100 g of lard per kg as a fat source rich in saturated fatty acids and supplemented additionally 5 g of cholesterol per kg of diet to mimic western diets of humans. Nevertheless, plasma cholesterol concentrations in the rats were relatively low. Other groups added cholic acid as a hyperlipidemic agent which increases absorption of dietary cholesterol and inhibits hepatic conversion of cholesterol into bile acids and thus results higher lipid concentration in plasma (Madani et al. 1998, Murphy et al. 2005). As dietary proteins can exert different

effects on the cholesterol metabolism in normolipidemic and in hyperlipidemic rats (Madani et al. 1998), it would be interesting to study the effects of dietary proteins in hyperlipidemic models in future studies.

5 Summary and Conclusions

It is well established that dietary proteins influence lipid metabolism. But the number of proteins examined in this connection, is so far very limited. Most of the studies are based on casein as a representative of animal proteins and soy protein as representative of plant proteins. Soy proteins are considered to be hypocholesterolemic in comparison to animal proteins mainly casein.

We performed three experiments using different animal and plant proteins. Growing male Sprague Dawley rats were used as model animal in all the experiments. In each experiment rats were fed for about 3 weeks. The diets varied only in protein source in first two experiments, but in third experiment all the plant protein containing diets (pea, lupin and soy protein) were supplemented with DL-methionine, and lupin protein containing diet was also supplemented with lysine to meet the AIN recommended amount. To mimic western diets, which are commonly rich in saturated fats and cholesterol, we chose lard as type of dietary fat and supplemented the diet with cholesterol (0.5 g/kg diet). As parameters we determined cholesterol and triglyceride concentrations in the liver, plasma and lipoproteins. To investigate the effects of dietary amino acid composition, we analyzed amino acid compositions of dietary proteins and plasma. To find out the mechanism at the genetic level we performed semiquantiative PCR for the selected genes using RNA isolated from liver.

The main aim of our first study was to investigate the effects of soy protein and its amino acids respectively, on lipid metabolism and the mechanism of action at the genetic level. To minimize the interference of other components, we decided to use ethanol washed soy protein. Soy protein isolate compared to casein led to a marked decrease of liver and VLDL cholesterol, liver and plasma triglyceride concentrations and increased bile acid excretion via faeces. Soy protein isolate lowered relative mRNA concentrations of SREBP-2, along with HMG-CoA reductase and LDL receptor, which are SREBP-2 down stream genes and play important role in the cholesterol metabolism. Soy protein also reduced the expression of SREBP-1c along with its target genes FAS and G6PDH compared to casein. The activities of FAS and G6PDH were also lowered by soy protein. This suggests that soy protein lowers fatty acid synthesis in the liver. Gene expression of insig 1 and 2, and were not different between the two groups.

Soy protein reduced MTP activity and mRNA concentration, and lowered Apo-B 100 protein and mRNA concentration thus resulting reduced assembly and secretion of VLDL particles and therefore reduced lipid concentration in plasma. But soy protein had no effect on VLDL particle size as indicated by core surface ratio.

In conclusion, this study suggests that soy protein affects cellular lipid homeostasis by down regulation of SREBP-2 and SREBP-1c and associated sterol-regulatory element regulated genes.

Besides casein, animal proteins such as those from beef, pork, poultry, or fish protein play an important role in human nutrition worldwide. Therefore, we planned a second experiment to investigate the effects of meat proteins isolated from pork, beef, and turkey, and fish protein isolated from Alaska pollack fillets and compared their effects on the lipid metabolism with casein and soy protein isolate. This study shows that proteins from beef, pork and turkey meat have similar effects as casein on the cholesterol metabolism of rats. In this study, most remarkable differences by the animal proteins were observed in their effects on triglycerides. An interesting result of this study was the triglyceride-lowering effect of pork protein compared to casein. Although we did not measure the mature fraction of SREBP-1c in nucleus and mRNA expression, the reduced activities of FAS and G6PDH in the liver suggest diminished lipogenesis via SREBP-1c. Proteins isolated from beef and turkey meats also slightly reduced concentrations of triacylglycerols in liver and plasma compared to casein, although the differences were not significant.

Proteins isolated from fish fillet had effects on cholesterol metabolism. Rats fed fish protein had higher concentrations of cholesteryl esters in liver, higher bile acid excretion via faeces, a lower concentration of cholesterol in the high-density lipoprotein fraction. Moreover fish protein feeding lowered triglyceride concentration in plasma compared to casein. As there is less knowledge available regarding the effects of fish protein on lipid metabolism, and to confirm these observations we performed a third experiment. Besides fish protein, soy protein and casein as a control, we used pea and lupin protein as additional plant proteins. In this experiment we performed additional gene expression analysis with cDNA arrays. Main effects of the fish protein on cholesterol metabolism compared to casein were an increase of liver cholesterol concentration, a reduction of the high-density lipoproteins, along with a stimulated gene expression of SR-B1, SREBP-2 and a slight increase of HMG-CoA synthase and LDL receptor. Both are target genes of SREBP-2 and indicate an activation of this transcription

factor by fish protein. However, despite the observed cholesterol accumulation in livers of rats fed fish protein the concentration of cholesterol in plasma was not increased compared to rats fed casein. The higher gene expression of LDL receptor in livers of rats fed fish protein could have possibly contributed to an increased removal of LDL from the circulation, thereby preventing a distinct LDL accumulation in plasma.

HDL is the principle vehicle for removal of surplus cholesterol from the peripheral tissues for disposal in the liver. Several genes are involved in HDL metabolism Apo-AI, a structural component of HDL, and SR-BI responsible for the selective uptake and unloading of HDL cholesterol in the liver whereas the esterification of cholesterol by LCAT is critical for optimal cholesterol uptake and maturation of HDL. Gene expressions of Apo-AI and LCAT were not different between rats fed casein and fish protein. Although the reduced concentration of HDL cholesterol observed in the fish protein fed rats could possibly be related to an increased expression of SR-BI.

In conclusion, the present findings suggest that the fish protein from Alaska Pollack exerts distinct effects on plasma and liver lipids which were at least in part caused by an altered expression of hepatic genes involved in lipid homeostasis.

Pea and lupin protein lowered cholesterol concentration in the liver and VLDL, and triglyceride in the liver, plasma, VLDL and LDL. But these proteins like soy protein could not affect plasma cholesterol concentrations. We suggest that like soy protein pea and lupin protein also lower cholesterol and triglyceride synthesis in the liver although we did not determine mRNA concentrations or enzyme activities in the liver of rats fed pea or lupin protein. Plasma taurine concentration was significantly higher in the plant protein fed rats compared to casein. Previous findings have shown that taurine supplementation reduced the concentration of triacylglycerols in liver and plasma of rats, but the mechanism is still to be investgated.

Amino acid compositions of the dietary proteins might be in part responsible for lipid lowering effects observed by these proteins. The marked differences in the amino acid composition of the dietary proteins were in cysteine, glycine, arginine, lysine aspartic acid, proline and valine.

Although the results cannot be directly interpolated to human nutrition, some effects of these proteins on the lipid metabolism could also occur in humans.

6. Zusammenfassung und Schlussfolgerungen

Es ist seit längerem bekannt, dass Nahrungsproteine den Lipidmetabolismus beeinflussen. Bisher ist allerdings die Zahl diesbezüglich untersuchter Proteine begrenzt. Die meisten Studien basieren auf Kasein als Vertreter tierischer Proteine und Sojaprotein als Vertreter pflanzlicher Proteine. Für Sojaprotein wurden im Vergleich zu Kasein hypocholesterinämische Wirkungen beschrieben.

Im Rahmen dieser Studie wurden 3 Fütterungsversuche durchgeführt, bei denen unterschiedliche tierische und pflanzliche Proteine eingesetzt wurden. In allen Versuchen wurden wachsende männliche Sprague Dawley-Ratten als Modelltier verwendet. Die Tiere wurden jeweils für ca. 3 Wochen gefüttert. Die Diäten variierten nur in der Proteinquelle; allerdings wurden im dritten Versuch die Diäten, die Erbsen-, Lupinen- oder Sojaprotein enthielten, mit DL-Methionin und die Lupinenproteindiät zusätzlich mit Lysin supplementiert, um den Empfehlungen des AIN zu entsprechen. Um die Diät westlicher Industrieländer, die reich an gesättigten Fettsäuren und Cholesterin ist, zu imitieren, wurde Schweineschmalz als Diätfett gewählt und der Diät Cholesterin (0,5 g/kg) zugesetzt. Als Parameter wurden Cholesterin- und Triglyceridkonzentrationen in Leber, Plasma und Lipoproteinen bestimmt. Um den Effekt der Aminosäurezusammensetzung der Diätproteine zu erforschen, wurde die Aminosäurezusammensetzung von Diätproteinen und Plasma analysiert. Um Mechanismen auf der Ebene der Genexpression aufzuklären, wurden von RNA-Proben der Leber PCR zu ausgewählten Genen durchgeführt.

Das Hauptziel des ersten Versuchs war, die Effekte von Sojaprotein bzw. seinen Aminosäuren auf den Lipidstoffwechsel zu untersuchen und Wirkmechanismen auf der Ebene der Genexpression aufzudecken. Um mögliche Beeinflussungen durch andere Komponenten zu minimieren, wurde Ethanol-gewaschenes Sojaproteinisolat verwendet. Im Vergleich zu Kasein führte Sojaprotein zu einer deutlichen Senkung der Cholesterinkonzentration in Leber und VLDL sowie der Triglyceridkonzentration in Leber und Plasma und zu einem Anstieg der Gallensäureexkretion über Fäces. Sojaprotein führte zu niedrigeren mRNA-Konzentrationen des SREBP-2 sowie der SREBP-2-Zielgene HMG-CoA-Reduktase und LDL-Rezeptor, die beide eine wesentliche Rolle im Cholesterinmetabolismus spielen. Sojaprotein führte außerdem zu einer niedrigeren Expression von SREBP-1c sowie seiner Zielgene FAS und G6PDH. Die Aktivität von FAS und G6PDH wurde ebenfalls durch Sojaprotein gesenkt. Dies lässt vermuten, dass Sojaprotein die Fettsäuresynthese in der Leber senkt. Die Genexpression von Insig 1 und 2 wurde nicht beeinflusst. Sojaprotein reduzierte mRNA-Konzentration und

Aktivität von MTP sowie mRNA- und Proteinkonzentration von ApoB-100, wodurch eine verringerte Bildung und Freisetzung von VLDL-Partikeln resultierte und somit verringerte Plasmalipidkonzentrationen. Sojaprotein hatte keinen Effekt auf die Partikelgröße der VLDL.

Zusammenfassend lässt sich sagen, dass Sojaprotein die zelluläre Lipidhomöostase über eine d*own regulation* von SREBP-2 und SREBP-1c sowie assoziierter Zielgene beeinflusst.

In der menschlichen Ernährung spielen bei den tierischen Proteinen neben dem Kasein vor allem Proteine aus Rind-, Schweine- und Geflügelfleisch sowie Fisch weltweit eine bedeutende Rolle. Deshalb sollten in einem zweiten Versuch Proteine, die aus Rind-, Schweine- und Putenfleisch sowie Filets vom Seelachs isoliert wurden, mit Kasein und Sojaprotein bezüglich ihrer Wirkung auf den Lipidstoffwechsel verglichen werden. Diese Studie zeigte, dass Proteine von Rind-, Schweine- und Putenfleisch ähnliche Wirkungen auf den Cholesterinstoffwechsel der Ratte haben wie Kasein. Die bemerkenswertesten Unterschiede zwischen den tierischen Proteinen zeigten sich bei ihren Wirkungen auf den Triglyceridstoffwechsel. Ein interessantes Ergebnis hierbei war der Triglycerid-senkende Effekt von Proteinen aus Schweinefleisch im Vergleich zu Kasein. Obwohl weder die Konzentration noch die Genexpression des SREBP-1c gemessen wurde, weist die verminderte Aktivität von FAS und G6PDH in der Leber auf eine verminderte Lipogenese hin, vermittelt über SREBP-1c. Proteine aus Rind- und Putenfleisch reduzierten ebenfalls die Triglyceridkonzentrationen in Leber und Plasma im Vergleich zu Kasein, die Unterschiede waren allerdings nicht signifikant.

Proteine, die aus Fischfilets isoliert wurden, beeinflussten den Cholesterinstoffwechsel. Ratten, die Fischproteine erhielten, hatten eine höhere Konzentration an Cholesterinestern in der Leber, eine höhere Gallensäureexkretion über Fäces und eine niedrigere Cholesterinkonzentration in den HDL. Außerdem führte die Fütterung von Fischprotein zu niedrigeren Triglyceridkonzentrationen im Plasma als die Fütterung von Kasein. Da erstens wenig über die Wirkung von Fischproteinen auf den Lipidmetabolismus bekannt ist und zweitens die beobachteten Effekte bestätigt werden sollten, wurde ein dritter Versuch durchgeführt. Neben Fischprotein und den beiden Kontrollen Sojaprotein und Kasein wurden Erbsen- und Lupinenproteine als weitere pflanzliche Proteine eingesetzt. In diesem Versuch wurden zusätzliche Genexpressionsanalysen mittels cDNA-Arrays durchgeführt. Die Haupteffekte des Fischproteins im Vergleich zu Kasein auf den Cholesterinstoffwechsel waren Anstieg der Cholesterinkonzentration in der Leber, Senkung der Cholesterinkonzentration in den HDL sowie stimulierte Genexpression von SR-BI, SREBP-2

und, in geringerem Umfang, von HMG-CoA-Synthase und LDL-Rezeptor, welche Zielgene von SREBP-2 sind und folglich auf eine Aktivierung dieses Transkriptionsfaktors hinweisen. Trotz erhöhter Cholesterinakkumulation in der Leber von Ratten, die Fischprotein erhielten, war die Cholesterinkonzentration im Plasma dieser Tiere nicht höher als bei denen, die Kasein erhielten. Möglicherweise hat die höhere Genexpression des LDL-Rezeptors in der Leber von Ratten der Fischproteingruppe zu einer höheren LDL-Aufnahme aus dem Plasma in die Leber beigetragen und somit eine Akkumulation von LDL im Plasma verhindert.

Die HDL vermitteln prinzipiell den Abtransport von überschüssigem Cholesterin aus den peripheren Geweben zur Leber. In den HDL-Metabolismus sind mehrere Gene involviert: Apo-AI, eine Strukturkomponente der HDL, SR-BI, verantwortlich für selektive Aufnahme von HDL-Cholesterin in die Leber, sowie LCAT, welches für die Veresterung des Cholesterins verantwortlich ist und somit für optimale Cholesterinaufnahme und Reifung der HDL entscheidend. Da die Expression von Apo-AI und LCAT bei den Tieren der Fischprotein- und der Kaseingruppe gleich waren, könnte bei der Fischproteingruppe die höhere Expression von SR-BI für das geringere HDL-Cholesterin verantwortlich sein.

Insgesamt weisen die Ergebnisse auf deutliche Effekte von Seelachsprotein auf Plasma- und Leberlipide hin, welche, zumindest teilweise, durch eine modifizierte Expression von Genen, die für den Lipidstoffwechsel relevant sind, bedingt sind.

Erbsen- und Lupinenprotein senkten die Cholesterinkonzentrationen in Leber und VLDL sowie die Triglyceridkonzentrationen in Leber, Plasma, VLDL und LDL. Wie auch für Sojaprotein festgestellt, konnten diese Proteine nicht die Cholesterinkonzentration im Plasma beeinflussen. Wir vermuten, dass die Wirkungen der beiden Proteine ebenfalls auf eine verminderte Lipidsynthese in der Leber zurückzuführen sind, obwohl weder Expression noch Aktivitäten von relevanten Proteinen gemessen wurde.

Da beschrieben wurde, dass Taurinsupplementierung die Triglyceridkonzentration in Leber und Plasma bei Ratten senkt, könnte auch die erhöhte Plasmataurinkonzentration, die bei den Tieren, die pflanzliche Proteine erhielten, gemessen wurden, zu den niedrigeren Lipidkonzentrationen beigetragen haben. Ebenso könnte die Aminosäurezusammensetzung der Diätproteine für die Effekte auf den Lipidstoffwechsel verantwortlich sein. Deutliche Unterschiede zwischen den einzelnen Proteinen betrafen hierbei Cystein, Glycin, Arginin, Lysin, Methionin, Asparaginsäure, Prolin und Valin.

Obwohl die Ergebnisse nicht direkt auf die menschliche Ernährung übertragen werden können, sind beim Menschen ähnliche Effekte auf den Lipidstoffwechsel zu erwarten.

References:

Acton S., Rigotti A., Landschulz K.T., Xu S., Hobbs H.H., & Krieger M. (1996) Identification of scavanger receptor SR-BI as a high density lioprotein receptor. *Science 271: 518-520.*

Adams M.R, Golden D.L., Anthony M.S, Register T.C., Williams J.K (2002) The inhibitory effect of soy protein isolate on atherosclerosis in mice does not require the presence of LDL receptors or alteration of plasma lipoproteins. *J. Nutr. 132: 43-49*.

Adlercreutz H. (1990). Western dietsand western diseases: Some hormonal and biochemical mechanisms and associations. *Scand. J. Lab. Clin. Invest. 50 (Suppl. 201): 3-23.*

Ait Yahita D., Madani S., Prost E., Bouchenak M. & Belleville J., (2004) Fish protein improves blood pressure but alters HDL2 and HDL 3 composition and tissue lipoprotein lipase activities in spontaneously hypertensive rats. *Eur. J. Nutr. 44, 10-17*.

Ali A.A., Velasquez M.T., Hansen C.T., Mohamed A.I., Bhathena S.J., (2004) Effects of soybean isoflavones, probiotics, and their interactions on lipid metabolism and endocrine system in an animal model of obesity and diabetes. *J. Nutr. Biochem. 15 583-590*.

Anderson J.W., Johnstone B. M., & Cook- Newwell M. E. (1995) Meta analysis of the effects of soy protein intake on serum lipids. *N. Engl. J. Med. 333: 276-282*.

Ascencio C, Torres N, Isoard-Acosta F, Gómez-Pérez FJ, Hernández-Pando R, Tovar AR. (2004) Soy protein affects serum insulin and hepatic SREBP-1 mRNA and reduces fatty liver in rats. *J Nutr 134: 522-529.*

Austin M.A. (1991) Plasma triglyceride and coronary heart disease. *Arterioscler. Thromb. 11: 2-14.*

Austin M.A., Hokanson J.E., and Edwards K.L. (1998) Hypertriglyceridemia as a cardiovascular risk factor. *Am. J. Cardiol . 81(4A): 7B-12B.*

Antony M.S., Clarkson T.B. & Williams J.K. (1998) Effects of soy isoflavones on atherosclerosis: potential mechanisms. *Am. J. Clin. Nutr. 68: 1390S-1393S.*

Anthony M.S., Clarkson T.B., B.C., Wagner J.D. (1997) Soy protein versus soy phytoestrogens in the prevention of diet induced coronary artery atherosclerosis of male cymologous monkeys. *Arterioscler. Thromb. Vasc. Biol. 17: 2524-2531*.

Bakhit R.M., Klein B.P., Essex-Sorlie D., Ham J.O., Erdman J.W., and Potter S.M. (1994) Intake of 25 g soybean protein reduces plasma cholesterol in men with elevated cholesterol concentrations. *J. Nutr. 124: 213-222*.

Balmir F., stack R., Jeffrey E., Berber-Jimenez M.D., Wang L., and Potter S.M. (1996) An extract of soy flour influences serum cholesterol and thyroid hormones in rats and hamsters. *J. Nutr. 126: 3046-3053*.

Barth C.A., Scholz-Ahrens K.E., de Vrese M., and Hotze A. (1990) Differences of plasma amino acids following casein or soy protein intake: significance for differences of serum lipid concentrations.

Bassler and Buchholz H (1993) Methodenbuch Band III. Die chemische Untersuchung von Futtermitteln, *VDLUFA-Verlag, Darmstadt, Germany.*

Bergeron N., Dishies Y., & Jacques H., (1992) Dietary fish protein modulates high density lipoprotein cholesterol and lipoprotein lipase activity in rabbits. *J. Nutr. 122: 1731-1737.*

Bergeron N. and Jacques H. (1989) Influences of fish protein as compared to casein and soyprotein on serumlipids, and serum lipoprotein cholesterol levels in the rabbits. *Atherosclerosis 78: 113-121*.

Bilia A.R., Salvini D., Mazzi G.,. Vincieri F.F, (2001) Characterization of Calendula Flower, Milk-Thistle Fruit, and Passion Flower Tinctures by HPLC-DAD and HPLC-MS. *Chromatographia 53 3-4.*

Bradford M.M. (1976) A rapid and sensitive method for the quantification of microgramquantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem. 72: 255-260.*

Brandsch C., Shukla A., Hirche F., Sangl G.I. & Eder K. (2006) Effects of proteins from beef, pork, and turkey meat on plasma and liver lipids of rats compared with casein and soy protein. *Nutr. 22: 1162-1170.*

Butte W. (1983) Rapid method for the determination of fatty acid profiles from fats and oils using trimethylsulfonium hydroxide for transesterification. *J. Chromatogr. 261: 142-145.*

Carroll K.K. (1991) Review of clinical studies on cholesterol-lowering response to soyprotein. *J. Am. Dietic assoc. 91: 820-827.*

Carroll K.K., Kurowska E.M. (1995) Soy consumption and cholesterol reduction: review of animal and human studies. *J. Nutr. 125: 594S-597S.*

Chirgwin J.M., Przybyla A.E., MacDonald R.J., Rutter W.J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochem. 18: 5294-5299.*

Christiansen E.N., Lund J.S., Rorveit T., Rustan A.C. (1991) Effect of dietary n-3 and n-6 fatty acids on fatty acid desaturation in rat liver. *J boil. Chem. 274: 471-477.*

Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham J. (1991) Hyperhomocysteinemia: an independent risk factor for vascular disease. N Engl J Med 324: 1149-55.

Claudia T.P., Susan M. P., Julie C. H. and Niel F. S. (1998) Intake of Soy protein and Soy protein Extracts Influences Lipid Metabolism and Hepatic Gene Expression in Gerbils. *J. Nutr. 128: 839-842.*

Damasceno N.R., Gidlund M.A., Goto H., Dias C.T., Okawabata F.S., Abdalla D.S. (2001) Casein and soy protein in experimental atherosclerosis: influence on hypercholesterolemia and lipoprptein oxidation. *Ann. Nutr. Metab 45: 38-46.*

De Hoff J.L., Davidson J.H. & Kritchevsky D. (1978) An enzymatic assay for determining free and total cholesterol in tissues. *Clin. Chem. 24: 433-435.*

Desvergne B, Wahli W. (1999) Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev. 20: 649-688*.

Deutsch J. (1995). Glucose-6-phosphate dehydrogenase, In Bergmeyer H.U. (ed) Methods of enzymatic analysis 3: 190-197, *verlag chemie, VCH, Weinheim.*

Dreyer C., Krey G., Mahfoudi A., Keler H., Givel F., Helftenbein G. and Wahli W. (1992) Control of the peroxisomal beta-oxidation pathway by a novel family of nuclear hormone receptors. *Cell. 61: 879-887.*

Eaton S. (2002) Control of mitochondrial β-oxidation flux. *Prog. Lipid. Res. 41: 197-239.*

Eder K. and Brandsch C. (2002) Effect of fatty acid composition of rapseed oil on plasma lipids, fatty acid composition of tissues and susceptibility of low-density lipoprotein to lipid peroxidation in cholesterol-fed hamsters. *Eur. J. Lipid SCi. Technol. 104: 3-13.*

Eder K.and Kirchgessner M. (1994) J. Trace Elem. Electrol. Health Dis. 8: 63.

Ericsson C.G., Hamsten A., Nilsson J., Grip L., Svane B., and de Faire U. (1996) Angiographic assessment of effects of bezafibrate on progression of coronary artery disease in young male postinfarction patients. *Lancet 347: 849-853*.

Field F.J., Albright E. & Mathur S.N. (1987) Regulation of cholesterol esterification by miceller cholesterol in CaCo-2 cells. *J. Lipid Res. 28: 1057-1066.*

Forsythe W.A (1995) Soy protein, thyroid regulation and cholesterol metabolism. *J. Nutr. 116: 1165-1171.*

Forsythe W.A., (1986) Comparison of dietary casein or soy protein effects on plasma lipids and hormone concentrations in the gerbil (Meriones unguiculatus). *J. Nutr. 116 1165-1171.*

Foufelle F, Girard J, Ferre P. (1996) Regulation of lipogenic enzyme expression by glucose in liver and adipose tissue: a review of the potential cellular and molecular mechanisms. *Adv Enzyme Regul 36: 199-226.*

Fukui K., Tachibana N., Fukuda Y., Takamatsu K., Sugano M., (2004) Ethanol washing does not attenuate the hypocholesterolemic potential of soy protein. *Nutrition 29: 984-990*.

Gardner C.D., Newell K.A., Cherin R., Haskell W.L. (2001) The effects of soy protein with or without isoflavones relative to milk protein on plasma lipids in hypercholesterolemic postmenausal women. *Am. J. Clin. Nutr. 73: 667-678*

Garg M.L, Thomson A.B., Clandinin M.T. (1998) Effect of dietary cholesterol and omega 3 fatty acids on lipid composition and deta 5-desaturase activity of liver microsomes. *J. Nutr. 118: 661-668*

Gascon A., Jacques H., Moorjani S., Deshaies Y., Brun LD & Julien P. (1996) Plasma lipoprotein profile and lipolytic activities in response to the substitution of lean white fish for other animal protein sources in premenausal women. *Am. J. Nutr. 63: 315-321.*

Genest J. Jr. Marcil M., Denis M., & Yu L. (1999) High density lipoprotein in health and disease. *J. Invest. Med. 47: 31-42.*

Giroux I., Kurowska E.M., Freeman D.J., Carroll K.K., (1999) Addition of arginine but not glycine to lysine plus methionine-enriched diets modulates serum cholesterol and liver phospholipid in rabbits. *J. Nutr. 129 1807-1813.*

Giudetti A.M., Beynen A.C. Lemmens A.G., Gnoni G.V. & Geelen M.J.H. (2003) Hepatic fatty acid metabolism in rats fed diets with different contents of C18:0, C18:1 cis and C18:1 trans isomers. *Br. J. Nutr. 90: 887-893*.

Glass C. K. and Witztum J. L. (2001) "Atherosclerosis: the road ahead*." Cell 104(4): 503-16.*

Grudy S. M. (1994) Second Report of the National Cholesterol Education Program (NCEP) expert panal on detection, evaluation and treatment of high blood cholesterol in adults (Adults treatment panal II). *Circulation 89: 1329-1445.*

Grundy S.M. (1996) Lipids, nutrition, and coronary heart disease. In *Atherosclerosis and Coronary Artery Disease*. *(Fuster, V., Ross, R., and Topol, E.J. eds) V.1: 45-48, Lippincott-Raven Publishers, Philadelphia.*

Grundy S.M., Cleeman J.I., Rifkind B.M., and Kuller L.H. (1999) Cholesterol lowering in the elderly population. *Arch. Intern. Med. 159: 1670-1678.*

Gudbrandsen O.A., Wergedahl H., Liaset B., Espe M., Berge R.K. (2005) Dietary proteins with isoflavone content or low methionine-glycine and lysine-arginine ratios are hypocholesterolemic and lower the plasma homocysteine level in male zucker fa/fa rats. *Br. J. Nutr. 94: 321-326*.

Hagemeiser H., Scholz-Ahrens K.E., Schulte-Coerne H., and Barth C.A. (1990). Plasma amino acids and cholesterol following consumption of dietary casein or soy protein in minipigs. *J. Nutr. 120: 1305-1311.*

Hara A. and Radin N.S. (1978) Lipid extraction of tissues with a low toxicity solvent. *Anal Biochem 90: 420-426.*

Hojnacki J.L., Nicolosi R.J. & Hayes K.C. (1976) Densitometric quantification of neutral lipids on ammonium sulphate impregnated thin layer chromatograms. *J. Chromatogr. 128: 133-139.*

Hokenson J.E. & Austin M.A. (1996) Plasma triglyceride level as a risk factor for cardiovascular disease independent of High-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J. Cardiovasc. Risk 3: 213-219*

Horigome T. and Cho Y.S. (1992). Dietary casein and soy bean protein affect the concentration of serum cholesterol, triglyceride and free amino acids in rats. *J. Nutr. 122: 2273-2282.*

Hortan J.D., Bashmakov Y., Shimomura I. & Shimano H. (1998) Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. *Proc. Natl. Acad. Sci. USA 95: 5987-5992.*

Horton J. D., Goldstein J. L. & Brown M. S. (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest. 109: 1125-1131.*

Hua X., Sakai J., Ho Y.K., Goldstein J.L., Brown M.S. (1995). *J. Bio. Chem. 270: 29422-29427.*

Hua X., Yokoyama C., Wu J., Briggs M.R., Admon A., Goldstein J.L., Brown M.S., (1993) SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. *Proc. Natl. Acad. Sci U.S.A. 90 11603-11607.*

Iritani N., Narita R., Fugita T. and Tanaka T. (1985) Effects of fish protein, soybean protein and casein on cholesterol turnover in rats. *J. Nutr. Sci. Vitaminol. 31: 385-392.*

Iritani N, Nagashima K, Fukuda H, Katsurada A., and Tanaka T., (1986) Effects of Dietary Proteins on Lipogenic enzymes in Rat Liver. *J. Nutr. 116: 190-197.*

Iritani N., Hosomi H., Fukuda H., and Tada K., (1996) Soybean Protein Suppresses Hepatic Lipogenic Enzyme Gene Expression in Wister Rats. *J. Nutr 126: 380-388.*

Jacques H., Gascon Bergeron N., Lavigne C., Hurley C., Deshaies Y., Moorjani S. and Julien P. (1995) Role of dietary fish protein in the regulation of plasma lipids. *Can. J. Cardiol. 11 (suppl G), 63G-71G.*

Jawie J, Nastalek P, Korbut R. (2004) Mouse models of experimental atherosclerosis. *J Physiol Pharmacol 55:503-17.*

Jenkins D.J, Kendall C.W., Jackson C.J., Connelly P.W., Parker T., Faulkner D, Vidgen E., Cunnane S.C., Leiter L.A., Josse R.G., (2002) Effects of high- and low-isoflavones soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. *Am. J. Clin. Nutr. 76: 365-372*.

Karpe F., Hamsten A., (1994) Determination of apolipoproteins B-48 and B-100 in triglyceride-rich lipoproteins by analytical SDS PAGE. *J. Lipid Res. 35: 1311-1317*.

Kern M., Ellison D., Marroquin Y., Ambrose M. & Mosier (2002) Effects of soy protein supplemented with methionine on blood lipids and adiposity of rats *Nutr. 18: 654-656.*

Kirk E.A., Sutherland P., Wang S.A., Chait A., and Le Boeuf R.C. (1998) Dietary isoflavones reduce plasma cholesterol and atherosclerosis in C 57BL/6 mice but not LDL receptor-deficient mice. *J. Nutr.128: 954-959*

Kjeldahl J., 1883, A new method for the determination of nitrogen in organic substances. *Ztschr. anal. Chem., 22, 366*,

Krauss R.M. (1998) Atherogenicity of triglyceride-rich lipoproteins. *Am. J. Cardiol. 81(4A): 13B-17B.*

Kris-Etherton P.M., Etherton T.D., and Yu S. (1997) Efficacy of multiple dietary therapies in reducing cardiovascular disease risk factors. *Am. J. Clin. Nutr. 65: 560-561*.

Kritchevsky D. and Czarnecki S.K. (2000) Influence of specific amino acids on experimental atherosclerosis in rabbits. *J. Nutr. 130: (Suppl.): 673S-4S*.

Kritchevsky D., Tepper S.A., Czarnecki S.K., Klurfeld D.M., (1982) Atherogenicity of animal and vegetable protein. Influence of the lysine to arginine ratio. *Atherosclerosis 41 429-431.*

Kurowska E.M., and Carroll K.K., (1991). Studies on the mechanism of induction of hypercholesterolemia in rabbits by high dietary levels of amino acids. *J. Nutr. Biochem. 2: 656-662*

Lapre J.A., West C.E., Lovati M.R., Sirtori C.R., Beynen A.C. (1989) Dietary animal proteins and cholesterol metabolism in rats. *Int J Vitam Nutr Res.59: 93-100.*

Lichtenstein A.H. (1998) Soy Protein, Isoflavones and Cardiovascular Disease Risk. *J. Nutr. 128: 1589-1592*

Lichtenstein A.H., Jalbert S.M., Adlercreutz H., Goldin B.R., Rasmussen H., Schaefer E.J. & Ausman L.M. (2002) Lipoprotein response to diets high in soy or animal protein with and without isoflavones in moderately hypercholesterolemic subjects. *Arterioscler. Thromb. Vasc. Biol. 22: 1852-1858.*

Li Z., Lamon-Fava S., Otvos J., Lichtenstein A. H., Velez-Carrasco W., McNamara J. R., Ordovas J.M., and Scaefer E.J. (2004) Fish consumption shifts lipoprotein subfractions to a less atherogenic Pattern in humans. *J.Nutr. 134: 1724-1728.*

Lovati M.R., Manzoni C., Agostinelli P., Ciappellano S., Mannucci L., Sirtori C.R., (1991) Studies on the mechanism of the cholesterol lowering activity of soy proteins. *Nutr. Metab. Cardiovasc. Dis. 1 18-24.*

Lovati M.R., Manzoni C., Canavesi A., Sirtori M., Vaccarino V., Marchi M., Gaddi G., Sirtori C.R., (1987) Soybean protein diet increases low density lipoprotein receptor activity in mononuclear cells from hypercholesterolemic patients. *J. Clin. Invest. 80 1498-1502.*

Lovati M.R., Manzoni C., Corsini A., Granata A., Frattini R., Fumagalli R., Sirtori C.R., (1992) Low density lipoprotein receptor activity is modulated by soybean globulins in cell culture. *J. Nutr. 122 1971-1978.*

Lovati M.R., Manzoni C., Corsini A., Granata A., Fumagalli R., Sirtori C.R., (1996) 7S globulin from soybean is metabolized in human cell cultures by a specific uptake and degradation system. *J. Nutr. 126 2831-2842.*

Lovati M.R., Manzoni C., Gianazza E., Arnoldi A., Kuroswka E., Carroll K.K., Sirtori C.R. (2000) Soy protein peptides regulate cholesterol homeostasis in Hep G2 cells. *J. Nutr. 130 2543-2549.*

Lovati M.R., Manzoni C., Gianazza E., Sirtori C.R., (1998) Soybean protein products as regulators of liver low-density lipoprotein receptors. I. Identification of active β--conglycinin subunits. *J. Agric. Food Chem. 46 2474-2480.*

Lucas E.A., Khalil D.A., Daggy B.P., and Arjmandi B.H. (2001) Ethaol-extracted soy protein isolate does not modulate serum cholesterol in golden Syrian hamsters: a modl of postmenopausal hypercholesterolemia. *J.Nutr. 131 (2): 211-4*

Luskey K.L. (1988) Regulation of cholesterol synthesis: mechanism for control of HMG CoA reductase. *Recent Prog. Horm. Res. 44: 35-51.*

Madani S., Lopez S., Blond J.P., Prost J., Belleville J. (1998) Highly purified soy protein is not hypocholesterolemic in rats but stimulates cholesterol synthesis and reduces polyunsaturated fatty acid biosynthesis. *J. Nutr. 128: 1084-1091.*

Madani S., Prost J., Norce M., & Belleville J. (2003) VLDL metabolism in rats is affected by the concentration and source of dietary protein *J. Nutr. 133: 4102-4106.*

Mandard S., Müller M., and Kersten S. (2004) Peroxisome proliferator-activated receptor α target genes. *CMLS, Cell. Mol. Life Sci. 61: 393-416*.

Manzoni C., Lovati M.R., Gianazza E., Sirtori C.R., (1998) Soybean protein products as regulators of liver low-density lipoprotein receptors. II. α-α' Rich commercial soy concentrate and α' deficient mutant differently affect low-density lipoprotein receptor activation. *J. Agric. Food Chem. 46 2481-2484.*

Marlett J.A. & Fisher M.H. (2002) A poorly fermented gel from psyllium seed husk increases excreta moisture and bile acid excretion in rats. *J. Nutr. 132: 2638-2643*.

Mason T.M. (1998) The role of factors that regulate the synthesis and secretion of very low density lipoprotein by hepatocytes. *Crit. Rev. Clin. Lab. Sci. 35: 461-487*

Meddings J., Spady D., Dietschy J., (1986) Kinetic constants for receptor-dependent and receptor-independent low density lipoprotein transport in the tissues of the rat and hamster. *J. Clin. Invest. 77: 1474-1481.*

Morita T., Oh-hasi A., Takai K., Ikai M., Kasaoka S., and Kiriyama S. (1997) Cholesterol-lowering effects of soybean, potato and rice protein depend on their low methionine contents in rats fed a cholesterol-free purified diet. *J.Nutr. 127: 470-477.*

Mullen E., Brown R.M., Osborne T.F., Shay, (2004) Soy isoflavones affect sterol regulatory element binding proteins (SREBPs) and SREBP-regulated genes in HepG2 cells. *J. Nutr. 134: 2942-2947.*

Murphy C, Parini P, Wang J, Björkhem I, Eggertsen G, Gäfvels M. (2005)Cholic acid as a key regulator of cholesterol synthesis, intestinal absorption and hepatic storage in mice. *Biochim Biophy Acta 1735: 167-75.*

Nagata Y., Ishiwaki, N. & Sugano M. (1982) Studies on the mechanism of antihypercholesterolemic action of soy protein and soy protein type amino acid mixtures in relation to the casein counterparts in rats. *J. Nutr. 112: 1614-1625*.

Nagata Y., Tanaka K., Sugano M. (1981) Serum and liver cholesterol levels of rats and mice fed soy-bean protein or casein. *J.Nutr. Sci. Vitaminol (Tokyo) 27: 583-93.*

Nagaoka S., Miwa K., Eto M., Kuzuya Y., Hori G & Yamamoto K (1999) Soy protein peptic hydrolysate with bound phospholipids decreases miceller solubility and cholesterol absorption in rats and caco cells. J. Nutr. 112: 1614-1625.

Nanami K., Oda H., & Yokogoshi H. (1996) Antihypercholesterolemic action of taurine on streptozotocin-diabetic trats or on rats fed a high cholesterol diet. *Adv. Exp. Med. Biol. 403: 561-568.*

Naumann C. and Bassler R. (1976) Methodenbuch Band III. Die chemische Untersuchung von Futtermitteln, *VDLUFA-Verlag, Darmstadt, Germany,1993.*

Nehler M.R. Taylor L.M. & Porter J.M. (1997) Homocysteine as a risk factor for atherosclerosis: a review *Cardiovasc. Pathol. 6: 1-9*

Nepokroef C.M., Lakshmanan M.R., Porter J.W. (1975). Fatty acid synthase from rat liver. *Methods Enzymol. 35: 37-44.*

Ni W, Tsuda Z., Sakono M. and Imaizumi K (1998) Dietary soy protein isolate, compared with casein, reduces atheroscerotic lesion area in Apolipoprotein E- deficient mice. *J. Nutr. 128: 1884-1889.*

Ni W., Yoshida S., Tsuda.,Y., Nagao K., Sato M & Imaizumi K. (1999) Ethanol extracted soy protein isolate results in elevation of sreum cholesterol in exogenously hypercholesterolemic rats. *Lipids 34: 713-716.*

Park T. and Lee K. (1998) Dietary taurine supplementation reduces plasma and liver cholesterol and triglyceride levels in rats fed a high-cholesterol or a cholesterol-free diet. *Adv. Exp. Med. Biol. 442:.319-25.*

Peluso M.R., Winters T.A., Shanahan M.F., Banz W.J. (2000) A cooperative interaction between soy protein and its isoflavone-enriched fraction lowers hepatic lipid in male obese Zucker rats and reduces blood platelet sensitivity in male Sprague-Dawley rats. *J Nutr 130: 2333-42.*

Pfeuffer M. and Barth C.A. (1986) Modulation of very low density lipoprotein secretion by dietary protein is age dependent in rats. *Ann. Nutr. Metab. 30: 281-288.*

Potter S. M. (1995) Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *J. Nutr. 125: 606S-611S*

Potter S.M. (1996) Soy protein and serum Lipids. *Curr Opin Lipidol.7: 260-264.*

Potter S.M., Baum J.A., Teng H., Stillman R.J., Shay N.F., and Erdman J.W. Jr. (1998) Soy protein and isoflavones: their effects on blood lipids and bone density in postmenopausal women. *Am. J. Clin. Nutr. 68 (Suppl): 1375S-1379S.*

Rawson R.B. (2003) The SEBP pathway-Insights from insig and insects. *nature 4: 631-640*

Reeves P.G, Nielsen F.H. and Fahey G.C. Jr. (1993) AIN-93 purified diets for laboratory rodents: Final report of the American Institute of Nutrition ad hoc Writing Committee on the reformulation of the AIN-76A rodent diet. *J Nutr 123: 1939-1951.*

Refsum H, Ueland PM, Nygård O, Vollset SE. (1998) Homocysteine and cardiovascular disease. *Annu Rev Med 49: 31-62.*

Ringseis R. and Kder K. (2005) Effects of dietary fat and oxidized cholesterol on gene expression in rat liver as assessed by cDNA array analysis. *Eur.J. Nutr. 44: 231-241.*

Sanchez A., Hubbard R.W., Smit E., and Hilton G.F., (1988). Testing a mechanism of control in human cholesterol metabolism: Relation of arginine and glycine to insulin and glucagons. *Atherosclerosis 71: 87-92.*

Sautier C., Dieng K., Flament C., Doucet C., Suquet J.P., Lemonnier D. (1983) Effects of whey protein, casein, soya-bean and sunflower proteins on the serum , tissue and faecal steroids in rats. *J. Nutr. 49 (3): 313-319.*

Sautier C., Flament C., Doucet C., Suquet J. P., (1986) Effect of eight dietary proteins and their amino acid content on serum, hepatic and fecal steroids in rats. *Nutr. Rep Int, 34: 1051-1061.*

Schuster R. (1988) Determination of amino acids in biological, pharmaceutical, plant and food samples by automated pre-column derivatization and high-performance liquid chromatography. *J. Chromatogr. 431: 271-284*.

Schaefer E.J., Lichtenstein A.H., Lamon-Fava S., Contois J.H., Li Z., Rossmussen H., McNamara J.R. & Ordovas J.M. (1995) Effect of National Cholesterol Education Program Step 2 diets in normolipidemic and hyperlipidemic middle aged and elderly men and women. *Arterioscler. Throm. Vasc. Biol.15: 1079-1085.*

Schaefer E.J., Lichtenstein A.H., Lamon-Fava S., Contois J.H., Li Z., Goldin B. R., Rossmussen H., McNamara J.R. & Ordovas J.M. (1996) Effect of National Cholesterol Education Program Step 2 diets relatively high or relatively low in fish-derived fatty acids on plasma lipoproteins in middle aged and elderly subjects. *Am. J. Clin. Nutr. 63: 234-241.*

Semenkovich C.F. (1997) Regulation of fatty acid synthase (FAS). *Prog. Lipid Res. 36: 43-53.*

Shelness G.S., Seller J.A (2001) very low density lipoprotein assembly and secretion. *Curr. Opin. Lipidol. 12: 151-157.*

Shimano H., Yahagik N., Amemiya-Kudo M., Hasty A.H., Osuga J., Tamura Y., Shionoiri F., Lizuka Y., Ohashi K., Harada K., Gotoda T., Ishibashi S. & Yamada N. (1999) Sterol regulatory element binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J. Biol. Chem. 274: 35832-35839*.

Shimomura I., Shimano H., Horton J.D., Goldstein J. L. & Brown M.S. (1997). *J. Clin. Invest. 99: 838-845.*

Shukla A., Bettzieche A., Hirche F., Brandsch C., Stangl G.I., Eder K. (2006 a) Dietary fish protein alters blood lipid concentrations and hepatic genes involved in cholesterol homeostasis in the rat model. *Br. J. Nutr. 96: 674-682.*

Shukla A., Brandsch C., Bettzieche A., Hirche F., Stangl G.I., Eder K. (2006 b) Isoflavone-poor soy protein alters lipid metabolism of rats by SREBP-mediated down regulation of hepatic genes. *J. Nutr. Biochem. (published online).*

Sirtori C.R., Galli G., Lovati M.R., Carrara P., Bosisio E., Kienle M.G., (1984) Effects of dietary proteins on the regulation of liver lipoprotein receptors in rats. *J. Nutr. 114: 1493-1502.*

Sirtori, C.R., Lovati, M.R. (2001) Soy Proteins and Cardiovascular Disease. *Curr Atherosaler Rep. 3: 47-53.*

Sirtori C.R., Lovati M.R., Castiglioni S., Duranti M., Magni C., Moranti S., D' Agostina A. & Arnoldi A. (2004) proteins of white lupin seed, a naturally isoflavone-poor legume, reduced cholesterolemia in rats and increase LDL receptor activity in HepG2 cells. *J. Nutr. 134: 18-23.*

Sirtori C.R., Lovati M.R., Manzoni C., Gianazza E., Bondioli A., Staels B., Auwerx J. (1998) Reduction of serum cholesterol by soybean proteins: clinical experience and potential molecular mechanisms*. Nutr Metab Cardiovasc Dis 8: 334-40*.

Sparks J. D., Phu1ng T.L., Bolognino M., Cianci J., Khurana R., Peterson R.G., Sowen M.P., Corsetti J.P. and Sparks C.E. (1998) Lipoproteins alterations in 10- and 20-weeks old Zucker diabetic fatty rats: hyperinsulinemic versus insulenopenic hyperglycemia. *Metabolism 47: 1315-1324.*

Stamler J., Wentworth D., and Neaton J.D. (1986) Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? indings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT*). J. Am. Med. Assoc. 256: 2823-2828.*

Sugano M., Tanaka K. and Idle T. (1982) Secretion of cholesterol, triglyceride and apolipoprotein A-I by isolated perfused liver from rats fed soybean protein and casein on their amino acid mixtures. *J. Nutr. 112: 855-862.*

Sugiyama K, Kanamori H., Akachi T, and Yamakawa A., (1996). Amino acid composition of dietary proteins affects plasma cholesterol concentration through alteration of hepatic phospholipids metabolism in rats fed a cholesterol free diet. *J. Nutr. Biochem. 7: 40-48*

Sugiyama K. and Muramatsu K. (1990) Significance of amino acid composition of dietary protein in regulation of plasma cholesterol. *J. Nutr. Sci. Vitaminol. 36: 105S-110S.*

Sugiyama K., Ohkawa S. & Muramatsu K. (1986) Relationship between amino acid composition of diet and plasma cholesterol level in growing rats fed a high cholesterol diet. *J Nutr Sci Vitaminol 32: 413-423.*

Sugiyama K., Yamakawa A.A., Kumazawa A., Saeki S. (1997) Methionine content of dietary proteins affects the molecular species composition of plasma phosphatidylcholine in rats fed a cholesterol-free diet. *J. Nutr. 127: 600-607.*

Sülzle A., Hirrche F., and Eder K., (2004) Thermally oxidized fat upregulates the expression of target genes of PPARα in rat liver. *J. Nutr. 134: 1375-1383.*

Tachibana N., Matsumoto I., Fukul K., Arai S., Kato H., Abe K. & Takamatsu K. (2005) Intake of soy protein isolate alters hepatic gene expression in rats. *J. Agric. Food Chem. 53: 4253-4257.*

Terpstra AHM, van Tintelen G., West C. (1982) The effect of semipurified diet containing different proportions of either casein or soybean protein on the concentration of cholesterol on whole serum, serum lipoproteins and liver in male and female rats. *Atherosclerosis 42: 85-92.*

Terpstra AHM, van Tintelen G., West C. (1983) Serum lipid lipoprotein composition and liver cholesterol in genetically obese zucker rat fed semipurified diets containing either casein or soy protein. *Ann. Nutr. Metab. 27: 132-136.*

Toborek M., Kopieczna-Gzebieniak E., Drozdz M. & Wieczorek M (1995) Increased lipid peroxidation as a mechanism of methionine- induced atherosclerosis in rabbits. *Atherosclerosis 115: 217-224.*

Tovar A., Manzano N., Torres N. (2005) Metabolism of cholesterol and fatty acids in nephrotic syndrome and its regulation by sterol regulatory element binding protein (SREBP's). Effect of soy protein consumption. *Gac Med Mex 141: 407-415.*

Tover A.R, Murguia F., Cruz C., et al. (2002) A Soy Protein Diet Alters Hepatic Lipid Metabolism, Gene Expression and Reduces Serum Lipids and renal Fibogenic Cytokines in Rats with Chronic Nephrotic Syndrome. *J. Nutr. 132: 2562-2569*.

Tsai Po-Jung & Huang Po-Chao (1999) Effects of isoflavones containing soyprotein isolate compared with fish protein on serum lipids and susceptibility of low density lipoprotein and liver lipids to in vitro oxidation in hamsters. *J. Nutr. Biochem. 10: 631-637.*

Tso P., Drake D.S., Black D.D. & Sabesin S.M. (1984) Evidence for separate pathways of chylomicron and very low-density lipoprotein assembly and transport by rat small intestine. *Am. J. Physiol. 247: G599-G610.*

Vallett S.M., Sanchez H.B., Rosenfeld J.M. & Osborne T.F. (1996) A direct role of sterol regulatory element binding protein in activation of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene. *J Biol Chem 271: 12247-12253.*

Van Raaij J.M.A., Katan M.B., West C.E., Hautvast J.G.A.J., and Hermus R.J.J. (1981) Effects of casein versus soy protein diets on serum cholesterol and lipoproteins in young healthy volunteers. *Am. J Clin Nutr. 34: 1261-1271*.

Van Raaij J.M.A., Katan M.B., West C.E., Hautvast J.G.A.J. (1982) Influence of diets containing casein, soy protein isolate, and soy protein concentrate on serum cholesterol and lipoproteins in middle- aged volunteers. *Am. J. Clin. Nutr. 35: 925-934.*

Vigna G.B., Pansini F., Bonaccorsi G., Albertazzi P., Donega P., Zanotti L., De Aloysio D., Mollica G., & Fellin R. (2000) Plasma lipoproteins in soy-treated postmenausal women: a double blind, placebo-controlled trial. *Nutr. Metab. Cardiovasc. Dis. 10: 315-322.*

Vlahecevic Z.R., Pandak W.M. & Stravitz R.T. (1999) Regulation of bile acid biosynthesis. *Gastroenterol Clin North Am 28: 1-25*.

Wang W.W., Smith E. O'Brian, Stuff J.E., Hachey D.L., Heird W.C. and Pownell H.J. (1998) Cholesterol lowering effect of soy protein in normocholesterolemic and hypercholesterolemic men. *Am. J. Clin. Nutr. 68 (suppl), 1385S-1389S.*

Welch G.N. and Loscalzo J. (1998) Homocysteine and atherothrombosis. *N Engl J Med 338: 1042-50.*

Wergedahl H., Liaset B., Gudbrandsen O.A., Lied E., Espe M., Muna Z., Mork S., & Berge R.K., (2004) Fish protein hydrolysate reduces plasma total cholesterol, increases the proportion od HDL cholesterol, and lowers acyl-CoA acyltransferase activity in liver of zucker rats. *J.Nutr. 134: 1320-1327.*

Woo CWH, Siow Y.L., Pierce G.N., Choy P.C., Minuk G.Y., Mymin D. & Karmin O. (2005) Hyperhomocysteinemia induces hepatic cholesterol biosynthesis and lipid accumulation via activation of transcription factors. *Am. J. Physiol. Endocrinol. Metab. 288, E1002-E1010.*

Wright S.M., Salter A.M. (1998) Effect of soy protein on plasma cholesterol and bile acid excretion in hamsters. Comp. Biochem. Physiol. *B. Biochem. Mol. Biol 119: 247-254.*

Yabe D., Brown M.S. & Goldstein J.L. (2002) Insig-2, a second endoplasmatic reticulum protein that binds SCAP and blocks export of sterol regulatory element-binding proteins. *Proc. Natl. Acad. Sci. U.S.A. 99: 12753-12758.*

Yan C.C., Bravo E., Cantafora A. (1993) Effect of taurine levels on liver lipid metabolism: an in vivo study in the rat. *Proc. Soc. Exp. Biol. Med. 202: 88-96.*

Yang T., Espenshade P.J., Wright M.E., Yabe D., Gong Y., Aebersold R., Goldstein J.L. & Brown M.S. (2002) Crucial Step in Cholesterol Homeostasis: Sterols promote Binding of SCAP to INSIG-1, a Membrane Protein that Facilitates Retention of SREBPs in ER. *Cell 110: 489-500.*

Zhang X. & Beynen A.C. (1993) Influence of dietary fish proteins on plasma and liver cholesterol concentrations in rats. *Br J Nutr 69: 767-777.*

Lebenslauf

Angaben zur Person

Name:	Shukla
Vorname:	Anjali
Geburtsname:	Bajpai
Geburtsdatum:	01.07.1977
Natioalität:	Indisch
Familienstand:	Verheiratet
Adresse:	Vogelpothsweg 34, 44149 Dortmund, Germany

Schulischer Bildungsgang

1982-1989	Allgemeinbildende Schule
1989-1991	Gymnasium
1991-1993	Abitur

Universitäter Bildungsgang

1993-1996	Bachelor of Science (B.Sc.), Kurse in Zoologie, Botanik und Chemie an der Lucknow Universität, Indien
1996-1998	Master of Science (M.Sc.), Kurse in Pflanzenwissenschaften an der Lucknow Universität, Indien
1999	Projektarbeit im Rahmen des Master of Science: “Response of iron in Rice and Maize seedlings” (Betreuer: Dr. B.D. Nautiyal)
2000-2001	Arbeit am Projekt “Role of Microbes in Green Clean Technology” im Industrial Toxicology Research Centre (ITRC) Lucknow, Indien (Betreuer: Prof. Dr. K.G. Dubey)
Seit 2002	Doktoradin an der Landwirtschaftlichen Fakultät der MLU Halle-Wittenberg am Institut für Ernährungwissenschaften (seit 1.9.2006 Naturwissenschaftliche Fakultät III) (Betreuer: Prof Dr. K. Eder)

Liste der Veröffentlichungen

Originalarbeiten

1. **Shukla, A.**, Brandsch, C., Bettzieche, A., Hirche, F., Stangl, G.I., Eder, K. (2006) Isoflavone-poor soy protein alters the lipid metabolism of rats by SREBP-mediated down-regulation of hepatic genes. J Nutr Biochem., [Epub ahead 2006 Sep 6].
2. Brandsch, C., **Shukla, A**., Hirche, F., Stangl, G.I., Eder, K. (2006) Effect of proteins from beef, pork and turkey meat on plasma and liver lipids of rats compared to casein and soy protein. Nutrition 11-12, 1162-1170.
3. **Shukla, A**., Bettzieche, A., Hirche, F., Brandsch, C., Stangl, G.I., Eder, K. (2006) Dietary fish protein alters blood lipid concentrations and hepatic genes involved in cholesterol homeostasis in the rat model. Br. J. Nutr. 96 (4), 674-682.
4. Brandsch, C., Sachs, H., **Shukla, A**., Hirche, F., Stangl, G.I., Eder, K. Fish protein lowers the arachidonate:eicosapentaenoate-ratio in membrane phospholipids and the concentrations of PGE2 in plasma and tissues of rats. Prostag. Leukotr. & Ess. Fatty Acids (eingereicht).

Abstarcts

1. **Shukla, A.**, Brandsch, C., Eder, K. (2004) Influence of dietary proteins on lipid concentrations in plasma and liver of weaning rats. Proceedings of the German Nutrition Society, Vol. 6, PI-16. Abstracts zum 41. Wissenschaftlichen Kongress vom 11. - 12. März 2004 an der TU München, Freising-Weihenstephan
2. Brandsch, C., Sachs, H., **Shukla, A.**, Eder, K. (2005) Wirkungen von Fischprotein auf den Lipidstoffwechsel von wachsenden Ratten. Proceedings of the German Nutrition Society, Vol. 7, PI-37. Abstracts zum 42. Wissenschaftlichen Kongress vom 17. - 18. März 2005 an der Universität Kiel
3. Eder, K., Sachs, H., **Shukla, A.**, Brandsch, C. (2005) Effects of dietary fish protein on the lipid metabolism in rats. Proc. Soc. Nutr. Physiol. 14, 59. Tagung vom 8.-10. März 2005 in Stuttgart-Hohenheim.
4. Brandsch, C., Bettzieche, A., **Shukla, A**., Stangl, G.I., Eder, K. (2006) Wirkung von isoflavonarmem Sojaprotein auf die Expression der Transkriptionsfaktoren SREBP1 und 2 sowie dessen Zielgene bei Ratten. Proceedings of the German Nutrition Society, Vol. 8, VI-19. Abstracts zum 43. Wissenschaftlichen Kongress vom 8. - 9. März 2006 an der Universität Hohenheim, Stuttgart

ERKLÄRUNG

Hiermit erkläre ich, dass ich die Arbeit selbständig angefertigt und keine anderen als die angegebenen Hilfsmittel verwendet habe.

Dortmund, den 20.11.2006

_____

Anjali Shukla

Acknowledgements

I would like to sincerely thank all people who made the work in this dissertation possible as well as enjoyable. First of all I express my deep gratitude to Prof. Dr. Klaus Eder for suggesting the interesting problem and his talented guidance, encouragement and assistance throughout this work. His expert knowledge, ideas and suggestions were very helpful for me. I benefited a lot from them.

I am very deeply grateful to Dr. Corinna Brandsch for her patience in unveiling me her vast experience in nutrition science and for the numerous fruitful discussions we have had. Without her permanent guidance, assistance and confidence in my work, none of my scientific achievements would have been possible.

I express my sincere thanks and gratefulness to Dr. Frank Hirche, for his guidance and helping hands.

I am also thankful to Dr. Bettina König, Dr. Norbert Nass and Dr. Robert Ringseis for their useful hints. I would like to thank all other colleagues from the Institute of Agricultural and nutrition sciences. They offered me a very nice environment which allowed me to work with high efficiency and great pleasure. I offer my sincere gratitude to those unmentioned persons who contributed at various stages in this work.

I want to thank my husband Dr. Anuj Shukla for his continuous help at the times of special efforts in writing this dissertation and moral support. I would like to express my gratitude and special thanks to my parents Mr. Ram Sagar Bajpai and Mrs. Suman Bajpai, and other family members for their love, support and encouragement throughout the years of my studies. If there is any success of mine, it belongs to them.

The financial support from the Bundesministerium für Bildung und Forschung (BMBF) - 0312750A is gratefully acknowledged.