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Lipoprotein lipid and protein responses to

dietary fat and diabetes in rats

by

Bernhard Hennig

A Dissertation Submitted to the

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INTRODUCTION

Elevation of plasma-triglyceride and cholesterol levels appears to be related independently to atherosclerotic disease (Carlson and Böttiger, 1981). Both abnormalities can be found in diabetes, but it has become increasingly evident that hypertriglyceridemia is much more common than hypercholesterolemia in diabetic subjects with atherosclerosis (Nikkilä, 1973; Reckless et al., 1978).

Effects of dietary fat composition on blood lipid metabolism is still not fully understood. In general, a diet rich in polyunsaturated fatty acids appears to be associated with lower lipid levels, including tri-glyceride and cholesterol levels, than one containing larger quantities of saturated fatty acids and/or cholesterol (Goodnight et al., 1982). Since apoproteins have structural and regulatory functions in lipid metabolism, they cannot be overlooked when studying effects of dietary fat on plasma lipid levels.

The main objective of the present study was to compare dietary and insulin-deficiency types of hyperlipidemia by investigating plasma glu-cose, insulin and lipoprotein lipids and proteins in adult normal and streptozotocin-induced diabetic male breeder rats. It is hypothesized that excess intake of high-saturated fat diets may contribute to a metabolic pattern resembling that of a diabetic state.

Adult male breeder rats were used as an animal model, since they have been shown to have higher plasma-triglyceride and cholesterol levels than virgin male rats of the same age (Wexler, 1976). One might speculate that changes in lipid metabolism due to diet manipulations will be more

severe in breeder rats than virgin rats.

In order to ensure that consumption of a high-saturated fat diet is responsible for changes in blood lipid metabolism, which resemble those in a diabetic state, effects of a high-polyunsaturated fat diet or a low-fat purified diet upon blood lipid metabolism were also investigated. To accentuate effect of type of fat, some normal rats were switched directly from a high-saturated to a high-polyunsaturated fat diet. A reverse switch (high-polyunsaturated to high-saturated fat diets) was also done.

Streptozotocin-induced diabetic rats were used to compare animals fed the various experimental diets with animals known to be metabolically different through chemical induction of diabetes.

REVIEW OF LITERATURE

Introduction

The scientific literature covering areas of lipoprotein and apoprotein metabolism is enormous and many detailed review articles have been published (Alaupovic, 1980; Brewer, Jr., 1981; Eisenberg and Levy, 1975; Getz and Hay, 1979; Schaefer et al., 1978b). This review of literature will be restricted primarily to the major lipoprotein classes (very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL)) including the major apoproteins (A, B, C, E) associated with them. Human and rat plasma have been the major sources for investigation of lipoprotein and apoprotein metabolism, and most of the following discussion will rely on information using research from humans and rats. Species differences have to be considered when comparing results of different investigations. Although quantitative differences in some aspects of lipoprotein and apoprotein biosynthesis and catabolism exist between the two species (Havel, 1980), most of the metabolic processes are rather similar (Chapman, 1980; Eisenberg and Rachmilewitz, 1975). For example, human plasma contains much more lipoprotein of all classes than rat plasma; there is twice as much HDL, 20-25 times as much LDL and four times as much VLDL in human plasma as there is in that of the rat (Getz and Hay, 1979). Rat plasma, on the other hand, contains more apo-E particularly in HDL than does human plasma (Havel, 1980).

The following literature review will include studies on normal lipoprotein and apoprotein metabolism, defects of lipoprotein and apo-

protein metabolism during a diabetic state, dietary effects (amount and degree of saturation of dietary fat) on lipoprotein and apoprotein metabolism, and effects of diet and diabetes on plasma glucose and insulin levels.

Normal Lipoprotein Metabolism

Introduction

Transport of lipids in plasma, an aqueous medium, is accomplished by water-soluble macromolecular complexes called lipoproteins. The nomenclature for lipoproteins (Alaupovic et al., 1972) was based on their ultracentrifugal separation at characteristic densities determined by the relative proportions of lipid and protein in the particles (Lindgren, 1980). The lipoprotein classes are customarily designated as chylomicrons, VLDL, LDL and HDL. These classes, however, do not constitute absolute categories since within each class, there is heterogeneity with respect to size, lipid and protein composition (Getz and Hay, 1979). Basically, all lipoproteins share the same structural profile: they consist of a hydrophobic core, mainly triglyceride and cholesteryl ester, surrounded by an amphipathic surface layer of phospholipid, cholesterol and specific proteins called apoproteins (Brewer, Jr., 1981). Chylomicrons and VLDL are primarily composed of triglyceride; LDL is the major cholesterol carrying vehicle, and HDL molecules have the highest protein and phospholipid levels compared to all other lipoproteins.

Chylomicrons and very low density lipoprotein

Chylomicrons and VLDL share many structural features and are, therefore, discussed together. Chylomicrons and VLDL transport chiefly exogenous and endogenous triglyceride from the intestine and liver, respectively, for utilization or storage in peripheral tissues (Getz and Hay, 1979). Both of these processes are markedly influenced by changes in hormones, such as insulin and glucagon levels (Getz and Hay, 1979; Nikkilä, 1973). Chylomicrons are synthesized in the intestinal mucosa where they combine with apo-B and enter the blood stream via the thoracic duct. Instead of dietary fat, precursors for VLDL-triglyceride synthesis are dietary carbohydrates, circulating fatty acids and other short-carbon-chain fractions. The major site of VLDL synthesis is the liver, where the lipids are combined with apo-B and the VLDL then released into the bloodstream (Levy, 1981).

Triglyceride-rich particles, intestinal chylomicrons and hepatic as well as intestinal VLDL are hydrolyzed by lipoprotein lipase at capillary surfaces of peripheral tissues such as adipose and muscle cells (Nilsson-Ehle et al., 1980). One of the apoproteins, HDL-derived apo-CII, is needed for optimal activity of lipoprotein lipase (Nilsson-Ehle et al., 1980). After initial hydrolysis, further modification of the particles produced can also occur by a triglyceride lipase originating in the liver (Nilsson-Ehle et al., 1980). Progressive delipidation of VLDL and chylomicrons leads to the formation of lipoproteins of intermediate density (IDL) and chylomicron remnants, respectively. IDL finally is degraded to LDL (Eisenberg and Rachmilewitz, 1975).

To determine the effect of activation of lipoprotein lipase on the

metabolism of [124]I-labeled VLDL, Eisenberg and Rachmilewitz (1975) injected heparin into rats which were preinjected with ^{125}I-labeled VLDL. The investigators determined that there was only one single mechanism of formation of IDL and LDL in humans and rats either in vivo or in vitro. Eisenberg and Rachmilewitz (1975) also found that the lipid and apoprotein composition of IDL more closely resembled a "triglyceride-rich" LDL rather than a "triglyceride-poor" VLDL. Thus, the IDL represents an unstable lipoprotein form that is either removed from the circulation by the liver (primarily in rat (Steinberg, 1979)) or converted into LDL (primarily in human (Getz and Hay, 1979)). Bierman et al. (1973) reported that IDL was catabolized more efficiently than VLDL by arterial smooth muscle cells in tissue culture. In the rat (Chapman, 1980), triglyceride concentration was higher in VLDL and lower in LDL compared to IDL.

Low density lipoprotein

The catabolism of LDL occurs in peripheral cells (e.g., smooth muscle cells, fibroblasts, endothelial cells) and in the liver (Brewer, Jr., 1981). A high affinity receptor process in peripheral cells has been elucidated by Goldstein and Brown (1977). The binding of LDL to these receptors and subsequent internalization and degradation of LDL has a regulatory effect on cellular cholesterol metabolism. In addition to LDL (which contain primarily apo-B), certain HDL (which contain relatively large amounts of apo-E) have been shown to interact with receptors on peripheral cells and, thus, influence intracellular regulation of cholesterol metabolism (Mahley, 1981).

High density lipoprotein

HDL has been called a "polydisperse collection of lipoprotein particles" (Brewer, Jr., 1981), and functions and metabolic pathways of the HDL system are therefore very intricate. The heterogeneity of HDL has been demonstrated primarily by techniques including hydrated densities (HDL_1, HDL_2 and HDL_3) and apoprotein composition (Brewer, Jr., 1981; Getz and Hay, 1979). Nascent HDL particles are released by the liver and intestine into the plasma where they are rapidly transformed into spherical HDL particles by plasma components (Getz and Hay, 1979).

Intravascular hydrolysis of triglyceride-rich lipoproteins (chylomicrons and VLDL) from the intestine and liver results in the transfer of lipids and apoproteins (except apo-B) to HDL (Alaupovic, 1981; Getz and Hay, 1979). As a result, lipids such as cholesterol and phospholipids are transferred to HDL where the enzyme lecithin-cholesterol-acyl-transferase (LCAT) catalyzes the transfer of fatty acids found in the β-position of phospholipids to free cholesterol. The cholesteryl ester produced can be transferred back to LDL and VLDL. Also, net transfer of cholesteryl ester from human or rabbit LDL to VLDL and a net transfer of triglyceride from VLDL to LDL has been reported in vitro (Barter et al., 1980).

Animal studies indicate that intestine, liver and kidney are major sites of HDL removal (Getz and Hay, 1979). HDL has been postulated to transport cholesterol from various peripheral tissues to the liver for final elimination from the organism (Glomset, 1979). Mahley (1981) showed in the canine that high cholesterol feeding resulted in the production of a particle he called HDL_c from typical HDL which became en-

riched in cholesterol and apo-E. Mahley (1981) suggested then that the function of HDL_c would be to transport cholesterol from peripheral tissues to the liver for excretion, with hepatic recognition of HDL_c mediated by apo-E.

Normal Apoprotein Metabolism

Introduction

In addition to their role in maintaining lipoprotein structure, apo-proteins play important regulatory roles by serving as cofactors for enzymes in lipid metabolism. For example, apo-Al and apo-CII are neces-sary cofactors for LCAT and lipoprotein lipase, respectively (Nilsson-Ehle et al., 1980); apo-B and apo-E function as binding ligands for cellular receptors (Mahley, 1981). Recently discovered apoproteins such as apo-D and apo-H are thought to function as cholesterol ester exchange protein and cofactor for lipoprotein lipase, respectively (Brewer, Jr., 1981). Metabolism of apo-B, E, C, and A will be discussed.

Apo-B

Apo-B which is synthesized in both the liver and intestine enters the circulation with either chylomicrons or VLDL (Schaefer et al., 1978b). Apo-B appears to be necessary for formation and secretion of triglyceride-rich particles (Getz and Hay, 1979), because without apo-B, triglyceride is not transported out of the liver and into the blood stream (Levy, 1981). Sparks and Marsh (1981) recently isolated two apo-B components (MW 300,000D and 80,000D) and reported preferential binding and catabolism of triglyceride-rich lipoproteins enriched in apo-B with MW of 80,000D.

In contrast to other apoproteins, physical properties of apo-B are very similar to membrane proteins which are not readily soluble in aqueous solutions (Schaefer et al., 1978b).

There exists a direct precursor-product relationship between apo-B in VLDL and LDL, respectively (Eisenberg and Rachmilewitz, 1975; Getz and Hay, 1979). Brewer, Jr. (1981) discussed two types of interactions between apoproteins and plasma lipoproteins. In the "quasi-irreversible" apoprotein-lipoprotein interaction, the same apoprotein remains attached to the same lipoprotein particle during metabolism. Chylomicron-apo-B and liver VLDL-apo-B are examples of the "quasi-irreversible" mechanism. Thus, apo-B remains associated with the VLDL-IDL-LDL delipidation cascade. On the other hand, "reversible" apoprotein-lipoprotein interactions are exemplified by apoproteins A, C and E (Brewer, Jr., 1981). These apoproteins are able to exchange between lipoprotein particles and, therefore, cannot be used as markers for the metabolism of particular lipoproteins. The clearance of apo-B from the circulation is thus thought to occur primarily in the form of LDL particles (Schaefer et al., 1978b).

The catabolism of LDL which occurs in peripheral cells is initiated by apo-B affinity receptors (Goldstein and Brown, 1977). Mahley and Innerarity (1977) also showed that both apo-B of human LDL and apo-E of canine HDL_c can interact with the same receptor sites in human fibroblasts. Trace amounts of apo-B can be found in human HDL_2 (Getz and Hay, 1979). Swaney et al. (1977), however, did not find apo-B in rat HDL.

Apo-E

Apo-E is found in VLDL, LDL and HDL, and the percent distribution

is species dependent (Chapman, 1980; Schaefer et al., 1978b). Apo-E can

be synthesized in the liver and intestine (Schaefer et al., 1978b) and the

major catabolic site of apo-E appears to be the liver (Havel, 1980).

Apo-E apparently enters the circulation from the liver with nascant HDL

particles and is then transferred to VLDL during cholesterol ester forma-

tion (Schaefer et al., 1978b). Norum et al. (1975) reported that apo-E

was transferred from HDL to VLDL when the plasma from LCAT deficient

patients was incubated with an LCAT enzyme preparation. Generally, the

concentration of apo-E is considerably higher in rats than humans (Havel,

1980). Lipolytic breakdown of VLDL causes a net mass transfer of apo-E

from VLDL to HDL particles (Alaupovic, 1981). Eisenberg and Levy (1975) and

Schaefer et al. (1978b), however, reported that apo-E may contribute

significantly to the apoprotein content of intermediate lipoproteins.

Reports on a possible role of apo-E in the metabolism of triglyceride-

rich VLDL or IDL particles are contradictory. Apo-E has been shown to

strongly inhibit apo-CII activated lipoprotein lipase (Ekman and Nilsson-

Ehle, 1975; Ganesan et al., 1976). On the other hand, Yamada and Murase

(1980) reported that apo-E significantly activated lipoprotein lipase,

while activity of this enzyme was inhibited by apo-E antibody. Despite

Mahley's claim of HDL$_c$ (rich in apo-E) being "anti-atherogenic" (Mahley,

1981), no association has been found between levels of plasma apo-E and

angiographically documented coronary artery disease (Miller et al., 1981).

Apo-C

As with apo-E, newly synthesized triglyceride-rich liver and intestine lipoproteins are relatively devoid of apo-C, which is synthesized in the liver (Schaefer et al., 1978b). However, both apo-E and apo-C rapidly associate with newly synthesized lipoprotein particles in the circulatory system (Brewer, Jr., 1981). Apo-C can also exchange between VLDL and HDL and is, therefore, an example of "reversible" apoprotein-lipoprotein particle interactions (Brewer, Jr., 1981). Several investigators (Alaupovic, 1981; Eisenberg and Rachmilewitz, 1975) have reported that the content of apo-C in VLDL was related to the content of triglycerides in VLDL and that it was independent of all other lipoproteins, especially apo-B. The rate of release of apo-C-containing lipoprotein particles from VLDL to HDL (particularly HDL_2) during lipolysis has been suggested to reflect the state of triglyceride metabolism (Alaupovic, 1981). Berman et al. (1981) found that calculated rate constants for transfer of triglyceride between VLDL and HDL were similar compared to those for apo-C exchanges. Thus, apo-C could be a marker for triglyceride clearance during lipolysis of triglyceride-rich particles (Mao and Kottke, 1981). Apo-C metabolism has been suggested to be independent of apo-B and apo-A since apo-C is found in the plasma of patients with either abetalipo-proteinemia (absence of circulating apo-B) (Gotto, Jr. et al., 1971) or Tangier disease (apo-A and HDL deficiency) (Assman et al., 1977).

The C apoproteins, CI, CII and CIII, have similar molecular weights but different functions related to lipid metabolism. Apo-CI has been reported to activate LCAT (esterification of plasma cholesterol) (Jackson et al., 1976) and both to inhibit (Ekman and Nilsson-Ehle, 1975) and

to activate lipoprotein lipase (Ganesan et al., 1975). Apo-CII, which
is provided primarily by HDL, is essential for intravascular lipolysis
as an activator of lipoprotein lipase (Nilsson-Ehle et al., 1980).
Apo-CIII has been shown to inhibit the activation of lipoprotein lipase
(Ganesan et al., 1976; Saudek and Eder, 1979). The inhibitory effect of
apo-CIII, however, could be reversed by increases in apo-CII or tri-
glyceride concentrations (Ganesan et al., 1976).

Apo-A

In man, apo-AI and apo-AII are the major apoproteins of HDL (Schaefer
et al., 1978b). Apo-AII, however, is only a minor component of rat HDL
(Swaney et al., 1977). Trace amounts of apo-A can also be detected in
other lipoprotein fractions (Curry et al., 1976). Rat VLDL and HDL also
contain apo-AIV (Swaney et al., 1977) which recently has been discovered
in human plasma (Beisiegel and Utermann, 1979). Apo-A is largely derived
from the intestine (Zilversmit, 1979) but also from the liver (Schaefer
et al., 1978b). Catabolic sites of apo-A are liver and kidney lysosomes
(Schaefer et al., 1978b). Some reports indicate that apo-A, like apo-B,
E and C, can move freely from one lipoprotein to another (Brewer, Jr.,
1981; Zilversmit, 1979). Alaupovic (1981), however, reported that apo-C
and apo-E but not apo-A and apo-B could exchange between VLDL and HDL.
Apo-A apoproteins are thought not to be involved in the transport of
lipids out into the plasma but rather of lipid clearance from the plasma
(Levy, 1981).

Apo-AI is the major activator of LCAT, and plasma levels of apo-AI
and apo-AII are low in patients with LCAT deficiency (Getz and Hay, 1979).

In the rat, chylomicron- and VLDL-cholesteryl esters are primarily synthesized in cells producing these lipoproteins but almost all of the HDL- and also LDL-cholesteryl ester is probably derived from the action of LCAT upon HDL (Havel, 1980). Both apo-AI and apo-AII also have been shown to inhibit the activation of human adipose tissue lipoprotein lipase (Ekman and Nilsson-Ehle, 1975).

Recently, much interest has been focused upon apo-AI, in addition to HDL-cholesterol levels, in relation to coronary artery disease and its risk factors. Fager et al. (1980) found lower levels of apo-AI in survivors of myocardial infarction compared to a control group. Maciejko et al. (1982) reported that, out of 84 male patients with severe coronary artery disease (diagnosed by coronary angiography), 34 had normal levels of HDL cholesterol while all had abnormally low levels of apo-AI. Maciejko et al. (1982) then suggested that apo-AI levels may be a better indicator of possible coronary artery disease than HDL-cholesterol levels. On the other hand, Miller et al. (1981) found no association between angiographically defined coronary artery disease and apo-AI levels, but HDL_2 levels were lower in patients with the disease than in the control group.

Defects of Lipoprotein and Apoprotein Metabolism during Diabetes

Introduction

Elevations of plasma triglyceride and cholesterol levels appear to be related independently to atherosclerotic vascular disease (Carlson and Böttiger, 1981). Both abnormalities, however, can be found in

diabetes, but it has become increasingly evident that hypertriglyceridemia is much more common than hypercholesterolemia in diabetic subjects with atherosclerosis (Nikkilä, 1973; Prager et al., 1980; Reckless et al., 1978). Some disagreement exists in the literature about alterations in individual lipoprotein and apoprotein patterns in diabetes. Interpretations can be difficult because of the many factors in diabetes that influence blood lipid levels, including insulin levels, obesity, age, sex, the type of diabetes and the genetic heritage of the diabetic patients under study (Saudek and Eder, 1979). One of the characteristics of untreated or poorly controlled juvenile-type diabetes (type I or insulin-dependent diabetes), as well as experimental diabetes induced, for example, by streptozotocin, is insulin deficiency. Hyperinsulinism, on the other hand, is a characteristic feature of many obese subjects with adult-onset diabetes (or type II or noninsulin-dependent diabetes) (Saudek and Eder, 1979). In general, the lack of insulin in insulin-dependent diabetes and the obesity and hyperinsulinemia in noninsulin-dependent diabetes could each affect factors regulating hepatic input of lipoproteins into plasma (such as hepatic lipogenesis, triglyceride and protein synthesis) and factors contributing to the catabolism of lipoproteins (such as lipoprotein lipase activity) (Nikkilä, 1973).

Lipoprotein-triglycerides

Diabetes is usually (Nikkilä, 1973) but not always (Taylor et al., 1981) accompanied by hypertriglyceridemia. Theoretically, the rise in plasma triglyceride levels could result from increased lipoprotein production or from decreased removal of circulating lipoproteins.

Enhanced lipolysis in adipose tissue has been suggested to contribute to hypertriglyceridemia by providing increased amounts of precursors (fatty acids) for triglyceride synthesis in the liver (Mancini et al., 1980). Plasma- and VLDL-triglyceride levels decreased in both obese nondiabetic and obese noninsulin-dependent diabetic Pima Indians after several months of consumption of a 500 calorie diet (Howard et al., 1979). Many noninsulin-dependent diabetic patients are obese, and Olefsky et al. (1974) proposed that hypertriglyceridemia may be the consequence of insulin resistance, which in turn leads sequentially to hyperinsulinemia, increased VLDL production and, hence, elevated triglyceride levels. Brunzell et al. (1979) have found no evidence for an increase in triglyceride production in untreated diabetes. In addition, perfused livers of diabetic rats secreted less VLDL-triglyceride and incorporated less amino acid into secreted VLDL-protein (Getz and Hay, 1979). Bar-On et al. (1976a), on the other hand, reported a fivefold increase in VLDL-protein in diabetic compared to control rats. Thus, in addition to increased synthesis, impaired catabolism or removal of lipoproteins could lead to diabetic hyperlipidemia.

A defect in lipoprotein removal has been reported in streptozotocin-induced diabetic rats (Chen et al., 1979; Reaven and Reaven, 1974; van Tol, 1977) and untreated diabetic humans (Brunzell et al., 1979). Impaired extrahepatic lipoprotein lipase (Brunzell et al., 1979; Getz and Hay, 1979; Pykälistö et al., 1975) and not hepatic triglyceride lipase (Nikkilä et al., 1977) may be, in part, responsible for the diabetic hyperlipidemia. Tritiated VLDL-palmitate was removed more slowly from the circulation of diabetic compared to normal rats (van Tol, 1977). Insulin therapy

increased lipoprotein lipase activity (Taskinen and Nikkilä, 1979) and

resulted in rapid decreases in chylomicrons and VLDL in patients with

diabetic ketoacidosis (Weidman et al., 1982).

Schonfeld et al. (1974) and Mancini et al. (1980) reported the ac-

cumulation of triglyceride-rich LDL in diabetic patients. Elevated LDL-

triglyceride and HDL-triglyceride levels were found in insulin-dependent

diabetic patients with electrocardiographic evidence of myocardial in-

farction (Aro et al., 1981). The elevated triglyceride levels in the

LDL fraction (d = 1.006 to 1.063 g/ml) have been related to a relative

increase in IDL and accumulation of remnant particles of similar density

(Mancini et al., 1980).

Lipoprotein-cholesterol

Reports about lipoprotein cholesterol levels in diabetes have not

been consistent. Elevation in total plasma cholesterol has been found in

streptozotocin-induced diabetic rats (Deutsch et al., 1980) and both in

insulin-dependent (Taskinen and Nikkilä, 1979; Mattock et al., 1979) and

noninsulin-dependent diabetic humans (Mattock et al., 1979). On the

other hand, Alaupovic et al. (1982) and Aro et al. (1981) reported no

change in total cholesterol in noninsulin-dependent diabetic patients

compared to control groups. Several investigators have reported increased

levels of VLDL-cholesterol in insulin-dependent (Lopes-Virella et al.,

1981; Taskinen and Nikkilä, 1979) and noninsulin-dependent diabetic

humans (Alaupovic et al., 1982; Aro et al., 1981).

Elevated levels of LDL-cholesterol have been shown both in insulin-

dependent (Loves-Virella et al., 1981; Reckless et al., 1978) and

noninsulin-dependent (Taskinen and Nikkilä, 1979) diabetic patients but

vascular disease was only associated with insulin-dependent (Reckless

et al., 1978) but not noninsulin-dependent diabetic patients (Aro et al.,

1981; Reckless et al., 1978). No changes in LDL-cholesterol levels in

noninsulin-dependent diabetic patients were found by Alaupovic et al.

(1982) and Aro et al. (1981). A decrease in LDL cholesterol levels has

been reported in the diabetic rat (van Tol, 1977). Increase in cholesterol

synthesis or decrease in removal could lead to increased cholesterol levels

in both VLDL and LDL. Nakayama and Nakagawa (1977) and Saudek and Eder

(1979) found increased HMG-CoA reductase activity in the intestine of

rats with streptozotocin-induced diabetes. Impaired lipoprotein lipase

activity in diabetic populations has been mentioned earlier.

In general, both insulin-dependent (Lopes-Virella et al., 1981;

Taskinen and Nikkilä, 1979) and noninsulin-dependent diabetic subjects

(Aro et al., 1981; Mattock et al., 1979; Taylor et al., 1981) have de-

creased HDL-cholesterol levels. Bar-On et al. (1976b), however, found un-

changed cholesterol content in HDL of streptozotocin-induced diabetic

rats, and Mattock et al. (1979) reported elevated HDL-cholesterol levels

in insulin-dependent patients. Insulin therapy tended to increase HDL-

cholesterol levels in diabetic patients (Prager et al., 1980; Weidman

et al., 1982), but Taskinen and Nikkilä (1979) did not find an increase

in HDL cholesterol after treating insulin-dependent patients for two weeks.

Most literature results, therefore, suggest an increase in VLDL- and LDL-

and a decrease in HDL cholesterol levels in diabetic compared to normal

populations. Thus, a mild increase in total cholesterol in diabetes could

be secondary to an increase in VLDL- and/or LDL-cholesterol (Saudek and
Eder, 1979).

Lipoprotein-protein

An elevation of total protein in lipoprotein fractions of diabetic
rats has been reported by Bar-On et al. (1976b). On the other hand, in-
corporation of radioactive amino acids into the major lipoproteins was
depressed in the perfused liver of alloxan-induced diabetic rats (Getz
and Hay, 1979). In general, individual apoproteins appear to be affected
by a diabetic state (Saudek and Eder, 1979), but findings reported in
the literature are rather inconsistent.

Apo-B

Plasma-apo-B levels were higher in both insulin-dependent (Alaupovic,
1980; Schonfeld et al., 1974) and noninsulin-dependent diabetic (Schonfeld
et al., 1974) compared to control subjects. Bar-On et al. (1976b) found
an increase in plasma apo-B by 86% in streptozotocin-induced diabetic com-
pared to control rats. However, Alaupovic et al. (1982) reported normal levels
in noninsulin-dependent diabetic subjects. Similar results were obtained
by Prager et al. (1980) who investigated apo-B levels both in insulin-
dependent and noninsulin-dependent patients. Apo-B levels tended to be
higher in children with diabetes but were statistically not different
from those of healthy controls (Tuvemo et al., 1981). Plasma-apo-B
levels decreased with insulin treatment due to a decrease in VLDL-apo-B
but not LDL- or IDL-apo-B levels (Weidman et al., 1982).

Apo-E

Increased plasma-apo-E levels were found in insulin-dependent
(Alaupovic, 1980), but noninsulin-dependent diabetic patients had normal
apo-E levels (Alaupovic et al., 1982). A significant decrease in apo-E
levels was observed in the diabetic rat (Bar-On et al., 1976b) due to
almost absence of apo-E in HDL of diabetic compared to control rats.
Deutsch et al. (1980) also found a major decrease in HDL-apo-E levels in
diabetic rats.

Apo-C

An increase in plasma-apo-C levels has been observed in diabetic
rats (Bar-On et al., 1976b) and insulin-dependent diabetic patients
(Alaupovic, 1980). The apo-C changes (diabetic vs. control) in the rat
(Bar-On et al., 1976b) appeared to be similar in VLDL and HDL. Alaupovic
(1980) also reported an increase in apo-CIII in noninsulin-dependent
diabetic patients compared to a control population.

Apo-A

Prager et al. (1980) found decreased plasma-apo-AI and apo-AII
levels in both insulin-dependent and noninsulin-dependent diabetic
patients compared to a control population. Similar results have been
reported by Alaupovic et al. (1982). On the other hand, Tuvemo et al.
(1981) reported significantly higher levels of apo-AI in diabetic com-
pared to normal children. Apo-AII levels also tended to be higher in
the diabetic children. Plasma apo-AI levels were not lower in noninsulin-
dependent diabetic patients with normal plasma triglyceride levels com-
pared to normal subjects (Taylor et al., 1981). Bar-On et al. (1976b)

found apo-AIV in diabetic rat VLDL. Apo-AIV which is normally present
in rat HDL could not be detected in HDL of diabetic rats (Bar-On et al.,
1976b). In human diabetic ketoacidosis (Weidman et al., 1982), apo-AI
levels were normal, decreased upon insulin treatment initially and
reached basal levels again 24 hours after treatment.

The various effects of the several forms of diabetes in rats and
humans upon lipoproteins are summarized in Table 1.

Dietary Fat Effects on Lipoprotein and Apoprotein Metabolism

Introduction

In the United States, about 42% of the energy in the diet comes from
fat (Rizek, 1981). About 15% of dietary energy comes from saturated
fatty acids and about 6% from polyunsaturated fatty acids (Hershcopf
et al., 1982). Since diets high in saturated fat and/or cholesterol
have been shown to be atherogenic (Kris-Etherton and Cooper, 1980), much
research has been focused on how the extent of changes of fatty acid
composition of dietary fats might affect plasma lipid levels. In
general, a diet rich in polyunsaturated fatty acids appears to be as-
sociated with lower lipid levels, including triglyceride and cholesterol
levels, than one containing larger quantities of saturated fatty acids
and/or cholesterol (Goodnight et al., 1982).

Lipoprotein-triglycerides

Stimulation of triglyceride output by the perfused liver has been
shown to increase with fatty acid chain length, at least up to 18 carbon
atoms (Kohout et al., 1971). Plasma triglyceride levels were higher

Table 1. Summary of recent reports of defects of apoprotein metabolism during diabetes

Apoprotein	Rat (streptozotocin-induced diabetes)	Reference
apo-B	↑(plasma)	Bar-On et al., 1976b
apo-E	↓ (plasma)	Bar-On et al., 1976b
	↓ (HDL)	Bar-On et al., 1976b
	↓ (HDL)	Deutsch et al., 1980
apo-C	↑ (plasma)	Bar-On et al., 1976b
apo-A		

[a]NS = not statistically significant.

Human	Insulin-dependent (ID) Noninsulin-dependent (NID)	Reference
↑(plasma)	ID	Alaupovic, 1980
		Schonfeld et al., 1974
NS[a] (plasma)	NID	Alaupovic, et al., 1982
NS (plasma)	NID	Prager et al., 1980
NS (plasma)	ID	Prager et al., 1980
↑(plasma)	NID	Schonfeld et al., 1974
NS (plasma)	ID	Tuvemo et al., 1981
↑(plasma)	ID	Alaupovic, 1980
NS (plasma)	NID	Alaupovic et al., 1982
↑(plasma)	ID	Alaupovic, 1980
↓(plasma)	ID	Alaupovic et al., 1982
↓(plasma)	NID	Alaupovic et al., 1982
↓(plasma)	ID	Prager et al., 1980
↓(plasma)	NID	Prager et al., 1980
↑(plasma)	ID	Tuvemo et al., 1981

in rats fed a diet high in beef tallow compared to rats fed a high-corn oil diet (Koh and Chi, 1981). A marked increase in VLDL-triglyceride was observed in human subjects who consumed high-coconut oil compared to high-corn oil diets (Fisher et al., 1981). On the other hand, plasma-triglyceride levels in rhesus monkeys (Ershow et al., 1981) were not altered by either high-coconut oil or corn oil diets (31% of energy each). Turner et al. (1981) also found no change in plasma-triglyceride levels in humans fed diets with P/S ratios of 8.0 or 0.2.

Polyunsaturated fat feeding caused a decrease in plasma-triglyceride levels in humans (Shepherd et al., 1978, 1980) and in both normal and diabetic monkeys (Howard, Jr., 1979). Chait et al. (1974) attributed a decrease in plasma-triglyceride levels during polyunsaturated fat feeding primarily to a decrease in VLDL-triglyceride levels. In chronically insulin deficient rats, however, hypertriglyceridemia was accentuated by consumption of a high-fat diet (30% of energy from corn oil) compared to a high-carbohydrate diet (Reaven and Reaven, 1974). High-carbohydrate diets, at least on a short-term basis, have been shown to induce elevated levels of VLDL-triglyceride (Brussaard et al., 1980; Falko et al., 1980; Saudek and Eder, 1979).

Feeding a diet high in coconut oil rather than corn oil to humans resulted in an increase in LDL-triglyceride levels (Fisher et al., 1981). Similar diets were fed to monkeys (Ershow et al., 1981), but no changes in LDL-triglyceride levels were observed. In another study, however, high polyunsaturated fat (P/S ratio of 2.4) compared to high saturated fat (P/S ratio of 0.2) diets had no significant effect on LDL-triglyceride levels (Chait et al., 1974). Brussaard et al. (1980)

reported that a diet low in fat (fat = 20% of energy) was more effective in increasing LDL-triglyceride levels in humans than high-saturated or high-polyunsaturated fat diets (40% of energy each).

There were no significant changes in HDL-triglyceride levels when feeding high-saturated or high-polyunsaturated fat diets (31% of energy each) either to humans (Fisher et al., 1981) or monkeys (Ershow et al., 1981). However, the addition of cholesterol to a high-saturated fat diet resulted in a significant elevation of HDL-triglyceride levels compared to a diet high in polyunsaturated fatty acids and cholesterol (Ershow et al., 1981). Dietary fat effects upon lipoprotein-triglycerides in various species are summarized in Table 2.

Lipoprotein-cholesterol

When comparing with a high-corn oil diet, feeding a diet high in coconut oil resulted in an increase in whole plasma- and VLDL-cholesterol levels in humans (Fisher et al., 1981). Similar results were observed in rats which were fed high-beef tallow and high-corn oil diets (Koh and Chi, 1981). Plasma-cholesterol levels were only slightly higher in diabetic compared to normal monkeys regardless of diet, and in both groups, cholesterol levels were lowest when a low-fat diet was fed but highest when a high-saturated fat diet (P/S ratio of 0.53) was fed (Howard, Jr., 1979). A high-polyunsaturated fat diet (P/S ratio of 2.4) compared to a high-saturated fat diet (P/S ratio of 0.2) significantly decreased total plasma-cholesterol levels which was largely due to a decrease in VLDL- and LDL-cholesterol in a study by Chait et al. (1974). Similar results were observed by Shepherd et al. (1978). Turner et al.

Table 2. Summary of recent reports of dietary fat effects upon lipoprotein-triglycerides

Plasma or lipoprotein	Saturated fat vs. polyunsaturated fat		Dietary cholesterol (if reported)	Reference
Plasma	NS[a]	(monkey)	None	Ershow et al., 1981
	↑	(normal and diabetic monkeys	< 0.01%	Howard, Jr., 1979
	↑	(rat)	—	Koh and Chi, 1981
	↑	(human)	400 mg/day	Shepherd et al., 1978, 1980
	NS	(human)	< 150 mg/day	Turner et al., 1981
VLDL	↑	(human)	—	Chait et al., 1974
	↑	(human)	None	Fisher et al., 1981
LDL	NS	(human)	—	Chait et al., 1974
	NS	(monkey)	None	Ershow et al., 1981
	↑	(human)	None	Fisher et al., 1981
HDL	NS	(monkey)	None	Ershow et al., 1981
	↑	(monkey)	300 mg/1000 kcal diet	Ershow et al., 1981
	NS	(human)	None	Fisher et al., 1981

[a]NS = not statistically significant.

(1981), however, reported no significant impact of polyunsaturated fat feeding (P/S ratio of 8.0 vs. 0.2) on VLDL-cholesterol levels in humans. Cholesterol levels were higher in VLDL when a low-fat diet rather than diets high in saturated or polyunsaturated fatty acids were fed to healthy humans (Brussaard et al., 1980). All diets contained about the same amount of cholesterol.

High-saturated fat compared to high-polyunsaturated fat feeding resulted in a marked increase in LDL-cholesterol levels both in humans (Fisher et al., 1981; Illingworth et al., 1981) and rats (Koh and Chi, 1981). The diets in the human studies were cholesterol-free. Illingworth et al. (1981) reported that compared to a high-saturated fat diet, a monounsaturated fat diet lowered LDL-cholesterol less than a polyunsaturated fat diet. After diets with P/S ratios of 0.25, 1, or 3 were fed to healthy humans for 3 to 4 weeks, LDL ratios of cholesteryl ester to free cholesterol were lowest at P/S ratio of 3 and highest at P/S ratio of 0.25 (Shore et al., 1981). Ershow et al. (1981) reported a significant rise in the cholesteryl ester fraction of LDL after feeding monkeys a diet high in coconut oil and cholesterol. Both Chait et al. and Shepherd et al. (1978, 1980) reported that feeding high-polyunsaturated compared to high-saturated fat diets to humans resulted in a significant decrease in LDL-cholesterol. As with the VLDL data, cholesterol was higher in LDL when a low-fat diet rather than diets high in saturated or polyunsaturated fatty acids were fed to humans (Brussaard et al., 1980).

Degree of saturation of high-fat diets (diets without cholesterol) had no effect on HDL-cholesterol levels either in humans (Fisher et al.,

1981; Illingworth et al., 1981) or rats (Koh and Chi, 1981). Shepherd et al. (1978, 1980) observed a decrease in human HDL-cholesterol levels by feeding a high-polyunsaturated fat diet (P/S ratio of 4.0) compared to a high-saturated fat diet (P/S ratio of 0.25). Shepherd's diets, however, contained 400 mg cholesterol per day. Dietary fat effects upon lipoprotein-cholesterol in various species are summarized in Table 3.

Fat vs. cholesterol in diet

Ershow et al. (1981) reported that neither saturated fat (coconut oil) nor cholesterol alone substantially altered plasma-cholesterol levels. Together, however, these dietary components interacted synergistically to produce a significant elevation in plasma-cholesterol. Anderson et al. (1976) compared addition of cholesterol to cholesterol-free diets in young men fed saturated or polyunsaturated fat as 40% of energy. The polyunsaturated fat resulted in about 30% reduction in serum-cholesterol compared to saturated fat while addition of 300 mg/day of dietary cholesterol caused a 5% increase compared to cholesterol-free diet regardless of fat type. Oh et al. (1982) also observed that degree of dietary fat saturation had greater effects on plasma-cholesterol levels than dietary cholesterol.

Lipoprotein-protein

Apo-B   Since apoproteins have structural and regulatory functions in lipid metabolism, they cannot be overlooked when studying effects of dietary fat on plasma-lipid levels. Cholesterol-free diets, which were either high in saturated (coconut oil, 14% per weight) or poly-unsaturated fat (safflower oil, 15% per weight), had no significant

Table 3. Summary of recent reports of dietary fat effects upon lipoprotein-cholesterol

Plasma or lipoprotein	Saturated fat vs. polyunsaturated fat	Dietary cholesterol (if reported)	Reference
Plasma	↑ (human)	None or 300 mg/day	Anderson et al., 1976
	↑ (human)	—	Chait et al., 1974
	NS[a] (monkey)	None	Ershow et al., 1981
	↑ (monkey)	300 mg/kcal diet	Ershow et al., 1981
	↑ (human)	None	Fisher et al., 1981
	NS (normal and diabetic monkeys)	< 0.01%	Howard, Jr., 1979
	↑ (rat)	—	Koh and Chi, 1981
	↑ (human	1000 mg or 300 mg/day	Oh et al., 1982
	↑ (human)	400 mg/day	Shepherd et al., 1978
VLDL	↑ (human)	None	Fisher et al., 1981
	↑ (rat)	—	Koh and Chi, 1981
	↑ (human)	400 mg/day	Shepherd et al., 1978
	NS (human)	< 150 mg/day	Turner et al., 1981
LDL	↑ (human)	—	Chait et al., 1974
	↑ (human)	None	Fisher et al., 1981 ·
	↑ (human)	None	Illingworth et al., 1981
	↑ (rat)	—	Koh and Chi, 1981
	↑ (human)	40 mg/day	Shepherd et al., 1978
HDL	NS (human)	None	Fisher et al., 1981
	NS (rat)	—	Koh and Chi, 1981
	NS (human)	None	Illingworth et al., 1981
	↑ (human)	400 mg/day	Shepherd et al., 1978, 1980

[a]NS = not statistically significant.

effects on apo-B levels in VLDL, LDL or HDL of Mongolian gerbils (Nicolosi et al., 1981). However, feeding the high safflower oil diet tended to increase apo-B levels in VLDL and HDL. Similar results in VLDL were obtained by Chait et al. (1974) in humans. On the other hand, feeding polyunsaturated compared to saturated fat diets with (Shepherd et al., 1980; Shore et al., 1981) and without cholesterol (Illingworth et al., 1981) resulted in a decrease in total apo-B and LDL-apo-B levels (Shepherd et al., 1980). Carbohydrate-induced hypertriglyceridemia was associated with elevations of VLDL-apo-B levels (Brussaard et al., 1980; Getz and Hay, 1979) but Brown (1980) found no changes in VLDL-apo-B production in humans consuming a high-carbohydrate diet.

Apo-E   The accumulation of apo-E in hypercholesterolemic rats fed a high-saturated fat diet (butter fat and cholesterol) compared to rats fed a Purina Chow diet (Wong and Rubinstein, 1979) has been attributed to an increase in apo-E secretion rather than a decrease in catabolism. In the Mongolian gerbil (Nicolosi et al., 1981), a high-coconut oil diet tended to increase VLDL-apo-E but decrease LDL-apo-E levels compared to a high-safflower oil diet. Both diets were cholesterol-free. Compared to a high-corn oil diet, plasma-apo-E levels were elevated in rhesus monkeys fed a cholesterol-free diet high in coconut oil (Zannis et al., 1981). Fisher et al. (1981) reported an increase in apo-E levels primarily in human VLDL but also in LDL but not in HDL when a high-coconut oil rather than a high-corn oil diet (31% of energy each) was consumed over a period of 18 days. The intake of a high-carbohydrate, liquid-formula diet (85% carbohydrate and 15% protein) resulted in an elevation of VLDL-apo-E but not plasma-apo-E levels (Falko et al., 1980).

Apo-C   Apo-C, or low molecular weight apoproteins, were significantly higher in HDL of Mongolian gerbils fed the high-saturated compared to the high-polyunsaturated fat diet (Nicolosi et al., 1981). Diet, however, had no effect on apo-C levels in VLDL and LDL (Nicolosi et al., 1981). Isocaloric substitution of dietary saturated, monounsaturated and polyunsaturated fatty acids had no effect on human plasma-apo-CIII levels (Illingworth et al., 1981). Compared to high-saturated fat diets, high-polyunsaturated fat diets tended to decrease levels of VLDL-apo-C (Chait et al., 1974) and HDL-apo-CIII (Illingworth and Connor, 1980). Among C apoproteins, apo-CII levels were higher in carbohydrate-induced VLDL than in control VLDL (Schonfeld et al., 1976).

Apo-A   Human plasma-apo-AI levels were not affected by feeding diets high in saturated, monounsaturated or polyunsaturated fatty acids (Illingworth et al., 1981). There was significantly less apo-AI in HDL of gerbils fed coconut oil (cholesterol-free) compared to safflower oil (cholesterol-free) containing diets (Nicolosi et al., 1981). On the other hand, Shepherd et al. (1978) reported a decrease in plasma-apo-AI levels in humans when polyunsaturated fat rather than saturated fat containing diets were consumed. In the latter study, cholesterol was present in both types of diets. Dietary fat effects upon apoproteins in various species are summarized in Table 4.

Lipoproteins and Disease

Triglyceride and cholesterol levels in the plasma appear to be related independently to atherosclerotic vascular disease. Epidemiological

Table 4. Summary of recent reports of dietary fat effects on apoproteins

Apoprotein	Saturated fat vs. polyunsaturated fat	Species	Dietary cholesterol (if reported)	Reference
Apo-B	↑ (plasma)	Human	None	Illingworth et al., 1981
	NS[a] (VLDL, LDL, HDL)	Gerbil	None	Nicolosi et al., 1981
	↑ (plasma, LDL)	Human	400 mg/day	Shepherd et al., 1980
	↑ (plasma)	Human	300 mg/day	Shore et al., 1981
Apo-E	↑ (VLDL, LDL)	Human	None	Fisher et al., 1981
	NS (HDL)	Human	None	Fisher et al., 1981
	NS (VLDL, LDL)	Gerbil	None	Nicolosi et al., 1981
	↑ (plasma)	Monkey	None	Zannis et al., 1981
Apo-C	NS (plasma-apo-CIII)	Human	None	Illingworth et al., 1981
	NS (VLDL, LDL)	Gerbil	None	Nicolosi et al., 1981
	↑ (HDL)	Gerbil	None	Nicolosi et al., 1981
Apo-A	NS (plasma-apo-AI)	Human	None	Illingworth et al., 1981
	↓ (HDL-apo-AI)	Gerbil	None	Nicolosi et al., 1981
	↑ (plasma-apo-AI)	Human	400 mg/day	Shepherd et al., 1978

[a]NS = not statistically significant.

studies, such as the Stockholm Prospective Study (Carlson and Böttiger, 1981) showed that elevated plasma-triglyceride levels were a risk factor for ischemic heart disease.

The catabolism of LDL, which is usually the major cholesterol carrying vehicle in the plasma, occurs in peripheral cells and in the liver (Brewer, Jr., 1981). Excess deposition of cholesterol in and around cells of the arterial wall can lead to atherosclerotic lesions. Thus, populations with high LDL levels are at greater risk of developing coronary heart disease (Getz and Hay, 1979).

On the other hand, results of epidemiological studies (Heiss et al., 1980) have suggested a protective role of HDL, which is mediated by a reverse transport of cholesterol from cells to HDL and ultimately to the liver for final excretion. Brewer, Jr. (1981) suggested that the protective effect of HDL could be primary via direct mediation by a component within HDL, or secondary via the effectiveness of lipolysis of triglyceride-rich lipoproteins of intestinal and liver origin. Calculations, however, from a computerized mathematical model (Verdery, 1981), which included fibroblasts, HDL and LCAT, did not prove or disprove that cholesterol loss from peripheral tissue proceeds as described by Glomset (1979).

Effects of Diabetes and Diet on Plasma Glucose and Insulin Levels

Plasma glucose tends to be abnormally elevated in all types of diabetes. In insulin-dependent diabetes (untreated juvenile-type diabetes and experimental diabetes), plasma insulin levels are subnormal

or deficient, and in noninsulin-dependent diabetes (adult-onset type diabetes), insulin levels are usually increased above normal (Nikkilä, 1973). In insulin-dependent diabetic patients, insulin therapy generally results in normalization of the plasma lipid profile (Weidman et al., 1982).

In monkeys (Howard, Jr., 1979), amount and degree of saturation of fat in the diet had no significant effect on fasting plasma glucose levels. Nevertheless, plasma glucose levels tended to be highest in animals fed high-saturated fat (coconut oil, P/S ratio of 0.53), intermediate in those fed low-fat and lowest in those fed polyunsaturated fat diets (safflower, P/S ratio of 1.18). Similar results were obtained with plasma insulin levels, which tended to be highest in low-fat-fed, intermediate in saturated-fat-fed and lowest in polyunsaturated-fat-fed monkeys (cholesterol concentration in all diets was less than 0.01%). In humans (Chait et al., 1974), polyunsaturated fat feeding (P/S ratio of 2.4) tended to decrease plasma insulin levels compared to a diet high in saturated fat (P/S ratio of 0.2).

MATERIALS AND METHODS

Experimental Plan

The main objective of this study was to make comparisons of dietary and insulin-deficiency types of hyperlipidemia by investigating plasma glucose, insulin and lipoprotein lipids and proteins in adult normal and streptozotocin-induced diabetic male breeder rats.   It is hypothesized that excess intake of a diet too high in saturated fat or too low in polyunsaturated fat may contribute to a metabolic pattern resembling that of a diabetic state.

Animal selection

The normal adult virgin rat is not very prone to hyperlipidemia probably because of its relatively low plasma-VLDL and LDL levels. Male breeder rats, which will develop advanced metabolic and degenerative changes as early as six to seven months of age, have been shown to have higher plasma-triglyceride and cholesterol levels than virgin male rats of the same age (Wexler, 1976). One might speculate that changes in lipid metabolism due to dietary fat will be more severe in breeder than virgin rats. Male breeder rats were, therefore, thought to be a proper model to study comparisons of dietary and insulin-deficiency types of hyperlipidemia. Six- to eight-month-old Wistar/Sprague-Dawley derived male breeder rats and streptozotocin-induced diabetic male breeder rats from the same stock colony were used as animal models of hyper-lipidemia. Male rats were used for mating every seven weeks starting at 10 weeks of age. Males were left with females for 10 days during each breeding period.

Experimental Protocol

The hypothesis to be tested was that excess intake of high-saturated fat diets may contribute to a metabolic pattern resembling that of a diabetic state. Insulin-dependent but also noninsulin-dependent diabetic subjects are often hyperlipidemic with defects primarily in triglyceride metabolism. If the hypothesis mentioned above is true, one would expect changes in blood lipid metabolism in subjects fed high-saturated fat diets that are similar to changes found in a diabetic state.

Changes from low-fat stock to high-fat diets

Prior to the study, all rats consumed a low-fat stock ration (Reeves and Arnrich, 1974). In order to ensure that consumption of a high-saturated fat diet is responsible for changes in blood lipid metabolism which resemble those in a diabetic state, one also has to investigate how a high-polyunsaturated fat diet or a low-fat purified diet might affect blood lipid metabolism. Thus, adult breeder rats were fed either a low-fat diet or diets high in beef tallow or high in corn oil for two months in order to assess a direct effect of amount and degree of saturation of dietary fat on blood lipid metabolism.

Switch of high-fat diets

To accentuate effect of type of fat, another group of rats was fed first a high-saturated fat diet for two months and then switched to a high-polyunsaturated fat diet for another two months. The reverse switch (first polyunsaturated fat then saturated fat) was done with an additional group of rats.

Total amount of fat effects

One also must consider the time factor of a feeding study. Does intake of high-fat diets, independent of P/S ratio, over four months have different effects on blood lipid metabolism than a two-month feeding study, compared to low-fat feeding?

Insulin-deficiency type of hyperlipidemia

The streptozotocin-induced diabetic rats were considered a control group. The diabetic rats were necessary to compare animals fed the various experimental diets with animals known to be metabolically different through chemical induction of diabetes.

Procedure

Animals

Animals were housed individually in meshwire cages at $24 \pm 1^{\circ}C$ with 12-hour light (0600 to 1800) and 12-hour dark (1800 to 0600) cycles. All rats had free access to water and were fed diets ad libitum. Cages were rotated within the animal room once a week.

Experiment 1

Twelve rats were made diabetic by injection of streptozotocin. The dose was sufficient to reduce plasma insulin to less than 5 $\mu U/ml$ and increase glucose to over 300 mg/dl plasma. Rats were then fed a stock ration (Reeves and Arnrich, 1974) for two weeks.

Experiment 2

Three groups of 24 rats each were fed either high-beef tallow, high-corn oil or low-fat diet for two months.

Experiment 3

Twelve rats were fed high-beef tallow diet for two months and then switched to high-corn oil diet for another two months.

Twelve rats were fed high-corn oil diet for two months and then switched to high-beef tallow diet for another two months.

Diets

Animals were randomly assigned to experimental diets which were instituted ad libitum. Composition of the diets is shown in Table 5. The approximate gross composition of the American diet (USA) was used for the weight percentage of the ingredients (U.S. Department of Agriculture, 1969). The beef tallow diet contained about 38% of energy as beef fat and 2% as corn oil which resulted in a polyunsaturated to saturated fat (P/S) ratio of about 0.2 (Dupont et al., 1972). The corn oil diet contained about 40% of energy as polyunsaturated fat which provided a P/S ratio of about 5 (Dupont et al., 1972).

Food Intake Study

Food intake was measured twice over a total period of two weeks. Twelve rats within each group were selected randomly and food intake

Table 5. Composition of diets, weight percent

Ingredient	Low fat	Beef tallow	Corn oil
Lactalbumin[a]	15.0	19.0	19.0
Corn oil[b]	1.0	1.2	21.2
Beef tallow[c]	—	20.0	—
Salt mix[d]	4.0	5.0	5.0
Cellulose[d]	2.0	2.5	2.5
Cornstarch[e]	39.9	25.3	25.3
Sucrose[f]	36.5	25.0	25.0
Vitamin mix[g]	1.6	2.0	2.0
Kcal/g diet[h]	4.229	5.383	5.383

[a]United States Biochemical Corporation, 21000 Miles Parkway, Cleveland, Ohio.

[b]Mazola obtained from local retail market.

[c]Beef tallow obtained from Carriage House Meat & Provision Co., Ames, Iowa.

[d]Teklad, Madison, Wisconsin. Composition reported in Williams et al. (1968). Chromium at 0.3 ppm and selenium at 0.1 ppm were added.

[e]Clinton Corn Processing, 1251 Beaver Channel, Clinton, Iowa.

[f]Friley Food Stores, Iowa State University, Ames, Iowa.

[g]Contained per 2 kg of vitamin mixture made up with cornstarch: vitamin A acetate, 0.8g (500,000 IU/g); vitamin D (calciferol), 0.5g (400,000 IU/g); alpha-tocopherol acetate, 40.0g (500 IU/g); menadione, 0.1g; vitamin B_{12}, 10g (0.1% trituration with mannitol); biotin, 0.05g; folic acid, 0.2g; p-aminobenzoic acid, 20.0g; inositol, 20.0g; Ca-pantothenate, 2.0g; niacin, 3.0g; pyridoxine-HCl, 1.0g; riboflavin, 1.0g; thiamin-HCl, 0.5g; choline chloride 400.0g; ascorbic acid, 10.0g.

[h]Calorie values of this formulation of diets were determined by bomb calorimetry (Parr Oxygen Bomb Calorimeter, Moline, Illinois).

was determined by weight of diet provided, minus that remaining and correcting for spillage.

Experimental Diabetes Mellitus

Twelve rats were rendered diabetic by a single i.v. injection of streptozotocin (gift of Dr. William Dulin, Upjohn Company, Kalamazoo, Michigan 49001, USA). Pilot studies with our rats suggested a streptozotocin concentration of 30 mg/kg to be sufficient in inducing experimental diabetes (about two weeks after injection, blood glucose concentration was approximately 340 mg/dl in 12-hour fasted rats). Streptozotocin was dissolved in 0.15M NaCl solution acidified to pH 4.5 with citrate and injected within five minutes after preparation of a fresh solution.

Blood Samples

Rats were fasted overnight (12 hours) and then anesthetized with ether. After exposure of the abdominal cavity, approximately 12 to 15 ml of blood per animal was drawn from the abdominal aorta into tubes containing EDTA (1 mg/ml blood). Blood was handled at 4°C and centrifuged at low speed (800xg) for 15 minutes to remove platelets and red blood cells. Plasma samples from two rats within each group were combined for further analyses. Plasma glucose concentration was always assayed immediately and a small plasma sample was stored at -16°C for insulin determination. Plasma was prepared for lipoprotein isolation within 12 hours after blood sampling.

Lipoprotein Isolation

Plasma lipoproteins were isolated into various density classes by repeated ultracentrifugation as previously described (Havel et al., 1955). Ultracentrifugation was performed in a Beckman L-3 model ultracentrifuge with a rotor type 50Ti. The very low density lipoproteins (VLDL) were isolated at plasma density $d < 1.006$ g/ml by centrifugation at 40,000 rpm (106,000 x g; R_{ave} = 6 cm) for 18 hours. To eliminate albumin contamination, isolated VLDL were washed in saline with a salt density of 1.006 g/ml by centrifugation at 40,000 rpm for 18 hours. Other lipoprotein fractions were isolated by sequentially raising the plasma density to 1.063 and 1.21 g/ml by the addition of crystalline KBr. The $d = 1.006$ to 1.063 g/ml density fraction was obtained following ultracentrifugation at 40,000 rpm for 24 hours. The $d = 1.063$ to 1.21 g/ml fraction was isolated by ultracentrifugation at 40,000 rpm for 48 hours. The $d = 1.063$ to 1.21 g/ml fraction was recentrifuged for 24 hours at $d = 1.21$ g/ml to eliminate albumin contamination. Rotor chamber temperature was always 4°C. All fractions were removed by the tube-slicing technique (National Heart and Lung Institute, 1974). The purity of the lipoproteins was judged by paper electrophoresis (Jencks and Durrum, 1955) in rats used in a separate study.

Quantitation of Apoproteins

Lipoproteins were extensively dialyzed against water with 0.01% EDTA pH 7.0 at 4°C and subsequently analyzed for their apoprotein content (Weisgraber and Mahley, 1978). Protein concentration was determined

using the Lowry method (Lowry et al., 1951). Proteins in each lipo-
protein fraction included primarily the apoproteins. Some samples, how-
ever, also contained trace amounts of albumin and other unidentified
contaminants. Samples containing 20 μg of protein were transferred into
15 ml conical centrifuge tubes. After a Kimwipe was fastened on top of
each tube, samples were frozen and then lyophilized for about 12 hours
in a freeze dryer (VirTis Research Equipment, Gardiner, New York).
Lyophilized samples were kept in the 15 ml conical centrifuge tubes
and delipidated at 4°C by adding 1.5 ml of chloroform:methanol, 2:1,
V/V. After 30 minutes at 4°C, 1.5 ml of chilled MeOH was added and the
samples were then centrifuged at 800xg for 15 minutes. Most of the
solvent was removed by suction and protein pellets were dried with a
gentle stream of nitrogen. Kimwipe was fastened on top of each centri-
fuge tube. As such, samples were stored in sealed jars containing
anhydrous $CaSO_4$ at -16°C for further analysis.

Prior to electrophoresis, 100 μl of a buffer containing 2.5 mM
tris, 19.2 mM glycine, 0.3% SDS-bromophenol blue solution was added to
each sample. Samples were then incubated for 90 minutes at 37°C. Six
to 8 grains of large crystal sucrose were added to each tube during the
last 15 minutes of incubation to increase the density of the solution.

The polyacrylamide gels contained 0.1% SDS (W/V), 0.19% N,N,N^1,N^1-
tetramethylethylenediamine (V/V) and 0.5% ammonium persulfate (W/V) in
a buffer containing 24 mM Tris in 19.2 mM glycine (pH 8.3). An acrylamide
to N,N^1-methylene acrylamide (bis) ratio of 23.9 to 1 was used in the
11% acrylamide gels (W/V). Reservoir solutions contained 0.5% SDS in a
buffer containing 25 mM tris in 192 mM glycine (pH 8.3).

Samples were subjected to electrophoresis toward the anode at 150 volts until one-half of the bromophenol blue dye marker had run off the bottom of the gel (about 1.5 to 2.0 hours). After electrophoresis, gels were fixed in a 40% methanol, 7% acetic acid aqueous solution for at least 10 hours, then stained by immersion for 2.0 hours in a solution containing 20 parts 50% aqueous methanol, 1 part glacial acetic acid and 0.1% (W/V) Coomassie blue. The gels were destained for about two days in an aqueous solution containing 7.5% acetic acid and 5% methanol.

The following standards of known molecular weight were used as reference proteins: Albumin (Bovine Plasma) — 66,000D; Albumin (Ovalbumin) — 45,000D; Pepsin (Porcine Stomach Mucosa) — 34,700D; Trypsinogen (Bovine Pancreas) — 24,000D; β-Lactoblobulin (Bovine Milk) — 18,400D; Lysozyme (Egg White) — 14,300D; Insulin (Bovine Pancreas) — 5,000D. All proteins were purchased from Sigma Chemical Company, Saint Louis, Missouri.

The gels were analyzed for their apoprotein content by scanning of the apoprotein bands at a wavelength of 590 nm in a single-beam spectrophotometer (Gilford Model 240, Oberlin, Ohio) equipped with a gel scanner (Gilford Model 2410, Oberlin, Ohio) and connected to a recorder (Gilford Model 242, Oberlin, Ohio) and a Supergrater-1 Computer Integrator (Columbia Scientific Industries, Austin, Texas).

With this method, apoproteins can be separated on the basis of size alone if they are first solubilized with the detergent sodium dodecylsulfate (SDS). SDS binds to the molecules converting them to rod-like shapes and masking their native charge with its own negative charge. The proteins then become equal in charge density. When subjected to

polyacrylamide gel electrophoresis, apoproteins are then separated according to size by the molecular sieving effects of the gel. In SDS electrophoresis, migration rate correlates quite accurately with molecular weight which makes this method suitable for molecular weight determinations of unknown proteins.

Analysis of apoprotein data

Apoproteins analyzed in VLDL were apo-B, apo-E and apo-C. The different C-apoproteins (CI, CII, and CIII) did not resolve distinctly by SDS-polyacrylamide gel electrophoresis and were, therefore, collectively called apo-C or low molecular weight apoproteins. LDL apoproteins analyzed were apo-B and apo-E, and apoproteins analyzed in HDL included apo-B, apo-E, apo-AI and apo-C.

The apoproteins of LDL and HDL fractions may be converted to mass units by multiplying the relative area of each apoprotein by the mass determined for each lipoprotein fraction by the Lowry procedure. Quantifying apoproteins by spectrophotometry, however, did not permit assessment of absolute apoprotein mass in the VLDL fractions because equal sample sizes (20 μg of protein) applied on the polyacrylamide gels did not always correlate with visual assessment of color density of protein bands after electrophoresis. Excess lipids in the VLDL fractions often caused cloudiness in the aqueous medium used in the protein determination assay. The extra cloudiness in addition to the color reagent (Lowry procedure) gave false high readings. Efforts were made to extract lipids from the VLDL fractions with ether before reading absorbances.

It appeared that relative densities of apoproteins in each gel did

not change with different sample sizes. It was, therefore, possible to calculate relative amounts of apoproteins in each lipoprotein fraction by using the sum of all major apoproteins as a total and computing the percent of each apoprotein.

Analysis of Lipids

Chemical analysis of isolated lipoproteins included total and free cholesterol, triglycerides, phospholipids and protein. Moni-Trol-1 Chemistry Control (DADE Division, American Hospital Supply Corporation, Miami, Florida) was used as a control to monitor accuracy and precision of individual assays.

Cholesterol

Total and free cholesterol were determined by sensitive enzymatic procedures which were slightly modified (Oh and Dupont, 1981) from the original methods described by Allain et al. (1974) and Carlson and Goldfarb (1977). In the reaction, cholesteryl esters are split by cholesteryl ester hydrolase and the total cholesterol is then oxidized by cholesterol oxidase. The liberated hydrogen peroxide is finally coupled with 4-aminoantipyrine and phenol in the presence of horseradish peroxidase to form a chromogenic quinoline dye with maximum absorption at 500 nm. Plasma- and lipoprotein-cholesterol can be quantitatively determined without extraction of lipids. Cholesteryl esters were assumed to be the difference between total and free cholesterol.

Triglycerides

Plasma- and lipoprotein-triglycerides were manually measured by a colorimetric procedure modified from the original method described by Giegel et al. (1975). With this method, lipids were first partitioned between a water/isopropanol and a heptane phase. A transesterifying agent, which consisted of NaOH in isopropanol, was used to liberate glycerol molecules from triglycerides. Glycerol was then oxidized by periodate (sodium periodate dissolved in acetic acid) and the formaldehyde produced was reacted with acetylacetone. The resulting chromogenic dye showed maximum absorption at 415 nm.

Phospholipids

Sandhu method   A slightly modified procedure originally described by Sandhu (1976) was used to determine phospholipid concentrations in lipoproteins in experiments 2 and 3. Lecithin was used as a standard. A 100 μl sample was added to 1.5 ml of isopropanol. The entire supernatant was then used for phospholipid analysis. After evaporation, chloroform, chromogenic reagent and nonane were added to each sample. The chromogenic solution was composed of ammonium molybdate, HCl, mercury, H_2SO_4, methanol, chloroform and water. Maximum absorption of Prussian blue complex formed was at 710 nm.

In Sandhu's method, intact phospholipids are complexed with the chromogenic reagent which is soluble in organic solvent (nonane). This eliminates interference from inorganic phosphorous coming from plasma, reagents or glassware.

<u>Bartlett method</u>   Phospholipids in lipoprotein fractions of the diabetic rats (experiment 1) were determined by a modified Bartlett phosphorous method (1959). Standards were prepared in absolute ethanol using Sigma Egg Yolk Lecithin III E. In the Bartlett method, acid digestion of lipids results in the conversion of all organic to inorganic phosphorous, making the chromogenic reagent complex water soluble. On the other hand, the phospholipid-chromogenic reagent complex in Sandhu's method is soluble in organic solvent. This prevents interference from inorganic phosphorous. Because of these methodological differences, phospholipid data from diabetic rats and animals fed the experimental diets were not compared.

Analysis of Total Lipoprotein-Protein

Protein was determined by the procedure of Lowry et al. (1951). Bovine serum albumin was used as the standard. Protein concentrations were used to calculate sample size for apoprotein quantitation. In this method, two distinct steps led to the final color with protein resulting in a maximum absorption at 660 nm: reaction with copper in alkaline solution and reduction of the phosphomolybdic-phosphotungstic reagent (Folin Reagent) by the copper treated protein.

Especially in VLDL, excess lipids caused cloudy samples. If this occurred, lipids were then extracted first with ether before reading absorbances.

Analysis of Glucose

Glucose was determined enzymatically using a Beckman Glucose Analyzer which is an electronic oxygen sensor device. When the sample is injected into an enzyme reagent solution, glucose from the sample combines dissolved O_2 from the solution. Gluconic acid and H_2O_2 are then formed via the reaction of glucose and O_2 in the presence of glucose oxidase. O_2 consumption is proportional to glucose concentration in the sample. To ensure destruction of the peroxide, ethanol, catalase, iodide and molybdate are added to the enzyme reagent solution.

Analysis of Insulin

An Insulin Radioimmunoassay Kit was used for the detection of insulin in plasma. The kit was purchased from Amersham Corporation, Arlington Heights, Illinois. A modification of the assay was necessary, since the concentrations of immunoreactive insulin in the present study were low. This modification can be obtained from Amersham Corporation. The range of the human insulin standard concentrations was from 0.5 to 160 μl insulin/ml. Sample size was 100 μl. In this modified procedure, samples of the unknown and standard insulin solutions were incubated with 0.1 ml of the insulin binding reagent at 4°C for 24 hours. Insulin-^{125}I (0.1 ml) was added and samples were incubated at 4°C for an additional 72 hours. The 700 μl of cold buffer (4°C), consisting of 0.4% (W/V) bovine serum albumin in phosphate buffered saline (pH 7.4), was added to all tubes. The insulin binding reagent used in this procedure was an insoluble complex of guinea pig antibody

to insulin which had been reacted with a second rabbit antibody to the guinea pig antibody. The insoluble antigen-antibody complex was separated from the soluble free insulin by centrifugation at 2,000xg for 20 minutes in a refrigerated centrifuge (Beckman J-21B, Palo Alto, California). Radioactivity in the precipitate of both standards and unknowns was counted in a Gamma Counter (Beckman, Biogamma II, Fullerton, California). A standard curve was constructed from which unknown insulin values were interpolated.

The basic principles of RIA utilize the reaction between antigen and antibody. Since both the labeled insulin and the insulin present in the sample will compete for the limited number of antibody sites, the amount of radioactivity in the antigen-antibody complex will be an inverse factor of the antigen (insulin) concentration in the sample.

Statistical Analysis

Data were analyzed by using the analysis of variance and Student's t-test. The statistical program used was Statistical Analyzer System (SAS) User's Guide (Helwig and Council, 1979). Statistical probability of $p < 0.05$ was considered significant. In tables, data are reported as means with a value for pooled standard deviation (SD). For better visual evaluation of differences, the standard error of the mean was used in figures.

RESULTS

Food Intake Study

Rats fed high-fat diets consumed similar amounts of food per day
(Table 6 and Figure 1). Dietary intake of low-fat (LF)-fed rats, however,
was greater than in beef tallow (BT)- (p < 0.005) and corn oil (CO)-
(p < 0.001) fed rats. There was no difference in food intake when
data were expressed as kcal consumed per day (Figure 2). This was ex-
pected since the energy value of both the BT and CO diets was higher
(5.383 kcal/g diet) than that of the LF diet (4.229 kcal/g diet).

Table 6. Food intake of male breeder rats

Treatment (n)	Food intake	
	g/day	kcal/day
BT (12)	20.5^a	110.1^a
CO (12)	18.8^a	101.2^a
LF (12)	25.1^b	106.0^a
Pooled SD:	3.3	11.7

Different superscripts indicate significantly different means (p<0.05).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.

Rat Body Weights

Ignoring diets, the average initial (beginning of feeding study)
body weight of the rats fed the experimental diets for two months (ex-
cluding NBT and NCO rats) was 619 ± 72g. Diets had no effect on the

Figure 1. Effects of dietary fat on food intake of male breeder rats. Each variable is expressed as mean ± standard error. There were 12 animals per variable. BT: rats fed high-beef tallow diet; CO: rats fed high-corn oil diet; LF: rats fed low-fat diet
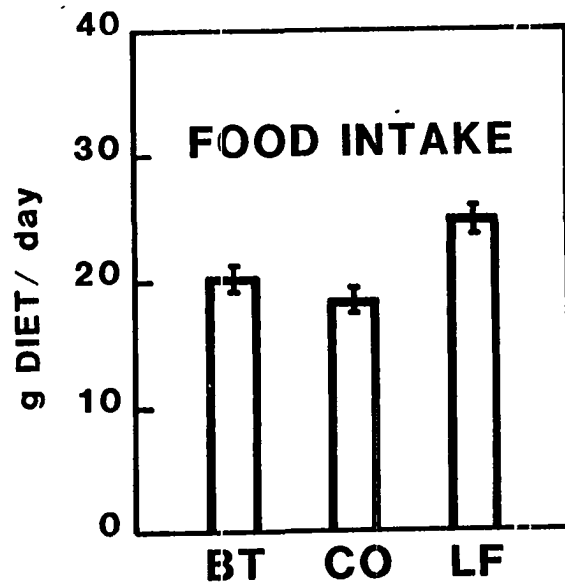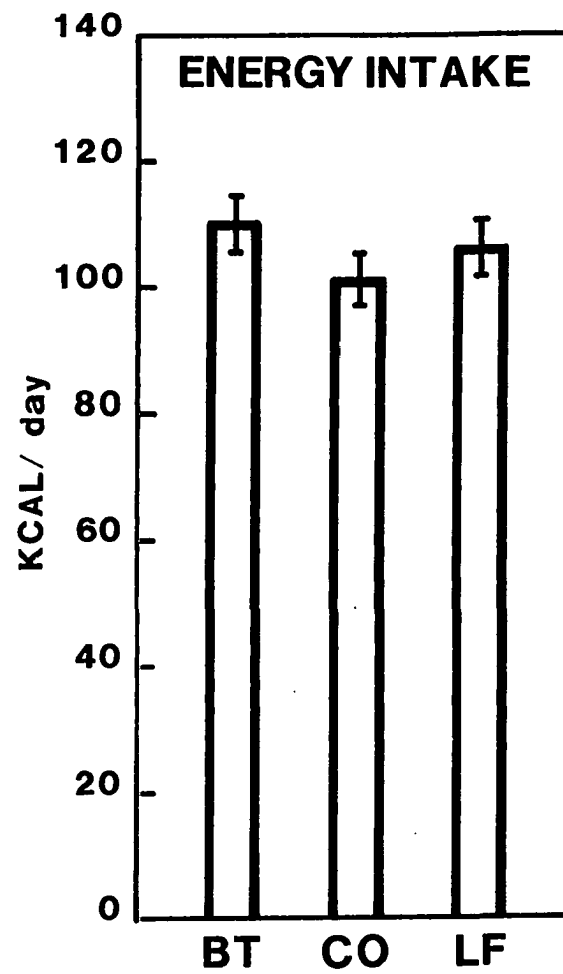
Figure 2. Effects of dietary fat on energy intake of male breeder rats. Each variable is expressed as mean ± standard error. There were 12 animals per variable. BT: rats fed high-beef tallow diet; CO: rats fed high-corn oil diet; LF: rats fed low-fat diet

Figure 1.

Figure 2.

initial and final (end of feeding study) body weights. In general, all animals gained weight over the two-month feeding period (Figure 3). However, rats fed the high-fat diets appeared to gain more weight than animals fed the low-fat diet. Average weight gain (final minus initial body weight) over the two-month feeding period was significantly higher in CO (53g) and BT (42g) than LF (13g) (p < 0.001) rats (Table 7).
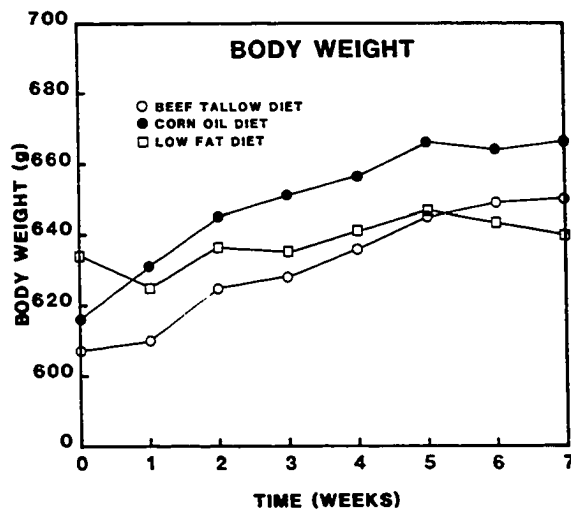

Figure 3. Illustration of effects of dietary fat on body weight of male breeder rats. Each data point represents the mean of 24 rats (experiment 2).

Plasma Glucose and Insulin

Plasma glucose

Diets had little effect on plasma glucose levels. However, plasma glucose was significantly higher (p < 0.001) in diabetic rats than in normal animals fed experimental diets (Table 8 and Figure 4).

Table 7. Body weights of male breeder rats at the beginning and end
of the two-month feeding study

Treatment (n)	Initial weight	Final weight	Difference
		Gram	
BT (24)	607^a	649^a	42^a
CO (24)	616^a	669^a	53^a
LF (24)	633^a	646^a	13^b
Pooled SD:	72	73	25

Different superscripts indicate significantly different means ($p < 0.05$).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.

Plasma insulin

Plasma insulin concentrations were not affected significantly by
amount and degree of saturation of dietary fat but tended to be
lower in BT-fed rats compared to CO- ($p < 0.2$) and LF-fed ($p < 0.1$)
animals (Table 8 and Figure 5). Fasting plasma insulin levels were
significantly lower ($p < 0.01$) in diabetic rats compared to all other
groups.

Lipoprotein-Protein

Protein values in lipoprotein fractions are summarized in Table 9.
Diet had no effect on protein concentrations in LDL and HDL. Protein
concentrations also were not different in the LDL and HDL between normal
animals fed the experimental diets and the diabetic rats. There was
significantly more VLDL-bound protein in BT- ($p < 0.025$), CO- ($p < 0.05$)

Table 8. Fasting plasma glucose and insulin concentrations of male
breeder rats

Treatment (n)	Glucose mg/dl	Insulin µU/ml
BT (12)	129^a	8.7^a
CO (12)	125^a	10.1^a
LF (12)	129^a	12.0^a
NBT (6)	121^a	8.0^a
NCO (6)	123^a	8.5^a
D (6)	343^b	4.8^b
Pooled SD:	19	4.0

Different superscripts indicate significantly different means
($p < 0.05$).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.

and LF-fed ($p < 0.025$) rats compared to NBT and NCO rats. There was,

however, no dietary effect on VLDL-protein concentration between BT-

and CO-fed rats and also between NBT and NCO animals. In contrast to

LDL and HDL, VLDL-protein concentration was significantly higher in

diabetic rats than BT- ($p < 0.05$), CO- ($p < 0.05$), NBT- ($p < 0.005$)

and NCO- ($p < 0.005$) but not LF-fed rats.

Lipoprotein-Phospholipids

Only the phospholipid data from normal rats fed the experimental

diets are reported. There was no dietary effect on phospholipid levels
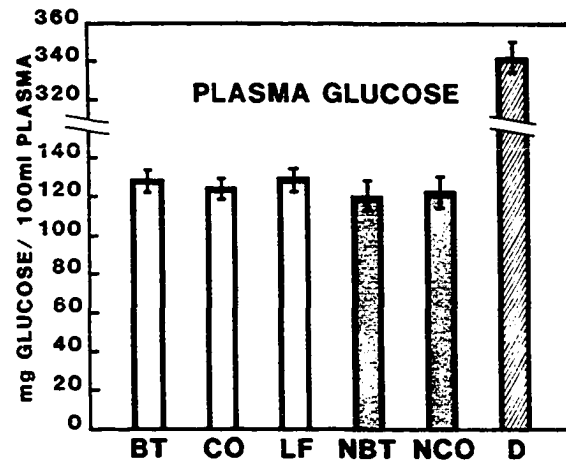
in any lipoprotein fraction (Table 10).

Figure 4. Effects of dietary fat and streptozotocin-induced diabetes
on plasma glucose in 12-hour fasted male breeder rats.
Each variable is expressed as mean ± standard error. There
were either 12 (BT, CO or LF) or 6 (NBT, NCO or D) animals
per variable. BT: rats fed high-beef tallow diet; CO:
rats fed high-corn oil diet; LF: rats fed low-fat diet;
NBT: rats switched from CO to BT diet; NCO: rats switched
from BT to CO diet; D: streptozotocin-induced diabetic
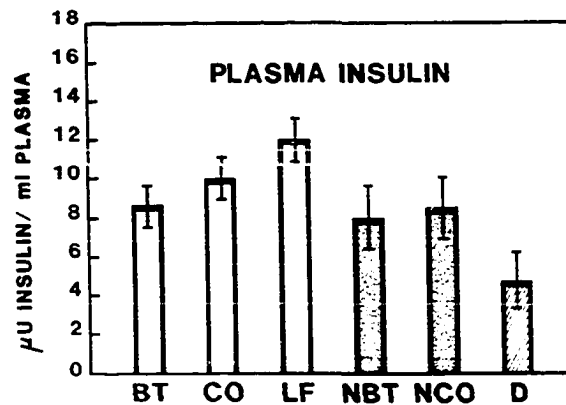rats.

Figure 5. Effects of dietary fat and streptozotocin-induced diabetes
on plasma insulin in 12-hour fasted male breeder rats. Each
variable is expressed as mean ± standard error. There were
either 12 (BT, CO or LF) or 6 (NBT, NCO or D) animals per
variable. BT: rats fed high-beef tallow diet; CO: rats
fed high-corn oil diet; LF: rats fed low-fat diet; NBT:
rats switched from CO to BT diet; NCO: rats switched from
BT to CO diet; D: streptozotocin-induced diabetic rats.

Table 9. Total protein content in lipoprotein fractions of male
breeder rats

Treatment (n)	VLDL	LDL	HDL
	mg/dl plasma		
BT (12)	7.1^a	7.5^a	51.0^a
CO (12)	6.2^a	6.0^a	55.4^a
LF (12)	7.0^{ac}	6.0^a	56.4^a
NBT (6)	2.8^b	4.6^a	47.2^a
NCO (6)	2.8^b	5.2^a	52.5^a
D (6)	12.1^c	6.8^a	45.1^a
Pooled SD:	4.1	2.8	9.2

Different superscripts indicate significantly different means ($p < 0.05$).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.

Lipoprotein-Cholesterol

VLDL-cholesterol

Animals fed the experimental diets appeared to contain more

cholesteryl ester than free cholesterol in the VLDL fraction (Table 11

and Figure 6). In diabetic rats, free cholesterol, however, pre-

dominated over cholesteryl ester. Diet had no effect on total cholesterol

but there was significantly more total cholesterol in VLDL of diabetic

rats compared to BT ($p < 0.05$) and NCO ($p < 0.05$) animals. Diet also

had no effect on free cholesterol but levels of free cholesterol were

significantly higher in diabetic rats ($p < 0.001$) compared to all other

Table 10. Lipoprotein phospholipids of male breeder rats

Treatment (n)	VLDL	LDL	HDL
	mg/dl plasma		
BT (12)	13.5^a	7.6^a	39.7^a
CO (12)	11.2^a	7.0^a	37.4^a
LF (12)	14.2^a	6.8^a	39.8^a
NBT (6)	12.2^a	6.6^a	35.2^a
NCO (6)	12.3^a	6.0^a	37.1^a
Pooled SD:	4.1	2.1	9.4

Different superscripts indicate significantly different means $(p<0.05)$.

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.

animals. There was no difference in cholesteryl ester levels among all animals. Dietary fat, differing in degree of saturation, therefore, did not affect VLDL-cholesterol levels.

LDL-cholesterol

In contrast to VLDL-cholesterol data, cholesteryl ester levels in LDL were higher than free cholesterol levels not just in animals fed the experimental diets but also in diabetic rats (Table 12 and Figure 7). LDL-total cholesterol levels were the same in normal animals fed the experimental diets but significantly lower in diabetic rats $(p < 0.001)$ compared to all other animals. There was no dietary effect on free cholesterol levels, and among rats fed the experimental diets only, CO-fed rats had significantly more LDL-free cholesterol $(p < 0.001)$

Table 11. Plasma very low density lipoprotein-cholesterol of male breeder rats

Treatment (n)	Total cholesterol	Free cholesterol	Cholesteryl ester
		mg/dl plasma	
BT (12)	5.3^a	2.0^a	3.3^a
CO (12)	5.5^{ab}	2.1^a	3.4^a
LF (12)	5.5^{ab}	2.7^a	2.8^a
NBT (6)	6.4^{ab}	2.5^a	3.9^a
NCO (6)	4.5^a	2.0^a	2.5^a
D (6)	8.5^b	6.1^b	2.4^a
Pooled SD:	3.1	1.9	1.8

Different superscripts indicate significantly different means $(p < 0.05)$.

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.

than diabetic rats. Cholesteryl ester levels also were not affected by diets but were significantly lower in diabetic $(p < 0.001)$ compared to all other rats. Thus, amount and degree of saturation of dietary fat had no effect on LDL-cholesterol; however, a diabetic state resulted in a decrease in cholesteryl ester and thus total cholesterol in LDL.

HDL-cholesterol

In HDL, all animals exhibited more cholesteryl ester than free cholesterol (Table 13 and Figure 8). Diet did not affect total cholesterol levels but HDL of diabetic rats contained significantly less total cholesterol $(p < 0.001)$ than HDL of the normal rats fed the experimental
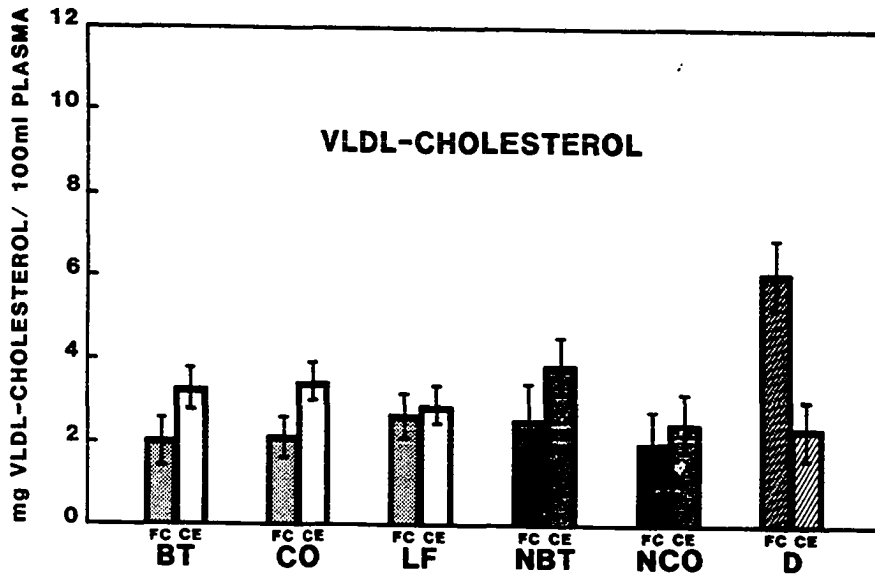
Figure 6. Effects of dietary fat and streptozotocin-induced diabetes on VLDL-cholesterol in 12-hour fasted male breeder rats. Each variable is expressed as mean ± standard error. There were either 12 (BT, CO or LF) or 6 (NBT, NCO or D) animals per variable. BT: rats fed high-beef tallow diet; CO: rats fed high-corn oil diet; LF: rats fed low-fat diet; NBT: rats switched from CO to BT diet; NCO: rats switched from BT to CO diet; D: streptozotocin-induced diabetic rats; FC: free cholesterol; CE: cholesteryl ester.

diets. Free cholesterol levels were significantly higher in LF compared to BT ($p < 0.025$) and NBT ($p < 0.005$) rats. Free cholesterol levels were also significantly higher in CO compared to BT ($p < 0.05$) and NBT ($p < 0.01$) rats. It appeared, therefore, that high-saturated fat but not high-polyunsaturated or low-fat diets caused a decrease in free cholesterol levels in HDL molecules. Free cholesterol levels were significantly lower in diabetic rats than all normal rats ($p < 0.001$). Diet had no effect on HDL-cholesteryl ester levels which, however, were significantly lower in diabetic ($p < 0.001$) than all other rats. Diabetes, therefore, decreased HDL-cholesterol levels.

Table 12. Plasma low density lipoprotein-cholesterol of male breeder rats

Treatment (n)	Total cholesterol	Free cholesterol	Cholesteryl ester
		mg/dl plasma	
BT (12)	9.7^a	2.5^{ab}	7.2^a
CO (12)	9.6^a	3.0^a	6.6^a
LF (12)	8.3^a	2.3^{ab}	6.0^a
NBT (6)	8.3^a	2.4^{ab}	5.9^a
NCO (6)	9.4^a	2.4^{ab}	7.0^a
D (6)	4.5^b	1.6^b	2.9^b
Pooled SD:	2.9	0.9	2.3

Different superscripts indicate significantly different means ($p < 0.05$).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.

Lipoprotein-Triglycerides (TG)

VLDL-triglycerides

There was no significant dietary effect on VLDL-TG levels (Table 14 and Figure 9). VLDL-TG levels were, however, significantly higher in diabetic rats ($p < 0.001$) compared to normal animals fed experimental diets.
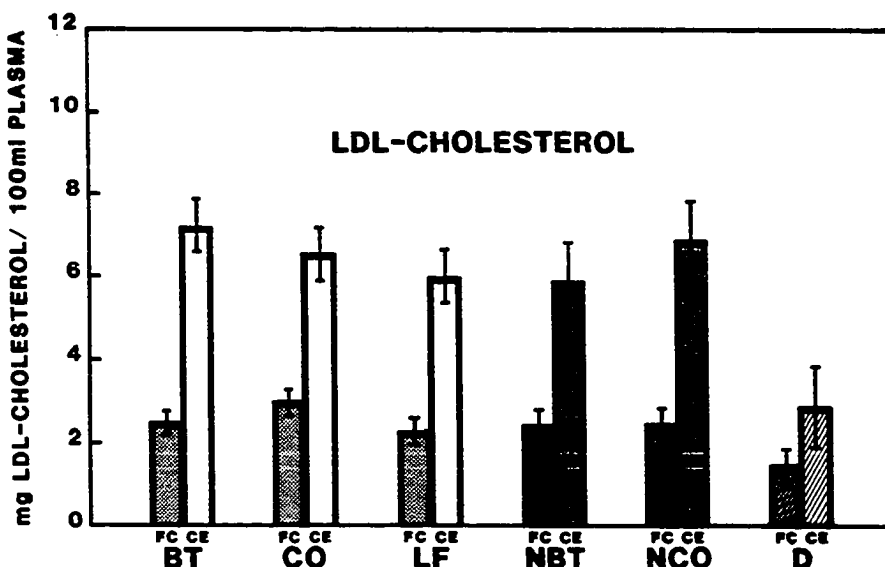
Figure 7. Effects of dietary fat and streptozotocin-induced diabetes on LDL-cholesterol in 12-hour fasted male breeder rats. Each variable is expressed as mean ± standard error. There were either 12 (BT, CO or LF) or 6 (NBT, NCO or D) animals per variable. BT: rats fed high-beef tallow diet; CO: rats fed high-corn oil diet; LF: rats fed low-fat diet; NBT: rats switched from CO to BT diet; NCO: rats switched from BT to CO diet; D: streptozotocin-induced diabetic rats; FC: free cholesterol; CE: cholesteryl ester.

LDL-triglycerides

LDL-TG levels were significantly lower in CO- (p < 0.025) and LF-fed (p < 0.001) rats compared to BT-fed rats (Table 14 and Figure 10). A dietary change from CO to BT (=NBT) resulted in a significant (p < 0.001) elevation of LDL-TG, with NBT similar to the diabetic rats. LDL-TG levels in NBT rats were also significantly higher than in BT- (p < 0.005), CO- (p < 0.001) and LF-fed (p < 0.001) rats. TG levels in LDL were significantly higher in diabetic rats compared to BT (p < 0.001), CO (p < 0.001), LF (p < 0.001) and NCO (p < 0.001) but not NBT rats. High-

Table 13. Plasma high density lipoprotein-cholesterol of male breeder rats

Treatment (n)	Total cholesterol	Free cholesterol	Cholesteryl ester
		mg/dl plasma	
BT (12)	30.8^a	6.2^a	24.6^a
CO (12)	31.0^a	8.0^b	23.0^a
LF (12)	33.5^a	8.2^b	25.3^a
NBT (6)	28.4^a	5.1^a	23.3^a
NCO (6)	35.8^a	7.2^{ab}	28.6^a
D (6)	17.7^b	2.6^c	15.1^b
Pooled SD:	7.5	2.0	6.1

Different superscripts indicate significantly different means ($p < 0.05$).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.

saturated fat feeding, therefore, led to an increase in LDL-TG levels similar to a diabetic state.

HDL-triglycerides

In contrast to VLDL- and LDL-TG, there was no dietary effect on HDL-TG levels (Table 14 and Figure 11). However, HDL-TG levels were significantly lower in diabetic ($p < 0.001$) compared to all other rats.
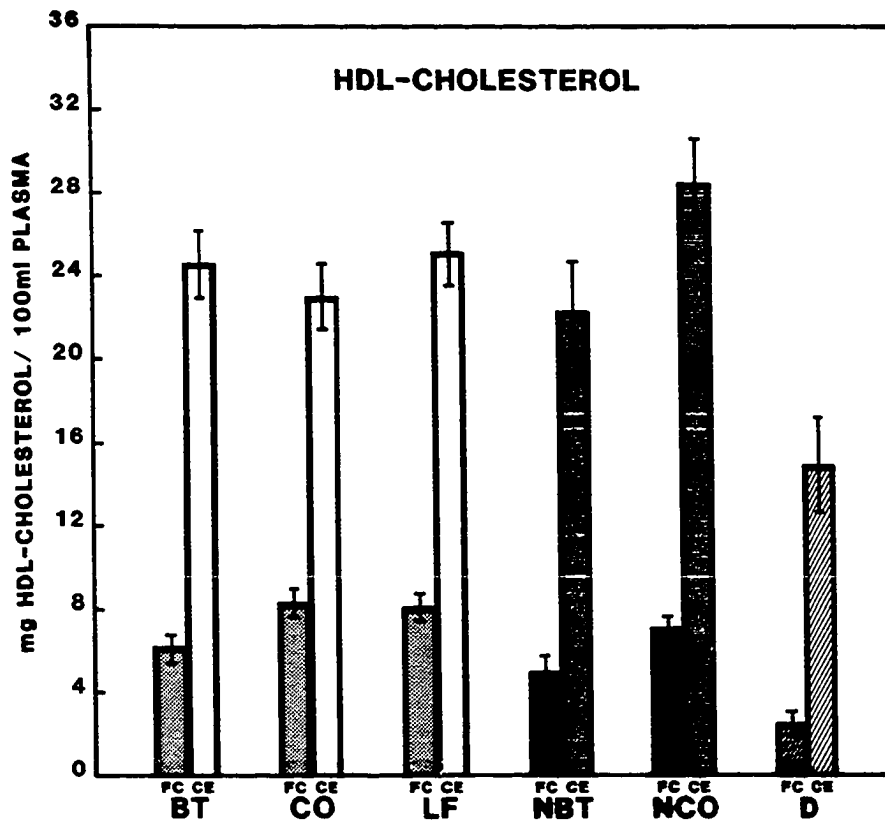
Figure 8. Effects of dietary fat and streptozotocin-induced diabetes on HDL-cholesterol in 12-hour fasted male breeder rats. Each variable is expressed as mean ± standard error. There were either 12 (BT, CO or LF) or 6 (NBT, NCO or D) animals per variable. BT: rats fed high-beef tallow; CO: rats fed high-corn oil diet; LF: rats fed low-fat diet; NBT: rats switched from CO to BT diet; NCO: rats switched from BT to CO diet; D: streptozotocin-induced diabetic rats; FC: free cholesterol; CE: cholesteryl ester.

Table 14. Plasma lipoprotein-triglycerides of male breeder rats

Treatment (n)	VLDL	LDL	HDL
	mg/dl plasma		
BT (12) .	55.5^a	3.4^{bd}	2.2^a
CO (12)	48.9^a	2.7^a	2.2^a
LF (12)	68.7^a	2.4^a	2.3^a
NBT (6)	51.7^a	4.5^c	2.1^a
NCO (6)	46.7^a	2.8^{ad}	2.4^a
D (6)	110.7^b	4.7^c	1.0^b
Pooled SD:	31.8	0.7	0.6

Different superscripts indicate significantly different means (p <0.05).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.

Lipoprotein-Apoproteins

Figures 12-14 illustrate samples of apoproteins from different lipo-
protein fractions as they appeared after electrophoretic migration.
Major detectable apoproteins were apo-B, apo-E and apo-C in VLDL, apo-B
and apo-E in LDL, and apo-B, apo-E, apo-AI and apo-C in HDL. A major
decrease in intensity of apo-E bands both in LDL and HDL was apparent
in diabetic rats compared to animals fed the experimental diets.

VLDL-apoproteins

Generally, diet and streptozotocin-induced diabetes had little
effect on apo-B (Table 15 and Figure 15). However, percent of apo-B

Figure 9. Effects of dietary fat and streptozotocin-induced diabetes on
VLDL-triglyceride level in 12-hour fasted male breeder rats.
Each variable is expressed as mean $\pm$ standard error. There
were either 12 (BT, CO or LF) or 6 (NBT, NCO or D) animals
per variable. BT: rats fed high-beef tallow diet; CO:
rats fed high-corn oil diet; LF: rats fed low-fat diet;
NBT: rats switched from CO to BT diet; NCO: rats switched
from BT to CO diet; D: streptozotocin-induced diabetic rats.

Figure 10. Effects of dietary fat and streptozotocin-induced diabetes
on LDL-triglyceride level in 12-hour fasted male breeder
rats. Each variable is expressed as mean $\pm$ standard error.
There were either 12 (BT, CO or LF) or 6 (NBT, NCO or D)
animals per variable. BT: rats fed high-beef tallow diet;
CO: rats fed high-corn oil diet; LF: rats fed low-fat
diet; NBT: rats switched from CO to BT diet; NCO: rats
switched from BT to CO diet; D: streptozotocin-induced
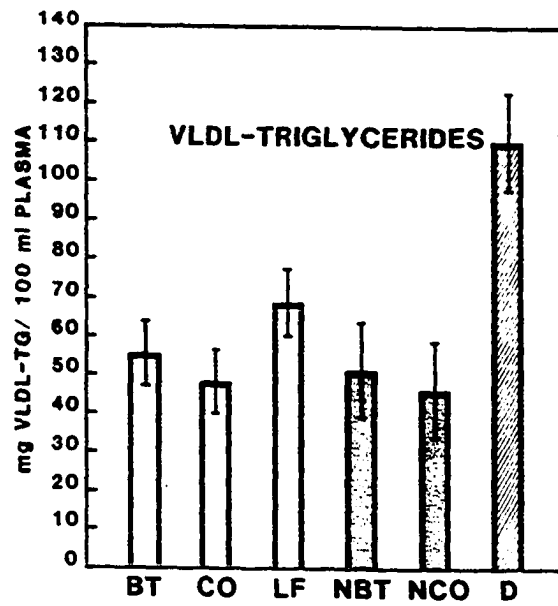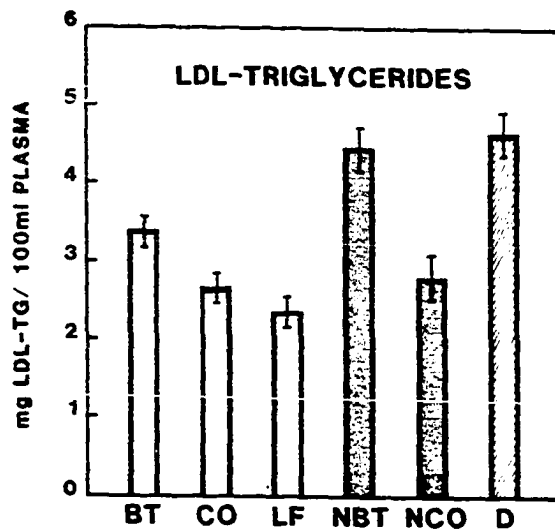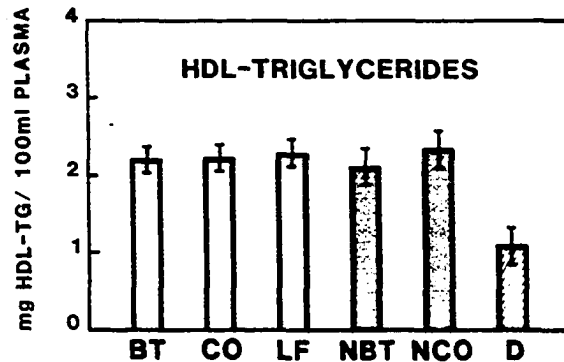diabetic rats.

Figure 9.

Figure 10.

Figure 11. Effects of dietary fat and streptozotocin-induced diabetes
on HDL-triglyceride level in 12-hour fasted male breeder
rats. Each variable is expressed as mean $\pm$ standard error.
There were either 12 (BT, CO or LF) or 6 (NBT, NCO or D)
animals per variable. BT: rats fed high-beef tallow diet;
CO: rats fed high-corn oil diet; LF: rats fed low-fat
diet; NBT: rats switched from CO to BT diet; NCO: rats
switched from BT to CO diet; D: streptozotocin-induced
diabetic rats.

was significantly lower in LF than BT ($p < 0.05$) and diabetic ($p < 0.005$)

rats. Overall, apo-B appeared to be higher in BT, NBT and diabetic

rats compared to all other animals. Feeding the three experimental

diets for two months caused no difference in apo-E levels in VLDL frac-

tions. Also, apo-E levels were the same in BT-, CO- and LF-fed and

diabetic rats. However, NBT and NCO rats which were fed high-fat diets

(differing only in degree of saturation) for a total of four months had

significantly higher apo-E levels than animals fed either BT (NBT vs.

BT, $p < 0.01$; NCO vs. BT, $p < 0.005$) or CO (NBT vs. CO, $p < 0.025$; NCO

vs. CO, $p < 0.005$) diets for two months. Apo-E levels also were higher

in NBT ($p < 0.01$) and NCO ($p < 0.005$) rats compared to diabetic animals.

Similar to the apo-E data, significant differences were observed

with the apo-C (or low molecular weight apoproteins) data between animals

Figure 12. Sodium dodecyl sulfate polyacrylamide gel electrophoresis
of rat VLDL. Approximately 25 µg of lipoprotein protein
was applied to each gel. Apoproteins are illustrated in
sample gels. BT: rats fed high-beef tallow diet; CO:
rats fed high-corn oil diet; LF: rats fed low-fat diet;
NBT: rats switched from CO to BT diet; NCO: rats switched
from BT to CO diet; D: streptozotocin-induced diabetic
rats.
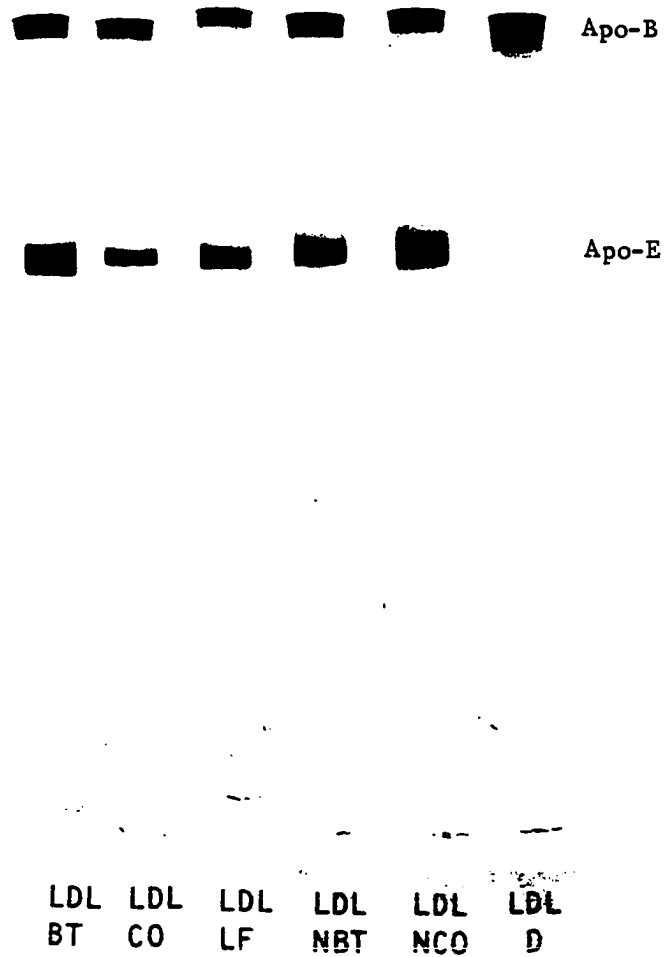
Figure 13. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of rat LDL. Approximately 25 µg of lipoprotein protein was applied to each gel. Apoproteins are illustrated in sample gels. BT: rats fed high-beef tallow diet; CO: rats fed high-corn oil diet; LF: rats fed low-fat diet; NBT: rats switched from CO to BT diet; NCO: rats switched from BT to CO diet; D: streptozotocin-induced diabetic rats
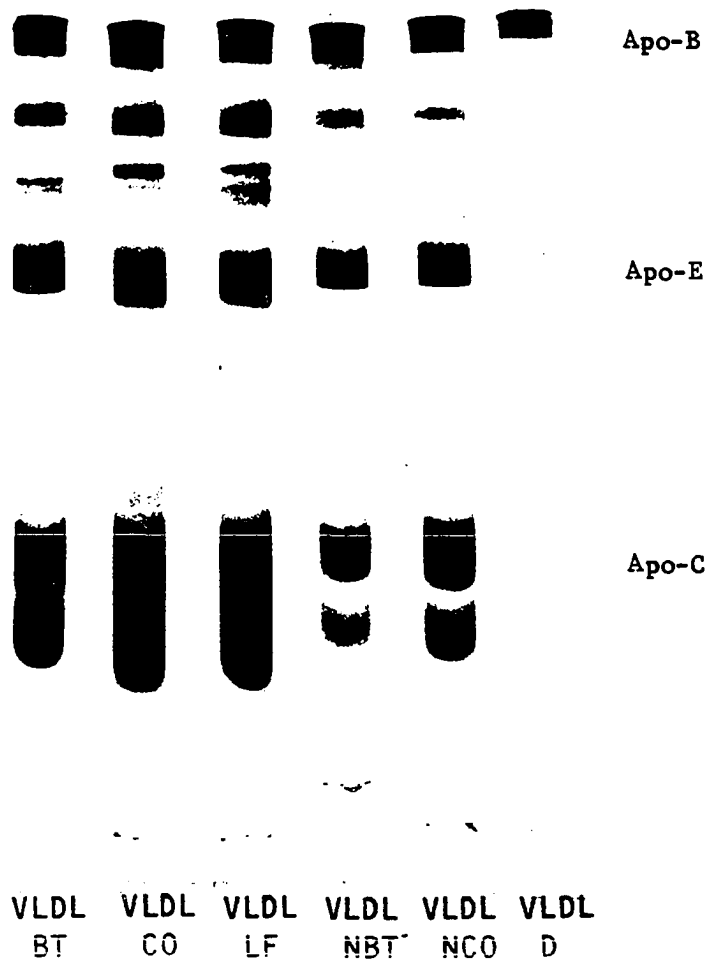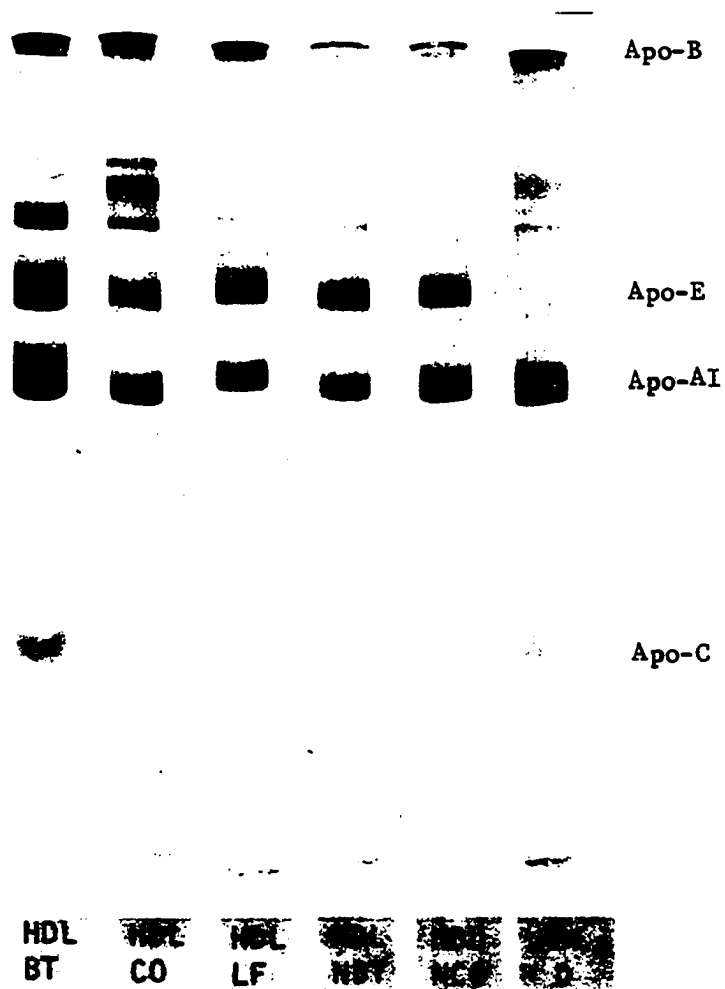
Figure 14. Sodium dodecyl sulfate polyacrylamide gel electrophoresis
of rat HDL. Approximately 25 μg of lipoprotein-protein
was applied to each gel. Apoproteins are illustrated in
sample gels. BT: rats fed high-beef tallow diet; CO:
rats fed high-corn oil diet; LF: rats fed low-fat diet;
NBT: rats switched from CO to BT diet; NCO: rats switched
from BT to CO diet; D: streptozotocin-induced diabetic
rats.

Table 15. VLDL-apoproteins of male breeder rats

Treatment (n)	Apo-B	Apo-E	Apo-C
	Percent of total apoproteins		
BT (10)	46.2^a	14.6^a	39.2^{ac}
CO (10)	41.8^{ab}	15.6^a	42.6^{ad}
LF (10)	30.5^b	20.0^{ab}	49.5^a
NBT (6)	45.0^{ab}	25.3^b	29.7^{bc}
NCO (6)	38.3^{ab}	27.0^b	34.7^{bcd}
D (6)	52.8^a	13.4^a	33.8^{cde}
Pooled SD:	14.0	7.0	11.6

Different superscripts indicate significantly different means ($p < 0.05$).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.

fed high-fat diets for two and four months. In contrast to apo-E, apo-C was lower in NBT ($p < 0.05$) than CO rats. Apo-C levels were highest in LF-fed animals and lowest in diabetic rats and animals fed high-fat diets over a four-month period. More specifically, LF-fed rats contained significantly more VLDL-bound apo-C than NBT ($p < 0.005$) and NCO ($p < 0.025$) and also diabetic rats ($p < 0.025$).

LDL-apoproteins

The major apoprotein in rat LDL appeared to be apo-B (Table 16 and Figure 16). As with the VLDL fraction, apo-B levels were not different in rats fed BT or CO diets. Feeding the LF diet caused, however, a significant decrease in apo-B levels compared to BT- ($p < 0.005$) or CO-

Figure 15. Effects of dietary fat and streptozotocin-induced diabetes
on VLDL-apoproteins in 12-hour fasted male breeder rats.
Each variable is expressed as mean ± standard error. There
were either 10 (BT, CO or LF) or 6 (NBT, NCO or D) animals
per variable. BT: rats fed high-beef tallow diet; CO:
rats fed high-corn oil diet; LF: rats fed low-fat diet;
NBT: rats switched from CO to BT diet; NCO: rats switched
from BT to CO diet; D: streptozotocin-induced diabetic
rats.

(p < 0.025) fed rats. Apo-B levels were significantly higher in diabetic

rats compared to all normal rats (p < 0.005).

In contrast to apo-B data, apo-E levels were significantly lower

in streptozotocin-induced diabetic animals compared to all normal rats

(p < 0.005). Apo-E levels were significantly higher in LF- than BT-

(p < 0.005) or CO- (p < 0.025) fed rats and percent of LDL-bound apo-E

was the same in rats fed the BT and CO diets. Therefore, only amount of

fat but not the degree of saturation of dietary fat affected apo-B and

apo-E levels in LDL. However, diabetes led to an increase in apo-B and

Table 16. LDL-apoproteins of male breeder rats

Treatment (n)	Apo-B	Apo-E
	Percent of total apoproteins	
BT (12)	78.3^a	21.7^a
CO (12)	73.0^a	27.0^a
LF (12)	55.0^b	45.0^b
NBT (6)	67.8^{ab}	32.2^{ab}
NCO (6)	67.8^{ab}	32.2^{ab}
D (4)	95.5^c	4.5^c
Pooled SD:	16.5	16.5

Different superscripts indicate significantly different means $(p<0.05)$.

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rat.

a decrease in apo-E levels which was significant compared to animals fed

the experimental diet.

HDL-apoproteins

Apo-B, apo-E, apo-AI and apo-C were the apoproteins analyzed in HDL

(Table 17 and Figure 17). Apo-AI appeared to be the major apoprotein in

rat HDL. Only trace amounts of apo-B were present in most samples. Diet

had no effect on apo-B but apo-B levels were significantly higher in

diabetic rats (p <0.005) compared to all other animals. Apo-E levels

were not different in rats fed the high-fat diets over a two- or four-

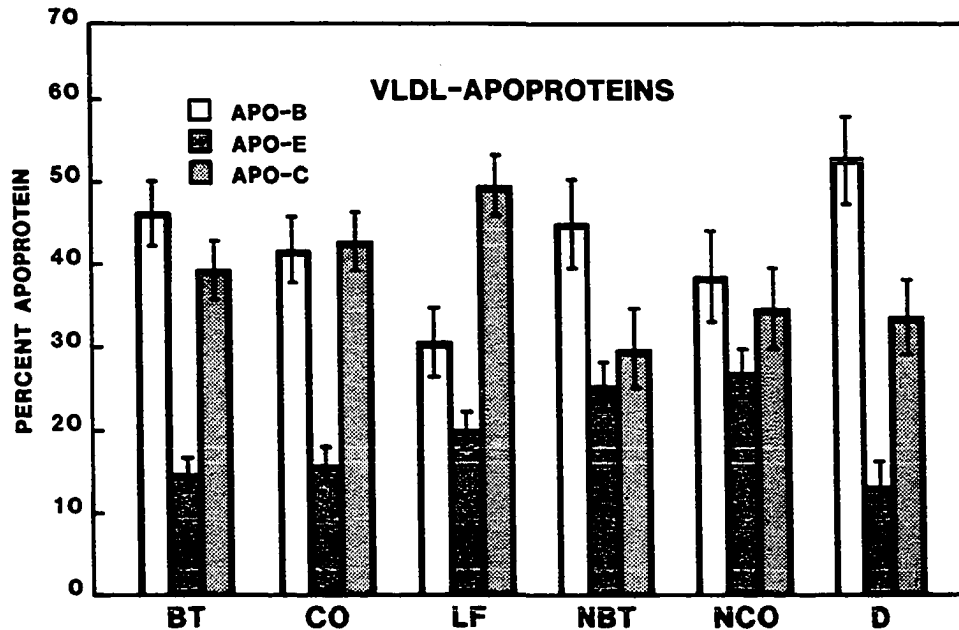month period. However, apo-E levels were significantly higher in LF-fed

Figure 16. Effects of dietary fat and streptozotocin-induced diabetes
on LDL-apoproteins in 12-hour fasted male breeder rats.
Each variable is expressed as mean ± standard error. There
were either 12 (BT, CO or LF), 6 (NBT or NCO) or 4 (D)
animals per variable. BT: rats fed high-beef tallow diet;
CO: rats fed high-corn oil diet; LF: rats fed low-fat
diet; NBT: rats switched from CO to BT diet; NCO: rats
switched from BT to CO diet; D: streptozotocin-induced
diabetic rats.

rats compared to BT (p < 0.001), CO (p < 0.001), NBT (p < 0.001) and

NCO (p < 0.001) rats. Apo-E was almost nondetectable in HDL of diabetic

rats, whereas HDL of all other rats contained major amounts of apo-E.

Thus, apo-E was significantly lower in diabetic (p < 0.001) compared to

all other animals. Apo-AI levels were always more than twice as high

as apo-E levels in all rats except the LF-fed animals in which there

was no difference. Percent of apo-AI appeared to be highest in diabetic

rats and lowest in LF-fed animals. Apo-AI was significantly lower in

Table 17. HDL-apoproteins in male breeder rats

Treatment (n)	Apo-B	Apo-E	Apo-AI	Apo-C
	Percent of total apoproteins			
BT (11)	0.6^a	24.7^a	65.5^{ac}	9.2^{ab}
CO (12)	2.1^a	30.0_b^a	57.5_b^a	10.4^{ab}
LF (12)	2.1^a	43.5^b	42.4^b	12.0^a
NBT (6)	1.4^a	26.8^a	62.8^{ac}	9.0^{ab}
NCO (6)	2.3_b^a	26.7^a	64.2^{ac}	6.8^b
D (6)	6.7^b	1.3^c	74.0^c	18.0^c
Pooled SD:	3.6	8.4	9.9	5.1

Different superscripts indicate significantly different means (p<0.05).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.

LF than BT (p < 0.001), CO (p < 0.001), NBT (p < 0.001) and NCO (p < 0.001) rats. Diabetic rats had significantly higher apo-AI levels than CO- (p < 0.005) and LF- (p < 0.001) but not BT-fed rats. There was no dietary effect on apo-C levels except in LF and NCO rats, with higher apo-C levels in LF (p < 0.05) than NCO rats. Apo-C levels were significantly higher in diabetic rats than all normal animals (p < 0.001).

Triglyceride Metabolism

Lipid and protein values in different lipoprotein fractions may reflect the status of triglyceride metabolism. For example, a comparison between cholesterol values in HDL (HDL-TC) and triglyceride values in
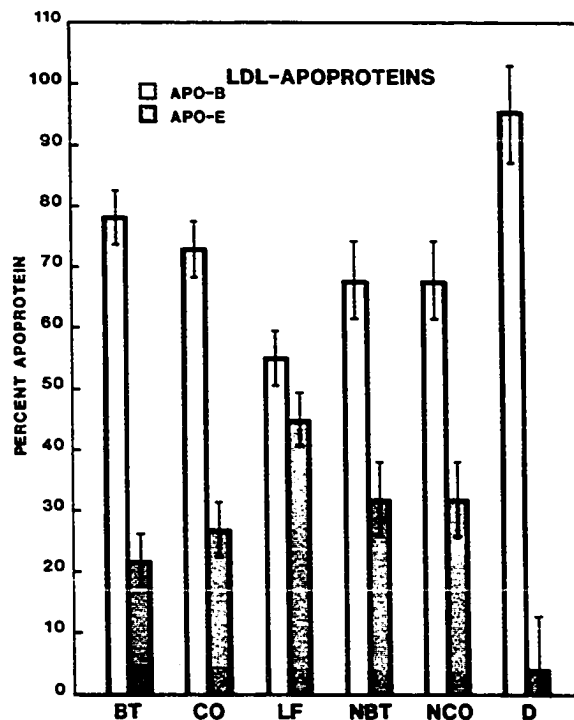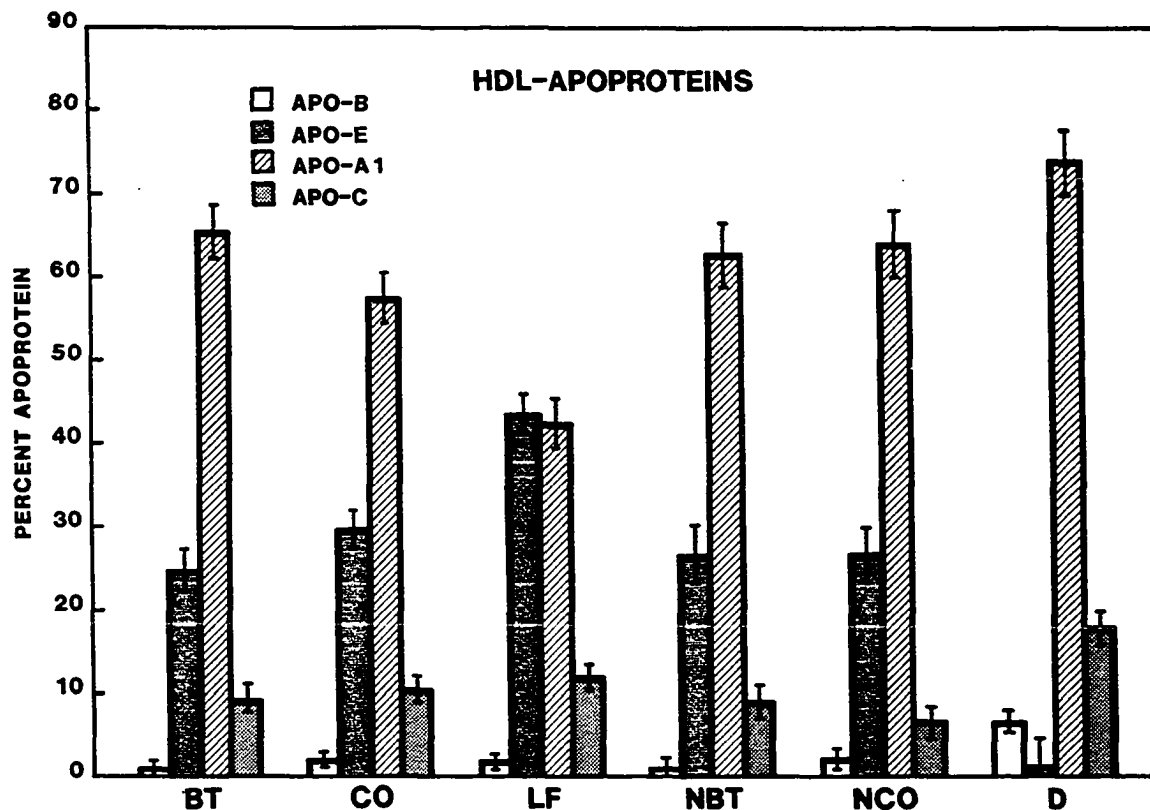
Figure 17. Effect of dietary fat and streptozotocin-induced diabetes on HDL-apoproteins in 12-hour fasted male breeder rats. Each variable is expressed as mean ± standard error. There were either 11 (BT), 12 (CO or LF) or 6 (NBT, NCO or D) animals per variable. BT: rats fed high-beef tallow diet; CO: rats fed high-corn oil diet; LF: rats fed low-fat diet; NBT: rats switched from CO to BT diet; NCO: rats switched from BT to CO diet; D: streptozotocin-induced diabetic rats.

VLDL (VLDL-TG) has been suggested to be an informative indicator of the efficiency of the degradation of triglyceride-rich lipoproteins (Alaupovic, 1981). Defective clearance of triglyceride-rich lipoproteins would result in lower concentrations of HDL-cholesterol.

During lipolysis, apo-B remains with VLDL to become LDL (Brewer, Jr., 1981). The ratios VLDL-TG/LDL-TG and VLDL-apo-B/LDL-apo-B, therefore, can be used to estimate lipolytic rate or lipoprotein lipase activity.

HDL-TC/VLDL-TG
_____

The ratio of total cholesterol in HDL divided by triglyceride in

VLDL was not affected by amount and degree of saturation of dietary fat

(Table 18). Comparing the two high-fat diets, however, CO feeding

tended ($p < 0.2$) to result in a larger ratio than BT feeding. The

HDL-TC/VLDL-TG ratio was significantly lower ($p < 0.001$) in diabetic

rats compared to all animals fed the experimental diet.

VLDL-TG/LDL-TG
_____

The VLDL-TG/LDL-TG ratio (Table 18) was significantly higher in LF

compared to BT ($p < 0.01$), NBT ($p < 0.005$), CO ($p < 0.05$) and NCO

Table 18. Ratios of selected lipoprotein lipids and proteins of male breeder rats

Treatment (n)	HDL-TC/VLDL-TG	VLDL-TG/LDL-TG	VLDL-apo-B/LDL-apo-B
BT (12)	0.61^a	16.9^a	0.60^a
CO (12)	0.75^a	19.9^a	0.59^a
LF (12)	0.58^a	29.6^b	0.64^a
NBT (6)	0.60^a	12.0^a	0.69^a
NCO (6)	0.80^a	16.7^a	0.62^a
D (6)	0.23^b	17.6^a	0.55^a
Pooled SD:	0.24	10.7	0.22

Different superscripts indicate significantly different means ($p < 0.05$).

BT: rats fed high-beef tallow diet.
CO: rats fed high-corn oil diet.
LF: rats fed low-fat diet.
NBT: rats switched from CO to BT diet.
NCO: rats switched from BT to CO diet.
D: streptozotocin-induced diabetic rats.
TC: total cholesterol.
TG: triglyceride.

(p < 0.025) rats. There was no difference between BT- and CO-fed rats and between rats fed the high-fat diets and diabetic animals. The VLDL-TG/LDL-TG ratio was, however, significantly higher in LF (p < 0.05) compared to diabetic rats.

VLDL-apo-B/LDL-apo-B

Diet and diabetes had no significant effects on VLDL-apo-B/LDL-apo-B ratio (Table 18).

SUMMARY OF RESULTS

Food Intake Study

LF-fed rats consumed more g diet/day than BT- and CO-fed rats. There was no difference when data were expressed as kcal consumed per day.

Rat Body Weight

Body weight of rats increased throughout the feeding periods. Rats fed the high-fat diets, however, appeared to gain more weight than animals fed the low-fat diet.

Plasma Glucose and Insulin

Plasma glucose

Diets had little effect on fasting plasma glucose levels which, however, were lower than those of diabetic rats.

Plasma insulin

As with the glucose data, diets had little effect on fasting plasma insulin levels which, however, tended to be lower in BT- than CO- and LF-fed rats. Insulin levels were lower in diabetic compared to all normal rats.

Lipoprotein-Protein

Rats fed the high-fat diets over a four-month period had lower VLDL-bound protein than animals fed high-fat or low-fat diets for two months each. In VLDL, protein concentration was higher in diabetic rats than animals fed high-fat diets but not LF-fed rats. Thus, duration of high-fat feeding and diabetes affected VLDL-protein levels. Diet and streptozotocin-induced diabetes had no effect on protein levels in LDL and HDL.

Lipoprotein-Phospholipids

There was no dietary effect on phospholipid levels in any of the lipoprotein fractions.

Lipoprotein-Cholesterol

VLDL-cholesterol

Experimental diets did not induce changes in TC, FC and CE levels. Generally, VLDL appeared to contain more CE than FC in rats fed the experimental diets. This was not true in diabetic rats where FC levels were higher than CE levels. An increase in TC in diabetic rats compared to animals fed experimental diets was due to a marked increase in FC and not CE levels.

LDL-cholesterol

In contrast to VLDL-cholesterol data, CE levels were always higher than FC levels in all animals, including diabetic rats. TC, FC and CE

levels were not affected by diets but were generally lower in diabetic compared to all normal rats.

HDL-cholesterol

In HDL, all animals exhibited higher CE than FC levels. Diet did not affect TC and CE levels in HDL. FC levels, however, were higher in CO- and LF- than BT-fed rats. The tendency for a decrease in TC levels in BT- compared to CO- and LF-fed rats was, therefore, due to a change in FC values. Diabetes resulted in a decrease in cholesterol levels compared to normal rats fed experimental diets.

Lipoprotein-Triglycerides (TG)

VLDL-triglyceride

There was no significant dietary effect on VLDL-TG. VLDL-TG levels were higher in diabetic than all normal rats.

LDL-triglyceride

LDL-TG levels were distinctly lower in CO- and LF- than BT-fed rats. A dietary change from CO to BT (=NBT) resulted in a marked elevation of TG, with NBT similar to diabetic rats. High-saturated fat feeding, therefore, led to an increase in LDL-TG levels similar to a diabetic state.

HDL-triglyceride

In contrast to VLDL- and LDL-TG data, there was no dietary effect on HDL-TG levels which were, however, lower in diabetic compared to all other rats.

Lipoprotein-Apoproteins

A major decrease in intensity of apo-E bands both in LDL and HDL
was noticeable in polyacrylamide gels of diabetic rats compared to nor-
mal animals fed the experimental diets.

VLDL-apoproteins

Generally, diet and diabetes had little effect on apo-B levels.
Percent of apo-B, however, appeared to be higher in BT, NBT and diabetic
rats compared to all other animals.

Apo-E levels were not different in BT, CO, LF and D rats. However,
feeding high-fat diets for four months compared to two months resulted
in an increase in apo-E levels.

In contrast to apo-E data, feeding high-fat diets for four months
compared to two months resulted in a decrease in apo-C levels. In
general, apo-C levels were highest in LF-fed rats and lowest in diabetic
rats and animals fed high-fat diets over a four-month period.

LDL-apoproteins

Apo-B levels were lower in LF-fed rats compared to animals fed high-
fat diets. Degree of saturation of dietary fat did not cause differences
in apo-B levels. Diabetic rats had higher apo-B levels than all other
animals.

Apo-E levels were lower and sometimes undetectable in diabetic rats
compared to animals fed experimental diets. In contrast to apo-B data,
apo-E was higher in LF-fed rats compared to animals fed high-fat diets.

Similar to apo-B data, degree of saturation of dietary fat did not affect apo-E levels in LDL.

HDL-apoproteins

Although present only in trace amounts, apo-B levels were higher in diabetic rats than in all normal animals.

Apo-E levels were higher in LF-fed rats compared to animals fed high-fat diets. Apo-E levels were not affected by degree of saturation of dietary fat. Apo-E was almost undetectable in HDL of diabetic rats.

Apo-AI levels were lower in LF compared to BT, CO, NBT and NCO rats. Diabetic rats had higher apo-AI levels than CO- and LF- but not BT-fed rats.

There was no dietary effect on apo-C levels except in LF and NCO rats, with higher apo-C levels in LF than in NCO rats. Apo-C levels were higher in diabetic rats than all normal animals.

Triglyceride Metabolism

HDL-TC/VLDL-TG

The ratio was not affected by amount and degree of saturation of dietary fat but tended to be larger in CO compared to BT- and LF-fed rats. Diabetes resulted in a decrease in the HDL-TC/VLDL-TG ratio, indicating a decrease in HDL-TC and/or increase in VLDL-TG levels in diabetic compared to rats fed experimental diets.

VLDL-TG/LDL-TG

The ratio was higher in LF-fed rats compared to animals fed high-fat diets and to diabetic rats. There was no difference in ratios between BT- and CO-fed rats.

VLDL-apo-B/LDL-apo-B

The ratio was not affected by diet and diabetes, indicating similar flux of apo-B from VLDL to LDL in all animals.

DISCUSSION

Food Intake and Body Weight

All rats appeared to adapt to a uniform calorie or energy intake.
The three experimental diets were a high-saturated fat or high-beef tallow
diet (BT), a high-polyunsaturated fat or high-corn oil diet (CO) and a
high-carbohydrate or low-fat diet (LF). No cholesterol was added to any
of the experimental diets. Rats fed LF diet consumed more food per day
than rats fed either high-BT or high-CO diets. This was expected be-
cause the energy value of the LF diet was lower than that of BT or CO
diets. Nevertheless, rats fed the high-fat diets gained more weight
than animals fed the low-fat diet. This diet-related difference in ap-
parent efficiency in fat deposition was previously observed in mice
(Hennig et al., 1980) and rats (Sclafani and Gorman, 1977). Dietary
induced thermogenesis (Sharief and Macdonald, 1982) may have been partially
responsible for the resistance to body weight increases in the rats fed
the low-fat diet.

Plasma Glucose and Insulin

In the present study, the amount and degree of saturation of dietary
fat had no significant effects on fasting plasma glucose levels. How-
ever, nonsignificance of plasma glucose differences after a 12-hour fast
does not rule out a possible alteration of glucose tolerance by feeding
diets high in saturated fat.

Due to necrosis of the pancreatic β-cells, caused by i.v. injection
of streptozotocin (Cooperstein and Watkins, 1981), plasma insulin levels

were lower in diabetic rats than in all other animals. This led to the
marked elevation of plasma glucose in the diabetic rats. As with the
glucose data, diet-induced changes in insulin levels were not signifi-
cant. Nevertheless, animals fed the high-BT diet tended to have lower
plasma insulin levels than rats fed the high-CO or LF diets. Whether
this was due to a decrease in synthesis or to a release of insulin from
the pancreas needs to be determined. Streptozotocin-induced diabetes
has been suggested to be due in part to a direct effect of streptozotocin
on the islet cell membranes, thereby changing membrane properties (Cooper-
stein and Watkins, 1981). Rapid changes in membrane fatty acid composi-
tion controlled by degree of saturation of dietary fatty acids have
been observed in inner membrane of cardiac mitochondria (Innis and
Clandinin, 1981) and liver (Flick, 1981). One might then speculate
that the tendency for lower plasma insulin levels in rats fed the high-
beef tallow diet was due in part to a change in the nature of β-cell
membrane fatty acids.

Lipoprotein-Cholesterol

Dietary fat effects

In the present study, the virtually cholesterol-free diets differing
in amount and degree of saturation of dietary fat did not induce changes
in total cholesterol, free cholesterol or cholesteryl ester levels,
either in very low density lipoprotein (VLDL) or low density lipoprotein
(LDL). Similar results were observed with male and female virgin rats
(Dupont et al., 1972, 1978). Up to about 12 months of age, serum
cholesterol levels were affected neither by age nor amount and degree of

saturation of dietary fat (Dupont et al., 1972). However, from about
12 to 21 months, serum-cholesterol increased linearly with age, regard-
less of dietary fat composition (Dupont et al., 1978). It appears, there-
fore, that changes in total plasma-cholesterol levels which may occur
during the lifetime of rats may be independent of amount and degree of
saturation of dietary fat.

As with LDL, cholesteryl ester levels in high density lipoprotein
(HDL) were always higher in all animals than HDL-free cholesterol levels.
In HDL, free cholesterol levels were higher in rats fed CO and LF diets
than in BT-fed rats, suggesting the possibility of an increase in
lecithin-cholesterol-acyl-transferase (LCAT) activity in BT-fed animals.
Generally, cholesterol transferred to HDL from other lipoprotein frac-
tions is rapidly esterified by LCAT (Getz and Hay, 1979). A tendency
for a decrease in total HDL-cholesterol levels in BT and NBT as compared
to CO, NCO and LF rats was, therefore, due to a change in free cholesterol
values.

Diabetes effects

In diabetic rats, VLDL-cholesterol levels were elevated compared to
normal animals fed the experimental diets. This was due to a marked in-
crease in free cholesterol and not cholesteryl ester. Increased HMG-CoA
reductase (rate limiting enzyme of cholesterol synthesis pathway)
activity in the intestine of rats with streptozotocin-induced diabetes
has been reported by Nakayama and Nakagawa (1977) and Saudek and Eder
(1979). The increase in VLDL-cholesterol thus may be due to increased
intestinal synthesis of chylomicron-cholesterol. The liver takes up

chylomicron remnant-cholesterol, thereby making cholesterol available for incorporation into VLDL.

LDL-cholesterol levels were generally lower in diabetic rats compared to animals fed experimental diets. Van Tol (1977) also reported a decrease in LDL-cholesterol levels in the diabetic rat. However, in most human studies, elevated levels of LDL-cholesterol have been shown both in insulin-dependent (Lopes-Virella et al., 1981; Reckless et al., 1978) and noninsulin-dependent patients (Taskinen and Nikkilä, 1979). This species discrepancy could be due to a quantitatively different transport mechanism of cholesterol since human plasma contains 20-25 times more LDL than rat plasma (Getz and Hay, 1979).

Diabetes resulted in a decrease in HDL-cholesterol levels compared to normal rats fed experimental diets. This was due both to a decrease in free cholesterol and cholesteryl ester, suggesting no difference in LCAT activity in diabetic compared to normal rats. The rat data are supported by human studies where LCAT activity was similar in diabetic patients and a control population (Prager et al., 1980). Salter et al. (1981) suggested that reduced levels of HDL-cholesterol (primarily HDL_2-cholesterol) were secondary to hypertriglyceridemia.

In humans, HDL-cholesterol levels have been shown to be positively correlated with lipoprotein lipase activity of adipose tissue (Nikkilä et al., 1978). HDL-cholesterol concentration may then be dependent on the efficiency of the removal of triglyceride-rich lipoproteins from the circulation since insulin therapy has been shown to increase lipoprotein lipase activity in humans (Taskinen and Nikkilä, 1979).

Lipoprotein-Triglycerides

<u>Dietary</u> <u>fat</u> <u>effects</u>

Amount and degree of saturation of dietary fat had no effect on VLDL-triglyceride levels. Because the fractional catabolic rate (FCR) of VLDL-apo-B is about 0.2 per hour (FCR is the fraction of total plasma pool of VLDL-apo-B catabolized per hour) (Huff et al., 1981), nonsignificance of VLDL-triglyceride difference after a 12-hour fast does not rule out shorter-term changes in VLDL synthesis or degradation due to high-saturated fat feeding.

The most pronounced effect of feeding high-saturated fat was on LDL-triglyceride levels which were significantly higher in rats fed the high-BT diet compared to rats fed the high-CO or -LF diets. Since LDL-protein levels were not affected by diets, the hypertriglyceridemia which resulted from saturated fat feeding was probably due to larger lipoprotein particles that transported more lipid, especially triglycerides.

The excess triglyceride in LDL could be due to a metabolic defect in the progressive delipidation of VLDL and chylomicrons. In addition to hormonal control of lipoprotein lipase via insulin concentrations (Taskinen and Nikkilä, 1979), it is conceivable that at very elevated triglyceride-rich lipoprotein levels, lipoprotein lipase becomes saturated resulting in diminished activity. This could lead to a less efficient delipidation of VLDL and chylomicrons with an accumulation of inter-mediate density lipoproteins (IDL) and chylomicron remnants of similar density as the LDL fraction (Kris-Etherton and Cooper, 1980). This phenomenon would explain in part the elevated triglyceride levels in

diabetic rats and animals fed high-BT diet.

A decrease in catabolism of LDL molecules also could have contributed to the excess LDL-triglyceride levels in BT-fed animals. However, the extent and nature of a possible defect in LDL removal in rats fed high-BT diet is not clear since both LDL-protein and LDL-cholesterol levels were not affected significantly by dietary treatment.

In contrast to LDL-triglyceride data, there was no dietary effect on HDL-triglyceride levels. Similar results were observed in humans (Fisher et al., 1981) who also received high-fat diets which were cholesterol-free.

Diabetes effects

VLDL-triglyceride levels were significantly higher in diabetic rats than normal animals fed experimental diets. This was probably due to an increased number of circulating VLDL particles since both VLDL-protein and VLDL-cholesterol levels were also elevated in diabetic rats. Theoretically, the rise in plasma-triglyceride levels could result from increased lipoprotein production or decreased removal of circulating lipoproteins, or from a combination of both factors. Mancini et al. (1980) suggested that enhanced lipolysis in adipose tissue of diabetics contributes to hypertriglyceridemia by providing increased amounts of precursors (fatty acids) for triglyceride synthesis in the liver. Impaired extrahepatic lipoprotein lipase in untreated diabetes (Brunzell et al., 1979) might also be partially responsible for diabetic hyper-triglyceridemia, because ^{3}H-palmitate labeled VLDL was removed more slowly from the circulation in diabetic rats than in normal rats

(van Tol, 1977).

A dietary change from high-CO to high-BT diets resulted in a marked elevation of LDL-triglycerides. This increased level of LDL-triglycerides was also seen in streptozotocin-induced diabetic rats. However, plasma-triglyceride levels have been reported to be related to the degree of diabetes and, therefore, to the amount of streptozotocin injected (Chen et al., 1979). Nevertheless, the resemblance of LDL-triglyceride levels in animals fed the high-BT diet to those in diabetic rats suggests that a long-term intake of diets high in saturated fats or low in polyun-saturated fats could contribute to a plasma lipid profile analogous to that of diabetes. Elevated LDL-triglyceride levels observed in diabetic patients (Schonfeld et al., 1974) have been correlated with a relative increase in IDL or remnant particle accumulation (Mancini et al., 1980).

HDL-triglyceride levels were lower in diabetic rats than in normal animals. Smaller sized circulating HDL particles appeared to be responsible for the decreased HDL-triglyceride levels in the diabetic rats since HDL-cholesterol but not HDL-protein levels were lower in diabetic rats than in normal animals.

Apoproteins

Apo-B

VLDL-apo-B tended to be higher in high-BT-fed and diabetic rats than in rats fed high-CO or LF diets. This suggested a possible in-crease in synthesis of VLDL in BT and diabetic rats since apo-B is neces-sary for formation and secretion of triglyceride-rich particles (Getz

and Hay, 1979). LDL-apo-B levels were lower in LF-fed rats compared to animals fed high-fat diets. Diabetic rats also had higher LDL-apo-B levels than all other animals which agrees with reports in the literature of elevated plasma-apo-B levels in insulin-dependent (Alaupovic, 1980) and noninsulin-dependent diabetic humans (Schonfeld et al., 1974).

There exists a direct precursor-product relationship between apo-B in VLDL and LDL (Eisenberg and Rachmilewitz, 1975; Getz and Hay, 1979), and a positive correlation ($r = 0.35$; $p = 0.01$) between VLDL-apo-B and LDL-apo-B levels supports the findings that apo-B remains associated with the VLDL-IDL-LDL delipidation cascade (Brewer, Jr., 1981). The removal of apo-B from the circulation is thought to occur primarily in the form of LDL particles (Schaefer et al., 1978b). Therefore, an elevation of LDL-apo-B in diabetic compared to normal rats was probably associated with a slower catabolic rate of LDL particles in diabetic animals.

Apo-E

VLDL-apo-E levels were not different in BT, CO, LF and diabetic rats. Degree of saturation of dietary fat did not affect apo-E levels in VLDL, LDL and HDL. Thus, the marked increase in LDL-triglyceride levels in BT and diabetic rats cannot be explained by possible inhibitory (Ekman and Nilsson-Ehle, 1975; Ganesan et al., 1976) or activating (Yamada and Murase, 1980) effects of apo-E on lipoprotein lipase.

Both in LDL and HDL, apo-E levels were higher in LF-fed rats compared to animals fed high-fat diets, suggesting a net mass transfer of apo-E from VLDL to HDL during lipolytic breakdown of VLDL in LF-fed rats (Alaupovic, 1981). Apo-E levels were much lower in LDL of diabetic rats

compared to normal animals and almost not detectable in HDL of diabetic rats. Similar results were observed by Bar-On et al. (1976b) and Deutsch et al. (1980). The lack of apo-E in LDL and HDL of diabetic rats could have been partially due to a decrease in apo-E synthesis since apo-E generally appears to enter the circulation from the liver with HDL particles (Schaefer et al., 1978b).

Apo-C

In VLDL, apo-C or low molecular weight proteins appeared to be higher in normal rats fed experimental diets for two months compared to diabetic rats. However, a decrease in VLDL-apo-C to levels similar to those seen in diabetic rats was observed in animals fed the high-fat diets for four months. These findings suggest that the duration of high-fat feeding had a more pronounced effect on VLDL-apo-C levels than the degree of saturation of dietary fat. In HDL, apo-C levels were significantly higher in diabetic rats than in normal animals. Alaupovic (1981) suggested that the rate of release of apo-C-containing lipoprotein particles from VLDL to HDL during lipolysis reflected the state of triglyceride metabolism. Judging from the apo-C data alone, the lipolytic rate would have been greater in the diabetic rats than in the normal animals. This, however, does not explain the fact that triglyceride levels were generally higher in VLDL and LDL and lower in HDL in diabetic rats than in normal animals fed experimental diets. Comparing lipolytic rate with the status of apo-C metabolism must be done with caution since the C apoproteins, CI, CII and CIII, have similar molecular weights but different functions related to lipid metabolism. Apo-CII is essential

for lipoprotein lipase activity (Nilsson-Ehle et al., 1980), whereas apo-CIII has been shown to inhibit the activation of lipoprotein lipase (Ganesan et al., 1976; Saudek and Eder, 1979). In addition, apo-C can exchange between lipoprotein particles and might not be a suitable marker for the metabolism of particular lipoproteins (Brewer, Jr., 1981).

Apo-A

In man, apo-AI and apo-AII are the major apoproteins of HDL (Schaefer et al., 1978b). However, apo-AII is only a minor component of rat HDL (Swaney et al., 1977). In this study, therefore, apo-A represents only apo-AI.

Amount but not degree of saturation of dietary fat had an effect on apo-AI levels in this study. Similar results were observed in humans (Illingworth et al., 1981). Apo-AI was significantly lower in LF-fed rats compared to animals fed the high-fat diets and diabetic rats. Apo-AI levels were significantly higher in diabetic rats than CO- and LF-fed but not BT-fed rats. These findings could reflect an increased LCAT activity in diabetic rats and normal animals fed high-BT diet since apo-AI is the major activator of LCAT (Getz and Hay, 1979). Tuvemo et al. (1981) reported significantly higher levels of apo-AI in diabetic children than in normal children. The increase in apo-A during a diabetic state could be partially due to lack of insulin in untreated diabetes. Weidman et al. (1982) reported a decrease in apo-AI levels upon insulin treatment in human diabetic ketoacidosis, with the largest decrease in apo-AI levels in the more hypertriglyceridemic subjects. Apo-AI status may reflect degree of control of diabetes.

Dietary Saturated Fat and Diabetes

Since apo-B remains associated with the VLDL-IDL-LDL delipidation cascade (Brewer, Jr., 1981), the ratios VLDL-TG/LDL-TG and VLDL-apo-B/ LDL-apo-B could be indicators of lipolytic activity. The ratios were not significantly different between BT- and CO-fed rats and between rats fed the high-fat diets and diabetic animals, suggesting no metabolic impairment in lipolytic activity. However, in addition to hormonal control via insulin concentration (Taskinen and Nikkilä, 1979), lipoprotein lipase (LPL) may become saturated at very high concentrations of triglyceride-rich lipoproteins (Nilsson-Ehle et al., 1980), which would explain in part elevated triglyceride levels in VLDL and LDL. To what extent increased triglyceride levels in LDL of BT-fed rats were due to a possible defect in LDL removal or to an accumulation of IDL or remnant particles (Mancini et al., 1980) is not clear. Compared to the high-BT diet, diabetes appeared to have a more pronounced effect on plasma triglyceride metabolism, with more significant metabolic changes.

Hypertriglyceridemia was associated with LDL in normal BT-fed rats and both with VLDL and LDL in diabetic animals. Although LDL usually carries less triglycerides than VLDL, triglyceride concentration in LDL during a fasting state may reflect effects of dietary fat composition on total plasma triglyceride metabolism, because the normal FCR of LDL is 0.3 to 0.8 per day (Hopkins and Williams, 1981).

The decreased HDL-cholesterol levels in diabetic rats when compared to normal rats and the tendency for a decrease in total HDL-cholesterol levels due to a significant decrease in free HDL-cholesterol in BT-fed

compared to CO- and LF-fed rats, also might have been partly due to a delay in chylomicron clearance (Grundy and Mok, 1976). HDL-cholesterol levels have been reported to be inversely correlated with plasma or VLDL-triglyceride levels, both in normolipidemic and hyperlipoproteinemic subjects (Schaefer et al., 1978a). Comparing the two high-fat diets, BT feeding tended ($p < 0.2$) to result in a smaller HDL-TC/VLDL-TG ratio than CO feeding. The ratio was significantly lower in diabetic rats compared to all normal animals, indicating both a decrease in HDL-total cholesterol and increase in VLDL-triglyceride levels in diabetic rats.

Apo-AI levels, which were significantly higher in diabetic rats than in CO- and LF- but not in BT-fed animals, also support a metabolic relationship between high-saturated fat feeding and diabetes. Since insulin treatment led to a decrease in apo-AI in human diabetic ketoacidosis (Weidman et al., 1982), low insulin levels in diabetic and normal BT-fed rats could have been responsible in part for the elevated apo-AI levels.

Apo-C and apo-E but not apo-A and apo-B can exchange between VLDL and HDL (Alaupovic, 1981), which would explain why there appeared to be no comparative relationship between apo-C and apo-E levels and lipoprotein lipids among normal animals fed experimental diets and diabetic rats.

The results related to lipoprotein composition support the hypothesis that excess intake of a diet too high in saturated fat (or too low in polyunsaturated fat) over a long period of time could contribute to a metabolic pattern resembling that of a diabetic state. In addition, the hyperlipidemia observed in diabetic rats and animals fed the high-BT diet appeared to be primarily due to a defect in triglyceride metabolism and not cholesterol metabolism.

CONCLUSION AND SUGGESTIONS FOR FURTHER STUDIES

A relationship with regard to plasma lipoprotein lipid and protein metabolism has been shown between high-saturated fat feeding and diabetes. Excess intake of a diet too high in saturated fat or too low in polyunsaturated fat may contribute to a metabolic pattern which resembles that of a diabetic state.

However, many questions remain unanswered and further research is needed to clarify some of the results. It is not really clear whether or not feeding the high-beef tallow and not the high-corn oil diet results in an increase in VLDL-triglyceride levels under nonfasting conditions. Elevated triglyceride-rich lipoprotein levels, which include chylomicrons and VLDL, could be due in part to an increase in synthesis at the intestinal level or in the liver. One might feed radioactively labeled triglycerides or perfuse livers with labeled fatty acids to explore dietary effects on lipoprotein synthesis.

Whether high-saturated fat diets can inhibit lipoprotein lipase activity directly or indirectly via affecting synthesis or availability of apo-CII is not known. Subclasses of apo-C (apo-CI, apo-CII and apo-CIII) could be separated and quantitated by isoelectric focusing gel electrophoresis. This technique would, however, eliminate the opportunity to quantitate all other apoproteins at the same time.

The most pronounced effect of feeding high-saturated fat was on LDL-triglyceride levels. A diminished removal of LDL from the circulation or the accumulation of IDL or remnant particles in the LDL density fraction (d = 1.006 to 1.063 g/ml) due to high-saturated fat feeding

cannot be ruled out. Since apo-B remains associated with the VLDL-IDL-LDL delipidation cascade (Brewer, Jr., 1981), one could analyze for radioactively labeled apo-B and compare relative concentrations in lipoprotein fractions and hepatic and extrahepatic tissues.

It also needs to be explored why plasma insulin tended to decrease in animals fed the high-beef tallow diet. Can different compositions of dietary fat lead to a change in the fatty acid composition of the pancreatic islet cell membranes? This would conceivably have an effect on insulin release. It is also not known whether synthesis of insulin can be altered by degree of saturation of dietary fat. Plasma insulin levels in relation to glucose tolerance may further define a relationship between saturated fat feeding and diabetes.

The breeder rats in the present study were probably too young to respond more obviously to changes in dietary fat composition. Thus, older breeder rats with a genetic tendency for obesity and diabetes should be used for further studies. Although larger animals (for example, pigs (Reitman et al., 1982)) might be a better model to study lipoprotein metabolism, high costs due to care and maintenance often prohibit their use.

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