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Effects of dietary beef tallow and soy oil of glucose and cholesterol homeostasis in normal and diabetic pigs

Laura A. Woollett

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EFFECTS OF DIETARY BEEF TALLOW AND SOY OIL ON GLUCOSE AND
CHOLESTEROL HOMEOSTASIS IN NORMAL AND DIABETIC PIGS

Iowa State University

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Effects of dietary beef tallow and soy oil on glucose and cholesterol homeostasis in normal and diabetic pigs

by

Laura A. Woollett

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY

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INTRODUCTION

Coronary heart disease (CHD) is the leading cause of death in Americans and is responsible for more deaths than all forms of cancer combined. Approximately 50% of all deaths in normal individuals and 75% of all deaths in diabetic individuals are a direct result of CHD. A large number of other individuals have undiagnosed coronary disease, thus increasing numbers of those with CHD even more. Atherosclerosis is most often associated with CHD, even though CHD involves all forms of micro- and macrovascular disease. Because heart disease is both life threatening and costly, over 6.5 billion dollars are spent per year in the U.S. on CHD, ways to defer its development must be studied.

Saturated fat was first implicated in development of atherosclerosis in the mid-1950s and since that time the literature has been replete with studies on effects of type of dietary fat on atherosclerosis development. Even though some controversy still exists, the general agreement is that dietary saturated fat will lead to elevated plasma cholesterol concentrations. Lipid content and rate of accretion of lipid in tissues, however, are more directly related to atherosclerosis development than is plasma
cholesterol and need to be studied in addition to plasma cholesterol concentrations.

Because of the interest on dietary fat in atherosclerosis development, effect of dietary fat on homeostasis of other metabolites, such as plasma glucose and insulin, largely have been ignored. Limited studies have demonstrated that different degrees of unsaturation of dietary fats will influence binding and action of insulin (Ginsberg et al., 1982; McCaleb and Donner, 1981; Gould et al., 1982) and concentrations of plasma insulin (Hulsmann and Kort, 1983; Weekes et al., 1986). Thus, dietary fats with different degrees of unsaturation may be important in regulation of glucose homeostasis in normal and diabetic animals.

The objectives of the present study were 1) to determine if different types of dietary fat will influence glucose homeostasis in normal and diabetic pigs, 2) to elucidate the mechanism for hypercholesterolemia and hypertriglyceridemia in normal pigs fed saturated fat, 3) to elucidate the mechanism for hypercholesterolemia and hypertriglyceridemia in diabetics and 4) to determine if different types of dietary fat will influence lipid homeostasis in diabetics.
REVIEW OF LITERATURE

Lipoprotein Metabolism

Lipids are transported in plasma of warm-blooded animals and birds as lipoproteins, which are water-soluble particles comprised of nonpolar lipids, polar lipids, and protein (Havel, 1972). The nonpolar components, triglyceride and cholesterol esters, are located in a hydrophobic core of the particle. Polar lipids, cholesterol and phospholipids, and protein form a hydrophilic shell around the hydrophobic core, making the insoluble lipid water-soluble (Havel, 1972).

There are several classes of lipoproteins: very low density lipoproteins (VLDL), chylomicrons, intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Each lipoprotein class is characterized by amount of lipid and protein and, thus, by density.

**VLDL and chylomicron composition**

The two classes of lipoproteins that are the primary carriers of triglycerides are VLDL and chylomicrons. Very low density lipoproteins transport endogenous triglycerides from liver to extrahepatic tissues. They are comprised of about 90% lipid (60% triglyceride, 12% cholesterol, and 18%
phospholipid) and 10% apolipoprotein (apoB, apoC, and apoE) and have a density in the range of 0.96-1.006 g/ml.

Chylomicrons transport dietary triglyceride from the small intestine to the rest of the body. Chylomicrons are comprised of about 98% lipid (90% triglyceride, 5% cholesterol, and 3% phospholipid) and 2% apolipoprotein (apoB and apoC) with a density less than 0.96 g/ml (Scanu, 1983).

**VLDL and chylomicron synthesis and secretion**

Very low density lipoproteins are synthesized most often in the liver. A small percentage of VLDL is synthesized in the small intestine, primarily during fasting (Byers and Friedman, 1960; Cendella et al., 1974).

Synthesis and secretion of VLDL are similar in the two tissues and occur by way of a series of membrane-bound compartments similar to those for other secretory proteins (Alexander et al., 1976). Apolipoproteins of VLDL are synthesized in the rough endoplasmic reticulum and are transported to smooth endoplasmic reticulum where the apolipoproteins associate with non-polar lipids, primarily triglycerides (Janero and Lane, 1983). The VLDL-like particles transverse to the Golgi apparatus where phospholipids and cholesterol are added to the outer shell. In Golgi-derived secretory vesicles, nascent VLDL are
shuttled to the plasma membrane and released into blood (Janero and Lane, 1983; Higgins and Hutson, 1984; Janero et al., 1984).

Chylomicrons are synthesized in small intestine. Components of this lipoprotein are derived primarily from exogeneous, dietary sources. During fasting, however, lipids and proteins are synthesized in the small intestine and used for VLDL synthesis (Shiau et al., 1985). Chylomicrons and VLDL of mucosal cells are synthesized and packaged similarly to VLDL of hepatocytes; the two intestinal lipoproteins are packaged and secreted into the lymphatic system separately from one another (Thomson, 1978; Tso et al., 1984).

Many effectors of VLDL and chylomicron synthesis exist, the most important being substrate availability. Hepatic fatty acids (Reaven and Greenfield, 1981; Fukuda and Ontko, 1984; Kazumi et al., 1985) and phospholipids (Mansbach et al., 1985) are the primary regulators of VLDL synthesis and secretion; insulin is required as a permissive factor for VLDL secretion (Reaven and Greenfield, 1981). Primary effectors of chylomicron synthesis are dietary sources of lipid (Zilversmit, 1978).
**VLDL and chylomicron catabolism**

Catabolism of VLDL and chylomicrons, like their synthesis, is a multi-step process. The enzyme that catalyzes hydrolysis of a majority of the triglyceride in lipoproteins is lipoprotein lipase (LPL; EC 3.1.1.3). Lipoprotein lipase is synthesized intracellularly and secreted to the endothelial lining of blood vessels of several tissues, such as adipose tissue, heart, skeletal muscle, and aorta. As apoC-II-containing lipoproteins, VLDL and chylomicrons, pass by LPL, they become bound to the enzyme, which is located in the lumen of blood vessels. ApoC-II-activated LPL catalyzes hydrolysis of triglycerides of lipoproteins to monoglycerides and free fatty acids. The hydrolyzed products of LPL activity cross the endothelial lining of blood vessels, enter the cells, and are stored as triglycerides or are oxidized. The removal of triglycerides from VLDL and chylomicrons does not occur all at once. Several different LPL molecules, located at different areas of the body, are involved in the hydrolysis of one lipoprotein particle (Cryer, 1981; Quinn et al., 1982).

Hepatic lipase is another enzyme, similar to LPL, that plays an important role in catabolism of triglyceride-containing particles. This enzyme, which is similar to LPL, is located on endothelial lining of
capillaries in liver. Hepatic lipase catalyzes hydrolysis of triglycerides of VLDL and chylomicrons similarly to LPL (Murase and Itakura, 1981), but its exact role in metabolism of triglyceride-carrying lipoproteins is still relatively unclear (Kinnunen, 1984).

As VLDL undergo delipidation by LPL, they become more dense; dense VLDL are IDL. The constituents of IDL are the same as VLDL; the difference between the two lipoproteins is that IDL have less triglyceride than do VLDL. As more triglycerides are removed from the core of the IDL, by way of LPL activity, the outer shell of the lipoprotein begins to buckle and fold into a star-like configuration. The projections, comprised of protein, phospholipids, and cholesterol, are transferred to HDL, leaving smaller, spherical lipoproteins. The composition of this new, denser lipoprotein is that of LDL (Hamilton, 1978; Eisenberg, 1984).

Conversion of VLDL to IDL and finally to LDL occurs in plasma of normal humans (Bilheimer et al., 1972; Sigurdsson et al., 1975; Reardon et al., 1978; Reardon and Steiner, 1982). In most other animals, such as pig (Hannan et al., 1980; Huff and Telford, 1985) and rat (Baker and Schotz, 1964) and in hypertriglyceridemic humans (Oschry et al., 1985), IDL are removed from plasma primarily by the liver;
LDL subsequently are synthesized and secreted from liver as newly synthesized particles.

Catabolism of chylomicrons is similar to that of VLDL. Lipoprotein products of chylomicron hydrolysis are chylomicron remnants and are "mirror images" of IDL and LDL (Cryer, 1981). Chylomicron remnants are taken up intact by tissues of most animals (Lippiello et al., 1985; Van Lenten et al., 1985).

Removal of VLDL, chylomicrons, and their products from the blood stream occurs by way of receptor-dependent and receptor-independent processes. Little is known about the receptor-independent process. There are three major receptor-dependent processes, and they are distinguishable from one another by their specificity for certain apolipoproteins (Mahley and Innerarity, 1983; Shepherd and Packard, 1986).

The LDL receptor is probably the most widely studied lipoprotein receptor. It is an apoB,E receptor and has been described in detail by Brown and Goldstein (1984). Chylomicron remnants and IDL, in addition to LDL, bind to this receptor (Bradley and Gianturco, 1986). Receptors for LDL are present in all tissues except nervous tissue (Miller, 1979), with a majority of receptor activity occurring in the liver (Dietschy et al., 1983; Dietschy,
Because most receptor activity is in the liver, the main route of cholesterol removal occurs by way of the liver as bile acids, which are synthesized primarily in liver (Salen and Shefer, 1983).

A second type of receptor involved in VLDL removal from plasma binds and internalizes VLDL before delipidation. These receptors have been found on hepatocytes (Gustafson et al., 1985). Little research has been done to determine which apolipoproteins are required for binding of lipoproteins to this receptor.

A third type of receptor that removes triglyceride-laden lipoproteins binds chylomicron remnants and cholesterol-laden VLDL. These receptors are found on hepatocytes and possibly macrophages and are specific for apoE (Mahley et al., 1981; Hui et al., 1984; Jones et al., 1984; Van Lenten et al., 1985). For an excellent review on the role of lipoprotein uptake and processing by liver, see the article written by Havel (1985).

Because the mechanism of LDL binding to the LDL receptor, internalization of LDL, and processing of LDL by cells is the most elucidated lipoprotein receptor mechanism, it will be the only mechanism reviewed here. After lipoproteins bind to receptors, lipoprotein receptor complexes aggregate in clathrin-coated pits. The pits
undergo endocytosis and fuse together. Because of the acidic nature of the newly formed endosomes, lipoproteins dissociate from receptors. Most receptors are recycled to the plasma membrane, and lipoproteins are transported to lysosomes where they are catabolized fully. The resultant amino acids, fatty acids, glycerol, and cholesterol are either used by the cell or are repackaged as new lipoprotein particles (Havel, 1985). Lipoprotein-derived cholesterol released from lysosomes is important in that it will regulate both de novo synthesis of cholesterol and translation of mRNA for LDL receptor by the cell (Brown and Goldstein, 1978, 1983).

**High density lipoprotein metabolism**

High density lipoproteins are synthesized and secreted by liver. Nascent, disc-shaped HDL contain little lipid and protein; they acquire most of their constituents from other tissues and from other lipoproteins. High density lipoproteins obtain free cholesterol from tissues, free cholesterol and phospholipids from VLDL, and apoA from chylomicrons. The esterification of HDL cholesterol to form cholesterol esters is catalyzed by lecithin cholesterol acyltransferase (LCAT; EC 2.3.1.43), an enzyme located on the lipoprotein. As HDL acquire more cholesterol and cholesterol esters, the discoidal shape becomes more
spherical. Subsequently, some of the cholesterol esters are transferred from HDL to VLDL and chylomicron remnants by transfer proteins, making the HDL discoidal again. High density lipoproteins also become discoidal after removal of cholesterol esters by liver (Hamilton, 1978; Rudel et al., 1983; Eisenberg, 1984).

**Pig as a Human Model**

The domestic pig (*Sus domesticus*) has been used by many as a model animal for several human diseases because of the similar physiology and anatomy of digestive and cardiovascular systems of pigs and humans (Dodds, 1982). Specifically, pigs have been used as model animals in studies on obesity (Houpt et al., 1979) and atherosclerosis (Vesselinovitch, 1979). Their relatively large size allows one to take repeated blood samples from the same animal and allows for samples of small tissues, such as aorta.

Vesselinovitch (1979) has reviewed the usefulness of pigs as an animal model for humans in atherosclerosis and lipoprotein metabolism. Pigs develop atherosclerotic lesions both spontaneously (Gottlieb and Lalich, 1954; Skold and Getty, 1961) or as a result of dietary manipulations (Reiser et al., 1959; Reitman et al., 1982). The lesions formed in pigs are similar in composition and formation to those in humans (Fritz et al., 1980). Fidge (1973) and
Terpstra and Beynen (1984) have found that composition of lipoproteins of pigs is very similar to that of humans, and, when fed a hypercholesterolemic diet, changes in lipoprotein composition of pigs occur that are similar to changes in humans fed high cholesterol diets (Reitman and Mahley, 1982).

There are also some disadvantages to the use of pigs as a model animal for lipid disorders in humans. Pigs synthesize a majority of their fatty acids in adipose tissue (O'Hea and Leveille, 1969; Huang and Kummerow, 1976), and humans synthesize most of their fatty acids in liver (Patel et al., 1975). Thus, VLDL synthesis and secretion are more extensive in humans. Also, humans have two transfer proteins that shuttle triglycerides and cholesterol esters between lipoprotein classes; a different transfer protein is specific for each lipid (Hopkins and Barter, 1980). Pigs do not have transfer proteins for either triglycerides or cholesterol esters (Barter et al., 1982). Finally, in normal humans, most VLDL is converted to LDL in plasma. In pigs, LDL are synthesized in liver and secreted as nascent particles into the blood stream (Sigurdsson et al., 1975; Reardon et al., 1978; Huff and Telford, 1985). In a recent review of literature by Havel (1984), however, it was concluded that humans may be more like other animals than
first believed, and humans may synthesize nascent LDL particles in liver like other animals.

The pig also has been used as a model animal for studies in type I diabetes in humans with the use of alloxan. Treatment with alloxan will result in preferential destruction of beta cells and will result in elevated plasma glucose concentrations, elevated plasma free fatty acid concentrations, and decreased plasma insulin concentrations (Romsos et al., 1971; Gabel et al., 1985).

Atherosclerosis Development

Atherosclerosis is any process that affects the arterial wall either through alterations in intracellular components, such as triglycerides and cholesterol esters, or alterations in extracellular components, such as collagen and fibrin. Early arterial wall damage involves mostly intracellular changes, whereas severe plaque formation involves a combination of intracellular and extracellular changes. Atherosclerotic plaque development in humans is normally a slow process. For reviews on atherosclerosis development, see Ganda (1980), Fielding (1981), and Moore (1985).

The first step in development of plaque is a breakdown of the endothelial lining, which acts as a barrier between blood and smooth muscle cells. Blood constituents, such as
lipoproteins and platelets, enter the damaged arterial wall and come in contact with smooth muscle cells (Schwartz, 1980). Platelets aggregate at the damaged area and release a platelet-derived growth factor that stimulates cell proliferation (Schwartz and Ross, 1984). As smooth muscle cells proliferate, they also accumulate lipids. Macrophages enter the damaged area and also begin to accumulate lipids. Thus, foam cells, which are lipid-laden smooth muscle cells and macrophages, begin to develop (Brown and Goldstein, 1984).

Lipid accretion occurs in smooth muscle cells and macrophages by several mechanisms. Lipoprotein receptors are present on both smooth muscle cells and macrophages; smooth muscle cells have apoB,E receptors and macrophages have apoB,E and apoE receptors (Brown and Goldstein, 1983; Van Lenten et al., 1985). In addition to functional LDL receptor activity, smooth muscle cells have functional LPL activity. Zilversmit (1973, 1979) believes that LPL activity in this vicinity is a leading contributor to atherosclerosis development. The hypothesis is that remnants are liberated in the vicinity of arterial endothelium after hydrolysis of VLDL and chylomicrons by LPL and subsequently are taken up by receptors on smooth muscle cells and macrophages (Pitas et al., 1983). Uptake of
remnants would be enhanced because of the high concentration of remnants in the vicinity of the receptors (Zilversmit, 1973).

Effects of Different Dietary Fats on Lipoprotein Metabolism and Atherosclerosis

Coronary heart disease, specifically atherosclerosis, is the leading cause of death in the U. S. Hypertriglyceridemia and hypercholesterolemia often times occur in conjunction with atherosclerosis and are considered to be two major risk factors of the disease. Different ways to manipulate plasma triglyceride and cholesterol concentrations, such as by diet, always are being evaluated to find a "cure" for atherosclerosis (Havel, 1983; Kritchevsky, 1983; Samuel et al., 1983).

Studies on effect of amount and type of dietary fat on plasma lipid concentrations and composition started in the mid-1950s. Nichols et al. (1957) and Hatch et al. (1955) demonstrated that high carbohydrate, low fat diets resulted in hypertriglyceridemia in which VLDL were the primary triglyceride carriers (Liu et al., 1983; Kazumi et al., 1985). Evidently, both triglyceride and apolipoprotein synthesis is elevated in hepatocytes of animals fed high carbohydrate, low fat diets, which result in increases in
VLDL synthesis and secretion (Boogaerts et al., 1984; Kazumi et al., 1985).

Occurring concurrently with an increase in VLDL secretion in animals fed high carbohydrate, low fat diets is an increase in removal of VLDL by tissues. Activity of LPL in white adipose tissue (Pokrajac and Lossow, 1967; deGasquet et al., 1977; Granneman and Wade, 1983) and uptake of triglyceride-containing lipoprotein remnants by liver (Kortz et al., 1984) was increased in animals fed high carbohydrate, low fat diets, resulting in increased removal of VLDL-triglyceride.

Effect of type, in addition to amount, of dietary fat on plasma triglyceride concentrations also has been evaluated. Baudet et al. (1984), Oh and Monaco (1985), Shepherd et al. (1980), and Schonfeld et al. (1982) found a decrease in plasma triglyceride concentrations in animals fed diets high in polyunsaturated fatty acids (PUFA). The changes in plasma triglycerides most often occurred as VLDL. Still unknown is whether differences in VLDL-triglyceride concentrations in animals fed different types of fat are the result of changes in secretion or removal of VLDL. Several investigators have studied effects of type of dietary fat on LPL activity; increases (Bagdade et al., 1970, Cryer et al.,
1978), decreases (Paik and Yearick, 1978), or no changes (Lawson et al., 1981) have been found.

Effect of type of dietary fat on plasma cholesterol concentrations has caused much more of a controversy than has effect of type of dietary fat on plasma triglyceride concentrations. It is commonly accepted that dietary PUFA lower plasma cholesterol; however, which lipoprotein class this change occurs in is still a widely-disputed topic. Grundy and Ahrens (1970) and Oh and Monaco (1985) have shown that diets high in PUFA will result in a decrease in LDL-cholesterol only. These results are not consistent with others, however, because Mattson and Grundy (1985), Jackson et al. (1984), and Rudel et al. (1983) have shown that high PUFA diets result in decreases in both LDL-cholesterol and HDL-cholesterol. Brussard et al. (1980) found an increase in HDL-cholesterol with a decrease in LDL-cholesterol of PUFA-fed animals.

In addition to effects on cholesterol and triglyceride concentrations, type of dietary fat affects composition of fatty acids and apolipoproteins of lipoproteins. Shore et al. (1983) found that fatty acid composition of lipoproteins follows the same pattern as does the fatty acid composition of diets. In addition, protein content, specifically apoB, of VLDL are elevated in animals fed saturated fat (Baudet et
al., 1984; Renner et al., 1986); lipoproteins from animals fed different fats even appear structurally different when observed with an electron microscope (Feldman et al., 1983). Lipoproteins from animals fed saturated fat were flatter when compared with lipoproteins from animals fed PUFA.

Similar to that of lipoproteins, fatty acid composition of plasma membranes is influenced by type of dietary fat. Composition of fatty acids of plasma membranes resemble the fatty acids of the diet (Blomstrand et al., 1985; Popp-Snijders et al., 1986). Differences in fatty acid composition and fluidity of plasma membrane will affect intramembrane protein function, such as lipoprotein receptor, or passive diffusion of fatty acids and cholesterol across plasma membranes and, thus, could have a large influence on lipoprotein metabolism. Recently, Spady and Dietschy (1985) demonstrated that LDL receptor function was obliterated in animals fed saturated fat in comparison with animals fed PUFA. This change evidently was because of a change in plasma membrane composition.

Cholesterol content in membranes changes as a result of changes in fluidity of plasma membranes. Cholesterol is added to plasma membranes in order to make them more rigid (Kummerow, 1983; Spector and Yorek, 1985). In pigs (Forsythe et al., 1980), rats (Awad, 1981), and squirrel
monkeys (Lofland et al., 1970) fed PUFA, increased cholesterol was found in aortas when compared with animals fed saturated fat, and this change possibly occurred as the result of a change in plasma membrane composition in animals fed PUFA.

Insulin

Structure, biosynthesis, and secretion

Insulin is synthesized in the endoplasmic reticulum of pancreatic beta cells by a mechanism similar to that of other secretory proteins (Steiner et al., 1974). The leader sequence of preproinsulin is cleaved as it enters the endoplasmic reticulum. The resultant proinsulin is transported to the Golgi apparatus and packaged into secretory granules. While in the endoplasmic reticulum, the polypeptide is folded into the proper conformation of proinsulin. The internal section of proinsulin, C-peptide, is cleaved, leaving a protein (insulin) containing two chains of amino acids linked by two disulfide bridges. Insulin and C-peptide are released from beta cells in a 1:1 molar ratio. About 50% of the insulin is taken up and degraded during the first pass through the liver (Samols and Ryder, 1961; Rubenstein et al., 1972b). C-peptide is not taken up and degraded by liver, but by kidney (Rubenstein et
al., 1972a), and thus its concentration in blood can be used to estimate insulin secretion (Horwitz et al., 1975).

Several mechanisms have been proposed to account for release of insulin by pancreatic cells. Responsible for stimulation of insulin secretion is acidification of islet cells produced as a consequence of intracellular catabolism of nutrients (Deleers et al., 1985), cytosolic NADPH to NADP⁺ ratios (Sener et al., 1984), ionic calcium (Vydelingum et al., 1978; Phang et al., 1984), and arachidonic acid catabolism (Turk et al., 1985; Metz, 1985). Nutrient substrates, such as carbohydrate, protein, and fat, affect secretion of insulin either directly or indirectly. Carbohydrates, specifically glucose, affect insulin secretion more than any other compound. To be more precise, metabolism of intracellular glucose by pancreas, and not glucose per se or glucose binding to the beta cells, is coupled to insulin secretion (Grodsky et al., 1963; Garfinkel et al., 1984).

Oral glucose stimulates insulin secretion to a greater degree than does intravenously administered glucose; this difference is a result of oral glucose stimulating secretion of intestinal hormones, which also stimulate insulin secretion. The intestinal hormone with the most potent
ability to stimulate insulin secretion is gastric inhibitory polypeptide (GIP; Dupre et al., 1973).

Other types of dietary components, such as amino acids and fats, also affect insulin secretion more when given orally than when infused. Dietary protein and amino acids, especially arginine and leucine, stimulate insulin secretion both directly and by way of intestinal hormone secretion (Schulz et al., 1982; Yoros et al., 1982). Dietary fat stimulates insulin secretion only through an indirect mechanism by way of GIP (Falko et al., 1972; Hampton et al., 1983).

Insulin secretion is influenced by other hormones besides the aforementioned intestinal hormones. Hormones that mediate their activity by way of cAMP, such as glucagon and β-adrenergic agonists, stimulate insulin secretion by increasing intracellular concentrations of Ca²⁺ (Vydelingum et al., 1978; Phang et al., 1984; Korman et al., 1985). A good review on insulin secretion has been written by Pfeifer et al. (1981).

Actions of insulin

The first step in the action of insulin is to bind to receptors on plasma membranes. After binding to the receptor, the insulin-receptor complexes aggregate into coated pits and are taken into cells by endocytosis. Either
insulin is degraded in lysosomes and receptors re-exteriorized or the whole receptor-insulin complex is re-exteriorized (Marshall, 1985a; Marshall, 1985b).

Binding of insulin to the receptor, and not internalization, seems to be required for insulin to exert its effect on intracellular metabolism. A mediator, like cAMP, probably is involved in action of insulin. Several groups have isolated and partially characterized a metabolite believed to be the mediator of insulin action (Walaas and Horn, 1981; Jarett et al., 1982; Larner et al., 1982; Seals and Czech, 1982; Cheng and Larner, 1985; Parker and Jarett, 1985).

Insulin influences metabolism of both hepatic and extrahepatic tissues and results in an anabolic state in liver, adipose tissue, skeletal muscle, and heart. In liver, insulin stimulates glycolysis, glycogenesis, and lipogenesis. Specifically, insulin induces synthesis of glucokinase, allowing greater uptake of glucose into hepatocytes, and of pyruvate kinase, allowing for greater rates of glycolysis. Insulin promotes dephosphorylation of phosphorylatable enzymes, such as glycogen phosphorylase, glycogen synthase, phosphofructokinase, pyruvate kinase, pyruvate dehydrogenase, acetyl-CoA carboxylase, and β-hydroxy β-methylglutaryl-CoA reductase. These enzymes are
active when dephosphorylated except for glycogen phosphorylase, which is active when phosphorylated.

In addition to the direct effect of insulin on activity of enzymes involved in glycolysis, glycogenesis, and lipogenesis, insulin also has an indirect effect on these processes by enhancing formation of metabolites that allosterically modulate metabolite fluxes through several metabolic pathways. For example, intracellular concentrations of fructose-1,6-diphosphate, a positive allosteric effector of pyruvate kinase, pyruvate, a positive effector of pyruvate dehydrogenase, and citrate, a positive effector of acetyl-CoA carboxylase, are elevated when insulin is present; these changes help maintain hepatocytes in a state of anabolism.

In peripheral tissues, insulin has several effects. First, insulin enhances uptake of glucose by increasing activity of transport mechanisms. In addition, glucose utilization is enhanced by increasing rates of glycolysis and lipogenesis in adipose tissue and by increasing glycolysis and glycogenolysis in skeletal muscle. Insulin also stimulates triglyceride deposition in adipose tissue by increasing LPL activity and by inhibiting hormone-sensitive lipase activity, which is an enzyme that needs to become
phosphorylated to be active (Harris, 1982; Harris and Crabb, 1982).

**Counter-regulatory hormones**

The lowering of plasma glucose concentrations by insulin is regulated closely by several counter-regulatory hormones that help maintain plasma glucose concentration. The primary counter-regulatory hormones are glucagon, catecholamines, cortisol, and growth hormone (Eaton and Schade, 1978). Glucagon is the counter-regulatory hormone correlated most closely on a day-to-day maintenance basis with insulin (Unger, 1971). The main function of catecholamines, cortisol, and growth hormone are during times of stress and when plasma glucose is lowered by more than 20 or 30 mg percent (Eaton and Schade, 1978).

Glucagon affects metabolism of hepatic and extrahepatic tissues by stimulating the phosphorylation of key regulatory enzymes and switching tissues to a state of catabolism. Tissues are affected indirectly by glucagon because of the influence that glucagon has on allosteric effectors of metabolism. In liver, glucagon enhances concentrations of fatty acyl-CoA, acetyl-CoA, and citrate, which inhibit acetyl-CoA carboxylase, pyruvate dehydrogenase, and phosphofructokinase, respectively. Uptake of gluconeogenic precursors by liver, such as amino acids, is enhanced by
glucagon. The liver is supplied with more gluconeogenic precursors because glucagon stimulates protein catabolism in skeletal muscle. Furthermore, glucagon stimulates lipolysis, which will supply liver with more fatty acids to be oxidized for energy and with more glycerol to be used for gluconeogenesis. Thus, glucagon promotes the body to be in a state of catabolism as a mechanism to elevate plasma glucose concentration (Mackrell and Sokal, 1969; Harris, 1982; Harris and Crabb, 1982).

Diabetes

Diabetes has been classified as either type I or type II diabetes. Type I diabetes or juvenile-onset diabetes usually occurs in individuals under 25 years of age. This type of diabetes is associated with a lack of insulin, usually from a lack of functional beta cells. Patients with type I diabetes are said to have insulin-dependent diabetes mellitus (IDDM). Type II diabetes or maturity-onset diabetes usually occurs in individuals over 40 years of age. This type of diabetes commonly is associated with hyperinsulinemia, obesity, and insulin resistance. Patients with this type of diabetes are said to have noninsulin-dependent diabetes mellitus (NIDDM). About 5% of the total U.S. population is diabetic. Of this incidence,
about 10% of the diabetics are type I and 90% are type II (Coughlin and Kahn, 1986).

Definition of diabetes and development of type I diabetes

There are numerous defects in metabolism of diabetics, but the two most underlying defects are decreased uptake of glucose by peripheral tissues and increased hepatic gluconeogenesis. Diabetics are literally "starving in the midst of plenty" because, even though there is an excess of extracellular glucose, a deficiency of intracellular glucose exists.

The primary cause of the lack of functional insulin in patients with IDDM is a lack of functional beta cells in the pancreas from pathogenic (Rerup, 1970; Wilson et al., 1984), immunologic (Kannazawa et al., 1984; Ziegler et al., 1984), genetic (Leiter, 1984), or viral (Notkins, 1979) destruction of pancreatic cells. Lack of beta cells is not the only reason for a decrease of functional insulin in IDDM, however. In some individuals with IDDM, beta cells are functional; antibodies are made against insulin, rendering the secreted insulin ineffective (Tager, 1984; Palmer et al., 1983).

An excess of glucagon almost always accompanies the lack of insulin in type I diabetes because, without insulin, no inhibition of glucagon secretion occurs (Unger and Orci,
1981). The resultant high ratio of glucagon to insulin leads to a catabolic state of the body in an attempt to elevate plasma glucose. In untreated diabetics, triglycerides and fatty acids are oxidized for energy and result in an excess of acetyl-CoA, which is used for synthesis of ketone bodies. An excess of ketones in plasma lowers the pH of the blood and, if untreated, will lead to ketoacidosis. Exogeneous insulin, therefore, is essential for the diabetic to maintain pseudo-normal conditions and prevent ketoacidosis.

Often times, the amount of insulin required to maintain normal glucose concentrations in diabetics is greater than would be expected, implying that tissues are resistant to insulin. Pedersen and Hjollund (1982), DeFronzo et al. (1982), and Del Prato et al. (1983) have shown cells and tissues to be resistant to insulin in IDDM. Del Prato et al. (1983) and Corin and Donner (1982) have shown that the insulin resistance in IDDM is primarily because of a post-receptor defect. Pedersen and Hjollund (1982) have shown that the resistance is the result of a combination of a defect in post-receptor metabolism and in insulin binding.

Diabetes and Atherosclerosis

Atherosclerosis is the major cause of death in diabetics (Ganda, 1980). Several major risk factors for
atherosclerosis, such as hyperglycemia, hypertension, and hyperlipidemia, often are associated with both type I and II diabetes. Exogeneous insulin and dietary modifications will correct hyperglycemia to a large degree. Hyperlipidemia, on the other hand, is not corrected by exogeneous insulin to the same extent as is hyperglycemia (Sosendo et al., 1980; Glasgow et al., 1981).

In diabetic hyperlipidemia, the most consistent alteration in plasma lipids is an elevation in VLDL-triglycerides (Van Tol, 1977; Chen et al., 1979; Reaven and Greenfield, 1981; Briones et al., 1984), even though at times triglyceride and cholesterol concentrations are elevated in all lipoprotein classes (Bar-On et al., 1976; Wilson and Brown, 1978). Changes that occur in other lipoprotein classes, such as LDL-cholesterol, are most likely a secondary effect of increases in VLDL. After reviewing the recent literature, Chen et al. (1980) came to the conclusion that the cause of hypertriglyceridemia in diabetes is because of alterations in both removal and secretion of VLDL-triglycerides.

Very low density lipoprotein-triglyceride hydrolysis, and thus removal of VLDL-triglycerides from plasma, is catalyzed by LPL activity. Adipose tissue LPL activity, which is stimulated by insulin, removes a majority of plasma
triglycerides. A lack of LPL activity results in a decrease in removal of VLDL-triglycerides from plasma and, hence, leads to elevated triglyceride concentrations (Cryer, 1981). Because insulin stimulates LPL activity in adipose tissue, a lack of insulin results in a lack of LPL activity (Cryer, 1981). Nikkila et al. (1977) and Brunzell et al. (1979) demonstrated that type I diabetics have depressed activity of LPL in adipose tissue, which could contribute to formation of hypertriglyceridemia (Chen et al., 1979; Reaven and Greenfield, 1981). Treatment of diabetic patients with insulin results in an increase and decrease in LPL activity and plasma lipids, respectively (Taskinen et al., 1981).

The depressed LPL activity measured in diabetics can not entirely account for the elevation in plasma triglyceride concentrations (Chen et al., 1979); secretion of VLDL-triglycerides must be altered in diabetics also. Plasma free fatty acid concentration, which is elevated in diabetics, has a large influence on VLDL-triglyceride secretion rates. Insulin, however, must be present as a permissive hormone for this effect to occur (Woodside and Heimberg, 1972; Reaven and Greenfield, 1981). In patients with IDDM, there may be enough insulin to stimulate VLDL-triglyceride secretion when found in combination with
elevated free fatty acid concentration (Reaven and Greenfield, 1981).

Hyperlipidemia and hyperglycemia in diabetics are treated with insulin. Because insulin is administered subcutaneously, a much higher dose of insulin is administered than would have been if the insulin were administered intraportally. The resultant, peripheral hyperinsulinemia, given to control diabetic hyperglycemia and hyperlipidemia, actually could accelerate atherosclerosis development because insulin stimulates smooth muscle cell proliferation of arterial cells (Stout, 1981; Nakao et al., 1985); proliferation of smooth muscle cells is believed to be the first step in atherosclerosis development (Stout, 1981). Exogeneous insulin also stimulates lipogenesis in arterial cells, which results in further lipid accretion in those cells (Capron et al., 1984; Falholt et al., 1985).

Dietary treatment, type II diabetes formation, and control of type I diabetes

Just as diet is believed to influence atherosclerosis development, diet also could influence type II diabetes formation. The sequence of events leading to development of type II diabetes is not well elucidated. One theory is that diabetes formation begins as resistance of tissues to
insulin either by defects in insulin binding and/or post-receptor mechanisms (Olefsky, 1981; Orchard et al., 1983; Reaven, 1984). Hyperinsulinemia will follow insulin resistance to compensate for the defects in insulin function.

Alternatively, hyperinsulinemia could be the primary defect in diabetes development. Down regulation of receptors would follow the hyperinsulinemia (Soman and DeFronzo, 1980), which subsequently would lead to insulin resistance and to a more exaggerated case of hyperinsulinemia. Thus, any secretogues of insulin have the ability to potentiate NIDDM formation. In a recent study, rats fed saturated fat in comparison to rats fed PUFA had greater concentrations of plasma insulin in response to a meal. Greater concentrations of plasma GIP also were found in plasma of rats fed saturated fat (Hulsmann and Kort, 1983). Hulsmann and Kort (1983) have postulated that saturated fat may be absorbed from the intestine more slowly than PUFA and that GIP secretion is stimulated for a longer time period in animals fed saturated fat; these changes could lead to hyperinsulinemia.

In addition, type of dietary fat can affect insulin receptor function directly, thus affecting type II diabetes formation. As previously mentioned, the binding of insulin
to its receptor is the first step in activation of intracellular mechanisms. If the receptor is not functional because of changes in physical characteristics of the plasma membrane, there will be less efficient binding and subsequent loss of activation of intracellular metabolic processes by insulin (Amatruda and Finch, 1979; Olefsky, 1981).

Because type of dietary fat alters plasma membrane composition and characteristics (Blomstrand et al., 1985; Popp-Snijders et al., 1986), type of dietary fat can influence insulin binding and action. Several researchers have shown tissues with varying degrees of fatty acid unsaturation to have different insulin binding properties. Ginsberg et al. (1982) and Gould et al. (1982) have demonstrated that plasma membranes of erythrocytes with a greater percentage of lipid as saturated fatty acids had decreased insulin receptor number and increased binding affinity. No measure of insulin action, however, was reported in these studies. In cultured 3T3-L1 cells, decreased insulin binding and insulin action accompanied an increase of saturated fatty acids in membranes (Grunfeld et al., 1981). Fatty acid composition of hepatocytes also affects properties of the insulin receptor (McCaleb and Donner, 1981). Because tissues react differently to varying
degrees of unsaturation, tissues need to be studied separately.

In addition to using dietary regime to hinder NIDDM formation, dietary treatment is used to help maintain IDDM patients at pseudo-normal glucose and lipid concentrations. High carbohydrate diets improve glucose tolerance of diabetic patients or animals (Olefsky and Saekow, 1978; Simpson et al., 1979; Portha et al., 1982). High carbohydrate diets, however, result in an increase in plasma triglyceride and a decrease in plasma cholesterol concentrations in patients with IDDM (Hollenbeck et al., 1985). High fat diets also lead to insulin resistance, which is already a problem in diabetics (Lavau et al., 1979). Thus, the issue on amount of dietary fat is unresolved. Effect of dietary saturated or unsaturated fat in diabetics has not been studied as of yet. Reaven (1986) stated in a review on dietary treatment of diabetics, "Based upon the data reviewed in this presentation, it seems that this advice is, at best, unlikely to have any useful metabolic effect, and, at worst, may aggravate the defects in carbohydrate and lipoprotein metabolism present in diabetes. The only conclusion consistent with this point of view is that widespread changes in dietary recommendations be avoided until they can be strongly supported by rigorous
data. What is required is less advice and more information."
LITERATURE CITED


EXPLANATION OF DISSERTATION FORMAT

This dissertation has been prepared according to the alternate dissertation format as described in the Iowa State University Graduate College Thesis Manual. Use of this format involves the presentation of two or more sections, each of which is in a form suitable for publication in a scientific journal. Two independent papers have been prepared from the data collected from research performed in partial fulfillment of the requirements for the Ph.D. degree. A general discussion will follow the papers.
SECTION I. EFFECTS OF DIETARY BEEF TALLOW AND SOY OIL ON GLUCOSE AND INSULIN HOMEOSTASIS IN NORMAL AND DIABETIC PIGS
ABSTRACT

To evaluate whether dietary fats that are greatly different in saturation of fatty acids can alter glucose homeostasis in normal and diabetic animals, 6-week-old normal and alloxan-diabetic pigs were fed diets containing either beef tallow (saturated) or soy oil (unsaturated) as the primary source of fat for 6 weeks. After an intra-arterial and oral dose of glucose, pigs fed soy oil had greater plasma insulin concentrations than did pigs fed soy oil, even though pigs fed different dietary fats had similar concentrations of plasma glucose. Insulin sensitivity and glucose effectiveness were determined from the intra-arterial glucose tolerance test by using a mathematical model for glucose-insulin dynamics. Feeding beef tallow to normal pigs did not affect insulin sensitivity and increased glucose effectiveness (0.07 versus 0.05 min⁻¹) (P<0.03) when compared with normal pigs fed soy oil. After consumption of a meal, concentrations of triglyceride (P<0.01), free fatty acids (P<0.05), and gastric inhibitory polypeptide (GIP) (P<0.04) in plasma were greater in diabetic pigs than in normal pigs. No dietary differences were found in plasma triglyceride and free fatty acid concentrations after a meal in either normal or diabetic pigs. No dietary effect was detected for GIP
concentrations in normal pigs. Diabetic pigs fed soy oil had approximately 80% greater GIP concentrations for the 8 hours after the meal than did pigs fed beef tallow (P<0.05). Our results suggest that dietary saturated and unsaturated fat could be an important regulator of glucose homeostasis in normal and diabetic pigs.
INTRODUCTION

Although saturated and polyunsaturated dietary fats may affect processes related to glucose homeostasis, the mechanism by which degree of unsaturation of dietary fatty acids regulates plasma glucose concentrations is poorly understood. In rats, dietary saturated fat leads to both increases (Hulsmann and Kort, 1983) and decreases in insulin concentrations (Hennig and Dupont, 1983; Weekes et al., 1986). The differences in insulin concentrations between animals fed different fats could be a result of changes in secretion or action of insulin. Different types of dietary fat can affect insulin secretion through different insulin secretagogues; Hulsmann and Kort (1983) found that rats fed saturated fat have elevated concentrations of the insulin secretagogue, gastric inhibitory polypeptide (GIP), in response to a meal (Falko et al., 1975; Williams et al., 1981). Additionally, diet-induced alterations in fatty acid composition of plasma membranes could alter insulin action in tissues; increasing the degree of fatty acid unsaturation in plasma membrane of cultured cells (Ginsberg et al., 1981; Gould et al., 1982) and cells of intact animals (Ginsberg et al., 1982) increases receptor number but decreases receptor affinity for insulin in some, but not all (McCaleb and Donner, 1981; Bar et al., 1984), studies, thereby affecting
insulin action. Thus, dietary saturated and polyunsaturated fat could affect both insulin secretion and insulin action.

The objective of the present study was to determine the effect of a diet high in saturated fat (beef tallow) and a diet high in polyunsaturated fat (soy oil) on regulation of plasma glucose and insulin homeostasis. In addition, effect of type of dietary fat on glucose homeostasis with minimal influence of insulin were studied in pigs made diabetic by alloxan injection.
MATERIALS AND METHODS

Animals

Twenty crossbred male pigs were purchased in groups of four littermates at approximately 6 weeks of age. Five replicate experiments were conducted over a period of 12 months. Each replicate consisted of two normal and two diabetic pigs, with one pig in each group being fed beef tallow-containing diets and the other pig in each group being fed soy oil-containing diets. Before the experiment, pigs were housed in individual pens and offered a commercial starter feed and water ad libitum. When pigs weighed 18 kg, catheters were implanted into the femoral artery of each pig, with the catheter end positioned in the aortic arch. Four days after catheterization, 10 of the 20 pigs were injected intra-arterially with alloxan at a dose of 125 mg/kg body weight. Alloxan was administered as a 20% solution in a sodium citrate (0.1 M):sodium phosphate (0.2 M) buffer, pH 4.0 (Dawson et al., 1969).

Diabetic pigs were maintained by subcutaneous injection of porcine insulin that was administered immediately after each meal. Pigs were given 5-20 Units of Regular insulin (Iletin I Insulin, Lilly, Indianapolis, IN) with the morning meal. In the evening, pigs were given 5-25 Units of Regular
insulin and 10-40 Units of NPH long-lasting insulin (Iletin I Insulin, Lilly, Indianapolis, IN).

Insulin doses were usually less than required for the two days after diabetes induction to insure that hypoglycemia did not occur; initial doses consisted of 8 Units of Regular insulin and 12 Units of NPH insulin. For the first week after diabetes induction, insulin doses were adjusted daily. If plasma glucose concentrations were greater than 240 mg/dl plasma, insulin doses were increased 2 Units with each meal until the proper insulin dose was reached; if plasma glucose concentrations were less than 240 mg/dl plasma, insulin doses were decreased 4 Units. After 1 week, insulin doses were adjusted on a weekly basis.

Diets

One week after catheterization of normal pigs and 1 week after alloxan-treatment of diabetic pigs, dietary treatments were begun. Dietary ingredients are shown in Table 1. Pigs were fed corn-based diets supplemented with either beef tallow or soy oil. Lean ground beef was the primary source of protein. Fat made up 41% of total calories; carbohydrate and protein made up 42% and 17% of total calories, respectively. Vitamins and minerals were supplemented to meet requirements established by the National Research Council (1979) for growing pigs.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef tallow</td>
<td>Soy oil</td>
</tr>
<tr>
<td>Ground shelled corn</td>
<td>61.00</td>
<td>61.00</td>
</tr>
<tr>
<td>Ground beef&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.60</td>
<td>18.60</td>
</tr>
<tr>
<td>Soybean oil&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.00</td>
<td>15.30</td>
</tr>
<tr>
<td>Beef tallow&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.30</td>
<td>0.00</td>
</tr>
<tr>
<td>Crystalline cholesterol&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Vitamin-mineral supplement&lt;sup&gt;e&lt;/sup&gt;</td>
<td>5.08</td>
<td>5.07</td>
</tr>
</tbody>
</table>

<sup>a</sup>Iowa State University Meats Laboratory, Ames, IA.

<sup>b</sup>Edsoy, Staley Mfg. Co., Decatur, IL, donated through the courtesy of T. Bessler.

<sup>c</sup>Beef tallow was prepared by Iowa State University Meats Laboratory, Ames, IA.

<sup>d</sup>Crystalline cholesterol was purchased from J.T. Baker Co., Phillipsburg, NJ.

<sup>e</sup>Supplements were added so that diets contained these nutrient concentrations, expressed as percentage or amount/kg diet (dry-matter basis): Ca, 1.28%; P, 1.11%; Na, 0.33%; Cl, 0.49%; K, 0.32%; Mg, 0.09%; Fe, 121.9 mg; Zn, 174.1 mg; Mn, 57.5 mg; Cu, 11.6 mg; I, 9.3 mg; Se, 0.2 mg; retinol, 22130 IU; cholecalciferol, 3410 IU; α-tocopherol, 32 IU; 2-methyl-1,4-naphthoquinone, 13.7 IU; riboflavin, 18.5 mg; niacin, 123.0 mg; pantothenic acid, 56.4 mg; cyanocobalamin, 136.7 mg; choline, 1388 mg; thiamine, 6.2 mg; pyridoxine, 8.2 mg; biotin, 0.1 mg; and folacin, 0.8 mg.
Crystalline cholesterol was added to diet to simulate amount of cholesterol that average adult Americans consume (Fisher et al., 1985) and to equalize amount of cholesterol fed to pigs on the two diets. Pigs were fed twice daily, and water was available at all times. Amount of feed was adjusted weekly so that daily consumption was 40 g dry matter per kg body weight.

Nutrient composition of diets is shown in Table 2. Samples of diets were collected every month and stored at -20°C. Protein, fat, and cholesterol concentrations were determined in lyophilized samples.

Intra-arterial Glucose Tolerance Tests

Glucose tolerance tests were performed on pigs after 6 weeks of dietary treatment. Diabetic pigs received their final injection of Regular insulin 24 hours and NPH insulin 40 hours before the tolerance test. Two blood samples were taken approximately 15 minutes before the injection of glucose to determine basal glucose and insulin concentrations. Glucose in a 50% solution (350 mg/kg body weight) was injected through the femoral artery catheter into the aorta at approximately 8 a.m. Serial blood samples were taken from the arterial catheter for the next 3 hours; samples were taken every minute for the first 19 minutes,
Table 2. Intake and nutrient composition of diets\textsuperscript{a}

<table>
<thead>
<tr>
<th>Component analyzed</th>
<th>Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beef tallow</td>
</tr>
<tr>
<td>Dry matter (DM), %</td>
<td>64.4</td>
</tr>
<tr>
<td>Intake, g DM/kg bwt\textsuperscript{b} * day</td>
<td>40.0</td>
</tr>
<tr>
<td>Gross energy, kcal/kg DM</td>
<td>5242.0</td>
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<tr>
<td>Protein, % DM</td>
<td>20.1</td>
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<tr>
<td>Fat, % DM</td>
<td>20.8</td>
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<tr>
<td>Ash, % DM</td>
<td>4.2</td>
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<tr>
<td>Cholesterol, % DM</td>
<td>0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Component</th>
<th>Calculated percentage of calories</th>
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<tbody>
<tr>
<td>Corn</td>
<td>49.8</td>
</tr>
<tr>
<td>Ground beef</td>
<td>19.3</td>
</tr>
<tr>
<td>Soy oil</td>
<td>0.0</td>
</tr>
<tr>
<td>Beef tallow\textsuperscript{c}</td>
<td>30.9</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Polyunsaturated fat to saturated fat ratio (w/w) was calculated from published values of fatty acid composition of soybean oil, beef tallow, and corn oil (Naito, 1982). Ratio for beef tallow diet was 0.65, and ratio for soy oil diet was 3.41.

\textsuperscript{b}bwt = body weight.
every other minute until 59 minutes, every 10 minutes until
120 minutes, and then at 150 and 180 minutes after glucose
injection. Blood was placed into centrifugation tubes
containing disodium ethylenediamine tetraacetate (EDTA; 15
mg/10 ml blood) and sodium fluoride (6 ug/10 ml blood).
Plasma was recovered by centrifugation at 7700 x g for 20
minutes at 4°C and was stored at -20°C until analysis.

Insulin Sensitivity and Glucose Effectiveness
A mathematical model of glucose-insulin dynamics,
developed by Bergman et al. (1979), was used to determine
insulin sensitivity and glucose effectiveness from
intra-arterial glucose tolerance studies done in normal
animals. Model parameters were evaluated by using two IMSL
subroutines from the IMSL Library. A Runga-Kutta routine
(DVERK) (IMSL, 1984a) was used to numerically integrate the
differential equations, whereas a Marquardt-Levenberg
regression routine (ZXSSQ) (IMSL, 1984b) fitted the model
unknowns. Three parameters were determined: insulin
sensitivity, glucose effectiveness, and initial glucose
concentration.

The same model was used to characterize the response of
diabetic pigs during glucose tolerance studies, except that
the endogenous insulin contributions were set to zero. This
model was integrated analytically, and values of glucose
effectiveness and initial glucose concentration were determined by using the NLIN program of the Statistical Analysis System (SAS, 1982).

Response to a Meal Study

After 7 weeks of dietary treatment, "response to a meal" studies were performed on pigs. Diabetic pigs received their final injection of Regular insulin 24 hours and NPH insulin 40 hours before the response to a meal study. Basal blood samples were collected 15 minutes before feeding. At approximately 8 a.m., pigs were fed their normal morning meal. Blood was collected into centrifugation tubes containing EDTA, EDTA and sodium fluoride, or EDTA and Trasylol (500 IU/ml plasma, Mobay Chemical Company, New York, NY). Samples were collected every 15 minutes for 2 hours starting 15 minutes after pigs began to consume their meal, every 30 minutes until hour 5, and then every hour until hour 8.

Plasma Membrane Fatty Acid Composition of Liver

Liver was collected after pigs were killed by magnesium sulfate injection. Liver was stored at -20°C until analysis. Plasma membranes were isolated by differential centrifugation (Hollenberg and Cuatrecasas, 1976), and fatty
acid composition of isolated plasma membranes was determined by gas-liquid chromatography (Arrendale et al., 1983).

Chemical Analyses

**Diet** Gross energy of diets was determined by bomb calorimetry (Parr Oxygen Bomb Calorimeter, Model 1231, Parr Instrument Co., Inc., Moline, IL). Protein content was determined by the Kjeldahl method (N x 6.25, Association of Official Analytical Chemists, 1975). Total fat content was determined gravimetrically after extraction with chloroform:methanol:water (10:20:8; v:v:v) (Bligh and Dyer, 1959), and cholesterol concentrations were determined enzymatically (Omodeo Sale et al., 1984).

**Plasma samples** Plasma glucose concentrations of all pigs were assayed enzymatically (Glucostat, Worthington Biochemicals, Freehold, NJ); plasma insulin concentrations were assayed by radioimmunoassay (Trenkle, 1970). Samples for assay of GIP were stored with Trasylol and were assayed by radioimmunoassay (Kuzio et al., 1974). Plasma samples that contained EDTA were assayed enzymatically for concentrations of triglycerides (Triglycerides 500 nm, Sigma Diagnostics) and free fatty acids (Shimizu et al., 1979).
Statistical Methods

Data were analyzed as a 2 x 2 factorial design. Data from glucose tolerance tests and response to a meal studies were analyzed by using a split-plot design with treatments as main plot effects and time as a subplot effect (Snedecor and Cochran, 1980b). Treatment mean squares were tested against mean squares for pigs within treatments to reduce animal-to-animal variation. Effects of time and treatment-by-time were tested against residual mean square by using conservative degrees of freedom. Differences in treatment-by-time effects indicated that the slopes of data curves were different between treatments. Insulin concentrations were measured only in normal animals, so, for this variable, treatments consisted only of the two types of fat. Data for concentrations of metabolites at each sampling time and composition of fatty acids were analyzed for main effects and interaction by using analysis of variance (Snedecor and Cochran, 1980a). Percentages of fatty acids also were analyzed with Duncan's multiple range test of the Statistical Analysis System (SAS, 1982).
RESULTS

Growth and Concentrations of Plasma Glucose and Insulin

Weekly body weights of pigs appear in Figure 1. Normal pigs gained weight at a faster rate than did diabetic pigs (P<0.02). Thus, at week 6, normal pigs weighed 25% more (P<0.01) than did diabetic pigs. Type of dietary fat did not affect weight gain in normal or diabetic pigs.

Plasma glucose and insulin concentrations were determined in 16-hour fasted pigs every 2 weeks after initiation of dietary treatment; data appear in Table 3. Even though diabetic pigs had been given exogenous insulin at the time of sampling, diabetic pigs had almost threefold greater plasma glucose concentrations than did normal pigs (P<0.01). Type of dietary fat had no effect on plasma glucose concentrations in normal or diabetic pigs. A trend was found (P<0.15) for plasma insulin concentrations of pigs fed beef tallow to be greater than those of pigs fed soy oil (4.88±0.91 versus 3.30±0.30 uUnits/ml) after 6 weeks of dietary treatment. Plasma insulin concentrations seem to increase either with age or dietary treatment.

Intravenous Glucose Tolerance Tests

Plasma glucose concentrations before and after an intra-arterial (IA) glucose injection in normal and diabetic
Figure 1. Growth of normal and diabetic pigs fed beef tallow- or soy oil-containing diets. Standard error of the mean (SEM) for all treatments at all time points is indicated.
Table 3. Plasma glucose and insulin concentrations in normal and diabetic pigs fasted 16 hours^a

<table>
<thead>
<tr>
<th>Type of pig</th>
<th>Dietary fat</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal pig</td>
<td></td>
<td>Beef tallow</td>
<td>96.2±7.5</td>
<td>98.2±4.2</td>
<td>103.6±9.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soy oil</td>
<td>93.7±9.0</td>
<td>99.9±8.1</td>
<td>98.3±6.5</td>
</tr>
<tr>
<td>Diabetic pig</td>
<td></td>
<td>Beef tallow</td>
<td>459±87</td>
<td>357±50</td>
<td>302±24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soy oil</td>
<td>435±56</td>
<td>290±20</td>
<td>281±18</td>
</tr>
<tr>
<td>Normal pig</td>
<td></td>
<td>Insulin (uU/ml)</td>
<td>1.75±0.58</td>
<td>2.91±1.00</td>
<td>3.36±0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Beef tallow</td>
<td>1.53±0.72</td>
<td>2.23±0.49</td>
<td>3.88±0.82</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Soy oil</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^aConcentrations are means ± SEM.
pigs are presented in Figure 2A. Data from only the first 60 minutes are presented; diabetic pigs had basal or zero time glucose concentrations that were approximately threefold greater than those of normal pigs (P<0.02). Furthermore, diabetic pigs removed IA glucose from plasma more slowly than did normal pigs (diabetes-by-time effect, P<0.01). Type of dietary fat did not alter the rate of removal of administered glucose in either normal or diabetic pigs.

Concentrations of plasma insulin in normal pigs in response to an IA dose of glucose appear in Figure 2B. Over the 180-minute sampling period, no significant effect of dietary fat or diet-by-time was observed in plasma insulin concentrations after an IA dose of glucose. Integrated area under the curve, which is representative of average insulin concentrations, tended (P<0.14) to be greater for pigs fed soy oil than those fed beef tallow (502±41 versus 362±76 uUnits/ml) for 0 to 23 minutes after IA injection of glucose. Concentrations of insulin in plasma, however, were different between pigs fed different fats at 1, 2, and 4 minutes after IA glucose and were 18.6±4.9 versus 43.6±16.3, 27.4±2.3 versus 40.5±8.7, and 18.5±5.4 versus 33.8±8.8 uUnits/ml for beef tallow- versus soy oil-fed pigs, respectively.
Figure 2. Plasma glucose and insulin concentrations after an IV dose of glucose in normal and diabetic pigs fed beef tallow- or soy oil-containing diets. Differences between normal pigs fed beef tallow and normal pigs fed soy oil are indicated by "a" (P<0.08). Standard error of the mean (SEM) for all treatments at all times is indicated.
Plasma insulin concentrations also were determined in diabetic pigs at 0, 4, and 59 minutes after IA glucose; insulin concentrations after the glucose injection were 1.11±0.11 versus 1.22±0.38, 1.14±0.10 versus 1.17±0.22, and 0.85±0.20 versus 1.16±0.28 uUnits/ml for beef tallow- and soy oil-fed diabetic pigs at 0, 4, and 59 minutes, respectively.

Mathematical Model for Glucose-Insulin Dynamics

Insulin sensitivity, glucose effectiveness, and initial glucose concentrations appear in Table 4. Insulin sensitivity in normal pigs was not influenced significantly by dietary treatment because of the large variation between pigs. Glucose effectiveness, the ability of elevated plasma glucose concentrations to affect the disappearance of plasma glucose, was 32% greater in normal pigs fed beef tallow than in normal pigs fed soy oil (P<0.03). No dietary effect was detected in diabetic pigs. Initial plasma glucose concentrations, determined as a parameter of the model and defined as extrapolation of the data curve for glucose disappearance to 0 time, also was greater (8.3%) for normal pigs fed beef tallow (P<0.06). No differences were detected in initial glucose concentrations of diabetic pigs fed different dietary fats.
Table 4. Insulin sensitivity, glucose effectiveness, and initial glucose concentrations of normal and diabetic pigs<sup>a,b</sup>

<table>
<thead>
<tr>
<th>Type of pig</th>
<th>Dietary fat</th>
<th>Insulin sensitivity (1000 x ml/min×uU)</th>
<th>Glucose effectiveness (min&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Initial glucose concentrations (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pigs</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Beef tallow</td>
<td>14.5±6.1</td>
<td>0.071±0.006**</td>
<td>292.6±7.1*</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>6.4±1.2</td>
<td>0.054±0.003**</td>
<td>270.3±7.1*</td>
</tr>
<tr>
<td>Diabetic pigs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Beef tallow</td>
<td>N.D.</td>
<td>0.032±0.012</td>
<td>576.0±41.8</td>
</tr>
<tr>
<td></td>
<td>Soy oil</td>
<td>N.D.</td>
<td>0.026±0.004</td>
<td>614.1±75.1</td>
</tr>
</tbody>
</table>

<sup>a</sup>Initial glucose concentrations are plasma glucose concentration determined after extrapolating the data curve for glucose disappearance to 0 time.

<sup>b</sup>Values are means ± SEM.

<sup>c</sup>N.D. = not determined.

*Dietary fat effects within type of pig are different (P<0.06).

**Dietary fat effects within type of pig are different (P<0.02).
Response to a Meal Study

Concentrations of plasma glucose after consumption of a meal in normal and diabetic pigs are presented in Figure 3A. Throughout the 480 minutes after the meal, plasma glucose concentrations were threefold to fourfold greater in diabetic pigs than in normal pigs (P<0.02). Furthermore, a diabetes-by-time interaction was detected (P<0.03); no effect of diet-by-time was found.

Insulin concentrations in plasma of normal pigs after consumption of experimental diets are shown in Figure 3B. Concentrations were greater in plasma of pigs fed soy oil than in pigs fed beef tallow at 240 (10.9+2.1 versus 19.2+3.5), 270 (10.7+1.4 versus 17.6+4.5), 300 (9.7+1.6 versus 17.1+3.2), and 420 (6.5+0.4 versus 10.1+1.6 uUnits/ml) minutes after consumption of the meal (P<0.07). Concentrations before 240 minutes were similar. No diet-by-time interaction was observed.

Plasma triglyceride concentrations after consumption of a meal are shown in Figure 4. Diabetic pigs had twofold to fourfold greater concentrations of plasma triglycerides than did normal pigs for 480 minutes after consumption of the meal (P<0.01). In addition, diabetic pigs had a more exaggerated response of plasma triglycerides to consumption
Figure 3. Plasma glucose and insulin concentrations after consumption of a meal of beef tallow- or soy oil-containing diets in normal and diabetic pigs. Differences between normal pigs fed beef tallow and normal pigs fed soy oil are indicated by "a" ($P<0.07$). Standard error of the mean (SEM) for all treatments at all times is indicated.
Figure 4. Plasma triglyceride concentrations after consumption of a meal of beef tallow- or soy oil-containing diets in normal and diabetic pigs. Differences between normal pigs fed beef tallow and normal pigs fed soy oil are indicated by "a" (P<0.08). Differences between diabetic pigs fed beef tallow and diabetic pigs fed soy oil are indicated by "b" (P<0.07). Standard error of the mean (SEM) for all treatments at all times is indicated.
of a meal than did normal pigs (P<0.05). Triglyceride concentrations at 0, 15, and 30 minutes after consumption of the meal were less in normal soy oil-fed pigs than in normal beef tallow-fed pigs (P<0.08); concentrations of plasma triglyceride were 137±35 versus 62±8, 155±65 versus 55±12, and 184±62 versus 62±3 mg/dl for beef tallow- and soy oil-fed pigs, respectively. The only dietary effect in diabetic pigs occurred at 180 minutes after consumption of the meal; pigs fed soy oil had greater concentration of triglyceride (P<0.09) than did pigs fed beef tallow.

Diabetic pigs had twofold to threefold greater concentrations of free fatty acids than did normal pigs throughout the 480 minutes after eating (P<0.05) (Figure 5). A significant diabetes-by-time interaction was observed (P<0.05) such that concentrations of plasma free fatty acids tended to increase in diabetics, whereas free fatty acid concentrations tended to decrease in normal pigs after consumption of the meal. No dietary effect was found in plasma free fatty acid concentrations of pigs during the 480 minutes after meal consumption.

Plasma GIP concentrations during the 480-minute period after the meal are presented in Figure 6. Diabetic pigs had approximately threefold greater concentrations of GIP than did normal pigs from 60 to 480 minutes after meal
Figure 5. Plasma free fatty acid concentrations after consumption of a meal of beef tallow- or soy oil-containing diets in normal and diabetic pigs. Standard error of the mean (SEM) for all treatments at all times is indicated.
Figure 6. Plasma GIP concentrations after consumption of a meal of beef tallow- or soy oil-containing diets in normal and diabetic pigs. Differences between diabetic pigs fed beef tallow and diabetic pigs fed soy oil are indicated by "a" (P<0.08). Standard error of the mean (SEM) for all treatments at all times is indicated.
consumption (P<0.04). Moreover, a diabetes-by-time interaction was observed in that GIP concentrations in plasma of diabetic pigs increased more than did those in normal pigs after consumption of a meal (P<0.05). Type of dietary fat did not influence GIP concentrations in normal pigs. Diabetic pigs fed soy oil, however, had greater average GIP concentrations (2084±235 pg/ml) than did pigs fed beef tallow (1407±183 pg/ml) (P<0.05).

Plasma Membrane Fatty Acid Composition

Composition of fatty acids in plasma membranes from livers of normal and diabetic pigs fed the two types of fat is presented in Figure 7. Fatty acids that were measured were palmitate (C16:0), palmitoleate (C16:1), stearate (C18:0), oleate (C18:1), and linoleate (C18:2). Type of dietary fat affected proportions of long-chained fatty acids in plasma membrane in all pigs. Pigs fed beef tallow had a greater percentage of membrane fatty acids as C16:0 (P<0.01), C16:1 (P<0.07), and C18:1 (P<0.001) and a lesser percentage as C18:0 (P<0.02) and C18:2 (P<0.001) when compared with pigs fed soy oil. Diabetic pigs had a greater percentage of fatty acids as C18:1 (P<0.03) than did normal pigs. Diabetes did not affect the percentage of C16:0, C16:1, C18:0, and C18:2. Total lipid and cholesterol concentrations in liver preparations were not determined.
Figure 7. Composition of long chain fatty acids in hepatic plasma membranes of normal and diabetic pigs fed beef tallow- or soy oil-containing diets. Data are presented as a weight percentage of total long-chained fatty acids measured. Within a specific fatty acid, bars with different letters are different (P<0.05). Standard error of the mean for each bar is indicated.
DISCUSSION

Response of plasma insulin concentration to exogenous stimuli was depressed in pigs fed beef tallow when compared with pigs fed soy oil; a smaller rise in plasma insulin concentrations in beef tallow-fed pigs was found in response to both intra-arterial glucose and a meal when compared with insulin concentrations of soy oil-fed pigs, even though plasma glucose concentrations were similar. Lower concentrations of plasma insulin, as found in beef tallow-fed pigs, are indicative of a decrease in secretion of insulin by pancreatic beta cells or an increase in removal of insulin from circulation by liver. Because insulin uptake and degradation by liver is fairly constant (Tranberg, 1979), changes in peripheral concentrations of insulin are representative of changes in insulin secretion, which are influenced by 1) insulin sensitivity of all tissues, 2) concentrations of insulin secretogues, and/or 3) sensitivity of pancreatic beta cells to insulin secretogues.

Insulin sensitivity is the amount of insulin needed to produce an effect and depends on binding of insulin to its receptor and the translation of insulin binding into action. If an animal is more insulin sensitive, less plasma insulin is required to achieve the same end metabolic result when compared with an animal that is less insulin sensitive.
Beef tallow-fed pigs had less plasma insulin and similar plasma glucose concentrations in response to exogenous stimuli and, thus, seemed to be more insulin sensitive than did soy oil-fed pigs. Quantitatively, however, insulin sensitivity, as determined by the mathematical model of glucose and insulin dynamics (Bergman et al., 1979), was similar between pigs fed different types of fat.

Insulin binding and action, and thus insulin sensitivity, can be influenced by fatty acid composition of plasma membranes (Amatruda and Finch, 1979; Grunfeld et al., 1981; Ginsberg et al., 1982; Gould et al., 1982; Bar et al., 1984). In the present study, fatty acid composition of plasma membranes from liver paralleled fatty acid composition of dietary fat such that pigs fed unsaturated fat had greater concentrations of linoleate and lesser concentrations of palmitate and oleate than did pigs fed saturated fat. Thus, a decrease in degree of unsaturation of plasma membrane fatty acids, as found in beef tallow-fed pigs, could lead to greater insulin sensitivity, possibly by way of a decrease in insulin receptor number and an increase in insulin binding to receptor as proposed by others (Ginsberg et al., 1981; Ginsberg et al., 1982; Gould et al., 1982).
The effect that plasma glucose has on its own removal from blood is just as important, if not more so, than is the effect of plasma insulin on glucose removal from blood (Ader et al., 1985). The ability of glucose to normalize its own concentration in plasma at basal concentrations of insulin is termed glucose effectiveness and can be quantified with the same mathematical model that is used to quantify insulin sensitivity (Bergman et al., 1979; Ader et al., 1985). In normal pigs, glucose effectiveness was greater in pigs fed beef tallow than in those fed soy oil, meaning that plasma glucose of pigs fed beef tallow are better able to inhibit glucose output by liver and/or enhance its own removal from plasma (Ader et al., 1985). The apparent qualitative difference in insulin sensitivity between pigs fed different types of fat actually could have been an artifact of differences in glucose effectiveness between pigs.

When exogenous insulin was removed from the system, as in diabetic pigs, no differences were detected in glucose effectiveness between pigs fed the two different types of fat; the same trend of dietary effect on glucose effectiveness in normal pigs also was present in diabetic pigs. A lack of any observed dietary effect on glucose effectiveness of diabetic pigs could have been a result of
the lack of sensitivity of the mathematical model because of low numerical values, making differences undetectable.

If beef tallow-fed pigs had similar action of insulin at lower concentrations, then concentrations of metabolites other than glucose, such as triglycerides and free fatty acids, should not differ between pigs fed the two diets. Indeed, after consumption of a meal, plasma triglyceride and free fatty acid concentrations were similar between pigs fed different types of fat. Plasma triglyceride concentrations of fasted beef tallow-fed pigs, however, were greater than were concentrations of fasted soy oil-fed pigs, even though plasma insulin concentrations were similar. This dietary-induced difference in basal triglyceride concentrations could have been a result of increased insulin sensitivity of liver or decreased activity of lipoprotein lipase activity in tissues of beef tallow-fed pigs than in soy oil-fed pigs (Bagdade et al., 1970; Cryer et al., 1978).

In addition to affecting insulin secretion indirectly by way of insulin sensitivity, different types of dietary fat also can affect insulin secretion by way of direct mechanisms. Dietary fat with different degrees of unsaturation could affect fatty acid composition of plasma membranes of beta cells of the pancreas, leading to changes in insulin or glucose receptor function, and could affect
secretion of gastrointestinal hormones, which, in turn, would stimulate insulin secretion; the most potent insulinotropic gastrointestinal hormone is GIP (Dupre et al., 1973; Falko et al., 1975). Hulsmann and Kort (1983) found that concentrations of plasma GIP paralleled plasma insulin concentrations and were greater in rats fed saturated fat in comparison with rats fed unsaturated fat.

In the present study, type of dietary fat influenced GIP concentrations differently in normal pigs than in diabetic pigs. Diet did not influence GIP concentrations in normal pigs; thus, influence of dietary fat on insulin secretion must have been through alterations of other less potent insulinotropic hormones or within the pancreatic beta cells. In diabetic pigs, those fed soy oil had greater concentrations of plasma GIP when compared with pigs fed beef tallow. Because plasma triglyceride concentrations did not vary to any large extent in diabetic pigs, dietary soy oil evidently stimulated GIP secretion to a greater degree than did dietary beef tallow in these diabetic animals. By deleting the negative feedback inhibition of insulin on GIP secretion in soy oil-fed normal pigs (Krurup et al., 1984), soy oil-fed diabetic pigs had greater GIP concentrations.

In summary, dietary beef tallow resulted in an increase in glucose effectiveness and a decrease in plasma insulin
concentrations in response to exogenous stimuli in normal pigs and a decrease in GIP concentrations after consumption of a meal in diabetic pigs. The overall results from the present experiment suggest that dietary saturated and polyunsaturated fats can be an important regulator of glucose homeostasis in normal and diabetic pigs.


SECTION II. EFFECTS OF DIABETES AND DIETARY SATURATED AND
UNSATURATED FATS ON VERY LOW DENSITY
LIPOPROTEIN-CHOLESTEROL HOMEOSTASIS
ABSTRACT

To study effects of diabetes and type of dietary fat on very low density lipoprotein-cholesterol (VLDL-CH) homeostasis, 6-week-old normal and alloxan-diabetic pigs were fed diets containing either beef tallow or soy oil as the primary source of fat. After 6 weeks of dietary treatment, pigs that had been fed every 4 hours for 48 hours were injected with autologous VLDL containing radioactively-labeled cholesterol. Blood samples were collected repeatedly for the first 30 minutes after injection of VLDL and less frequently thereafter. Twenty-four hours after injection, pigs were killed, and liver, skeletal muscle, adipose tissue, heart, and aorta were collected and analyzed. Diabetes caused half-lives of the primary component of VLDL-CH disappearance curves to double and half-lives of the secondary component to be 60-fold greater when compared with half-lives of normal pigs; pool sizes of both primary and secondary components of VLDL-CH disappearance curves were several times greater in diabetic than in normal pigs. The primary component probably represents VLDL-CH, and the secondary component probably represents VLDL-derived cholesterol in other lipoprotein classes and tissues in rapid equilibrium with VLDL-CH. In normal pigs, dietary beef tallow resulted in a
threefold increase in the half-life of both components of VLDL-CH disappearance and a similar pool size of both components in comparison with dietary soy oil. In diabetic pigs, feeding beef tallow fat resulted in a similar half-life of the primary component of VLDL-CH disappearance, a twofold smaller half-life of the secondary component, a threefold larger pool size of the primary component, and a similar pool size of the secondary component when compared with feeding soy oil. Diabetes resulted in greater accretion of plasma cholesterol in liver and other extrahepatic tissues analyzed and an increase in cholesterol content of liver and skeletal muscle. Feeding beef tallow resulted in greater accretion of plasma cholesterol by skeletal muscle and less uptake in carcass than did feeding soy oil. Net transfer of lipoprotein-cholesterol into tissues was influenced by diabetes or dietary fat only in liver where diabetes resulted in greater net transfer of lipoprotein cholesterol into liver when compared with normal pigs. In summary, diabetic pigs exchange VLDL-CH among vascular pools more slowly than do normal pigs, and net transfer of lipoprotein-cholesterol into tissues seems directly related to plasma cholesterol concentrations.
INTRODUCTION

Consumption of saturated fat is often associated with atherosclerosis (Mahley, 1979), primarily because of the hypocholesterolemic effect of dietary polyunsaturated fat when compared with dietary saturated fat. Because fatty acid composition of lipoproteins and tissues is altered by consumption of saturated fat (Baudet et al., 1984; Blomstrand et al., 1985; Renner et al., 1986; Woollett, Section I), diet-induced changes in the metabolism of lipoproteins could be responsible for observed changes in concentrations of plasma and tissue cholesterol and triglyceride (Cryer et al., 1978; Walsh et al., 1983; Baudet et al., 1984; Spady and Dietschy, 1985). Because VLDL are one of the two major triglyceride-containing lipoproteins, dietary fats have the potential to affect composition and metabolism of VLDL to a large extent (Green et al., 1984). Indeed, dietary saturated fat depresses disappearance of VLDL and chylomicrons when compared with dietary unsaturated fat (Nestel and Scow, 1964; Floren and Nilsson, 1977).

Lipoprotein metabolism also is regulated by insulin. Insulin influences plasma lipid concentrations primarily through regulation of VLDL metabolism (Chen et al., 1979; Reaven and Greenfield, 1981), and a lack of insulin is believed to be one of the primary reasons for decreased
catabolism of VLDL in diabetic animals (Chen et al., 1979; Reaven and Greenfield, 1981).

The present experiment was designed to study in vivo metabolism of VLDL-CH in nonfasted normal and diabetic pigs fed either beef tallow or soy oil. Metabolism of VLDL-CH was studied instead of the more commonly studied VLDL-TG because VLDL-CH is more of an indication of metabolism of the VLDL particle, including transfer of cholesterol from VLDL to other lipoproteins and tissues, whereas VLDL-TG metabolism is more of an indication of lipase activity (Chen et al., 1979; Reaven and Greenfield, 1981). In addition, effects of dietary fat on cholesterol deposition in normal and diabetic pigs were evaluated.
MATERIALS AND METHODS

Animals and Diets

Pigs and dietary treatments used in this study have been described in Section I.

Plasma and Lipoprotein Cholesterol and Triglyceride Concentrations

At weeks 0, 2, 4, and 6, pigs were fasted 16 hours, and arterial blood samples were collected from catheters of pigs. Diabetic pigs were maintained on exogenous insulin at the time of sampling. Disodium ethylenediamine tetraacetate (EDTA) was used as an anticoagulant, and plasma was collected by centrifugation. Lipoproteins were isolated from plasma by density gradient ultracentrifugation at 18°C (L8-70 ultracentrifuge, Ti 50.3 rotor, Beckman Instruments, Inc., Irvine, CA) (Havel et al., 1955). Protein (Bradford, 1976), cholesterol (Omodeo Sale et al., 1984), and triglyceride (Triglycerides 500 nm, Sigma Diagnostics) concentrations were determined in plasma, VLDL, LDL, and HDL.

VLDL Labeling

Forty-nine days after dietary treatment was begun, 35 ml of blood was collected from each pig after a 16-hour fast. Very low density lipoproteins were separated from
plasma by density ultracentrifugation (Havel et al., 1955). Even though pigs were fasted overnight, chylomicrons seemed to be present in this lowest density fraction because the cholesterol to triglyceride ratio was very low and, thus, was more similar to chylomicrons than to VLDL; henceforth, solutions of chylomicrons and VLDL will be referred to as VLDL.

About 50 uCi of [4-14C]-cholesterol (55.7 mCi/mmol, Amersham, Arlington Heights, IL) was incubated with VLDL following the protocol of Schwartz et al. (1978) with the following changes. Cholesterol had been tested previously for purity, and repurification was not necessary. Cholesterol, dissolved in 100 ul of ethanol, was added slowly to VLDL and incubated at 30°C for 30 min. The radioactively-labeled VLDL-CH solution was dialyzed for 18 hours against a solution of 0.01% EDTA:0.9% NaCl, pH 7.4. Autologous solutions of labeled VLDL were reinjected into pigs the same morning that VLDL were removed from dialysis. Purity of radioactively-labeled VLDL was verified by 1) staining the radioactively-labeled VLDL preparations, freshly prepared VLDL, and plasma with Sudan Black, 2) separating lipoproteins again by ultracentrifugation, and 3) comparing the banding of radioactively-labeled VLDL
preparations with those for freshly prepared VLDL and plasma (Terpstra et al., 1981).

VLDL Kinetics

To maintain steady state conditions for kinetic studies, pigs were fed one-sixth of their daily allotment of food every 4 hours from day 49 to day 52 after dietary treatment was begun. Diabetic pigs received injections of Regular insulin (Iletin I Insulin, Lilly, Indianapolis, IN) with every other meal on day 49. No injections of insulin were given on days 50, 51, and 52.

Two hours after the morning meal on day 51, 12 ml of radioactively-labeled VLDL were injected through the femoral artery catheter. Serial blood samples were collected at 1, 3, 5, 7, 9, 12, 15, 20, 30, 45, 60, 90, 120, 180, 240, 360, 720, and 1440 minutes after injection of radioactively-labeled VLDL. Plasma was collected, and lipoproteins were separated from each sample. Cholesterol concentrations (Omodeo Sale et al., 1984) were determined in all samples. Radioactivity was quantified in plasma, VLDL, LDL, and HDL by standard liquid scintillation counting techniques (Beckman LS-8000, Beckman Instruments, Inc., Irvine, CA) and using a commercial liquid scintillation cocktail (Beckman, Ready-Solv EP). Additionally, insulin
concentrations were determined in plasma of normal pigs at 0-time (Trenkle, 1970).

The decreases in specific radioactivities of VLDL-CH \((\text{dpm/mg VLDL-CH})/(\text{total dpm injected/kg body weight})\) with time were fitted to an exponential decay curve for a two-pool system for the first 60 minutes after injection of radioactively-labeled VLDL by using the NLIN program of the Statistical Analysis System (SAS, 1982). Data also were analyzed by using one- and three-pool models, but residual sum of squares were not improved over those from the two-pool model. Half-lives and pool sizes of VLDL-CH were determined from specific radioactivity decay curves (Shipley and Clark, 1972); half-lives were determined from overall turnover rates. Specific radioactivities of cholesterol in VLDL, LDL, and HDL also were determined from 60 to 1440 minutes post-injection.

**Tissue Analyses**

Twenty-four hours after injection of radiolabeled VLDL, pigs were killed by magnesium sulfate injection. After exsanguination, liver, aorta, heart, bilateral half-car cass (including head, skin, and hair but minus kidneys, spinal cord, and brain), and samples of skeletal muscle (longissimus dorsi) and subcutaneous adipose tissue were collected from each pig and weighed. Liver, heart, and
half-carcass were ground in a meat grinder, and subsamples were frozen with liquid nitrogen and pulverized in a blender.

Samples of each tissue were extracted with chloroform:methanol:water (10:20:8; v:v:v) (Bligh and Dyer, 1959). Amount of total lipid was determined gravimetrically. Lipids were saponified, and sterols were separated from saponified lipids by modifications of the procedure of Bachman et al. (1976). A known amount of $[^{14}C]$-cholesterol was added to each sample to determine recovery of cholesterol in the nonsaponified lipids. After heating lipid extracts at 55°C for 20 minutes in 2 ml of 95% ethanol and 2 ml of 50% KOH, 5 ml of hexane and 15 ml of water were added; then, sterols in the hexane layer were separated from saponified lipids in the aqueous fraction. The sterol fraction was dried, dissolved in absolute ethanol, and quantified enzymatically (Omodeo Sale et al., 1984).

Lipids from a second set of tissue samples were extracted with chloroform:methanol:water as before. Extracts were dried, and scintillation fluid was added to samples. Total radioactivity was quantified by standard liquid scintillation counting techniques. Net uptake of plasma cholesterol in tissues was calculated by dividing
specific radioactivity of tissue cholesterol by average specific radioactivity of plasma cholesterol for the entire 24-hour sampling period.

**Statistical Methods**

Concentrations, slopes, and intercepts of VLDL-CH of the 2 x 2 factorial design were analyzed for diet effects within a type of pig and for diabetes effects by using a Student's t-test (Assaf and Assaf, 1974). Tissue data were analyzed for main effects of diet and diabetes and interactions by using analysis of variance (Snedecor and Cochran, 1980a). Specific radioactivity curves of VLDL-, LDL-, and HDL-CH from 60 minutes to 24 hours post-injection were analyzed by using a split-plot design with treatments as main plot effects and time as a subplot effect (Snedecor and Cochran, 1980b). Pigs were nested within treatment to reduce animal-to-animal variation; treatment mean squares were tested against mean squares for pigs within treatments. Effects of time and treatment-by-time were tested against residual mean square by using conservative degrees of freedom. Treatment-by-time effects indicate that slopes of decay curves are different between treatments.
In Vivo Kinetic Analyses

Some common terms of in vivo kinetic analyses need to be defined before discussion of the data. A "pool" or "compartment" is defined as a specific metabolite homogeneously distributed in a given physical environment (Gurpide et al., 1963). The amount of metabolite in a compartment is its pool size. Therefore, when a metabolite is distributed into two distinct physical environments, it is considered to be distributed into two pools. If the rate of exchange of a given metabolite between two pools is very rapid or undistinguishable, the pools are considered as one. Associated with each compartment are rate constants for influx and outflux of the metabolite, and overall turnover rate constants can be determined for each compartment.

To conduct kinetic analyses, a model must be defined that distinguishes important compartments from one another. Analyses of lipoprotein kinetics is very complex and has been a widely studied field over the past 10 years; as of yet, a commonly accepted model for VLDL metabolism has not been defined. Data in the present study fit best to a two-pool model and are represented as:
Pool A is the VLDL-CH pool. The assumption is that instantaneous mixing occurs in Pool A after injection of the labeled VLDL-CH. Unfortunately, mixing is rarely instantaneous. Pool B is representative of all compartments that equilibrate rapidly with VLDL-CH, such as other lipoproteins and tissues that readily take up lipoprotein-cholesterol by way of receptor-mediated and receptor-independent processes. If the sampling period was longer than 1 hour, a third compartment consisting of slowly equilibrating tissues would have been present. Overall rate constants for each pool, defined as turnover of VLDL-CH of Pool A and turnover of VLDL-derived cholesterol of Pool B, were determined from combinations of $k_1$, $k_2$, $k_3$, and $k_4$. 
RESULTS

Plasma and Lipoprotein Cholesterol and Triglyceride Concentrations

Cholesterol and triglyceride concentrations were determined every 2 weeks in plasma; distribution of the two lipids among VLDL, LDL, and HDL of plasma from overnight-fasted pigs was determined also. Data from normal pigs appear in Table 1. At weeks 4 and 6, normal pigs fed beef tallow had approximately 30% greater (P<0.10) concentrations of cholesterol in plasma and in HDL and approximately 45% greater (P<0.10) concentrations in LDL. Concentrations seemed to decrease with time in soy oil-fed pigs and remained constant over time in beef tallow-fed pigs. Triglyceride concentrations were greater in plasma, VLDL, and LDL of beef tallow-fed than in soy oil-fed pigs by week 6 (P<0.10). Ratios of lipid to protein did not differ between diets; ratios for VLDL, LDL, and HDL were 7.59±0.62 versus 7.00±1.01, 1.82±0.30 versus 1.54±0.19, and 0.09±0.02 versus 0.07±0.02 for beef tallow- versus soy oil-fed normal pigs, respectively, by week 6. Ratios of LDL-CH to HDL-CH also were similar between beef tallow- and soy oil-fed normal pigs and were 1.76±0.19 versus 1.56±0.18, respectively, by week 6.
Table 1. Distribution of plasma cholesterol and triglycerides among plasma and lipoproteins of normal pigs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time fed diets (weeks)</th>
<th>Cholesterol (mg/dl plasma)</th>
<th>Triglyceride (mg/dl plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Dietary fat</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>121.1±9.9</td>
<td>158.4±14.2</td>
<td>149.2±9.9*</td>
</tr>
<tr>
<td>Soy oil</td>
<td>122.2±7.2</td>
<td>139.3±14.4</td>
<td>112.8±8.5</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>70.4±3.2</td>
<td>94.4±7.2*</td>
<td>82.1±11.0*</td>
</tr>
<tr>
<td>Soy oil</td>
<td>70.3±8.3</td>
<td>72.3±8.2</td>
<td>57.8±4.0</td>
</tr>
<tr>
<td><strong>HDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>15.2±2.1</td>
<td>21.2±1.2</td>
<td>23.5±1.4*</td>
</tr>
<tr>
<td>Soy oil</td>
<td>14.2±0.9</td>
<td>18.1±2.7</td>
<td>15.7±1.8</td>
</tr>
<tr>
<td><strong>Plasma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>124±12</td>
<td>106±21</td>
<td>96±25</td>
</tr>
<tr>
<td>Soy oil</td>
<td>109±27</td>
<td>88±3</td>
<td>68±9</td>
</tr>
<tr>
<td><strong>VLDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>81±12</td>
<td>76±12</td>
<td>70±10*</td>
</tr>
<tr>
<td>Soy oil</td>
<td>71±10</td>
<td>66±6</td>
<td>46±10</td>
</tr>
<tr>
<td><strong>LDL</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>16±2</td>
<td>18±6</td>
<td>12±3</td>
</tr>
<tr>
<td>Soy oil</td>
<td>18±4</td>
<td>10±2</td>
<td>8±2</td>
</tr>
</tbody>
</table>

\(^{a}\)Concentrations are means ± SEM.

*Concentrations are different between pigs fed different diets (P<0.10).
Cholesterol concentrations in plasma and lipoproteins of diabetic pigs appear in Table 2. Diabetic pigs were receiving exogenous insulin injections at the time of sampling. Cholesterol concentrations in plasma and lipoproteins usually were similar between pigs fed two different diets. Triglyceride concentrations were variable (data not shown), and no differences were found in triglyceride concentrations between pigs fed the two diets.

Nonfasted diabetic pigs had approximately a sevenfold greater concentration of VLDL-CH than did nonfasted normal pigs (P<0.002); see Table 2. No effect of soy oil or beef tallow were found in VLDL-CH concentrations in normal or diabetic pigs.

Lipoprotein Kinetics

To describe the effects of dietary fat and diabetes more thoroughly, several parameters of in vivo kinetics of VLDL-CH were calculated from standard equations (Shipley and Clark, 1972). Data are presented in Table 3. Intercepts and slopes of both components were greater in normal pigs than in diabetic pigs (P<0.05). Diabetic pigs fed soy oil had greater intercepts of the primary component than did diabetic pigs fed beef tallow (P<0.05), and normal pigs fed soy oil had greater slopes of the primary component than did normal pigs fed beef tallow (P<0.05). Diabetic pigs had
Table 2. Distribution of plasma cholesterol among plasma and lipoproteins of diabetic pigs

<table>
<thead>
<tr>
<th>Sample</th>
<th>Time fed diets (weeks)</th>
<th>Cholesterol (mg/dl plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Dietary fat</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>172±14</td>
<td>170±17</td>
</tr>
<tr>
<td>Soy oil</td>
<td>151±13</td>
<td>140±10</td>
</tr>
<tr>
<td>LDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>106±13*</td>
<td>102±15</td>
</tr>
<tr>
<td>Soy oil</td>
<td>75±12</td>
<td>76±7</td>
</tr>
<tr>
<td>HDL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beef tallow</td>
<td>17±3</td>
<td>14±3</td>
</tr>
<tr>
<td>Soy oil</td>
<td>17±2</td>
<td>19±2</td>
</tr>
</tbody>
</table>

*Concentrations are different between pigs fed different diets (P<0.10).

aConcentrations are means ± SEM.
Table 3. Kinetic parameters of VIDL-CH of normal and diabetic pigs fed beef tallow- and soy oil-containing diets

<table>
<thead>
<tr>
<th>Kinetic parameter</th>
<th>Normal Beef tallow</th>
<th>Normal Soy oil</th>
<th>Diabetic Beef tallow</th>
<th>Diabetic Soy oil</th>
<th>Diab Diet Int</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration C</td>
<td>2.0±.2</td>
<td>2.1±.2</td>
<td>15.0±3.2</td>
<td>16.2±2.2</td>
<td>**</td>
</tr>
<tr>
<td>Intercept, 1st comp d</td>
<td>.494±.116</td>
<td>.501±.250</td>
<td>.137±.017</td>
<td>.422±.017</td>
<td>**</td>
</tr>
<tr>
<td>Intercept, 2nd comp e</td>
<td>.411±.138</td>
<td>.439±.043</td>
<td>.007±.019</td>
<td>.001±.001</td>
<td>**</td>
</tr>
<tr>
<td>Slope, 1st comp f</td>
<td>.410±.132</td>
<td>1.201±.676</td>
<td>.119±.029</td>
<td>.107±.011</td>
<td>**</td>
</tr>
<tr>
<td>Slope, 2nd comp g</td>
<td>.104±.023</td>
<td>.149±.012</td>
<td>.004±.057</td>
<td>.002±.042</td>
<td>**</td>
</tr>
<tr>
<td>t₁/₂, 1st comp h</td>
<td>2.57±ND</td>
<td>.99±ND</td>
<td>6.15±ND</td>
<td>6.62±ND</td>
<td>ND</td>
</tr>
<tr>
<td>t₁/₂, 2nd comp i</td>
<td>2.85±ND</td>
<td>1.08±ND</td>
<td>69.5±ND</td>
<td>173.2±ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pool size, 1st comp j</td>
<td>1.1±ND</td>
<td>1.1±ND</td>
<td>7.2±ND</td>
<td>2.4±ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pool size, 2nd comp k</td>
<td>1.3±ND</td>
<td>1.2±ND</td>
<td>80.2±ND</td>
<td>63.4±ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Values are means ± SEM.
b Main diabetes and dietary effects and interactions.
c Data are presented as mg VIDL-CH/dl VLDL.
d Data are presented as specific radioactivity VIDL-CH/dose*kg body weight.
e Data are presented as minutes.
f Data are presented as /minute and are representative of half-lives of overall turnover rates.
g ND = not determined.
h Data are presented as mg VIDL-CH/kg body weight.
i *Effects are different (P<0.05).
j **Effects are different (P<0.01).
threefold longer half-lives of the primary component and about 60-fold longer half-lives of the secondary component of VLDL-CH disappearance than did normal pigs. Feeding a highly saturated fat to normal pigs resulted in nearly a threefold increase of half-life of both components over that of feeding a highly unsaturated fat. In diabetic pigs, type of dietary fat did not influence half-life of the primary component; half-life of secondary component was two- to threefold less than that of soy oil-fed pigs. Diabetic pigs had a fourfold and an 18-fold greater pool size of the primary and secondary components, respectively, of VLDL-CH disappearance than did normal pigs. Pool sizes of the primary and secondary components of normal pigs fed different fats were similar. In diabetic pigs, pool size of the primary and secondary components of beef tallow-fed pigs were threefold greater than and similar to, respectively, those of soy oil-fed pigs.

Disappearance/reappearance curves for VLDL-CH of normal and diabetic pigs fed beef tallow- and soy oil-containing diets are shown in Figure 1. Data are presented as natural logarithm of percentage of initial specific activity as a function of time after administration of radioactively-labeled VLDL-CH. Diabetic pigs fed beef tallow had a greater percentage of radioactive cholesterol
Figure 1. Natural logarithm of percentage of initial specific radioactivity of VLDL-CH as a function of time after injection of $^{14}$C-VLDL-CH in normal and diabetic pigs fed beef tallow- and soy oil-containing diets. Standard error of the mean (SEM) for all treatments at all times is indicated.
reappear in VLDL during the 1440 minutes after injection than did diabetic pigs fed soy oil (P<0.05). No dietary effect in normal pigs and no diabetes effect was found in reappearance of VLDL-CH.

Transfer of cholesterol from VLDL into other lipoproteins also was observed, and disappearance/reappearance curves for LDL-CH and HDL-CH are shown in Figures 2 and 3, respectively. A diabetes effect was found in specific radioactivity curves of LDL-CH and HDL-CH between 60 and 1440 minutes post-injection (P<0.05) in that diabetic pigs had 70% more VLDL-derived cholesterol reappear in LDL and HDL when compared with that for normal pigs. Diet did not affect specific radioactivity curves of LDL-CH or HDL-CH.

A comparison of specific radioactivity curves for VLDL-, LDL-, and HDL-CH, based on specific activity values of initial VLDL-CH, are shown in Figure 4. Even though radioactivity was found in LDL and HDL fractions 1 minute after injection of radiolabeled VLDL, specific radioactivity of LDL-CH and HDL-CH was very low when compared with specific radioactivity of VLDL-CH; specific radioactivities of LDL- and HDL-CH were 4% and 1% of specific radioactivities, respectively, of VLDL-CH at 1 minute post-injection. At 1440 minutes after the injection of
Figure 2. Natural logarithm of percentage of initial specific radioactivity of LDL-CH as a function of time after injection of $^{14}$C-VLDL-CH in normal and diabetic pigs fed beef tallow- and soy oil-containing diets. Standard error of the mean (SEM) for all treatments at all times is indicated.
Figure 3. Natural logarithm of percentage of initial specific radioactivity of HDL-CH as a function of time after injection of $^{14}$C-VLDL-CH in normal and diabetic pigs fed beef tallow- and soy oil-containing diets. Standard error of the mean (SEM) for all treatments at all times is indicated.
Figure 4. Natural logarithm of specific radioactivity of VLDL-, LDL-, and HDL-CH as a function of time after injection of $^{14}$C-VLDL-CH based on specific radioactivity of VLDL-TG in normal and diabetic pigs fed beef tallow- and soy oil-containing diets. Standard error of the mean (SEM) for all treatments at all times is indicated.
radiolabeled VLDL, specific radioactivity of VLDL-, LDL-, and HDL-CH accounted for 20%, 55%, and 25% of the total radioactivity in plasma.

Plasma insulin concentrations tended to be greater in soy oil-fed pigs than in beef tallow-fed pigs (P<0.18). Mean insulin concentrations were 4.14±0.62 and 6.57±1.56 uUnits/ml of plasma for beef tallow- and soy oil-fed pigs, respectively.

Tissue Analyses

Percent lipid, amount of cholesterol, amount of $^{14}$C-cholesterol per gram tissue, and net transfer rate of plasma cholesterol into tissues are shown in Table 4. Only main treatment effects will be discussed here; interactions are indicated in Table 4. Liver of diabetic pigs had greater percentage of lipid (P<0.04), more radioactively-labeled cholesterol (P<0.001), and greater net transfer rate of plasma cholesterol (P<0.09) than did liver of normal pigs. No main diabetes or dietary effects were found in cholesterol content of liver. Skeletal muscle of diabetic pigs had more cholesterol per gram of tissue (P<0.02) and more radioactively-labeled cholesterol (P<0.02) than did muscle of normal pigs. Pigs fed beef tallow had more radioactively-labeled cholesterol than did pigs fed soy oil (P<0.10). No treatment effects were found in
Table 4. Kinetic parameters of VLDL-CH of normal and diabetic pigs fed beef tallow- and soy oil-containing diets

<table>
<thead>
<tr>
<th>Tissue parameter</th>
<th>Treatment</th>
<th>Effects²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal Beef tallow</td>
<td>Normal Soy oil</td>
</tr>
<tr>
<td>Liver weight, %</td>
<td>1.96±0.11</td>
<td>1.89±0.25</td>
</tr>
<tr>
<td>lipid, %</td>
<td>3.93±0.07</td>
<td>4.02±0.11</td>
</tr>
<tr>
<td>CH, mg</td>
<td>41.41±3.45</td>
<td>33.31±1.56</td>
</tr>
<tr>
<td>NT CH*</td>
<td>26985±3570</td>
<td>25668±2799</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>3.18±0.33</td>
<td>2.74±0.32</td>
</tr>
<tr>
<td>lipd, %</td>
<td>12.01±0.30</td>
<td>10.22±1.22</td>
</tr>
<tr>
<td>CH, mg</td>
<td>632±87</td>
<td>449±34</td>
</tr>
<tr>
<td>NT CH*</td>
<td>43.9±2.4</td>
<td>38.9±4.0</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lipd, %</td>
<td>74.56±1.71</td>
<td>78.43±1.71</td>
</tr>
<tr>
<td>CH, mg</td>
<td>2.91±0.61</td>
<td>3.62±2.10</td>
</tr>
<tr>
<td>CH*</td>
<td>1172±132</td>
<td>1003±86</td>
</tr>
<tr>
<td>NT CH*</td>
<td>89.3±15.6</td>
<td>79.1±6.9</td>
</tr>
<tr>
<td>Aorta</td>
<td>1.72±0.71</td>
<td>1.76±0.06</td>
</tr>
<tr>
<td>lipd, %</td>
<td>16.89±0.90</td>
<td>19.34±3.96</td>
</tr>
<tr>
<td>CH, mg</td>
<td>524±85</td>
<td>529±71</td>
</tr>
<tr>
<td>NT CH*</td>
<td>34.6±4.5</td>
<td>72.4±8.0</td>
</tr>
<tr>
<td></td>
<td>Heart</td>
<td>Carcass</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------------------------------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td></td>
<td>weight, %</td>
<td>lipid, %</td>
</tr>
<tr>
<td></td>
<td>0.43±0.04</td>
<td>0.50±0.04</td>
</tr>
<tr>
<td></td>
<td>3.18±0.15</td>
<td>3.89±0.27</td>
</tr>
<tr>
<td>CH, mg</td>
<td>23.68±0.51</td>
<td>24.90±1.38</td>
</tr>
<tr>
<td>CH*</td>
<td>2816±408</td>
<td>2205±180</td>
</tr>
<tr>
<td>NT CH*</td>
<td>189.7±19.4</td>
<td>171.0±9.6</td>
</tr>
<tr>
<td></td>
<td>16.50±0.35</td>
<td>17.64±0.40</td>
</tr>
<tr>
<td>CH, mg</td>
<td>37.65±2.40</td>
<td>33.30±1.50</td>
</tr>
<tr>
<td>CH*</td>
<td>1207±135</td>
<td>818±69</td>
</tr>
<tr>
<td>NT CH*</td>
<td>73.9±8.7</td>
<td>65.5±8.0</td>
</tr>
<tr>
<td>protein, %</td>
<td>46.05±1.25</td>
<td>43.53±1.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^Values are means ± SEM.

^Main diabetes and dietary effects and interactions.

^Data are presented as a percentage of body weight.

^Data are presented as a percentage of tissue, wet tissue basis.

^CH, mg = mg cholesterol/100 g tissue, wet tissue basis.

^CH* = dpm 14C/g tissue, wet tissue basis.

^NT CH* = net transfer rate of plasma cholesterol/g tissue, wet tissue basis.

Data are presented as specific radioactivity of cholesterol in g tissue/average specific radioactivity of cholesterol in plasma x 1440 minutes.

^hData are presented as percentage of tissue, dry matter basis.

^iCH, Ave SA = average specific radioactivity of cholesterol in plasma for the 24-hour study.

*Effects are different (P<0.10).

**Effects are different (P<0.05).
percentage of lipid or net transfer rate of plasma cholesterol of this tissue. Diabetic pigs had a smaller percentage of lipid in adipose tissue (P<0.05) and had less radioactively-labeled cholesterol (P<0.005) than did normal pigs. In subcutaneous adipose tissue, dietary soy oil resulted in greater percent fat (P<0.03) than did dietary beef tallow. Net transfer rate of plasma cholesterol into adipose tissue was not affected by treatment. Aortas of diabetic pigs tended to have a greater percentage of lipid (P<0.20) and did have more radioactively-labeled cholesterol (P<0.002) than did normal pigs. Neither amount of cholesterol per gram aorta or net transfer rate of plasma cholesterol were influenced by treatment. Diabetic pigs had more radioactively-labeled cholesterol in heart than did normal pigs (P<0.007). Treatment did not affect percentage of lipid, cholesterol content, and net transfer rate of plasma cholesterol in heart. In carcass, pigs fed soy oil had greater percentage of lipid (P<0.03), less radioactively-labeled cholesterol (P<0.09), less cholesterol content (P<0.002), and a smaller percentage of protein (nitrogen content x 6.25) (P<0.04) than did pigs fed beef tallow. Carcass of normal pigs had a greater percentage of lipid (P<0.003), less radioactively-labeled cholesterol (P<0.002), and a smaller percentage of protein (P<0.05) than
did carcass of diabetic pigs. No treatment effects were found in net transfer rate of plasma cholesterol into carcass.
DISCUSSION

In the present study, feeding saturated fat to normal pigs resulted in hypercholesterolemia and hypertriglyceridemia when compared with feeding pigs unsaturated fat. Cholesterol concentrations were increased uniformly across all lipoprotein classes so that ratios of HDL-CH to LDL-CH did not change with diet. Additionally, no changes were found in protein to lipid ratios in pigs. Thus, different types of dietary fat did not influence amount of triglyceride, cholesterol, and protein of each lipoprotein particle in these pigs, which agrees with some previous studies (Walsh et al., 1983;) and disagrees with others (Mahley et al., 1975; Renner et al., 1986). A change in fatty acid composition and type of apolipoprotein in lipoproteins may have been different between pigs fed different types of fat but were not determined. Cholesterol and triglyceride concentrations were not affected to a large degree by dietary fat in diabetic pigs.

Literature is replete with studies on VLDL metabolism because VLDL metabolism is so closely linked to cholesterol and especially to triglyceride concentrations. A number of different models have been proposed to explain in vivo synthesis, secretion, and catabolism of VLDL (Reardon et al., 1978; Zech et al., 1979; Phair, 1982; Green et al.,
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Different models are used depending on whether cholesterol, triglyceride, phospholipid, or protein of VLDL is labeled. When cholesterol of VLDL is labeled, as in this study, disappearance of label from VLDL is an indicator of both receptor-mediated uptake of VLDL by tissues and exchange of cholesterol with other lipoproteins and tissues.

The in vivo kinetics of VLDL-CH metabolism in the present study were analyzed by a two-pool model (Shipley and Clark, 1972). The primary pool, or component, is cholesterol in VLDL. Pool size of this first component was similar to concentrations of VLDL-CH in normal pigs and diabetic pigs fed beef tallow. Diabetic pigs fed soy oil had smaller pool sizes than would be expected from concentrations of VLDL-CH, possibly because mixing was not as complete in these pigs as in pigs on other treatments. Half-lives of the primary pool were rapid (1.3 minutes in normal and 6.4 minutes in diabetic pigs) and were an indication of removal of cholesterol from VLDL into other lipoproteins or tissues.

The secondary pool of VLDL-CH is actually a combination of several different pools that readily exchange cholesterol with VLDL-CH, such as other lipoproteins, red blood cells, and tissues (Kovanen and Nikkila, 1976; Aigueperse et al., 1983). The pools that make up the secondary component were
"lumped" together because they were indistinguishable from one another in the present study. Thus, the secondary pool of VLDL-CH is comprised of VLDL-derived cholesterol.

Disappearance of cholesterol from VLDL and exchange of VLDL-CH with other cholesterol pools were drastically different between normal and diabetic pigs. The large differences in pool sizes and half-lives of the secondary pool of VLDL-CH between normal and diabetic pigs indicated that the secondary pool was different between the two types of pigs. In normal pigs, the small secondary pool probably represents cholesterol within the vascular system, such as lipoproteins or red blood cells. In diabetic pigs, the secondary pool probably represents cholesterol in tissues in addition to cholesterol of the vascular system.

Lipoproteins of normal pigs seem to undergo a rapid, more extensive exchange of VLDL-CH with other vascular pools of cholesterol than do lipoproteins of diabetic pigs. Lopes-Virella et al. (1985) have shown that lipoproteins of diabetics are metabolized by tissues slower than are lipoproteins of normal animals, possibly because the lipoproteins are glycosylated (Steinbrecher and Witzum, 1984) or have different compositions of fatty acids (Lopes-Virella et al., 1985). Thus, the different exchange rates of cholesterol within vascular pools of cholesterol
between normal and diabetic pigs also could be a result of changes in glycosylation or fatty acid composition of lipoproteins.

Dietary fat, in addition to diabetes, influenced VLDL-CH metabolism. In normal pigs, feeding beef tallow resulted in longer half-lives of the primary and secondary components when compared with feeding soy oil. These results are consistent with past research (Nestel and Scow, 1964; Green et al., 1984) and could be part of the reason for the elevated concentration of cholesterol in plasma of fasted pigs. Even though half-lives of both components were longer in beef tallow-fed pigs, pool sizes of both components as well as concentrations were similar between pigs fed two different types of fat. From the present study, the mechanism that caused the change in half-lives with no change in pool size is unknown and occurred during the first 6 weeks of dietary treatment of pigs.

In diabetic pigs, half-life of the initial component of VLDL-CH was not affected by diet. Half-life of the second component was longer in soy oil-fed pigs than in beef tallow-fed pigs, which was opposite of that found in normal pigs. Additionally, pool size of the primary pool was greater in beef tallow-fed pigs than in soy oil-fed pigs, and the pool size of the secondary pool was similar between
pigs fed two different types of fat. As stated before, the mechanism that caused the change in half-lives and pool sizes is unknown and can not be determined from the present study.

Additionally, diabetic pigs fed beef tallow recirculated more cholesterol as VLDL than did diabetic pigs fed soy oil and, as a possible result, retained more total VLDL-derived cholesterol in their body at death (85%) when compared with diabetic pigs fed soy oil and normal pigs fed either diet (70%). Evidently, diabetic pigs fed beef tallow did not remove cholesterol from their bodies as well as did other pigs, possibly because of a decrease in synthesis and/or secretion of bile acids. In agreement, feeding saturated fat to rats also leads to a greater retention of cholesterol in their body as a result of depressed secretion of bile acids when compared with feeding unsaturated fat (Oh and Monaco, 1985).

A direct result of altered lipoprotein metabolism is a change in accretion of cholesterol and triglyceride in tissues. Entry of cholesterol, which is one of the primary constituents of atherosclerotic plaque, into vascular epithelia occurs by way of a combination of de novo synthesis, receptor-mediated uptake, receptor-independent uptake, and turnover. By knowing specific radioactivity of
cholesterol in tissues and plasma, net transfer rate of plasma cholesterol into tissues can be estimated. 

Diabetes resulted in more radioactively-labeled cholesterol in all tissues of pigs in comparison with tissues of pigs that were normal. Greater uptake of plasma cholesterol in diabetic animals seems to be a function of cholesterol availability in plasma. Greater cholesterol content in tissues of diabetic pigs were not found most likely because de novo cholesterol synthesis was depressed in the diabetic pigs when compared with normal pigs (Brown and Goldstein, 1978). When specific radioactivities of plasma cholesterol were considered, liver was the only tissue of diabetic pigs that had greater net transfer rates of plasma cholesterol when compared with tissues of normal pigs. Lipoproteins were more available to liver of diabetic pigs (Green et al., 1984) as a consequence of slower catabolism of lipoproteins by extrahepatic tissues (Chen et al., 1979; Reaven and Greenfield, 1981; Lopes-Virella et al., 1985), resulting in greater net transfer rates in liver. Diabetics recirculated more cholesterol as LDL and HDL because they had larger pools of radioactive cholesterol in liver to synthesize new lipoproteins with.

Unlike past studies (Diersen-Schade, 1984; Walsh-Hentges et al., 1985; Baldner-Shank, 1986), diet did
not influence composition or net uptake of cholesterol by
tissues to any large degree. The largest influence of
dietary fat was that pigs fed soy oil had more lipid in
adipose tissue and carcass. An explanation for the increase
in percentage of lipid in tissues is that pigs fed soy oil
may have received more digestible energy than did pigs fed
beef tallow; saturated fat is less digestible than is
unsaturated fat (Mattson, 1959; Baldner-Shank, 1986).

It is commonly believed that the observed changes in
metabolism between normal and diabetic pigs fed two
different types of fat could be mediated by compositional
changes in lipoprotein and plasma membranes. Insulin
concentrations in plasma, however, are a major stimulus of
VLDL catabolism and could affect half-lives and pool sizes
of VLDL. One of the primary ways insulin regulates VLDL
catabolism is by way of lipoprotein lipase activity (Cryer,
1981). Others have shown that activity of lipoprotein
lipase in animals fed unsaturated fat is greater than
activity of lipoprotein lipase in animals fed saturated fat
(Bagdade et al., 1970; Cryer et al., 1978), as a result of
changes in insulin concentrations or fatty acid composition
of plasma membranes. In the present study, insulin
concentrations in plasma tended to be greater in soy oil-fed
pigs. Thus, a lack of plasma insulin in beef tallow-fed
pigs could have led to depressed removal of VLDL-CH as seen by a longer initial half-life of VLDL-CH when compared with pigs fed soy oil; moreover, initial half-lives in diabetic animals were not affected by diet and supports this theory.

In conclusion, the removal of VLDL-CH from circulation and exchange of VLDL-CH between vascular cholesterol could be dependent on insulin concentrations and other diet-induced factors such as fatty acid composition of lipoproteins and plasma membranes. Additionally, net transfer of plasma cholesterol into peripheral tissues seems to be directly related to availability of plasma cholesterol, and net transfer of cholesterol into liver seems to be indirectly related to net transfer of cholesterol into peripheral tissues.
LITERATURE CITED


GENERAL DISCUSSION

The overall conclusion of the present study was that dietary saturated and unsaturated fat, when fed for 6 weeks, can play an important role in regulation of glucose and VLDL-CH homeostasis. Several follow-up experiments could be completed to improve and better quantify the results of this study and possibly to elucidate the primary effects that led to the differences we detected.

To study glucose homeostasis in greater detail, metabolism should be studied in both fasted and fed conditions. With constant infusion of radioactively-labeled glucose, production rate and clearance of glucose can be determined, which would be a better indication of glucose homeostasis and glucose effectiveness. In the present study, insulin sensitivity could not be quantified as accurately as needed; either more than five animals needed to be used or insulin sensitivity should have been determined in vitro. Insulin sensitivity is essentially a combination of insulin binding and action, and thus, one could easily study these parameters in cell preparations. Because degree of unsaturation in plasma membranes do not affect insulin receptors similarly in different tissues, sensitivity should studied in several tissues, such as adipocytes, hepatocytes, and erythrocytes. A limitation of
the present study was that we could not determine if the increase in plasma insulin concentrations throughout the study were age- or diet-related. Thus, a control set of pigs, possibly pigs on a low-fat diet, should be used in follow-up studies.

Lipoprotein metabolism also can be evaluated to a greater extent in follow-up studies than in the present study with a few changes in protocol. Because of the lack of understanding of in vivo metabolism of lipoproteins, models are often oversimplified and much good data are "wasted", as was the case with data in this experiment. The problem in modeling in this experiment, and in other experiments, occurs when pools are "lumped" together. To get around some of the problems associated with bolus injections of radioactively-labeled lipoproteins, such as pool "lumping", one could perform kinetic studies during a constant infusion of labeled-lipoproteins. From these studies, estimation of production rate and clearance of the lipoproteins in fasted and fed conditions could be determined and would have more physiological significance than does pool size.

During constant infusions, as during bolus injections, lipoproteins are taken up by tissues and catabolized. Products of catabolism could be used in synthesis of new
lipoproteins and secreted back into the circulation as metabolites other than the metabolite injected; for example, cholesterol could be converted to cholesterol ester or bile acids. Specific metabolites under study should be separated from the sample, with digitonin in the case of cholesterol, and specific radioactivities determined from those data.

The choice of pigs as our animal model to use in this experiment was excellent. The large size of the pigs allowed repeated sampling of blood during glucose tolerance studies and measurements of in vivo lipid kinetics. Because of our ability to position catheters in the aortic arch, low doses of alloxan were used to render pigs diabetic, and our success rate for making pigs diabetic was very good. Pigs also were fairly easy to maintain, and we did not lose a pig from hypo- or hyperglycemia. The only drawback of using pigs was that pigs do not metabolize lipoproteins as do humans; VLDL-remnants are taken up as such in pigs, and VLDL-remnants are converted to LDL in plasma in humans. Nevertheless, pigs can and are often used in studies on lipoprotein metabolism; care must be taken when extrapolating results to humans, however.
Congratulations

I would like to thank the Iowa Beef Industry Council and the American Diabetes Association, Iowa Affiliate, for financial support of my research.

My two co-major professors, Drs. Donald Beitz and Steven Nissen, are very deserving of my deepest appreciation for all their help, encouragement, and support of my graduate education, both in and out of the laboratory. They have set a precedent in my mind for professor/student relationships that I will try to maintain throughout my career. I also would like to thank members of my committee, Drs. Jerry Young, James Thomas, Carl Tipton, and Allen Trenkle. Several individuals helped me with various aspects of my research and need to be recognized: Beth Jensen for assistance in the lab, Mike Dowd for assistance with the insulin sensitivity model, and Dr. Go of Mayo Clinic, Minnesota, for assaying my samples for GIP concentrations. My fellow Nutritional Physiologists also deserve thanks for being a major part of my home away from home.

Most of all, I would like to thank my family. Their endless supply of love and support played a large role in my graduate career, more than I think they will ever realize. You guys are the greatest!

Thanks to all for everything; it's a great feeling.
APPENDIX
Table A1. Analysis of variance on plasma glucose concentrations after consumption of a meal

<table>
<thead>
<tr>
<th>Sources of Variation</th>
<th>Degrees of Freedom (Conservative)</th>
<th>Sum of Squares</th>
<th>Mean Squares</th>
<th>F Value</th>
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<tr>
<td></td>
<td>Degrees of Freedom (Type III)</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>4822752</td>
<td>19.7</td>
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<td>14708260</td>
<td>14708260</td>
<td>60.2</td>
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<tr>
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<td>30064</td>
<td>30064</td>
<td>0.1</td>
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<tr>
<td>interaction</td>
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<td>22823</td>
<td>22823</td>
<td>0.1</td>
</tr>
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<td>3909161</td>
<td>244322</td>
<td></td>
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<tr>
<td>Time</td>
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<td>.1</td>
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<tr>
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<td>20875</td>
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<tr>
<td>interaction*time</td>
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<td>19176353</td>
<td>1198522</td>
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Animal(Trt) = Error A.