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Canada

The effects of dietary protein and fat

on

cholesterol metabolism

in

the Golden Syrian hamster

Nathalie Goyette

School of Dietetics and Human Nutrition

Macdonald Campus of McGill University, Montréal

December, 1992

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfilment of the requirements for the degree of Master of Science.

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ABSTRACT

Dietary fats and animal proteins have been shown to exert different lipidemic responses in many animals, including humans. Oxidative stress has been associated with the development of several diseases including atherosclerosis. The hypotheses of this study were that: 1) the degree and type of dietary fatty acid unsaturation will influence the type of cholesterolemic responses via the induction of differential levels of oxidative stress; and 2) hyperlipidemia induced by casein in a butterfat-based diet is related to increased tissue lipid peroxidation. Male Golden Syrian hamsters were fed diets containing butterfat/casein (BF/CAS), butterfat/egg white (BF/EGG), safflower oil/egg white (SAFF/EGG) or menhaden oil/egg white (MHO/EGG) for 27 days. In comparison to the BF/EGG and SAFF/EGG diet groups, the MHO/EGG hamsters exhibited higher levels of total serum cholesterol, serum triglyceride, serum apolipoprotein B and serum lipid peroxides. These results suggest that MHO induced-hyperlipidemia could be exerted through increased concentrations of serum apolipoprotein B and increased serum lipid peroxidation. The BF/CAS diet, in comparison to the BF/EGG diet, increased serum total cholesterol and increased serum and hepatic levels of lipid peroxides. These results suggest that dietary casein-induced hypercholesterolemia could be related to increased oxidative stress. The protective effect of dietary egg white on lipid peroxidation may involve sulfur amino acids which are found in greater amounts in egg white than casein.

RESUME

Chez les animaux ainsi que les humains, les gras et les protéines alimentaires sont reconnus pour posséder différent effects sur le métabolisme des lipides. Le stress oxidatif a été associé avec le développement de plusieurs maladies incluant l'athérosclérose. Les hypothèses de cette étude étaient que 1) le degré et le type de saturation des acides gras influenceront différentes réponses cholestérolémiques par une induction de différents niveaux de stress oxidatif et 2) l'hyperlipidemie causée par la caséine dans une diète à base de beurre sera reliée à une augumentation de peroxidation des lipides dans les tissus. Les hamsters mâles Syriens ont été nourris des quatre différentes diètes contenant du beurre et de la caséine (BF/CAS), du beurre et de l'albumine d'oeuf (BF/EGG), de l'huile de carthame et de l'albumine d'oeuf (SAFF/EGG) et de l'huile de menhaden et de l'albumine d'oeuf (MHO/EGG) pendant une période de 27 jours. En comparaison aux groupes reçevant les diètes BF/EGG and SAFF/EGG, les hamsters obtenant la diète MHO/EGG avaient des plus hauts taux de cholésterol, triglycéride, apolipoprotéine B et de peroxidation des lipides au niveau du sérum. Ces résultats suggèrent que l'hyperlipidémie causée par l'huile de menhaden peut être dû à une augmentation des apolipoprotéines B et de la peroxidation des lipides sérumique. La diète BF/CAS, en comparaison à la diète BF/EGG, a élevé le taux hépatique et sérique de la peroxidation des lipides. Ces résultats suggèrent que l'hypercholéstérolemie causée par la caséine alimentaire peut être reliée à une augmentation du stress oxidatif. L'effet protecteur de l'albumine d'oeuf sur la peroxidation des lipides peut impliquer les acides aminées contenant du souffre qui sont retrouvées en plus grande quantité dans l'albumine d'oeuf que dans la caséine.

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ABBREVIATIONS

Apo	Apolipoprotein
BF	Butterfat
CAS	Casein
DHA	Docosahexanoic acid
EGG	Egg white
EPA	Eicosapentanoic acid
FAME	Fatty acid methyl ester
HDL	High-density lipoprotein
HMB	Hemoglobin-methylene blue
LDL	Low-density lipoprotein
LPO	Hydroperoxides
MHO	Menhaden oil
SAFF	Safflower oil
TBA or TBARS	Thiobarbituric acid-reactive substances
TC	Total serum cholesterol
VLDL	Very-low density lipoprotein

Section 1: Literature review

1.1 Dietary animal proteins: Effects on cholesterol metabolism

Dietary protein is generally not considered to be involved in the process of hypercholesterolemia and atherosclerosis in humans. Most researchers have rather looked at the effects of fat and fibre ingestion on serum cholesterol. Epidemiological studies, however, have shown a strong positive correlation between the amount of animal protein in the diet and mortality from coronary heart disease in different countries (Connor et al., 1972 and Stamler, 1979).

Laboratory animal studies have demonstrated that different dietary proteins differentially affect serum cholesterol levels in most but not all animal species. Carroll and co-workers (1975, 1976, 1977) carried out a series of feeding studies using cholesterol-free; semi-purified diets to study the effects of various animal or vegetable proteins on plasma cholesterol levels in the New Zealand white rabbit. These workers found that there were striking differences between animal and vegetable proteins in their ability to alter plasma cholesterol. Some protein sources, such as extracted whole egg, skim milk powder, lactalbumin and casein produced rather high concentrations of serum cholesterol, whereas other protein sources such as raw egg white, peanut and soy protein were able to maintain low levels of serum cholesterol in rabbits (see Table 1.1). Out of eleven animal proteins studied, egg white albumin was found to be the least hypercholesterolemic

Table 1.1. Concentration of plasma cholesterol in rabbits fed semi-purified diets containing different animal and plant proteins (Carroll and Hamilton 1975, Hamilton and Carroll 1976)[1]

Dietary protein	Number of rabbits	Plasma cholestrol mg/dl, mean ± SEM
Animal protein		
Extracted whole egg	4	235 ± 89
Skim milk	6	230 ± 40
Lactalbumin	5	215 ± 69
Casein	20	200 ± 22
Beef protein concentrate	5	160 ± 60
Pork protein concentrate	6	110 ± 17
Raw egg white	6	105 ± 28
Plant protein		
Wheat gluten	6	80 ± 21
Peanut protein concentrate	6	80 ± 10
Peanut meal	4	75 ± 27
Soybean protein concentrate	6	25 ± 5
Soybean protein isolate	6	15 ± 5

[1]The diets were fed for a period of 28 days.

animal protein whereas casein compared to egg white increased total plasma cholesterol by 50%.

Hypercholesterolemia is typically observed when rats (Nath et al., 1959), hamsters (Terpstra et al., 1991), chickens (Kritchevsky et al., 1959), pigs (Forsythe et al., 1980), monkeys (von Duvillard et al., 1992) and humans (Terpstra et al., 1983) are fed a cholesterol-enriched casein diet compared to a cholesterol-free diet. Other researchers, however, have found contrasting results. Lovati et al. (1990) found that rabbits fed low cholesterol diets (80 mg cholesterol/kg feed) with either soy protein, casein or ovalbumin did not have significantly increased serum cholesterol levels from the initial value after four weeks of experimental feeding, regardless of the protein fed. On the other hand, rabbits fed the casein diet in the presence of high cholesterol content (1500 mg/kg feed) showed a significant rise in serum cholesterol levels compared to the animals fed the low cholesterol diet (80 mg cholesterol/kg feed). Thus, in rabbits, casein appeared to exhibit hypercholesterolemic properties only in the presence of high levels of cholesterol in diets.

In rabbits, recent work has investigated the effects of the level of protein in cholesterol-free semi-purified diets on serum cholesterol levels (Hrabek-Smith et al., 1989). When the dietary level of protein was fed at the level found in chow (16%), the animals receiving the casein diet had a moderately elevated total serum cholesterol level compared to animals fed the soy protein and chow diets. Moreover, a much larger

proportion of the serum cholesterol of the casein-fed rabbits was present in the low-density lipoprotein (LDL) fraction. When the dietary protein was increased to 27%, rabbits fed either the soy protein or casein diets had an increase in the cholesterol and protein content of LDL as well as an increase in total serum cholesterol concentrations. Increasing the proportion of casein to 27% in the diet produced greater elevations in the LDL and total cholesterol concentration relative to animals fed the soy protein. On the other hand, Okita and Sugano (1990) also observed that rats fed casein had a similar total plasma cholesterol levels as soy protein-fed rats when fed protein at 20% and 30% in cholesterol-free, semi-purified diets. Furthermore, when the level of dietary protein equalled 10% in the cholesterol-free diets, the casein-fed animals had a lower plasma cholesterol level than the rats given the soy protein diet.

Hrabek-Smith (1989) reported that the degree of hypercholesterolemia induced by dietary casein can also be modified by other dietary components. Earlier studies have shown that the addition of casein to commercial chow caused a moderate elevation of serum cholesterol in rabbits but that response was less than that obtained with semi-purified diets containing an equivalent amount of casein (Carroll, 1971). This may be due to the blunting of the hypercholesterolemic effect of casein by some other ingredients in the chow diet such as fibre, complex carbohydrate and fat sources (Hamilton and Carroll, 1976). Kritchevsky et al. (1989) investigated in the rabbit the interaction between the type of fat (saturated or unsaturated) and the type of protein (casein or soy protein) on serum cholesterol concentrations. When the rabbits were fed the soybean oil diet, they

exhibited a decrease in plasma cholesterol in both the casein and soy protein groups. The casein-fed animals had higher plasma cholesterol levels than did the soy protein group; however, the drop in plasma cholesterol levels was more marked in the casein-fed animals switched from a saturated fat diet to an unsaturated fat diet than for the soy protein-fed rabbits. There were no aortic atherosclerotic lesions in the unsaturated fat-casein group whereas the saturated-casein animals exhibited some lesions. The effects of dietary protein on serum cholesterol concentrations therefore interact with, and are dependent on, the degree of dietary fat saturation. Lofland et al. (1966) compared the interaction among different levels of casein-lactalbumin (85:15) and wheat gluten with butter, corn oil, margarine and shortening in White Carneau pigeons fed diets containing 30 mg of cholesterol per 100 g fat. No consistent effects were observed although birds fed 8% wheat gluten and corn oil had the lowest serum cholesterol levels and the incidence of lesions was observed to be highest in the 30% wheat gluten-butterfat group. The effect of feeding cholesterol-enriched; semi-purified diets containing two levels (20% and 50% w/w) of either casein or soy protein in rats was investigated by Terpstra et al. (1982). In female rats, the feeding of casein diets resulted in significantly higher levels of serum cholesterol when compared with the soybean protein diet. Moreover, the hypercholesterolemic effect of casein was enhanced by increasing the level of protein in the diet. In males, no such differential effects by the level or the kind of proteins were observed. Upon feeding of casein diets, both the female and male rats exhibited a shift of cholesterol from the high density lipoproteins to the low density lipoproteins.

When high cholesterol diets were fed, marked effects of the type of protein on the levels of serum cholesterol were found in rats (Yadav et al., 1977; Jaya et al., 1981; Terpstra et al., 1982 and Terpstra et al., 1983) whereas, no effect or only a small effect was observed when cholesterol-free diets were fed (Sautier et al., 1979; Pathirana et al., 1980; Nagata et al., 1980). Neves et al. (1980) reported no consistent differences in plasma total cholesterol, HDL or total triglycerides between rats fed pure animal proteins (casein, lactalbumin, egg albumin) and those fed plant proteins (soy and alfalfa) in cholesterol-free diets. There were no consistent differences in plasma lipids in rats among any of the proteins. They concluded that plant proteins are not universally hypocholesterolemic compared with animal proteins. Terpstra et al. (1991) observed that Golden Syrian hamsters fed a cholesterol-free diet containing 25% casein had higher levels of plasma total triglycerides, cholesterol and VLDL (very low density-lipoprotein)+LDL cholesterol than did hamsters fed a 25% soybean protein diet. Addition of cholesterol to the diets caused even greater differences in plasma lipid levels between the animals fed the two different types of protein; however, there was also an increased inter-animal variability of response to the added cholesterol. The authors concluded that the hamster may be a useful model to examine the effect of different types of protein and their interactions with dietary cholesterol on various plasma lipids and lipoproteins.

In humans, several studies have shown that plants proteins (soybean protein) compared with animal proteins (casein, milk, beef, turkey) lowered plasma total

cholesterol and LDL levels in hypercholesterolemic patients (Siroti et al., 1977; Descovich et al., 1980; Wolfe et al., 1981; Goldberg et al., 1982; Verrillo et al., 1985). In general, these researchers have used high protein diets containing limited amounts of saturated fat (P/S ratio of 1.5-2.5) and cholesterol (less than 250 mg of cholesterol/day). An exception to these human dietary studies were the experiments performed by Siroti et al. (1977, 1979) who fed diets with high P/S ratio. In a cross-over study, they observed that soybean protein diets with a low P/S ratio (0.1) were less effective in lowering the serum cholesterol levels than diets with a high P/S ratio (Siroti et al., 1979). In addition, the dietary intake of cholesterol was higher in diets containing animal protein than in the soybean protein diets although these researchers reported that an intake of an additional 500 mg of cholesterol per day did not affect their results. In contrast, more recent work using hypercholesterolemic subjects showed that diets high in plant and animal proteins but low in saturated fat and cholesterol did not result in striking differences in plasma lipoprotein levels and that casein had no LDL-cholesterol-elevating effect compared to soy protein in these diets (Holmes et al., 1980; Huff et al., 1984; Wolfe et al., 1985). In another study, Shorey et al. (1981) explored the effects of soy protein and animal protein in mixed diets given to young men with mildly elevated plasma cholesterol. A portion of the individuals showed a decrease of plasma cholesterol to initial baseline values; however, the reduction of plasma cholesterol occurred on both diets and there were no differences in cholesterol response between diets containing soy protein and animal protein. Thus, it is possible that the cholesterol-lowering effect of vegetable proteins becomes obvious only when patients have a high initial concentation

of serum cholesterol.

Studies performed in hyperlipidemic subjects have used quite different types of animal protein: pork, sausage, beef, turkey, ham, skimmed milk, dairy protein or a mixture of all of these animal protein sources. Wolfe et al. (1985) suggested that some of the variability in response between studies may have been due to differences in the digestibility of the various protein preparations. Different levels of dietary protein, cholesterol and the degree of saturated fats (Sirtori et al., 1979) in the diets may also have been factors contributing to the differential effects observed.

Animal proteins (i.e., dietary casein) do not have marked effects on plasma lipoprotein levels in normolipidemic subjects when compared to hypercholesterolemic subjects. Grundy and Abrams (1983) showed that the substitution of soy protein for casein protein produced no consistent change in plasma concentrations of cholesterol, triglycerides, low-density lipoprotein or high-density lipoprotein. van Raaij et al. (1981, 1982) found no effects on serum cholesterol between casein and soy protein in young and middle-aged healthy male and female subjects. One study investigated the effect of egg white (animal protein) and gluten (vegetable protein) on serum lipids in male subjects (Anderson et al., 1971). The results indicated no significant differences in serum cholesterol and triglycerides between the two dietary situations tested in young normolipidemic men. However, only half of the total protein intake was as egg white or gluten; the other half was coming from other sources in the diet. Anderson et al. (1971)

concluded that changes in the protein of the diet are of no particular value in designing diets for the reduction of serum cholesterol in normolipidemic males. The results of Meinertz et al. (1988, 1989), however, indicate that casein had a more distinct hypercholesterolemic effect when dietary cholesterol is high. When normolipidemic individuals were consuming a low-cholesterol diets (100 mg cholesterol/day), these workers reported that the concentration of each of the major lipoprotein classes was similar for either casein or soy proteins (Meinertz et al., 1988). On the other hand, when the dietary cholesterol was increased to a level of 500 mg/day, casein was found to be hypercholesterolemic (Meinertz et al., 1989). Carroll et al. (1978) examined the effects of the type of dietary protein on cholesterol levels in young, healthy, normolipidemic women. A diet rich in soy protein was compared to one containing animal protein; dietary cholesterol did not seem to be accounted for. Even on the animal protein diet, plasma cholesterol levels were relatively low (191 mg/dl) but the women on the plant protein diet had a significant fall in the serum cholesterol concentration to 175 mg/dl.

Many researchers have proposed that the effect seen on serum cholesterol by different proteins is due to differences in amino acid composition (Kritchevsky, 1979; Huff and Carroll, 1980; Sugano et al., 1982; Vahouny et al., 1985; Jacques et al. 1986). However, feeding amino acid mixtures corresponding to amino acid composition of dietary proteins did not always reproduce the effect of dietary protein on plasma cholesterol (Huff et al., 1977). Furthermore, Critchfield et al. (1990) showed that supplementation of essential amino acids at equivalent levels could not account for

differences in serum cholesterol levels observed between beef, casein, soy and zein proteins. Others have suggested that these effects could be due to changes in cholesterol turnover (Huff and Carroll, 1980), cholesterol absorption (Vahouny et al., 1984), faecal steroid excretion and saponin formation (Sautier et al., 1979 and 1983; Sidhu and Oakenfull, 1986; Sugano et al., 1988), bile acid synthesis and excretion and hepatic cholesterol synthesis (Roberts et al., 1981; Sirtori et al., 1984), changes in hormonal status: insulin/glucagon (Sugano et al., 1983) and thyroid hormones (Valdermarsson, 1983), and the phosphorylation state of the protein (Van der Meer, 1983).

In summary, it has been known for a long time that the type of dietary protein can influence cholesterol metabolism in experimental animals. In addition, the cholesterolemic effect of a dietary protein can be enhanced by increasing the proportion of that protein in the diet, though not all experiments are consistent in this respect, and by feeding diet diets with large amounts of cholesterol. In human subjects, the marked effects of the type of dietary protein can only be observed in hypercholesterolemic patients. On the other hand, in normolipidemic subjects dietary protein has been demonstrated to exert either a small effect or no effect at all.

1.2 **Dietary fats: Effects on cholesterol metabolism**

Many epidemiological studies have shown a link between dietary fats, plasma lipid levels and coronary heart disease (Keys, 1980; Stallones, 1983; Stamler, 1983).

Furthermore, it is well known that the risk of cardiovascular disease is increased in hypercholesterolemic subjects (Kannel et al., 1971; Stamler et al., 1986; Kannel, 1988). Many clinical, epidemiological and animal studies have shown a relationship between the degree of saturation of dietary fatty acids, the level of dietary fats and the concentration of plasma cholesterol. It has been widely accepted that saturated fats generally increase plasma cholesterol and that polyunsaturated fats have a hypocholesterolemic action.

Dietary cholesterol and saturated fats such as butterfat have been positively correlated with atherosclerosis in many animal species as well as in humans. The major cause of high serum levels of LDL is believed to be reduced activity of LDL receptors (Grundy, 1987). When the number of receptors are decreased, two changes in the metabolism of LDL occur. First, the clearance of LDL itself is decreased; second, because VLDL remnants are cleared by LDL receptors, more VLDL remnants are converted to LDL. Another important discovery was the finding that the synthesis of LDL receptors is under feedback control by amounts of cholesterol in cells (Goldstein and Brown, 1977). When concentrations of cholesterol in liver cells increase, the formation of LDL receptors is suppressed and plasma levels of LDL rises. Thus, any factors that will increase the liver's content of cholesterol should suppress the activity of LDL receptors. Such factors include inherited hyperabsorption of cholesterol, a defect in the conversion of cholesterol into bile in the liver and the total caloric intake. When the intake in energy is high, there are more VLDL produced and because VLDL is a precursor to LDL, an overproduction of VLDL can increase the concentration of LDL.

It has been proposed that the uptake of ß-VLDL and LDL by macrophages produces foam cells in blood vessels which lead to arteriosclerotic lesions. Another possible mechanism for the development of atherosclerosis is the infiltration of post-absorptive LDL or VLDL cells into arterial walls (Grundy, 1987).

Numerous clinical studies have reported LDL cholesterol levels and total cholesterol concentrations are raised in humans by dietary saturated fats (Mattson and Grundy, 1985; Grundy and Vega, 1988; Bonanome and Grundy, 1988). Wardlaw and Snook (1990) studied the effect of saturated, polyunsaturated and monounsaturated fats on serum lipids in men. They found that the feeding of a butterfat-based diet for two weeks did not increase total serum cholesterol, LDL cholesterol, HDL cholesterol and total triglycerides from the levels seen before the study started. Furmeron et al. (1991) investigated the effects of changing the P:S ratio on serum lipids in young healthy subjects with a genetic predisposition to cardiovascular disease. The two prescribed diets were consumed successively for three weeks; one diet contained butter (P:S 0.2), the other contained sunflower margarine (P:S 1.1). Cholesterol and apolipoprotein B in serum and in low-density lipoproteins were lower with the margarine diet. However, the HDL_2 subfraction and apolipoprotein A-I were significantly higher in the butter diet. The authors concluded that the undesirable effect of a diet with a high P:S ratio could cancel the benefits of lowering the LDL-cholesterol concentrations. Baudet et al. (1984) have observed a large increase in the serum level of all lipoproteins with a milk fat diet (butter and cream) fed to nuns. Such an increase in human has also been noted by many other

researchers (Shepherd et al., 1978; Schonfed et al., 1982; Turner et al., 1981; Vega et al., 1982 and Kukis et al., 1982). There was a large increase in the serum VLDL due not only to an increase in the number of circulating particles but also to an increase in the amount of apolipoproteins. Milk fat induced the appearance of VLDL particles having a higher protein to triglyceride ratio because of an increased protein level and decreased triglyceride level. Therefore, there was an increase in these particles which were enriched with cholesterol and triglycerides. There was also a decrease in plasma HDL induced by the high milk fat diet. The catabolism of LDL was markedly decreased with a palm oil and milk fat diet. With the milk fat diet, Baudet et al. (1984) obtained LDL particles enriched with esterified cholesterol, which would explain the decreased binding, internalization, and protein degradation. Thus, it is suggested that a modification of the structure, composition and viscosity of LDL according to the degree of saturation of the diet alters its cellular metabolism. St Clair et al. (1980) have shown that in monkeys fed hypercholesterolemic diets, the number of LDL particles bound on receptors was reduced by half. These hypercholesterolemic diets induced formation of LDL particles enriched with esterified cholesterol, which are larger and require more space on the cell surface leading to a decrease internalization of the LDL particle in the tissues and increasing total serum cholesterol.

Rabbits fed semi-synthetic diets without added cholesterol developed hypercholesterolemia and a form of atherosclerosis when butter was the sole dietary fat (Wigand, 1960; Funch et al., 1960b and Funch et al., 1962). Vesselinovitch et al. (1974)

studied the severity of atherosclerotic lesions in Rhesus monkeys fed one of three diets, each containing 2% cholesterol and 25% lipid, either corn oil, butterfat or peanut oil over a period of 50 weeks. The butterfat diet induced an elevation of serum lipids and the highest serum cholesterol concentrations relative to animals fed corn oil. The peanut oil fed animals had similar elevations in serum lipids to those monkeys fed the butterfat diet. The butterfat diet produced severe aortic lesions, characterized by abundant lipid deposition and relatively little cell proliferation or collagen deposition. The most widespread and advanced atherosclerosis, however, was observed in the peanut oil-fed monkeys. They concluded that not all types of saturated fats produce the same atherogenic effect. Rowsell et al. (1958) obtained similar results when swine were either fed a low fat diet or isocaloric diets rich in butterfat or margarine (40% calories as fat). The high fat diet using margarine as the fat source caused little increase in the amount of atherosclerosis produced, while a high fat diet containing butter produced a considerable amount of atherosclerosis. There was no increase in blood cholesterol and phospholipid levels in the swine on either the butterfat or margarine diets. The coagulation indices studied were most active in the butterfat-fed swine with more atherosclerosis. In contrast, Toda et al. (1984) did not see any significant difference in the incidence and extent of intimal thickening of the coronary arteries between swine fed diets containing 8.3% butter or margarine for four months.

Most investigators have established that diets high in solid, saturated or animal fats generally increase levels of circulating cholesterol in many species including humans.

The proposed mechanisms have included: increased absorption of exogenous cholesterol from the small intestine, increased synthesis of cholesterol, decreased excretion of faecal steroids perhaps secondary to secretion into bile, decreased synthesis of bile, a change in the distribution of cholesterol between plasma and various extrahepatic pools. Altered hepatic and intestinal lipoprotein synthesis and altered catabolism reflected in peripheral uptake of sterols, lipoproteins, cholesterol and bile turnover (Feldman et al., 1979) are also potential mechanisms that modify cholesterol metabolism. Lipoprotein structure (relative to triglyceride composition) may be influenced by dietary fat saturation (Witcox et al., 1975). Lipoprotein size in turn can influence catabolic rates of lipoprotein degradation including lipoprotein lipase activity (Quarfordt and Goodman, 1966). Dietary saturated fats (trilaurin, trimyristin, tripalmitin, tristearin) fed to rats down regulated hepatic HMG-CoA reductase activity in proportion to chain length. The mechanism was postulated to be related to the fate of absorbed fatty acids perhaps via fluidity of microsomal lipids or an effect on bile flow (Feldman et al., 1979).

The action of dietary saturated fatty acids (such as butterfat) as a lipid class to raise total cholesterol is well established. Dietary saturated fats raise LDL-cholesterol levels as well as total cholesterol concentrations in the blood of both animals and humans.

The ingestion of unsaturated fat by man is known to increase the proportion of unsaturated fatty acids in the lipids of plasma lipoproteins (Bragdon et al., 1961 and

Kayden et al., 1963) and to bring about changes in the positional distribution of acyl groups in plasma glycerolphospholipids (Gordon et al., 1975) and triglycerides (Gordon, et al., 1975 and Parijs et al., 1976). Furthermore, there is a decrease in plasma total cholesterol (Keys et al., 1957; Grundy, 1975 and Shepherd et al., 1978) and triglyceride (Grundy, 1975; Shepherd et al., 1978 and Engelberg, 1966) concentrations.

A significant reduction in plasma VLDL, LDL and HDL in normolipidemic human subjects fed polyunsaturated fatty acid diets (omega-6) has also been reported (Shepherd et al., 1978 and Kurkis et al., 1982). Similar results have been reported in experimental animals including rabbits (Wigand, 1960), rats (Narayan et al., 1974) and chimpanzees (Rosseneu et al., 1979). Hepatic production of high-density lipoprotein (HDL) was decreased in non-human primates fed omega-6 fatty acids compared to the butter-fed animals (Johnson et al., 1986).

Linoleic acid has been reported to decrease serum triglycerides in some patients with hypertriglyceridemia (Grundy, 1975 and Chait, 1974). Grundy and Denke (1990) reported that this reduction can occur relative to carbohydrates or saturated fatty acids. Mattson and Grundy (1985) hypothesized that high intake of omega-6 fatty acids reduces hepatic synthesis of VLDL triglycerides. Alternatively, serum triglycerides containing polyunsaturated fatty acids could be a better substrate for lipoprotein lipase than those high in saturated acids, contributing to the lowering of serum triglycerides. However, the triglyceride-lowering action of polyunsaturated fatty acids is not consistent and many

patients with hypertriglyceridemia do not respond with a reduction in serum triglycerides when fed a diet high in linoleic acid (Mattson and Grundy, 1985).

There are several proposed mechanisms for the action of omega-6 fatty acids on plasma lipids. An increased faecal excretion of neutral steroids and endogenous cholesterol has been reported with omega-6 fatty acid feeding in some subjects with normal plasma lipid levels (Moore et al., 1968; Wood et al., 1966 and Connor et al., 1969) but not in subjects with hypercholesterolemia (Spritz et al., 1965 and Grundy and Ahrens, 1970). Bile salt excretion increases with omega-6 fatty acid ingestion (Campbell et al., 1972). Omega-6 fatty acids can decrease the rate of synthesis of VLDL apolipoprotein B (Shepard et al., 1978) and may decrease the rate of hepatic VLDL secretion (Chait et al., 1974). A decrease in the synthetic rate and an increase in fractional catabolic rate of LDL apolipoprotein B (Turner et al., 1981) have also been reported. Higher fractional catabolic rates have been reported for apolipoproteins A-I and A-II with diets high in omega-6 fatty acids (Chong et al., 1987).

In summary, vegetable oils rich in polyunsaturated fatty acids (omega-6) are known to lower plasma cholesterol levels when substituted for dietary saturated fatty acids in both experimental animals and humans. On the other hand, omega-6 fatty acids fed at high levels also possess a plasma HDL-lowering action which is not always advantageous.

The beneficial effects of omega-3 fatty acids on cardiovascular disease have been promoted by Dyerberg et al. (1975, 1981) who correlated the low incidence of coronary heart disease in the Greenland Eskimos with a high intake of omega-3 fatty acids. These Eskimos consumed a diet high in animal fat but large quantities of omega-3 fatty acids (14% of total fat) coming from their marine diet. The Eskimos living in Greenland had longer bleeding times, reduced platelet aggregation, and lower serum cholesterol and triglycerides compared to Danes and to Eskimos living in Denmark who were eating diets containing similar total fat content (40% kcal as fat) but with only 3% of total fat as omega-3 fatty acids.

In normolipidemic subjects, fish oil (salmon oil) lowered plasma triglyceride and VLDL levels as well as plasma cholesterol and LDL although levels of HDL were not affected (Harris and Connor, 1980; Harris et al., 1983 and Failor et al., 1988). Similar results were observed in type IIa hyperlipidemia (Connor, 1982 and Phillipson et al., 1985). On the other hand, Sanders et al. (1981) have reported a significant rise in HDL cholesterol when 20 ml of cod liver oil were administered daily to normolipidemic men for six weeks. Childs et al. (1990) reported a significant decrease in plasma HDL levels when normolipidemic subjects were consuming marine oils diets for three weeks compared to subjects eating butterfat-based diets. Simons et al. (1985) noted no change in plasma cholesterol in types IIa and IIb patients following a diet supplemented with fish oil. The results in type V patients were similar in showing a lowering of serum cholesterol and triglyceride and a the disappearance of chylomicrons and remnants and

a decrease in VLDL (Phillipson et al., 1985). On the other hand, Demke et al. (1988) reported a significant increase in total cholesterol, LDL, HDL and HDL_2 compared to baseline values in hypercholesterolemic patients given a supplement of 5 g of fish oil daily. Zucker et al. (1986) reported significantly increased LDL and HDL cholesterol in type IIb patients given fish oil dietary supplements. Similar results were found by Wilt et al. (1989) who gave 1 g of MaxEPA daily to middle-aged hypercholesterolemic men. No change in total cholesterol and HDL cholesterol in type IIa patients following dietary supplements was seen by Brox et al. (1983).

In animal feeding trials where fish oil is given in large amounts, plasma triglyceride levels have been demonstrated to be generally decreased (Wong et al., 1984 and Ruiter et al., 1978). Rats fed 15% wt% MaxEPA for two weeks had 40% lower concentration of plasma triglycerides compared to the same amount of saffiower oil but no differences were observed in total plasma cholesterol levels (Wong et al., 1984). Feeding an omega-3 fatty acid rich mixture from liver squid or a combination of the mixture from liver squid and methyloleate to rats decreased plasma cholesterol concentrations when compared to rats fed diets containing lard. The greatest alteration in plasma cholesterol levels was observed with a 5% liver squid oil and no added methyloleate; mixtures of the liver squid oil mix and methyloleate produced changes proportional to their PUFA content (Kobatake, 1983). Evidence from animal feeding trials suggests the HDL level may be more sensitive to docosahexanoic acid (DHA) than eicosapentanoic acid (EPA) intake.

Conflicting results with respect to effects of fish oils on serum LDL and HDL levels exist in rabbits (Field et al., 1987) and rats (Morisaki et al., 1983). Nestel (1986) reported that habitual consumption of fish oils effectively lowered cholesterol and triglyceride levels in VLDL, LDL and HDL. Field et al. (1987) reported that dietary cholesterol was less hypercholesterolemic when rabbits were fed 1% cholesterol diets including menhaden oil than 1% cholesterol diets including cocoa butter. HDL cholesterol increased by 11% when rats where given 100 mg of DHA daily for two weeks whereas no significant change was observed when the same quantity of EPA was given (Morisaki et al., 1983). No significant difference in HDL was noted among rats fed a commercial diet with or without EPA ethylester (81 mg/kg body weight) (Hamazaki et al., 1982).

The mechanisms whereby dietary omega-3 fatty acid lowers plasma triglyceride appear to involve inhibition of triglyceride synthesis in the liver (Wong et al., 1984; Nossen et al., 1986 and Topping et al., 1987), suppression of apolipoprotein B production (Nestel et al., 1984) and an increased fatty acid oxidation as indicated by increased ketogenesis (Wong et al., 1984 and Topping et al., 1987). Additionally, Parks et al. (1989) reported that fish oils reduce the cholesteryl ester content of lipoprotein, but not the number of particles secreted into serum.

Examination of the effect of dietary fish oil on serum lipids and the development of atherosclerosis in animals has also led to conflicting results. No significant changes

in plasma cholesterol levels were observed when a 10% mackerel oil diet was consumed by pigs (Ruiter et al., 1978). Similarly, no significant differences were seen in plasma triglycerides or cholesterol between control and treatment rats which received either 81 mg EPA or 100 mg/kg body weight of EPA (Hamazaki et al., 1982) and 100 mg of DHA daily (Morisaki et al., 1983). Weiner et al. (1986) reported a reduction in lesion formation in both intact coronary arteries and vessels subjected to balloon abrasion in a hypercholesterolemic swine model brought about by the addition of 30 ml of cod liver oil to a hyperlipidemic diet. Compared to the control group (without the supplement of cod liver oil) , the swine fed the omega-3 supplement did not experience a decrease in plasma total lipid levels (i.e. total plasma cholesterol, triglycerides, LDL, VLDL, LDL and HDL). Kim et al. (1989) conducted a similar study in swine and found a striking reduction in atherosclerotic lesion development in butterfat-fed animals given a fish oil supplement (30 ml cod liver oil) with a decrease in total plasma cholesterol and lipoprotein fractions as compared to butterfat-fed animals which did not receive the supplement. These workers also reported greater plasma thiobarbituric acid reactive substances (TBARS) levels in the fish oil treated swine than in the untreated group. The authors concluded that since the lesions were smaller and showed less necrosis in the treated groups, the presence of TBARS had no serious effects on the development of these lesions. This study contrasts with the results of Thiery and Seidel (1987) who showed that dietary fish oil results in an enhancement of cholesterol-induced atherosclerosis in rabbits, resulting in 60% more extensive thoracic aorta lesions than hyperlipidemic control animals. It was suggested that the increase in aortic lesions was

due to the cytotoxic effect of lipid peroxidation products as reflected by the higher plasma level of TBARS in the fish oil-treated group. Kristensen et al. (1988) reported no change in atherogenesis and thrombopoiesis in cholesterol-fed rabbits receiving fish oil supplementation compared to the control group. Rogers and Karnovesky (1988) supplemented diets with either 5% MaxEPA fish oil or 5% safflower oil for a period of two weeks to normolipidemic, moderately and severely hypercholesterolemic rats. In normolipidemic rats, fish oil did not influence the morphology of blood vessel walls, although both total plasma and HDL cholesterol levels were reduced. In moderately hypercholesterolemic rats, monocyte adhesion was the same irrespective of dietary oil; however, intimal foam cell formation was two-fold higher in the fish oil-fed animals despite a reduction in serum cholesterol levels. In severely hypercholesterolemic rats, monocyte adhesion to the vessel wall and intimal foam cell formation were both four-fold higher in the fish oil compared to the safflower oil-fed animals. These workers concluded that dietary fish oil, when fed to hypercholesterolemic rats for a period of two weeks, enhances the rate of monocyte adhesion and fatty streak formation in the thoracic aorta.

The exact effect of dietary fish oils on cholesterol metabolism remains to be elucidated. Fish oils appears to have a hypotriglyceridemic action in normolipidemic animals (including humans). On the other hand, omega-3 fatty acids exert a hypercholesterolemic effect in certain types of hypercholesterolemia in humans.

1.3 **Lipid oxidation products: Implications in cardiovascular disease**

Free radicals are molecules with an unpaired electron. The presence of the unpaired electron can carry reactivity to the free radical which can damage biological material such as DNA, nucleotide co-enzymes, proteins and lipids. Cellular lipid peroxidation can naturally occur by autoxidation, enzymatic reactions and chemical catalysis by heme groups. Lipid hydroperoxides and secondary products are created during lipid peroxidation process. Polyunsaturated fatty acids are susceptible to free radical mediated peroxidation leading to disturbances in membrane structure and function and to damage to the arterial endothelium.

Kaneda and Ishii (1953) reported that hydroperoxides were found in some tissues after oral administration of lipid hydroperoxides, so they concluded that hydroperoxides might be absorbed from the intestinal wall and transported to the tissues. However, Andrew et al. (1960) did not observe any lipid hydroperoxides in the lymph of rats when they were administered and concluded that peroxides were decomposed at the intestinal wall. A later study reported that lipid hydroperoxides are very toxic when administered intravenously but oral administration was less toxic because hydroperoxides are poorly absorbed, probably due to some conversion before or during absorptive process (Bergan and Draper, 1970). Glavind et al. (1971) could not detect lymphatic methyllinoleate hydroperoxide after it had been administered intragastrically to rats. They concluded that methyllinoleate hydroperoxide is converted to hydroxyoctadecadieneoate, which is

partially recovered in the lymph. Hydroperoxides are broken down in the presence of oxygen and form a mixture of secondary autoxidation products such as aldehydes (including malonaldehyde), polymers, hydroperoxy alkenals and hydroperoxy epoxides. It has been found by Kanazawa et al. (1985b) that oral doses of secondary autoxidation products are absorbed into the circulatory system and can accumulate at high levels particularly in the liver. Piché et al. (1988) obtained indirect evidence of malonaldehyde absorption by measuring urinary malonaldehyde in subjects consuming either a control or unstabilized (no preservatives) cod-liver oil, or either a control or stabilized cod-liver oil. The authors demonstrated a clear increase in urinary malonaldehyde excretion after the first comparison but not the second. They concluded that the unpreserved cod-liver oil contained malonaldehyde, and that consumption resulted in absorption and urinary excretion of malonaldehyde. An excessive accumulation of these secondary products can lead to an elevation of serum transaminase activities, an increase in hepatic lipid peroxide and a hypertrophy of the liver (Oarada et al., 1986).

Lipid hydroperoxides derived from polyunsaturated fatty acids are capable of causing damages to the arterial endothelium and they are known to be present in atherosclerotic plaques (Glavind et al., 1952; Duthie et al., 1989). Hennig et al. (1986) reported that the presence of linoleic acid hydroperoxides in the blood circulation damaged the monolayers of porcine pulmonary artery endothelial cells. In rabbits, when serum lipid hydroperoxide levels are high due to injection of linoleic acid hydroperoxides into the blood stream, marked damage and denudation of aortic endothelial cells was

observed (Yagi, 1987).

Yalcin et al. (1989) observed that the lipid peroxide levels and erythrocyte susceptibility to lipid peroxidation were significantly increased in hyperlipidemic individuals compared to the normolipidemic subjects. Furthermore, it has been observed that diabetic patients have a serum lipid peroxide level higher than normal subjects (Nishigaki et al., 1981). These workers suspected that high plasma levels of lipid peroxides could in part be the cause of angiopathy in patients suffering from diabetes. Hagihara et al. (1984) demonstrated that lipid peroxide levels in low density lipoprotein were higher in old people than in young individuals.

It has been shown that oxidized LDL are more susceptible to be taken up by macrophages. LDL conditioned by incubation with endothelial cell was degraded by macrophages three to five times more rapidly than lipoprotein incubated in the absence of endothelial cell (Henriksen et al., 1983). Macrophages incubated with cell-modified LDL acquire the morphological characteristics of foam cells, the typical cells of fatty streak. The absence of uptake and degradation of cell-modified LDL by fibroblasts suggest that during the oxidation process the binding domain to the LDL receptor is modified. At the same time, new epitopes are generated or exposed on the apolipoprotein B-100, which are recognized by the scavenger receptor (Zawadzki et al., 1989). The macrophages present in the fatty streak originate from blood monocytes. The first event is the adherence of monocytes to the endothelial cells. This adherence is followed by the

migration of monocytes into the intima. The adhesion followed by penetration is influenced by chemotactic factors. Oxidized LDL exhibits a chemotactic activity for human monocytes.

Tissue lipid peroxidation levels appear to be higher when animals are fed fish oil. Hammer and Will (1978) demonstrated that the lipid peroxide content of the liver endoplasmic reticulum and the rate of lipid peroxidation in the incubated suspensions were both greater after the feeding of fish oil (herring oil) than after the feeding of lard, corn oil or a fat-free diet . The lipid peroxide content of the liver microsomal fraction was also found to be greater after feeding corn oil diets (Iritani et al., 1980). A relationship between lipid peroxidation and atherogenic diets has been indicated by Aviram et al. (1991). The feeding of an atherogenic diet composed of 8% coconut oil with the addition of 1% cholesterol has been shown to increase the plasma TBARS content in rats (Aviram et al., 1991). Furthermore, the ingestion of fish oil by rabbits enhanced cholesterol-induced atherosclerosis and increased serum lipid peroxides (Thiery and Seidel, 1987). Kritchevsky et al. (1967) demonstrated that heated polyunsaturated oils were more atherogenic that unheated oils in the rabbit. Naruszewicz et al. (1987) reported that the oral administration of thermally oxidized soy bean oil increased the level of lipid peroxides in human plasma, mainly chylomicrons. Furthermore, human chylomicrons obtained after the ingestion of oxidized oil were degraded more rapidly by cultured mouse macrophages than chylomicrons after fresh oil intake.

A recent study demonstrated that normolipidemic individuals consuming a diet high in polyunsaturated fatty acids had less resistance to lipid peroxidation than the subjects consuming a high monounsaturated fatty acids diet. The peroxidation rate of plasma LDL was inversely correlated with the oleic acid to lin[illegible] acid ratio present in the LDL particle (Bonamone et al., 1992). Lipid peroxide concentrations were significantly higher in patients with ischaemic heart disease and peripheral arterial disease than in control humans (Stringer et al., 1989).

In summary, fatty acids of the omega-3 family are good substrates for lipid oxidation products since thay have more double bonds, on average, than omega-6 fatty acids. The toxicity of dietary lipid oxidation products has been correlated in several diseases, including hyperlipidemia and atheroclerosis in some animals and humans.

Section 2: Hypotheses and Objectives

2.1 Hypotheses

The hypothesis for the first part of the study is that hyperlipidemia induced by casein in a butterfat-based diet is related to increased tissue lipid peroxidation. It has been shown that casein induces hyperlipidemia in numerous animal models, including the male Golden Syrian hamster, (Terpstra, 1991), whereas egg white in a cholesterol-free diet is normolipidemic (Carroll and Hamilton, 1975; Hamilton and Carroll, 1976). In addition, rats fed casein-based diets exhibited higher levels of plasma cholesterol and TBARS than rats fed soy protein-based diets (Sugano et al, 1982).

The hypothesis for the second part of the experiment is that the degree and type of dietary fatty acid unsaturation will induce different cholesterolemic responses mediated through differences in oxidative stress. Butterfat has been demonstrated to induce hypercholesterolemia after four weeks of feeding in male Golden Syrian hamsters (Nistor et al, 1987), whereas safflower oil and menhaden oil are known to decrease plasma lipids and plasma triglycerides, respectively. However, lipid peroxidation has been associated with hyperlipidemia (Yalcin et al, 1989) and lipid peroxidation is induced by dietary polyunsaturated fatty acids, particularly, via omega-3 fatty acids. Thiery and Seidel (1987) demonstrated that the feeding of dietary fish oils in rabbits enhanced cholesterol-induced atherosclerosis and increased plasma TBARS.

2.2 **Objectives**

The male Golden Syrian hamster model of dietary-induced hyperlipidemia will be used to investigate the following objectives in parts one and two of the experiment.

Part 1: Comparison of egg white versus casein on butterfat-induced hyperlipidemia

a) to validate the choice of the experimental animal to induce hyperlipidemia under dietary protein challenge

b) to study the relative effects of dietary casein and egg white (15% w/w) on total serum cholesterol, serum HDL, serum triglycerides, serum apolipoprotein A-I and serum apolipoprotein B

c) to examine the relationship between *in vivo* oxidative stress in serum and liver and dietary protein-induced hyperlipidemia

d) to examine the relationship among dietary lipid oxidation products, *in vivo* oxidative stress and dietary protein-induced hypercholesterolemia

Part 2: Comparison of dietary fats on blood lipids

a) to validate the choice of the experimental animal to induce hyperlipidemia under dietary fat challenge

b) to investigate the influence of dietary butterfat, safflower and menhaden oil (15% w/w) on total serum cholesterol, serum HDL, serum triglycerides, serum apolipoprotein A-I and serum apolipoprotein B

c) to examine the relationship between *in vivo* oxidative stress in serum and liver and dietary fat-induced hyperlipidemia

d) to study the effect of dietary lipid oxidation products on *in vivo* oxidative stress on dietary fat-induced hypercholesterolemia

Section 3: Materials and Methods

3.1 Experimental model: The hamster

The hamster was chosen as the experimental model because, as in humans, its major plasma cholesterol carrier is low-density lipoprotein (LDL). As determined by electrophoresis in agrose gel, about 50% of hamster's plasma cholesterol occurs in the LDL fraction. Moreover, hamster LDL cross-reacts with antibody raised against human LDL (Nistor et al., 1987). These researchers also reported that the atherosclerotic plaques developed in male Golden Syrian hamster by the feeding of a hypercholesterolemic diet resembled human atherosclerotic plaques with huge cholesterol crystal deposits, calcium deposits and necrosis. Furthermore, Spady and Dietchy (1983) demonstrated that hamster hepatic cholesterol synthesis *in vitro* is lower than in rabbits, squirrel monkey, rats and guinea pigs and very close to human hepatic cholesterol synthesis *in vitro* and *in vivo*. The rat is quite resistant to diet-induced hypercholesterolemia and most of the plasma cholesterol (60-80%) is transported in HDL and only 5-10% in LDL (Day et al., 1979). Dietary supplementation with cholesterol is mainly transported by the ($d < 1.006$) lipoprotein fraction composed of chylomicrons, VLDL and possibly chylomicron remnants and ß-VLDL (Mahley and Holcombe, 1977). In normocholesterolemic rabbits, 50% of plasma cholesterol is carried by HDL, 35% by LDL and 15% by VLDL (Brattsand, 1976). Thus, the cholesterol distribution among lipoproteins in hamsters compared to rats and rabbits is closer to that of human where

27-35% of cholesterol is in the HDL and 60-80% is in the LDL fraction (Myant, 1981).

3.2.1 **Animals**

Male Syrian hamsters, weighing 110-120 g, were purchased at seven weeks of age from Charles River Inc., St-Constant, Québec. The animals were housed in individual plastic bottom wired top cages and kept in a temperature controlled room (23-25°C) with a 12 hour automatically maintained light-dark cycle (light cycle 0700 to 1900). After three weeks of feeding a non-purified diet (Mouse Chow 5015, Purina, Montréal, Québec) hamsters (ten weeks old) were weighed and randomly assigned to one of four dietary regimens of semi-purified diet. The hamsters (110-130 g) were allowed two days to adapt to their respective semi-purified diets prior to the start of the experiment. Food and water were given ad libitum throughout the study. The diets were kept in plastic bags at 4°C and bags were flushed with nitrogen after each opening during the trial. Forty-eight hamsters were fed one of four semi-purified diets for four weeks. The diets contained identical amounts of carbohydrate, protein and fat but differed in the type of protein and fat fed. Tap water was dispensed from plastic bottles and fresh diet was provided every day in stainless steel cups. Food intake was recorded every day and the uneaten food was discarded each day. Very little food spillage was recorded. The body weights were measured every second day.

3.2.2 **Diets**

The diets consisted of either: (I) butterfat and casein (BF/CAS), (II) butterfat and egg white (BF/EGG), (III) safflower oil and egg white (SAFF/EGG) and (IV) menhaden oil and egg white (MHO/EGG) (see Tables 3.1, 3.2 and 3.3 for the basal diet composition, fatty acid composition and amino acid composition respectively). Fat was supplied at a level of 15% instead of the 5% recommended by NRC for hamsters since it has been reported that hamsters fed a diet containing 15% of commercial butterfat developed high levels of serum cholesterol and atherosclerosis (Sima et al., 1990). Safflower oil was supplied at 15% in the diet because it is known to decrease serum lipids in both animals and humans (Herman et al., 1991). Menhaden oil was chosen for its capability to decrease serum triglycerides due to its high omega-3 content (Herman et al., 1991). Polyunsaturated fatty acids with different degrees of saturation, omega-6 (safflower oil) and omega-3 (menhaden oil), were also under investigation to determine their action on oxidative stress. Two different types of dietary protein, casein and egg white, were chosen 1) to validate the hypolipidemic effect of egg white and the hyperlipidemic action of casein in the hamster, and 2) to observe any differences between dietary casein and egg white on tissue lipid oxidation levels.

Butterfat (Lanctancia, Victoriaville, Québec) contains 18.4% of water. This level of moisture was reported by Lactancia, Victoriaville, Québec. Therefore, this factor was taken into account in the formulation of the butterfat diets (i.e. 177.6 g/kg of butter was

added into the diet instead of 150.0 g/kg of lipid that was added in the other diets) in order to make the diets isocaloric.

To the butterfat and the safflower oil-based diets, 0.0440% and 0.0864% cholesterol was added, respectively, to match the amount of cholesterol present in menhaden oil. However, the amount of cholesterol found in egg white and casein, 121.2 μg/g and 34.3 μg/g respectively, was not adjusted for (see Table 3.10). The cholesterol content in the butterfat/casein diet was 0.087% and 0.088% for the butterfat/egg white, safflower oil/egg white and menhaden oil/egg white diets.

As the dietary requirement for choline is influenced by the lipid content of the diet (Best et al., 1954), the dietary choline content (6 g/kg diet) was increased threefold above the NRC requirements. This was done to account for the threefold increase in fat intake (15%) from the NRC requirements for fat (5%).

Safflower oil (linoleic acid) was supplied to 1.6 % in all diets in order to avoid any essential fatty acid deficiency (EFA) (Hamilton, 1944) and thus met the essential fatty acid requirements (2-3% of total energy as EFA).

An antioxidant, butylated hydroxytoluene (BHT; ICN Biomedicals, Cleveland, OH), was also added to all dietary fat sources at a level of 0.01% of lipid weight to protect the fats from oxidation.

Cornstarch was fed at a higher level (53.7-53.8%) than the amounts supplied by Arrington et al. (1966), Banta et al. (1975) and Rogers et al. (1974) to hamsters in semi-purified diets. The reason for the lower cornstarch levels in these diets was due to added sucrose which was used to complement the cornstarch. Purified and simple sugars were not used in the present study as the sugars can cause a rapid rise in blood VLDL levels (Melish et al., 1980).

Casein and egg white were used as protein sources at the level of 15% in the diets. This level of dietary protein is sufficient for adequate growth and reproduction and to provide for the essential amino acids needs for the hamster (NRC, 1978). The protein levels (N X 6.25, dry basis) of casein and egg white albumin were minimally 90% and 80% respectively (US Biochemical Corporation, Cleveland, Ohio).

Cellulose was added at a level of 5% to the diet which is considered to be the satisfactory level of fibre in the diet (NRC, 1978).

All vitamins and minerals were added at 1.5 times the NRC requirements (see Tables 3.4, 3.5, 3.6 for vitamin and mineral composition). These vitamin and mineral formulations were calculated from the NRC hamster requirements. These concentrations provided enough vitamins and minerals to the hamsters in case of anticipated (30%) reduction in feed intake since no reports were done on the feed acceptibilty and intake of a menhaden oil containing diet in hamsters prior to the feeding trial. Vitamin A

naturally occurs in butterfat at a level of 30.07 IU/g of butterfat (Health and Welfare Canada, 1987), thus, only 4619 IU of vitamin A/kg of diet was added to the butterfat-based diet in order to keep vitamin A constant in all diets.

3.3 **Experimental techniques**

3.3.1 **Blood sampling**

On day 1 (after two days of adaptation on dietary treatments) and day 27, blood was collected using heparinized capillary tubes by retro-orbital sinus puncture, from 14 hour fasted hamsters anaesthetized with CO_2. Approximately 1.0 ml of blood was collected at day 1 whereas 2.0 ml of blood was taken at day 27. Whole blood was put in 1.5 ml polyethylene eppendorf tubes, left at room temperature to allow clotting and then centrifuged in a microcentrifuge at room temperature (Microspin 12S, Sorvall Instruments, Biomedical Products, Mississauga, ON) at 6000 X g for 10 minutes. The serum was then transferred into other 1.5 ml polyethylene eppendorff tubes for subsequent analysis. The serum was kept for no more than 2 days at 4°C during which time analyses for total cholesterol, total triglyceride and HDL content were performed. The remaining serum was stored at -20°C for later determination of serum apolipoprotein A-I, serum apolipoprotein B, serum alanine aminotransferase and serum lipid peroxides.

3.3.2 Necropsy

At day 27, after the blood was taken, the hamsters were killed by decapitation under CO_2 anaesthesia. Their hearts and livers were excised and cleaned of blood with ice cold saline. The livers were placed in pre-weighed polypropylene test tubes, which were reweighed to determine their weight. The livers were dipped in liquid nitrogen to freeze rapidly and then stored at -80°C for subsequent analysis.

The heart was taken out of the thorax area and was freed of surrounding tissue in order to obtain an intact aorta. Any blood clots in the aorta were flushed gently with saline using a glass pasteur pipette which was inserted into the left ventricle. Each heart was transferred into a 3.0 ml plastic cylinder which contained enough saline to cover the heart to store the organ overnight. After overnight storage, the aorta was placed side up on a wine cork disk of 2 mm thick with embedding medium to maintain the aorta in place. Some 15.0 ml of 2-methyl butane was placed in a 30.0 ml beaker and pre-chilled in liquid nitrogen. The cork and aorta were dropped in 2-methyl butane and dipped into liquid nitrogen for 2.0 minutes. The aorta was transferred into a storage plastic tube containing 1.5 ml of 2-methyl butane and then stored at -80°C.

3.4 Analytical procedures

3.4.1 Determination of cholesterol content in dietary proteins and fats

The cholesterol content of dietary fats was determined by the method of Heuck et al. (1977) and this procedure was performed in an external laboratory (Dr Chavez, Macdonald Campus of McGill University, Ste-Anne-de-Bellevue, Québec) by the use of gas-liquid chromatography (GLC). On the other hand, dietary protein cholesterol concentrations were determined using the Park and Addis (1985) method. The cholesterol was extracted from the proteins using the Folch (1957) method. The cholesterol was then injected on a high performance liquid chromatography (Beckman model 126) equipped with a UV detector (System Gold diode array detector module 168, Beckman). The detection was done using a wavelength of 206 nm. Injection was done through an automatic injector (Varian 9095) onto a u-Porasil 10 um pore size 30 x 0.39 cm column (Waters Associates, Mass). Elution was performed with an isocratic gradient of 7% isopropanol and 93% hexane at a flow rate of 0.95 ml/minute. All solvents used were omnisovl grade (BDH). Standard curves of cholesterol, ranging from 25 ppm to 500 ppm remained linear with a minimum r^2 value of 0.99 for each individual standard curve (n=4). These standard curves were used to quantify cholesterol from dietary proteins.

3.4.2 **Fatty acid analysis of dietary fats**

Fatty acid composition of the dietary fats was determined by gas-liquid chromatography after methyl esterification. The samples were done in triplicates for fatty acid methyl esters (FAME) formation by the method of Morrison and Smith (1964). Heptadecanoic acid (C17:0) was added as an internal standard to each sample before esterification. FAME were then separated and quantified by GLC (3700 Gas Chromatograph, Varian, Montréal, Québec) with a 1.82m X 6.3 mm glass column packed with 10% silar 10CP on 100/120 chromosorb Supelcoport (Supelco, Oakville, Ontario) and a Shimadzu C-R6A Chromatopac integrator (Fisher Scientific, Montréal, Québec). The carrier gas was helium which was set at a flow rate of 20 ml/minute. Injector and detector temperatures were maintained at 220°C and 230°C, respectively. The column oven temperature program ran from 160°C to 200°C at a rate of 2°C per minute. FAME were identified by comparing peak retention times with those of standards (Nu-Chek Prep, Elysian, MN) and quantified by comparison with the internal standard response.

3.4.3 **Serum alanine aminotransferase**

Serum alanine aminotransferase was measured because it is an enzyme found in high concentrations in the liver. This enzyme is present in high levels in the blood when liver toxicity occurs. Serum alanine aminotransferse was analyzed by an enzymatic assay

(Sigma, St. Louis, Missourri) using the Abbott VP Super System (Irving, Texas). This method is based on the procedure of Wroblewski and LaDue (1956) who were the first to report the spectrophotometric determination of alanine aminotransferase in serum. This enzymatic reaction involves alanine animotransferase (ALT) which catalyzes the transfer of the amino group from alanine to 2-oxoglutarate, to form glutamate and pyruvate. The pyruvate formed is then reduced to lactate in the presence of lactate dehydrogenase with simulaneous oxidation of reduced nicotinamide adenine dinucleotide (NADH). The rate of decrease in absorbance at 340 nm is directly proportional to ALT activity. On the carousel of the discrete analyzer, one sample cup was filled with 50 microliter of deionized water, two cups carried controls and the other cups were filled with serum. The reconstituted alanine animotransferase reagent was dispensed to the samples and the absorbance was read using a 340/380 filter.

3.4.4 **Serum total cholesterol**

Serum total cholesterol was determined by an enzymatic assay (Abbott) using the Abbott VP Super System (Irving, Texas). This method is based on the procedure of Allain et al. (1974) and involves hydrolysis of cholesterol esters by cholesterol esterase to cholesterol and fatty acids. The cholesterol is then oxidized by cholesterol oxidase to yield cholest-4-en-3 one and hydrogen peroxide. The hydrogen peroxide reacts with chromagen, 4-aminoantipyrine and phenol in the presence of peroxidase to produce quinomeimine dye which has an absorbance maximum at 500 nm. The intensity of the

colour is proportional to the total amount of cholesterol in the serum. On the carousel of the discrete analyzer, one sample cup was filled with 50 microliter of deionized water, two cups contained standards, two cups carried controls and the other cups were filled with serum. The reconstituted cholesterol reagent was dispensed to the samples and the absorbance was read using a 500/600 filter.

3.4.5 **Total serum triglycerides**

Serum triglyceride levels were measured enzymatically by kit assay (Abbott) using the Abbott Super System (Irving, Texas). The triglycerides are hydrolysed by lipoprotein lipase to yield glycerol and free fatty acids. The glycerol is phosphorylated with ATP by glycerol kinase to glycerol 1-phosphate and ADP. The ADP is then coupled with phosphoenolpyruvate in the presence of pyruvate kinase to form pyruvate and ATP. The pyruvate is coupled with NADH with lactic dehydrogenase to yield NAD and lactate. The disappearance of NADH observed at 340 nm is a stoichiometric measure of the glycerol present which in turn is related to the triglyceride content of the sample. On the carousel of the discrete analyzer, one sample cup was filled with 50 microliter of deionized water, two cups carried controls and the other cups were filled with serum. The reconstituted triglyceride reagent was dispensed to the samples and the absorbance was read using a 340/380 filter.

3.4.6 Serum HDL-cholesterol

Serum HDL-cholesterol (HDL-c) was measured by determining its cholesterol content by the enzymatic assay described in section 3.3.1. The HDL fraction is separated from other lipoprotein fractions using polyanions and divalent cations precipitation method (dextran sulfate and magnesium). At neutral pH sulfated polysaccharides form insoluble complexes with serum LDL and VLDL in the presence of divalent cations. The formation of an insoluble lipoprotein-polyanion-metal ion complex occurs and it is dependent on the protein:lipid ratio in the lipoprotein. The insoluble complexes form more readily when the protein:lipid ratio is low. Therefore, VLDL precipitated more readily than LDL and LDL more readily than HDL. Selection of the proper concentration of polyanion and cation (0.02 mmole/liter of reagent of dextran sulfate and 1000 mmole/liter of reagent of magnesium chloride) allows the selective precipitation of lipoprotein fractions. A modification was brought to this method in that half of the serum (250 ul) and half the reagent (25 ul) were used to determine the HDL-c level in serum. The samples were vortexed for 3 seconds and were allowed to stand for five minutes at room temperature. The 1.0 ml polyethylene eppendorf tubes were centrifuged for 15 minutes at 1000xg. The supernatant was carefully removed and used for the HDL cholesterol determination. On the carousel of the discrete analyzer, one sample cup was filled with 50 microliter of deionized water, two cups contained standards, two cups carried controls and the other cups were filled with serum. The reconstituted cholesterol reagent was dispensed to the samples and the absorbance was

read using a 500/600 filter.

3.4.7 **Serum apolipoprotein A-I**

Serum apolipoprotein A-I (Apo A-I) was measured by an immunoturbidity assay (Sigma, St. Louis, Missourri). Apo A-I in serum combines with specific antibody present in the reagent and forms an insoluble complex resulting in turbidity of the assay mixture. The amount of turbidity formed is proportional to the Apo A-I concentration in the sample. The turbidity was measured in a Du-40 Beckman spectrophotometer at 340 nm and the concentration of Apo A-I in the sample was determined from a calibration curve obtained using the multi-level Apo A-I calibrators. To 2.5 ul of blank (deionized water), controls, calibrators and serum, 0.5 ml of working Apo A-I antibody reagent was added in a 1.5 ml polystyrene cuvette in a 20 second time interval and mixed promptly. The samples were incubated at room temperature (23°C) for 15 minutes. The absorbance was read on a DU-40 Beckman spectrophotometer at 340 nm and recorded using the same 20 second time interval. The blank absorbance value of each control, calibrator and serum was subtracted from the respective test value to remove absorbance caused by sample turbidity.

3.4.8 **Serum apolipoprotein B**

Serum apolipoprotein B (Apo B) was measured by an immunoturbidity assay

(Sigma, St. Louis, Missourri). Apo B in serum combines with specific antibody present in the reagent and forms an insoluble complex resulting in turbidity of the assay mixture. The amount of turbidity formed is proportional to the Apo B concentration in the sample. The turbidity was measured by a DU-40 Beckman spectrophotometer at 340 nm and the concentration of Apo B in the sample was determined from a calibration curve obtained using the multi-level Apo B calibrators. To 5.0 ul of blank (deionized water), controls and serum, 0.5 ml of working Apo B antibody reagent was added in a 1.5 ml polystyrene cuvette in a 20 second time interval and mixed promptly. The samples were incubated at room temperature (23°C) for six minutes. The absorbance was read and recorded using the same 20 second time interval. The blank absorbance value of each control, calibrator and serum was subtracted from the respective test value to remove absorbance caused by sample turbidity.

3.4.9 **Lipid peroxidation**

3.4.9 a **Fat extraction**

The fat from the diets and livers was extracted using the Folch (1959) method. This procedure involved the addition of a chloroform:methanol solution (2:1) at a level of 20 fold and water at a level of 0.2 fold to a tissue. The livers were previously homogenized using about 4.0 g of liver with 4.0 ml of saline (0.9%). From the liver homogenate, 200 ul was taken and vortexed with the cholroform:methanol solution (2:1)

and water. The solution was then centrifuged for five minutes at 580Xg and the supernatant was discarded. From the chloroform layer, 600 ul were placed in a 13x100 glass test tube and dried down under nitrogen. The same treatment was applied to the diet samples except that 1.5 g of diet were used with the appropriate solvent ratio.

3.4.9 b **Liver, serum and dietary lipid peroxidation determination**

Liver, serum and dietary lipid peroxidation were determined using the Kamiya Biomedical's Determiner LPO (Thousand Oaks, CA) for quantitative determination of lipid peroxides. Haemoglobin catalysed the reaction of hydroperoxides with methyl blue derivative (MBDP), forming an equal molar concentration of methyl blue. Lipid peroxides were quantitatively determined by measuring, colorimetrically at 675 nm, the methylene blue formation. The fat was extracted from the diet and liver using the Folch (1959) method previously described. The fat obtained was reconstituted in different quantities of isopropanol (different dilution) because of the various amount of fat in the samples. To 10 ul of the isopropanol mixture or to 10 ul of serum, 100 ul of lyophilized reagent was added. The mix was incubated for 3 minutes at 30°C. To the mixture, 200 ul of methylene blue derivative was added and then the blend was incubated for 10 minutes at 30°C. The samples were placed on a microplate and scanned. The absorbance of the sample was determined with a microplate reader (Titertek Multiskan Plus MKII, ICN Biomedicals, Cleveland, Ohio) using a 620 filter. The standard curve was obtained by using cumene hydroperoxide as a reference standard. The advantage of this

hemoglobin-methylene blue method (HMB) is its increased specificity and sensitivity for unsaturated lipid peroxides as opposed to measuring thiobarbituric acid-reactive substances. The HMB method measures early events (hydroperoxides) in the mechanism of lipid peroxidation.

3.4.10 **Thiobarbituric acid assay**

Liver and dietary malonaldeyhde and malonaldehyde secondary products were determined using the 2-thiobarbituric acid assay for quantitative determination of lipid peroxide contents. Free malonaldehyde and its degradation products reacts with 2-thiobarbituric acid (TBA) to produce a red pigment. The reaction with TBA occurs by attack of the monoenolic form of malonaldehyde on the active methylene group of TBA, following ring closure. The method used was adapted from the one described by Dahle et al. (1962). The fat was extracted in duplicates from the diet and liver using the Folch (1959) method previously described. The fat samples were diluted to a concentration of 0.5 mg/ml in 40 % ethanol. Then, 500 ul of this dilution was taken and mixed with 500 ul of 40 % pure ethanol in a 16x100 test tube. To this 1.0 ml solution, 2.0 ml of phosphate buffer (pH=6) was added to provide a homogeneous colloidal dispersion. This was followed by the addition of 1.0 ml of 20 % tricholoracetic acid and 2.0 ml of a 0.067 % acetic acid solution of TBA. The samples were then incubated for 15 minutes at 100°C and allowed to cool down for 15 minutes. The heating of the samples and the addition of glacial acetic acid to dissolve TBA is essential for the liberation of

malonaldehyde from precursor as well as for the condensation of malonaldehyde with TBA. The absorbance was measured at 535 nm on a DU-40 Beckman spectrophotometer. Standard curves were run using malonaldehyde bis (diethyl acetate) as a standard reference. The micromoles of malonaldehyde and its degradation products were calculated from the standard curves. The TBARS method is an indicator of the end results of lipid peroxidation since it measures a relatively late event of lipid peroxidation. The thiobarbituric acid reagent reacts with compounds other than malonaldehyde and therefore, the TBARS method is less specific and sensitive than the HMB method.

3.4.11 **Grading atherosclerosis**

The heart was sectioned midway through the atria and the aorta was cut distal to the sinus valves and fixed on a cork. Cryostat frozen sections, 10 um thick, were cut at three different levels and stained with oil red O and counterstained with hematoxylin. The sections were examined microscopically, and a similar section of each aorta was scored from 0 to 10 according to a modification of the method of Roberts and Thompson. The histology was performed on 50% of hearts, taken randomly, in each diet group and the grading of the aorta was performed in Dr Stewart-Phillips laboratory (Montréal General Hospital Research Institute, Montréal, Québec).

3.5 Statistical analyses

A two-way analysis of variance containing the four diet groups (time and dietary treatments) was used when parameters were measured at the begining and at the end of the experiment: body weight, serum total triglyceride, serum total cholesterol, serum HDL, total cholesterol/HDL ratio, dietary LPO and dietary TBARS. Cumulative food intake, liver weight, liver weight as a percentage of body weight, serum alanine aminotransferase, body weight change, serum total triglyceride change, serum total cholesterol change, serum HDL change, total cholesterol/HDL ratio, serum apolipoprotein A-I, B and the ratio between apolipoprotein A-I/B, serum LPO, liver LPO and liver TBARS were analyzed by a one-way analysis of variance (ANOVA) in order to determine if there was a main effect of diet on these parameters. When there was a main effect, treatment mean differences were identified by LSMEANS. The values were expressed as means with appropriate standard error for all parameters measured except for dietary lipid peroxidation. This parameter was expressed as LSMEANS with LSMEANS standard error because means are not given by the SAS program when there is an interaction (time*dietary treatment). Model statement, F and p values from the statistical analysis of each independant variable are listed in Appendix 1, 2.1-2.3. Pearson's correlation was also run for many parameters and the p values are given in Appendix 2.4-2.8. A probability of $p < 0.05$ was accepted as the minimal level of significance for all analyses. All analyses were performed using the Statistical Analysis System for personal computers (SAS Institute, Cary, NC; Version 6.04, 1992).

Table 3.1 Composition of experimental diets

Ingredients	Butterfat/casein diet (g/kg)	Butterfat/egg white diet (g/kg)	Safflower oil/egg white diet (g/kg)	Menhaden oil/egg white diet (g/kg)
Vitamin free casein[1]	150	-	-	-
Egg white[2]	-	150	150	150
Cornstach[3]	538	538	537.5	538.3
Butterfat[4]	177.6	177.6	-	-
Safflower oil[5]	16	16	166	16
Menhaden oil[6]	-	-	-	150
Cholesterol[7]				
Naturally occurring	0.430	0.443	0.0182	0.882
Added	0.440	0.440	0.864	0.00
Total	0.870	0.883	0.882	0.882
BHT[8]	1.7	1.7	1.7	1.7
Cellulose[9]	50	50	50	50
Vitamin mix[10,11]	14	14	14	14
Mineral mix[12]	80	80	80	80
Total weight	1027.74	1027.74	1000.0	1000.0

[1] Vitamin free casein, US Biochemical Corporation, Cleveland, Ohio.

[2] Albumin egg, US Biochemical Corporation, Cleveland, Ohio.

[3] Cornstarch, ICN Biochemicals Canada Ltd., Montréal, Québec.

[4] Butter, unsalted "My Country" (Lactancia Ltd, Victoriaville, Québec)

[5] Safflower oil (US Biochemical Corporation, Cleveland, Ohio).

[6] Menhaden oil (US Biochemical Corporation, Cleveland, Ohio).

[7] Cholesterol USP (ICN Biochemicals Canada Ltd., Montréal, Québec) added to safflower oil and butterfat diets at 864 and 440 (mg/kg diet) respectively, no cholesterol added to menhaden oil diet because it naturally contains 5.76 mg of cholesterol/g of oil. Butterfat naturally contains 2.39 mg of cholesterol/g of butterfat and safflower oil has 0 mg of cholesterol/g of safflower oil (refer to Table 3.10) The naturally occurring cholesterol content of diets were not calculated in the total weight of the diets.

[8] Butylated hydroxytoluene, ICN Biomedicals, Cleveland, OH.

[9] Alphacel, ICN Biochemicals Canada Ltd., Montréal, Québec.

[10] Vitamin mix for butterfat-based diets provided the following (mg/kg diet): niacin 135.0; calcium pantothenate 60.0; riboflavin 22.5; pyridoxine 9.0; thiamin 30.0; folic acid 3.0; biotin 0.9; cyanocobalamine 0.015; menaquinone 6.0; choline 6000.0; alpha-tocopheryl acetate (1 350 IU/g) 6.1; cholecalciferol (400 000 IU/g) 9.32; vitamin A palmitate (250 000 IU/g) 18.5.

[11] Vitamin mix for safflower and menhaden oil diets provided the following (mg/kg diet): niacin 135.0; calcium pantothenate 60.0; riboflavin 22.5; pyridoxine 9.0; thiamin 30.0; folic acid 3.0; biotin 0.9; cyanocobalamine 0.015; menaquinone 6.0; choline 6000.0; alpha-tocopheryl acetate (1 350 IU/g) 6.1; cholecalciferol (400 000 IU/g) 9.32; vitamin A palmitate (250 000 IU/g) 40.2.

[12] Mineral mix provided the following (g/kg diet): $CaCO_3$ 22.48; K_2HPO_4 25.16; NaCl 5.72; $MgSO_4$ 4.46; $CuCO_3.Cu(OH).H_2O$ 0.0045; $CoCl_2.6H_2O$ 0.0666; NaF 0.00008; KIO_3 0.0040; $FeSO_4.7H_2O$ 1.045; $MnCO_3$ 0.0115; $NaSeO_3$ 0.00033; $ZnCO_3$ 0.0265.

Table 3.2 Total fatty acid composition of dietary fats (wt % of total fatty acids) as measured by the method of Morrison and Smith (1964).

	Dietary fats		
Fatty acids[1]	Butterfat	Safflower oil	Menhaden oil
C4:0	3.3		
C6:0	2.3		
C8:0	1.6		
C10:0	3.9		
C12:0	4.0		
C14:0	12.5	0.3	15.2
C14:1	1.2		
C16:0	33.1	11.9	22.3
C16:1 n - 9	1.7		12.7
C18:0	11.6	2.3	3.9
C18:1 n - 9	21.0	14.1	10.0
C18:2 n - 6	3.8	70.9	2.8
C18:3 n - 3		0.5	1.1
C18:4 n - 3			4.5
C20:1 n - 9			1.4
C20:4 n - 6			0.5
C20:5 n - 3			12.7
C22:1 n - 9			1.3
C22:4 n - 3			0.5
C22:5 n - 3			1.5
C22:6 n - 3			9.6

[1] Fatty acids are designated by chain length, number of double bonds and position of first bond from the methyl end of the molecule.

Table 3.3 Amino acid composition of casein and egg white (g amino acid/100 g protein) given by US Biochemical Corporation

	Dietary proteins	
Amino Acids	Casein	Egg white
Alanine	2.6	4.9
Arginine	3.6	4.6
Aspartic acid	6.4	8.2
Cystine	0.3	2.2
Glutamic acid	20.2	10.5
Glycine	2.4	2.8
Histidine	2.7	1.9
Isoleucine	5.5	4.3
Leucine	8.3	6.8
Lysine	7.4	5.1
Methionine	2.5	3.0
Phenylalanine	4.4	4.7
Proline	10.2	3.1
Serine	5.7	5.5
Threonine	4.4	3.6
Tryptophane	1.1	1.3
Valine	6.5	5.6
Arginine/lysine ratio	0.49	0.9

Table 3.4 Composition of vitamin mixture

Ingredient	mg/kg diet	NRC requirement mg/kg diet	Supplier
Niacin	135.0	90.0	ICN
Calcium pantothenate	60.0	40.0	US Biochemical
Riboflavin	22.5	15.0	ICN
Pyridoxine	9.0	6.0	ICN
Thiamin	30.0	20.0	ICN
Folic acid	2.0	2.0	ICN
Biotin	0.9	0.6	ICN
Cyanocobalamine	0.015	0.01	ICN
Menaquinone	6.0	4.0	ICN
Choline	6 000.0	2 000.0	ICN
Alpha-tocopheryl acetate (IU)	6.1	4.05	ICN
Cholecalciferol (IU)	3 726.0	2 484.0	ICN
Vitamin A palmitate[1] (IU)	4 619-10 050.0	6 700.0	ICN
Inositol	150.0	100.0	Anachemia

[1] Butter already contains 5431 IU per 177.6 g of butter therefore only 4619 IU was added so that all diets would have the same amount of vitamin A.

Table 3.5 Composition of vitamin mix for butterfat-based diets

Ingredient	Grams	g/kg diet
	Fat soluble	
Vitamin A palmitate (250 000 IU/g)	0.92	
Cholecalciferol (400 000 IU/g)	0.46	
Alpha-tocopheryl acetate (1 350 IU/g)	0.23	
Menaquinone	0.3	
Cellulose	198.09	
Total	200.0	4
	Water soluble	
Niacin	13.5	
Calcium pantothenate	6	
Riboflavin	2.25	
Thiamin	3	
Pyridoxine	0.9	
Folic acid	0.3	
Biotin	0.09	
Cyanocobalamine	0.0015	
Inositol	15	
Cellulose	358.96	
Total	400.00	4
	Choline chloride	6

Table 3.6 Composition of vitamin mix for safflower oil and menhaden oil diets

Ingredient	Grams	g/kg diet
	Fat soluble	
Vitamin A palmitate (250 000 IU/g)	2.01	
Cholecalciferol (400 000 IU/g)	0.46	
Alpha-tocopheryl acetate (1 350 IU/g)	0.23	
Menaquinone	0.3	
Cellulose	197.0	
Total	200.0	4
	Water soluble	
Niacin	13.5	
Calcium pantothenate	6	
Riboflavin	2.25	
Thiamin	3	
Pyridoxine	0.9	
Folic acid	0.3	
Biotin	0.09	
Cyanocobalamine	0.0015	
Inositol	15	
Cellulose	358.96	
Total	400.00	4
	Choline chloride	6

Table 3.7 Composition of mineral mix

Ingredient	g/kg salt	g/kg diet @ 8.0%	Supplier
$CaCO_3$	281.00	22.48	Anachemia
K_2HPO_4	314.50	25.16	ICN
NaCl	71.50	5.72	Anachemia
$MgSO_4$	55.75	4.46	ICN
$CuCO_3 \cdot Cu(OH) \cdot H_2O$	0.05625	0.0045	Anachemia
$CoCl_2.6H_2O$	0.0530	0.00666	ICN
NaF	0.0010	0.00008	Anachemia
KIO_3	0.0506	0.00405	Anachemia
$FeSO_4.7H_2O$	13.175	1.054	Anachemia
$MnCO_3$	0.144	0.0115	Anachemia
$NaSeO_3$	0.004125	0.00033	ICN
$ZnCO_3$	0.331	0.0265	ICN
subtotal	739.18	59.13	
Citric acid	260.82	20.87	
Total	1000.00	80.00 = 8.0%	

Table 3.8 Elemental composition of salt mixture

Ingredient (MW)	mg/kg diet	Cu	Co	F	I	Fe	Mn	Se	Zn
$CuCO_3.Cu(OH)$ H_2O (239.19)	4.5	2.4							
$CoCl_2.6H_2O$ (237.84)	6.66		1.65						
NaF (41.99)	0.08			0.036					
KIO3 (214.02)	4.05				2.4				
$FeSO_4.7H_2O$ (278.01)	1045.35					210			
$MnCO_3$ (114.94)	11.46						5.48		
$NaSeO_3$ (172.92)	0.33							0.15	
$ZnCO_3$ (125.38)	26.47								13.8
Total	1098.9	2.4	1.65	0.036	2.4	210	5.48	0.15	13.8
NRC-reqt (1978)		1.6	1.1	0.024	1.6	140	3.65	0.1	9.2
Ratio		1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5

Table 3.9 Elemental composition of macroelements

Ingredient (MW)	mg/100g diet	Ca	K	Na	P	Mg
$CaCO_3$ (100.09)	2248.00	900				
K_2HPO_4 (174.16)	2516.25		1135.5		450	
NaCl (58.44)	571.95			225		
$MgSO_4$ (120.38)	445.76					90
Total	5781.96	900	1135.5	225	450	90
NRC Reqt.1978		590	610	150	300	60
Ratio		1.52	1.86	1.5	1.5	1.5

Table 3.10 Cholesterol content[1] of dietary fats and proteins 59

Dietary fats and proteins	Cholesterol content (mg/g of fat or protein)
Dietary fats	
Butterfat 1	2.46
Butterfat 2	2.32
Average cholesterol content of butterfat	2.39
Safflower oil 1	0.00
Safflower oil 2	0.00
Average cholesterol content of safflower oil	0.00
Menhaden oil 1	5.74
Menhaden oil 2	5.79
Average cholesterol content of menhaden oil	5.76
Dietary proteins	
Casein 1	0.0343
Casein 2	0.0313
Average cholesterol content of casein	0.0343
Egg white 1	0.13179
Egg white 2	0.11070
Average cholesterol content of egg white	0.12145

[1] Each cholesterol determination listed on the table was done in duplicates.

Section 4: Results

4.1 Part one: Comparison of egg white versus casein on butterfat-induced hyperlipidemia

4.1.1 Food intake and body weight change:

Cumulative food intake and body weight change were analyzed and the data are found in Table 4.1.

Cumulative food intake during the 27 days of experiment did not differ significantly between the animals fed the either casein or egg white (Table 4.1). The initial body weight as well as the final body weight were not significantly different between the two diet groups. Body weight change (body weight final - body weight initial) was significantly greater with the butterfat/casein diet compared to the butterfat/egg white diet (Table 4.1).

4.1.2 Liver weight:

Liver weight was expressed as an absolute value and calculated as a percent of total body weight (Table 4.1).

Liver weight as an absolute value and as a percent of body weight were not statistically different between the butterfat/casein and the butterfat/egg white diets (Table 4.1).

4.1.3 **Serum alanine aminotransferase:**

Serum alanine aminotransferase was analyzed and the data are found in Table 4.1.

Serum alanine aminotransferase was not significantly different between the casein and egg white diets (Table 4.1). There was no correlation between the serum alanine aminotransferase and the ratio between liver weight/body weight for the casein and egg white butterfat-based diets but there was a correlation when all the dietary treatments (butterfat-based and egg white-based diets) were grouped together ($r=0.29$; $p<0.05$).

4.1.4 **Total serum triglycerides:**

Total serum triglycerides was analyzed to examine the effects of different proteins on lipid metabolism; the data are described in Table 4.2.

Initial total serum triglycerides were not different between butterfat/casein and butterfat/egg white diets (Table 4.2). However, there was a significant increase in final total serum triglycerides ($p<0.05$) in the butterfat/casein group. Although close to significance ($p=0.07$), there was no effect of the kind of protein upon serum triglyceride concentration when the change in total serum triglycerides (total serum triglycerides final - total serum triglycerides initial) was analyzed.

4.1.5 **Total serum cholesterol:**

Total serum cholesterol was analyzed to examine the effects of different diets on lipid metabolism. The data are described in Table 4.2.

There was no statistical differences in initial total serum cholesterol between the

butterfat/casein and butterfat/egg white diets (Table 4.2). Final total serum cholesterol and the change in total serum cholesterol was significantly greater in the butterfat/casein group than in the butterfat/egg white group.

4.1.6 **Serum HDL-c**:

Serum cholesterol of high density lipoprotein (HDL-c) was analyzed to determine the effects of different dietary proteins on lipid metabolism; the data are summarized in Table 4.3.

Initial serum HDL-c, final serum HDL-c and the change in HDL-c (serum HDL-c final - serum HDL-c initial) were not affected by the kind of protein in the diet (Table 4.3).

4.1.7 **Ratio of total serum cholesterol and high density lipoprotein TC/HDL-c**

The ratio between total serum cholesterol and HDL-c was analyzed and the data are presented in Table 4.3.

Initial TC/HDL-c, final TC/HDL-c and the change in TC/HDL-c (TC/HDL-c final - TC/HDL-c initial) was not significantly different for the two diet groups (Table 4.3).

4.1.8 **Serum apolipoprotein A-I, apolipoprotein B and apolipoprotein A-I/apolipoprotein B ratio**

Serum apolipoprotein A-I, serum apolipoprotein B and the ratio between serum

apolipoprotein A-I/apolipoprotein B were analyzed to examine the effects of dietary proteins on lipid metabolism; the data are summarized in Table 4.4.

The source of protein did not affect the serum apolipoprotein A-I, apolipoprotein B levels and the ratio between serum apolipoprotein A-I/apolipoprotein B (Table 4.4).

4.1.9 **Serum oxidation products (LPO):**

Serum oxidation products (LPO) was analyzed to examine the lipid oxidation; the data are described in Table 4.5.

The level of serum oxidation products was affected by the kind of protein in the diet ($p < 0.05$). The butterfat/casein group had a significantly higher concentration of serum LPO than did the animals fed the butterfat/egg white diet. There were no correlations between serum LPO and liver TBA, liver LPO, total serum cholesterol (difference), total serum triglycerides (difference), serum HDL-c (difference), total serum cholesterol/serum HDL-c ratio (difference), body weight (difference), liver weight, serum apolipoprotein A-I, serum apolipoprotein B and the ratio between serum apolipoprotein A-I and apolipoprotein B in individual butterfat-based diets (see Appendix 2.7a and 2.7b). As a group, the only variables that were shown to correlate with serum LPO were serum apolipoprotein B ($r=0.52$; $p<0.011$) and the ratio between serum apolipoprotein A-I and apolipoprotein B ($r=-0.84$; $p<0.002$). When all the dietary treatments (butterfat-based and egg white-based diets) were grouped together, there were correlations between serum LPO and total serum cholesterol (difference) ($r=0.42$; $p<0.004$), total serum triglycerides (difference) ($r=0.47$; $p<0.0009$), total serum cholesterol/serum HDL-c

ratio (difference) ($r=0.43$; $p<0.007$), serum apolipoprotein B ($r=0.69$; $p<0.0001$) and the ratio between serum apolipoprotein A-I and apolipoprotein B ($r=-0.72$; $p<0.0004$).

4.1.10 **Liver oxidation products (LPO):**

Liver oxidation products were analyzed to examine the lipid oxidation; the data are presented in Table 4.5.

The concentration of lipid peroxides in the liver was affected by the source of protein ($p<0.05$). Animals fed the butterfat/casein diet had significantly higher liver oxidation product concentrations than did the animals fed the butterfat/egg white diet (Table 4.5). There were no significant correlations between liver LPO and total serum cholesterol (difference), serum triglycerides (difference), serum HDL-c (difference), ratio of total serum cholesterol/serum HDL-c (difference), serum apolipoproteins A-I, B and apolipoprotein A-I/B ratio, body weight, liver weight and liver weight/body weight ratio when butterfat/casein and butterfat/egg white were grouped together (see Appendix 2.5a and 2.5b).

4.1.11 **Liver TBA**

Liver TBA was analyzed to examine the lipid oxidation; the data are presented in Table 4.5.

TBA concentration in the livers was not affected by the source of protein in the diet (Table 4.5). There was also no correlation between liver TBA concentration and total serum cholesterol (difference), total serum triglycerides (difference), serum HDL-c

(difference), ratio of total serum cholesterol/serum HDL-c (difference), serum apolipoprotein A-I, B and apolipoprotein A-I/B ratio, body weight, liver weight and liver weight/body weight ratio in the butterfat/casein and butterfat/egg white diets (see Appendix 2.6a and 2.6b).

4.1.12 **Dietary lipid oxidation products:**

The dietary lipid oxidation products were analyzed and the data are found in Tables 4.6.

Dietary LPO differed significantly between the diets, time and the diet-time interaction (Table 4.6). From week 0 to week 2, there were no significant differences between the two diet groups, but at week 3 and 4, the butterfat/casein diet had a more elevated concentration of LPO than the butterfat/egg white diet. The difference between week 4 and week 0 (LPO concentration week 4 - LPO concentration week 0) was significantly higher ($p < 0.035$) in the butterfat/casein diet than in the butterfat/egg white diet (Table 4.7).

4.1.13 **Dietary TBA:**

There were no by-products of lipid degradation present in any of the two dietary treatments at any time.

4.1.14 **Atherosclerotic lesions:**

No aortic atherosclerotic lesions of any form were observed in any animals fed

the butterfat/casein diet and the butterfat/egg white diet.

4.2 Part two: Comparison of dietary fats on blood lipids

4.2.1 Food intake and body weight change:

Cumulative food intake and body weight change were analyzed and the data are found in Table 4.1.

There were no significant differences in cumulative food intake among the butterfat/egg white, safflower oil/egg white and menhaden oil/egg white diets. The initial body weights and the final body weights were not significantly different between the three diet groups (Table 4.1). Body weight change (body weight final - body weight initial) significantly decreased in the menhaden oil group compared to the butterfat and the safflower groups. No differences in body weight change were found between the butterfat and the safflower groups.

4.2.2 Liver weight:

Liver weight was expressed as an absolute value and calculated as a percent of total body weight (Table 4.1).

Absolute liver weight was not affected by the type of fat. Liver weight as a percent of body weight, however, was significantly greater in the hamsters fed the menhaden oil diet versus those fed the two other diets (Table 4.1).

4.2.3 Serum alanine aminotransferase:

Serum alanine aminotransferase was analyzed and the data are found in Table 4.1.

Serum alanine aminotransferase was not significantly different among the three dietary groups (Table 4.1). There was also no correlation between the serum alanine aminotransferase and the ratio between liver weight/body weight within each of the three diets. There was, however, a correlation when all the three dietary treatments were grouped together ($r=0.43$; $p<0.009$) and when all of the dietary groups were assembled together($r=0.29$; $p<0.05$) (see Appendix 2.8).

4.2.4 Total serum triglycerides:

Total serum triglycerides was analyzed to examine the effects of the different dietary fats on lipid metabolism; the data are described in Table 4.2.

There were no significant differences in the initial serum triglycerides among the three dietary groups (Table 4.2). Final total serum triglycerides and the change (total serum triglycerides final - total serum triglycerides initial) of total serum triglycerides were both greater in the menhaden oil diet than in the two other diets. There were no significant differences in final and change total in serum triglycerides for the butterfat and safflower groups.

4.2.5 **Total serum cholesterol:**

Total serum cholesterol was analyzed to examine the effects of different type of fat on lipid metabolism; the data are described in Table 4.2.

There were no statistical differences in the initial total serum cholesterol among the three dietary treatments (Table 4.2). Final total serum cholesterol and the cholesterol change were affected by the kind of fat. Hamsters fed the menhaden oil diet had the greatest concentration of final total serum cholesterol and total serum cholesterol change than the hamsters fed the safflower oil and butterfat diets. The butterfat-fed hamsters had a higher level of final total serum cholesterol and total serum cholesterol change than did the safflower-fed animals.

4.2.6 **Serum HDL-c:**

Serum cholesterol of high density lipoprotein (HDL-c) was analyzed to examine the effects of the different dietary fats on lipid metabolism; the data are summarized in Table 4.3.

The kind of fat had no significant effect on initial serum HDL-c, final serum HDL-c and the change in HDL-c (Table 4.3).

4.2.7 **Ratio of total serum cholesterol and high density lipoprotein TC/HDL-c**

The ratio between total serum cholesterol and HDL-c was analyzed to examine the effects of different fats on lipid metabolism; the data are presented in Table 4.3.

There were no significant differences in initial TC/HDL-c ratio among the three

dietary treatments (Table 4.3). The source of fat affected the final TC/HDL-c ratio ($p < 0.0003$) and TC/HDL-c ratio change with safflower oil-fed hamsters displaying a significantly lower ratio than the butterfat and the menhaden oil-fed animals.

4.2.8 Serum apolipoprotein A-I, apolipoprotein B and apolipoprotein A-I/apolipoprotein B ratio

Serum apolipoprotein A-I, apolipoprotein B and the ratio between apolipoprotein A-I/apolipoprotein B were analyzed to examine the effects of different dietary fats on lipid metabolism; the data are summarized in Table 4.4.

The source of fat affected the concentration of apolipoprotein B in serum and the ratio between apolipoprotein A-I and apolipoprotein B but not of apolipoprotein A-I (Table 4.4). Hamsters fed the menhaden oil diet had concentrations of apolipoprotein B significantly greater than did hamsters fed the butterfat and safflower oil diets. There were no significant differences between the apolipoprotein B concentrations of hamsters fed the butterfat and safflower diets. The menhaden oil fed animals had a significantly lower ratio of serum apolipoprotein A-I/B than the two other groups.

4.2.9 Serum oxidation products (LPO):

Serum oxidation products (LPO) were analyzed to examine the lipid oxidation; the data are described in Table 4.5.

The source of fat influenced the concentration of serum LPO ($p < 0.05$). The animals fed the menhaden oil diet had a significantly higher level of serum LPO than the

hamsters fed the safflower oil and butterfat diets. There were no differences in serum LPO between the hamsters fed the safflower oil and butterfat diets. There were no correlations between serum LPO and liver TBA, liver LPO, total serum cholesterol (difference), total serum triglycerides (difference), serum HDL-c (difference), total serum cholesterol/serum HDL-c ratio (difference), body weight (difference), liver weight, serum apolipoprotein A-I, serum apolipoprotein B and the ratio between serum apolipoprotein A-I and apolipoprotein B in individual butterfat and safflower oil diets. On the other hand, there were correlations with serum LPO and serum HDL-c (difference) ($r=-0.87$; $p<0.0021$), total serum cholesterol/serum HDL-c ratio (difference) ($r=0.85$; $p<0.004$), serum apolipoprotein B ($r=0.66$; $p<0.026$) and the ratio between serum apolipoprotein A-I and apolipoprotein B ($r=-0.93$; $p<0.023$) in the menhaden group alone. When the three dietary treatments were grouped together, the variables that were shown to possess a correlation with serum LPO were total serum cholesterol (difference) ($r=0.52$; $p<0.00143$, total serum triglycerides (difference) ($r=0.64$; $p<0.0001$), total serum cholesterol/serum HDL-c ratio (difference) ($r=0.53$; $p<0.0032$), serum apolipoprotein B ($r=0.77$; $p<0.0001$) and the ratio between serum apolipoprotein A-I and apolipoprotein B ($r=-0.71$; $p<0.003$). When all the four dietary treatments were grouped together, there were correlations between serum LPO and total serum cholesterol (difference) ($r=0.42$; $p<0.004$), total serum triglycerides (difference) ($r=0.47$; $p<0.0009$), total serum cholesterol/serum HDL-c ratio (difference) ($r=0.43$; $p<0.007$), serum apolipoprotein B ($r=0.69$; $p<0.0001$) and the ratio between serum apolipoprotein A-I and apolipoprotein B ($r=-0.72$; $p<0.0004$) (see Appendix 2.7a and 2.7b).

4.2.10 **Liver oxidation products:**

Liver oxidation products were analyzed to examine the lipid oxidation; the data are presented in Table 4.5.

Liver lipid oxide concentrations were not affected by the source of fat (Table 4.5). Although there was a tendency towards significance ($p=0.08$), no correlation was found between liver LPO and total serum cholesterol (difference) when the three dietary treatments were grouped together. No significant correlations between liver LPO and total serum triglycerides (difference), serum HDL-c (difference), ratio total serum cholesterol/serum HDL-c (difference), serum apolipoproteins A-I, B and the apolipoprotein A-I/B ratio, body weight, liver weight and liver weight/body weight ratio were observed from the three diets.

4.2.11 **Liver TBA**

Liver TBA was analyzed to examine the lipid oxidation; the data are presented in Table 4.5.

The type of fat did not influence significantly the level of hepatic TBA (Table 4.5). No correlation between liver TBA concentration and total serum cholesterol (difference), total serum triglycerides (difference), serum HDL-c (difference), ratio total serum cholesterol/serum HDL-c (difference), serum apolipoproteins A-I and B, body weight, liver weight and liver weight/body weight ratio was found in the three dietary treatments.

4.2.12 **Dietary lipid oxidation products:**

The dietary lipid oxidation products were analyzed and the data are found in Table 4.6.

Dietary LPO differed significantly between the diets, time and the diet-time interaction (Table 4.6). From week 0 to week 4, the safflower oil diet had a higher concentration of LPO than the menhaden oil diet which in turn had a significantly higher level of LPO than the butterfat diet. The difference between week 4 and week 0 (LPO concentration week 4 - LPO concentration week 0) was significantly higher ($p < 0.041$) in the butterfat diet than in the safflower oil diet. However, there were no significant differences between the safflower oil and the menhaden oil diets and between the butterfat and menhaden oil diets (Table 4.7).

4.2.13 **Dietary TBA:**

There were no by-products of lipid degradation present in any of the three dietary treatments at any time.

4.2.14 **Atherosclerotic lesions:**

No aortic atherosclerotic lesions of any form were observed in hamsters fed any of the three dietary treatments.

Table 4.1 **Effects of dietary proteins and fats on cumulative food intake, body weight, liver weight and serum alanine aminotransferase in hamsters[1,2,3]**

Diets	BF/CAS	BF/EGG	SAFF/EGG	MHO/EGG
Food intake[4] (g) Cumulative	(12) 175.13±8.73[a]	(12) 170.97±3.70[a,c]	(12) 162.98±3.73[c]	(12) 155.14±3.18[c]
Body weight initial[5] (g)	(12) 125.63±2.57[a]	(12) 126.80±2.46[a,c]	(12) 127.12±2.56[c]	(12) 126.93±2.42[c]
final[6] (g)	(12) 134.38±4.51[a]	(12) 128.60±4.15[a,c]	(12) 130.63±3.52[c]	(12) 125.00±2.33[c]
change[7] (g)	(12) 11.76±2.75[a]	(12) 4.40±2.10[b,c]	(12) 5.32±1.87[c]	(12) -1.92±1.44[d]
Liver weight[8] (g)	(12) 8.21±0.190[a]	(12) 8.14±0.205[a,c]	(12) 7.92±0.15[c]	(12) 8.34±0.091[c]
weight as a % body weight[9] (%)	(12) 6.15±0.138[a]	(12) 6.35±0.107[a,c]	(12) 6.08±0.067[c]	(12) 6.70±0.12[d]
Serum alanine aminotransferase[10] (U/L)	(12) 62.23±5.09[a]	(12) 72.02±8.36[a,c]	(12) 50.75±3.49[c]	(12) 60.77±4.21[c]

[1]Values are means ± SE. Number in parentheses equals number of hamsters. [2]Means within each parameters with different letters are significantly different ($p<0.05$) where [a] and [b] refer to comparisons between BF/CAS and BF/EGG, and [c,d,c] refer to comparisons amongst BF/EGG, SAFF/EGG and MHO/EGG. [3]Hamsters were fed butterfat/casein (BF/CAS), butterfat/egg white (BF/EGG), safflower oil/egg white (SAFF/EGG) and menhaden oil/egg white (MHO/EGG) diets for 27 days. [4-10]Significance of main effects from analysis of variance (ANOVA) refer to Appendix 2.1: [4]Dietary treatment (NS). [5-6]ANOVA: Dietary treatment (NS); time (NS); dietary treatment * time (NS). [7]ANOVA: Dietary treatment ($p<0.0005$).[8]ANOVA: Dietary treatment (NS). [9]ANOVA: Dietary treatment ($p<0.0013$). [10]ANOVA: Dietary treatment (NS).

Table 4.2 Effects of dietary proteins and fats on total serum triglycerides and total serum cholesterol in hamsters[1,2,3]

Diets	BF/CAS	BF/EGG	SAFF/EGG	MHO/EGG
Total serum triglycerides				
initial[4]	(12) 241.95±18.29[a]	(12) 230.33±10.19[a,c]	(12) 205.80±11.61[c]	(12) 200.03±14.15[c]
final[5] (mg/dl)	(12) 290.54±25.40[a]	(12) 223.96±21.35[b,c]	(12) 222.35±15.90[c]	(12) 303.95±25.65[d]
change[6] (mg/dl)	(12) 48.59±19.65[a]	(12) -6.37±22.25[a,c]	(12) 16.56±10.36[c]	(12) 103.92±20.06[d]
Total serum cholesterol				
initial[7] (mg/dl)	(12) 116.94±4.81[a]	(12) 108.87±5.73[a,c]	(12) 97.29±6.58[c]	(12) 100.58±4.49[c]
final[8] (mg/dl)	(12) 178.45±6.06[a]	(12) 154.67±7.54[a,c]	(12) 101.62±5.81[d]	(12) 183.86±6.97[c]
change[9]	(12) 61.51±6.36[a]	(12) 45.81±8.81[b,c]	(12) 4.33±8.72[d]	(12) 83.28±7.68[c]

[1]Values are means ± SE. Number in parentheses equals number of hamsters. [2]Means within each parameter with different letters are significantly different ($p<0.05$) where [a] and [b] refer to comparisons between BF/CAS and BF/EGG, and [c,d,e] refer to comparisons amongst BF/EGG, SAFF/EGG and MHO/EGG. [3]Refer to Table 4.1 for the meaning of acronyms. [4-9]Significance of main effects from analysis of variance (ANOVA); refer to Appendix 2.2: [4-5]ANOVA: Dietary treatment ($p<0.027$), time ($p<0.003$); dietary treatment * time ($p<0.025$). [6]ANOVA: Dietary treatment ($p<0.008$). [7-8]ANOVA: Dietary treatment ($p<0.0001$); time ($p<0.0001$); dietary treatment * time ($p<0.001$).[8]ANOVA: Dietary treatment (NS). [9]ANOVA: Dietary treatment ($p<0.0001$).

Table 4.3 Effects of dietary proteins and fats on serum HDL-c and total serum cholesterol TC/HDL-c ratio in hamsters[1,2,3]

Diets	BF/CAS	BF/EGG	SAFF/EGG	MHO/EGG
Serum HDL cholesterol				
initial[4] (mg/dl)	(10) 64.60±1.96[a]	(10) 59.12±2.33[a,c]	(10) 54.52±2.68[c]	(10) 54.29±2.00[c]
final[5] (mg/dl)	(12) 77.49±4.55[a]	(12) 71.38±3.86[b,c]	(12) 76.17±8.18[c]	(12) 83.04±8.80[c]
change[6] (mg/dl)	(10) 9.36±3.21[a]	(10) 6.05±2.65[a,c]	(10) 6.62±3.18[c]	(10) 10.87±5.52[c]
Ratio TC/HDL-c				
initial[7]	(10) 1.82±0.06[a]	(10) 1.82±0.06[a,c]	(10) 1.74±0.05[c]	(10) 1.86±0.05[c]
final[8]	(12) 2.37±0.15[a]	(12) 2.21±0.13[a,c]	(12) 1.45±0.11[d]	(12) 2.48±0.24[c]
change[9]	(10) 0.36±0.07[a]	(10) 0.40±0.17[a,c]	(10) -0.20±0.14[d]	(10) 0.74±0.28[c]

[1]Values are means ± SE. Number in parentheses equals number of hamsters. [2]Means within each parameter with different letters are significantly different ($p<0.05$) where [a] and [b] refer to comparisons between BF/CAS and BF/EGG, and [c,d,e] refer to comparisons amongst BF/EGG, SAFF/EGG and MHO/EGG. [3]Refer to Table 4.1 for the meaning of acronyms. [4-9]Significance of main effects from analysis of variance (ANOVA); refer to Appendix 2.3: [4-5]ANOVA: Dietary treatment (NS); time ($p<0.0001$); dietary treatment * time (NS). [6]ANOVA: Dietary treatment (NS). [7-8]ANOVA: Dietary treatment ($p<0.0002$); time ($p<0.0013$); dietary treatment * time ($p<0.0037$).[9]ANOVA: Dietary treatment ($p<0.0063$).

Table 4.4 Effects of dietary proteins and fats on serum apolipoprotein A-I, apolipoprotein B and on the ratio of apolipoprotein A-I/B in hamsters[1,2,3]

Diets	BF/CAS	BF/EGG	SAFF/EGG	MHO/EGG
Serum Apo A-I[4] (mg/dl)	(5) 59.80±1.71[a]	(5) 64.50±2.04[a,c]	(5) 62.80±1.53[c]	(5) 61.50±2.32[c]
B[5] (mg/dl)	(12) 18.58±1.70[a]	(12) 16.35±1.57[a,c]	(12) 15.21±1.20[c]	(12) 37.85±2.33[d]
Ratio Apo A-I/Apo B[6]	(5) 4.49±0.20[a]	(5) 5.32±0.43[a,c]	(5) 5.10±0.88[c]	(5) 1.77±0.17[d]

[1]Values are means ± SE. Number in parentheses equals number of hamsters. [2]Means within each parameter with different letters are significantly different ($p < 0.05$) where [a] and [b] refer to comparisons between BF/CAS and BF/EGG, and [c,d,e] refer to comparisons amongst BF/EGG, SAFF/EGG and MHO/EGG. [3]Refer to Table 4.1 for the meaning of acronyms. [4-6]Significance of main effects from analysis of variance (ANOVA) refer to Appendix 2.3: [4]Dietary treatment (NS). [5]ANOVA: Dietary treatment ($p < 0.0001$). [6]ANOVA: Dietary treatment ($p < 0.0005$).

Table 4.5 Effects of dietary proteins and fats on serum LPO, liver LPO and liver TBA in hamsters[1,2,3]

Diets	BF/CAS	BF/EGG	SAFF/EGG	MHO/EGG
Serum				
LPO[4] (nmol/ml)	(11) 33.89±10.11[a]	(12) 13.56±3.88[b,c]	(12) 20.23±5.90[c]	(12) 62.00±7.73[d]
Liver				
LPO[5] nmol/mg fat	(12) 4.77±0.73[a]	(12) 2.50±0.53[b,c]	(12) 1.36±0.23[c]	(12) 2.11±0.44[c]
TBA[6] nmol/mg fat	(11) 7.23±0.76[a]	(12) 8.98±2.02[a,c]	(11) 9.71±3.38[c]	(11) 10.78±1.82[c]

[1]Values are means ± SE. Number in parentheses equals number of hamsters. [2]Means within each parameter with different letters are significantly different ($p<0.05$) where [a] and [b] refer to comparisons between BF/CAS and BF/EGG, and [c,d,c] refer to comparisons amongst BF/EGG, SAFF/EGG and MHO/EGG. [3]Refer to Table 4.1 for the meaning of acronyms. [4-6]Significance of main effects from analysis of variance (ANOVA) refer to Appendix 2.4: [4]Dietary treatment ($p<0.0001$). [5]ANOVA: Dietary treatment ($p<0.0002$). [6]ANOVA: Dietary treatment (NS).

Table 4.6 Effects of oxidation (LPO) on diets[1,2,3]

Diets	Dietary LPO[4] nmol/g diet Week 0	Dietary LPO[5] nmol/g diet Week 1	Dietary LPO[6] nmol/g diet Week 2	Dietary LPO[7] nmol/g diet Week 3	Dietary LPO[8] nmol/g diet Week 4
BF/CAS	(2) 395.03±92.96[a]	(2) 476.24±92.96[a]	(2) 396.09±92.96[a]	(2) 1157.65±92.96[a]	(2) 1281.39±92.96[a]
BF/EGG	(2) 555.16±92.96[a,c]	(2) 536.33±92.96[a,c]	(2) 377.54±92.96[a,c]	(2) 827.82±92.96[b,c]	(2) 985.28±92.96[b,c]
SAFF/EGG	(2) 1554.51±92.96[d]	(2) 1367.72±92.96[d]	(2) 1664.19±92.96[d]	(2) 1776.71±92.96[d]	(2) 1514.31±92.96[d]
MHO/EGG	(2) 999.32±92.96[c]	(2) 1136.99±92.96[d]	(2) 967.17±92.96[c]	(2) 1170.26±92.96[c]	(2) 1212.31±92.96[c]

[1]Values are LSmeans ± LSmean SD. Number in parentheses equals number of diets. [2]Means within each parameter with different letters are significantly different ($p<0.05$) where [a] and [b] refer to comparisons between BF/CAS and BF/EGG, and [c,d,e] refer to comparisons amongst BF/EGG, SAFF/EGG and MHO/EGG. [3]Refer to Table 4.1 for the meaning of acronyms. [4-6]Significance of main effects from analysis of variance (ANOVA); refer to Appendix 2.4: [4-6] ANOVA: Treatment ($p<0.0001$); time ($p<0.0001$); treatment * time ($p<0.0011$).

Table 4.7 Effects of oxidation on diets[1,2,3]

Diets	Difference of dietary LPO[4] (week 4- week 0) (nmol/g of diet)
BF/CAS	(2) 22.07±3.22[a]
BF/EGG	(2) 9.42±0.22[b,c]
SAFF/EGG	(2) -2.63±3.39[d]
MHO/EGG	(2) 3.55±3.29[c,d]

[1]Values are means ± SD. Number in parentheses equals number of diets. [2]Means within each column with different letters are significantly different ($p<0.05$) where [a] and [b] refer to comparisons between BF/CAS and BF/EGG, and [c,d,e] refer to comparisons amongst BF/EGG, SAFF/EGG and MHO/EGG. [3]Refer to Table 4.1 for the meaning of acronyms. [4]Significance of main effects from analysis of variance (ANOVA); refer to Appendix 2.4: [4]ANOVA: Treatment ($p<0.015$).

Section 5: Discussion

5.1 Part one: Comparison of egg white versus casein on butterfat induced-hyperlipidemia

In this present study, the ability of dietary casein to induce hypercholesterolemia in the hamster has been investigated. The feeding of dietary casein produced a similar rise (34%) in total serum cholesterol levels to that reported in a previously published study exploring the effect of dietary casein in hamsters (Terpstra et al., 1991). In the earlier study, however, higher protein (25%) and cholesterol (0.1%) levels were fed than the in the present experiment (15% protein and 0.086% cholesterol). The increase in total serum cholesterol levels in the casein-fed hamsters is also in agreement with reports of casein-induced hypercholesterolemia using other animal species fed either with and without cholesterol (Carroll and Hamilton, 1975; Hamilton and Carroll, 1976; Yadav et al., 1977; Jaya et al., 1981; Terpstra et al., 1982; Terpstra et al., 1983; Meinertz et al., 1988, 1989; Lovati et al., 1990). Dietary egg white caused a smaller rise in serum cholesterol levels than did the casein feeding and this result is also in agreement with reports using other animal species such as rabbits (Carroll and Hamilton, 1975; Hamilton and Carroll, 1976) and rats (Neves et al., 1980; Sautier et al., 1986; Sugiyama and Muramatsu, 1990). Thus, the effects of dietary proteins on serum cholesterol levels in the present experiment validate that the hamster is a good experimental model of casein-induced hypercholesterolemia.

The hamsters fed the casein diet had a greater change in body weight compared to the animals fed egg white. This could be attributed to the higher nitrogen content found in the casein diet. The change in body weight of animals was put as a covariate in the statistical model for every lipid parameters. In this study, the age of the hamsters at the beginning of the experiment was ten weeks old (this age is considered "adult") and the hamsters were fed their respective diets for four weeks. The four weeks aging period probably did not create any disturbances in lipid metabolism since Terpstra et al. (1991) had used male Golden Syrian hamsters, which were nine weeks old at the start of the experiment, and fed the animals for a period of five weeks without reporting any disturbances in lipid metabolism.

Although casein feeding was associated with an increase in total serum triglycerides at the end of the four week feeding period, the difference between the total serum triglycerides at the beginning and the end of the experiment was not statistically significant ($p=0.07$). The lack of statistical significance in the present study could have been due to the large inter-animal variability of response. Casein compared to soy prtoein has been reported to be hypertriglyceridemic in hamsters (Terpstra et al., 1991).

The mechanism(s) by which the type of dietary protein affects plasma cholesterol levels in the hamster remains to be elucidated. Studies done in other experimental animals have indicated that various factors such as amino acid composition and digestibility of the proteins, excretion of faecal steroids and changes in liver cholesterol

synthesis (hepatic enzymes and lipoprotein receptor modifications) might be involved. The amino acid composition of egg white showed that it contained more cystine, alanine and arginine than casein; the latter comprised more glutamic acid, histidine, lysine and proline than egg white. Sugiyama et al. (1984, 1985, 1986a, 1986b) and Sautier et al. (1983) reported a significant negative correlation between plasma cholesterol and the cystine content in diets. Their results indicated that a higher cystine content in dietary proteins irrespective of animal or plant origin led to lower plasma cholesterol levels in rats fed the diets enriched with cholesterol. In rats fed cholesterol-free diets, Sautier et al. (1983, 1986) have shown that there were significant correlations between serum cholesterol levels and the content of several amino acids in dietary proteins. They found that serum cholesterol levels had a significant positive correlation with tyrosine and glutamic acid (casein has twice as much glutamic acid than egg white) and a negative correlation with cystine and alanine when four types of animal and plant proteins were fed (Sautier et al., 1983). They also found that serum cholesterol levels had a positive correlation with proline (casein has three times as much proline than egg white) and a negative correlation with arginine when eight kinds of protein were fed (Sautier et al., 1986). The addition of methionine along with cholesterol has also been found to be hypercholesterolemic by other researchers (Sugiyama et al., 1984, 1986b, 1986c). Histidine has also been shown to increase plasma cholesterol in rats when supplemented at 2.5% to a 25% casein cholesterol-enriched diet (Sugiyama et al., 1985), at 5% to a 10% casein cholesterol-free diet (Katayama et al., 1990) and at 5% to a chow diet (Solomon and Geiser, 1978). Some of the results on addition of amino acids to diets are

not always consistent because of the absence or presence of exogenous cholesterol in the diets fed and the level of amino acid fed. In the present study, the increased presence of cystine and alanine in egg white and the higher content of glutamic acid, histidine, lysine, proline in casein correlates well with the hypocholesterolemic and hypercholesterolemic properties of these two proteins.

Sugiyama and Muramatsu (1990) suggested several mechanisms underlying the effects of sulfur-containing amino acids on blood cholesterol. These authors stated that cystine is reduced to cysteine and in turn cysteine is metabolized to taurine or incorporated into glutathione. Taurine participates in bile acid conjugation and glutathione is one of the stimulators for the activity of 7-α hydroxylase, the rate limiting enzymes for bile biosynthesis. Therefore, the plasma cholesterol-lowering effect of cystine might act by stimulation of bile acid excretion via enhanced taurine and glutathione synthesis.

Sugiyama et al. (1989b) found that feeding a egg albumin diet containing high amounts of both methionine and cystine led to a decrease in plasma cholesterol levels. It was suggested that the plasma cholesterol-lowering effect of cystine (when present at approximately 2% in the diet) is stronger in the presence of about 3% methionine in rats fed cholesterol-enriched diets. When dietary cystine is added to a level of 1% to a 25% casein diet (which naturally contains a large amount of methionine), the total plasma cholesterol concentrations in rats decreased. The mechanism of how methionine exerts its hypercholesterolemic action has not yet been elucidated.

The arginine/lysine ratio of casein and egg white are 0.49 and 0.9 respectively. Although Kritchevsky (1979) suggested that a decreased arginine/lysine ratio of dietary protein is an important factor in the elevation of total plasma cholesterol concentrations, reports are conflicting as to the importance of this ratio especially when the single amino acids, lysine or arginine, were added to diets of rats (Sugano et al., 1982) and rabbits (Huff and Carroll, 1980). In contrast, the ratio appeared to determine plasma cholesterol levels when proteins having different ratios were fed to experimental animals (Kritchevsky et al., 1982 and Sugano et al., 1984). A negative correlation between the arginine concentrations of dietary proteins and hypercholesterolemia has been observed in various experimental animals (Ecklund and Sjoblom, 1980; Huff and Carroll, 1980; Vahouny et al., 1985 and Sautier et al., 1986). Vahouny et al. (1985) showed that the supplementation of arginine to a casein diet resulted in a reduction of serum d < 1.006 lipids and an elevation in hepatic 7α-hydroxylase activity therefore, suggesting that arginine increased bile acid synthesis. Lysine inhibits liver arginase activity, which leads to an accumulation of arginine at the hepatic level when lysine is present in high concentration (casein possesses 45% more lysine than egg white). Kritchevsky (1979) hypothesized that more arginine might be available in casein-fed animals to be incorporated into arginine-rich apolipoprotein E, a constituent of LDL which is atherogenic. Thus, dietary lysine would increase LDL production in the liver leading to increased total plasma cholesterol and prossibly atherosclerosis.

Histidine might increase serum cholesterol through an increase incorporation of

acetate and octanoate into cholesterol by the liver (Solomon and Geison, 1978).

Therefore, in the present study, the higher arginine and arginine/lysine ratio (0.90) in egg white and lower arginine/lysine ratio (0.49) in casein may explain the observed hypocholesterolemic and hypercholesterolemic effects of egg white and casein, respectively.

Dietary casein induced higher levels of serum, liver and dietary lipid peroxidation as measured by the haemoglobin-methylene blue method than did dietary egg white in this study. A similar finding was observed by Sugano et al. (1982) who reported higher levels of plasma TBARS in rats fed casein diets than in animals receiving a soy protein diet. Neither the addition of arginine to casein nor lysine to soy protein influenced lipid peroxidation. These workers postulated that the difference in lipid peroxidation may have been related to the observed changes in the serum cholesterol concentrations and liver lipids, since lipid peroxidation has been implicated as a possible cause of hypercholesterolemia and atherosclerosis (Vladimirov et al., 1980). The role of lipid peroxides in dietary induced hypercholesterolemia has been further suggested by Avriam et al. (1991) who reported that the feeding of a cholesterol-enriched coconut oil (saturated fat), casein based diet increased the plasma TBARS and macrophage uptake of plasma cholesterol in rats. Aviram et al. (1991) postulated that the enhanced peroxidation in the hypercholesterolemic rats might have resulted from the relatively reduced levels of lipoprotein antioxidants or from the occurrence of increased plasma

levels of polyunsaturated fatty acids which are highly susceptible to peroxidation. Dietary tryptophan supplementation has also been implicated in the elevation of plasma TBARS, in the enhancement of macrophage cholesterol esterification (Avriam et al., 1991) and in the platelet aggregation (Mokady et al., 1990) in rats. However, the effects of dietary tryptophan on cholesterol metabolism does not apply in this present study since the concentration of tryptophan was approximately the same in casein (1.1%) and in egg white (1.3%).

Methionine (Braddock and Duggan, 1973 and Gamage and Matsushita, 1973), lysine (Chiba et al., 1976), cystine and cysteine (Roubal, 1971) and tryptophan (Kanazawa et al., 1975) have been reported to be amino acids most sensitive to oxidation in the presence of lipid peroxidation. The sulfur amino acids (cystine and methionine) are assumed to be oxidized by the hydroperoxides (Tannenbaum et al., 1969; Yong et al., 1980 and Finley et al., 1981), whereas lysine is thought to react with the secondary products (Kanazawa et al., 1975). The methyl sulfide side chain of methionine and the sulfhydryl group of cystine, which may be soluble in the lipid phase or aligned at the water-lipid interface, could decompose hydroperoxides and form carbonyl compounds (Tannenbaum et al., 1969). However, these experiments have been performed *in vitro*, and with regard to food processing and food storage. Whether or not such reactions exits *in vivo* is not yet known. The antioxidant action of sulfur-containing amino acids however, might explain the observed findings in this present study of increased serum, liver and dietary lipid peroxidation levels in hamsters fed dietary casein relative to egg

white, as egg white appears to possess more sulfur containing amino acids (5.5%) than casein (2.8%). There would be less tissue and dietary lipid peroxides because the latter might react with sulfur amino acids. Another possible explanation of the decreased degree of tissue lipid peroxidation might be through the increased synthesis of glutathione induced by dietary cystine. It has been demonstrated that supplementation of cystine in diets of chicks increased liver and plasma glutathione concentrations (Boebel and Baker, 1983) and induced glutathione peroxidase activity. It is known that glutathione protects proteins and cell membranes against peroxides and free radicals and that glutathione peroxidase catalyses the reduction of organic peroxides. Thus, egg white, which possesses almost a two-fold higher concentration of cystine compared to casein could have led to increased liver and serum glutathione concentrations and decreased the level of lipid peroxidation in these two tissues. However, the application of these findings *in vivo* has not been investigated.

Liver lipid peroxides were elevated in the casein-fed hamsters but not the hepatic secondary products (TBARS). As secondary oxidation products are absorbed through the gut (Kanazawa et al., 1985) but not hydroperoxides (Bergan and Draper, 1970), the elevation in both liver and serum hydroperoxides in the casein-fed animals probably comes from *in vivo* metabolic changes since no dietary TBARS were detected. There were no differences in the liver weight, liver weight as a percentage of body weight and serum alanine aminotransferase levels between the two butterfat-based diets. Other reports showed that ingested secondary oxidation products can accumulate in the liver to

cause an elevation of serum transaminase activities and liver hypertrophy (Kanazawa et al., 1985; Oorada et al., 1986). The results in this present study may indicate low levels of hepatic secondary oxidation products which were not sufficiently elevated by the diets to cause hepatic cytotoxicity and hypertrophy in these hamsters. On the other hand, the lack of relationship between lipid peroxides and hepatic toxicity could be due to the fact that lipid peroxide concentrations, as detected by the highly specific and sensitive haemoglobin-methylene blue (HMB) method used in this study, reflects early events in lipid peroxidation (Kanazawa et al., 1985). In contrast, the TBARS method measures end stage breakdown products of lipid peroxidation and therefore is better correlated with hepatotoxicity.

Correlations between cytotoxic aldehydes, such as malonaldehyde, and several diseases, including vascular disorders, have been described (Yagi, 1987). Lipid hydroperoxides have been shown to accelerate the atherosclerotic process in terms of initiation of endothelial injury; the progression of the phase in which there is an accumulation of plaque and the final termination phase of thrombosis (Yagi, 1987). The presence of oxidized lipids in atherosclerotic lesions has been reported (Duthie et al., 1989) and a study has detected higher levels of lipid peroxides in the serum of patients with cardiovascular disease than in controls (Stringer et al., 1989).

In the present study, the lower levels of tissue lipid peroxides found in the egg white-fed hamsters might have been due to higher levels of dietary sulfur-containing

amino acids (cystine and methionine) in egg white relative to casein. Another possible explanation for the decreased degree of lipid peroxidation in egg white-fed hamsters might be the to increased synthesis of glutathione induced by higher levels of cystine in egg white.

5.2 Part two: Comparison of dietary fats on blood lipids and lipid peroxidation levels

Dietary fat composition affected both cholesterol metabolism and lipid peroxidation levels in the hamster. An increase in total serum cholesterol but not in total serum triglycerides, serum HDL-c, serum apolipoproteins A-I and B was observed in the animals fed butterfat compared to those fed safflower oil. Nistor et al. (1987) reported that male Golden Syrian hamsters fed a standard chow diet supplemented with 3% cholesterol and 15% butter increased total serum cholesterol and LDL by four-fold after four weeks of feeding. Furthermore, at the end of the four week feeding interval, fatty streaks and foam cells began to appear in the aortic arch. In the present study, no foam cells or fatty streaks were observed at the end of the four week feeding trial. These results might have been due to the lower amount of cholesterol (0.088%) provided in the diets of the hamsters. Even if no atherosclerotic lesions developed in hamsters, however, these animals appear to be a good model to study cholesterol metabolism since they responded to dietary fat challenge. The fact that the butterfat diet produced a higher total serum cholesterol without an increase in serum apolipoprotein B can not be explained fully; even if the cholesterol content of low density-lipoproteins (LDL-c) were not

measured, it is probable that this indicates larger LDL-c size which has been reported to be less atherogenic than a small one (Sniderman et al., 1980).

The hamsters fed the menhaden oil diet had a lower change in body weight than the animals fed the butterfat and safflower oil diets. The menhaden oil-fed hamsters ate less than the hamster receiving the butterfat and safflower oil diets eventhough the cumulative feed intake of the hamsters was not statistically significant (model $p < 0.07$). The strong and displeasing odour of fish oil could have induced the hamsters to eat less. The change in body weight of animals was put as a covariate in the statistical model for every lipid parameters.

There were no differences in serum and liver lipid peroxidation (both hydroperoxides and TBARS) between the animals fed the butterfat or the safflower oil diet. However, there were higher levels of lipid peroxides in the diet (itself) containing safflower oil when the experiment started and throughout the experiment than in the butterfat diet. Polyunsaturated fatty acids are known to readily oxidized at ambient or subambient temperatures (Chan, 1987) and when subjected to light and air (Kaunitz et al., 1955 and Andrews et al., 1970). Thus, the higher levels of lipid peroxidation in the safflower oil diet in this present experiment could have been caused by prior poor storage conditions. Again, this could not have increased tissue lipid peroxidation as lipid peroxides are not absorbed (TBARS were not detectable). The lipid peroxides in the tissues of safflower oil-fed animals were not elevated relative to the butterfat-fed group

probably because the animals possessed a sufficiently adequate antioxidant status which protected tissue linoleic acid from peroxidation.

The menhaden oil diet contained less lipid peroxides (hydroperoxides) than the safflower oil diet at the beginning and at the end of the experiment. This was probably due to the 50% higher degree of peroxidation exhibited by the safflower oil diet at the beginning of the experiment. However, the difference in the levels of lipid peroxides between the start and at the end of the experiment was higher in the fish oil diet. This is probably due to the higher degree of unsaturation of fatty acid in fish oil which makes them more susceptible to oxidation. It is unlikely that the increase in dietary lipid peroxides caused an increase in oxidative stress *in vivo* since lipid hydroperoxides are poorly absorbed and TBARS were not detectable in all the diets.

Dietary menhaden oil induced higher total serum cholesterol and triglyceride levels in normolipidemic hamsters than did the butterfat diet. These results are in contradiction to most previous studies which have shown decreased or unchanged plasma cholesterol levels and reduced plasma triglycerides in both experimental animals and humans upon omega-3 fatty acid ingestion (Connor and Connor, 1990). Some studies using hypercholesterolemic subjects, however, have reported an increase in plasma cholesterol and triglyceride levels induced by fish oils (Zucker et al., 1986; Demke et al., 1988; Wilt et al., 1989). Moreover, Loo et al. (1991) demonstrated that rabbits made hypercholesterolemic and fed menhaden oil for five to six weeks demonstrated an

increase in total plasma cholesterol concentrations. In addition, the LDL fractions were higher in the fish oil group than in the groups fed coconut oil or corn oil. Menhaden oil feeding did not affect lipoprotein and liver membrane fluidity even though eicosapentaenoic and docosahexaenoic acids were incorporated in both the hepatic microsomal and heart phospholipids. Furthermore, Loo et al. (1991) reported that LDL binding to hepatic membranes was not related to membrane fluidity such that substitution of corn oil or menhaden oil for coconut oil affected blood cholesterol independently of changes in hepatic receptor LDL binding capacity. Roach et al. (1987) have observed that feeding fish oil to rats lowered blood cholesterol, but LDL receptor binding activity was reduced in liver membrane. Thus, the effects of membrane fatty acid composition on plasma lipids appeared to result from modulation of membrane localized enzymes as opposed to changes in LDL receptor binding activity. In this study, an increase in serum triglyceride levels were observed in hamsters fed the menhaden oil diet. In rabbits, Kristensen et al. (1988) reported that animals supplemented with MaxEPA compared to rabbits receiving olive oil exhibited higher serum triglyceride concentrations. The authors concluded that the rabbit might not tolerate high dose of fish oil supplementation.

In the present experiment, an increase in plasma lipid peroxidation (lipid hydroperoxides) levels were observed in the hamsters fed menhaden oil. These results are in accordance with those of Thiery and Seidel (1987) who reported higher serum lipid peroxidation levels, expressed as malondialdehyde equivalents, and increased aortic atherosclerosis in rabbits fed a cholesterol-enriched diet (1.5%) supplemented with two

ml per day of MaxEPA than in the group receiving the cholesterol-enriched diet alone. An increase in serum triglycerides in the fish oil-fed group was observed but no changes in serum cholesterol were reported between the two groups. In the present study, a strong correlation was obtained between serum lipid peroxidation concentration and total serum triglyceride levels ($r^2=0.47$, $p < 0.0009$). L'Abbé et al. (1991) demonstrated that the consumption of menhaden oil diet by rats increased TBARS in the urine, heart and liver and decreased superoxide dismutase activities in the liver, heart and aorta. These researchers suggested that enhanced tissue lipid peroxidation occurred as a result of increased susceptibility to oxidation of tissue omega-3 fatty acids. In the present experiment, serum lipid peroxide levels in the menhaden oil group was higher that in the safflower oil hamsters probably because fish oils fatty acids possess a higher degree of unsaturation than safflower oil and therefore are more susceptible to oxidation. Vladimirov et al. (1980) reported that peroxidation may increase the cell membrane rigidity as well as increased cholesterol content in biomembranes. This could be another possible mechanism for the increase in total serum cholesterol observed in the menhaden oil-fed hamsters. Meydani et al. (1987) demonstrated that mice fed fish oil had significantly lower plasma vitamin E levels than those fed corn and coconut oils at three different levels of dietary tocopherol tested. Therefore, this study suggested that the vitamin E needs were increased when mice were fed fish oil, probably to protect omega-3 fatty acids against lipid peroxidation.

Fish oil can possess high levels of oxysterols (Addis, 1986) and therefore, the

cholesterol-elevating effects of menhaden oil may have been exerted via these substances. An indication for a serum cholesterol-raising effect of oxysterols has come from a recent study which demonstrated that dietary commercial cholesterol containing 5% of oxidized cholesterol derivatives (0.2 g cholesterol/kg body weight) caused a greater elevation of plasma cholesterol than purified cholesterol in the rabbit (Kosykh et al., 1989). Cholesteryl ester levels in hepatocytes and serum apolipoprotein B and E from the commercial cholesterol diets were significantly increased over the purified cholesterol group. The rapid development of hypercholesterolemia induced by dietary commercial oxidized cholesterol was associated with the stimulated production of VLDL and cholesteryl esters by hepatocytes. The presence of oxidized cholesterol derivatives in cholesterol-rich diets, which has also been found capable of blocking hepatic secretion of free cholesterol (Stone et al., 1985), and bile acid synthesis (Kubaska et al., 1985, Kapke et al., 1978). Peng et al. (1985) have also reported a strong inhibition of cholesterol uptake by cultured aortic smooth muscles in the presence of cholesterol oxides. These workers suggested that cholesterol oxides may have induced the inhibition of cholesterol biosynthesis and caused membrane cholesterol to decrease, resulting in a decreased functionality of receptor-dependent processes and that cholesterol oxides could have competed with cholesterol for cell-surface binding sites (Peng et al., 1985). Such mechanisms have been proposed to explain the rapid increase in the hepatic cholesterol level and development of hypercholesterolemia in oxidized cholesterol-fed rabbits as compared with purified cholesterol-fed animals.

The presence of oxysterols in menhaden oil can be an important hypercholesterolemic factor in the present study because the cholesterol oxides found in large amounts in fish oils can be absorbed postprandially in both rats (Bascoul et al., 1986) and humans (Emanuel, 1989). Emanuel (1989) demonstrated the chylomicron-associated absorption of cholesterol oxidation products in humans. Subjects fed a meal consisting primarily as powered eggs with high levels of cholesterol oxides exhibited a pronounced postprandial increase in chylomicron and plasma cholesterol oxides. Humans consuming fresh eggs (containing only traces of cholesterol oxides) demonstrated a minor increase in postprandial plasma cholesterol oxides.

The role of cholesterol oxides in the elevation of total serum cholesterol in the menhaden oil-fed hamsters is suggested as menhaden oil contained a large amount of cholesterol (8.64 mg of cholesterol/g of oil) and the oil was highly oxidized prior to the experiment. The addition of pure cholesterol (free of cholesterol oxides) was added to the other diets in order to match the cholesterol content of menhaden oil. The cholesterol found in fish oil could have been readily oxidized prior to the experiment, since storage duration of the oil at the supplier is unknown, and this may have caused disturbances in lipid metabolism. Whether or not cholesterol oxides were present and/or absorbed is not known in the present experiment. An attempt to measure several cholesterol oxides was made according to the methods of Csallany et al. (1989), Sander et al. (1989), Park and Addis (1985, 1986, 1987), Kou and Holmes (1985) and Tsai et al. (1985) without any success mainly due to the poor recovery rate of cholesterol oxides after the saponification

of lipids from the diets and livers or after the application of fats on silica gel column.

It is possible that the increase in serum lipids observed in the hamsters fed menhaden oil could be attributed to their high serum levels of lipid peroxides since serum lipid peroxide levels were correlated with total serum cholesterol concentrations (0.42; $p < 0.004$). The liver lipid peroxidation levels (hydroperoxides and secondary oxidation products) in menhaden oil-fed animals were not significantly different from the other diets. Liver weight as a percentage of body weight, which can be used as an indicator of liver toxicity, was increased in fish-oil fed animals; however, the serum amino alanine transferase levels were not. The present results could indicate that the fish oil-induced hyperlipidemia was due to a decrease in hepatic binding of oxidized lipoproteins. In addition, it is possible that the increase in serum lipid peroxidation relative to the unchanged hepatic levels of lipid peroxides was due to the hepatic high content of detoxifying enzymes and/or to high levels of hepatic antioxidants relative to that of the serum.

Dietary fish oils have been reported to increase the tissue suceptibility to lipid peroxidation. Leibovitz et al. (1990) fed rats for five weeks either with a 10% menhaden oil or a 10% corn oil-lard mixture in diets supplemented with high or low levels of vitamin E. These authors reported that slices of liver and heart from fish oil-fed rats released significantly more TBARS *in vitro* than did those from the corn oil-lard mixture-fed rats, regardless of the vitamin E levels. It was suggested that there mignt be an

increased requirement for dietary antioxidants by animals consuming fish oil. Furthermore, Horwitt (1991) observed higher levels of plasma tocopherols in male subjects after being fed a saturated fat diet than in the group consuming polyunsaturated fatty acids.

The level of serum apolipoprotein B was also higher in the menhaden oil group than in the butterfat and safflower animals. Although total serum cholesterol was elevated in the butterfat-fed hamsters (but not as high as in the menhaden oil group), no increase in serum apolipoprotein B was observed in the butterfat-fed hamsters. This observation could indicate that there was an elevation in the number of smaller and more atherogenic serum LDL particles (eventhough not mesured) in the menhaden-fed hamsters (Sniderman et al., 1980). The relatively higher levels of serum apolipoprotein B in the fish oil-fed group could also be due to the presence of cholesterol oxides which have been reported to increase serum apolipoprotein B levels in rabbits (Kosykh et al., 1989).

Dietary fat composition influenced both cholesterol and lipid oxidation concentrations in the hamsters. The feeding of menhaden oil to hamsters induced higher serum triglyceride and apolipoprotein B levels than did the butterfat and safflower oil diets. Furthermore, serum cholesterol concentrations were similar in both the menhaden oil and butterfat groups. This increase in serum lipid levels in the menhaden oil-fed hamsters could be attributed to higher serum lipid peroxidation concentrations and may also involve cholesterol oxides since they are known to be in high levels in oxidized fish

oils.

Section 6: Concluding remarks

These experiments were designed to study the effects of dietary proteins and fats on cholesterol metabolism in the male Golden Syrian hamster. Part one of the experiment verified that the hamster responded to dietary protein challenge by altering blood cholesterol concentations. This result confirms an earlier report (Terpstra et al, 1991) on the hypercholesterolemic effect of dietary casein in hamsters fed cholesterol-enriched semi-purified diets. In addition the hypocholesterolemic effect of egg white compared to casein was also validated in part one which confirms the work done by Carroll and Hamilton, 1975. These results suggest that the amino acid composition of dietary protein may play a role in cholesterol metabolism. Furthermore, the type of dietary protein caused differential effects with respect to oxidative stress. This is the first study demonstrating that hamsters fed casein had a higher lipid peroxidation concentrations in serum and liver compared to animals fed egg white. Moreover, since no dietary TBARS were found in the diets, this study demonstrated that the oxidative stress resulted from *in vivo* metabolic changes and not from the absorption of dietary lipid peroxidation products. These results lead to the suggestion that the protective effect of dietary egg white may involve sulfur amino acids which are found in greater concentrations in egg white than in casein and play a role in detoxification of free radicals and lipid peroxidation. These results provide an interesting lead for further research on sulfur amino acids, cholesterol metabolism and oxidative stress.

Part two of the experiment validated the choice of the hamster as the experimental animal, since the hamster responded to dietary fat challenge by altering blood lipid concentrations. The second section of the study also showed that dietary menhaden oil (n-3 fatty acids) can induce hypertriglyceridemia and hypercholesterolemia. The hypercholesterolemia could be induced through an increase in serum apolipoprotein B and serum lipid peroxidation. This experiment demonstrated a correlation of serum cholesterol and serum triglyceride concentrations with serum lipid peroxidation levels. Furthermore, it is suggested that the hypercholesterolemic response of hamsters by feeding n-3 fatty acids may involve cholesterol oxides since they are known to be in high amounts in oxidized fish oils (Addis, 1986) and can be absorbed from the gut (Bascoul et al, 1986; Emanuel, 1989). The high oxidative stress observed in tissues of omega-3 fed hamsters occurs from *in vivo* metabolic events rather than from lipid peroxidation products absorbed from the diet.

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Appendix 1: Statistical methods

a)cumulative food intake obtained by individual hamster:

```
PROC SORT;
BY TRT;
PROC MEANS MEAN STDERR N PRT;
BY TRT;
VAR FOOD;
PROC GLM;
CLASS TRT;
MODEL FOOD = TRT;
LSMEANS TRT/PDIFF STDERR;
```

b)body weight, total serum cholesterol, serum triglyceride, serum HDL, ratio TC/HDL recorded for each hamster

```
PROC SORT;
BY TRT TIME;
PROC MEANS MEAN STDERR N PRT;
BY TRT TIME;
VAR BWT TC TG HDL TCHDL;
PROC GLM;
CLASS TRT TIME;
MODEL BWT TC TG HDL TCHDL = TRT TIME TRT*TIME;
LSMEANS TRT TIME TRT*TIME/PDIFF STDERR;
```

c)change between initial and final data for body weight for each hamster

```
PROC SORT;
BY TRT;
PROC MEANS MEAN STDERR N PRT;
BY TRT TIME;
VAR DBWT;
PROC GLM;
CLASS TRT;
MODEL DBWT = TRT;
LSMEANS TRT/PDIFF STDERR;
```

d)change between initial and final data for total serum cholesterol, serum triglyceride, serum HDL, ratio TC/HDL recorded for each hamster; body weight integrated as a covariate

```
PROC SORT;
BY TRT;
```

```
PROC MEANS MEAN STDERR N PRT;
BY TRT;
VAR DTC DTC DHDL DTCHDL;
PROC GLM;
CLASS TRT;
MODEL DTC DTC DHDL DTCHDL = TRT BWT;
LSMEANS TRT/PDIFF STDERR;
```

e)serum apolipoprotein A-I and serum alanine aminotransferase

```
PROC SORT;
BY TRT;
PROC MEANS MEAN STDERR N PRT;
BY TRT;
VAR APO A ALT;
PROC GLM;
CLASS TRT;
MODEL APOA ALT = TRT;
LSMEANS TRT/PDIFF STDERR;
```

f)apolipoprotein B with TC/HDL (final) as a covariate

```
PROC SORT;
BY TRT;
PROC MEANS MEAN STDERR N PRT;
BY TRT;
VAR APO B;
PROC GLM;
CLASS TRT;
MODEL APOB = TRT HDL;
LSMEANS TRT/PDIFF STDERR;
```

g)ratio serum apolipoprotein A-I/B

```
PROC SORT;
BY TRT;
PROC MEANS MEAN STDERR N PRT;
BY TRT;
VAR APOAB;
PROC GLM;
CLASS TRT;
MODEL APOAB = TRT;
LSMEANS TRT/PDIFF STDERR;
```

h)liver weight, liver weight/body weight ratio, liver LPO, liver TBA obtained from individual hamster

```
PROC SORT;
BY TRT;
PROC MEANS MEAN STDERR N PRT;
BY TRT;
VAR LWT LWTBWT LLPO LTBA;
PROC GLM;
CLASS TRT;
MODEL LWT LWTBWT LLPO LTBA = TRT;
LSMEANS TRT/PDIFF STDERR;
```

i)LPO obtained from each diet

```
PROC SORT;
BY TRT TIME;
PROC MEANS MEAN STDERR N PRT;
BY TRT TIME;
VAR DLPO;
PROC GLM;
CLASS TRT TIME TRT*TIME;
MODEL DLPO = TRT TIME;
LSMEANS TRT TIME TRT*TIME/PDIFF STDERR;
```

j)LPO obtained from each diet difference between week4 - week 0

```
PROC SORT;
BY TRT;
PROC MEANS MEAN STDERR N PRT;
BY TRT;
VAR DDLPO;
PROC GLM;
CLASS TRT;
MODEL DDLPO = TRT;
LSMEANS TRT/PDIFF STDERR;
```

k)correlation between liver lpo and total serum cholesterol with each diet

```
PROC CORR;
BY TRT;
WITH LLPO;
VAR TC;
```

l)correlation between liver lpo and total serum cholesterol with all diets together

```
PROC CORR;
WITH LLPO;
VAR TC;
```

m)correlation between liver weight/body weight ratio with serum alanine aminotransferase with all diets together

```
PROC CORR;
WITH LWTBWT;
VAR ALT;
```

Appendix 2: Analyses of variance

Appendix 2.1 ANOVA results[1] for food intake, body weight, difference between body weight final - body weight intial, liver weight and liver weight as a percentage of body weight

Dependent variable	Effects in the model statment	F value	P>F
Cumulative food intake (g)	Trt	2.75	0.054
	Trt	2.75	0.054
Body weight (g)	Trt time (trt*time)	0.88	0.53
	trt	0.73	0.53
	time	1.54	0.80
	trt*time	0.80	0.50
Body weight difference (g)	Trt	7.16	0.0005
	trt	7.16	0.0005
Liver weight (g)	Trt	1.13	0.35
	trt	1.13	0.35
Liver weight as a % body weight (%)	Trt	6.23	0.0013
	trt	6.23	0.0013

[1] Analyses were executed by PROC GLM in SAS-PC, version 6.04

Appendix 2.2 ANOVA results[1] for serum alanine aminotransferase, total serum triglycerides, total serum triglycerides (difference), total serum cholesterol and total serum cholesterol (difference)

Dependent variable	Effects in the model statment	F value	P > F
S. alanine aminotrans-ferase (U/L)	Trt	2.41	0.079
	trt	2.41	0.079
Total serum triglycerides (mg/dl)	Trt time (trt*time)	4.14	0.0006
	trt	3.20	0.027
	time	9.50	0.003
	trt*time	3.28	0.025
Total serum triglycerides difference (mg/dl)	Trt bwt[2]	5.10	0.0019
	trt	6.74	0.0008
	bwt	0.78	0.38
Total serum cholesterol (mg/dl)	Trt time (trt*time)	35.64	0.0001
	trt	25.24	0.0001
	time	128.61	0.0001
	trt*time	15.06	0.0001
Total serum cholesterol difference (mg/dl)	Trt bwt[2]	14.84	0.0001
	trt	18.60	0.0001
	bwt	3.57	0.067

[1] Analyses were executed by PROC GLM in SAS-PC, version 6.04

[2] Body weight (difference) was put as a covariate in the model statement

Apoendix 2.3 ANOVA results[1] for serum HDL, serum HDL (difference) ratio between total serum cholestrol and HDL (TC/HDL), ratio between total serum cholestrol and HDL (TC/HDL) (difference), serum apolipoprotein A-I, serum apolipoprotein B and ratio apolipoprotein A-I/B

Dependent variable	Effects in the model statment	F value	P>F
Serum HDL (mg/dl)	Trt	4.16	0.0006
	trt	0.54	0.66
	time	24.34	0.0001
	trt*time	1.05	0.37
Serum HDL difference (mg/dl)	Trt bwt[2]	0.26	0.90
	trt	0.35	0.79
	bwt	0.01	0.94
Ratio TC/HDL	Trt time (trt*time)	7.33	0.0001
	trt	7.32	0.0002
	time	11.16	0.0013
	trt*time	4.86	0.0037
Ratio TC/HDL difference	Trt bwt[2]	4.40	0.0055
	trt	4.86	0.0063
	bwt	3.03	0.090
Serum apo A-I (mg/dl)	Trt	1.07	0.39
	trt	1.07	0.39
Serum apo B (mg/dl)	Trt TC/HDL diff	27.36	0.0001
	trt	36.44	0.0001
	TC/HDL diff	0.12	0.73
Ratio Apo A-I/B	Trt	10.42	0.0005
	trt	10.42	0.0005

[1] Analyses were executed by PROC GLM in SAS-PC, version 6.04
[2] Body weight (difference) was put as a covariate in the model statement

Appendix 2.4 ANOVA results[1] for serum LPO, liver LPO, liver TBA, diet LPO and the difference between diet LPO week 4 - diet LPO week 0

Dependent variable	Effects in the model statment	F value	P>F
Serum LPO (umol/ml)	Trt	8.99	0.0001
	trt	8.99	0.0001
Liver LPO (nmol/mg fat)	Trt	8.21	0.0002
	trt	8.21	0.0002
Liver TBA (nmol/mg fat)	Trt	0.45	0.72
	trt	0.45	0.72
Diet LPO (nmol/mg fat)	Trt time trt*time	23.01	0.0001
	trt	101.12	0.0001
	time	19.27	0.0001
	trt*time	4.73	0.0011
Diet LPO (difference) (nmol/mg fat)	Trt	13.51	0.015
	trt	13.51	0.015

[1] Analyses were executed by PROC GLM in SAS-PC, version 6.0.

Appendix 2.5a Correlation results[1] with liver LPO

	Variables							
Diets	S. triglycerides[2]		S.cholesterol[2]		S. HDL[2]		Ratio TC/HDL[2]	
	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>F
BF/CAS	-0.35	0.27	-0.13	0.69	0.42	0.2[illegible]	-0.44	0.20
BF/EGG	-0.0036	0.99	-0.34	0.28	0.0099	0.98	0.064	0.86
SAFF/EGG	0.0078	0.98	0.70	0.011	0.19	0.59	0.62	0.055
MHO/EGG	0.36	0.24	-0.076	0.81	-0.38	0.28	0.53	0.11
ALL DIETS	0.096	0.51	0.075	0.61	0.064	0.69	0.12	0.46
BF/CAS + BF/EGG	-0.18	0.38	-0.22	0.29	0.22	0.36	-0.089	0.71
BF/EGG + SAFF/EGG + MHO/EGG	0.29	0.09	0.17	0.32	-0.038	0.84	0.24	0.20

[1]Analyses were executed by PROC CORR in SAS-PC, version 6.04.

[2]The correlations were run on the triglycerides, cholesterol, HDL, TC/HDL differences (final measurement - initial measurements).

Appendix 2.5b Correlation results[1] with liver LPO (con't)

	Variables									
Diets	Body weight		Liver weight		S. Apo A-I		S. Apo B		Ratio Apo A/B	
	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>f
BF/CAS	0.15	0.63	-0.35	0.25	-0.15	0.81	0.31	0.32	0.66	0.22
BF/EGG	0.0062	0.98	0.055	0.87	0.90	0.04	0.55	0.065	-0.55	0.33
SAFF/EGG	0.096	0.77	0.10	0.75	0.17	0.78	0.36	0.25	0.69	0.20
MHO/EGG	0.40	0.20	0.048	0.88	0.14	0.82	0.11	0.74	-0.95	0.012
ALL DIETS	0.13	0.37	-0.093	0.53	-0.065	0.78	0.16	0.28	0.080	0.73
BF/CAS + BF/EGG	0.11	0.60	-0.15	0.47	-0.15	0.68	0.43	0.036	-0.42	0.22
BF/EGG + SAFF/EGG + MHO/EGG	0.022	0.88	0.046	0.79	0.11	0.69	0.19	0.25	-0.096	0.73

[1]Analyses were executed by PROC CORR in SAS-PC, version 6.04.

Appendix 2.6a Correlation results[1] with liver TBA

	Variables							
Diets	S. triglycerides[2]		S.cholesterol[2]		S. HDL[2]		Ratio TC/HDL[2]	
	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>F
BF/CAS	0.12	0.71	0.22	0.51	0.04	0.91	0.26	0.27
BF/EGG	-0.25	0.43	-0.26	0.41	-0.15	0.69	0.10	0.78
SAFF/EGG	-0.15	0.65	-0.0097	0.98	-0.14	0.72	-0.026	0.95
MHO/EGG	-0.23	0.31	-0.23	0.50	-0.15	0.68	0.038	0.92
ALL DIETS	-0.093	0.54	-0.065	0.67	-0.039	0.81	0.23	0.16
BF/CAS + BF/EGG	-0.20	0.36	-0.21	0.320	-0.038	0.87	0.13	0.57
BF/EGG + SAFF/EGG + MHO/EGG	-0.11	0.53	-0.05	0.77	-0.053	0.78	0.23	0.22

[1]Analyses were executed by PROC CORR in SAS-PC, version 6.04.
[2]The correlations were run on the triglycerides, cholesterol, HDL, TC/HDL differences (final measurement - initial measurements).

Appendix 2.6b Correlation results[1] with liver TBA (con't)

	Variables									
Diets	Body weight		Liver weight		S. Apo A-I		S. Apo B		Ratio Apo A/B	
	Corr. coef.	p > F	Corr. coef.	p > F	Corr. coef.	p > F	Corr. coef.	p > F	Corr. coef.	p > F
BF/CAS	-0.64	0.033	-0.30	0.37	-0.61	0.39	-0.093	0.78	0.86	0.14
BF/EGG	0.11	0.74	-0.047	0.88	0.59	0.30	-0.073	0.82	-0.38	0.53
SAFF/EGG	-0.21	0.53	-.019	0.96	0.47	0.43	-0.32	0.32	0.33	0.58
MHO/EGG	0.046	0.89	0.082	0.81	-0.48	0.52	-0.33	0.31	0.49	0.50
ALL DIETS	-0.17	0.28	-0.024	0.87	0.31	0.21	-0.018	0.90	0.24	0.33
BF/CAS + BF/EGG	-0.15	0.47	-0.097	0.66	0.38	0.31	-0.11	0.62	-0.12	0.75
BF/EGG + SAFF/EGG + MHO/EGG	-0.073	0.68	-0.0034	0.98	0.36	0.19	-0.039	0.82	0.24	0.41

[1]Analyses were executed by PROC CORR in SAS-PC, version 6.04.

Appendix 2.7a Correlation results[1] with serum LPO

	Variables											
Diets	Liver LPO		Liver TBA		S. triglycerides[2]		S.cholesterol[2]		S. HDL[2]		ratio TC/HDL[2]	
	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>F	Corr. coef.	p>f	Corr. coef.	p>F
BF/CAS	-0.13	0.70	-0.45	0.16	-0.066	0.85	-0.041	0.90	0.17	0.65	-0.028	0.94
BF/EGG	0.11	0.73	0.027	0.93	0.072	0.82	-0.083	0.80	0.084	0.82	-0.22	0.54
SAFF/EGG	-0.43	0.16	-0.12	0.72	0.52	0.082	0.060	0.85	-0.32	0.35	0.39	0.27
MHO/EGG	0.49	0.12	0.24	0.51	0.56	0.070	0.55	0.081	-0.87	0.0021	0.85	0.004
ALL DIETS	0.059	0.69	0.027	0.86	0.47	0.0009	0.42	0.004	-0.10	0.54	0.43	0.007
BF/CAS + BF/EGG	0.15	0.49	-0.17	0.46	0.13	0.55	0.074	0.73	0.17	0.47	-0.11	0.66
BF/EGG + SAFF/EGG + MHO/EGG	0.14	0.41	0.090	0.62	0.64	0.0001	0.52	0.0013	-0.17	0.37	0.53	0.0032

[1]Analyses were executed by PROC CORR in SAS-PC, version 6.04.
[2]The correlations were run on the triglycerides, cholesterol, HDL, TC/HDL differences (final measurement - initial measurements).

Appendix 2.7b Correlation results[1] with serum LPO (con't)

	Variables									
Diets	Body weight		Liver weight		S. Apo A-I		S. Apo B		Ratio Apo A/B	
	Corr. coef.	p > F	Corr. coef.	p > F	Corr. coef.	p > F	Corr. coef.	p > F	Corr. coef.	p > f
BF/CAS	0.26	0.43	0.27	0.43	0.25	0.69	0.56	0.071	-0.83	0.082
BF/EGG	0.40	0.20	-0.44	0.15	0.49	0.40	0.39	0.21	-0.83	0.079
SAFF/EGG	0.27	0.39	-0.18	0.57	-0.26	0.67	-0.084	0.79	0.16	0.79
MHO/EGG	-0.16	0.64	0.31	0.35	0.74	0.15	0.66	0.026	-0.93	0.023
ALL DIETS	0.039	0.80	0.042	0.78	0.15	0.54	0.69	0.0001	-0.72	0.0004
BF/CAS + BF/EGG	0.36	0.095	0.060	0.79	-0.23	0.51	0.52	0.011	-0.84	0.0024
BF/EGG + SAFF/EGG + MHO/EGG	-0.12	0.50	-0.034	0.85	0.093	0.74	0.77	0.0001	-0.72	0.0027

[1]Analyses were executed by PROC CORR in SAS-PC, version 6.04.

Appendix 2.8 Correlation results[1] with serum alanine aminotransferse

Variables		
	Liver wt/body wt	
Diets	Corr Coef.	P>F
BF/CAS	-0.11	0.72
BF/EGG	0.51	0.09
SAFF/EGG	0.55	0.06
MHO/EGG	0.38	0.21
ALL DIETS	0.29	0.047
BF/CAS+ BF/EGG	0.26	0.23
BF/EGG+ SAFF/EGG+ HO/EGG	0.42	0.0094

[1]Analyses were executed by PROC CORR in SAS-PC, version 6.04.