INTERACTIVE EFFECTS OF DIETARY FAT SOURCE AND STEROL INTAKE ON LIPID METABOLISM IN SPONTANEously HYPerTENSIVE AND NORMOTENSIVE RATS

By

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We accept this thesis as conforming to the required standard

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ABSTRACT

The effect of dietary fat source and cholesterol intake level on plasma, lipoprotein and tissue lipid composition, lipid balance, and red blood cell (RBC) antioxidant status in rats was studied. Spontaneously hypertensive rat (SHR) and Wistar Kyoto normotensive rat (WKY) were randomly assigned to one of the six semi-purified diets containing 16% lipid. The fat blends consisted of 13% from either butter, soybean oil or menhaden oil, plus 3% from canola oil. Cholesterol was added to dietary fat sources at final concentrations of either 0.05% or 0.5% (wt/wt). During the 6th week of the experiment, animals were placed in individual metabolic cages for a 5 day balance study, where feed intake was measured and excreta collected and measured daily. Animals were returned to individual aluminum cages and at the end of 10 weeks the experiment was concluded. Rats were anaesthetized and blood was drawn from the heart. Liver tissue was also removed. RBC, plasma, lipoprotein fractions and liver tissue were assayed for lipid composition. Malondialdehyde (MDA) production and glutathione depletion assays were used to evaluate the oxidative status of RBC, while copper (Cu^{2+})-induced oxidation was performed on low density (LDL) lipoprotein fractions to evaluate LDL-oxidation potential.

In SHR, blood pressure measurements at 12 weeks of age confirmed elevated pressures in all dietary groups. High cholesterol menhaden-fed animals of both strains produced lower plasma cholesterol concentrations compared to animals fed high cholesterol butter or soybean based diets (p <0.001).
Dietary cholesterol feeding increased other physiologic parameters, including liver cholesterol (p < 0.001), total crude lipid concentrations (p < 0.001), RBC cholesterol concentration (p = 0.031), and RBC triacylglycerol (p < 0.001), while it lowered MDA production at 5 mM H$_2$O$_2$ (p = 0.004). RBC-cholesterol was raised in high cholesterol fed SHR (p ≤ 0.05), while MDA production in the same treatment group was reduced (p ≤ 0.001). Cu$^{2+}$-induced LDL oxidation increased with time, but there were no significant differences between the individual dietary treatment groups. Reduced MDA production in high cholesterol fed SHR points to a protective effect of dietary cholesterol against oxidative stress induced in the RBC membranes collected from these particular animals. Of all high cholesterol fed groups, liver cholesterol was lowest (p ≤ 0.05) in menhaden-fed SHR. Equivalent differences were not noticed in WKY. No differences were observed between liver cholesterol values from high cholesterol butter-fed and high cholesterol soybean-fed animals of either strain. Lower liver cholesterol concentrations combined with elevated VLDL-cholesterol levels produced in high cholesterol menhaden-fed SHR, indicated a differential effect of very long chain n-3 PUFAs on hepatic cholesterol metabolism in those animals. WKY rats, on the other hand, exhibited no differences in liver cholesterol among high cholesterol fed animals, but produced lower VLDL-cholesterol concentrations in menhaden-fed rats. All animals increased fecal cholesterol and fecal total crude lipid excretion when fed the high cholesterol diets, however SHR exhibited a relatively greater ability to excrete cholesterol than WKY. SHR appeared to adapt differently to high cholesterol intake than the WKY rats.
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Definitions:

AA = araquidonic acid
ANOVA = analysis of variance
ApoA = apolipoprotein A
ApoAI = apolipoprotein AI
ApoB = apolipoprotein B
ApoE = apolipoprotein E
AR = aldose reductase
ATP = adenosine triphosphate
$^{14}$C = carbon isotope with atomic mass 14
CHD = coronary heart disease
CNS = central nervous system
DHA = docosahexaenoic acid
DOCA = deoxycorticosterone acetate
Dyslipidemia = abnormality in the serum lipoprotein pattern
EDRF = endothelium relaxing factor
EPA = eicosapentanoic acid
GSH = glutathione = L-$\gamma$-glutamyl-L-cysteinylglycine
GSSG = oxidized glutathione
$^3$H = tritium
HDL = high density lipoprotein
HDL$_3$ = high density lipoprotein subfraction 3
SOD = superoxide dismutase

SHR = spontaneously hypertensive rat

SHR-SP = stroke-prone spontaneously hypertensive rat

TBARS = thiobarbituric acid reactive substances

TC = total cholesterol

TG = triacylglycerol

VLDL = very low density lipoprotein

WKY = Wistar Kyoto normotensive rat
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Introduction

Hypercholesterolemia is one of a number of factors included in the cluster of risk factors commonly found in individuals who develop coronary heart disease (Cheung et al., 1994; Barnard et al., 1993; Rubattu et al., 1993; Nickerson et al., 1992). Others are obesity, diabetes, hypertriglyceridemia and hypertension (Giugliano et al., 1995; Cunnane et al., 1993; Roberts, 1992).

There is evidence that a portion of the human population is genetically susceptible to the development of hypertension (Hsia, 1968). Recent studies from this laboratory have also identified the importance of a genetic factor in the expression of hyperlipidemia which was not related to HT in SHR and WKY rats (Yuan et al., 1996). On the other hand, new knowledge about the effect of diet on a number of the metabolic parameters cited above has led to the recognition of dietary habits as a tool for modifying various risk factors for CHD (Demoz et al., 1994; Woodward and Limacher, 1993; Kritchevsky, 1995). For example, dietary fat and cholesterol intake are two of the underlying causes for dyslipidemia (Grundy and Denke, 1990; Lin et al., 1995; Sanders et al., 1994), which in turn has been associated with CHD (Tzonou et al., 1993; Upasani and Wasir, 1994; Hrboticky and Sellmayer, 1996). Blood pressure, a risk factor for CHD, is also said to be affected by dietary habits. For example, it has been suggested that by increasing the intake of linolenic acid (C18:3 n-3), a drop in blood pressure could be achieved (Berry and Hirsch, 1986). Findings regarding the relationship of linoleic acid (C18:2 n-6) with hypertension and CHD are controversial. While some researchers have found that linoleic acid is positively associated with CHD (Hodgson et al., 1993), others have concluded that
it does not result in an independent effect (Pietinen, 1994), nor that it decreases the incidence of sudden cardiac death (Roberts et al., 1995). Alternatively, a greater proportion of polyunsaturated fatty acids in the diet may also increase the risk of lipid oxidation and thus further the development of CHD (Regnistroem and Nilsson, 1994). On the other hand, supplementing dietary intake with fish oil, rich in n-3 fatty acids, has been found to reduce the increase in blood pressure of CHD patients whose medication had been discontinued (Bairati et al., 1992).

These associations between diet and CHD motivated this research, which was designed to determine the interactions of different dietary fat blends and cholesterol consumption levels on specific metabolic parameters such as plasma and tissue lipids, red cell antioxidant status and hypertension.

Coronary heart disease (CHD) is one of the major causes of death in Canada (Wigle, 1995). A number of conditions including obesity, diabetes, hypertension and elevated plasma cholesterol concentrations, are frequently associated with CHD. Epidemiological studies established a link between dietary lipid intake and CHD (Linscheer and Vergroesen, 1988; Rasanen et al., 1992). In Japan, the average diet is relatively rich in polyunsaturated fats, and the incidence of heart disease is relatively low (Tanaka et al., 1996). In contrast, in Scandinavian countries and in North America, the average diet has traditionally contained more fat, approximately 40% of total calories, with a high saturated fatty acid content (Spencer, 1995). The incidence of coronary heart disease in these countries is relatively high. There is clinical evidence that the level of dietary lipid intake affects the incidence of dyslipidemia in humans (Grundy and Denke, 1990; Sanders et al.,
Blood pressure in humans also appears to be influenced by dietary lipid composition (Hodgson et al., 1993; Berry and Hirsch, 1986; Regnstroem and Nilsson, 1994). Researchers have shown through animal studies that plasma total cholesterol (TC) levels can be increased by a diet high in saturated fatty acid intake (Lindsey et al., 1990; Khosla and Hayes; 1993). Consumption of diets rich in long chain n-6 polyunsaturated fatty acids (PUFA) have resulted in lowered plasma triacylglycerol (TG) and TC (Weintraub et al., 1988), whereas n-3 enriched diets have been found to reduce plasma TG in rodent species (Singer et al., 1990). Individual lipoprotein fractions such as very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL), have been used as indicators for CHD status in both human and animal studies and have been shown to be altered by dietary PUFA intake (Hayes et al., 1994; Stangl et al., 1994). Fat is, however, usually ingested with cholesterol and this combination has been shown to exert an influence on both plasma lipid and liver lipid composition. The cholesterol concentration concurrently present in a food will modulate the effect of a particular fatty acid composition on liver lipids (Fungwe et al., 1994; Lin et al., 1995). The effect of diet induced changes in plasma and tissue lipids on oxidative status has however not been evaluated.

This study was designed to examine the effects of different dietary fat sources, with particular fatty acid compositions, in combination with a high and a low cholesterol level on several physiological parameters. Three main dietary fat sources, butter - with a high saturated fatty acid content, soybean oil - rich in n-6 fatty acids, and menhaden oil - with a high n-3 very long chain polyunsaturated fatty acid content, were incorporated into
fat blends with high (0.5%) and low (0.05%) cholesterol concentrations and fed to two different rat strains. Liver and plasma lipid changes, alterations in RBC lipid composition were also examined to assess the effect of diet-induced changes on RBC and lipoprotein composition relative to the resistance to oxidative stress in the tissues. Since LDL oxidation has been linked to the severity of CHD (Regnstroem and Nilsson, 1992), diet-induced changes in the susceptibility of both isolated LDL and RBC to oxidative stress were used to further identify exogenous factors for CHD. Finally, spontaneously hypertensive rats (SHR) have genetically determined high blood pressure (Okamoto and Aoki, 1963), while Wistar Kyoto (WKY) normotensive rats do not exhibit that characteristic. Therefore this study also attempted to look at the impact of dietary fat sources and cholesterol intake levels on modifying the predisposition to hypertension, an important contributor to CHD. The experimental assessment of the interaction of different effects of dietary lipid intake on the expression of both exogenous (dietary) and endogenous (genetic) variables was utilized in attempting to define a relationship between dietary intake and CHD.
Thesis Hypotheses:

1- Different sources of dietary fats influence characteristically the development of metabolic changes associated with oxidative status in the spontaneously hypertensive rat and its normotensive control.

2- Metabolic factors associated with hypertension are influenced by genetic makeup and dietary fats, and relate to the susceptibility to oxidative stress.

Thesis Objectives:

1- To determine if there is a relationship between genetic predisposition to hypertension and diet induced hyperlipidemia.

2- To determine if there is a cause-effect relationship between dietary fat composition and oxidative status of a target tissue (e.g. RBC).

Thesis Aims:

1- To use the spontaneously hypertensive rat (SHR) and its normotensive control Wistar Kyoto (WKY) as a model in feeding studies designed to determine possible interactions between dietary fat and cholesterol on RBC lipid composition, plasma lipid and lipoprotein constituents.

2- To assess oxidative stress as defined by relevant parameters in tissue lipid and lipoprotein composition and oxidative status.

3- To integrate the findings of the two previous aims in an evaluation of endogenous (genetic) and exogenous (dietary) parameters related to dyslipidemia and to hypertension.
LITERATURE REVIEW

1 (i) Dietary Fats and Dyslipidemia

The human diet consists of a great variety of foods from diverse sources. As a consequence we ingest many different fats containing a variety of fatty acids (Kitts and Jones, 1996). Comparing the composition of diets in North America and Northern Europe, versus diets in Mediterranean countries and in Asia, there are considerable differences between the percentage of total calories contributed by fat, and between the degree of saturation of the triacylglycerols.

The North American diet has traditionally contained 40% of its calories in fat (Linscheer and Vergroesen, 1988), most of which is saturated. Scandinavian countries, for example Finland, show a similar pattern of fat type consumption (Rasanen et al., 1992; Nissinen et al., 1987). A diet providing an average intake of 40% calories from fat, containing a high percentage of saturated fatty acids, may be considered an atherogenic diet. The Mediterranean diet, on the other hand, is relatively rich in olive oil containing oleic acid, (C18:1), and thus contributes to the high unsaturated fat content of the diet in those countries. In Japan, fish is ingested on a regular basis and the Japanese diet is relatively rich in long chain n-3 polyunsaturated fatty acids. Individual response variability to a given dietary factor, as well as differences in experimental design, make comparisons of experimental results from different animal species difficult to interpret. Furthermore, individual responses in plasma lipoprotein levels vary according to the influences of other dietary inputs and genetic predisposition.
I (ii) Digestion and Absorption of Fat

One of the first stages of digestion for dietary fats occurs in the stomach. Due to the action of lingual and gastric lipases, which act preferentially on fatty acids in the sn-3 position, and the time fat remains in the stomach, approximately 30% of the triacylglycerols are hydrolyzed by the time they leave the stomach. The major products resulting from the action of lipases are monoacylglycerols and free fatty acids of medium and short chain length (C:6 - C:12). Free short chain fatty acids are absorbed directly from the stomach by passive diffusion into the circulation. The entry of the digestive bolus into the duodenum stimulates the secretion of bile, which contains bile acids, salts, and pancreatic lipase (Small, 1991). Pancreatic lipase cleaves preferably fatty acids in the sn-1 and sn-3 positions. The action of an isomerase converts 2-acylglycerols into 1-acylglycerols, which are then hydrolyzed. Colipase and bile contribute to the degradation of long-chain (C-14 - C-18) fatty acids in the small intestine. The function of colipase is to form a complex with bile salts and lipase, stabilizing the enzyme. When long-chain fatty acids are present in the triacylglycerol unit, they are removed by a carboxyl ester hydrolase.

Some of the medium chain fatty acids go through the portal circulation, while others are incorporated into triacylglycerol (TG). Triacylglycerols resynthesized in the intestinal cells are largely similar to those ingested in the diet. Long-chain fatty acids are re-esterified to triacylglycerols through the glycerol-3-phosphate pathway. Chylomicrons, composed of triacylglycerols, cholesterol esters, apo B48, and other lipoproteins in lesser proportions, are released into the lymphatic system and enter the circulation through the
vena cava (Bach and Babayan, 1982). When chylomicrons reach the capillaries, triacylglycerols are hydrolyzed by lipoprotein lipase and apoproteins are transferred to nascent high density lipoproteins (HDL). The proportion of free cholesterol increases in this particle.

Long-chain fatty acids present at the sn-1 and sn-3 positions of triacylglycerols are preferentially hydrolysed by hepatic lipase. In the liver these are re-esterified into triacylglycerols and together with apo-B100 and apo-E are secreted as part of very low density lipoproteins (VLDL), which are then released into the circulation. This occurs because the liver has limited capacity to store triacylglycerols (Norum, 1992). Fatty acids are hydrolysed from triacylglycerols in capillary beds. Lipoprotein transfer occurs, forming HDL and low density lipoprotein (LDL) particles, closing the cycle of lipid transport.

I (iii) Dietary Fatty Acids and Lipidemia

Polyunsaturated fatty acids have been shown to have a postprandial lipoprotein lowering effect (Weintraub et al., 1988). Fish oils, with a relatively high content of very long chain polyunsaturated fatty acids have been found to lower plasma VLDL-cholesterol concentration (Phillipson et al., 1985). Plasma VLDL and LDL cholesterol, however, have been shown to increase significantly when Syrian hamsters are fed a fish oil diet (Lin et al., 1995), despite the presence of n-3 PUFA. Results from the same study suggested that, based on a comparison between cholesterol loaded and cholesterol free diets, a decrease in lipoprotein lipase and LDL receptor activity could be the cause for
increased VLDL-cholesterol and VLDL-triacylglycerol. In a study where African Grivet monkeys were fed menhaden oil, a reduction in plasma cholesterol and HDL cholesterol of 33%, and in LDL molecular weights by 15% was observed. Taking into account that menhaden oil contained 89 mg cholesterol/g oil compared to 8.6 mg cholesterol/g for lard, a reduction in total plasma cholesterol may seem surprising.

Other plasma lipid parameters can also be affected. An increase in plasma triacylglycerols with a parallel reduction in LDL size has been observed in humans fed a fish oil diet (Crouse et al., 1985). The underlying mechanism suggested, involves an exchange of cholesterol esters in LDL for VLDL triacylglycerols, followed by hydrolysis of LDL-triacylglycerols by lipoprotein lipase, thereby reducing LDL size (Parks et al., 1987). An increase in dietary n-3 PUFAs has been shown to reduce triacylglycerol levels in normal (Harris et al., 1983), but more so in hypertriglyceridemic individuals (Phillipson et al., 1985). Raising n-3 PUFA intake has been thus implicated in the reduction of cardiovascular risk factors (Foerste, 1987). Competitive inhibition of PUFAs at hepatic receptor sites and relatively poor incorporation of polyenoic fatty acids into triacylglycerols explain those observations (Nestel and Wong, 1986).

The efficacy of n-3 PUFAs in reducing the risk of cardiovascular disease is likely also based on effects on blood platelets and vascular tissue. Arachidonic acid (AA), a n-6 fatty acid, is converted into thromboxane A2 which has platelet aggregatory and vasoconstrictory functions. When n-3 eicosapentaenoic acid (EPA) is ingested, the amount of AA available for conversion is reduced (Ciu, 1994), because these particular fatty acids compete for enzymes, such as cyclooxygenase (Drevon, 1992). The
thromboxane produced from EPA has significantly less platelet aggregatory function and vasoconstricting properties. Moreover, interaction of AA and EPA affects leukotrienes in leukocytes in a similar manner. However, leukotriene receptors have 20% the affinity for Leukotriene B5 (LTB5), derived from EPA, than that expressed for LTB4 (from AA).

There are other fatty acids that can have analogous effects. Linoleic acid (C18:2 (n-6)) can be converted to AA, while linolenic acid (C18:3 (n-3)) can be converted to EPA through a series of elongation and desaturation reactions (Nelson, 1992). C18:3 is found in leafy green vegetables and vegetable oils. The main sources of linoleic acid are seeds. It is important to balance the intake of n-3 and n-6 fatty acids. Some suggested levels for physiological requirements of these fatty acids are: I) n-3 fatty acids at 2-5% concentration in dietary fat to enhance immune function, reduce blood clotting and lower serum triacylglycerol; II) a concentration of 3-4% of n-6 fatty acids in dietary fat is enough to fulfill essential fatty acid requirements. The greater affinity of Δ6 desaturase is significant in an in vivo environment only when the diet provides less than 5% of calories as C18:2 (n-6), and/or a very high proportion of C18:3 (n-3). Alternatively, less than 0.4% kcal 18:3 n-3 and no n-3 long chain polyenoic acids (LCP) respectively also is effective (Innis, 1991). In human infants the requirement for 18:2 n-6 stems from the high rate of synthesis of structural lipids (Innis, 1991). The synthesis of C22:6 (n-3) from 18:3 n-3, is suggested to be crucial for central nervous system (CNS) associated membrane function (Innis, 1991).

Comparing the effects of olive oil and soybean oil on HDL cholesterol and lipoproteins in young men, Bruin et al. (1993) found that total cholesterol concentrations
did not change significantly. In that study soybean oil decreased HDL cholesterol, while olive oil did not. Olive oil utilized in this experiment contained 13.7% C16:0, 71.1% C18:1, and 10% C18:2, while soybean oil contains 11.0% C16:0, 23.4% C18:1, and 53.2% C18:2 (White, 1992). Olive oil had a higher content of saturated fatty acids than soybean oil. After a meal, chylomicrons derived from olive oil appeared in the plasma before those derived from soybean oil. This observation pointed to a slower removal rate of soybean chylomicron remnants or the presence of a greater number of chylomicrons (Bruin, 1993). The same workers concluded that hepatic lipase (HL) had a greater affinity for olive oil. At an intake level of 40% of total energy intake, oleic and linoleic acid lowered plasma LDL-cholesterol concentrations in normotriglyceridemic individuals, individuals consuming lowered LDL concentrations more frequently (Pronczuk et al., 1994). Neither mono- nor polyunsaturated fatty acids changed triacylglycerol levels in this study. In hypertriglycerideremic patients HDL-cholesterol levels were not affected by intake of unsaturated fats, but HDL cholesterol was found to be reduced in the presence of the presence of saturated (palm oil) fat diet (Mattson and Grundy, 1985). A reduction in plasma triacylglycerol levels has also been reported with n-3 PUFAs (Harris et al., 1983), especially when administered to hypertriglycerideremic patients (Phillipson et al., 1985).

The cholesterol-raising effect of saturated fatty acids has been recognized to be mainly due to an increase in LDL-cholesterol (Keys et al., 1965). Furthermore, fatty acid chain length of the fatty acid has been found to influence the lipoprotein composition and level. Oleic- and linoleic acid have been found to lower LDL cholesterol compared to palmitic acid (Mattson and Grundy, 1985; Mensink and Katan, 1989). meanwhile stearic
acid has been found to be neutral in regard to a cholesterol raising effect (Keys et al., 1965; Bonanome and Grundy, 1988). Evidence has indicated that high intakes of ω-3 PUFAs supplied on a long-term basis, reduce fasting lipoprotein levels more than ω-6 PUFAs (Weintraub et al., 1988). In the same study postprandial lipoprotein concentrations, chylomicron and non-chylomicron retinyl palmitate, were reduced by 56% and 38%, and 67% and 53%, respectively, compared to saturated fat in response to an acute fat load (Weintraub et al., 1988). The chronic effect of PUFA on plasma lipids appears to be due to a change in the concentration of an endogenous competitor for the system that catabolizes the lipoprotein. Meanwhile, chylomicrons containing saturated fatty acids (SFA) are more susceptible to lipolysis than those produced when a diet rich in PUFA is consumed, so that observed lipoprotein levels are the result of chronic and acute load interactions. When the effects of purified EPA and DHA at a dose of 1g/kg body weight per day, were compared to the effects of corn oil and C16:0, EPA resulted in lower plasma triacylglycerol, phospholipid, free fatty acid (FFA) and cholesterol concentrations. DHA led to reduced levels of cholesterol and FFA (Demoz et al., 1994). While the dosage of 1g/kg body weight per day administered orally could be of therapeutic value, it is not likely to be a common dietary choice.

It has been shown, on the other hand, that the cholesterol raising effect of saturated fatty acids, C16:0 in particular, is significant when considered in combination with cholesterol intake (Pronczuk et al., 1994). With a cholesterol-free diet plasma triacylglycerol levels were significantly higher with a C16:0 rich diet than with a C18:1 rich diet. When cholesterol was added, the saturated fat diet resulted in higher total
cholesterol and LDL-cholesterol than the C18:1 diet (Khosla and Hayes, 1994). Additionally, there are differential effects when saturated fatty acids of varying chain lengths are ingested (MacDougall et al., 1996; Hayes et al., 1991). Similar infant formulas containing either medium chain fatty acids or C16:0 located in the sn-2 position of the triglycerol, and containing similar C18:1 and C18:2 n-6 content, were fed to piglets. Plasma lipid concentrations of C18:1 and C18:2 n-6 have been reported higher in piglets fed medium chain triacylglycerols. Piglets fed formula containing C16:0 had a higher cholesteryl ester content of that fatty acid as well as higher plasma total cholesterol and HDL-cholesterol (Innis, 1991).

It is important to keep in mind that plasma lipid effects of dietary fatty acids depend to a large extent on the species that is being studied in a given experiment. Syrian hamsters that were fed a fish oil diet, compared to diets containing soybean and coconut oil, showed lowered plasma and hepatic triacylglycerol concentration (Liu et al., 1995). These researchers pointed out that the fish oil high cholesterol diet lowered hepatic cholesterol relative to the soybean and coconut oil diets. Whether these results are transferrable to humans is uncertain. The level of fish oil and cholesterol - 10% and 1% respectively - may not be relevant and therefore studies on human subjects are necessary.

The Relationship Between Dietary Cholesterol and Plasma Cholesterol

I (iv) Absorption and Metabolism of Cholesterol

When cholesterol is ingested in the diet, it is absorbed through the intestinal wall. In the smooth endoplasmic reticulum of the intestinal lining, cholesterol and
triacylglycerols are combined with apo B-48. Subsequently apo A is incorporated and chylomicrons are secreted into the lymphatic system, where they acquire apo C-II and apo E from other lipoproteins. Chylomicrons enter the circulation through the subclavian vein. Lipoprotein lipase in skeletal muscle and adipose tissue hydrolyze fatty triacylglycerol, and excess surface material as for example unesterified cholesterol, phospholipids and some apolipoproteins, leave the particles, rendering chylomicron remnants (Norum, 1992). Remnants are then absorbed by the liver, where cholesterol is further processed (Laker, 1996).

Plasma cholesterol and bile in rats fed cholesterol at 1% wt/wt have been compared to those in rats fed a cholesterol-free diet (Smit et al., 1993). Plasma chylomicron remnant clearance was significantly delayed in the former, likely due to competition with βVLDL for hepatic uptake sites. Bile acid radioactivity, from [3H] cholesteryl-ester labelled chylomicron remnants, appeared in cholesterol-fed rats more quickly, showing that this route of cholesterol excretion is coupled to chylomicron remnant uptake by the liver (Smit et al., 1993).

In the liver, endogenous and exogenous fatty acids are utilized to synthesize triacylglycerols which, together with esterified and non-esterified cholesterol, phospholipids, and apo B, C-III, and E, are combined in VLDL particles. These particles are then secreted into the circulation. The transfer of cholesterol esters from HDL and enrichment with apoE, along with the action of lipoprotein lipase in peripheral tissues results in the conversion of VLDL to IDL and further to LDL (Spady et al., 1993; Glickman and Sabesin, 1994). LDL, with its main apoprotein B-100, is absorbed by the
liver via a receptor pathway in a complex process, since the number of receptors and the enzymes involved are regulated by feedback mechanisms. LDL can also be formed through the action of hepatic lipase and can be taken up by hepatic LDL receptors or incorporated by cells in peripheral tissues. The process of conversion of VLDL to LDL is dependent on hormone-sensitive lipase to the extent that an increase in its activity leads to an increase in the uptake of free fatty acids in the liver generating more VLDL and more LDL. (Norum, 1992).

Excess circulating cholesterol additionally represses LDL receptor synthesis at the level of gene transcription (Russell et al., 1983). Alternatively, researchers (Ordovas et al., 1995) have shown that genotype effects can be modulated via alterations in both the amount and type of dietary fat and dietary cholesterol. Dietary cholesterol, at 1% wt/wt, was found to affect cholesterol synthesis and LDL receptor activity differently (Roach et al., 1993). While cholesterol synthesis as measured by hydroxymethylglutarate-coenzyme A (HMG-CoA) enzyme activity, or ratio of plasma lathosterol to cholesterol were inhibited in both Wistar and Sprague-Dawley rats, hepatic LDL receptor activity and mass were increased in Wistar, but remained unchanged in Sprague-Dawley rats. HMG-CoA reductase is the key enzyme in the most regulated step of cholesterol synthesis.

While LDL contributes significantly to plasma cholesterol levels in humans, HDL is also important with regard to cholesterol transport. There are two main mechanisms through which HDL is formed. Surface phospholipids and apoproteins from VLDL are released and combine with a pre-existing pool, raising HDL levels (Glickman and Sabesin, 1994). Alternatively, discoidal HDL particles can dissociate from chylomicrons. Nascent
discoidal HDL excreted by the liver consists of a lipid bilayer and apoE. A reduction of intermediate density HDL on the other hand, can be explained in two ways: 1) a decrease in lecithin-cholesterol-transferase (LCAT) activity; 2) a decrease in the hepatic synthesis of precursor particles (Parks et al., 1987). LCAT progressively esterifies the free cholesterol in discoidal HDL. These cholesterol esters move to the center of the particle, giving it a spherical shape (Laker, 1996).

While HDL catabolism has not been quantitatively characterized, it is known that cholesterol esters transfer to other lipoproteins (Glickman and Sabesin, 1994). One of the main roles of HDL is to carry cholesterol from peripheral tissues, where HDL$_3$ takes up free cholesterol, which is esterified by LCAT, thereby creating HDL$_2$. Free cholesterol from HDL is taken up by the liver, regenerating HDL$_3$ which goes on to a new cycle. Preferential uptake of free cholesterol from HDL by the liver is supported by results from a study (Bravo et al., 1994) comparing the uptake of esterified vs. non-esterified HDL cholesterol. For example it was demonstrated that $[^3]$H-labelled non-esterified cholesterol (52.8%) was measured in the liver, exceeding 6.8% tritiated label recovered in esterified cholesterol. The response pattern in acutely cholesterol loaded peripheral tissues was observed by means of labelled acetyl-LDL and measurement of effluxed cholesterol in the HDL fractions. Researchers (Fragoso and Skinner, 1996) suggested that the distribution pattern of cholesterol in the various HDL fractions pointed to the existence of two routes for reverse cholesterol transport: e.g. one for intermediate size particles and the other for excess cholesterol. With labelled acetyl-LDL loading there was an increase in the
cholesterol fraction of HDL and a 5.1% recovery of $^{14}$C in the liver. In the absence of cholesterol loading this recovery was not observed.

It has been shown that the liver is the major site for HDL uptake, with one third of total HDL protein and two thirds of cholesterol esters taken up by this organ (Pittman and Steinberg, 1984). The kidney is the second major site for HDL apoprotein removal (Pittman and Steinberg, 1984).

The cholesterol taken up by the liver can be converted to bile acids. There is evidence that two pathways contribute to the formation of bile acids (Axelson and Sjovall, 1990). Cholesterol may be degraded either under an acidic pathway, or mitochondrial 27-hydroxylase pathway, yielding chenodeoxycholic acid. It can also be catabolized via the neutral pathway, or microsomal 7α-hydroxylase pathway (Vlahevic et al, 1994), forming cholic and chenodeoxycholic acid. Chenodeoxycholate is converted to lithocholate and is mostly excreted in the feces. Cholate is dehydroxylated to deoxycholate and mostly reabsorbed through the enterohepatic circulation (Laker, 1996).

It is difficult to establish a definite cause-effect relationship for dietary cholesterol intake and plasma cholesterol levels. With respect to the pattern of response to dietary cholesterol consumption, humans can be grouped into “responders” and “non-responders”. The consumption of dietary cholesterol in “responders” leads to a fall in the fractional catabolic rate of LDL, which may be due to a reduction in hepatic LDL receptor activity. It has also been suggested that there is no significant reduction in the LDL receptor independent pathway. Non-responders keep a constant plasma LDL level by decreasing synthesis of cholesterol in the liver, and increasing conversion of cholesterol
into bile acids (Myant, 1990). Additionally there are considerable differences between species. While dietary cholesterol decreased clearance of LDL in hamsters (Spady et al., 1985), feeding up to 0.5% wt/wt was shown to have no measurable effect in rats (Srivastava, 1996).

In humans, dietary cholesterol has been found to lead to an increase in LDL cholesterol concentration as a consequence not only of the presence of a greater number of cholesterol particles (Rudel et al., 1985), but also larger lipoproteins, as the cholesterol ester content increases. Down-regulation of synthesis of LDL receptors (Brown and Goldstein, 1975), as well as suppression of LDL-receptor activity can contribute further to an increase in LDL cholesterol levels (Kovanen et al., 1981).

I (v) The Effect of Dietary Intake on Plasma Cholesterol

Plasma cholesterol levels depend on the interaction of a number of variables, as total plasma cholesterol is the sum of the lipoprotein fractions and each one can be altered differently.

Some studies appear to indicate that exposure early in life to high cholesterol intake can contribute to the development of cardiovascular disease later on in life (Cunnane, 1993). A study comparing several parameters in cholesterol metabolism in low birthweight infants was performed by feeding fortified breast milk (cholesterol intake (ci.) 28.7± 6.5 mg/kg/day), standard pre-term formula (ci. 10.5± 0.5 mg/kg/day) or a high cholesterol formula (ci. 57.3± 2.3 mg/kg/day. Cholesterol excretion and cholesterol
balance were higher in the high cholesterol group (35.5± 8.5 and 21.8± 3.4 mg/kg/day respectively). Fortified breast milk fed infants had intermediate values for cholesterol excretion and balance (20.1± 7.2 mg/kg/day and 8.6±1.2, respectively). Meanwhile those infants put on a low cholesterol formula were in negative cholesterol balance (-7.7±2.4 mg/kg/day), yet they excreted cholesterol at 18.2 ± 5.5 mg/kg/day. As serum cholesterol concentrations were similar in the group fed fortified milk and high cholesterol formula, it was concluded that preterm infants were able to adapt to a cholesterol intake higher than that of regular breast milk, by increasing fecal excretion and reducing endogenous synthesis (Boehm et al., 1995).

In the adult, the effect of dietary cholesterol on plasma lipids can be compounded by the presence of dietary fats. In a study where Syrian hamsters were fed cholesterol at a concentration of 0.12% wt/wt added to a low fat diet, the cholesterol concentrations in most lipoprotein fractions increased (Sessions and Salter, 1995). This effect was not observed when cholesterol was not supplied in the diet (Sessions and Salter, 1995). Various species involved in studies on the effects of dietary cholesterol on plasma cholesterol levels are affected differently. Humans and hamsters, both species with relatively low rates of hepatic cholesterol synthesis, when fed cholesterol, tend to down-regulate their rates of cholesterol synthesis (Roach et al., 1993; Wong et al., 1993; Gylling and Miettinen, 1992). Dietary cholesterol levels are therefore reflected in plasma cholesterol concentration. Feeding cholesterol to vegetarians, in the form of three egg yolks per day, who commonly have low plasma cholesterol concentrations (Nestel et al., 1981), resulted in an increase in total serum and LDL cholesterol concentration. These
changes can be explained by an increase in cholesterol absorption, combined with an unchanged rate of cholesterol synthesis (Vuoristo and Miettinen, 1994). The potential for change in plasma cholesterol through an alteration in dietary cholesterol intake is maximized in individuals with primary hypercholesterolemia. In this instance, an increase in 100 mg dietary cholesterol can lead to an increase in LDL cholesterol concentration of 3 mg/dl, supporting a maximal reduction in dietary cholesterol intake (Illingworth et al., 1995).

**Hypertension and Dyslipidemia**

I (vi) **Human Studies**

Hypertension (HT) generally implies that a person’s arterial pressure is above the upper limit of a normal range. Arterial systolic hypertension is defined by systolic blood pressure above 140 mm Hg, while diastolic hypertension corresponds to diastolic blood pressure above 90 mm Hg (Perera, 1959). Elevated blood pressure is also frequently associated with decreased renal function, leading to salt and water retention. This in turn can lead to a further increase in blood pressure. These parameters are regulated in a hormonal feedback manner to the point where the pressure exerted on the kidneys is such that they are forced to excrete at normal levels. The physiological basis for increased blood pressure can be either an increase in blood volume or vasoconstriction.

Ninety percent of all persons who do have HT, are said to have essential hypertension, which means the HT is of unknown origin. Many people do have some kind of genetic predisposition to HT. Some of the characteristic features of HT include a
decrease in renal blood flow in the later stages of the disease, combined with a nearly normal filtration rate due to increased pressure. Cardiac output is also near normal values, as is the secretion of salt and water. The main consequences of high blood pressure are an increased workload for the heart, resulting in the hypertrophy of the left ventricle over time. The high blood pressure also increases the risk for arteriosclerosis. Sclerosis of blood vessels occurs, leading to formation of blood clots and to weakening of the blood vessel, thereby enhancing the probability of thrombosis (Guyton, 1987). These can manifest as myocardial infarction or cerebro-vascular accident.

I (vi-1) The Relationship Between LDL and Vascular Lesions

The mechanism through which hypertension promotes damage is as follows:

As mentioned previously several factors contribute to the development of lesions in the arterial lining. High plasma LDL levels and oxidized LDL are two of the factors that can be influenced by dietary factors. It is known that glycosylated endproducts in diabetes and circulating vasoactive amines can lead to the initial injury that allows entry of lipids into the vessel’s intima (Picard, 1995). LDL represents the primary lipid source for atherosclerotic lesions (Fuster et al., 1992). It recognizes dysfunctional layers in the vessel wall. Once LDL enters the intima it is oxidized. At this stage, the production of adhesive molecules is initiated and migration of macrophages to the site occurs. These take up modified LDL and are gradually converted into foam cells during the process. Some of the excess LDL may be removed by HDL; however, if this does not occur, and more LDL enters than exits, the foam cells explode, releasing oxidized LDL and free radicals. These
products have cytotoxic properties and can cause further injury (McLaughlin and Fuster, 1995). Smooth muscle cells concentrate at the locus of an injury and form an atherosclerotic plaque composed of macrophages, fibrous tissue and lipids.

I (vi-2) **Cholesterol Biosynthesis**

Diet provides a portion of the cholesterol utilized in various metabolic processes. If the quantity of ingested cholesterol increases, the maintenance of circulating serum cholesterol values depends on the body’s ability to down-regulate endogenous synthesis. A brief outline of cholesterol synthesis therefore aids in clarifying which steps are critical in this process.

Two molecules of acetylCoA combine to form acetoacetil CoA. The addition of another acetylCoA yields HMG-CoA. This step is regulated by feedback inhibition. The reduction of HMG-CoA yields mevalonate in what is the most highly regulated step of the pathway. From mevalonate, isopentylpyrophosphat is produced, then geranylpyrophosphate followed by farnesylpyrophosphate are produced as precursors for squalene, a membrane bound cholesterol precursor, which yields, after several steps, cholesterol. The enzyme HMG-CoA reductase is the rate limiting enzyme for cholesterol synthesis and is a common target site for cholesterol-lowering medication.

Cholesterol can be excreted as biliary cholesterol or further metabolized into bile acids, steroid hormones or other lipoprotein fractions. Most cholesterol is excreted in the feces as neutral sterols or as a component of bile acids, the synthesis of which is controlled by 7α-hydroxylase (Jelinek et al., 1990).
I (vi-3) **Plasma Lipids as Risk Factors for Coronary Heart Disease**

Coronary heart disease (CHD) has been associated with elevated blood lipid levels, in particular with increased LDL cholesterol levels. One condition that determines high plasma cholesterol levels is a reduced number of hepatic LDL receptors or the presence of receptors with reduced affinity. A number of epidemiological studies (Rosengren et al., 1989; Neaton and Wentworth, 1992; Pocock et al., 1989) have shown that a reduction of 10% in total plasma cholesterol was associated with a 54% lower incidence of CHD (Law et al., 1994). The values for reduction in incidence were lower as more advanced age groups were studied (Gotto, 1995).

A direct cause-effect relationship between hypertension and dyslipidemia has not been clearly established. Indirect mechanisms through which serum lipids modify physiologic parameters that directly modulate hypertension have been better defined (Giuliano, 1995; Karanja et al., 1989). For example, both blood clotting factors VII and PAI-1 strongly correlate to serum triacylglycerol levels (Gotto, 1995). Elevated LDL levels are thought to inhibit release of endothelium-derived relaxing factor (EDRF). Low HDL-cholesterol levels are thought to interfere with endothelial production of prostacyclin and may promote the degradation of prostacyclin. Even these short-term mechanisms could affect vasodilation and thrombotic events (Gotto, 1995).

There are three ways through which one can acquire a risk factor to CHD (Cunnane, 1993). These are: i) genetic predisposition, which is relatively rare; ii) environmental susceptibility, which includes diet and life-style induced elevation of serum cholesterol; iii) a combination of both. Among the life-style factors are poor dietary habits,
lack of exercise and excessive alcohol consumption (Liu et al., 1995). These could result in excessive energy intake compared to output leading to obesity. Within dietary habits, excessive fat intake is a major contributing factor. Interestingly, it is known that especially when there is a family history of CHD, children from the family are already susceptible to these factors (Holman, 1961). This brings attention to the importance of proper dietary and exercise habits from an early time in life.

Familial predisposition can also be the underlying cause for both hypertriglyceridemia and hypercholesterolemia. In these cases fasting triacylglycerols levels are above the 90th percentile and HDL-cholesterol levels are in the lowest 10th percentile (Genest et al., 1992). While the efficacy of cholesterol-lowering drugs has been confirmed in a number of studies (Furberg et al., 1994; Gotto, 1994; S.S.S.G., 1994), it is worth noting that among the side effects of some older anti-hypertensive drugs are increased triacylglyceride and lower HDL levels (Simons et al., 1992). A study on untreated hypertensives showed that total serum cholesterol was 4% higher in males and 5% higher in females when compared with the normotensive population. Total serum triglycerol levels were 10% higher in males and 17% higher in females, but there was no difference in HDL levels among the hypertensive individuals (Simons et al., 1992).

Although depressed plasma HDL levels correlate with increased incidence of CHD, there are only few data on the benefits of raising plasma HDL cholesterol (Manninen et al., 1988; Gordon et al., 1986).

Diabetes mellitus is another risk factor for CHD found in combination with dyslipidemia. Diabetes that develops at an adult age, frequently in middle age, is often the
consequence of life-style factors. Researchers (Samuelsson et al., 1994) determined that the presence of diabetes mellitus, elevated serum cholesterol and triacylglycerols at the outset of a 15 year follow-up study on middle aged hypertensive treated men were associated with CHD. Diabetes mellitus that developed during the study did not show the same correlation. Some risk factors are interrelated and the progression of an individual factor can neutralize the benefits of lowering blood pressure. Hypertension and insulin resistance, as observed in diabetics, were found to have a highly significant positive association, independent of confounding factors such as age, sex, and obesity (Modan et al., 1985). These workers have also shown that hyperinsulinemia is a common pathophysiologic feature of obesity, glucose intolerance and hypertension. Of the hypertensive individuals in that study, 83.4% were overweight. The relevance of these findings lies in that some inter-related contributing factors can be modified by dietary fat intake. For example, although a direct effect of food intake on hypertension has not been unequivocally confirmed, it appears that hypertension can be influenced indirectly by dietary food habits.

I (vi-4) Dietary Fats and Hypertension

One theory on the effect of unsaturated fatty acids on blood pressure suggested that the incorporation of those fatty acids into membranes increases cell permeability, which in turn stimulates the activity of the sodium pump and cation transport (Morris, 1994). However supplementation with linoleic and oleic acid has not been shown to change sodium pump activity (Sacks et al., 1987).
Biochemical studies on the effects of fatty acids on blood pressure are more accurate than diet recording, due to limited record of food selection. Equilibration times required for fatty acid modelling of lipid constituents range from 3-4 weeks in plasma phospholipids to 1-2 years in adipose tissue. However, according to Morris (1994) neither the biochemical, nor the cross sectional studies conclusively showed that dietary fats affect blood pressure directly. A cross-over dietary intervention study showed a surprising decrease in diastolic blood pressure with a measured intake of saturated fat (McIver et al. 1990). Interestingly, it appears that the most promising results have been observed in hypertensive patients who had a reduction in blood pressure from 3.4 mmHg to 2.8 mm Hg when given a fish-oil dose of 5.6 g/day.

An intake of 5 % (wt/wt) of fish oil administered to SHR and WKY rats was found to decrease the spontaneous basal tone of the aorta (Smit et al., 1993). In a study on the long-term effects of four diets, with different levels of saturated, monounsaturated and polyunsaturated fatty acids, it was shown that an increase in the intake of saturated fatty acids raised blood pressure in men (Uusitupa et al., 1994). A diet low in saturated fats, with a high linoleic acid content resulted in a lower blood pressure (Uusitupa et al., 1994). In vitro studies on perfused vascular mesenteric beds of SHR and SHR-WKY backcross rats, produced an attenuation of blood pressure between 10-26 mm Hg. The magnitude of change was dependent on the EPA and DHA content of the fish oils (Mano et al., 1995).

The content of EPA and DHA in a given oil per se appear to play a role in modifying blood pressure, but it has also been shown to lower plasma lipid concentration. The proportion of saturation to unsaturation in dietary fats is also an important factor to
consider in hypertensive individuals. Some Western population groups with a relatively high rate of fish consumption still exhibit a high incidence of CHD. Some researchers (Nordoy et al., 1992) compared hemostatic parameters, e.g. systolic blood pressure, in men who were given a diet containing two levels of saturated fat (5% wt/wt and 19% wt/wt) equivalent to 25% and 39% calories from fat, combined with n-3 polyunsaturated or monounsaturated fatty acids, mostly from oleic acid, at 2% of energy. The effects of EPA and DHA on platelet, vascular function and eicosanoid production were modulated by saturated fatty acid content. For example, bleeding time increased with a diet low in saturated fat. The cholesterol-lowering effect of a reduction in saturated fat intake from 19% to 5% of energy intake was not altered by the addition of n-3 fatty acids as assessed by cross-over design (Nordoy et al., 1992). This study had however, at least two shortcomings. The timespan covered by the experiment, three weeks, was likely insufficient to determine whether the observations were truly longterm effects. Secondly the number of subjects was very small and may not have allowed for a true estimate of the outcomes, given that inter- and intra-individual variation can be considerable.

I (vii) Animal Studies

In Milan hypertensive rats (MHS), the development of hypertension is linked to a molecular abnormality that induces faster cell ion transport and increased renal tubular reabsorption, probably mediated by an accelerated Na⁺-K⁺-pump activity (Bianchi and Ferrari, 1995). An endogenous Na⁺-K⁺-inhibitor, ouabain-like factor (OLF), is simultaneously increased in MHS (Ferrandi et al., 1992). While OLF levels have been
shown to be increased in MHS, as well as during experimental and human hypertension, the role of OLF in SHR has not been described in depth (Ferrari et al., 1993). Simultaneously, the occurrence of diabetes and hypertension exacerbated early vascular pathology (Todd et al., 1993). The renal artery of both streptozotocin-induced diabetic Wistar rats and diabetic deoxycorticosterone acetate (DOCA) hypertensive Wistar rats has been shown to have a significantly thickened tunica media. Additionally, the diabetic/hypertensive animals showed evidence of subendothelial macrophage invasion with foam cells of varying shapes and densities (Todd et al., 1993). These examples demonstrate the underlying pathology of hypertension induced by insulin resistance can be further modified by dietary intake of selected fats.

While the effects of minerals on the manifestation of hypertension in SHR have been partly elucidated (Kitts et al., 1992), the influence of dietary fatty acids has not been as clearly defined. Saturated fatty acids increased platelet function in hypertensive animals, but polyunsaturated fatty acids did not eliminate platelet reactivity associated with hypertension (McGregor et al., 1981). These changes were associated with an enhancement of cellular tissue C20:3ω9 content in hypertensive animals fed saturated fat, while those on polyunsaturated fat showed an increase in 20:4 fatty acids. Fish oil diets, when compared to olive oil, safflower oil and beef fat, reduced the blood pressure of stroke-prone SHR by 20-25 mmHg. n-3 polyunsaturated fatty acids modulated the contractile responses of blood vessels mediated by sympathetic nerves in isolated perfused mesenteric vascular bed preparations (Head et al., 1991). Hypercholesterolemic diets, including 5% cholesterol, caused an increase in hepatic fat deposition, aortic cholesterol
deposits, and changes in monounsaturated/saturated and linoleate/arachidonate ratios in tissue lipids. These changes did not correlate positively with blood pressure. This comparative study on WKY, SHR and SHR-SP rats indicated that hypertension alone was not the only result in the development of hypercholesterolemia and arterial cholesterol deposition (Mori et al., 1993). Extreme diets have been used to elicit a number of risk factors for CHD. High fat/high sucrose diet at 39.5% each respectively led to the development of obesity, hypertension, hyperinsulinemia and hypertriglyceridemia in inbred Fisher rats. Their counterparts fed a low fat/complex carbohydrate diet (6% and 58%, respectively) did not demonstrate the same changes (Barnard et al., 1993).

Fish oil has been found to lower blood pressure in SHR, when compared to butter and corn oil (Karanja et al., 1989). However, the supplementation of butter diets with calcium lowered blood pressure in animals when compared to counterparts fed corn oil, but resulted in no significant difference when compared to both high and low calcium fish oil diets (Karanja et al., 1989). Stroke-prone SHR fed a soyprotein diet enriched with EPA and methionine, at 0.5% cholesterol, increased the EPA to arachidonic acid ratio in plasma and lowered total plasma cholesterol (Chang et al., 1990).

Male Sprague-Dawley rats on an extremely high fat diet (66.4%) with their food intake limited to prevent obesity, did not develop hypertension. Their counterparts fed a high glucose diet did develop high blood pressure without becoming either obese or hyperinsulinemic. The rats that developed hypertension also had higher norepinephrine excretion, indicating that the sympathetic nervous system is involved in mediating the effects of dietary fat or glucose on blood pressure (Kaufman et al., 1991). Norepinephrine
stimulated calcium influx in arterial smooth muscle cells was faster in SHR than in WKY (Cauvin and Van Breemen, 1986). Therefore, a change in blood pressure associated with feeding a particular fat type may be norepinephrine-based calcium flux. At a level of 6 g\% menhaden oil, both reduced plasma cholesterol and triacylglycerol concentration, as well as reduced the hypertensive damage to the renal and the cardiovascular system (Shimamura and Wilson, 1991). Although the data on the effects of dietary fats on risk factors for CHD are contradictory, evidence shows that dietary fats do influence prostaglandin production. They also alter blood lipid parameters, as described later on.

I (vii-1) **The Relationship Between Antioxidant Status and Hypertension Enhancing Factors**

Glutathione, L-γ-glutamyl-L-cysteinylglycine (GSH), is known to function directly or indirectly in many biological functions including metabolism and protection of cells. GSH forms conjugates with prostaglandins and leukotrienes and thus participates in their metabolism (Meister and Johnson, 1983). GSH is also involved in the protection of cells against reactive oxygen compounds and free radicals, by means of reducing reactions. GSH turnover is linked to its transport out of cells. Intracellular GSH is converted to GSSG by GSH peroxidase, which catalyzes the reduction of $\text{H}_2\text{O}_2$ and other peroxides. GSSG can also be produced by transhydrogenation. The conversion of GSSG to GSH is mediated by GSSG reductase, which uses NADPH as a cofactor (Meister and Johnson, 1983). Intracellular glutathione is composed of over 99\% GSH, although that value may vary. Repletion of intracellular GSH in cell preparations is accomplished by degradation of
extracellular GSH and intracellular resynthesis (Meister and Johnson, 1983). Transport of GSH allows for the transfer of cysteine sulfur between cells and in the process protects the cell against oxidative processes.

More specifically, GSH peroxidase catalyzes GSH-dependent reduction of H$_2$O$_2$. The reaction is coupled to the oxidation of glucose-6-phosphate and 6-phosphogluconate providing NADPH for the function of GSSG reductase. At the same time, other peroxides can also be reduced through this pathway and cells are protected against their toxic effects (Meister and Johnson, 1983). GSH peroxidase also plays a role in the regulation of prostacyclin formation (Flohe, 1979).

GSH reductase occurs only intracellularly and there does not appear to be an extracellular mechanism for GSSG reduction (Anderson and Meister, 1980). This suggests that GSH may be a major source of thiol for the blood plasma compartment. Intracellular GSH levels can thus be considered as an indicator of the degree of oxidative stress that cells are exposed to and may be a marker for cell damage leading to hypertensive changes, as discussed below.

The difference in antioxidant status and plasma lipid levels in SHR and WKY on a standard diet was evaluated by Yuan et al. (1996). GSH depletion in heart tissue was not different between animal strains, but thiobarbituric acid reactive substances (TBARS) production was greater in SHR. RBC-GSH in SHR was relatively less depleted, but MDA production was enhanced at higher levels of pro-oxidant (Yuan et al., 1996)
I (vii-2) **Oxidative Stress**

Reactive oxygen molecules are generated through the activation of triplet oxygen. Triplet oxygen itself can react with free radicals, form complexes with oxygen and metal ions and thus generate active peroxide radicals, initiating an auto-oxidation process in unsaturated fatty acids (Namiki et al., 1990). Activated oxygen is obtained by excitation or reduction of triplet oxygen (Namiki et al., 1990). Singlet oxygen also reacts with unsaturated bonds, but it does not dehydrogenate compounds, nor does it initiate chain reactions (Wasserman and Murray, 1979). With heme iron or chlorophyll active oxygen radicals, auto-oxidation of unsaturated fatty acids is initiated in the presence of light.

Endothelium-derived nitric oxide (NO) is a potent endogenous vasodialator. Oxygen-derived free radicals (OFR) have been found to interfere with NO action, thus contributing to hypertension in diabetic patients. Vitamin E has been shown to reduce oxidative stress and red blood cell microviscosity in diabetic patients (Giugliano et al., 1995). Depleted GSH levels can therefore serve as indicators for potential of further deteriorative developments. There are several pathways, which when positively enhanced, can lead to a decrease or increase in NADPH or NADH concentrations respectively, ultimately causing an imbalance between $O_2^-$ and NO. For example, a hyperactive polyol pathway, caused by an increase in the activity of aldose reductase (AR) will lead to a drop in NADPH levels. This will promote a decrease in the generation of NO from arginine and an increase in $O_2^*$, leading to the formation of OH. The role of free radicals in the development of hyperglycemia is explained in the figure below.
Fig. 1. Hyperglycaemia may cause hyperactivity in these pathways and lead to an imbalance between the radicals $O_2^-$ and NO.

(AR = aldose reductase; SDH = sorbitol dehydrogenase; Cox = cyclooxygenase; AA = arachidonic acid; $\text{PGG}_2 = \text{prostaglandin G}_2$; $\text{PGH}_2 = \text{prostaglandin PGH}_2$; OH = hydroxyl radical; $\text{NO}_2 = \text{nitrogen dioxide}$; adapted from Picard (1995).

In vitro treatment of erythrocytes from non insulin dependent diabetes mellitus (NIDDM) patients with glucose has been shown to result in reduced $\gamma$-glutamylcysteine synthetase activity, glutathione concentration and thiol transport. Treatment of patients with this disorder using an antidiabetic agent can restore the levels of GSH parameters to
normal values, suggesting that oxidative stress can be the cause for some complications of diabetes mellitus (Yoshida et al., 1995).

I (viii) Lipid Peroxidation

There are two pathways for lipid peroxidation in vivo. One involves autoxidation of catecholamines, thiols and quinone redox reactions of heme compounds. The second pathway involves active oxygen from the action of enzymes such as NADPH oxidase and other oxidative enzymes, e.g. cytochrome P\textsubscript{450}. The presence of lipid peroxides has been linked to the degradation of membrane phospholipids through the action of hydroperoxides, free radical intermediates and other secondary products. Peroxidation can alter the structure of biological membranes and change functional properties, which results in greater susceptibility to injury (Recknagel et al., 1982; Ungemach et al., 1987). In a study on essential hypertensive individuals an inverse correlation was obtained between systolic and diastolic blood pressure, and GSH concentrations and superoxide dismutase (SOD) activity (Giugliano et al., 1995; Kimoto et al., 1995). NADPH is located in the cell membrane of polymorphonucleocytes (PMNL) and is involved in the generation of oxygen derived free radicals (Sagar et al., 1992). PMNL from essentially hypertensive patients have greater chemiluminescence, an indication of the presence of oxidized compounds (Miazawa et al., 1992), and thus also greater depletion of antioxidants. This implies that with essential hypertension, increased membrane NADPH oxidase activity occurs (Sagar et al., 1992). Low density lipoprotein oxidation could also contribute to accelerated atherogenesis (McQuinn, 1987). An enhanced rate of lipoprotein peroxidation
was observed in PMNL of hypertensive patients, indicating a link between antioxidant status, lipoprotein lipid composition and susceptibility to CHD (Rhaman and Nath, 1988).

MDA (malondialdehyde) derived from thromboxane A2 was increased in patients with CHD in comparison to the concentrations measured in healthy subjects (Buczynsky et al., 1993). GSH in PMNL from hypertensive patients was significantly decreased when compared to that of normotensive patients (Rhaman and Nath, 1988). Conjugated dienes, a sign lipid oxidation which could lead to oxidative stress, were found to be increased in PIH (pregnancy induced hypertension), when compared to normotensive pregnancies, as was the activity of glutathione peroxidase. Fetuses of women with PIH showed lower levels of antioxidants which appeared to reflect the extent of maternal oxidative stress (Uotila et al., 1993). Studies on LDL of hypertensive subjects have revealed enhanced susceptibility to oxidation in vitro as indicated by the forced peroxidation of lipids after exposure to CuSO4 (Maggi et al. 1993). Furthermore a higher plasma antioxidized LDL antibody concentration was found in hypertensive patients. No differences in vitamin E concentration in plasma were found when hypertensive hypercholesterolemic patients and normotensive controls were compared (Uotila et al., 1993).

Young male survivors of myocardial infarction had LDL assessed for its susceptibility to oxidative modification by measuring the lag phase for the formation of conjugated dienes from exposure to copper ions. This information was combined with that on the severity of coronary atherosclerosis (CA). An inverse relationship between the lag phase for diene formation and coronary atherosclerosis was found (Regnstroem, 1992). LDL cholesterol and LDL triacylglycerol levels also correlated with the severity of CA.
and with the susceptibility to oxidative changes (Regnstroem and Nilsson, 1992). Further
evidence supporting the interdependence of hypertension and atherosclerosis with the
influence of dietary input has been reviewed (Alexander, 1995).

1 (ix) Animal Models and CHD

The choice of an animal model for the study of coronary heart disease (CHD) rests
on the fulfillment of a number of requirements. The species of choice should be easy to
acquire and maintain, easy to handle, and have a size that is adequate to facilitate the
procedures it will undergo. Furthermore, the animal model should ideally confirm results
in a laboratory setting and have well-defined genetic characteristics to minimize
intraspecies variation. The animal should also share some of the key-features of the disease
process under study with the human species (Jokinen et al., 1985). There is no animal that
fulfills all these requirements. The appropriate model is therefore often used to investigate
one particular aspect of a disease or, alternatively, to study an associated condition. The
interdependence of the environment and phenotypes at various levels of cellular
organization from the perspective of the development of a particular trait defined by its
genetic make-up is shown in a diagram below.
According to this diagram, hypertension is a complex final phenotype that involves many body control systems operating at each level of biological organization (Cusi and Bianchi, 1992).

Hypertension has been known to be one of several factors contributing to the development of atherosclerosis. There are a number of avian species, such as pigeon, chicken, turkey, and quail, that lend themselves to the study of atherosclerosis. Characteristics they share are the development of atherosclerotic lesions which can be aggravated by atherogenic diets, and the fact that they are easy to maintain. However as these are non-mammal species, plasma lipoprotein profiles in pigeons and chickens, for
example, tend to be different than for humans. The specific combination of advantages and disadvantages as seen in the context of a particular study of human disease using animal models is then the decisive factor in making an appropriate choice. Mammalian species are often used in CHD research. Rabbits, dogs, pigs, mice and rats have the potential to develop lesions under experimental conditions. Rats have several advantages. They have a short reproductive cycle and are easily maintained. More importantly, there are several strains with specific genetic disorders available, including spontaneously hypertensive and spontaneously hypertensive-stroke prone strains. Rats have some disadvantages as well, such as their small body size, which increases the difficulty of manipulative procedures, in particular those involving blood vessels (Jokinen et al., 1985). The amount of sample material obtained is often limited. Important features, such as arterial lesions, may be difficult to induce and visualize.

The spontaneously hypertensive rat (SHR) of the Okamoto strain was developed in 1963 by Okamoto and Aoki in Kyoto, Japan (Okamoto and Aoki, 1963). Animals from this strain establish hypertension by ten weeks of age. In humans essential hypertension is predominant, consequently the origin of 90% of hypertension in man cannot be determined with certainty. SHR on the other hand were specifically bred for the development of hypertension over a time span. The breeding process has also led to a high degree of genetic definition. It is known that hypertension in SHR is determined by several genetic loci (Louis et al., 1969). There is evidence that hypertension in humans can also be influenced by dietary fats (McCarty et al., 1996; Simon et al., 1996; Bexis et al., 1994) in
susceptible individuals; however the origin of this symptom is unknown and more difficult to trace in a cause-effect relationship.

Another advantage of the rat model is that it is haemodynamically similar to humans. Baseline measurements of plasma cholesterol and triacylglycerides in rats have been found to resemble human values. These values can vary significantly, depending on the diet supplied and the rat strain studied (Tobian et al., 1991). For example, SHR fed a Ca$^{2+}$ deficient diet showed increased activity of Na$^{+}$K$^{-}$-ATPase, Ca$^{2+}$-ATPase, and alkaline phosphatase in basolateral membranes and Ca$^{2+}$-ATPase and alkaline phosphatase in brushborder membranes in the intestine when compared to rats fed a diet with adequate Ca$^{2+}$ content (Blakeborough et al., 1990). Wistar Kyoto (normotensive controls) (WKY) did not show these alterations. Similarly blood pressures were higher in SHR fed a Ca-deficient diet than those on an adequate Ca-diet, and WKY did not show blood pressure changes. Other minerals, for example P, Mg, and Na, have been found to have an effect on hypertension in SHR (Linas and Marzec-Calvert, 1986; Schleifer et al., 1984; Stern et al., 1984). Intestinal Mg-malabsorption has been linked to hypertension in SHR (Lau and Oasa, 1984), and Mg depletion has been found to decrease blood pressure in SHR but not in WKY rats (Linas and Marzec-Calvert, 1986).
II - Materials and Methods

Male spontaneously hypertensive rats (SHR) and Wistar Kyoto (WKY) rats, 5 weeks old, eight animals per batch, for a total of 96 animals, were purchased from Charles River (Quebec) and weighed upon arrival before being housed in individual wire-mesh bottom stainless steel cages. The room temperature was set at 23°C and the light/darkness regimen was timed in 12 h intervals, with light from 8:00 am to 8:00 pm. Animals were fed standard rat chow (Ralston - Purina) ad libitum for 7 days. On day eight, the experimental diets were introduced ad libitum. From day 15, animals were habituated to a meal-feeding regimen with feed supplied at 9:00 am and feeders removed at 3:00 pm. Feeders were weighed before being placed in the cages and after their removal. Base feed was kept in a freezer at -20°C. Experimental diets were prepared freshly every week and kept refrigerated at 4°C. Animals had access to deionized distilled water ad libitum.

The animals were weighed three times a week. On week 10 of the experiment, the animals were placed in metabolic cages (Nalgene, Rochester, NY) for 150 - 300 g rats. The cages were equipped with collection funnels and separation cones to separate feces and urine, to eliminate urine washover and contamination of feces. Excreta, urine and feces, were collected, and weighed for 5 days, pooled for each animal and frozen until analysis. The weighing regimen proceeded as before. Feed intake and water consumption during the metabolic study was monitored according to the protocol of the entire experimental period. Animals were cared for according to the principles of the Guide to
the Care and Use of Experimental Animals, Vol.1, of the Canadian Council of Animal Care (Olfert et al., 1993).

II (i) Experimental Diets

A basal diet was prepared by adding dry components. Then a different fat source, as well as cholesterol and cholic acid, were added. Diets were mixed in a Hobart mixer for 20 min. Vitamin-free casein, corn starch, and cholesterol were purchased from ICN Biomedicals Inc., Aurora, Ohio. Calcium carbonate was added as garden lime from Pacific Gardens Galore & Co., Delta, B.C. AIN mineral mix (a calcium free mineral mix), and D,L-methionine were acquired from US Biochemical Corporation, Cleveland, Ohio. Alphacel, non-nutritive bulk, and a vitamin mix were purchased from ICN Biochemicals Inc., Cleveland, Ohio. Sucrose and canola oil were supplied by Neptune Food Services, Richmond, B.C. Amonium monophosphate was supplied by Van Waters & Rogers in Abbotsford, B.C. Unsalted butter was supplied by Dairyworld Foods, Burnaby, soybean oil by Bioforce Canada, Burnaby, B.C., and menhaden oil by Zapata Haynie, Reedville, VA.

Total cholesterol content of the diets was standardized to either 0.5% or 0.05% (wt/wt), for a total of six different diets. Cholic acid was added at 0.25% or 0.025% (wt/wt) to facilitate the absorption of dietary cholesterol. Detailed composition of the diets can be found in Table 1.
II (ii) **Dietary Fatty Acid Composition Analysis**

This analysis was carried out by SGS Canada Inc. according to AOAC method 991.39. Fatty acids were identified as their methylesters by gas-liquid chromatography using a Varian Model 3700 GC, fitted with a SP-2330 silica capillary column (30 m x 0.25 mm ID, Supelco Inc., USA). Nitrogen was the carrier gas at 40 ml/min. The analysis was performed at 180°C for 10 min, followed by a temperature gradient that increased by 5°C/ min to 195°C. The proportion of principal fatty acids was calculated based on the mass of their methyl esters from retention times of known standards using a Varian Model CDS 401 integrator. Fatty acid composition, as described above, and total crude lipid content of the experimental diets, utilizing Folch’s extraction, shown in Table 2 and 3, were assessed in order to confirm the actual make-up of the blend resulting from the combination of the main dietary fat with canola oil. Included in Table 2 are those fatty acids present at concentrations of 0.1% wt/wt or more.
### Table 1: Composition of Experimental Diets

<table>
<thead>
<tr>
<th>Component</th>
<th>Content in % weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein&lt;sup&gt;1&lt;/sup&gt;</td>
<td>25.0</td>
</tr>
<tr>
<td>CaCO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.0</td>
</tr>
<tr>
<td>Ca free mineral mix&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.5</td>
</tr>
<tr>
<td>Vitamin mix&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3.0</td>
</tr>
<tr>
<td>Choline chloride&lt;sup&gt;6&lt;/sup&gt;</td>
<td>0.2</td>
</tr>
<tr>
<td>D,L-methionine&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.3</td>
</tr>
<tr>
<td>Alphacel&lt;sup&gt;4&lt;/sup&gt;</td>
<td>5.0</td>
</tr>
<tr>
<td>Sucrose&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3.0</td>
</tr>
<tr>
<td>Canola&lt;sup&gt;5&lt;/sup&gt;</td>
<td>3.0</td>
</tr>
<tr>
<td>Test fat source</td>
<td></td>
</tr>
<tr>
<td>Butter&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Soybean Oil&lt;sup&gt;8&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Menhaden Oil&lt;sup&gt;9&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Monophosphate&lt;sup&gt;6&lt;/sup&gt;</td>
<td>3.0</td>
</tr>
<tr>
<td>Corn Starch&lt;sup&gt;1&lt;/sup&gt;</td>
<td>39.0</td>
</tr>
<tr>
<td>Cholesterol (free)&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.5 or 0.05</td>
</tr>
<tr>
<td>Cholic Acid&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.25 or 0.025</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

---

<sup>1</sup> ICN Biomedicals Inc., Aurora, Ohio.
<sup>2</sup> Pacific Gardens Galore & Co., Delta, B.C.
<sup>3</sup> US Biochemical Corporation, Cleveland, Ohio.
<sup>4</sup> ICN Biochemicals Inc., Cleveland, Ohio.
<sup>5</sup> Neptune Food Services, Richmond, B.C.
<sup>6</sup> Van Waters and Rogers, Abbotsford, B.C.
<sup>7</sup> Dairy World Foods, Burnaby, B.C.
<sup>8</sup> Bioforce Canada, Burnaby, B.C.
<sup>9</sup> Zapata Haynie, Reedville, Va.
Table 2  Fatty Acid Profile of Experimental Diets

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>Diets</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Butter</td>
<td>Soybean</td>
<td>Menhaden</td>
</tr>
<tr>
<td>Tetranonic</td>
<td>C4:0</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Hexanonic</td>
<td>C6:0</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>Octanonic</td>
<td>C8:0</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>Decanonic</td>
<td>C10:0</td>
<td>2.1</td>
<td>-</td>
</tr>
<tr>
<td>Dodecanonic</td>
<td>C12:0</td>
<td>2.9</td>
<td>-</td>
</tr>
<tr>
<td>Tetradecanonic</td>
<td>C14:0</td>
<td>9.1</td>
<td>0.3</td>
</tr>
<tr>
<td>Tetradecaenoic</td>
<td>C14:1</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Pentadecanonic</td>
<td>C15:0</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>Hexadecanonic</td>
<td>C16:0</td>
<td>26.7</td>
<td>22.7</td>
</tr>
<tr>
<td>Hexadecaenoic</td>
<td>C16:1</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Heptadecanonic</td>
<td>C17:0</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Heptadecaenoic</td>
<td>C17:1</td>
<td>-</td>
<td>0.2</td>
</tr>
<tr>
<td>Octadecanonic</td>
<td>C18:0</td>
<td>8.3</td>
<td>9.4</td>
</tr>
<tr>
<td>Octadecaenoic</td>
<td>C18:1</td>
<td>29.6</td>
<td>50.5</td>
</tr>
<tr>
<td>Octadecadienoic</td>
<td>C18:2</td>
<td>9.0</td>
<td>8.3</td>
</tr>
<tr>
<td>Octadecatrienoic</td>
<td>C18:3</td>
<td>2.4</td>
<td>0.5</td>
</tr>
<tr>
<td>Eicosanoic</td>
<td>C20:0</td>
<td>0.3</td>
<td>1.1</td>
</tr>
<tr>
<td>Eicosenoic</td>
<td>C20:1</td>
<td>0.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Eicosadienoic</td>
<td>C20:2</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eicosapentaenoic</td>
<td>C20:5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dodecanonic</td>
<td>C22:0</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>Docosaenoic</td>
<td>C22:1</td>
<td>-</td>
<td>0.4</td>
</tr>
<tr>
<td>Docosapentaenoic</td>
<td>C22:5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Docosahexaenoic</td>
<td>C22:6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

- Indicates concentration present in the individual dietary fat blend 0.1% or less.
Table 3  Dry Matter (DM), Total Crude Lipid (TCL) and Gross Energy (GE) of Experimental Diets

<table>
<thead>
<tr>
<th>Diet</th>
<th>DM (%)</th>
<th>TCL (%)</th>
<th>GE (cal/g DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butter L*</td>
<td>91.24</td>
<td>15.8</td>
<td>5293</td>
</tr>
<tr>
<td>H**</td>
<td>91.62</td>
<td>16.1</td>
<td>5445</td>
</tr>
<tr>
<td>Soybean L</td>
<td>91.27</td>
<td>16.0</td>
<td>5294</td>
</tr>
<tr>
<td>H</td>
<td>91.32</td>
<td>15.9</td>
<td>5445</td>
</tr>
<tr>
<td>Menhaden L</td>
<td>91.14</td>
<td>16.0</td>
<td>5283</td>
</tr>
<tr>
<td>H</td>
<td>91.34</td>
<td>16.8</td>
<td>5370</td>
</tr>
</tbody>
</table>

L* = low cholesterol diet  
H** = high cholesterol diet

II (iii) Blood Pressure Measurement

Systolic blood pressure recordings were obtained from the SHR and WKY animals at 12 weeks of age, after a 1 week training period, to confirm the presence of hypertension in the SHR animals and to verify the possible effect of a diet on that parameter. Measurements were taken between 3 p.m. and 5 p.m. in conscious rats by an indirect tail-cuff method (Harvard Apparatus Ltd., South Natick, MA; Kitts et al., 1992). Each recorded value represents the mean of three successive determinations over a period of 10 - 15 min.

II (iv) Tissue Sample Preparation

At 15 weeks of age the animals were sacrificed under halothane (Fluothane Halothane B.P. N°3125, Ayerst Laboratories, Montreal, Que.) anaesthesia. Blood was
collected by cardiac puncture (syringes and needles-Becton Dickinson, Rutherford, NJ) into cooled heparinized tubes for plasma separation at 1000 x g, 5 min, 4°C (refrigerated centrifuge, IEC CENTRA-7R, International Equipment Co., Massachusetts, US). RBC were collected and washed twice with isotonic (0.88%) saline. Plasma aliquots were dispensed into Eppendorf tubes and frozen at -35°C until analysis. Hearts and livers were collected into chilled 50 mM Tris (Tris (Hydroxymethyl) Aminomethane, BioRad Laboratories, Richmond, CA) 0.1 mM EDTA (ethylenediamine-tetraacetic acid, Sigma Grade, St. Louis, MS) pH 7.6 homogenizing buffer, blotted dry and frozen in a - 30 °C freezer until analysis. The following analyses were then performed.

II (v) Plasma Analyses

Plasma aliquots were thawed at refrigeration temperature. They were analyzed for total cholesterol based on the method of Siedel et al. (1983), for phospholipids according to Takayama et al. (1977) and Trinder (1969), and for triacylglycerols following the method of Trinder (1969) and Wahlefeld (1974) using biochemical assay kits (Boehringer Mannheim, Laval, Quebec).

II (vi) RBC-Glutathione (GSH) Assay Forced Peroxidation

RBC-GSH content was assayed by measuring sulphydryl group content in the sample according to the method of Moron et al. (1979).

Fifty microliter aliquots of packed RBC were diluted into a 10% suspension of 0.9% NaCl - 2 mM NaN₃ and incubated at 37°C for 5 min. Cold peroxidizing agent (500 μl H₂O₂,
freshly prepared in saline azide) was added, followed by incubation at 37°C for 30 min. The reaction was stopped by centrifugation at 12,000 x g (Eppendorf centrifuge 5402), 5 min, 4°C. Pelleted RBC were washed once with 1 ml cold saline azide. The cells were then lysed with the addition of 50 μl cold destilled deionized H2O and 325 μl cold 5% TCA (trichloroacetic acid, analytical grade, BDH Chemicals, Toronto, On), and vortexed. The supernatant was assayed as follows. 1.04 ml 0.1 mM pH 8.0 phosphate buffer, 120 μl cold supernatant, and 40 μl 3 mM 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma, St. Louis, MS) in phosphate buffer, pH 8.0. The absorbance was read after 10 min at 412 nm.

II (vii) RBC Malondialdehyde (MDA) Assay Forced Peroxidation

This assay followed the methodology developed by Gilbert and Stump (1984).

Aliquots (50 μl) of packed RBC were diluted into a 10% suspension with 0.9 % NaCl - 2 mM NaN3 and incubated at 37°C for 5 min. A peroxidizing agent (500 μl H2O2, freshly prepared in saline azide) was added, then incubated at 37°C for 30 min. The reaction was stopped by addition of 0.5 ml cold 28% TCA- 0.1 M Na-arsenite (BDH Chemicals, Poole, England) followed by centrifugation at 12,000 x g, 5 min, 4°C. A 1.0 ml aliquot of supernatant was assayed with 0.5 ml 0.5% 2-thiobarbituric acid (TBA, Eastman Organic Chemicals, Rochester, NY) freshly prepared in 0.025 M NaOH for the formation of malondialdehyde. After boiling for 15 min and cooling to room temperature, absorption was read at 532 nm and 453 nm.

The standard curve was determined by the method of Kwon and Watts (1963). A 10 mM MDA standard was made by adding 240 μl 1,1,3,3 tetraethoxypropane to 100.0
ml in a volumetric flask with 1 % H₂SO₄. After mixing well and allowing to stand in a dark cupboard at room temperature overnight (12 h), the standard was ready for use. Several dilutions from 0.005 mM to 10 mM were made by dilution with 0.9 % NaCl - 2 mM NaN₃. To confirm the actual concentration of the dilutions, molar absorptivity of 0.01 mM at 245 nm was measured and determined to be 1.37.

For the assay, 50 μl MDA standard were added to 0.95 ml NaCl-NaN₃ and 0.5 ml 28 % TCA -arsenite, and vortexed. A 1.0 ml aliquot was taken and 0.5 ml TBA reagent (0.5 % TBA in 0.025 M NaOH) were added. Test tubes were boiled for 15 min and then cooled to room temperature. Absorbances (abs) were read at 532 nm and 453 nm. The concentration of MDA in the samples was based on the standard MDA curve (Kwon and Watts, 1963) and on delta (Δ) values according to the equation below:

\[
\text{abs sample} @ 532 - \text{blk} @ 532) - 20\% (\text{abs sample} @ 453 - \text{blk} @ 453) = \text{delta (Δ)}
\]

\[
\Delta - 0.069981 \times \frac{50 \mu l}{3.26522 \text{ wt(g)}} = \text{n mole MDA/g RBC}
\]

where blk = blank (equation 1)

Absorption at both 532 nm and 453 nm were both corrected for possible interference of the blank. Gilbert et al. (1983) demonstrated that erythrocyte peroxidation produces compounds that react with TBA, interfering with the assay. That interference is equivalent at 532 nm is equivalent to 20 % of maximum absorbance at 453 nm.
coefficients 1 and 2 are respectively slope and intercept as determined through a standard curve.

**II (viii) RBC Fragility Test**

This assay was performed according to the method developed by Hawks (1965), was designed to measure the resistance of RBC to osmotic changes. Packed RBC (150 µl) were pipetted into glass test tubes containing 2.25 ml 15 mM Tris buffer, pH = 7.0, and vortexed. RBC (75 µl) suspended in buffer were dispensed into saline dilutions with concentrations varying from 0.01 % to 0.90 %, mixed and allowed to stand for 15 min. Absorbance was read at 540 nm.

**II (ix) RBC Lipid Extraction**

RBC lipid extraction was performed according to the method by Periago et al. (1990). RBC (0.5 g) were pipetted into a glass test tube. After adding 4 ml methanol, the tubes were sealed with caps and placed in a water bath (55°C) for 15 min. Five milliliters hexane: chloroform 4:1 (v/v) (ACS reagents, Fisher Scientific, Nepean, On) were added and the tubes were returned to the waterbath for 15 min. Tubes were removed from the waterbath, 1 ml H₂O was added and the tubes were centrifuged at 1500 x g for 15 min at 4°C. The supernatant was removed with a Pasteur pipette, and the extraction process repeated. The supernatant from the second extraction was added to the previous and the tubes were dried under N₂ gas. The lipid extract obtained was resuspended in 1.0 ml Folch’s solution (Folch et al., 1957).
For RBC cholesterol measurement, aliquots (100 μl) of RBC extract were dried under N\textsubscript{2} and 100 μl Tween 80 was added for assay by adding 2 ml cholesterol reagent, from assay kits from Boehringer Mannheim. Absorbances measured at 540 nm corresponded to μg cholesterol /tube.

For the standard curve, 2.0 ml cholesterol reagent was added to 20 μl aliquots of known cholesterol concentration and absorbance was measured within 60 min at 540 nm. RBC triacylglycerol and phospholipid analyses were performed as described for RBC cholesterol, utilizing assay kits from Boehringer Mannheim.

II (x) Plasma - Lipoprotein Fraction Separation

The separation of plasma lipoprotein fractions was carried out according to the method of Terpstra et al. (1981). Plasma fractions (2 ml) were extracted, as described under tissue sample preparation, and stored at 4°C to be processed for lipoprotein separation within 1 day. In preparation for ultracentrifugation 0.770 g KBr and 0.050 g sucrose were dispensed into a SW41 centrifuge tube. Serum (2.0 ml) was pipetted into the tube, followed by 0.2 ml Sudan Black solution. The contents of the tube were then mixed by vortexing. The mixture was overlayed subsequently with 2 ml of a salt solution $p_{20}$ = 1.225 g/ml, prepared by adding NaCl at 11.42 x 10\(^{-3}\) g/ml and KBr at 315.54 x 10\(^{-3}\) g/ml. Then 4 ml of a salt solution $p_{20}$ = 1.10 g/ml, prepared by adding NaCl at 11.42 x 10\(^{-3}\) g/ml and KBr at 133.48 x 10\(^{-3}\) g/ml. The solutions above contained 10\(^{-4}\) g/ml Na\textsubscript{2}EDTA. Lipoprotein separation was performed by sucrose-gradient ultracentrifugation (Beckman Ultracentrifuge Model L3-50, SW 41 rotor, Beckman Ultra clear centrifuge tubes 14 x 89
mm, Palo Alto, CA) at 100,000 x g for 24 h. Sudan black (0.2 ml) was added to the tubes to prestain the plasma. Sudan black solution was prepared according to the method of Narayan (1975), by adding 0.1 g of Sudan black to 100 ml ethylene glycol at 65°C and stirring the mixture vigorously for 2 min, followed by filtration. Lipoprotein fractions collected were: VLDL (1.0063<p_{20}<1.019), LDL (1.019<p_{20}<1.063) and HDL (1.063<p_{20}<1.21). Fractions were dialyzed against cold, degassed, N₂- saturated HEPES-NaCl buffer, pH = 7.4 overnight. The protein content of each fraction was determined by the method of Lowry et al. (1951) using serum albumin as a standard. Cholesterol, triacylglycerol and phospholipid analyses of the different fractions were performed as outlined above.

II (xi) Lipoprotein Oxidation

Oxidation of lipoprotein fractions was carried out according to the method of Comminacini et al., (1991). Aliquots of each fraction, corresponding to an equal amount of protein (0.5 mg/ml), were placed in a sealed test-tube containing copper (50 µM) which was used to catalyze the reaction. Reaction tubes were kept in a water bath (37°C) for up to 4 h. at prefixed time intervals (0, 1 h, 2 h and 4 h). Aliquots were removed for fluorescence measurements at 430 nm, with excitation at 360 nm using a Shimadzu spectropho-fluorometer (Shimadzu, UV-visible recording spectrophotometer, UV-160, Tekscience, Oakville, On).
Liver Tissue Analysis

Liver tissue was removed from the freezer and thawed at refrigeration temperature (4°C). Lipid extraction was performed according to Folch's method (1957) with minor modifications. Liver homogenates were prepared by weighing 2 g aliquots into chilled centrifugation tubes (Falcon, polypropylene) with 35 ml chloroform-methanol (2:1) (ACS reagents, Fisher Scientific, Nepean, On) (Folch's solution) and using a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY) at 25% maximum speed for 30 s (2 x 15s). Folch's solution (10 ml) was added and the cap-sealed tubes were placed in a shaking waterbath for 15 min, at 37°C and 125 rpm, before undergoing centrifugation at 1000 x g, 4°C, 15 min. The supernatant, collected with a pipette pump, was dispensed into 100 ml glass-stoppered graduated cylinders. The cylinders had been washed twice with detergent, followed by an acid wash to remove detergent residues and rinsed twice with destilled deionized water. This procedure was designed to maximize removal of fat residues. 30 ml Folch's solution was again added to the pellet. Samples were put through a second shaking/centrifugation cycle. The supernatant was removed and added to the separation cylinder. Saline at 0.9 % (w/v) was added to 25 % volume of supernatant in the cylinder. The cylinders were inverted gently three times and left overnight to allow for phase separation. The top layer was removed, and the volume of the bottom layer was recorded.
II (xiii) **Total Crude Lipid Extraction**

A 5 ml aliquot of extract was pipetted into a pre-weighed aluminum dish (Fisherbrand, Fisher Scientific, 57 mm, Pittsburgh, PA) and left to dry at room temperature overnight in a fume hood. The remainder of the extract was collected into polypropylene tubes (Falcon, Fisher Scientific, Pittsburgh, PA) and stored at -10°C until further analysis. The weight of dried aluminum dishes was recorded after 24 h. Total crude lipid (TCL) percentage of liver sample was calculated as follows:

\[
\% \text{ TCL (g lipid/g liver)} \times 100 \% = \frac{\text{Volume of organic phase (ml)}}{\text{volume of sample (ml)}} \times \frac{x}{\text{weight of sample (g)}} \times 100 \%
\]

where \(x\) = weight of lipid in aluminum dish  

(\text{equation 2})

II (xiv) **Liver Lipid Extract Analyses**

Liver lipid extracts were removed from the freezer and allowed to reach room temperature before further analysis. Aliquots of liver lipid extracts of 100 μl and 50 μl, from low cholesterol and high cholesterol diets, respectively, were dispensed into glass test tubes. The extract was dried under a stream of \(N_2\) (Praxair, Mississauga, Ont.), resuspended with Tween 80 (Difco Laboratories, Detroit, Michigan) and dried down again.

Tubes were assayed for total cholesterol according to Siedel et al. (1983) and triacylglycerols according to Ziegenhorn et al. (1975) using biochemical assay kits (Boehringer Mannheim).
II (xv) Fecal Lipid Extraction

Fecal material collected from balance studies was ground, whilst kept on ice and freeze dried (freeze-dryer Model 10-145-MRBA, Virtis Research Equipment, Gardner, NY). Samples (2 g) were weighed into a wide, dried glass tube. Freeze-dried samples were further ground using a Polytron after the addition of 10 ml Folch’s solution. Samples in Folch’s were dispensed into 125 ml Erlenmeyer flasks and 40 ml Folch’s were added. The Erlenmeyer flasks were sealed with aluminum foil and left for lipid extraction overnight in a shaker at 60 rpm (shaking waterbath without water). The solution was then filtered (Whatman #1) into fat-free 100 ml glass-stoppered graduated cylinders. Fifteen milliliters Folch’s was used to rinse flasks, which were then filtered into a separate vessel. Twelve milliliters of 0.9 % saline were added (first wash) and the cylinders were stoppered and gently tilted to mix well. The methanol-aqueous layer was suctioned off and 10 ml of 3:47:48 (CHCl₃:CH₃OH:dH₂O) was added (second wash). Cylinders were stoppered, gently tilted to mix the solutions and left overnight for phase separation. The final bottom layer volume was recorded and aliquots were recorded for further analysis.

II (xvi) Fecal Total Crude Lipid Extraction

The extract (20 ml) was dispensed into pre-weighed aluminum dishes and left at room temperature over night to evaporate off the solvent in a fumehood. Weights of aluminum dishes with dried extracts were recorded and lipid content calculated as above for liver lipid extract.
(xvii) **Fecal Lipid Analysis**

Analysis of the fecal lipid extract for cholesterol was performed according to the method described above for the liver extract.

(xviii) **Statistical Analyses**

Values are expressed as means with their standard errors. Differences between treatment means were tested by one-way analysis of variance (SPSS Inc., Chicago, IL.). Where differences existed, the sources were identified by the Student-Newman-Keuls multiple range test at p < 0.05 (SPSS). Interactions between treatments were identified by two-way ANOVA (MANOVA; SPSS). Correlation coefficients were determined by the method of least squares.
III Results:

III (i) Feed Intake and Animal Growth

No cholesterol, fat type or animal strain effect was observed on animal feed intake over the entire experimental period (Table 4). Similarly, no significant interactions between dietary cholesterol intake and fat source on animal feed intake and growth parameters were observed. No significant differences in weight gain between the different main experimental treatments were observed (Table 5). This outcome was attributed to similar feed intakes between SHR and WKY rats, and resulted in no differences in the feed efficiency ratio, calculated over the period of the balance study.

III (ii) Balance Study

Results from the balance study, using metabolic cages, confirmed findings obtained over the length of the experiment, in which animals were housed in individual aluminum cages. There were no differences between dietary treatments on feed intake during the 5-day period of the balance study (Table 6). The fecal output collected from animals during the balance study was significantly different (p=0.015) between rat strains, with WKY exhibiting a greater fecal output than its SHR counterpart. Cholesterol feeding also had a significant (p=0.001) effect on fecal output, as animals on high cholesterol diets exhibited greater fecal outputs. High cholesterol butter fed SHR excreted on average 17.1 g of
Table 4 Feed Intake by SHR and WKY Rats Fed Experimental Diets\(^1\)\(^2\)

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) w/w</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>807.40 (\pm) 18.92</td>
<td>813.10 (\pm) 5.61</td>
</tr>
<tr>
<td>Soybean</td>
<td>753.40 (\pm) 32.90</td>
<td>779.10 (\pm) 41.80</td>
</tr>
<tr>
<td>Menhaden</td>
<td>766.70 (\pm) 30.87</td>
<td>757.60 (\pm) 25.67</td>
</tr>
</tbody>
</table>

Interactions:

MANOVA p-value\(^3\):

SxF: N.S.
SxC: N.S.
CxG: N.S.

1 Values indicate mean \(\pm\) SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 Feed intake (g wet weight) over 10 weeks
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
Table 5  Weight Gain by SHR and WKY Rats Fed Experimental Diets\(^1,2\)

| Rat Strain | SHR | | SHR | | WKY | | WKY |
| --- | --- | | --- | | --- | | --- | | --- |
| Dietary Cholesterol | | | | | | | | |
| (%) w/w | 0.05 | 0.5 | 0.05 | 0.5 |
| Dietary Fat Source | | | | | | | | |
| Butter | 146.20 ± 7.40 | 148.20 ± 7.50 | 132.20 ± 6.91 | 149.60 ± 5.66 |
| Soybean | 142.30 ± 7.04 | 143.10 ± 4.58 | 139.50 ± 5.29 | 149.10 ± 8.66 |
| Menhaden | 143.50 ± 8.96 | 142.50 ± 2.50 | 136.60 ± 14.20 | 150.30 ± 5.06 |
| Interactions: | | | | | | | | |
| MANOVA p-value\(^3\): | | | | | | | | |
| SxF | N.S | | | | | | | |
| SxC | N.S. | | | | | | | |
| CxF | N.S. | | | | | | | |

1  Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2  Weight gain (g wet weight) over 10 weeks
3  Interactions:  S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
Table 6  Feed Intake by SHR and WKY Rats Fed Experimental Diets During Balance Study

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th></th>
<th>WKY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) w/w</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>72.10 ± 2.40</td>
<td>75.70 ± 2.40</td>
<td>65.40 ± 3.49</td>
<td>65.80 ± 3.19</td>
</tr>
<tr>
<td>Soybean</td>
<td>67.40 ± 2.70</td>
<td>70.40 ± 2.90</td>
<td>61.10 ± 3.43</td>
<td>65.50 ± 2.93</td>
</tr>
<tr>
<td>Menhaden</td>
<td>68.30 ± 3.80</td>
<td>67.80 ± 2.50</td>
<td>68.50 ± 3.49</td>
<td>68.50 ± 0.90</td>
</tr>
<tr>
<td>Interactions:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MANOVA p-value³</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SxF</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SxC</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CxF</td>
<td>N.S.</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Value indicates mean ± SEM, SHR = spontaneously hypertensive rats, WKY = Wistar Kyoto normotensive rats
2 Feed intake (g wet weight) during balance study (5 days)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
feces, while low cholesterol butter fed animals excreted on average 11.5 g (Table 7). Dietary fat type also produced a significant effect (p = 0.006) on total fecal output.

Total crude lipid balance, that is lipid retained, (Figs. 3 and 4) from different experimental diets was calculated from the difference between dietary lipid ingested and the total crude lipid excreted in feces. There were no significant differences noted between the various main treatments, nor were there interactions between the main treatment effects.

Lipid digestibility was calculated as the ratio of the difference between lipid ingested minus lipid excreted over lipid ingested. It was significantly affected by dietary cholesterol intake (p < 0.001) and dietary fat source (p = 0.048). Lipid digestibility was lowered by cholesterol intake in both rat strains (Table 8).

Total crude lipids excreted during the balance study were significantly (p < 0.001) different between cholesterol treatments. Total weight of total crude lipids secreted in the feces showed a trend to increase with the level of dietary cholesterol. While low cholesterol fed animals from both rat strains excreted between 0.42g and 0.49g total crude lipids during the balance study, high cholesterol fed rats eliminated fat in feces at a rate of 0.69g to 0.82g fat during the same period of time for WKY and SHR, respectively (Table 9).

Differences in fecal cholesterol output over the 5 day period of the balance study, were highly significant (p < 0.001) for such treatment effects as rat strain, dietary cholesterol level and fat type. Total cholesterol excretion over the period of the balance study ranged from 1.3 mg cholesterol/ g dried feces in WKYs on a low cholesterol butter
Table 7  Total Fecal Output by SHR and WKY Rats Fed Experimental Diets During Balance Study\textsuperscript{1,2,4}

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>11.5 ± 0.9\textsuperscript{a,x}</td>
<td>17.1 ± 1.5\textsuperscript{a,y}</td>
</tr>
<tr>
<td>Soybean</td>
<td>10.2 ± 0.4\textsuperscript{a,x}</td>
<td>13.7 ± 1.6\textsuperscript{a,xy}</td>
</tr>
<tr>
<td>Menhaden</td>
<td>11.0 ± 0.3\textsuperscript{a,x}</td>
<td>14.2 ± 1.0\textsuperscript{a,xy}</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value\textsuperscript{3}:
- SxF: N.S.
- SxC: N.S.
- CxF: N.S.

1. Value indicates mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2. Fecal output (g wet weight) during balance study (5 days)
3. Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4. a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistical significance for differences between dietary cholesterol levels p ≤ 0.05.
Fig. 3 Total Crude Lipid Balance in SHR Fed Different Dietary Fat Sources$^{1,2}$

1-Values indicate lipid balance (g) = (lipid ingested(g) - lipid excreted(g)) over 5 days; mean ± SEM; n = 8.

2- SHR = spontaneously hypertensive rat.

Fat Sources Identified: Butter = ; Soybean = ; Menhaden =

No significant treatment effects were observed.
Fig. 4 Total Crude Lipid Balance in WKY Fed Different Dietary Fat Sources

1- Values indicate lipid balance (g) = (lipid ingested(g) - lipid excreted(g)) over 5 days; mean ± SEM; n = 8.
2- WKY = Wistar Kyoto normotensive rat
   Fat Sources Identified: Butter = ; Soybean = ; Menhaden = 
   No significant treatment effects were observed.
**Table 8**

Lipid Digestibility of Experimental Diets Fed to SHR and WKY Rats$^{1,2}$

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>0.964 ± 0.002</td>
<td>0.931 ± 0.008</td>
</tr>
<tr>
<td>Soybean</td>
<td>0.955 ± 0.004</td>
<td>0.936 ± 0.010</td>
</tr>
<tr>
<td>Menhaden</td>
<td>0.965 ± 0.004</td>
<td>0.905 ± 0.030</td>
</tr>
</tbody>
</table>

Interactions:

MANOVA p-value$^3$:

- SxF: N.S.
- SxC: N.S.
- CxF: p = 0.032

1. Value indicates mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2. Lipid digestibility = 1- (g lipid excreted/ g lipid ingested)
3. Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Dietary Cholesterol</th>
<th>Dietary Fat Source</th>
<th>Interactions:</th>
<th>MANOVA p-value³:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%) w/w</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>0.42 ± 0.9³</td>
<td>0.82 ± 0.2³</td>
<td>0.45 ± 0.2³</td>
<td>0.69 ± 0.1³</td>
</tr>
<tr>
<td></td>
<td>0.49 ± 0.1³</td>
<td>0.70 ± 0.3³</td>
<td>0.45 ± 0.1³</td>
<td>0.80 ± 0.2³</td>
</tr>
<tr>
<td></td>
<td>0.44 ± 0.1³</td>
<td>0.72 ± 0.1³</td>
<td>0.43 ± 0.1³</td>
<td>0.69 ± 0.1³</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value³:

SxF           N.S.
SxC           N.S.
CxS           N.S.

1 Value indicates mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 Total crude lipid (g dried weight) excreted in feces during balance study (5 days)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4 a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
diet, to 6.3 mg cholesterol/g dried feces in the SHR fed a high cholesterol menhaden diet (Table 10).

Cholesterol balance (Figs. 5 and 6) was calculated as the difference between cholesterol ingested and cholesterol excreted over a 5 day period. There was a significant (p ≤ 0.05) main effect for dietary cholesterol concentration. Dietary fat sources also had a significant (p ≤ 0.05) effect on cholesterol balance. SHR fed the high cholesterol menhaden diet had a lower cholesterol balance than either SHR fed a high cholesterol soybean or rats fed a high cholesterol butter diets. Among high cholesterol fed WKY, butter fed animals expressed a higher cholesterol balance than either of the groups fed other dietary fat sources.

Liver Lipid Analysis

III(iii) Total Crude Lipids

Total crude lipid content of livers varied significantly (p<0.001) according to the level of dietary cholesterol intake and fat source fed to rats. There was no difference in liver lipid content between SHR and WKY animals. Livers from rats consuming high cholesterol diets had a total crude lipid content that was approximately three times that observed in rats fed corresponding low cholesterol diets. The intake of individual fat blends in animals fed high cholesterol diets resulted in lower (p<0.05) % crude lipid content in livers from menhaden fed animals, with 22.7 % and 23.1 % in SHR and WKY, respectively. Rats fed either butter or soy diets, in combination with high cholesterol had a liver total crude lipid content that ranged from 27.7 % liver in butter fed SHR to 29.8 % in...
Table 10  Fecal Cholesterol Output from SHR and WKY Rats Fed Experimental Diets During Balance Study$^{1,2,4}$

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) w/w</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Butter</td>
<td>2.0 ± 0.1$^a$x</td>
<td>3.4 ± 0.5$^b$y</td>
</tr>
<tr>
<td>Soybean</td>
<td>2.9 ± 0.2$^a$x</td>
<td>5.6 ± 0.5$^b$y</td>
</tr>
<tr>
<td>Menhaden</td>
<td>2.5 ± 0.3$^a$x</td>
<td>6.3 ± 0.5$^b$y</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value$^3$:
- SxF  
  p = 0.032
- SxC  
  p = 0.05
- CxF  
  p = 0.004

1 Value indicates mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 Fecal cholesterol concentration (mg / g dried feces / 5 days)
3 Interactions: S x F = strain x fat, S xC = strain x cholesterol, C x F = cholesterol x fat.
4 a,b,c - denotes statistical significance between dietary fat sources, p ≤ 0.05,
  x,y - denotes statistical significance for differences between dietary cholesterol levels, p < 0.05.
Fig. 5  Cholesterol Balance in SHR Fed Different Dietary Fat Sources

1-Values indicate cholesterol balance (mg): (chol. ingested (mg) - chol. excreted (mg)) over 5 days; mean ± SEM; n = 8.
2- SHR = spontaneously hypertensive rat
Fat Sources Identified: Butter = □; Soybean = ■; Menhaden = □

a,b,c - denotes statistical significance for differences between dietary fat sources, $p \leq 0.05$

x,y - denotes statistical significance for differences between dietary cholesterol levels, $p \leq 0.05$
Cholesterol Balance in WKY Fed Different Dietary Fat Sources$^{1,2}$

1-Values indicate cholesterol balance (mg): (chol. ingested (mg) - chol. excreted (mg)) over 5 days; mean ± SEM; n = 8.

2 - WKY = Wistar Kyoto normotensive rat

Fat Sources Identified: Butter = ; Soybean = ; Menhaden =

$^{a,b,c}$ denotes statistical significance for differences between dietary fat sources, $p \leq 0.05$

$x,y$ - denotes statistical significance for differences between dietary cholesterol levels, $p \leq 0.05$
soybean oil fed SHR. The interaction between dietary cholesterol level and fat type was significant for liver total crude lipid (p=0.021; Fig. 7 and 8).

III (iv) Liver Cholesterol

Liver cholesterol concentrations corresponded to the proportions of dietary cholesterol consumed by the rats. For example, low cholesterol soybean fed SHR rats had an average of 8.19 ± 0.94 mg liver cholesterol per g tissue wet weight, while those on a high cholesterol diet had 88.18 ± 5.61 mg cholesterol/g tissue wet weight (Fig. 9 and 10). Of the high cholesterol fed animals, the menhaden fed SHR group exhibited the lowest liver cholesterol concentration (67.88 ± 5.02 mg cholesterol/g tissue wet weight), compared to WKY animals (87.05 ± 5.76 mg cholesterol/g tissue wet weight) in the menhaden group. Both cholesterol intake (p<0.001) and fat type (p=0.016) produced significant effects on liver cholesterol concentration. The interaction between fat type and dietary cholesterol level in total liver cholesterol concentration was significant (p=0.012).

III (v) Liver Triacylglycerol

Liver triacylglycerol concentrations were lowest in SHR fed a high cholesterol menhaden diet (86.63 ± 2.76 mg/g liver wet weight). Comparing soybean and menhaden fed rats, those groups fed menhaden diets had lower (p<0.05, Figs. 11 and 12) triacylglycerol concentration than their soybean fed counterparts. Animals fed butter diets showed no change in triacylglycerol levels with an increase in cholesterol intake. All main treatment effects were significant (p<0.001), as were the interactions between animal
Fig. 7 Liver Total Crude Lipid Content in SHR Fed Different Dietary Fat Sources$^{1,2}$

1- Values indicate total crude lipid content (g/g liver wet weight) x 100%; mean ± SEM; n = 8.

2- SHR = spontaneously hypertensive rat
Fat Sources Identified: Butter = ; Soybean = ; Menhaden =

a, b, c - denotes statistical significance for differences between dietary fat sources, $p \leq 0.05$

x, y - denotes statistical significance for differences between dietary cholesterol levels, $p \leq 0.05$
Fig. 8 Liver Total Crude Lipid Content in WKY Fed Different Dietary Fat Sources$^{1,2}$

1 - Values indicate total crude lipid content (g/g liver wet weight) $\times$ 100%; mean ± SEM; $n = 8$.
2 - SHR = spontaneously hypertensive rat
Fat Sources Identified: Butter = ]; Soybean = ]; Menhaden = ]
$a,b,c$ - denotes statistical significance for differences between dietary fat sources, $p \leq 0.05$.
$x,y$ - denotes statistical significance for differences between dietary cholesterol levels, $p \leq 0.05$. 
Fig. 9 Liver Cholesterol Concentration in SHR Fed Different Dietary Fat Sources$^{1,2}$

1- Values indicate liver cholesterol concentration (mg/g liver wet weight); mean ± SEM; n = 8.
2- SHR = spontaneously hypertensive rat.

Fat Sources Identified: Butter = ; Soybean = ; Menhaden =

$^{a,b,c}$ - denotes statistical significance for differences between dietary fat sources, $p < 0.05$

$x,y$ - denotes statistical significance for differences between dietary cholesterol levels, $p < 0.05$
Fig. 10 Liver Cholesterol Concentration in WKY Fed Different Dietary Fat Sources

1-Values indicate liver cholesterol concentration (mg/g liver wet weight); mean ± SEM; n = 8.
2- SHR = spontaneously hypertensive rat
Fat Sources Identified: Butter = ; Soybean = ; Menhaden =
 a,b,c - denotes statistical significance for differences between dietary fat sources,
p ≤ 0.05.
x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
Fig. 11 Liver Triacylglycerol Concentration in SHR Fed Different Dietary Fat Sources

1- Values indicate liver triacylglyceride concentration (mg/g liver wet weight); mean ± SEM; n = 8.
2- SHR = spontaneously hypertensive rat
Fat Sources Identified: Butter = ; Soybean = ; Menhaden = 

a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05.
x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
Fig. 12 Liver Triacylglycerol Concentration in WKY Fed Different Dietary Fat Sources

1- Values indicate liver triacylglyceride concentration (mg/g liver wet weight); mean ± SEM; n = 8.
2- WKY = Wistar Kyoto normotensive rat.

Fat Sources Identified: Butter = ; Soybean = ; Menhaden =

a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05.

x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
strain and fat type (p=0.001) and animal strain and cholesterol intake (p<0.001). A significant interaction between cholesterol intake and fat type with liver triacylglycerol concentration was also observed (p=0.010; Figs. 11 and 12).

**RBC Lipid Analysis**

**III (vi) RBC-Triacylglycerol**

RBC-triacylglycerol concentrations were significantly (p<0.001) affected by the level of cholesterol intake. Animals fed high cholesterol diets produced RBC-triacylglycerol concentrations ranging from 2.1 ± 0.11 (mg/g RBC) in WKYs on the menhaden based diet, to 2.45 ± 0.20 (mg/g RBC) for SHR fed the butter diet. Those SHR groups fed butter diets also had the highest RBC-triacylglycerol levels for both high and low cholesterol diets respectively (Table 11).

**III (vii) RBC Cholesterol**

RBC cholesterol concentrations show a significant dietary fat type treatment effect (p = 0.031) and a cholesterol intake level effect (p = 0.032). There was no significant strain effect. Menhaden-fed animals had higher RBC cholesterol levels than those animals fed other fat sources. Cholesterol concentration varied from 5.11 ± 0.49 mg/g RBC in low cholesterol soybean-fed SHR to 7.29 ± 0.43 mg/g RBC in high cholesterol menhaden-fed SHR. The interaction of rat strain with dietary cholesterol intake was, however, significant (p=0.01; Table 12).
<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th></th>
<th>WKY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>1.70 ± 0.3^a,x</td>
<td>2.45 ± 0.2^a,x</td>
<td>1.78 ± 0.4^a,x</td>
<td>2.26 ± 0.2^a,x</td>
</tr>
<tr>
<td>Soybean</td>
<td>1.29 ± 0.1^a,x</td>
<td>2.17 ± 0.1^a,x</td>
<td>1.31 ± 0.1^a,x</td>
<td>2.30 ± 0.2^a,x</td>
</tr>
<tr>
<td>Menhaden</td>
<td>1.34 ± 0.2^a,x</td>
<td>2.29 ± 0.1^a,y</td>
<td>1.31 ± 0.1^a,x</td>
<td>2.10 ± 0.1^a,x</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value:
SxF
SxC
CxF

1. Value indicates mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2. RBC Triacylglycerol concentration (mg / g RBC)
3. Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4. a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistical significance for differences between dietary cholesterol levels, p < 0.05.
Table 12  RBC- Cholesterol Concentration in SHR and WKY Rats Fed Experimental Diets$^{1,2,4}$

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td>% w/w</td>
<td>0.05</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>5.52 ± 0.3$^{a,x}$</td>
<td>6.40 ± 0.5$^{a,x}$</td>
</tr>
<tr>
<td>Soybean</td>
<td>5.11 ± 0.5$^{a,x}$</td>
<td>5.93 ± 0.2$^{a,x}$</td>
</tr>
<tr>
<td>Menhaden</td>
<td>5.32 ± 0.4$^{a,x}$</td>
<td>7.29 ± 0.4$^{a,y}$</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value$^3$:
SxF N.S.
SxC $p = 0.01$
CxF N.S.

1 Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 RBC Cholesterol concentration (mg / g RBC)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4 a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05; x,y - denotes statistical significance for differences between dietary cholesterol levels, p < 0.05.
III (viii) **RBC Phospholipids**

RBC phospholipid concentrations were higher (p<0.001) in rats fed high cholesterol diets in SHR animals. Dietary fat sources produced a significant (p = 0.004) treatment effect. In high cholesterol soybean-fed SHR, RBC-phospholipid levels were lower than in those animals fed other dietary fat sources at a high cholesterol level. There were no significant interactions between the different main treatments for this parameter (Table 13).

**Plasma Lipid Analysis**

**Whole Plasma Analysis**

III (ix) **Total Plasma Cholesterol**

Total plasma cholesterol concentration was elevated (p=0.001) in animals fed high cholesterol diets. This effect was especially pronounced in WKY animals fed the butter and soybean high cholesterol diets. Comparing the values for this parameter between SHR and WKY, it was noted that the differences in plasma cholesterol concentration from animals fed high and low dietary cholesterol were relatively smaller. For example WKY animals had higher plasma cholesterol levels than SHR counterparts, indicating a strain effect (p < 0.001). Menhaden oil fed animals generally had lower plasma cholesterol levels when compared to those fed other fats, but that difference reached statistical significance for high cholesterol fed menhaden WKY. The animal strain and fat source (p = 0.010), and cholesterol and fat source interactions (p = 0.001) were also significant (Table 14).
<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th></th>
<th></th>
<th>WKY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>6.00 ± 0.4&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;x&lt;/sup&gt;</td>
<td>10.50 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;&lt;sup&gt;y&lt;/sup&gt;</td>
<td>7.26 ± 0.7&lt;sup&gt;x&lt;/sup&gt;</td>
<td>9.69 ± 0.7&lt;sup&gt;y&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>6.30 ± 0.7&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;x&lt;/sup&gt;</td>
<td>8.02 ± 0.8&lt;sup&gt;y&lt;/sup&gt;</td>
<td>5.80 ± 0.4&lt;sup&gt;x&lt;/sup&gt;</td>
<td>8.95 ± 0.7&lt;sup&gt;y&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Menhaden</td>
<td>5.95 ± 0.8&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;x&lt;/sup&gt;</td>
<td>11.36 ± 0.4&lt;sup&gt;ab&lt;/sup&gt;&lt;sup&gt;y&lt;/sup&gt;</td>
<td>7.19 ± 0.7&lt;sup&gt;x&lt;/sup&gt;</td>
<td>10.69 ± 0.9&lt;sup&gt;y&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Interactions:

MANOVA p-value<sup>3</sup>:

<table>
<thead>
<tr>
<th>F</th>
<th>SxF</th>
<th>SxC</th>
<th>CxF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

1. Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat.
2. RBC phospholipid concentration (mg / g RBC).
3. Interactions:  S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4. a,b,c - denotes statistically significant differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistically significant differences between dietary cholesterol levels, p ≤ 0.05.
Table 14: Total Plasma Cholesterol Concentration in SHR and WKY Rats Fed Experimental Diets\(^1,2,4\)

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Dietary Cholesterol (% w/w)</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>86.2 ± 8.9(^a)x</td>
<td>112.9± 14.3(^a)x</td>
<td>118.6 ± 4.3(^a)x</td>
</tr>
<tr>
<td>Soybean</td>
<td>102.8 ± 9.0(^a)x</td>
<td>111.3± 21.3(^a)x</td>
<td>135.7 ± 6.4(^a)x</td>
</tr>
<tr>
<td>Menhaden</td>
<td>83.8 ± 5.9(^a)x</td>
<td>97.2 ± 6.1(^a)x</td>
<td>121.8 ± 9.0(^a)x</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value\(^3\):
SxF  \(p=0.010\)
SxC  \(p<0.001\)
CxF  \(p=0.001\)

1 Values indicate mean ± SEM, \(n=8\), SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 Total plasma cholesterol concentration (mg / dl plasma)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat.
4 a,b,c - denotes statistical significance for differences between dietary fat sources, \(p \leq 0.05\).
x,y - denotes statistical significance for differences between dietary cholesterol levels, \(p \leq 0.05\).
Plasma triacylglycerol concentrations, expressed as mg triacylglycerol/dl plasma, were significantly modified by dietary cholesterol (p<0.001) and fat type (p<0.001). The interaction of dietary cholesterol and fat type on plasma triacylglycerol was also highly significant (p=0.005). Plasma triacylglycerol levels were highest in low cholesterol butter fed animals (66.60 ± 3.59 mg/dl for the SHR; 70.47 ± 7.56 mg/dl for the WKY group). The lowest triacylglycerol levels observed were in high cholesterol, soybean fed SHR (36.87 ±1.84 mg/dl, Table 15).

Plasma phospholipid concentrations were significantly altered by animal strain (p<0.001) and dietary cholesterol intake level (p=0.005). Phospholipid levels were higher in WKY than in SHR. Animals fed low cholesterol levels had higher plasma phospholipid concentration than counterparts fed high cholesterol diets. The interaction between rat strain and cholesterol level on plasma phospholipids was significant (p=0.004; Table 16).

Plasma Lipoprotein Fraction Lipid Analysis

Plasma lipoprotein fractions were assayed for their cholesterol, triacylglycerol and phospholipid contents.
Table 15: Plasma Triacylglycerol Concentration by SHR and WKY Rats Fed Experimental Diets$^{1,2,4}$

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol (%) w/w</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.05</td>
<td>0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>66.60 ± 3.6$^{x}$</td>
<td>42.97 ± 2.8$^{y}$</td>
</tr>
<tr>
<td>Soybean</td>
<td>45.28 ± 5.7$^{x}$</td>
<td>36.87 ± 1.8$^{y}$</td>
</tr>
<tr>
<td>Menhaden</td>
<td>45.30 ± 4.8$^{x}$</td>
<td>40.23 ± 1.3$^{y}$</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value$^{3}$:
SxF                 N.S.                  
SxC                 N.S.                  
CxF                 p = 0.005

1 Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 Plasma triacylglycerol concentration (mg / dl plasma)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4 a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
x,y - denotes statistical significance for differences between dietary cholesterol, p ≤ 0.05.
### Table 16

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>110.83 ± 9.4^a^x</td>
<td>79.90 ± 2.0^a^x</td>
</tr>
<tr>
<td>Soybean</td>
<td>115.98 ± 15.5^a^x</td>
<td>75.93 ± 4.3^a^x</td>
</tr>
<tr>
<td>Menhaden</td>
<td>100.53 ± 5.1^a^x</td>
<td>84.96 ± 3.1^a^x</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value:
- SxF: N.S.
- SxC: p = 0.004
- CxF: N.S.

1. Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2. Plasma phospholipid concentration (mg / dl plasma)
3. Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4. a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05.
   - x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
III (xii) **VLDL-Cholesterol**

VLDL-cholesterol content was significantly (p<0.001) influenced by dietary cholesterol intake, animal strain and fat type. WKY animals had higher VLDL-cholesterol concentration than SHR, for both high and low cholesterol diets. Both WKY and SHR soybean fed animals consuming the low cholesterol diet displayed the lowest plasma VLDL-cholesterol concentrations (7.94 ± 1.47 mg/dl plasma and 5.98 ± 0.74 mg/dl plasma, respectively). The highest VLDL-cholesterol concentrations in all treatments occurred in the WKY animals fed the high cholesterol butter and soybean diets. Interactions between the main treatment factors and plasma VLDL-cholesterol were all significant (p<0.001; Table 17)

III (xiii) **VLDL Triacylglycerol**

VLDL-triacylglycerol concentrations were significantly different for both animal strain (p=0.020), and fat type (p=0.017). Among the WKY animals, those fed soya oil exhibited the lowest VLDL-triacylglycerol concentrations (e.g. 8.92 ± 0.99 mg/dl for the low cholesterol treatment). Triacylglycerol concentrations in WKY - VLDL were higher (p= 0.05) in menhaden fed than those observed in SHR, with the exception of low cholesterol butter fed WKY rats. The interaction between dietary cholesterol and fat type was also significant (p=0.044; Table 18).
Table 17: VLDL Cholesterol in SHR and WKY Rats Fed Experimental Diets\(^1,2,4\)

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th></th>
<th>WKY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>9.18 ± 2.3(^a)</td>
<td>16.63 ± 1.5(^b)</td>
<td>15.10 ± 0.8(^c)</td>
<td>81.95 ± 14.7(^d)</td>
</tr>
<tr>
<td>Soybean</td>
<td>5.98 ± 0.7(^a)</td>
<td>19.92 ± 2.1(^b)</td>
<td>7.94 ± 1.5(^c)</td>
<td>71.65 ± 3.3(^d)</td>
</tr>
<tr>
<td>Menhaden</td>
<td>6.11 ± 0.9(^a)</td>
<td>18.15 ± 2.0(^b)</td>
<td>13.17 ± 1.6(^c)</td>
<td>26.42 ± 3.4(^d)</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value\(^3\):

- SxF  \(p<0.001\)
- SxC  \(p<0.001\)
- CxF  \(p<0.001\)

1 Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 VLDL cholesterol concentration (mg / dl plasma)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4 a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
Table 18  VLDL Triacylglycerol in SHR and WKY Rats Fed Experimental Diets<sup>1,2,4</sup>

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) w/w</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>11.01 ± 1.1&lt;sup&gt;x&lt;/sup&gt;</td>
<td>11.26 ± 1.1&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soybean</td>
<td>10.68 ± 1.0&lt;sup&gt;x&lt;/sup&gt;</td>
<td>11.53 ± 0.6&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>Menhaden</td>
<td>13.30 ± 0.4&lt;sup&gt;x&lt;/sup&gt;</td>
<td>12.17 ± 0.7&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value<sup>3</sup>:
- SxF  N.S.
- SxC  N.S.
- CxF  p=0.044

1  Value indicates mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2  VLDL triacylglycerol concentration (mg / dl plasma)
3  Interactions:  S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
4  a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
III (xiv) **VLDL-Phospholipids**

VLDL-phospholipid concentrations were all significantly (p<0.001) affected by rat strain, and dietary cholesterol intake level. WKY animals generally exhibited higher VLDL-phospholipid concentrations than SHR. Among the different fat treatments, within the SHR groups, those fed high cholesterol menhaden diets exhibited the highest VLDL-phospholipid concentration. Among the WKY rats fed high cholesterol diets, it was those animals fed the butter diet that exhibited the highest VLDL-phospholipid concentrations. High cholesterol fed animals from butter fed WKY groups produced higher VLDL-phospholipid concentrations than counterparts fed low cholesterol diets (e.g. 44.12 ± 6.77 mg/dl and 14.13 ± 1.40 mg/dl fraction respectively). The interactions between all main treatment effects and VLDL-phospholipid were significant (p<0.001 - between rat strain and fat type, and rat strain and cholesterol level; and p=0.008 - between cholesterol and fat type; Table 19).

III (xv) **LDL-Cholesterol**

LDL-cholesterol concentrations were not affected by the different treatment effects, except for the rat strain effect. WKY animals showed higher (p<0.001) LDL-cholesterol levels compared to their SHR counterparts. The interactions between main treatments were not significant (Table 20).

III (xvi) **LDL-Triacylglycerol**

LDL-triacylglycerol did not show any significant main treatment effects or interactions (Table 21).
<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>6.26 ± 1.1&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;x&lt;/sup&gt;</td>
<td>7.76 ± 1.1&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soybean</td>
<td>5.86 ± 1.0&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;y&lt;/sup&gt;</td>
<td>10.12 ± 1.2&lt;sup&gt;a&lt;/sup&gt;&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>Menhaden</td>
<td>12.70 ± 0.6&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;x&lt;/sup&gt;</td>
<td>15.12 ± 1.7&lt;sup&gt;b&lt;/sup&gt;&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Interactions:

MANOVA p-value<sup>3</sup>:

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SxF</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>SxC</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>CxF</td>
<td>p = 0.008</td>
<td></td>
</tr>
</tbody>
</table>

1 Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 VLDL phospholipid concentration (mg / dl plasma)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat.
4 a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
Table 20  LDL-Cholesterol in SHR and WKY Rats Fed Experimental Diets$^{1,2}$

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th></th>
<th>WKY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol (%) w/w</td>
<td>0.05</td>
<td>0.5</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>12.04 ± 1.78</td>
<td>6.33 ± 1.14</td>
<td>15.09 ± 2.54</td>
<td>19.40 ± 2.80</td>
</tr>
<tr>
<td>Soybean</td>
<td>13.98 ± 5.40</td>
<td>6.02 ± 1.68</td>
<td>21.60 ± 2.15</td>
<td>16.87 ± 3.47</td>
</tr>
<tr>
<td>Menhaden</td>
<td>13.59 ± 2.47</td>
<td>13.15 ± 2.50</td>
<td>19.71 ± 3.04</td>
<td>20.23 ± 2.28</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value$^3$:
SxF | N.S.
SxC | N.S.
CxF | N.S.

1 Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 LDL cholesterol concentration (mg / dl plasma)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant
Table 21  LDL-Triacylglycerol in SHR and WKY Rats Fed Experimental Diets\(^1,2\)

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>2.55 ± 0.28</td>
<td>2.66 ± 0.50</td>
</tr>
<tr>
<td>Soybean</td>
<td>2.26 ± 0.29</td>
<td>2.76 ± 0.71</td>
</tr>
<tr>
<td>Menhaden</td>
<td>2.39 ± 0.25</td>
<td>2.13 ± 0.34</td>
</tr>
</tbody>
</table>

Interactions:

MANOVA p-value\(^3\):

<table>
<thead>
<tr>
<th>Term</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SxF</td>
<td>N.S.</td>
</tr>
<tr>
<td>SxC</td>
<td>N.S.</td>
</tr>
<tr>
<td>CxF</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

1 Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2 LDL triacylglycerol concentration (mg / dl plasma)
3 Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
III (xvii) **LDL Phospholipids**

LDL-phospholipid plasma concentrations were significantly affected by both cholesterol intake level (p<0.001) and rat strain (p=0.012), but not by dietary fat type. Low cholesterol diets fed to SHR produced higher (p=0.05) phospholipid values than those observed in rats fed high cholesterol diets, with the exception of SHR fed a low cholesterol butter diet. Soybean based diets resulted in the highest phospholipid concentrations in the low cholesterol treated animals. There were no significant interactions between the different main effects (Table 22).

III (xviii) **HDL-Cholesterol**

HDL-cholesterol plasma concentrations were significantly different for rat strain (p=0.002) and cholesterol intake (p<0.001). WKY rats had higher HDL cholesterol concentrations than SHR. The highest HDL-cholesterol concentrations were measured in the low cholesterol fed WKY soybean diet group. Interactions between main treatment effects and HDL-cholesterol were not significant (Table 23).

III (xix) **HDL-Triacylglycerol**

HDL-triacylglycerol plasma concentrations were not affected by animal strain, but dietary cholesterol level (p<0.001) and fat source (p = 0.048) effects were significant. HDL fractions collected from animals fed low cholesterol containing diets produced higher triacylglycerol concentrations than animals fed a high cholesterol diet. Among SHR
### Table 22

**LDL Phospholipids in SHR and WKY Rats Fed Experimental Diets**

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>11.87 ± 1.5&lt;sup&gt;x&lt;/sup&gt;</td>
<td>3.46 ± 0.6&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soybean</td>
<td>15.58 ± 4.5&lt;sup&gt;x&lt;/sup&gt;</td>
<td>4.63 ± 0.7&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>Menhaden</td>
<td>12.23 ± 2.4&lt;sup&gt;x&lt;/sup&gt;</td>
<td>12.30 ± 2.4&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Interactions:**

MANOVA p-value<sup>3</sup>:

- SxF: N.S.
- SxC: N.S.
- CxF: N.S.

1. Value indicates mean ± SEM, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
2. LDL phospholipid concentration (mg / dl plasma)
3. Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat.
4. a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>Dietary Cholesterol (% w/w)</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peanut</td>
<td>59.23 ± 3.5(^{xx})</td>
<td>53.31 ± 6.9(^{xx})</td>
<td>66.54 ± 5.5(^{xx})</td>
</tr>
<tr>
<td>Soybean</td>
<td>58.46 ± 4.2(^{xx})</td>
<td>48.08 ± 6.2(^{xx})</td>
<td>81.20 ± 7.0(^{xy})</td>
</tr>
<tr>
<td>Menhaden</td>
<td>52.46 ± 3.7(^{xx})</td>
<td>56.44 ± 3.9(^{xx})</td>
<td>66.97 ± 6.5(^{xx})</td>
</tr>
</tbody>
</table>

Interactions:
MANOVA p-value\(^3\):
SxF: N.S.
SxS: N.S.
CxF: N.S.

1. Values indicate mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat.
2. HDL cholesterol concentration (mg / dl plasma).
3. Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat.
4. a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
   x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
animals there were no significant differences with either cholesterol feeding or fat source. WKY rats fed the low cholesterol soybean oil diet yielded the highest triacylglycerol concentrations (90.11 ± 6.62 mg/dl plasma) (Table 24).

III (xx) **HDL-Phospholipids**

HDL-phospholipids were significantly (p<0.001) affected by dietary cholesterol intake. Those SHR animals fed the high cholesterol diets exhibited the highest HDL-phospholipid concentrations. The interaction between rat strain and cholesterol intake level was significant (p<0.001, Table 25).

III (xxi) **Red Blood Cell Fragility**

This parameter (Figs. 13 and 14) was defined by a detectable increase in absorbance at 412 nm, proportional to a decrease in the concentration of the saline environment, leading to cell lysis. Analysis of variance did not reveal any significant main treatment effects. There were no significant interactions between main effects either.

**Oxidative Stress**

III (xxii) **RBC Glutathione Depletion (RBC-GSH)**

RBC-GSH depletion, calculated as the percent ratio of the difference between initial and final RBC-GSH concentration, was significantly (p<0.001) different between rat strains. RBC-GSH depletion was greater in WKY animals compared to SHR counterparts, when fed low cholesterol diets. The interactions between rat strain and
Table 24  HDL -Triacylglycerol Concentration from SHR and WKY Rats Fed Experimental Diets\textsuperscript{1,2,4}  

<table>
<thead>
<tr>
<th>Rat Strain</th>
<th>SHR</th>
<th>WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dietary Cholesterol (%) w/w</td>
<td>0.05</td>
<td>0.5</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>67.30 ± 5.2**</td>
<td>53.28 ± 4.8**</td>
</tr>
<tr>
<td>Soybean</td>
<td>64.73 ± 6.4**</td>
<td>54.68 ± 6.9**</td>
</tr>
<tr>
<td>Menhaden</td>
<td>53.91 ± 4.7**</td>
<td>56.48 ± 3.0**</td>
</tr>
</tbody>
</table>

Interactions: MANOVA p-value\textsuperscript{3}:
SxF \quad N.S.
SxC \quad p = 0.05
CxF \quad p = 0.032

\textsuperscript{1} Value indicates mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
\textsuperscript{2} HDL triacylglycerol concentration (mg / dl plasma )
\textsuperscript{3} Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
\textsuperscript{4} a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05;
x,y - denotes statistical significance for differences between dietary cholesterol sources, p ≤ 0.05.
<table>
<thead>
<tr>
<th>Table 25</th>
<th>HDL-Phospholipids in SHR and WKY Rats Fed Experimental Diets&lt;sup&gt;1,2,4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat Strain</td>
<td>SHR</td>
</tr>
<tr>
<td>Dietary Cholesterol</td>
<td></td>
</tr>
<tr>
<td>(% w/w)</td>
<td>0.05</td>
</tr>
<tr>
<td>Dietary Fat Source</td>
<td></td>
</tr>
<tr>
<td>Butter</td>
<td>6.33 ± 0.6&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soybean</td>
<td>4.55 ± 0.5&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>Menhaden</td>
<td>5.23 ± 1.0&lt;sup&gt;x&lt;/sup&gt;</td>
</tr>
<tr>
<td>Interactions:</td>
<td></td>
</tr>
<tr>
<td>MANOVA p-value&lt;sup&gt;3&lt;/sup&gt;:</td>
<td></td>
</tr>
<tr>
<td>SxF</td>
<td>N.S.</td>
</tr>
<tr>
<td>SxC</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>CxF</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

<sup>1</sup> Value indicates mean ± SEM, n = 8, SHR = spontaneously hypertensive rat, WKY = Wistar Kyoto normotensive rat
<sup>2</sup> HDL phospholipid concentration (mg / dl plasma)
<sup>3</sup> Interactions: S x F = strain x fat, S x C = strain x cholesterol, C x F = cholesterol x fat, N.S. = non significant.
<sup>4</sup> a,b,c - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05;
x,y - denotes statistical significance for differences between dietary cholesterol levels, p ≤ 0.05.
Fig. 13 RBC Fragility in SHR Fed Different Dietary Fat Sources\textsuperscript{1,2}

1- Values indicate RBC fragility; mean ± SEM; n = 8
2- SHR = Spontaneously hypertensive rat

Fat Sources Identified: Butter = \(\square\); Soybean = \(\blacktriangle\); Menhaden = \(\blacklozenge\)

No significant treatment effects were observed.
Fig. 14 RBC Frailty in WKY Fed Different Dietary Fat Sources$^{1,2}$

1- Values indicate RBC frailty; mean ± SEM; n = 8.
2- SHR = Spontaneously hypertensive rat
   Fat Sources Identified: Butter = , Soybean = , Menhaden =
   No significant treatment effect was observed.
dietary fat type, and rat strain and dietary cholesterol level were not significant. Cholesterol intake and fat type resulted in a significant interaction (p<0.001). These results indicated that, fat type and cholesterol level did not independently produce a difference in RBC-GSH depletion; however the interaction between cholesterol intake and dietary fat source did contribute to the degree of depletion observed in the various animal treatment groups (Figs. 15 and 16).

III (xxiii) RBC-MDA Assay

Red blood cell malondialdehyde formation was measured at two concentrations of oxidizing agent $H_2O_2$ - 1.0 mM and 5.0 mM. The measurement of MDA through the formation of a chromatophore did not reveal any significant main treatment effects or interactions thereof at the lower concentration of peroxide challenge. At 5.0 mM $H_2O_2$, dietary cholesterol level was found to significantly (p=0.004) influence the MDA concentration (Figs. 18 and 20). RBC-MDA content was highest in animals fed the lower cholesterol containing diets. There were no significant strain or dietary fat type effects observed with RBC-MDA measurements (Figs. 17 - 20).

III (xxiv) LDL Oxidation

This assay was carried out using two levels of copper sulfate. At a low level of copper sulfate, no significant differences were found for the main treatment effects. No significant interactions between the main treatment effects were detected either. The results for LDL oxidation at the higher copper sulfate concentration were analogous to
Fig. 15 Glutathione Depletion in SHR Fed Different Dietary Fat Sources²

1 - Values indicate % glutathione depletion (C₅₀⁺₀ / C₅₀⁻₀) × 100; mean ± SEM, n = 8.
2 - SHR = spontaneously hypertensive rat
   Fat Sources Identified: Butter = a; Soybean = b; Menhaden = c
   a, b, c - denotes statistical significance for differences between dietary fat sources; p ≤ 0.05
   x, y - denotes statistical significance for differences between dietary cholesterol levels; p ≤ 0.05
Fig. 16 Glutathione Depletion in WKY Fed Different Dietary Fat Sources$^{1,2}$

1-Values indicate % glutathione depletion ($C_{\text{initial}} - C_{\text{final}} / C_{\text{initial}}$) x 100; means ± SEM; n = 8.
2- WKY = Wistar Kyoto normotensive rat
Fat Sources Identified: Butter = ; Soybean = ; Menhaden =

a,b,c - denotes statistical significance for differences between dietary fat sources, p ≤ 0.05.

x,y - denotes statistical significance for differences between dietary levels, p ≤ 0.05.
Fig. 17 RBC-MDA Concentration at 1 mM H$_2$O$_2$ in SHR Fed Different Dietary Fat Sources$^{1,2}$

1- Values indicate malondialdehyde (MDA) concentration (nmol/g RBC); mean ± SEM; n = 8.
2- SHR = spontaneously hypertensive rat.

Fat Sources Identified: Butter = □; Soybean = ■; Menhaden = □□□

No significant treatment effects were observed.
Fig. 19 RBC-MDA Concentration at 1 mM H$_2$O$_2$ in WKY Fed Different Dietary Fat Sources$^{1,2}$

1- Values indicate malondialdehyde concentration (nmol/ g RBC); mean ± SEM; n = 8.
2- WKY = Wistar Kyoto normotensive rat
   Fat Sources Identified: Butter = ; Soybean = ; Menhaden =
No significant treatment effects were observed.
**Fig. 20 RBC-MDA Concentration at 5 mM H$_2$O$_2$ in WKY Fed Different Dietary Fat Sources$^{1,2}$**

1. Values indicate malondialdehyde concentration (nmol/g RBC); mean ± SEM; n = 8.
2. WKY = Wistar Kyoto normotensive rat
   
   Fat Sources Identified: Butter = []; Soybean = ■; Menhaden = □

No significant treatment effects were observed.
those obtained at the low Cu-concentration. There were neither significant treatment
effects nor significant interactions thereof with forced peroxidation of LDL (Figs. 21 - 24).

III (xxv) Blood Pressure

Blood pressure readings showed that SHR exhibited significantly (p<0.001) higher blood
pressure than WKY rats (Figs. 25 and 26). There were no other significant main treatment
effects or interactions.
Fig. 21 Cu$^{+2}$-Induced LDL Oxidation in SHR Fed Different Dietary Fat Sources Containing 0.05% Cholesterol (wt/wt)$^{1,2}$

1- Values indicate LDL oxidation index; mean ± SEM; n = 8.
2- SHR = spontaneously hypertensive rat

Fat Sources Identified: Butter = - - - ; Soybean = - - - ; Menhaden = - - - -

No significant treatment effects were observed.
Fig. 22 Cu$^{+2}$-Induced LDL Oxidation in SHR Fed Different Dietary Fat Sources Containing 0.5% Cholesterol (wt/wt)$^{1,2}$

1-Values indicate LDL oxidation index; mean ± SEM; n = 8.
2- SHR = spontaneously hypertensive rat
   Fat Sources Identified: Butter = ---; Soybean = -----; Menhaden = ······
   No significant treatment effects were observed.
Fig. 23 Cu^{2+}-Induced LDL Oxidation in WKY Fed Different Dietary Fat Sources Containing 0.05% Cholesterol (wt/wt)^12

1. Values indicate LDL oxidation index; mean ± SEM; n = 8.
2. WKY = Wistar Kyoto normotensive rat
   Fat Sources Identified: Butter = ---; Soybean = ---; Menhaden = ---
   No significant treatment effects were observed.
Fig. 24 Cu$^{2+}$-Induced LDL Oxidation in WKY Fed Different Dietary Fat Sources Containing 0.5% Cholesterol (wt/wt)$^{1,2}$

1. Values indicate LDL oxidation index; mean ± SEM; n = 8.
2. WKY = Wistar Kyoto normotensive rat
   Fat Sources Identified: Butter = – – –; Soybean = – – ; Menhaden = ... 
   No significant treatment effects were observed.
Fig.25 Blood Pressure in SHR Fed Different Dietary Fat Sources$^{1,2}$

1- Values indicate blood pressure (mmHg); mean ± SEM; n = 8.

2- SHR = Spontaneously hypertensive rat

Fat Sources Identified: Butter = ; Soybean = ; Menhaden =

A significant (p < 0.001) treatment effect for animal strain was observed.
Fig. 26 Blood Pressure in WKY Fed Different Dietary Fat Sources$^{1,2}$

1-Values indicate blood pressure (mmHg), mean ± SEM; n = 8.
2- Fat Sources Identified: Butter = , Soybean = , Menhaden = 

A significant (p < 0.001) treatment effect for animal strain was observed.
IV Discussion:

IV (i) The Effect of Dietary Lipid Intake on Plasma and Lipoprotein Cholesterol and Triacylglycerols

In this experiment, all diets were made isocaloric and since no significant differences in feed intake were observed, the amount of calories ingested by each treatment group was therefore similar. There was no difference in energy intake between high and low cholesterol diets. The lack of variation in feed intake was reflected in the similar weight gain of the various groups. A variation in caloric intake between groups can therefore be dismissed as the basis for differences in plasma lipid concentration in the different experimental treatment groups.

Comparing total plasma cholesterol concentration values of rats fed different dietary fat blends with variable saturated and unsaturated fatty acid content, demonstrated that high cholesterol-butter fed WKY consistently had a higher total plasma cholesterol than counterparts fed menhaden. In other studies with guinea pigs fed beef tallow, corn oil and olive oil at 15 g dietary fat/100 g diet, it was reported that only those animals fed corn oil had a lower plasma cholesterol level (Fernandez and McNamara, 1993; De Schrijver et al., 1984). Results from this study show that fat blends containing a higher saturated fatty acid content raised plasma cholesterol in comparison with those fat sources containing more unsaturated fatty acids; however, this occurred only when dietary cholesterol intake was a factor for WKY rats. Other workers have obtained similar results. For example, normocholesterolemic cebus monkeys consuming diets enriched with palmitic acid (C16:0) generated significantly higher plasma total cholesterol values, when compared to
monkeys fed diets enriched with oleic acid (C18:1), but only when cholesterol was present at a concentration of 0.3% (w/w) (Khosla and Hayes, 1993). Feeding a diet rich in C16:0 was found to significantly raise plasma total cholesterol in hamsters, specifically HDL-cholesterol. The substitution of C12:0 and C14:0 for C16:0 (Lindsey et al., 1990), at a constant saturate/monounsaturate/polyunsaturate fatty acid ratio was shown to raise total plasma cholesterol. Similar results were obtained in monkeys when C16:0 was substituted for C12:0 and C14:0, while replacing C16:0 for C18:2 only produced a slight raise in plasma total cholesterol and the LDL-HDL ratio (Hayes et al., 1991).

A study conducted with human subjects showed that diets containing fat equivalent to 23% energy, where C18:0 was substituted for C16:0 at a 1:1 proportion, produced no measurable differences in plasma cholesterol levels (Ng et al., 1992). The analyses of the present study revealed that low cholesterol treatments, for both SHR and WKY, produced no differences between butter and menhaden fed animals. In Ng’s experiment, where palm oil and olive oil were used, palm oil had approximately equal C16:0 and C18:1 content. The fatty acid composition of our butter and menhaden based diets in this study closely resembled the fatty acid profile of the experimental diet used by Ng et al. (1992).

It is not only the fatty acid composition of a dietary fat source that exerts an influence on physiologic parameters, but the position of the fatty acids on the glycerol backbone that is important in lipid transport and further metabolism (Renaud et al., 1995).

The particular composition of triacylglycerols has been found to influence chylomicron remnant uptake by the liver. As pointed out by Small (1991) and Norum
(1992), the presence of saturated long chain fatty acids, particularly in the sn-2 position, retards cholesterol uptake by the liver, due to reduced affinity of lipoprotein lipase for the triacylglycerols containing those fatty acids. The basis for the diminished affinity may stem from a compact surface configuration of those lipoproteins which have a long chain saturated (LCS) fatty acid in the sn-2 position. This feature appears to be involved in reducing access of lipase, or a change in configuration of the triacylglycerols, making binding to the hepatic receptor more difficult (Redgrave et al., 1988).

In other studies supplementing butter or margarine based swine diets with additional magnesium (Mg) was shown to produce similar plasma total cholesterol in both treatment groups (Kummerow et al., 1993). That finding was explained by the fact that Mg, a cofactor for fatty acid desaturase and aid in the conversion of linoleic to arachidonic acid was limiting in the diet. Thus other nutritional factors, in addition to fatty acid composition play a role in lipidemia.

Research on PUFA metabolism has shown that C20:4 and C20:5 acids present on positions 1 and 3 of glycerol are not readily hydrolyzed by lipoprotein lipase (LPL) (Ekstroem et al., 1989; Nilsson et al., 1987; Balsinde et al., 1991). Hepatic lipase, however, appears to have a relatively high affinity for diacylglycerols with the forementioned configuration. This indicates a key role of triacylglycerol composition, as well as geometric position of key fatty acids have a role in lipoprotein metabolism and plasma cholesterol levels. In the present experiment, the butter based diet contained approximately 12.6% (wt/wt) saturated fatty acids, while the menhaden diet contained 6.3% and the soybean diet 5.1% saturated fatty acids. Oleic acid constituted 50.5%
(wt/wt) of the fatty acids in the soybean diet, whereas butter and menhaden diets had 29.4% and 26.4%, C18:1, respectively. The menhaden diet, in addition, contained EPA (6.4%) and docosahexaenoic (DOHA) (4.7%), which were not present in the other fat blends. Fatty acids in the sn-2 position are absorbed as monoacylglycerols and tend to maintain that configuration as they are incorporated into lipoproteins (Small, 1991). When rats were fed a diet using lard, in which 80 % of the sn-2 positions are esterified with palmitic acid, as a fat source, random rearrangement of the fatty acids on the glycerol backbone through interesterification led to a decrease in plasma triacylglycerols (Renaud et al., 1995). The decline in plasma triacylglycerols observed in this study, when feeding a high (0.5%) cholesterol concentration, is likely due to a metabolic adjustment to the hyperlipidemia associated with hypercholesterolemia.

The key to the cholesterol lowering effect of unsaturated fatty acids, and very long chain PUFAs in particular, appears to be with the hepatic metabolism of free fatty acids. VLDL, synthesized in and released from the liver, contain mainly triacylglycerols, along with about 17 % cholesterol (Glickman and Sabesin, 1994). VLDL-cholesterol and triacylglycerol concentrations can, however, be altered through modifications in dietary intake (Hau et al., 1996; Jaques et al., 1995; Nelson et al, 1995). PUFA (n-3) can suppress triacylglycerol synthesis, as well as the assembly of nascent VLDL (Nestel et al., 1984). Therefore, reducing VLDL may contribute to a reduction of total cholesterol. A smaller amount of triacylglycerols to be transported in VLDL, as well as a smaller number of particles assembled, would be a consequence of less cholesterol being transported via
VLDL. This is one possible explanation for the distinct differences in animal lipid profiles derived from menhaden diets.

Plasma triacylglycerol levels were shown to be reduced in animals fed menhaden diets, in comparison with in butter and soybean in WKY, in this study. The triacylglycerol lowering effect of the menhaden diet can be explained by decreased peripheral utilization of n-3 fatty acids (Singer et al., 1990). While the soybean diet did not contain a similar proportion of very long PUFAs, its n-6 polyunsaturated fatty acid content has also been found to reduce fasting plasma cholesterol (Weintraub et al., 1988). On the other hand, dietary intake of soybean phytosterols has been shown to reduce plasma total cholesterol and LDL-cholesterol, while increasing HDL-cholesterol in normocholesterolemic humans (Pelletier et al., 1995). It has been suggested that phytosterols decrease cholesterol absorption in the intestine (Laraki et al., 1993; Slota et al., 1983) by limiting the access of cholesterol to the transport protein.

Plasma triacylglycerol levels were highest in animals fed butter based diets and low dietary cholesterol level 0.05% (wt/wt) in this experiment. Plasma lipid levels measured in samples obtained from fasted normocholesterolemic cebus monkeys fed 10% energy from fat C18:1 or C16:0 (Khosla and Hayes, 1993), were shown to be affected by the level of saturated fat in the diet. In the absence of dietary cholesterol, plasma triacylglycerol concentration was found to be higher in C16:0 rich diets, but produced comparable plasma cholesterol levels. In Syrian hamsters, plasma triacylglycerol concentrations of animals fed fish oil were found to be similar to those in hamsters fed coconut oil and soybean oil in the presence of 0.5% cholesterol (Lin et al., 1995). Meanwhile, a high concentration of
dietary cholesterol cancelled the differences in the triacylglycerol raising effects of individual fatty acids observed with a lower cholesterol concentration. A diet containing 0.5% (wt/wt) cholesterol and 5% (wt/wt) corn oil was found to increase hepatic triacylglycerol as well as cholesterol synthesis (Fungwe et al., 1994). These results agree with our observations, as an increase in dietary cholesterol intake led to a rise in liver triacylglycerols in WKY animals fed high cholesterol diets. In contrast to Lin et al. (1995), in this experiment high cholesterol soybean-fed WKY rats produced higher liver triacylglycerol values than those fed the other dietary fat sources. Those differences point to the importance of the fatty acid composition of the individual dietary fat source. While the butter and menhaden based diets in this experiment both had similar myristic acid contents, the latter diet had a very long chain PUFA content that was unmatched in either of the two other diets. The combination of a significant C14:0 content (8.9%) with the very long PUFA could explain the lower liver triacylglyceride concentrations observed in animals fed menhaden diets. While C14:0 is metabolized more quickly than long-chain fatty acids, the preferential uptake of very long PUFA by the liver, could thus lead to a lowering of hepatic triacylglycerol.

Diets varying in particular fatty acids tend to influence individual lipoprotein fractions differently. For example, a diet rich in monounsaturated fatty acids may decrease LDL cholesterol (Demacker et al., 1994), while the same diet may also increase triacylglycerol fractions (Mata et al., 1992). In this study plasma VLDL-cholesterol from high cholesterol fed WKY was significantly altered by the dietary fat source. A similar trend was observed in low cholesterol fed rats of both strains, but it was not statistically
significant. While WKY rats exhibited a differentiated response to fat sources with a high dietary cholesterol content, SHR did not respond differently to varying levels of unsaturation when given a high cholesterol diet. Low cholesterol butter fed SHR had higher VLDL-cholesterol values than counterparts fed fats from other sources. This was also observed in low cholesterol butter fed WKY. These findings stand in agreement with the inhibition of VLDL synthesis by n-3 polyunsaturated fatty acids (Nestel et al., 1984) as illustrated above. The trend observed among low cholesterol fed SHR was reversed in animals fed high cholesterol diets. Both the menhaden and soybean fed SHR groups had higher VLDL-cholesterol values than the butter group from the same treatment. This finding indicates that when cholesterol intake is high, the fat source unsaturation influence on VLDL-cholesterol is overridden by the concurrent ingestion of cholesterol.

In the study by Weintraub et al. (1988), ω-3 fatty acid rich diets lowered plasma HDL-cholesterol levels, when compared to diets containing predominantly saturated fatty acids (Weintraub et al., 1988). In this study, no dietary fat source effect was observed in HDL-cholesterol in any animal group. One possible explanation for this finding is that the proportion of dietary saturated to unsaturated fatty acids in rat diets used in this experiment was not sufficient to promote a difference in HDL-cholesterol between different groups of animals fed diets with characteristically distinct fat blends.

There was however a significant animal strain effect, as evidenced by the fact that WKY rats had higher plasma HDL cholesterol levels than SHR and low cholesterol fed WKY had higher HDL-cholesterol levels than high cholesterol fed WKY animals. These
results point to animal strain differences in cholesterol transport between the SHR and WKY rats. Taking into consideration that rats carry most of their plasma cholesterol in the HDL fraction (Jokinen et al., 1985), the absence of a difference in HDL-cholesterol concentration between low and high cholesterol fed SHR, points to a lack of response in cholesterol transport when dietary cholesterol supply is increased.

IV (ii) **Relationship between Dietary Fat Sources and Liver Lipids**

Further to the animal strain influence on cholesterol handling, dietary lipid intake was found to be a factor. When long chain fatty acids are absorbed in the gut, they are incorporated into chylomicrons. While these circulate, triacylglycerols are hydrolyzed by lipoprotein lipase until the chylomicron remnants are taken up by the liver. Cholesterol clearance is achieved by receptor mediated endocytosis (Vlahevic et al., 1994). Excess cholesterol suppresses the biosynthesis of LDL receptors at the level of gene transcription (Russell et al., 1983). In rats, in particular, cholesterol delivered to the liver via chylomicrons will up-regulate 7α-hydroxylase, suppress HMG-CoA reductase and cause a rapid decrease in cholesterol biosynthesis (Carrella and Cooper, 1979). In this study, liver cholesterol concentration results were significantly influenced by dietary cholesterol feeding. High cholesterol fed animals exhibited liver cholesterol concentrations that were relative to the dietary cholesterol intake. While plasma cholesterol concentration paralleled the increase in dietary cholesterol intake, this effect was not of the same magnitude as the difference in liver cholesterol concentration between the low and high cholesterol fed
animals. Although cholesterol synthesis may have been suppressed and 7α-hydroxylase activity enhanced, these results indicated that possible hepatocellular adaptive changes were not sufficient to compensate for the greater dietary supply, resulting in hypercholesterolemia in high cholesterol-fed animals.

There was also a significant dietary fat source effect on liver cholesterol content. High cholesterol menhaden fed rats of both animal strains produced lower hepatic cholesterol concentration values than those rats fed other dietary fat sources. Dietary fats rich in n-3 fatty acids, such as linseed and menhaden oil, have been shown to reduce both total and HDL cholesterol in Wistar rats (L’Abbe et al., 1991). These differences appear to be the result of a combination of factors. Preferred hepatic uptake of PUFA from chylomicrons could have led to an inhibition of hepatic cholesterol synthesis and a reduction of triacylglycerol synthesis, as well as a reduction in ACAT activity (Smit et al., 1994). N-6 PUFA, but not n-3 PUFA, have been found to stimulate the disposition of cholesterol into bile by potentiating bile-acid dependent cholesterol secretion and by facilitating the diversion of bile-destined cholesterol (Smit et al., 1994; Berr et al., 1993). Some researchers suggest, on the other hand, that remnant cholesterol reaches bile mainly in the form of bile acids under conditions of stimulated bile-acid synthesis (Carrella et al., 1995). The difference in findings may be due to the fact that the latter authors evaluated short term cholesterol metabolism. With the addition of cholesterol at 1% (wt/wt) to a diet containing 5% rubber seed oil, a rich source of n-3 fatty acids, a hypercholesterolemic effect was observed in Wistar rats compared to a hypocholesterolemic effect in the absence of cholesterol (Nwokolo et al., 1988). These authors suggested that, the increase
of serum VLDL-cholesterol observed concurrently with rubber seed oil was in response to increased absorption of cholesterol by altering its transport across the intestinal cells. No difference in liver cholesterol concentrations was observed between high and low cholesterol butter-fed and soybean-fed animals in our experiment. High cholesterol menhaden-fed SHR produced lower liver cholesterol than their counterparts fed other dietary fats. This finding stands in agreement with that of Nwokolo et al. (1987). In this instance, however, the cholesterol lowering effect was observed in SHR, suggesting that, although there may be differences between SHR and WKY lipid metabolism, the combination of n-3 fatty acid intake concurrent with a high cholesterol level overrides the genetic predisposition. In other studies, where rats were fed olive oil, hepatic cholesterol homeostasis was maintained through an equilibrium between ACAT and HMG-CoA activities (Fernandez and McNamara, 1993). Sprague-Dawley rats fed a diet containing 12% fat (wt/wt) derived from palm oil, corn oil, tallow or safflower oil, as well as 0.4% CHL, exhibited higher liver cholesterol than those on cholesterol free diets (Rule et al., 1996). These workers showed that animals fed the safflower/cholesterol diet produced the highest liver cholesterol concentrations. This finding was traced to a low C18:0 content of the dietary fat source. The butter and soybean based diets in our study contained similar proportions of C18:0, a fatty acid noted for its relatively poor bioavailability. Exogenous C18:1 on the other hand has been shown to stimulate cholesterol synthesis in the perfused rat liver (Goh and Heimberg, 1979). This explains the liver cholesterol values obtained in soybean oil fed rats, where the diet had 3.3% eicosenoic (C20:1) acid, which would tend to reduce liver cholesterol, as explained above. The diet also contained 50.5% C18:1
which could have stimulated cholesterol synthesis in high cholesterol-soybean fed rats. This hypothesis is further supported by a former observation, that feeding 0.5% (wt/wt) cholesterol in combination with highly hydrogenated soybean oil, rich in unevenly distributed C18:0, resulted in greater cholesterol excretion (Kamei et al., 1995). Furthermore, with an increase in Apo-B and Apo-AI, but not Apo-E, in VLDL in the isolated hamster liver, Apo-AI has been said to enhance hepatic export of cholesterol and triacylglycerols (Chen et al., 1996). Cholesterol fed at a concentration of 0.5% (wt/wt) raised hepatic cholesterol concentration by 100% in rats and 60% in mice (Srivastava, 1996). The author concluded that, based on the increases observed in hepatic apo-E and plasma apo-B levels, dietary cholesterol regulates Apo-E by a transcriptional mechanism and dietary saturated fat by a posttranscriptional mechanism. The results stand in agreement on the experiment, as saturated fats increased plasma cholesterol and cholesterol feeding raised hepatic cholesterol levels, while no difference between hepatic cholesterol levels between butter and soybean fed rats was observed.

Fish oil has been shown to reduce VLDL secretion in rats (Topping et al., 1987). Eicosapenatenoic acid (EPA), one of the characteristic PUFA present in fish oil, increases intracellular degradation of apolipoprotein B, the lipoprotein of VLDL, when added to hepatocytes (Wang et al., 1993). Fish oil concentrate can also reduce fatty acid synthetase (FAS) activity as a consequence of a proportionate decrease in FAS protein mass, as shown by Western Blot analysis (Benhizia et al., 1994). Singer et al., (1990) reported that n-3 PUFA supplementation resulted in decreased free fatty acid concentrations in hyperlipidemic patients, thus explaining the triacylglycerol-lowering effect of n-3 fatty
acids. A similar effect would be expected to contribute to lowering VLDL concentration as was observed in high cholesterol fed WKY in this study. The findings herein compare with other studies where diets enriched in monounsaturated fatty acids, comprising 60% of the energy from fat (diets containing 30% of energy as fat) and 0.1% cholesterol, resulted in higher liver triacylglycerol content than the saturated fat diets (Brousseau et al., 1995). Therefore, while n-3 PUFA have been shown to influence lipid metabolism, the effect appears to depend on the tissue being analyzed. The presence of other dietary fatty acids, as well as other factors contributing to the metabolic status, such as the occurrence of hyperlipidemia, also play an important role in the evaluation of the n-3 PUFA intake.

Analysis of liver lipids revealed that Wistar rats fed a lard soybean oil diet with a PUFA/ SFA ratio of 1, and enriched with 1% cholesterol, accumulated an excess triacylglycerol due to an increased synthesis and decreased secretion of triacylglycerol (Liu et al., 1995). In the present study, all main treatments showed a significant effect on liver triacylglycerol concentrations. While high cholesterol-menhaden fed rats produced the lowest triacylglycerol values in both strains, high cholesterol-soybean fed rats produced the greatest response in plasma triacylglycerol. In contrast, baboons fed a saturated fat diet and high cholesterol exhibited a liver triacylglycerol concentration that was higher than that in animals fed a monounsaturated fatty acid rich diet (Fox et al., 1987). The conflicting results suggest a synergism between saturated fats and cholesterol at a high cholesterol intake level (Brousseau et al., 1995) in regard to liver triacylglycerol. Dietary fat content was 16 % (wt/wt) and cholesterol content was 1 % (wt/wt) in the study by Fox.
et al. (1987). Comparisons of results were difficult, since cholesterol content was
different.

The exact mechanism through which dietary fatty acids alter the profile of hepatic
lipids is still the subject of much research. At present, it is not known precisely how
saturated fatty acids affect liver cholesterol and triacylglycerol concentrations. To what
extent C18:0 will reduce fatty acid incorporation into hepatocytes is also unknown.
Moreover, it is unknown to what degree dietary cholesterol level neutralizes the
cholesterol lowering effect of a diet rich in PUFA, and which pathway would mediate
these changes. It is clear however, that a low dietary cholesterol level affects liver lipids
differently with a low fat intake and when it is associated with a high dietary fat intake.
Liver triacylglycerols were elevated in rats fed monounsaturated diets compared with rats
fed a saturated diet (Brousseau et al., 1993). Supplying equal amounts of lard and fish oil
in a diet produced no difference in liver triacylglycerols (Chiang and Tsai, 1995). Lard has
a high C18:1 content (45%) (White, 1992). Fish oil contains a relatively high amount of
monounsaturated fatty acids, over 20% in menhaden oil (Jandacek, 1992) and it is the
PUFA/monounsaturated fatty acid ratio that appears to be relevant to liver triacylglycerol
concentration. Additionally, the PUFA content of the diet has been found to modulate
liver cholesterol content. Rats fed diets with different n-6/n-3 ratios were found to
produce lower liver cholesterol with increasing dietary n-6 content (Lee et al., 1989).
When Sprague-Dawley rats were fed n-3 enriched egg yolks, liver cholesterol levels were
found to be lower than those of rats fed n-6 enriched diets (Jiang and Sim, 1992). Since
the fat sources in the former experiment were of plant origin, the different outcomes point
to the importance of taking dietary cholesterol intake into account, as confirmed by the results of our experiment.

IV (iii) Dietary Lipid Intake and Red Blood Cell Composition

Blood is intimately involved in mediating the effects of lipid metabolism to different organ systems. Evaluating changes occurring in RBC membrane lipid composition further illustrates the consequences of our experimental treatments on lipid metabolism homeostasis. Dietary fat is reflected in plasma and RBC composition over a long time (Dougherty et al., 1987). Membrane lipid content and anion transport by old human erythrocytes, when compared to newly synthesized cells, has been reported to increase with fish-oil supplementation (Mills et al., 1995). However, although the fatty acid composition of the diet was monitored in the former study, dietary cholesterol intake was not and therefore its specific influence on membrane composition was not assessed. If there were neither significant animal strain, nor significant dietary fat source effects on RBC-triacylglycerol composition, then the influence of dietary cholesterol intake on RBC-triacylglycerol in the present experiment is likely the consequence of only sterol intake. The RBC phospholipid content of Sprague-Dawley rats has also been shown to be unaltered by dietary fat sources with varying unsaturated fatty acid content (Lands et al., 1990). The hypocholesterolemic effect of vegetable oil intake has been linked to the increased uptake of C20:4n-6 (Theret et al., 1993). Therefore, these results stand in agreement with the RBC-phospholipid analysis in this study, which revealed a significant
RBC-phospholipid effect with increased level of dietary cholesterol intake. Furthermore, the effect of elevated dietary cholesterol intake on RBC-cholesterol in rats fed high cholesterol diets occurred in both strains, suggesting that this effect is enhanced by external environmental factors such as diet, rather than genetic make-up. The decrease in RBC phospholipid/cholesterol ratio was primarily due to the relatively greater increase in the RBC-cholesterol content. As plasma cholesterol expressed significant main treatment effects, the role of individual plasma lipoprotein fractions in mediating the dietary cholesterol effect on the RBC is therefore of special importance.

IV (iv) Lipid Balance Study

The balance study conducted herein helped establish the extent to which lipid excretion contributed to whole body fat homeostasis and thus to the concentrations of cholesterol measured in the liver, RBC, and plasma. While there were no differences in feed intake during the balance period study, the fact that total fecal output showed a significant main treatment effect, indicated potential differences in diet digestibility. High cholesterol fed rats of both strains exhibited greater excreta output than those fed low cholesterol diets. This outcome points to a possible reduction in feed absorption efficiency in the presence of a high cholesterol intake. More specifically, the assessment of lipid digestibility from the different experimental diets in both rat strains indicates that cholesterol feeding lowered lipid digestibility in those animals fed high cholesterol diets. This observation did not seem to have an influence on total crude lipid excreted or weight
gain over the period of the entire experimental period, suggesting that the difference in cholesterol intake in the high cholesterol diets may have been compensated for by increased fecal cholesterol excretion over time. This is further underscored by the analysis of total crude lipid excretion, which was raised in high cholesterol fed animals. For example, while high cholesterol fed rats had higher fecal output and cholesterol excretion than low cholesterol fed animals, lipid digestibility, on the other hand, was not altered by animal strain. This result indicates that endogenous lipid synthesis in SHR is possibly greater than in WKY.

The finding that dietary fat sources affected total fecal output significantly, was largely attributed to the animals fed the soybean diets, which produced the lowest fecal output. High cholesterol butter fed rats of both strains had the lowest fecal cholesterol excretion compared to counterparts fed other dietary fat sources. These results indicate that fecal cholesterol excretion was not determined by total fecal output in these rats.

The digestibility of dietary fats is primarily dependent on the amount and chain lengths of the saturated fatty acids esterified to the glyceride structure (Kitts and Jones, 1996; Calloway et al., 1956; Hayes, 1995; Kritchevsky, 1995). The degree of fatty acid saturation has been observed to affect specific aspects of lipid absorption and transport. For example, there is evidence that fatty acids may precipitate Ca$^{2+}$ and Mg$^{2+}$ ions, important for micellization (Brink et al., 1994; Lien, 1994; Mattson et al., 1979). Moreover, decreases in fatty acid chain length have been associated with an increase in homogenous triacylglycerides, saturated with fatty acids of C12-C18 (Bracco, 1994).
Rats fed diets containing 20% dietary fat, of which 50% was present in the form of highly hydrogenated soybean oil, along with 0.5% cholesterol, have been reported to increase fecal cholesterol excretion, compared to control animals (Kamei et al., 1995).

In this experiment menhaden fed animals had lower lipid digestibility than their counterparts fed more saturated fat sources. This partly explains the significant interaction of dietary cholesterol level with the fat source effect observed in this study. Lipid digestibility was not significantly affected by animal strain in this study. Yet, given the presence of the genetic differences in these two rat strains relative to other parameters related to lipid metabolism, the results described above could represent an indirect expression of the noted differences in lipid digestion. Alternatively, a heightened 7α-cholesterol hydroxylase response would be expected to lower liver and plasma lipid parameters by enhancing cholesterol driven bile acid production (Russell, 1992). Since there were no significant differences in feed intake or weight gain between SHR and WKY, differences in lipid balance, including cholesterol, could have occurred only between groups fed high dietary cholesterol concentration. Additionally, greater fecal cholesterol excretion was observed in rats fed high cholesterol diets in this study. This indicates a positive response to increased bile acid production and excretion in those animals.

A shift in dietary consumption, from a high fat, high P:S ratio to a low fat high P:S ratio diet, has been shown to increase whole-body oxidation of $^{13}$C16:0 in human subjects (Clandinin et al., 1995). These authors suggested that an increase in a P:S ratio of a diet
could alter the differential utilization of individual dietary fats. Varying the chain length of saturated fatty acids was found to lead to a proportional increase in the net oxidation of those fatty acids (MacDougall et al., 1996\textsuperscript{a}). Most recently the presence of C\textsubscript{16}:0 and C\textsubscript{14}:0 in dietary tallow, butter and corn oil did not result in differences in dietary energy production or substrate utilization after a meal (MacDougall et al., 1996\textsuperscript{b}), although net dietary fatty acid oxidation was influenced by dietary fatty acid content.

In the present study, a high dietary cholesterol concentration was shown to reduce lipid digestibility, which therefore may have further compounded the effect of varied P:S ratios. As a consequence, reduced utilization of these fats for energy would be expected.

IV (v) **Oxidative Stress**

Alterations in tissue lipid levels caused by differences in the digestibility of the various diets or by the specific effect of individual fat blends are likely to have changed the potential for fatty acid oxidative processes.

Cells have protective mechanisms against reactive oxygen species and free radicals, and glutathione is an important component of such mechanisms (Meister and Anderson, 1983). When GSH is utilized within cells, it is converted to an oxidized form (GSSG). The status of reduced glutathione (GSH) in a cell therefore reflects its potential for protection against oxidative injury. Various membrane bound sulphydryl enzymes are protected by glutathione (Arias and Jakoby, 1976). In this study, glutathione depletion in RBC was
significantly lower in SHR than in WKY. Consequently, RBCs from SHR responded to a lesser extent to the forced oxidative stress applied in vitro compared to their WKY counterparts. Several studies have indicated a correlation between reduced antioxidant status and the development of coronary artery disease (Riemersa et al., 1991; Ytrehus et al., 1987; Basu et al., 1987). Vitamin E and selenium have been observed to have a protective effect on glutathione-dependent enzymes by increasing the capacity to form glutathione conjugates and improving the recovery of glutathione (Ip, 1984). It follows that, rats exposed to diets with varying potential for oxidative stress, either through unsaturation of dietary fat sources or cholesterol level, appear to have compensated for dietary differences in oxidative potential. It may be important however, to discern the source of oxidative stress, as exercise and hypertension were found to affect antioxidant status differently (Hong and Johnson, 1995). SHR have genetically determined high blood pressure, which in turn has been shown to affect tissue antioxidant as per increased TBARS production in both liver and plasma (Bui et al., 1995).

The fact that RBC-MDA was significantly decreased by cholesterol intake was an important finding in this study. RBC-MDA production at 5 mM H₂O₂ was lower in rats fed high cholesterol diets. The production of TBARS has been widely used as an indicator of oxidative processes. For example, an increase in MDA concentration of PMNL in hypertensive patients, indicated a rise in peroxidative reactions (Rahman and Nath, 1988). In patients with CHD, blood platelet MDA values were higher than those of healthy subjects, pointing to a low antioxidative defense status, possibly due to induced lipid peroxidation in those patients (Buczynski et al., 1993). On the other hand, a study on the
effects of smoking on antioxidant systems in rats revealed that hypertension had a stronger
effect on tissue antioxidants than nicotine (Bui et al., 1995).

As pointed out previously, the cholesterol content of RBC in this experiment
increased with cholesterol feeding. A rise in plasma LDL levels in humans carries an
increased risk for oxidative stress due to the increase of a particularly susceptible LDL
fraction (Benz et al., 1995; Chiu et al., 1994; Sevanian et al., 1996). An increase in rat
RBC-cholesterol in high cholesterol-fed animals could alternatively reflect compensation
to oxidative stress. Furthermore, low density lipoprotein-phospholipid content decreased
in fractions from high cholesterol fed rats in our study, indicating that cholesterol
displaced phospholipids, possibly as a consequence of their increased uptake by the RBC.
This would also reflect a potential for greater stability towards oxidative stress. Dietary
cholesterol thus appears to have acted as an antioxidant in protecting RBCs from
peroxidation.

IV (vi) The Influence of Dietary Fat on LDL Oxidation

Copper-based LDL oxidation of the samples from the rat specimens in this
experiment did not reveal significant main treatment effects. This outcome can be
explained with the help of the following evidence. In humans, diabetes has been suggested
to contribute to the development of CHD via two routes (Picard, 1995). High levels of
glucose lead to the formation of glycated LDL which aggravates atherosclerosis directly
and contributes to the formation of free radicals. These give rise to oxidized LDL which
also contributes to atherosclerosis. Increased plasma LDL levels have been associated with
an increased risk of atherosclerosis (Brown and Goldstein, 1983). While cholesterol that
has not been oxidized does not significantly enhance lipid accumulation in arterial walls
(O’Brien and Chait, 1994; Hennig and Chow, 1988), oxidative alteration of LDL favours
uptake by endothelial cells, macrophages and smooth muscle cells (Steinberg et al., 1989).
Mildly oxidized LDL stimulates the expression of genes for adhesion molecules for
leukocytes (Berliner et al., 1990). Further oxidation of LDL causes chemotaxis of
monocytes and stimulates their differentiation into macrophages, while also inhibiting their
motility at the site (O’Keefe et al., 1995). Additionally, recognition by scavenger
receptors, present on endothelial cells and macrophages, is triggered and foam cell
formation occurs as the accumulation of oxidized LDL progresses (Ginsberg, 1994). At
the same time smooth muscle cells proliferate, synthesize collagen, and cytokines are
released (Witztum and Steinberg, 1992; O’Brien and Chait, 1994, Ginsberg, 1994).
Arterial medial cells also have been observed to proliferate when stimulated by factors
found in serum of Mg deficient animals (Bussiere et al., 1994). Foam cells rupture and an
inflammatory process takes place (Jang et al., 1993), leading to plaque formation and
hardening of the blood vessel. Recent research has revealed the existence of an
association between the presence of antibodies to an oxidized form of LDL and the risk of
atherosclerosis (Salonen et al., 1992; Cushing et al., 1990), especially in the case of
hypertensive patients (Maggi et al., 1993).

Dietary fat and/or cholesterol intake can provoke an increase in plasma LDL and over
time promote LDL oxidation as discussed above (Chiu et al., 1994; Benz et al., 1995).
Several studies have shown a correlation between LDL oxidation and atherosclerosis.
(Regnstroem et al., 1992). For instance, dietary olive oil, rich in C18:1, has been shown to limit LDL lipid peroxidation and uptake by macrophages (Aviram and Eias, 1993). While the effect of fatty acid unsaturation on LDL oxidation has been evaluated (Bonanome et al., 1996; Regnstroem and Nilsson, 1994), there has been great interest in the role of antioxidants relative to CHD. In vitro studies of endothelial cells have shown that oxidative changes in LDL can be completely prevented with the presence of antioxidants (Steinbrecher et al., 1984). Population studies, such as the WHO/MONICA (Koenig et al., 1992), have revealed a negative correlation between plasma vitamin E levels and mortality from CHD (Gey et al., 1991) in both women (Stampfer et al, 1993) and men (Rimm et al., 1993). Some have pointed out that vitamin E is likely the most important antioxidant in regard to atherosclerosis (Gey et al., 1991; Jialal and Grundy, 1993; Esterbauer et al., 1991). Vitamin E, more specifically α-tocopherol, is lipid soluble. LDL oxidation has been shown to be inhibited by vitamin E in vivo (Riemersma et al., 1991, Lavy et al., 1993), while the trans-isomer of β-carotene was shown to be particularly effective (Lavy et al., 1993). Alternatively vitamin E depleted LDL becomes more susceptible to oxidation (O’Keefe, 1995).

Vitamin C is also an antioxidant. It is, however, water soluble and therefore active mostly in plasma. Furthermore, it promotes the absorption of iron, which increases the potential for oxidative stress. It is therefore not surprising that some researchers have found favourable effects of vitamin C (Enstrom et al., 1992), while others have not (Stampfer et al., 1993; Rimm et al., 1993). Minerals such as Mg have been suggested to be a factor in pathological processes mediated by lipid peroxidation products. Mg-deficient
diets fed to weanling Wistar rats were observed to produce VLDL- and HDL susceptible to lipid peroxidation as verified by TBARS (Rayssiguier et al., 1993). The antioxidant status of plasma and the lipoprotein fraction can thus have different effects on LDL oxidation.

The susceptibility of a given animal species or strain to CHD or atherosclerosis can also be genetically determined. Some species are relatively resistant to atherosclerosis and can be utilized in the comparison of conditions that modulate susceptibility (Jokinen et al., 1985). While dietary fat source and cholesterol are important factors in the development of atherosclerosis in rabbits (Kritchevsky, 1970), the levels of hypercholesterolemia involved in promoting the expression of these symptoms was extreme (Jokinen et al., 1985). Alternatively, hypertension may be induced surgically, while hyperlipidemia can be genetically determined in Watanabe heritable hyperlipidemic rabbits (Nickerson et al., 1992). Cynomolgous monkeys and humans display a difference in the incidence of coronary atherosclerosis where males and females are concerned, which reflects its relationship to plasma lipoprotein profiles (Hamm et al., 1983; Rudel and Pitts, 1978). The use of rats in the study of CHD had been motivated partly by the possibility to produce lesions through dietary manipulation, and the existence of strains that develop HT such as the spontaneously hypertensive rat (SHR) and the stroke-prone spontaneously hypertensive rat (SP-SHR) (Mori et al., 1993). Rats are nevertheless considered to be relatively resistant to atherosclerosis (Joris et al., 1980).

The fact that we did not observe significant differences in LDL oxidation, neither due to dietary fat source, cholesterol or animal strain, may thus be an indirect
manifestation of this species' lack of susceptibility to atherosclerosis. LDL oxidation is an important component in the development of sclerotic lesions. The absence of significant alterations in this factor via dietary input shows, that rats are able to compensate in some way to minimize the potential for LDL oxidation due to dietary fat and cholesterol sources. Dietary factors therefore affect other metabolic parameters associated with hypertension rather than atherosclerosis in the rat model.

IV (vii) Dietary Fat Sources and Hypertension

In the present experiment blood pressure in the SHR was higher than corresponding WKY at 10 weeks of age. There were no significant main treatment effects related to diet. Similarly blood pressure in the WKY was not altered during the experimental period. The effect of dietary fat source composition on blood pressure in rats has been evaluated in a number of studies (McGregor et al., 1980; Karanja et al., 1989).

The alterations observed in platelet function, an increase in the platelets' thrombin-induced aggregability, of hypertensive rats following saturated dietary fat intake were found to be similar to those induced by hypertension (McGregor et al., 1980). Ca\(^{2+}\) transport system abnormalities were cited as a plausible cause for the latter findings (Hamet et al., 1978). In SHR supplied with diets containing butterfat, corn oil and fish oil, those diets containing butterfat and Ca\(^{2+}\) produced blood pressures similar to the fish oil diets (Karanja et al., 1989). Lowering fat intake significantly, while increasing relative polyunsaturated fatty acid content, effectively reduced systolic and diastolic blood
pressure in humans (Iacono and Dougherty, 1985). This effect was reversed upon returning to the regular diet. In humans, a diet enriched by 1% in C18:3 was found to decrease blood pressure, while C18:2 supplemented in the same proportion did not (Berry et al., 1986). In that study higher blood pressure observed in SHR was not attributable to the effect of dietary fatty acid composition on prostaglandins.

The fact that blood pressure was not altered in either SHR or WKY by dietary fat in the present experiment could be due to the dietary fatty acid composition of the fat blends supplied in this experiment providing sufficient C18:2 to normalize the change in blood pressure observed in this study on rats. Alternatively, as has been outlined above, high blood pressure in SHR is likely more so related to other dietary factors such as an imbalance in Na⁺/K⁺ transport and/or in Ca metabolism (Kitts et al., 1992). Therefore, to exert a dietary fat source effect on these mechanisms may require a dietary fat source that would for example reduce mineral bioavailability.
CONCLUSIONS

These results demonstrate that genetic makeup has a stronger influence on promoting the development of dyslipidemia and altered antioxidant status in the presence of a dietary fat source than the dietary fat source alone. An important interaction between fat source and cholesterol intake was found to influence the degree of susceptibility to change in lipid metabolism. For example, the influence of dietary fat source unsaturation level on VLDL-cholesterol was overridden by high cholesterol intake. VLDL-cholesterol levels were higher in those groups fed a high cholesterol diet, while at a lower dietary cholesterol concentration, butter-based diets produced higher VLDL-cholesterol values. Furthermore, cholesterol feeding promoted an increase in plasma cholesterol in WKY, while in SHR that effect was not evident.

While there were no differences in total crude lipid balance, lipid digestibility was lower in high cholesterol-fed animals and total crude lipid and cholesterol excretion were greater in SHR. Cholesterol feeding increased liver total crude lipid content. Dietary fat sources modulated the change, as seen in high cholesterol menhaden fed animals, which produced lower levels than animals fed the other dietary fat sources. At the same time liver triacylglycerides were lower in low cholesterol fed WKY rats than in low cholesterol fed SHR.

Therefore it is concluded that while SHR expressed increased blood pressure, this abnormality was determined by a genetic predisposition more than by the dietary fat source or cholesterol intake level.
The RBC-MDA assay showed that dietary input from fat sources does have an impact on oxidative stress. RBC-MDA production showed a trend to lower values in high cholesterol fed animals in both SHR and WKY, although it appears to be more pronounced in SHR, indicating a protective effect of cholesterol against oxidative stress. Additionally, the response in RBC-MDA production amongst groups fed different dietary fat sources at a specified cholesterol level was relatively uniform. The absence of a dietary fat source effect alone thus confirms the importance of dietary cholesterol in susceptibility to oxidative stress in RBC. However dietary fat unsaturation was not equally relevant in regard to the level of oxidative stress in RBC. The difference in response pattern between the two rat strains may offer clues as to the indirect effects of dietary fat unsaturation on factors associated with heart disease. For example, while dietary fat sources elicited similar levels of oxidative stress in SHR RBC, LDL oxidation showed a constant trend for higher levels of oxidation in soybean fed animals, although intraspecies variation did not allow for statistical significance. WKY rats on the other hand expressed a trend for greater variation within a given level of cholesterol feeding in both the RBC-MDA assay at high peroxide level and in LDL peroxidation irrespective of dietary fat source.

Finally it is concluded that while dietary fat sources may have an impact on factors associated with CHD, such as hyperlipidemia, concurrent cholesterol intake must be taken into consideration to assess the potential for modification of tissue composition as occurred in liver lipids and RBC cholesterol and phospholipids. Hypertension was however not modulated by dietary lipid source in this study.
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