

GLUCOCORTICIDS AND HYPERCHOLESTEROLEMIA
IN THE OBESE ZUCKER RAT

A DISSERTATION

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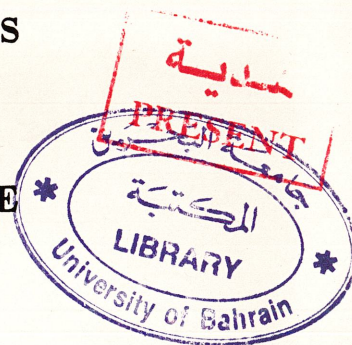
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This study is dedicated to the fond memories of my
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ABSTRACT

The obese Zucker rat is hypertriglyceridemic, hypercholesterolemic, and hypercorticosteronemic. It is also hyperinsulinemic and insulin resistant. We tested the hypothesis that hypercorticosteronemia causes the hypercholesterolemia. Four to five week old obese and lean rats were adrenalectomized and sacrificed 5 weeks post-adrenalectomy.

Obese control rats had higher serum free/total cholesterol ratio and their insulin levels correlated positively with serum free cholesterol and triglyceride (TG) levels. Their serum TG and free cholesterol levels also correlated positively. This indicates higher serum levels of free cholesterol and TG enriched very low density lipoproteins (VLDL). Hepatic hydroxymethylglutaryl(HMG)-CoA reductase activity was greater and microsomal free cholesterol content lower in obese rats compared to lean rats. We suggest that hepatic VLDL oversecretion reduces microsomal free cholesterol content which enhances reductase activity by alleviating negative-feedback inhibition by free cholesterol. Greater reductase activity, in turn, contributes to the hypercholesterolemia. Obese rats absorbed more cholesterol when expressed as percentage

of ^{14}C -cholesterol dose or as weight absorbed but not as weight absorbed normalized to body weight.

Adrenalectomized obese rats had lower serum TG and phospholipid levels compared to control obese rats and were normoinsulinemic. This indicates that adrenalectomy ameliorated insulin resistance and hepatic VLDL hypersecretion. Nevertheless, adrenalectomy exacerbated the hypercholesterolemia in obese rats and induced it in lean rats. Liver microsomal free cholesterol content was elevated. In obese adrenalectomized rats reductase activity was lower. We suggest that accumulation of free cholesterol lowered reductase activity. Adipose tissue size and adipocyte cholesterol content were drastically lower in adrenalectomized animals. We suggest that curtailment of adipose tissue storage capacity contributes to the hypercholesterolemia in adrenalectomized rats. Hepatic 7-alpha hydroxylase activity was not enhanced by accumulation of free cholesterol; this also contributes to the hypercholesterolemia. Adrenalectomized animals absorbed less cholesterol when expressed as weight absorbed but not when data were normalized to body weight.

We conclude that hypercorticoesteronemia induces insulin resistance and hyperinsulinemia which in turn enhance hepatic TG synthesis directly and/or by

providing free fatty acids and thus cause VLDL oversecretion and hypercholesterolemia. Additionally adrenalectomy in the rapid growth period in rats causes hypercholesterolemia by under-growth of adipose tissue.

INTRODUCTION

It is well established that coronary heart disease is the leading cause of death in the United States and probably world wide. The incidence of atherosclerosis which underlies the development of coronary heart disease is highly correlated with the plasma levels of cholesterol (hypercholesterolemia) and triglyceride (TG) (hypertriglyceridemia) (Tzagournis et al. 1968; Carlson and Bottiger, 1972; NIH Consensus Development Conference Statement 1985). In many instances hypercholesterolemia and hypertriglyceridemia are expressed concomitantly, in which case the perturbation is referred to as hyperlipidemia.

Because cholesterol and TG are secreted into the plasma in the form of lipoprotein particles, it follows that changes in the metabolism of either cholesterol or TG that may potentially affect lipoprotein secretion will affect the serum levels of both. Additionally defects in lipoprotein removal from the plasma are likely to cause elevated plasma levels of both cholesterol and TG. Therefore investigating the etiology of any hyperlipidemia should include examination of changes in the metabolism of both TG and cholesterol.

Hyperlipidemia can generally be classified under two categories, disturbances of input or disposal. Disturbances of input include high levels of dietary intake or enhanced gastrointestinal absorption of cholesterol, TG, or carbohydrate. Increases in endogenous lipogenesis, such as increases in hepatic cholesterol or TG synthesis would also be included under disturbances of input. Disturbances of disposal would include deficient lipoprotein lipase (LPL) activity. LPL hydrolyzes the TG moiety in TG-rich lipoproteins (very low density lipoprotein (VLDL) and chylomicrons) and thus promotes TG removal from the blood stream. It also enhances cholesterol removal by transforming the TG-rich lipoproteins (very low density lipoproteins (VLDL) and chylomicrons) into higher density lipoproteins (low, intermediate and high density lipoproteins, LDL, IDL and HDL respectively). Those higher density lipoproteins can be removed by receptor mediated mechanisms (Brown et al., 1981; Eisenberg et al., 1983). Inadequate TG removal is one of the mechanisms thought to be responsible for the higher cholesterol and TG levels observed in type IV hyperlipidemia. Among disturbances of removal one can also include deficiencies and defects of and cellular receptors for lipoproteins as well as deficiencies and defects of apoproteins that mediate the interaction of

lipoproteins with these receptors. Among these perturbations are type IIa hyperlipidemia (familial hypercholesterolemia) which is caused by hereditary deficiency of LDL receptors (Goldstein and Brown, 1985), and Type III hyperlipidemia, which is thought to be produced by abnormal apo E structure (Ehnholm et al., 1984). Additionally any decrease in cholesterol excretion such as decreased levels of bile acid production would also lead to cholesterol retention and hypercholesterolemia.

Hormonal disturbances can lead to hyperlipidemia or hypolipidemia through many of the mechanisms mentioned above. More specifically, disturbances of insulin and glucocorticoid secretion potentially provide an explanation for a good part, if not most, of the hyperlipidemias expressed in humans. Those include the hyperlipidemias observed in obesity, type 2 diabetes mellitus, Cushing's syndrome, and possibly even that precipitated by the exposure to stress.

High levels of circulating glucocorticoids invariably lead to hyperinsulinemia. Glucocorticoids are antagonistic to many of the actions of insulin. Thus, in excess, glucocorticoids cause insulin insensitivity and hyperinsulinemia. Receptor and post-receptor mechanisms have been proposed to explain

the glucocorticoid antagonism to insulin actions. Most evidence suggests that it is probably not due to down regulation of insulin receptors and is distinct from that found in obesity (Kahn et al. 1978; Nosadini et al. 1983; Karnieli et al. 1985; Cohn et al. 1986)

The obese Zucker rat (the animal model in this study) is hyperglucocorticoidemic, hyperinsulinemic, and hyperlipidemic and thus provides a good model for the study of involvement of excess glucocorticoid state in the development of hyperlipidemia.

In the literature review that follows, the mechanisms by which insulin and glucocorticoid excess - independently and in combination - may lead to the development of hyperlipidemia will be discussed. The first section will discuss experimental evidence from humans and animals other than the obese Zucker rat. The second section will discuss in depth, the nature of the hyperlipidemia displayed by the obese Zucker rat, as well as the mechanism by which the hyperinsulinemia and excess secretion of glucocorticoids may lead to such a hyperlipidemia.

LITERATURE REVIEW

SECTION 1. INSULIN AND GLUCOCORTICOID INDUCED
HYPERLIPIDEMIAS : EVIDENCE FROM HUMAN STUDIES AND
ANIMALS OTHER THAN THE OBESE ZUCKER RAT

1.1 EFFECT OF GLUCOCORTICOID AND INSULIN ON HEPATIC
LIPOPROTEIN PRODUCTION:

The majority of evidence suggests that glucocorticoid excess leads to hyperlipidemia. Glucocorticoid administration in rats and mice increases hepatic VLDL and TG secretion at lower doses but is without effect at high doses (Reaven et al. 1974; Krausz et al. 1981). Plasma HDL cholesterol and HDL protein are elevated by glucocorticoid treatment in a dose dependent manner (Krausz et al. 1981; Mitamura 1987). In perfused rat liver, in which any effect of hyperinsulinemia is precluded, TG secretion is increased by glucocorticoid inclusion in the perfusion buffer (Klausner and Heimberg, 1967). Patients on prolonged glucocorticoid therapy are hypercholesterolemic and hypertriglyceridemic (Adlersberg et al. 1950; Bagdade et al., 1970; Stern et al. 1973) Furthermore, Cushing's syndrome patients are hyperlipidemic and hyperlipoproteinemic (Taskinen et al., 1983).

Some investigators have proposed a relationship between stress, glucocorticoids, hyperlipidemia, and coronary heart disease (Williams et al., 1982; Schwertner et al., 1984; Troxler and Schwertner, 1985). This proposed relationship is based on the finding that serum cholesterol in individuals that display type A behavioral pattern (defined as individuals in a relatively chronic excessive struggle to obtain unusually unlimited things from their environment in the shortest period of time against the opposing efforts of other persons or things in this same environment) (Roskies et al., 1978) correlates positively with cortisol levels (Schwertner et al., 1984). This correlation is also found in individuals with minimal or significant coronary artery disease (Schwertner et al., 1984). Furthermore behavioral modification in individuals with type A behavioral pattern decreases serum cholesterol levels (Roskies et al., 1978).

The effect of insulin on VLDL serum levels and hepatic secretion is a complex and controversial issue. This reflects the multifaceted nature of insulin action. Insulin can influence VLDL production and serum levels by any one or combination of the following mechanisms:

1. Insulin inhibits lipolysis in adipose tissue and thus lowers the availability of serum free fatty acids (FFA) thus decreasing the availability of substrate for hepatic TG synthesis.

2. Insulin can directly enhance hepatic TG synthesis and thus potentially increase VLDL-TG secretion (see section on insulin effect on hepatic TG synthesis below).

3. Insulin enhances LPL activity, and thus enhances the removal of VLDL-TG from the plasma compartment (see section on insulin effect on LPL activity, below).

In human studies, evidence of insulin involvement in hepatic VLDL and TG secretion came from correlation studies in endogenous hypertriglyceridemia (type IV hyperlipoproteinemia) patients and patients with type II diabetes mellitus (Olefsky et al., 1974; Laakso et al., 1987). These studies demonstrate the presence of a strong positive correlation between serum insulin levels and serum concentrations of TG and VLDL. Positive correlation is also found between the insulin response to a glucose dose and serum VLDL levels in healthy male volunteers (Capaldo et al.; 1985). This positive correlation however, is not proof of a causative relationship. To the contrary, these

correlation studies may indicate that the VLDL levels are positively correlated with the degree of insulin resistance manifested in these individuals, which in turn may be causatively involved in the hepatic over production of VLDL particles. Insulin resistance is manifested in most patients with endogenous hypertriglyceridemia (Olefsky et al., 1974; Reaven et al., 1983), and is the hallmark of type II diabetes mellitus (Reaven, 1984).

Some investigators assume that despite the presence of an insulin resistant state (defined as the presence of hyperglycemia or normoglycemia despite the hyperinsulinemia), hepatic TG synthesis remains responsive to the stimulatory action of insulin. It is plausible that hyperglycemia might not be caused by hepatic resistance to insulin action on glucose uptake or release but rather resistance to insulin action on glucose uptake by peripheral tissues. This is especially true in view of the fact that insulin does not stimulate glucose uptake in the liver by a carrier mediated system as it does in other tissues. Thus in certain cases of "alleged insulin resistance", in which such resistance can only be determined by an exaggerated insulin response to an exogenous glucose dose; and in which the individuals are normoglycemic

and normoinsulinemic in the postabsorptive state (as is the case for endogenous hypertriglyceridemia patients) (Vogelberg et al., 1980; Reaven et al., 1983) the liver might not be resistant to insulin action on TG synthesis or on glucose metabolism. Accordingly postprandial hyperinsulinemia caused by a selective resistance to insulin action on glucose uptake in peripheral tissues may enhance hepatic TG synthesis and thus lead to oversecretion of VLDL. This mechanism is in agreement with that suggested by Olefsky et al. (1974) for the development of hypertriglyceridemia in endogenous hypertriglyceridemia patients.

Alternatively, some investigators suggest that the enhanced VLDL secretion is caused by the removal of insulin inhibitory effect on lipolysis in adipose tissue by the insulin resistant state. Accordingly the resulting enhancement of FFA release from adipose tissue would provide substrate and thus stimulate hepatic TG synthesis and hepatic VLDL production (Nikkila, 1985). This mechanism may be operational in diabetes mellitus type II patients and patients with endogenous hypertriglyceridemia (Olefsky et al., 1974; Nikkila, 1985). Additionally, the enlarged mass of adipose tissue combined with insulin resistance can potentially provide ample FFA substrate for hepatic TG

synthesis and thus lead to the hypertriglyceridemia often seen in obese individuals (Olefsky, 1976; Kesaniemi, 1983).

In Cushing's syndrome, increased hepatic VLDL secretion, hyperinsulinemia, and insulin resistance are expressed. (Kahn et al., 1978; Johnston et al., 1980; Marja-Ritta et al., 1983; Nosadini et al., 1983; Karnieli et al., 1985; Cohen et al., 1986). The nature of insulin resistance in Cushing's syndrome patients, however, is unique in that it is induced by glucocorticoid antagonism. In the liver, glucocorticoids induce gluconeogenesis and stimulate hepatic glucose secretion (Krausz et al., 1981). This leads to hyperglycemia and hyperinsulinemia. Glucocorticoids on the other hand do not antagonize and in fact promote insulin stimulatory effects on hepatic TG synthesis (see section on glucocorticoid effect on TG synthesis below). In peripheral tissues on the other hand, glucocorticoids antagonize insulin stimulation of glucose uptake (Munck, 1971). Glucocorticoids also antagonize insulin stimulatory action on TG synthesis and inhibitory action on lipolysis (see effect of glucocorticoids on TG synthesis below). Furthermore most reports suggest that the glucocorticoid antagonism to insulin action on glucose uptake by peripheral

tissues is manifested at a post-receptor level, which indicates that there is no effect on receptor number (Nosadini et al., 1983; Karnieli et al., 1985; Cohen et al., 1986). Thus the evidence strongly indicates that in a case of hyperglucocorticoidemia, hepatic TG synthesis will remain responsive to insulin action. This together with enhanced lipolysis in adipose tissue (Johnston et al., 1980), would lead to elevated rates of hepatic TG synthesis and enhanced VLDL secretion.

Insulin resistance may also lead to elevation of serum VLDL and TG levels by lowering the rate of their removal in the periphery. Thus resistance to insulin stimulatory action on lipoprotein lipase activity in adipose tissue, skeletal muscle and heart would lead to accumulation of serum VLDL and TG. This mechanism is probably also functional in hypertriglyceridemic patients (Bazelmans et al., 1983), patients with type II diabetes mellitus (Dunn et al., 1984; Nikkila, 1985), and Cushing's syndrome patients (Taskinen, 1983).

Insulin treatment in vivo in type II diabetic patients has been shown to decrease TG levels in the plasma. This effect was explained on the basis of enhanced of TG removal and decreased hepatic production by inhibiting lipolysis in adipose tissue (Dunn et al.

1984; Nikkila, 1985). This finding was also observed in normal volunteers (Bazelmans, 1983). Vogelberg et al. (1980) observed a decrease in hepatic VLDL production in normal individuals and an increase in endogenous hypertriglyceridemia patients in response to acute insulin administration via the portal vein. Insulin administration was associated with decreased hepatic uptake of FFA in both groups. In rats chronic insulin treatment enhances TG production as well as removal. However, the removal is enhanced to a greater extent than the production, with a net effect of a decrease in plasma TG levels (Steiner et al., 1984; Kazumi et al., 1986). In one study Kruszynska et al. (1987) suggests that acute insulin administration (euglycemic clamp) inhibits TG production rate to a greater degree in rats chronically over-treated with insulin relative to normal rats despite a similar drop in plasma FFA. Studies in perfused rat livers are conflicting, with some showing a decrease (Topping and Mayes, 1972; Spydevold et al., 1983) and some an increase in VLDL output when insulin is included in the perfusion buffer. Evidence from cultured rat hepatocytes indicate that insulin stimulates TG synthesis but inhibits VLDL secretion (Durrington et al., 1982; Patch et al., 1983). Beynen and Geelen (1982), however, showed that insulin does not affect secretion of the pre-stored TG

while stimulating secretion of the newly synthesized.

It is clear from the above discussion that there is no conclusive evidence to indicate that insulin has a direct (as opposed to indirect through decreasing the flow of FFA) inhibitory effect on hepatic VLDL production.

To summarize, in light of the above discussion, the following conclusions can be drawn:

1. Insulin enhances TG synthesis in the liver. Whether this effect persists when insulin resistance is present depends on the nature of the perturbation. This mechanism could be operational in Cushing's syndrome patients and in patients of endogenous hypertriglyceridemia and thus may contribute to the hyperlipidemia observed in these two perturbations.

2. In some diseases wherein insulin resistance is expressed, enhanced lipolysis and thus FFA supply in addition to the hyperglycemia may enhance hepatic TG synthesis and thus lead to the hypertriglyceridemia and enhanced hepatic VLDL secretion. These diseases include type II diabetes mellitus, endogenous hypertriglyceridemia, and Cushing's syndrome.

3. In some diseases, Insulin-resistance may lead to impairment of VLDL removal and thus contribute to the

accompanying hyperlipidemia. These diseases include type II diabetes mellitus, endogenous hypertriglyceridemia and Cushing's syndrome.

4. Chronic insulin treatment probably increases hepatic VLDL output if substrate supply is not limiting. The effect on plasma TG levels depends on the balance between production and removal. The findings on acute insulin administration are inconclusive.

1.2 EFFECT OF GLUCOCORTICOIDS AND INSULIN ON TRIGLYCERIDE SYNTHESIS:

Glucocorticoid administration enhances hepatic lipogenesis by increasing the activities of acetyl-CoA carboxylase, fatty acid synthetase, and NADP-malate dehydrogenase (Diamant and Shafrir, 1975; Krausz et al., 1981). This seems to be due to the concomitant hyperinsulinemia because it was not observed in alloxan-diabetic animals. In adipose tissue however, glucocorticoids decrease the activity of the lipogenic enzymes acetyl-CoA carboxylase, fatty acid synthetase, and NADP malate dehydrogenase (Diamant and Shafrir, 1975). Glucocorticoids enhance liver TG synthesis through inducing the synthesis of the enzyme phosphatidate phosphohydrolase. (Brindly 1981)

Phosphatidate phosphohydrolase converts phosphatidate to diacylglycerol. This provides the substrate for the final step in TG synthesis.

Insulin lipogenic effects in the liver and adipose tissue are well established (Block and Kramer, 1948; Brady and Gurin, 1950; Brady et al., 1951; Altman et al., 1951; Haft and Miller, 1958; Dannenburg et al., 1965; Ball, 1966; Haft, 1967; Ontko, 1972; Tulloch et al., 1972; Nikkila, 1974; Alberti et al., 1978; Geelen et al., 1978; Beynen et al., 1979). Briefly, insulin induces acetyl-CoA carboxylase (the rate limiting enzyme in fatty acid synthesis) and fatty acid synthetase. In doing so insulin also inhibits beta-oxidation of fatty acids. This is because malonyl-CoA the product of the acetyl-CoA corboxylase reaction inhibits carnitine acyltransferase which translocates fatty acids into the mitochondrial compartment where fatty acid oxidation takes place. Insulin also enhances glycolysis thus providing a source for alpha-glycerophosphate. Alpha-glycerophosphate is needed for TG synthesis. Insulin also enhances fatty acid synthesis from glucose by enhancing glucose uptake, the generation of pyruvate by glycolysis, and the conversion of pyruvate to acetyl-Co A which then can be converted to fatty acids.

Insulin also enhances free fatty acid synthesis by providing the needed reducing potential through activating the pentose cycle. In adipose tissue insulin promotes FFA uptake by enhancing LPL activity and inhibits fatty acid release by inhibiting hormone-sensitive lipase (Severson, 1979).

Based on the evidence presented above, in a condition in which hyperinsulinemia and hyperglucocorticoidemia coexist, one is likely to find enhanced hepatic lipogenesis. The effect on adipose tissue lipogenesis is difficult to predict however, and would depend on the severity of each perturbation.

1.3 EFFECT OF GLUCOCORTICOIDS AND INSULIN ON CHOLESTEROL SYNTHESIS AND EXCRETION :

Administration of glucocorticoids to rats in physiological doses increases hepatic hydroxymethylglutaryl(HMG)-CoA reductase activity (Lin and Snodgrass, 1982). At supra-physiological doses, glucocorticoids decrease reductase activity (Edwards 1973; Nepokroeff et al., 1974). In cultured cells glucocorticoids increase reductase activity (Cavenee and Melnykovych 1977; Henze et al. 1981; Lin and Snodgrass, 1982). In cultured hepatocytes, the increase

in reductase activity is reflected in an increase in cholesterol synthesis (Lin and Snodgrass, 1982). This, however, is not the case for cultured human fibroblasts and HeLa cells wherein the increase in reductase activity is accompanied by a decrease in cholesterol synthetic activity (Cavenee and Melnykovich, 1977; Henze et al., 1981). This indicates that in the latter two cell types HMG-CoA reductase might not be rate limiting. The inductive effect of glucocorticoids on HMG-CoA reductase activity in cultured cells is dependent on protein synthesis (Cavenee and Melnykovich, 1977; Lin and Snodgrass, 1982). The inductive effect was also shown not to depend upon dephosphorylating an existing enzyme protein (the dephosphorylated form of the enzyme is the active form) or inducing the synthesis of protein activator-factors (Lin and Snodgrass, 1982).

Adrenalectomy abolishes the diurnal rhythm of HMG-CoA reductase with the activity remaining at the level of the diurnal nadir (morning level) (Edwards, 1973; Mitropoulos and Blasubramaniam, 1976). Glucocorticoid administration to adrenalectomized animals 3 hrs before the expected diurnal high restored the enzymatic activity to levels of control animals. Glucocorticoid administration to adrenalectomized rats

3 hrs prior to the diurnal nadir failed to increase reductase activity to levels above that of control animals. The restoration of reductase activity when glucocorticoids are administered is dependent on protein synthesis because cyclohexamide blocked the effect (Mitropoulos and Blasubramaniam, 1976).

Insulin has also been shown to augment the activity of HMG-CoA reductase in rats and isolated rat hepatocytes. The mode of activation by insulin, however, is thought to be post-translational. Two models have been proposed for insulin action; one is by enhancing the reduced free sulfhydryl form of the enzyme (Sample and Ness, 1986); and the other is by enhancing the dephosphorylated form of the enzyme (Ingebristen et al., 1979). The model proposed by Ingebristen et al. (1979) is that insulin brings about the dephosphorylation of both HMG-CoA reductase and the reductase kinase. This activates the former and deactivates the latter. In humans with type 2 diabetes mellitus (hyperinsulinemia is usually manifested in cases of mild type 2 diabetes mellitus), hepatic cholesterol production is elevated. This finding is complicated by the hyperphagia that usually accompanies diabetes mellitus however (Bennion and Grundy, 1977).

To the author's best knowledge only one study examined the possible involvement of glucocorticoids in the regulation of 7-alpha hydroxylase activity. This enzyme is the rate limiting enzyme in bile acid synthesis. Mitropoulos and Blasubramaniam (1976) examined the effect of adrenalectomy in the rat on 7-alpha hydroxylase activity. The study indicated that adrenalectomy decreased the activity of the enzyme. The decrease was most prominent on the activity of the enzyme at the diurnal high (mid-dark period). Cortisol restored the activity to control levels if infused 3 hours before the diurnal high but was without effect if infused before the diurnal low. The response to cortisol was probably dependent on protein synthesis because it was inhibited by actinomycin D. (Mitropoulos and Blasubramaniam, 1976). The possible involvement of insulin in the regulation of 7-alpha hydroxylase has not been investigated.

1.4 EFFECT OF GLUCOCORTICOID AND INSULIN ON LIPOPROTEIN LIPASE ACTIVITY:

Chronic treatment of rats (5 to 8 days) with glucocorticoids depresses LPL activity in adipose tissue and increases it in lung , heart, and skeletal

muscle (Hamosh et al., 1976; Krausz et al., 1981). In the lung it also increases the heparin releasable enzyme activity. The fact that food intake also decreased may explain the decrease in adipose tissue LPL activity. Starvation in the rat, has been shown to increase heart and diaphragm LPL activity while decreasing that in adipose tissue (Borensztajn et al., 1970; De Gasquet and Pequignot, 1973; De Gasquet et al., 1975). In contrast, chronic administration of glucocorticoids to mice (Galpin et al., 1983) increases their food intake. Similarly, Cushing's syndrome patients are hyperphagic (Dallman, 1984). In contrast to the chronic effect of glucocorticoids in rats, acute administration increases LPL activity in both heart and adipose tissue (De Gasquet et al., 1975).

Insulin administration to rats for 2-8 weeks increases the heparin-releasable LPL activity. (Steiner et al., 1984; Kazumi et al., 1986; Kruszynska et al., 1987) Adipose tissue LPL activity is directly related to the plasma insulin levels (Steiner et al., 1984; Kazumi et al., 1986). The enhancement of adipose tissue LPL activity by insulin administration is also observed in man (Sadur and Eckel, 1982.; Yki-Jarvinen et al., 1984).

Insulin and glucocorticoids enhance LPL activity in isolated fat bodies from rats starved for 24 hours (Ashby and Robinson, 1980). Insulin's effect is observed independently, but it is potentiated in the presence of glucocorticoids (Ashby and Robinson, 1980). The stimulatory action of insulin correlates well with its stimulatory effect on protein synthesis (Hollenberg et al., 1970; Vydellingum et al., 1978). Glucocorticoids also potentiate LPL activity in the absence of insulin but their effect is much more dramatic in the presence of insulin (Ashby and Robinson, 1980). Despite the fact that glucocorticoids lead to an overall decrease in protein synthesis, their effect on LPL activity can be explained by selective enhancement of the LPL protein synthesis. Such specific enhancement of protein synthesis due to glucocorticoids has been observed previously (Higgins and Gehring, 1978). The effect of glucocorticoids on protein synthesis is probably at the transcriptional level (Schimke, 1969; Higgins and Gehring, 1978), while that of insulin is thought to be post-transcriptional (Borensztajn et al., 1970).

The evidence presented above suggests that LPL activity probably increases in response to higher glucocorticoid levels and hyperinsulinemia, providing

that food intake is not decreased and peripheral tissue insulin resistance is not expressed.

1.5 EFFECT OF GLUCOCORTICOID AND INSULIN ON ABSORPTION OF DIETARY CARBOHYDRATE AND LIPID:

Reports on the effect of adrenalectomy on carbohydrate absorption are conflicting, with some showing a decrease and some showing no change (Levin, 1969). The uptake of glucose into everted intestinal sacs from animals treated with ACTH or hydrocortisone prior to sacrifice is increased (Benerjee and Varma, 1966). Triglyceride absorption is decreased in adrenalectomized rats and in patients with adrenal insufficiency (Isselbacher, 1967; Nutrition reviews, 1967). The author is not aware of any reports on the effect of adrenalectomy or glucocorticoid administration on cholesterol absorption.

The effects of experimental diabetes on the absorption of sugars have been studied extensively in animals. Most reports show an increase in hexose transport across the intestinal mucosa in animals in which diabetes has been induced by alloxan or streptozotocin injection. This effect has been observed in both in vivo and in vitro preparations (Axelrad et

al., 1970; Levinson et al., 1970; Caspary, 1973; Thomson, 1981) This same observation has been noted in patients with juvenile onset diabetes (Vinnik et al., 1965) and patients with diabetes mellitus due to pancreatic insufficiency (Caspary, 1973). The disaccharidase activity has also been shown to be increased by streptozotocin or alloxan treatment in in vitro intestinal preparations (Caspary, 1973). Thomson et al. (1980) showed an increase in fatty acid and cholesterol transport across the intestinal mucosa as well. This is in agreement with findings of Nervi et al. (1974) in which cholesterol absorption was shown to be increased in alloxan diabetic rats.

Insulin administration In vivo, in both human subjects and experimental animals, either decreases or is without effect on intestinal glucose absorption (Caspary, 1973) . This, however, is in contradiction to in vitro studies, wherein enhanced glucose absorption has been demonstrated in intestinal segments obtained from animals treated with insulin prior to sacrifice (Caspary, 1973). The effect of long term insulin treatment has not been adequately studied however.

It is difficult to predict what the effect of a combined hyperinsulinemia and hyperglucocorticoidemia would be on carbohydrate and lipid absorption because

insulin seems to inhibit, whereas glucocorticoids stimulate these functions. The closest approximation is the experimental model in which ACTH or hydrocortisone was administered (Benerjee and Varma, 1966). In this model glucose absorption was increased.

1.6 THE EFFECT OF INSULIN AND GLUCOCORTICIDS ON LIPOPROTEIN BINDING AND RECEPTORS:

In rat hepatocytes, glucocorticoids decrease LDL binding via the classical LDL receptor (Salter et al., 1987; Salter et al., 1988). In cultured human fibroblasts and arterial smooth muscle cells, hydrocortisone decreases the uptake and degradation of LDL but is without effect on the binding (Henze et al.; 1983). Furthermore, dexamethasone has been shown to decrease LDL degradation by human monocyte-derived macrophages (Hirche and Mazzone, 1986).

In contrast, insulin increases the binding and degradation of LDL in cultured human skin fibroblasts (Chait et al., 1978) and increases the binding of LDL via the classical LDL receptor in rat hepatocytes (Salter et al. 1987; Salter et al. 1988). The insulin-induced increase in LDL binding was shown to be due to increasing the number, not the affinity of LDL

receptors (Chait et al., 1979). The stimulatory effect of insulin on LDL binding and degradation was also demonstrated in hepatoma G2 cells (Wade et al., 1988). Additionally, the VLDL binding activity of peritoneal macrophages isolated from alloxan-treated mice is reduced. This is corrected by insulin treatment (Kreamer, 1986). Suresh et al. (1986), however, reported an insulin-induced decrease in LDL binding to human lymphocytes. In vivo, insulin administration in humans, has been shown to enhance LDL clearance. Monocytes isolated from human subjects, after undergoing euglycemic clamp, had enhanced LDL catabolic activity (Mazzone et al., 1984).

It is evident that most of the work on insulin and glucocorticoid presented above has been performed on cultured cells. This leads to some uncertainty as to the physiological conclusions that can be drawn from these studies. With that in mind however, there seems to be agreement that insulin enhances LDL uptake and degradation by all cells studied, with the exception of human lymphocytes. This conclusion is strengthened by the agreement with the in vivo human study of Mazzone et al. (1984). Moreover the contribution of lymphocytes to total lipoprotein removal is small (Havel, 1986). There also seems to be agreement that glucocorticoids

inhibit LDL internalization and degradation. Finally, these studies of LDL receptor activity imply that the same effect would be observed with VLDL because the classical LDL receptor (which is specific for the apo B and apo E proteins) also recognizes VLDL and VLDL remnants (Havel, 1986).

SECTION 2. THE NATURE OF HYPERLIPIDEMIA IN THE OBESE
ZUCKER RAT : POSSIBLE INVOLVEMENT OF HYPERINSULINEMIA
AND HYPERGLUCOCORTICOIDEMIA

The obese Zucker rat, the animal model in this study, is most fittingly described as being severely hypertriglyceridemic and modestly hypercholesterolemic. It is also hyperinsulinemic and displays an insulin resistant state. Similar to the obese Zucker rat, these features are manifested by a broad spectrum of hyperlipidemic patients. These include patients with type II diabetes mellitus, patients with endogenous hypertriglyceridemia (type IV hyperlipoproteinemia), patients with Cushing's syndrome and patients whose hyperlipidemia is thought to be obesity-induced. However, the obese Zucker rat bears more resemblance to Cushing's syndrome patients and obese patients. In both obese Zucker rats and Cushing's syndrome patients, the hyperinsulinemia and insulin resistance is ameliorated by adrenalectomy. As in Cushing's syndrome patients, the obese Zucker rat maintains high levels of circulating glucocorticoids with no apparent diurnal rhythm. Similarly obese patients have increased production rates of corticosterone and share with the Zucker obese rat the metabolic complications of

maintaining enlarged adipose tissue depots. Obese patients however have normal levels of circulating glucocorticoids and maintain a normal diurnal rhythm of glucocorticoid secretion. Thus the hyperlipidemic state of the Zucker rat would most probably resemble that of Cushing's syndrome patients with complications due to obesity. The metabolic features discussed above make the obese Zucker rat an excellent model for studying the mechanism of induction of hyperlipidemia in diseases of glucocorticoid excess and those associated with hyperinsulinemic-insulin-resistant states.

2.1 LIPOPROTEINS IN THE OBESE ZUCKER RAT:

Obese Zucker rats are hyperlipoproteinemic, hypertriglyceridemic, and hypercholesterolemic compared to lean Zucker rats. The hypertriglyceridemia is due to high serum levels of TG-rich VLDL particles (Schonfeld et al., 1974; Durstine et al. 1985; Lin, 1985; Shepherd et al., 1985). Additionally the total cholesterol content is greater in all lipoproteins fractions in obese rats relative to lean rats. However, lipoprotein cholesterol distribution is different between male and female rats. In the obese male rat most of serum cholesterol is contained in HDL (Lin, 1985). In obese

female rats, on the other hand, the serum cholesterol distribution is more uniform, with VLDL containing a substantial part of serum total cholesterol (Durstine et al., 1985; Lin, 1985; Shepherd et al., 1985). High density lipoprotein cholesterol is mostly carried in the ester form while that in VLDL is mostly free (Durstine et al., 1985; Lin, 1985; Shepherd et al., 1985). This was true regardless of sex. The higher levels of VLDL cholesterol in female obese rats imply reduced levels of VLDL conversion into HDL in female rats. In support of this, Redgrave (1977) demonstrated lower fractional clearance rates of chylomicrone TG and cholesterol ester in female obese rats compared to lean female rats. This aspect has not been examined in male obese rats.

The hyperlipoproteinemia in obese rats seems to be age dependent. In younger rats the hyperlipoproteinemia is mostly due to elevated levels of serum VLDL. As the animals age serum levels of all lipoprotein classes become elevated (Durstine et al., 1985; Mathe et al., 1987).

Differences between hepatic perfusate (in isolated liver perfusions) and plasma lipoproteins provide insight as to how lipoproteins are modified in the circulation or the degree to which extrahepatic tissues

contribute to the final make up of plasma lipoproteins or both. There is agreement that perfusion of livers from obese rats results in hypersecretion of TG-enriched VLDL (Schonfeld and Pflieger, 1971; Fukuda et al., 1982). The very low density lipoprotein fraction in hepatic perfusates from obese rats is also cholesterol enriched relative to lean rats and contains most of the hepatic perfusate cholesterol (Fukuda et al., 1982). These findings imply that the fasting hypercholesterolemia and the hypertriglyceridemia in the obese rats are caused by hepatic oversecretion of VLDL. Furthermore the observation that most of the hepatic perfusate cholesterol is in VLDL indicates that the plasma cholesterol-rich HDL is either of non hepatic origin or is the catabolic byproduct of hepatic VLDL.

The VLDL particles of obese rats are larger, TG enriched and are less dense compared to those of lean control rats. The VLDL fractions are protein enriched as well, with the increased amount of apo C and lower amounts of apo E and apo B compared to lean control animals (Witztum and Schonfeld, 1979). Apo C is considered to be an activator of lipoprotein lipase while apo B and apo E are thought to mediate tissue uptake of LDL by tissue receptors. Since LDL levels are

very low in obese rat and not different from lean rats, it is unlikely that the hypercholesterolemia is caused by impaired receptor removal of LDL as would the lower VLDL content of apo B and apo E imply (Durstine et al., 1985; Lin, 1985; Shepherd et al., 1985). .

2.2 POSSIBLE MECHANISMS AND CONTRIBUTING FACTORS IN THE GENESIS OF HYPERLIPIDEMIA IN THE OBESE ZUCKER RAT:

2.21 HEPATIC TRIGLYCERIDE SYNTHESIS:

One mechanism that may underlie the increased VLDL secretion and thus the hyperlipidemia is the elevated hepatic TG synthesis in the obese rat. The supply of both exogenous and endogenous FFA to support the elevated levels of TG synthesis is abundant. The obese rat has elevated levels of plasma FFA (Martin et al., 1978) and maintains high levels of hepatic fatty acid synthesis as measured by radioactive substrate incorporation (Sullivan et al., 1977; Godbole and York, 1978; Kannan et al., 1980; Azain et al., 1985). Cleary (1986 a, b) showed elevated hepatic lipogenesis reflected by increased activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate

dehydrogenase, malic enzyme, and fatty acid synthetase in mature (44 weeks) and 15-week old obese rats. Taketomi et al. (1975) showed that the level of acetyl Co-A carboxylase was elevated in the liver and adipose tissues of 8-week old obese rats. Jamdar et al. (1978) showed increased glycerolipid formation in the liver and adipose tissue in obese rats starting at 32 days of age .

De novo fatty acid synthesis in male obese Zucker rats was shown to account for approximately 50% of the total fatty acids secreted in triglycerides by the liver. In comparison fatty acid synthesis accounted for only 10% of the fatty acids secreted in TG in lean rats. The rest of the fatty acids secreted in triglycerides originated from circulating fatty acids (Azain et al., 1985). When the partitioning of circulating FFA between oxidation and re-esterification was examined, the obese rat showed a greater proportion channeled into re-esterification despite similar rates of uptake between lean and obese rats (Fukuda et al., 1982). The obese rats consequently had lower rates of ketogenesis. Enhanced re-esterification of FFA derived from the plasma thus provide extra impetus for the increased TG secretion. Such a shift in FFA metabolism suggests insulin influence on this process.

To summarize, livers of obese rats display enhanced fatty acid synthesis, enhanced esterification of fatty acids, and depressed catabolism of fatty acid. These changes together with rich exogenous supply of fatty acids contribute to the elevated rates of hepatic TG synthesis. The elevated rate of hepatic TG synthesis probably leads to the higher rates of VLDL secretion by livers of obese rats.

2.22 LIPOPROTEIN REMOVAL:

There is no difference between lean and obese rats in adipose tissue lipoprotein lipase activity when expressed per gram of tissue or per millimole of tissue TG. However the activity is markedly elevated when expressed per fat pad or per cell (Gruen et al., 1978; Hartman, 1981; Maggio and Greenwood, 1982; Walberg et al., 1983). Reports on LPL activity in homogenates of heart and muscle are contradictory. Hartman (1981) reported lower LPL activity in heart and red muscle in 9-11 week old obese animals and no differences in 26-30 week old animals compared to lean control animals. Walberg et al. (1983) reported elevated levels of heart LPL activity at 7 weeks old and levels comparable to lean controls in 15 and 23 week old obese animals. Walberg et al. (1983) also showed no differences in the levels of LPL from gastrocnemius muscle of 7, 15, and 23 week old obese and lean rats.

Perhaps a more reliable measure of physiological lipolytic activity is heparin-extractable tissue LPL activity. This is because heparin releases only the LPL activity that is associated with the capillary endothelium which is thought to be operational in hydrolyzing the TG in TG-rich lipoproteins. The post

heparin LPL activity of heart, adipose tissue and skeletal muscle in 14-18 week old obese Zucker rat, expressed per gram of tissue, is not different from its lean control. There is no difference in post heparin plasma LPL activity between lean and obese rats while post heparin hepatic triglyceride lipase is elevated in obese rats(Wang et al., 1984).

Apparently the obese state does not affect adipose tissue LPL qualitatively. This can be concluded from the observation that the affinity of LPL for its substrates as measured by the K_m , is similar in lean and obese rats (Gruen et al., 1978). In agreement with this observation in LPL activity derived from tissue homogenates, heparin extractable LPL activity shows similar kinetic properties between lean and obese rats, i.e., they display similar K_m values. When VLDL from obese and lean rats are used as lipolytic substrates the rates of lipolysis are similar indicating no change in VLDL composition that might affect the LPL activity. In addition, VLDL in the obese rat has either increased or at least sufficient amounts of apo C (an apoprotein that is recognized as an LPL activator) to support lipolysis (Wang et al., 1984; Witztum et al., 1979).

Thus one can conclude from the above discussion that total adipose tissue LPL activity in the obese rat

is increased due to adipocyte hypertrophy and increased depot size. Heart and skeletal muscle LPL activity is either decreased or not different depending on age. Hepatic TG lipase activity is also increased. Furthermore there seems to be no perturbation in VLDL composition that renders it resistant to lipolysis. It thus seems unlikely, based on the aforementioned findings, that a defect in VLDL removal would play a major role in the development of hyperlipidemia. However Redgrave et al. (1977) reported that the fractional clearance rate of chylomicrone TG and cholesterol ester is lower in obese female rats relative to lean female rats. This finding is not necessarily contradictory to the findings on LPL activity. This is because the rate of production of TG-rich lipoproteins may overwhelm the rate of removal albeit it might be increased. Thus, at least in female rats, a defect in TG-rich lipoprotein removal may play a factor in the development of hyperlipidemia.

2.23 CHOLESTEROL SYNTHESIS AND EXCRETION:

Elevated levels of HMG-CoA reductase activity may contribute to the hypercholesterolemia in obese female rats. It has been shown that the obese female rat lacks the diurnal rhythm characteristic of the enzyme

activity. The enzymatic activity is maintained at the levels observed during the diurnal high of lean rats. The male obese rat lacks the diurnal rhythm of enzymatic activity as well, however the activity persist at levels similar to those observed during the diurnal low of lean rats. Because obese rats possess larger livers, expressing the enzymatic activity on whole liver basis amplifies the differences between female animals and restores that of the male animal to levels similar to its lean control (Lin, 1985). This is in agreement with the study of Triscari et al. (1980) in which 15-16 month old obese female but not male rats displayed elevated hepatic cholesterol synthesis relative to lean control. Pector et. al (1981), however, reported a small increase in hepatic cholesterol synthesis per liver in 3-month old male obese rats. The fact that increased cholesterol synthesis may contribute to the observed hypercholesterolemia is demonstrated by the report of McCune and Jurin (1987) in which mevinoлин (HMG-Co A reductase inhibitor) was shown to reduce the hypercholesterolemia in female obese rats but not in female lean rats. In the same report, hepatocytes isolated from lean and obese rats showed inhibition of HMG-Co A reductase activity to the same extent. This indicates that de novo hepatic cholesterol synthesis is

not a major determinant of plasma cholesterol levels in lean rats but does play such a role in obese rats.

Fecal bile acid excretion (expressed as mg of bile acids excreted per day) in 3-month old male obese rats is elevated relative to lean control rats. This argues against a defect in cholesterol excretion as a causative factor in the observed hypercholesterolemia Subbiah and Connely (1976). However, due to the marked difference in liver and body size between lean and obese rats, it would have been more meaningful if the data were normalized to body or liver weight. Normalization of the data allows one to conclude if bile acid excretion would affect cholesterol concentration in body storage compartments.

In liver perfusion studies bile production in livers from obese animals was not significantly different from that of lean controls (Fukuda et al., 1982; Azain et al., 1985). The composition of the bile was not examined in these studies however. In a recent publication, Tang et al. (1988) found that 7 alpha-hydroxylase activity in livers of obese rats was significantly lower at mid-dark cycle (diurnal high) than that of lean rats. Furthermore they found that the activity in livers of obese animals persisted at that low level with no apparent diurnal rhythm. In

comparison the activity in lean rats displayed a normal diurnal rhythm. The discrepancy between the results on 7-alpha hydroxylase activity of Tang et al. (1988) and that on bile acid excretion of (Subbiah and Connely, 1976) are not necessarily contradictory. The enhanced bile acid excretion reflects total hepatic production while the decreased 7-alpha hydroxylase activity reflects specific activity per mg of microsomal protein. Accordingly, the difference in liver size between lean and obese animals may well account for the observed discrepancy.

It is noteworthy to mention that the studies on HMG-Co A reductase and 7-alpha hydroxylase activity in the Zucker rat were published while the work for this study was in progress.

2.24 HYPERPHAGIA:

It is unlikely that the plasma cholesterol level in the obese Zucker rat is affected by the hyperphagia. When food intake was reduced to below maintenance levels to induce weight loss the hypercholesterolemia was not reversed or reduced in obese rats (Rogers and Kris-Etherton, 1983). Intermittent dietary restriction in mature (32-56 weeks) and growing (9-15 weeks) obese

rats does not reduce the hypercholesterolemia (Cleary, 1986 a, b). Additionally hypercholesterolemia is most pronounced in mature obese Zucker rats at an age at which these rats are not considered hyperphagic (Bach et al., 1981). Starvation for 3 days, however, reduced the plasma cholesterol levels substantially in both lean and obese rats regardless of sex, and the obese rats were not hypercholesterolemic when compared to lean controls (Lin, 1985). This observation is not consistent, however, with the report of Triscari et al. (1980) in which 15-16-month old male and female obese rats maintained elevated cholesterol levels compared to lean controls after starving for 2-12 days.

Hyperphagia does not seem to be a major factor in the development of the hypertriglyceridemia either. Mature obese rats that are no longer considered hyperphagic maintain high levels of plasma TG relative to age matched controls (Bach et al., 1981). Dietary restriction before weaning by increasing the number of rats per litter, followed by restricting food intake to that of lean litter mates after weaning, does not affect the development of the hypertriglyceridemia in the obese Zucker rat (Cleary et al., 1980). Severe caloric restriction does lead, however, to a substantial decrease in plasma TG levels although they

remain slightly above normal (Koletsy and Puterman, 1976). Intermittent dietary restriction in obese rats 32-44 weeks of age and 9-15 weeks of age decreases but does not abolish the hypertriglyceridemia when rats are sacrificed at the end of the last restriction period (Cleary, 1986 a,b). Fasting in 15-16 month old obese male and female rats for 2-12 days did not abolish the hypertriglyceridemia compared to lean controls (Triscari et al., 1980).

2.3 THE STATUS OF GLUCOCORTICOIDS AND INSULIN IN THE OBESE ZUCKER RAT

2.31 GLUCOCORTICOIDS:

Adrenal function in the obese Zucker rat is abnormal in that the plasma level of corticosterone is maintained at the level of the diurnal high of lean rats with no apparent rhythm. In 12 week old obese male rats plasma corticosterone levels remain higher than those of lean controls throughout most of the day. The exception is just prior and through the early stage of the dark cycle when the diurnal surge in lean animals takes place (Martin et al., 1983). This was also observed in 4-5 week old female rats (Fletcher, 1985). In pre-weaned rats (18 days) there seems to be no difference in corticosterone levels between the two genotypes (Fletcher, 1985). However, The serum levels of corticosterone in pre weaned rats is determined by maternal serum corticosterone levels as well endogenous production. This introduces a confounding factor and thus such values can not be used as a measure of endogenous plasma corticosterone levels. Cunningham et al. (1986) reported increased basal and dexamethasone-suppressed urinary corticosterone

excretion (dexamethasone is a potent synthetic glucocorticoid) in female obese rats. They also reported decreased pituitary responsiveness to corticotropin releasing factor (CRF) which they attributed to chronic hypercorticoesteronemia in the obese rat. In the same study, however, there was no difference in plasma corticosterone levels between lean and obese rats. This is probably due to the fact that they used an indwelling catheter to collect plasma samples, a procedure that probably introduced appreciable stress.

To summarize, the obese animal displays increased corticosterone production, lack of diurnal rhythm, and maintains serum corticosterone concentration at the level of the diurnal high of lean rats. The increase in glucocorticoid production rate and the lack of diurnal rhythm resembles adrenal function in Cushing's syndrome. However the adrenal function in Cushing's syndrome is different in that the levels of cortisol remain elevated well above the diurnal high in normal individuals. The adrenal function in human obesity is similar to that in obese Zucker rats in that it displays an elevated production rate. It however, displays normal diurnal rhythm and plasma cortisol levels.

2.32 INSULIN:

The plasma insulin level in the obese Zucker rat is elevated and remains so throughout the day. In contrast, the plasma insulin level in the lean rat follows the pattern of food intake, i.e., tends to be elevated during the dark cycle (Martin et al., 1978). A significant elevation in insulin levels is evident as early as 3 weeks of age (Zucker and Antoniades, 1972). Although no hyperinsulinemia could be detected in pre-weaned pre-obese pups, they showed a significant elevation in insulin response to an intragastric or intravenous glucose load (Rohner-Jeanrenaud et al., 1983; Fletcher, 1985,). Islet cells isolated from pancreata of obese rats release more insulin per unit time of incubation than those of lean animals (Stern et al., 1972; Stern et al., 1975). Furthermore, pancreata of obese rats showed islet hypertrophy and B cell hyperplasia (Shino et al., 1973). The islet size correlated well with its insulin secretion rate (Hayek and Woodside, 1979). The insulin and proinsulin of obese and lean rats are identical in their immunoreactivity and biological potency and represent the same proportion of total immunoreactive insulin extracted from the pancreas (Laburthe et al., 1975).

Theoretically, insulin resistance can lead to hyperinsulinemia. The Zucker obese rat is insulin resistant in the sense that high levels of insulin are needed to maintain normal or even slightly elevated levels of glucose as compared to those of lean controls (Zucker and Antoniades, 1972). Stern et al. (1972) found that insulin stimulated glucose uptake by isolated adipocytes is reduced in obese rats, 24 weeks of age, relative to lean control rats, despite similar basal uptake rates. They also showed decreased insulin stimulated glucose incorporation into diaphragm glycogen in obese rats compared to lean rats. Adipocyte preparations isolated from 10 week old obese Zucker rats exhibit greater basal and insulin stimulated glucose utilization and transport per cell but decreased insulin sensitivity (as manifested by a rightward shift in the dose response curve) relative to lean controls (Czech et al., 1978). Also in the same study, basal glucose utilization in soleus muscle from obese rats was depressed and insulin sensitivity as well as binding was decreased. These observations in soleus muscle were also reported in 5-6 week old animals by Crettaz et al. (1980). Adipose tissue in vivo did not show reduced response to insulin induced glucose uptake in 5 and 20 weeks old rats. However, a reduced response was observed in the diaphragm muscle

(Zucker and Antoniades, 1972). Penicaud et al. (1987) reported that in vivo insulin-stimulated glucose utilization is depressed in skeletal muscle and brown but not white adipose tissue in rats 4 and 12 weeks of age.

Based on the above findings, one can conclude that white adipose tissue does not contribute to the insulin resistance in vivo, and the insensitivity observed in isolated adipocytes probably does not have any physiological implications. Skeletal muscle insensitivity however is manifested in vivo and in vitro regardless of age and probably contributes considerably to the resistance observed in vivo.

The liver of the obese rat is insensitive to insulin in that it maintains glucose production rates similar to those of lean rats despite the hyperinsulinemia. Insulin infusion decreases the hepatic glucose production in lean rats but is without effect in obese rats (Terretaz and Jeanrenaud, 1983). This insulin insensitivity is in contradiction to the shift in hepatic fatty acid metabolism and TG synthesis toward a pattern that resembles enhanced insulin influence. This effect might be due to glucocorticoid antagonism to insulin action.

Insulin binding to liver plasma membranes from young obese (9-10 week old) rats is not reduced compared to lean rats (Clark and Clark, 1982, Clark et al., 1982). Findings in older rats (4 month old and above) are controversial however, with some showing a decrease (Clark and Clark, 1982, Figlewicz et al., 1985) and others showing no change (Steel et al., 1979). Karakash and Jeanrenaud (1983) showed that insulin removal per gram of liver is decreased in 9-10 week old obese rats compared to lean controls and that insulin binding and degradation is decreased in isolated hepatocytes from obese rats compared to lean rats. Broer et al. (1977) on the other hand showed that insulin binding is not different in isolated hepatocytes from 12-15 week old lean and obese rats. Sodoyez et al. suggested that despite the possible downregulation, because of the sustained hyperinsulinemia in obese rats, insulin binding to livers of obese rats is effectively elevated compared to lean rats. Cushing's syndrome patients are similar to obese rats in that they show decreased insulin removal (Karnieli et al., 1985). Hepatic insulin binding has not been examined in Cushing's syndrome patients, however.

The hyperinsulinemia in the obese Zucker rat may be related to the perturbed glucocorticoid status. Adrenalectomy prevents the development of hyperinsulinemia in obese Zucker rats (York and Godbole 1979; Freedman et al., 1986 a, b), decreases insulin levels in older (22 weeks) lean animals (Freedman et al., 1986 b), and does not affect insulin levels in younger (8-9 weeks) lean rats (York and Godbole 1979; Freedman et al. 1986 a). Adrenalectomy also corrects the insulin insensitivity as measured by steady state serum glucose (SSSG) as well as enhances hepatic insulin removal in the obese rats. An observation that lends support to possible glucocorticoid involvement in the observed hyperinsulinemia is the fact that insulin resistance is not manifested on hepatic TG synthesis which is consistent with glucocorticoid enhancement of hepatic lipogenesis. The metabolic features mentioned above, i.e normoglycemia or limited hyperglycemia in the presence of hyperinsulinemia, insensitivity of hepatic glucose production to insulin, increased hepatic fatty acid synthesis and reesterification at the expense of oxidation, and increased hepatic secretion of TG enriched VLDL are consistent with metabolic disturbances seen in diseases of glucocorticoid excess such as Cushing's syndrome.

The hyperphagia can serve as a stimulus for increased insulin secretion in the obese animal. However, several studies have shown this explanation to be invalid. Obese rats in which food intake was restricted prior to weaning by increasing the litter size, and after weaning by restricting their food intake to levels of lean animals, have insulin levels that are not different or even significantly higher than ad libitum controls (Cleary et al., 1980). Additionally, pair feeding obese rats to lean controls does not reverse the hyperinsulinemia in the obese rat (Martin, 1976; Clark and Clark, 1982). Following a 24 hour fast, serum insulin levels remained ten folds higher in the obese animal as that in lean controls although it decreased significantly if compared to the non-fasted state in both lean and obese animals (Martin and Gahagan, 1977). Obese rats under intermittent dietary restriction from 9 to 15 weeks of age or from 28 to 44 weeks of age have insulin levels that are several fold higher than lean controls when sacrificed at the end of the last food restriction period. The obese food-restricted rats, however had serum insulin levels that were markedly lower than those of obese rats fed ad libitum. (Cleary, 1986 a, b) One can conclude from the above that increased dietary intake

while contributing to the hyperinsulinemia is not a sine qua non for its existence.

Other factors that might be implicated in the increased insulin secretion by the pancreas include adipocyte hypertrophy (Stern et al., 1972), and increased plasma levels of branched chain amino acids (Stern et al., 1972; Finkelstein et al., 1982). Depressed negative feedback by insulin on isolated pancreatic islets from obese rats was also suggested. This suggestion was based on the observation that EC50 (half maximal effective concentration) is increased and the number of insulin high affinity binding sites is decreased in pancreatic islets from obese rats (Versphol et al., 1986). And finally Jeanrenaud suggests that the hyperinsulinemia in the obese rat is partly vagus nerve mediated. Total reversal of hyperinsulinemia by vagotomy has not been demonstrated, however (Rohner-Jeanrenaud et al., 1983).

2.4 POSSIBLE INVOLVEMENT OF THE DERANGED

GLUCOCORTICOID STATUS AND HYPERINSULINEMIA IN THE GENESIS OF HYPERLIPIDEMIA IN THE OBESE ZUCKER RAT:

Glucocorticoids play a central role in the development of obesity in the Zucker rat. Adrenalectomy in mature obese rats, 20 weeks and older, inhibits weight gain, and enhanced lipid deposition, but not food intake (Yukimura and Bray, 1978; Freedman et al., 1985). Obese rats, however, are not hyperphagic at that age compared to age matched lean controls (Bach et al., 1981). In younger rats adrenalectomy does inhibit food intake (Castonguay et al., 1986). If obese Zucker rats are adrenalectomized shortly after weaning, the development of obesity is severely impaired. Body weight gain, food intake and lipid deposition are severely reduced. Fat cell size is decreased but not fat cell number (Freedman et al., 1986 a).

Adrenalectomy prevents the expression of the insulin resistant state in obese rats in that they become normoglycemic and normoinsulinemic relative to lean control animals. This was demonstrated when adrenalectomy was performed on obese rats ranging in age from 3-12 weeks (Fletcher, 1986; Freedman et al., 1986 a, b).

In obese adrenalectomized animals, the hypertriglyceridemia is reversed as is the hyperinsulinemia. It is plausible to attribute the amelioration of hypertriglyceridemia to the lowered insulin levels and the decreased food intake or both. One observation that argues for the decreased insulin levels as the major factor underlying the hypertriglyceridemia is that obese, adrenalectomized rats with replacement therapy of high levels of glucocorticoids, become hyperinsulinemic relative to sham operated controls as well as hypertriglyceridemic but remain hypophagic (Freedman et al., 1986 a). The elevated hepatic fatty acid synthesis is also ameliorated by adrenalectomy; this may be caused by the reversal of hyperinsulinemia and may contribute to the amelioration of the hypertriglyceridemia (York and Godbole, 1979).

Adipose tissue LPL activity is depressed by adrenalectomy in both obese and lean adrenalectomized rats (Freedman et al., 1986 a).

In comparison, adrenalectomy does not affect food intake in the lean rat (Freedman et al., 1986 a; Fletcher, 1985; Castonguay et al., 1986). It affects body weight gain either marginally (Castonguay et al., 1985; Freedman et al., 1986 a) or not at all (York and

Godbole, 1979). Adrenalectomy also decreases lipid deposition and fat cell size in the lean rat if it is performed shortly after weaning (4 weeks) but not to the same extent as in the obese rat (Freedman et al., 1986 a). However, adrenalectomy in older lean rats (11 weeks) does not affect lipid deposition (Castonguay et al., 1986). Plasma TG are not affected (Castonguay et al., 1986; Freedman et al., 1986 a) Serum insulin levels are not affected (York and Godbole, 1979; Fletcher, 1985; Fletcher, 1986; Freedman et al., 1986 a) or lowered slightly (Freedman et al., 1986 b). In general the effects of adrenalectomy in lean rats are either not significant or less pronounced than in the obese animal.

The effect of adrenalectomy on plasma lipoprotein concentration and cholesterol levels in lean and obese Zucker rats has not been examined.

In rats treated with the anti-beta cell agent alloxan and replaced with low doses of insulin, fat deposition is lowered but not abolished (Chan et al., 1982). Furthermore when obese and lean rats are treated with the diabetogenic agent streptozotocin and replaced with equal doses of insulin, hepatic lipogenic enzymes activities remain elevated in obese rats (Martin and Stolz, 1982). However, in another study Godbole and

York (1978) demonstrated that hepatic FFA synthesis is restored to levels of lean control rats after streptozotocin treatment without insulin replacement. It should be noted that streptozotocin treatment does not abolish insulin secretion entirely. In fact, in the study of Godbole and York (1978), serum insulin levels remained higher in obese treated rats than lean control rats. Additionally the serum insulin levels reported by Martin and Stolz were exceedingly high in both lean and obese treated and replaced rats (almost 10 fold higher than those of control lean rats). If one had to conclude anything from the above discussion, it would be that while the excessive availability of insulin may play a major role in promoting the enhanced hepatic lipogenic activity, it is probably not an absolute requirement for its expression. Serum lipid levels were not measured in these studies.

It is likely that the hyperlipidemia in the obese Zucker rat is simply a manifestation of the hyperinsulinemic-insulin resistant state. If true, similarly to the mechanism proposed for the development of hyperlipidemia in many diseases in which insulin resistance and hyperinsulinemia is expressed, the hyperlipidemia (both the hypertriglyceridemia and the hypercholesterolemia) would be caused mostly by hepatic

oversecretion of VLDL. However it is possible that elevated serum levels of insulin and glucocorticoids lead to hypercholesterolemia by interfering with steps other than hepatic secretion. These include, enhancing cholesterol synthesis and/or enhancing dietary cholesterol absorption and/or depressing cholesterol excretion. The present study examined the possible involvement of the excess-glucocorticoid state in the development of hypercholesterolemia in the obese Zucker rat. Effects on cholesterol synthesis, excretion, dietary absorption, and liver and adipose tissue storage are investigated.

2.5 SPECIFIC AIMS:

To test the hypothesis that the excess glucocorticoid state leads to the expression of the observed hypercholesterolemia in the obese Zucker rat, two approaches were adopted:

1. Pharmacological suppression of glucocorticoid secretion.
2. Surgical removal of the adrenal glands.

We determined that the pharmacological approach was not successful in suppressing corticosterone

secretion chronically. Consequently, the major part of this study was dedicated to examining the effect of adrenalectomy on the following aspects of cholesterol metabolism:

1. Hepatic cholesterol synthesis as measured by HMG-Co A reductase activity.
2. Cholesterol excretion as measured by hepatic cholesterol 7-alpha hydroxylase activity.
3. Dietary cholesterol absorption.
4. Cholesterol content of the major storage depots namely, serum, liver, and adipose tissue.

Additionally, the findings in this study provide insight in the effect of genotype (lean vs. obese) on these aspects of cholesterol metabolism.

MATERIALS AND METHODS

1. SMITH, KLINE, AND FRENCH (SKF)-12185 COMPOUND,
PROPERTIES AND ADMINISTERED DOSE:

Compound SKF-12185 has been shown to be a specific inhibitor of the last step in cortisol and corticosterone synthesis both in vitro and in vivo (Saunders et al., 1966; Hewick and Young, 1973; Touitou et al., 1976). This step is catalyzed by the cytochrome P-450 enzyme 11-beta hydroxylase. Smith, Kline and French-12185 compound inhibited the stress induced rise in corticosterone concentration in rats both acutely and chronically (Saunders et al., 1966). Using rat adrenal homogenate, SKF-12185 was shown to be 250 fold as potent as another therapeutically used 11-beta hydroxylase inhibitor, metyrapone (Sprunt and Hannah, 1969). In the in vivo study of Saunders et al. (1966) maximum inhibition of corticosterone response to cold stress was obtained by an oral dose of 8 mg/kg of body weight. The dose they used for chronic inhibition was 20 mg/kg of body weight, administered orally. In the present study the drug was administered in the diet (0.1% weight/weight). This percentage was chosen, based on estimates of food intake, so that the administered dose would be approximately 100 mg/kg. This dose was chosen to ensure completeness of inhibition of

corticosterone secretion. The effective calculated dose, based on food intake and body weight data did not differ between lean and obese rats except in week 5 of the study in which food intake was severely low in obese animals. There was also a gradual decrease in the effective dose administered with time in both genotypes. This gradual decrease with time was caused by the decrease in food intake with time in treated animals of both genotypes coupled with a consistent increase in body weight. The decrease due to time was significantly lower in weeks 4 and 5 of the study relative to week 1 in both genotypes. Despite the decrease in the dose per kg of body weight, the lowest dose attained (55.18 ± 11.0 mg/kg, MEAN $\pm$ S.E.M, in obese treated animals, week-5) remained more than twice as high as that used in the study of Saunders et al. (1966) (Fig A , Appendix). The effective dose of SKF-12185 data was analyzed by two way ANOVA, genotype (lean and obese) vs. weeks in study (weeks 1-5). The F ratios are as follows: genotype: 1.0 , non significant, weeks of study: 39.80, significant at $\alpha = 0.01$, interaction: 3.3, non significant.

2. ANIMALS:

Zucker rats were identified as lean or obese by inguinal fat pad biopsy at 3 to 4 weeks of age. Because of sex-related differences in some aspects of cholesterol metabolism, only female rats were used. At 4 to 5 weeks of age the rats were either adrenalectomized or transferred to a diet containing 0.1 % (w/w) SKF-12185 (11 β -hydroxylase inhibitor, a generous gift from Smith Kline & French company, Philadelphia, PA).

Control animals for the adrenalectomy study were sham-operated. Control animals for the inhibitor study were kept on regular chow.

After surgical procedures were complete, or reaching 4 to 5 weeks of age in the case of inhibitor-treated rats, the rats were housed individually in a temperature controlled room (23° C to 25°C) with 6 AM-6 PM light-dark cycle for 5 weeks. Regular chow was provided ad libitum to all rats except the inhibitor treated rats as mentioned above. All treatment rats were provided with 1% NaCl (to compensate for the loss of aldosterone secretion) and

all control rats with tap water ad libitum. Daily food intake was determined during the fifth week of the study. Body weight gain was followed throughout the 5 week period. At the end of the 5 weeks follow-up period, the rats were sacrificed by decapitation at 9-11 AM and the tissues handled as mentioned below. The rats were fasted for 11-12 hours before sacrifice. To minimize novelty stress at the time of sacrifice the rats were handled frequently throughout the study, they were brought to the sacrifice room one night before sacrifice and housed individually, and the time between leaving the cage and decapitation was kept under 30 seconds for each animal. Novelty stress has been shown to increase corticosterone secretion and thus mask differences that may exist due to genotypes in serum corticosterone levels.

3. SURGICAL PROCEDURES:

It was necessary to determine animal phenotype at or before 4 weeks of age prior to inclusion in the study. Before 4 weeks of age it is not possible to determine the phenotype (lean or obese) of the animal visibly or by body weight with accuracy. Thus an inguinal fat pad biopsy was performed under light ether

anesthesia. Briefly, a small skin incision was made in the inguinal area, and one inguinal fat pad was quantitatively removed. The fat pads were weighed, and homogenized in 5 ml of isopropanol. The total volume of the isopropanol extract was made to 10 ml to insure complete lipid extraction. The triglyceride (TG) concentration of the isopropanol extract was measured by an automated procedure (see analytical methods). The TG content of total fat pads was calculated as follows :

$$\text{Fat pad TG content} = \text{TG concentration (mg/ml)} \times \text{total volume of extract (10 ml)}$$

The mean fat pad weight and fat pad TG content were calculated for each litter. Values above the mean of each litter were assumed to belong to obese animals. Genotype prediction was 100% accurate starting at 17 days of age based on fat pad TG content and at 18 days of age based on fat pad weight (Fig. B, appendix).

Adrenalectomy was performed under light ether anesthesia by the dorsal approach. Briefly, a midline skin incision was made in the dorsal area below the rib cage. Another incision was made through the muscle wall immediately below the rib cage, adjacent to the spinal cord, on both sides. The adrenal glands were visible

through the incision and could be removed along with the connective tissue that connects it to the kidney. The muscle layer and the fascia were sutured, and the skin incision closed with wound clips. The site of the skin incision was sprayed with topical antibacterial powder (Furazolidone, Veterinary Products Laboratories, Phoenix, Arizona). Sham-adrenalectomy was performed exactly as above except that the adrenal glands were not removed. Adrenalectomized rats were supplemented with 100 ug of dexamethasone per day per rat for 3 days. The dexamethasone was administered as a suspension in corn oil subcutaneously. The 1% NaCl solution and the tap water provided to the adrenalectomized rats and the sham adrenalectomized rats respectively, were supplemented with tetracycline hydrochloride powder (Polyotic, American Cyanamid Company, Wayne, NJ) for 3 days post-operative to prevent possible infections.

4. PROCESSING OF TISSUES:

After decapitation blood was collected and allowed to clot on ice for 15 minutes followed by centrifugation at 2300 to 2500 RPM for 10 minutes. Serum was extracted in isopropanol for cholesterol, TG,

and phospholipid determinations, and the rest divided in 0.3 ml fractions and stored below -20°C for hormonal and glucose determination.

After decapitation the liver was immediately excised and placed in ice-cold homogenization buffer. Subsequently the liver was washed 3 times with fresh homogenization buffer and weighed. Two liver pieces (2-4 g) were homogenized in homogenization buffer, with and without KFl (1:5), using a loose fitting motor-driven teflon pestle. KFl was added to inhibit phosphoprotein phosphatase, which activates the HMG-Co A reductase protein by dephosphorylation. To prepare the microsomal fraction, the homogenate was centrifuged at 12,000 g and the pellet discarded. The supernatant was transferred to Beckman ultracentrifuge tubes (Beckman polyallomer tubes No. 326823), and centrifuged at 109,000 g. The resulting pellet was resuspended in homogenization buffer with or without KFl (1ml), divided in 0.3 ml aliquots and stored at -70°C until assayed for HMG-Co A reductase within 2 to 3 weeks and 7 α -hydroxylase within 4 to 5 weeks. All microsomal isolation procedures were carried at $0-4^{\circ}\text{C}$. The buffer used for homogenization was a Tris buffer (40 mM, PH 7.4) containing KFl (50 mM) KCl (50 mM) or KCl only (100 mM), dithiothreitol (5 mM), EDTA (1 mM), and

sucrose (300 mM). Microsomal suspensions were extracted in isopropanol for cholesterol determination. The remainder of the liver was homogenized 1:10 in saline and the homogenate extracted in isopropanol for cholesterol, TG and phospholipid determination.

The perimetrial and retroperitoneal-perirenal depots were excised and transferred to plastic weigh boats containing saline at room temperature. The pads were then blotted on filter paper and weighed. One gram or less of each pad was transferred to a flask containing 10 ml of 0.95% Minimal Essential Medium (MEM) (Grand Island Biological Company, Grand Island, NW) containing 2 mg/ml Na HCO₃, 2 mg/ml dextrose, 10 mg/ml bovine serum albumin (fraction V, Sigma Chemical Co., St. Louis, MO), and 1 mg/ml collagenase (type 2, Sigma Chemical Co. St. Louis, MO) PH 7.4. The mixture was incubated in a 37° C water bath with shaking until digestion was complete. The mixture was then filtered through nylon mesh to exclude remaining connective tissue and centrifuged at low speed briefly (10-15 seconds). The centrifugation separates the adipocytes which float from fibrocytes and remnants of the digested connective tissue. As much of the supernatant as possible was discarded and the upper adipocyte layer was resuspended in fresh MEM containing no collagenase.

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Part of the washed adipocyte suspension was extracted in isopropanol for TG and cholesterol determination. The adipocytes were also sized using a computer program written for the Apple (II e) computer by Dr. Arthur D. Hartman. Briefly, a magnified microscopic image of a sample of the adipocyte suspension is projected on a graphic tablet (model A2M029, Apple Computer Inc., Cupertino, CA) connected to an interface card in the computer mother board. By using a pen also connected to the interface card one inputs the two points that demarcate the diameter of adipocytes which appear round in the projected image. This distance (the diameter) was stored and the program automatically converts the inputted information into adipocyte volumes and surface areas (assuming the adipocytes are perfect spheres) and gives the TG content per cell (ug) using the formula $TG \text{ weight(ug)} = \text{mean cell volume (pl)} \times 0.915 \times 10^{-3}$. This formula assumes that the entire adipocyte volume is occupied by TG and uses the density of triolein to convert the volume to TG content (weight). Since part of the adipocyte suspension was extracted for TG determination, one can determine the total number of adipocytes extracted as follows :

Total cells extracted = Total TG content of the extract/average TG content per adipocyte.

In this manner one can express the cholesterol content per million cells as follows :

Cholesterol content per million cells = Total extract cholesterol content/total number of cells per extract (millions).

5. ANALYTICAL PROCEDURES:

Liver homogenate, serum, and adipocyte suspension were extracted for lipids 1:20 in isopropanol (Lefeller, 1959). Stigmasterol (Sigma Chemical Co., St. Louis, MO) was added as an internal standard to the tissue extracts for cholesterol quantification. A fraction of the extract was dried and resuspended in methylene chloride and used directly for the determination of free cholesterol by gas chromatography. A fraction of the extract was dried and saponified in ethanolic KOH (Abelle et al., 1952). The non-saponifiable lipids were extracted in petroleum ether, dried, resuspended in methylene chloride and used for the determination of total cholesterol by gas chromatography. The gas chromatograph used was Perkin-Elmer, model 3920, equipped with 3% SE 30 on 120/140 Gas Chrom Q column, at a column temperature of

288° C, a detector temperature of 340° C, and an injector temperature of 340° C, with nitrogen as the mobile phase at a flow rate of 35 ml/min. The column length is 6 feet and the internal diameter 2 mm.

TG content of all extracts was determined by an automated colorimetric method (Kessler and Lederer, 1965). Phospholipid content was determined by a colorimetric method (Skoloff and Rothblat, 1974). Serum glucose was measured by an enzymatic assay. (Lowery and Passonneau, 1972)

6. HORMONAL MEASUREMENTS:

Serum insulin concentration was determined by radioimmunoassay (RIA) commercial kit (ICN Micromedical Systems, Horsham, PA 19644, Cat No. D1804 or Radioassay Systems Laboratories, Carson, CA 90746, Cat No. 134). Serum corticosterone levels were measured with an RIA commercial kit (Radioassay systems laboratories, Carson, CA 90746, Cat. No. 10070R, or Cambridge Medical Technology, 575 Middlesex TNPK Billerica, MA 01865),

7. ENZYMATIC ASSAYS:

Microsomal HMG-Co A reductase was assayed by the method of Shapiro et al. (1974) as modified by Goodwin and Morgalis (1976) with some modification. Briefly, the microsomal preparation was diluted in assay buffer. The assay buffer was a Tris buffer (40 mM) PH 7.4 containing dithiothreitol (5 mM), EDTA (1 mM), KCl (50 mM) and KF1 (50 mM) or KCl only (100 mM) so as to arrive at a protein concentration of 0.5 to 2 mg/ml in a total reaction mixture of 0.2 ml. This range of protein concentration was used to insure that the enzymatic activity was a linear function of the microsomal protein concentration. The microsomes isolated in the presence of KF1 were also assayed in the presence of KF1. This was done to maintain the inhibition of phosphoprotein phosphatase. After incubating 0.15 ml of the diluted microsomal suspension at 37° C for 20 min, 0.025 ml of HMG-Co A preparation containing 70 nmol. of ¹⁴C-HMG-Co A (NEN Research Products, Boston, MA) (specific activity =0.5 to 0.1 mCi/mM) in assay buffer was added. To start the reaction 0.025 ml of substrate preparation containing NADP (80 mM) (Boehringer Mannheim, Indianapolis, IN) glucose-6-phosphate (800 mM) (Boehringer Mannheim, Indianapolis, IN) , glucose-6-phosphate dehydrogenase

(60 I.U./ml) (Grade I suspension, Boehringer Mannheim, Indianapolis, IN), EDTA (72 mM) all in assay buffer were added. The reaction was carried out for 20-30 min in screw-capped conical centrifuge tubes (13 ml capacity) in a shaking water bath at 37°C. The reaction was stopped by adding 100 μ l of HCl (6N). To insure the lactonization of the mevalonate, the reaction mixture was incubated for an additional 30 min under the above incubation conditions. After lactonization, 20 μ M of ^{3} H-mevalonolactone (NEN Research Products, Boston, MA) (specific activity = 0.005 mCi/mM) in ethanol (0.025 ml) was added as internal standard to test the completeness of the extraction process. Na_2SO_3 (0.5 to 0.6 g) per incubation was added to enhance the extraction, and the reaction mixture was extracted in 7.5 ml of benzene or toluene twice. The extract was then dried, resuspended in scintillation cocktail for aqueous samples (Beckman, Ready-Solve EP). In some samples the reaction mixture was extracted with 2 volumes (10 ml) of non-aqueous scintillation cocktail (Beckman, Ready-Solve NA). The two extraction procedures had similar extraction efficiencies. The samples were counted in a Beckman scintillation counter, model LS-3145 T, and the ^{3} H and the ^{14}C counts were determined using a program written for the Apple-IIe computer by the author. Control

incubations for each sample were acid inactivated microsomes (100 uL of HCl (6N) added before starting the incubation). The enzymatic activity was expressed as picomoles of mevalonate formed per minute per ug of microsomal protein.

Cholesterol 7 alpha-hydroxylase was assayed according to the method of Shefer et al. (1968) as modified by Waxman (1986) with some modification. The microsomal preparation containing KF1 (50 mM) was used. KF1 was reported to decrease loss of enzymatic activity due to storage (Waxman 1986). ^{14}C -cholesterol (NEN Research Products, Boston, MA) (0.04 mCi/mM) was introduced into the microsomal preparation as a suspension in Tween-80 (Sigma Chemical Co., St. Louis, MO). This was done by dissolving the cholesterol in Tween-80-acetone suspension (7.5 mg of Tween-80 per ml of acetone). 40 ul of this suspension containing ^{14}C -cholesterol (80 nm) was transferred to 12x75 glass disposable culture tube. The acetone was dried under a stream of nitrogen. The Tween-80-cholesterol suspension was then resuspended in 350 ul of the microsomal preparation. Prior to addition, the microsomal preparation was diluted in assay buffer so that 300 ul contained 0.1-0.2 mg of protein. This was done to insure that the measured enzymatic activity is linearly

related to the protein concentration in the incubation mixture. The assay buffer was a Tris buffer (40 mM, PH 7.4) containing beta-mercaptoethylamine (5mM), KCl(50 mM), dithiothreitol (5 mM), KFl (50 mM), EDTA (10 mM). After resuspension the tubes were incubated for 10 min at 37°. The reaction was started by adding NADPH regenerating system (50 ul) composed of assay buffer containing NADP (8 mM), glucose-6-phosphate (40 mM), and glucose-6-phosphate dehydrogenase (20 IU/ml). The reaction was carried out for 40-50 min in a 37° C water bath with shaking. The reaction was then stopped by adding 3 ml of 1.5% isoamyl alcohol in ethyl acetate. After adding 0.3 g of NaCl to enhance the extraction, the mixture was vortexed at maximal speed for 15 sec. The organic solvent phase was removed and the mixture reextracted with 2 ml of the same solvent mixture. The two extracts were then combined, the solvent evaporated, and the extracted lipids were resuspended in chloroform (100 ul). The chloroform was spotted on flexible silica gel-G plates (Aluminum-backed, 250 um thickness). The tubes were washed with 50 ul of chloroform twice and the chloroform extract spotted to ensure complete transfer. The plates were then developed using ethyl acetate-toluene mixture (3:2). The radioactive bands were localized by exposure to X-ray film (Kodak X-OMAT AR) for 36-48 hr. The 7

alpha-hydroxycholesterol band was identified by spotting authentic 7 alpha-hydroxycholesterol standard (Steraloids Inc., Wilton, NH). The 7 alpha-hydroxycholesterol bands were then transferred with aluminum backing to a scintillation vial containing scintillation cocktail for non-aqueous samples (Beckman Ready-Solv NA). The enzymatic activity was expressed as nanomoles of cholesterol converted per minute per mg of microsomal protein.

Lecithin:cholesterol acyltransferase (LCAT) was measured by the methods of Stokke & Norum (1971) with modification by Albers et al. (1981) with some modification. To prepare the substrate 200 nmol of ^{14}C cholesterol in 0.2 ml of acetone was injected in 1 ml of 5% human serum albumin (HSA) in 100 mM phosphate buffer PH 7.4 with continuous stirring. The acetone was then evaporated under a stream of nitrogen. To initially inhibit LCAT activity, the serum (100 ul) was incubated with 20 ul of 5,5' dithiobis nitrobenzoic acid in 100 mM phosphate buffer PH 7.4 at 37° C for 30 min. The substrate preparation (25 ul) was then added and the mixture was incubated at 37° C for 4 hours to allow equilibration of substrate with serum lipoproteins. The reaction was started by adding 20 ul of 100 mM 2-mercaptoethanol in 100 mM phosphate buffer

PH 7.4. The reaction was stopped after 40 minutes by adding 0.25 ml of absolute ethanol and vortexing. The reaction mixture was extracted by adding 0.75 ml of absolute ethanol, 0.5 ml of saline, and 2 ml of hexane and vortexing. The hexane layer was transferred and the reaction mixture was extracted two more times in 2 ml of hexane. The hexane extract was combined, dried under a stream of nitrogen, and resuspended in 0.5 ml of chloroform. A known portion of the chloroform solution was spotted on silica gel-G plates (Aluminum-backed, 250 um thickness) and the plates developed in a solvent system of petroleum ether:ethyl acetate, (70:30). The cholesterol ester and cholesterol spots were identified by spotting authentic standards. The spots were cut with aluminum backing and transferred to scintillation vials containing 10 ml of non-aqueous scintillation cocktail (Beckman Ready-Solv NA). The LCAT activity (umoles of cholesterol esterified per hour per liter of serum) was calculated as follows:

$$\text{LCAT activity} = (\text{cpm CE} \times 60 \text{ min/hr} \times \text{serum FC} \times 10^6 \text{ umoles/mole}) / (\text{cpm FC} \times (\text{M.W. FC}) \times 40 \text{ min})$$

cpm CE : counts per minutes in the cholesterol ester spot.

serum FC : serum free cholesterol concentration
g/liter.

cpm FC : counts per minutes in free cholesterol spot.

M.W. FC : molecular weight of free cholesterol (g).

8. CHOLESTEROL ABSORPTION:

Cholesterol absorption was measured according to Borgstrom et al. (1969) adapted to the rat by Balmer and Zilversmit (1974) with modification. The method is based on the determination of the percentage of ^{14}C -cholesterol absorbed using ^3H -beta-sitosterol as internal standard to adjust for loss of cholesterol by means other than absorption in the intestinal tract. A meal composed of 5g of finely ground purina rodent chow containing 1 mg of cholesterol per 100 g of body weight, 50 mg of corn oil per 100 g of body weight, 1 μCi of ^{14}C -cholesterol per meal and 2 μCi of ^3H -sitosterol per meal was fed to the animals. The rats were housed individually in metabolic cages equipped with metal nets for fecal collection. The rats were fasted over night (9-10 hr fast). At the end of the fasting period, the meals were presented to the rats in special containers that collect the spillage so that

accurate determination of the amount consumed can be made. The meals were replaced with regular chow at the beginning of the dark cycle. The feces was collected daily for 6 days. The feces were then pooled, dried in a vacuum oven and finely ground using a conventional blender. The feces was then extracted in isopropanol (80 ml). When scintillation counting was performed directly after saponification, the counting was severely affected by color quenching and could not yield statistically significant ^{14}C counts. Thus, decolorization was carried according to Borgstrom (1964) with modification. Accordingly, to 10 ml of the isopropanol extract, 2 ml of 20 % $\text{Ba}(\text{Cl})_2$, 4 ml of 10 % ZnSO_4 , and 4 ml of $\text{Ba}(\text{OH})_2$ was added. The mixture was vortexed thoroughly followed by centrifugation at 2500 RPM for 5 min. The procedure above was repeated twice and the supernatant, which contained less than 2% of the total counts, was discarded. The precipitates were resuspended and extracted in 20 ml of petroleum ether twice. The petroleum ether extract was pooled and evaporated under a stream of nitrogen. The dry sediment was saponified by resuspending in 5 ml of 6% KOH (50 %) in absolute ethanol and heating at 75°C for 1 hr. To the ethanol-water mixture, 5ml of distilled water and 10 ml of petroleum ether were added. The tubes were vigorously shaken for 30 seconds, and the petroleum

ether phase which contained the non-saponifiable lipids was transferred to scintillation vials. The petroleum ether was dried and resuspended in scintillation cocktail for non-aqueous samples (Beckman Ready-Solv NA). Dual-label counting was performed in a Beckman scintillation counter. The counts per minute measured were converted to disintegrations per minute using a computer program written for the Apple IIe computer by the author. Cholesterol absorption expressed as a percentage of the total dose administered was determined using the following formula :

$$\text{Percentage of the total dose of cholesterol absorbed} = 1 - \left[(\% \text{ } ^{14}\text{C-cholesterol in feces})^a / (\% \text{ } ^3\text{H-sitosterol in feces})^b \right]$$

a: percentage of the total administered dose of ^{14}C -cholesterol recovered in feces.

b : percentage of the total administered dose of ^3H -sitosterol recovered feces.

9. STATISTICAL ANALYSIS:

Serum corticosterone data in the adrenalectomy study were analyzed by 2-tailed T test, lean vs. obese. All other data were analyzed by 2-way analysis of variance (ANOVA), lean vs. obese, and adrenalectomized or drug treated vs. control. Before proceeding with ANOVA or T test, data groups were analyzed for homogeneity of variance by Bartlett's test. Data groups whose variances were not homogeneous required log transformation. The 2-way ANOVA was followed by Student Newman Keuls test to test differences between individual means.

RESULTS

1. SKF-12185 STUDY:

1.1 BODY WEIGHT GAIN AND FOOD INTAKE:

Table 1 shows body weights, body weight gain, and food intake in lean and obese control and SKF-12185 treated animals. As expected, body weight gain of obese animals was significantly greater than that of lean control animals (98 ± 5 g in obese vs. 76 ± 4 g in lean control rats). Body weight gain was markedly decreased in obese treated animals (98 ± 5 g in obese control vs. 51 ± 4 g in obese treated animals). Treatment in obese animals decreased their body weight gain to levels significantly below that of lean control animals. There was also a less marked effect in lean animals. Food intake was severely decreased due to treatment in both genotypes (Table 1)

1.2 ADRENAL GLANDS AND FAT DEPOT WEIGHT:

Adrenal gland weight was higher in SKF-12185 treated rats in both genotypes (33.0 ± 1.4 and 28.3 ± 1.4 mg in lean and obese control animals vs. 45.6 ± 3.1 and 52.6 ± 4.0 mg in lean and obese treated animals respectively) (Table 1). This probably was due

Table 1

Body weights, adrenal gland weights, fat depot weights, body weight gain from 5 to 9 weeks of age, and average daily food intake from 8 to 9 weeks of age: The effect of genotype and SKF-12185 treatment

	BODY WEIGHT (g)	ADRENAL GLAND WEIGHT (mg)	PM FAT PAD WEIGHT (g)	PR FAT PAD WEIGHT (g)	(PM+PR) DEPOT WEIGHT (g) PER 100 GRAM OF BODY WEIGHT	BODY WEIGHT GAIN (g)	AVERAGE DAILY FOOD INTAKE (g)
LEAN n=	136 ± 6^1 11	33.0 ± 1.4^2 11	$0.5 \pm 0.1^{1,2,3}$ 11	$0.40 \pm 0.04^{1,2}$ 11	$0.6 \pm 0.1^{1,2,3}$ 11	$76 \pm 4^{1,2,3}$ 10	$14.4 \pm 0.4^{1,2,3}$ 11
OBESE n=	167 ± 6^2 10	28.3 ± 1.4^2 10	3.4 ± 0.5 10	2.2 ± 0.2 10	3.2 ± 0.4 10	98 ± 5^2 10	17.0 ± 0.6^2 11
LEAN/SKF n=	126 ± 5 5	45.6 ± 3.1 5	0.1 ± 0.03 5	0.10 ± 0.04 5	0.5 ± 0.1 5	53 ± 4 5	9.6 ± 0.8 4
OBESE/SKF n=	116 ± 7 5	52.6 ± 4.0 5	1.8 ± 0.4 5	1.3 ± 0.3 5	2.6 ± 0.4 5	51 ± 4 6	6.8 ± 1.3 5
F Lean vs Obese	2.17, NS	1.01, NS	61.42, $\alpha=.01$	44.98, $\alpha=.01$	238.43, $\alpha=.01$	3.61, NS	0.04, NS
F No Drug vs SKF	17.50, $\alpha=.01$	86.52, $\alpha=.01$	23.0, $\alpha=0.1$	19.23, $\alpha=.01$	33.64, $\alpha=.01$	51.12, $\alpha=.01$	96.16, $\alpha=.01$
F Interaction	8.30, $\alpha=.01$	10.93, $\alpha=.01$	11.30, $\alpha=.01$	7.30, $\alpha=.05$	18.63, $\alpha=.01$	5.62, $\alpha=.05$	12.29, $\alpha=.01$

¹ = Lean vs Obese, $\alpha=.05$, Student Newman Keuls

² = No Drug vs SKF treated of the same genotype, $\alpha=.05$, Student Newman Keuls

³ = Lean vs Obese/SKF, $\alpha=.05$, Student Newman Keuls

Values represent mean $\pm$ S.E.M.

PM: Perimetrial depot

PR: Perirenal depot

to oversecretion of adrenocorticotrophic hormone (ACTH) in response to inhibition of corticosterone secretion by the adrenal glands. As expected fat depot weight was higher in obese animals relative to lean control animals (3.4 ± 0.5 g in obese vs. 0.5 ± 0.1 g in lean control animals, in the perimetrial (PM) depot) (Table 1). The over-all effect of treatment (as determined by the F ratio) on fat pad weights, both perimetrial and perirenal, was a significant decrease. The post-hoc analysis showed a significant difference in the lean but not the obese group. Additionally fat pads from obese treated animals remained significantly larger than those of lean control animals in the perimetrial depot.

1.3 SERUM INSULIN AND CORTICOSTERONE CONCENTRATION:

Treatment with SKF-12185 did not produce to a detectible lowering of serum corticosterone concentration (Table 2). This indicates that the SKF-12185 treatment was not effective in suppressing corticosterone secretion at or before the time of sacrifice. Additionally, there was no difference between lean and obese control animals in serum corticosterone concentration. This is in contrast to the marked difference between lean and obese control

Table 2

Serum corticosterone, insulin, and cholesterol concentrations and serum LCAT activity: The effect of genotype and SKF-12185 treatment

	CORTICOSTERONE CONCENTRATION (ng/ml)	INSULIN CONCENTRATION (μ IU/ml)	SERUM LCAT ACTIVITY (μ m/hr/L)	CHOLESTEROL (mg/dl)		(FREE/TOTAL) $\times$ 100
				FREE	ESTER	TOTAL
LEAN n=	$104 \pm \frac{6}{7}$	$32 \pm \frac{7^{1,2}}{4}$	$55 \pm \frac{4^2,3}{5}$	$15.3 \pm \frac{1.0^{1,3}}{5}$	$48.4 \pm \frac{3.1^{1,3}}{5}$	$65.3 \pm \frac{3.6^{1,3}}{5}$
OBESE n=	$126 \pm \frac{15}{10}$	$73 \pm \frac{8^2}{5}$	$59 \pm \frac{2^2}{6}$	$28.3 \pm \frac{2.4^2}{6}$	$66.9 \pm \frac{3.2}{6}$	$95.2 \pm \frac{4.7}{6}$
LEAN/SKF n=	$84 \pm \frac{8}{5}$	$11 \pm \frac{2}{5}$	$32 \pm \frac{1}{5}$	$14.6 \pm \frac{1.0}{5}$	$49.4 \pm \frac{3.3}{5}$	$63.3 \pm \frac{3.8}{5}$
OBESE/SKF n=	$103 \pm \frac{14}{4}$	$34 \pm \frac{8}{4}$	$45 \pm \frac{3}{5}$	$21.1 \pm \frac{1.2}{4}$	$76.5 \pm \frac{4.4}{4}$	$97.6 \pm \frac{4.7}{4}$
F Lean vs Obese	2.23, NS	24.68, $\alpha=.01$	8.73, $\alpha=.01$	32.87, $\alpha=.01$	43.4, $\alpha=.01$	55.33, $\alpha=.01$
F No Drug vs SKF	2.48, NS	20.94, $\alpha=.01$	42.03, $\alpha=.01$	5.53, $\alpha=.05$	2.3, NS	1.39 $\times 10^{-3}$, NS
F Interaction	0.03, NS	1.71, NS	3.12, NS	3.70, NS	1.5, NS	0.24, NS
¹ = Lean vs Obese, $\alpha=.05$, Student Newman Keuls						10.56, $\alpha=.01$
² = No Drug vs SKF treated of the same genotype, $\alpha=.05$, Student Newman Keuls						3.87, NS
³ = Lean vs SKF treated Obese, $\alpha=.05$, Student Newman Keuls						9.60, $\alpha=.01$

Values represent mean $\pm$ S.E.M.

animals observed in the adrenalectomy study. This discrepancy is probably due to the smaller number of animals in the control groups in the SKF-12185 study and the high variability in the data.

In agreement with other studies, obese animals were hyperinsulinemic relative to lean animals (73 ± 8 ng/ml in obese vs. 32 ± 7 micro IU/ml in lean control animals) (table 2). Obese treated animals had insulin levels similar to those of lean control animals. Insulin levels were also significantly lower in lean treated animals compared to those of lean control animals.

1.4 SERUM LCAT ACTIVITY AND CHOLESTEROL CONCENTRATION:

Table 2 also shows serum LCAT activity, free and total cholesterol concentration, and free to total cholesterol ratio. There was no genotype differences in serum LCAT activity, and drug treatment decreased LCAT activity in both genotypes (55 ± 4 and 59 ± 2 um/hr/l in lean control animals vs. 32 ± 1 and 46 ± 3 um /hr/l in lean and obese treated animals, respectively). Obese control animals were hypercholesterolemic relative to lean control animals (95.2 ± 4.7 mg/dl in obese vs. 65.3 ± 3.6 mg/dl in lean control animals). Serum total cholesterol levels were not affected by the drug

treatment in both genotypes. Serum free cholesterol concentrations followed the same pattern as total cholesterol. The free/total cholesterol ratio was significantly greater in obese relative to lean control animals ($30.0 \pm 1.6 \%$ in obese vs. $23.0 \pm 0.6 \%$ in lean control animals). Because there was no difference in serum LCAT activity between obese and lean control animals, the greater free/total cholesterol ratio indicates that the free cholesterol in plasma of obese animals was inaccessible to esterification by LCAT. Obese drug treated animals had free/total cholesterol ratios similar to those of lean control animals.

1.5 LIVER CHOLESTEROL AND TG CONCENTRATION:

Table 3 shows the hepatic TG and cholesterol content. Liver TG content in obese animals was approximately twice that of lean animals (26.8 ± 4.0 mg/g wet wt. in obese vs. 12.6 ± 1.0 mg/g wet wt. in lean animals). This is an indication of higher lipogenic activity in livers of obese animals. The TG content of livers of obese drug treated animals remained greater than that of lean control animals. Free cholesterol content was greater in lean relative to obese control animals (1.7 ± 0.1 mg/g wet wt. in obese vs. 2.4 ± 0.1 mg/g wet wt. in lean control

Table 3

Liver TG and cholesterol concentrations: The effect of genotype and SKF-12185 treatment

CHOLESTEROL (mg/g wet wt.)					
		TG (mg/g wet wt)	FREE	ESTER	TOTAL
LEAN		$12.6 + 1.0^{1,3}$	$2.4 + 0.1^1$	$0.3 + 0.1^2$	$2.6 + 0.1^2$
n=		6	6	5	6
OBESE		$26.8 + 4.0$	$1.7 + 0.1^2$	$0.7 + 0.1^2$	$2.1 + 0.1^2$
n=		6	6	6	6
LEAN/SKF		$13.2 + 1.2$	$2.7 + 0.2$	$1.5 + 0.4$	$4.4 + 0.2$
n=		5	5	3	3
OBESE/SKF		$29.6 + 2.5$	$2.3 + 0.4$	$1.3 + 0.5$	$3.3 + 0.5$
n=		5	5	4	4

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F Lean vs Obese	57.66, $\alpha=.01$	12.22, $\alpha=.01$	0.04, NS	8.93, $\alpha=.01$
F No Drugs vs SKF	0.89, NS	6.07, $\alpha=.05$	22.48, $\alpha=.01$	31.53, $\alpha=.01$
F Interaction	0.20, NS	0.92, NS	0.7, NS	0.66, NS

¹ = Lean vs Obese, $\alpha=.05$, Student Newman Keuls

² = No Drug vs SKF treated of the same genotype, $\alpha=.05$, Student Newman Keuls

³ = Lean vs Obese/SKF, $\alpha=.05$, Student Newman Keuls

Values represent mean + S.E.M.

animals) Post-hoc analysis showed no genotype differences in esterified or total cholesterol content in control animals, although total cholesterol content tended to be greater in lean animals. The drug treatment resulted in greater hepatic total cholesterol content in both genotypes (2.6 ± 0.1 and 2.1 ± 0.1 in lean and obese control animals vs. 4.4 ± 0.2 and 3.3 ± 0.5 in lean and obese treated animals). This elevation of total cholesterol content, unlike that in adrenalectomized animals, can be mostly accounted for by an increase in the ester fraction.

2. ADRENALECTOMY STUDY:

2.1 SERUM INSULIN, CORTICOSTERONE, AND GLUCOSE CONCENTRATIONS:

Table 4 shows serum corticosterone, insulin, and glucose concentrations. Obese animals had significantly higher levels of corticosterone than lean animals (265 ± 30 ng/ml in obese vs. 144 ± 21 ng/ml in lean control animals) (Fig 2). All adrenalectomized animals had a serum corticosterone concentration of 30 ng/ml or less. Similar levels of plasma corticosterone have been reported in adrenalectomized animals by other investigators (Mitropoulos and Blasubramaniam, 1976). In agreement with the data from SKF-12185 study, the obese animals were hyperinsulinemic relative to lean controls (91 ± 10 in obese vs 12 ± 2 in lean control animals) (Fig 1). Adrenalectomized obese animals were not hyperinsulinemic relative to lean control animals. Adrenalectomy did not affect serum insulin levels in lean animals. The obese animals were also hyperglycemic relative to lean controls (8.70 ± 0.30 mM in obese vs. 7.7 ± 0.5 mM in lean control animals). Obese adrenalectomized animals, however, had serum glucose levels similar to lean controls and adrenalectomy had no effect on serum glucose levels of lean animals. The hyperglycemia in the face of

Table 4

Serum corticosterone, insulin, and glucose concentrations:
The effect of genotype and adrenalectomy

	CORTICOSTERONE (ng/ml)	INSULIN (uIU/ml)	GLUCOSE (mM)
LEAN n=	144 $\pm$ 21* 19	12 $\pm$ 2 ¹ 19	7.7 $\pm$ 0.2 ¹ 9
OBESSE n=	265 $\pm$ 30 24	91 $\pm$ 10 ² 24	8.7 $\pm$ 0.3 ² 6
LEAN/ADRX n=	< 30	10 $\pm$ 3 8	7.3 $\pm$ 0.2 4
OBESSE/ADRX n=	< 30	15 $\pm$ 2 21	7.7 $\pm$ 0.2 11
T Lean vs Obese	3.13, α =.05		
F Lean vs Obese		35.21, α =.01	8.34, α =.01
F Sham vs Adrx		44.89, α =.01	9.81, α =.01
F Interaction		16.78, α =.01	1.80, NS

¹= Lean vs Obese, α =.05, Student Newman Keuls

²= Sham vs Adrx of the same genotype, α =.05, Student Newman Keuls

*= Lean vs Obese, α =.05, T-test

Values represent mean $\pm$ S.E.M.

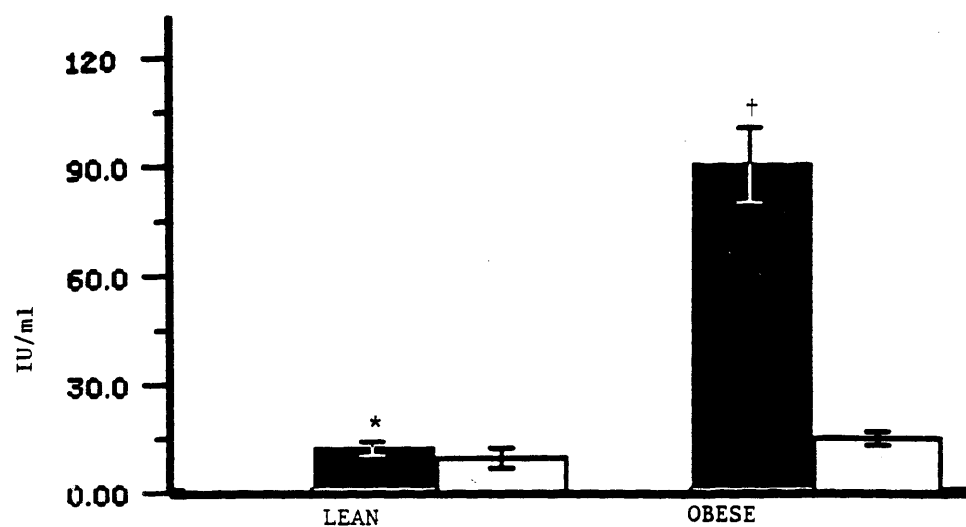


Fig. 1. Serum insulin concentration (IU/ml). The effect of genotype and adrenalectomy. Closed bars represent Sham animals, open bars represent Adrx animals. *: significant, Lean vs. obese, †: significant, Sham vs. Adrx of the same genotype, $\alpha=.05$, Student Newman Keuls. For details of 2-way ANOVA see table 4. Error bars represent S.E.M.

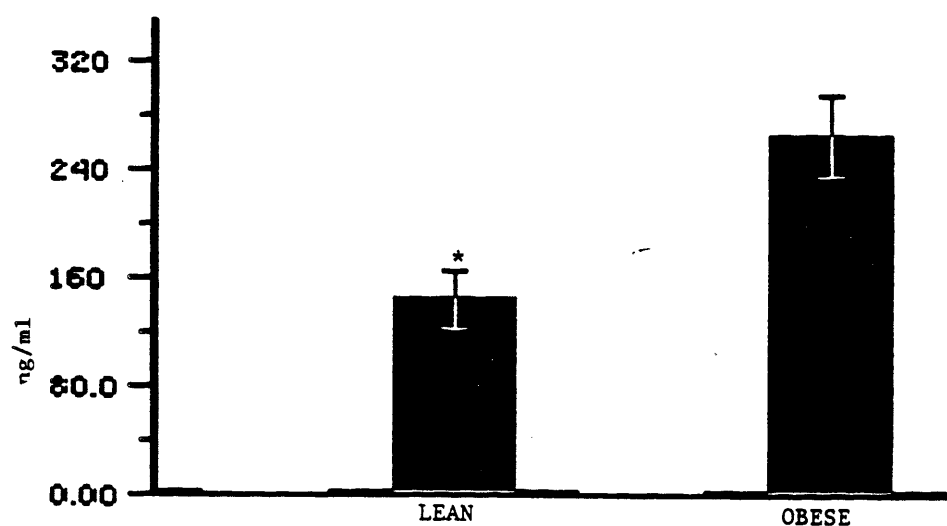


Fig. 2. Serum corticosterone concentration (ng/ml): The effect of genotype. *: significant, Lean vs. obese, $\alpha=.05$, T-test. Error bars represent S.E.M.

hyperinsulinemia in obese control animals indicates an insulin resistant state.

2.2 BODY WEIGHT GAIN AND FOOD INTAKE:

As expected, body weight gain of obese animals was much greater than that of lean animals (145 ± 5 in obese vs. 94 ± 3 in lean control rats) Body weight gain was lower in adrenalectomized animals of both genotypes compared to their respective control groups (Table 5). The body weight gain of obese adrenalectomized animals was significantly lower than that of even lean control animals. The over-all effect of adrenalectomy (as determined by the F ratio) on daily food intake was a significant decrease (Table 5). Post-hoc analysis, however, shows that the difference in daily food intake was significant only in obese adrenalectomized animals relative to obese control animals (23 ± 1 in obese control vs. 16 ± 1 in obese adrenalectomized animals)

2.3 SERUM LIPIDS:

Table 6 shows serum TG, cholesterol and phospholipid concentrations. Obese animals were markedly hypertriglyceridemic relative to lean control

Table 5

Body weights at the time of sacrifice, body weight gain from 5 to 9 weeks of age, and average daily food intake from 8 to 9 weeks of age: The effect of genotype and adrenalectomy

	BODY WEIGHT (g)	BODY WEIGHT GAIN (g)	AVERAGE DAILY FOOD INTAKE (g)
LEAN n=	$171 \pm 5^{1,2,3}$ 19	$94 \pm 3^{1,2,3}$ 12	17 ± 1^1 12
OBESE n=	237 ± 8^2 24	145 ± 5^2 12	23 ± 1^2 17
LEAN/ADRX n=	145 ± 6 10	61 ± 4 6	15 ± 1 6
OBESE/ADRX n=	148 ± 4 21	69 ± 5 13	16 ± 1 9
F Lean vs Obese	21.89, $\alpha=.01$	38.0, $\alpha=.01$	21.03, $\alpha=.01$
F Sham vs Adrx	71.84, $\alpha=.01$	131.0, $\alpha=.01$	40.71, $\alpha=.01$
F Interaction	16.75, $\alpha=.01$	21.19, $\alpha=.01$	13.14, $\alpha=.01$

¹ = Lean vs Obese, $\alpha=.05$, Student Newman Keuls

² = Sham vs Adrx of the same genotype, $\alpha=.05$, Student Newman Keuls

³ = Lean vs Obese Adrx, $\alpha=.05$, Student Newman Keuls

Values represent mean $\pm$ S.E.M.

Table 6

Serum lipids: The effect of genotype and adrenalectomy

	TG (mg/dl)	FREE (mg/dl)	ESTER (mg/dl)	TOTAL (mg/dl)	CHOLESTEROL (FREE/TOTAL) x 100	PHOSPHOLIPIDS (mg/dl)
LEAN n=	$28.6 \pm 3.3^{1,3}$ 8	$19.8 \pm 1.1^{1,2}$ 12	$45.3 \pm 3.1^{2,3}$ 11	$67.9 \pm 3.9^{1,2}$ 14	30 ± 1^1 11	131.1 ± 6.3^1 12
OBESE n=	232.4 ± 4.8^2 13	30.8 ± 2.4 14	51.9 ± 3.7^2 13	84.6 ± 4.0 17	38 ± 3^2 13	210.2 ± 15.0^2 11
LEAN/ADRX n=	29.0 ± 2.3 3	31.9 ± 2.6 9	79.1 ± 4.2 8	111.4 ± 5.7 8	29 ± 2 8	119.8 ± 6.6 6
OBESE/ADRX n=	51.2 ± 4.1 14	28.3 ± 1.5 17	65.9 ± 2.6 14	95.8 ± 4.7 16	31 ± 1 14	131.1 ± 4.9 11
F Lean vs Obese	$11.04, \alpha=.01$	3.46, NS	0.93, NS	0.01, NS	$5.53, \alpha=.05$	$20.1, \alpha=.01$
F Sham vs ADRX	$43.7, \alpha=.01$	$5.84, \alpha=.05$	$48.21, \alpha=.01$	$34.62, \alpha=.01$	3.92, NS	$20.1, \alpha=.01$
F Interaction	$13.3, \alpha=.01$	$13.50, \alpha=.01$	$8.24, \alpha=.01$	$13.05, \alpha=.01$	1.91, NS	$11.35, \alpha=.01$

¹= Lean vs Obese, $\alpha=.05$, Student Newman Keuls²= Sham vs ADRX of the same genotype, $\alpha=.05$, Student Newman Keuls³= Lean Sham vs Obese ADRX. $\alpha=.05$, Student Newman KeulsValues represent mean $\pm$ S.E.M.

animals (232.40 ± 4.8 mg/dl in obese vs. 28.6 ± 3.3 mg/dl in lean control animals) (Fig 3). Because the rats were sacrificed after 10-11 hr fast, the higher serum TG levels indicate elevated liver VLDL-TG secretion rate in obese animals. Serum TG concentrations were lower in obese but not lean animals due to adrenalectomy. Furthermore, TG levels in obese adrenalectomized animals remained significantly higher than those of lean control animals (51.2 ± 4.1 mg/dl in obese adrenalectomized vs. 28.6 ± 3.3 mg/dl in lean control animals).

As observed in the SKF-12185 study the obese animals were hypercholesterolemic relative to lean controls (84.6 ± 4.0 mg/dl in obese vs. 67.9 ± 3.9 mg/dl in lean controls) (Fig 4). Unlike the effect of adrenalectomy on serum TG levels, adrenalectomized animals of both genotypes had higher serum cholesterol levels than their respective control animals. Post-hoc analysis showed higher serum cholesterol concentrations due to adrenalectomy in lean animals only (67.9 ± 3.9 in lean control animals vs. 111.4 ± 5.7 in lean adrenalectomized animals). This effect cannot be explained on the basis of greater hepatic lipoprotein release because the effect on serum levels of TG and phospholipids suggest the opposite. Free cholesterol followed the same pattern as total cholesterol. In agreement with the finding in SKF-12185 study the

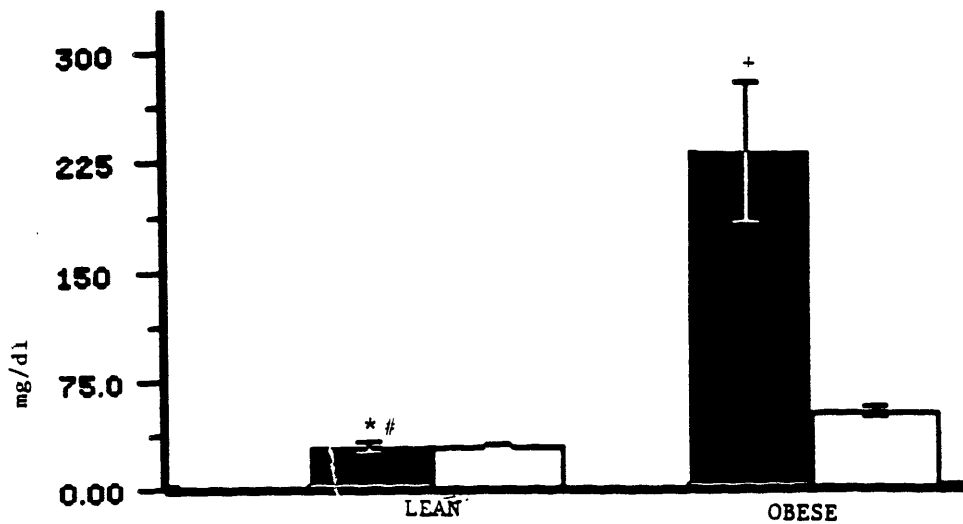


Fig. 5. Serum phospholipid levels (mg/dl): The effect of genotype and adrenalectomy. Closed bars represent Sham animals and open bars represent Adrx animals. *: significant, Lean vs. Obese, †: significant, Sham vs. Adrx of the same genotype, $\alpha=.05$, by Student Newman Keuls test. For details of 2-way ANOVA see table 6. Error bars represent S.E.M.

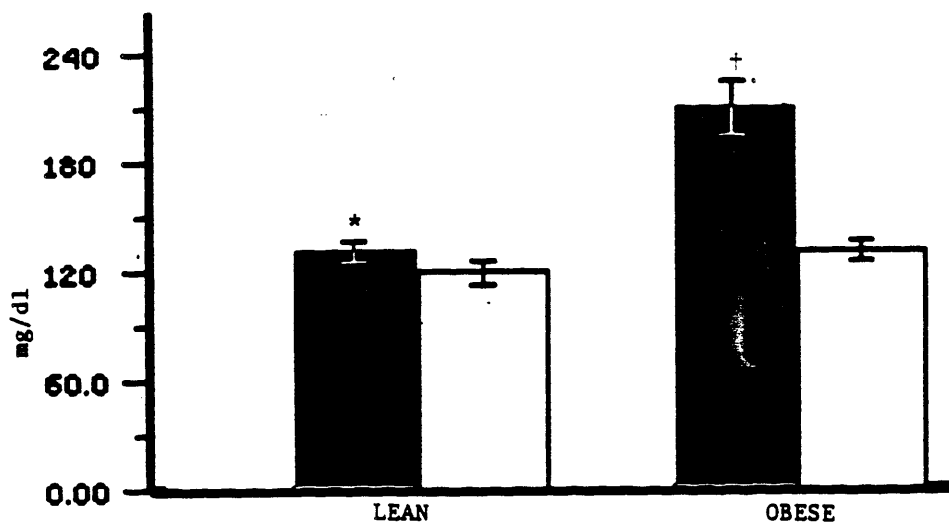


Fig. 3. Serum TG levels (mg/dl): The effect of genotype and adrenalectomy. Closed bars represent Sham animals and open bars represent Adrx animals. *: significant, Lean vs. Obese, †: significant, Sham vs. Adrx of the same genotype, #: significant, Lean Sham vs. Obese Adrx, $\alpha=.05$, by Student Newman Keuls test. For details of 2-way ANOVA see table 6. Error bars represent S.E.M.

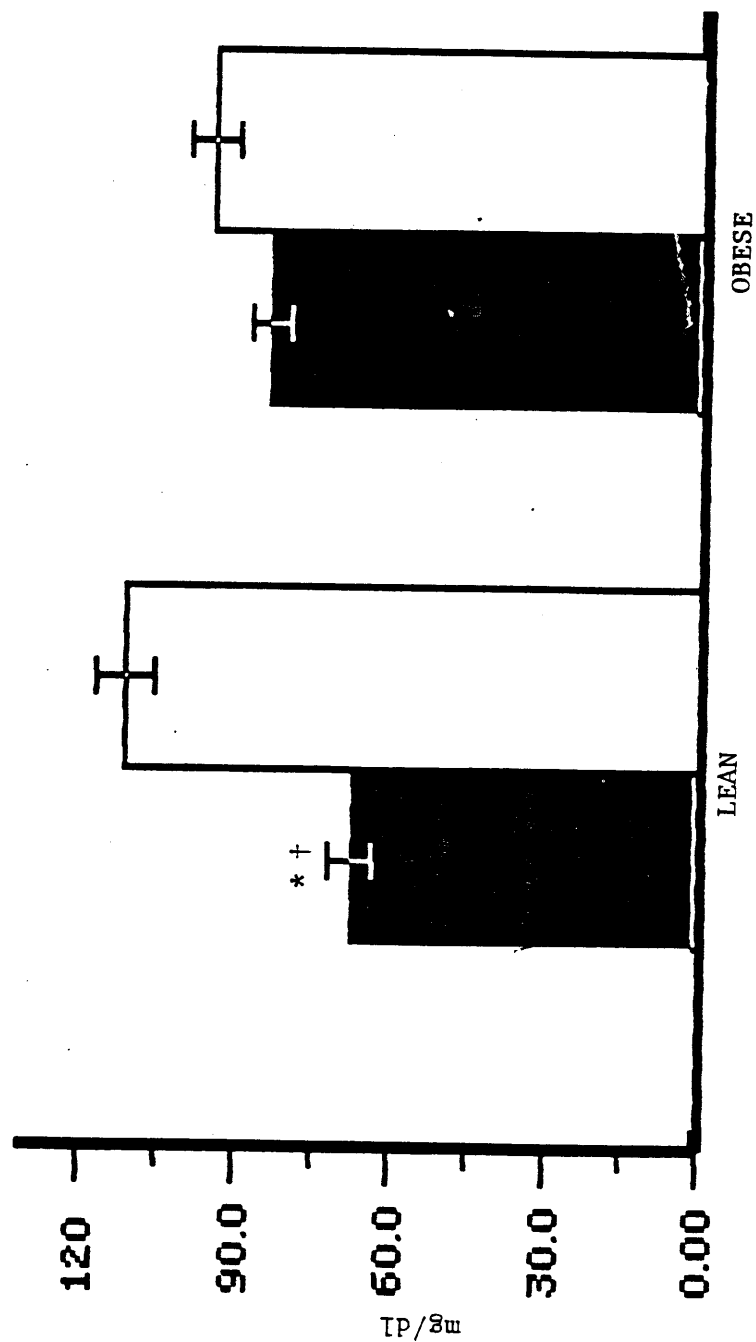


Fig. 4. Serum total cholesterol (mg/dl): The effect of genotype and adrenalectomy. Closed bars represent Sham animals and open bars represent Adrx animals. *: significant, Lean vs. Obese, +: significant, Sham vs. Adrx of the same genotype, $\alpha=.05$, by Student Newman Keuls test. For details of 2-way ANOVA see table 6. Error bars represent S.E.M.

free/total cholesterol ratio was elevated in obese animals relative to lean controls ($38 \pm 0.3 \%$ vs. $30 \pm 1 \%$ in lean control animals). Serum phospholipid levels in obese animals were markedly higher than those of lean control animals (210.2 ± 15.0 mg/dl in obese vs. 131.1 ± 6.3 mg/dl in lean control animals) (Fig 5). This indicates general hyperlipoproteinemia. Adrenalectomized obese animals had serum phospholipid concentrations similar to those of lean controls. Adrenalectomy did not affect the serum levels of phospholipids in lean animals.

2.4 LIVER LIPIDS:

Table 7 shows liver TG, cholesterol, and phospholipid content, and liver weights. Livers from obese animals were markedly larger than those from lean control animals (8.4 ± 0.3 g in obese vs. 6.0 ± 0.3 g in lean control animals). Adrenalectomized animals of both genotypes had lower liver weights relative to their respective control animals. Liver weights in obese adrenalectomized rats were significantly lower than those of lean control animals (4.9 ± 0.1 g in obese adrenalectomized vs. 6.0 ± 0.2 g in lean control animals) Liver weight per 100 gram of body weight was lower in adrenalectomized animals than control animals. This reflects a disproportionate decrease in liver

Table 7

Liver weights and liver lipids: The effect of genotype and adrenalectomy

	LIVER WEIGHT (g) PER 100 GRAM OF BODY WEIGHT	TC (mg/g wet wt.)	CHOLESTEROL (mg/g wet wt.)	CHOLESTEROL/PHOSPHOLIPID MOLAR RATIO x 100			
			FREE	ESTER	TOTAL	PHOSPHOLIPID (mg/g wet wt.)	
LEAN n=	6.0 ± 0.2 ^{1,2,3} 19	6.0 ± 1.3 ¹ 10	2.9 ± 0.2 9	0.5 ± 0.1 9	3.4 ± 0.2 ^{1,2} 9	37.6 ± 0.6 4	16.8 ± 1.5 ² 4
OBESE n=	8.4 ± 0.3 ² 24	13.3 ± 2.2 ² 15	2.4 ± 0.1 ² 12	0.3 ± 0.1 12	2.7 ± 0.1 ² 12	36.3 ± 1.2 4	14.8 ± 1.8 4
LEAN/ADRX n=	4.5 ± 0.3 7	4.0 ± 0.6 5	3.4 ± 0.2 3	0.6 ± 0.1 3	4.1 ± 0.2 3	34.7 ± 3.5 3	26.0 ± 2.3 3
OBESE/ADRX n=	4.9 ± 0.1 22	4.7 ± 0.9 11	3.2 ± 0.2 11	0.3 ± 0.1 11	3.6 ± 0.2 11	36.5 ± 0.1 5	20.0 ± 2.1 5
F Lean vs Obese	25.70, α=.01	5.56, α=.05	2.47, NS	7.02, α=.05	7.41, α=.05	0.3, NS	3.96, NS
F Sham vs ADRx	100.64, α=.01	11.78, α=.01	9.99, α=.01	1.10, NS	13.9, α=.01	0.81, NS	13.02, α=.01
F Interaction	9.86, α=.01	4.84, α=.05	0.38, NS	0.39, NS	0.16, NS	10.3, NS	0.99, NS

¹= Lean vs Obese, α=.05, Student Newman Keuls²= Sham vs ADRX of the same genotype, α=.05, Student Newman Keuls³= Lean Sham vs Obese ADRX, α=.05, Student Newman Keuls

Values represent mean ± S.E.M.

weight. This effect might contribute to the concentration of cholesterol in the liver as will be discussed below.

Liver TG content was markedly greater in obese animals relative to lean control animals (13.3 ± 2.2 mg/g wet wt. in obese vs. 6.0 ± 1.3 mg/g wet wt. in lean control animals). Liver TG content was significantly lower in obese adrenalectomized animals compared to obese control animals. Hepatic total cholesterol content was significantly greater in lean control animals relative to obese control animals. The free and esterified cholesterol content both tended to be greater in lean animals but did not reach significance. Adrenalectomized animals of both genotypes had significantly higher total cholesterol concentrations than their respective control groups (3.4 ± 0.2 and 2.7 ± 0.1 mg/g wet wt. in lean and obese control vs. 4.1 ± 0.2 and 3.6 ± 0.2 mg/g wet wt. in lean and obese adrenalectomized animals respectively). The difference was mostly caused by elevation of free cholesterol with very little change in esterified cholesterol.

Whole liver cholesterol content was significantly greater in control animals relative to their adrenalectomized counterparts (24.1 ± 1.0 and 22.2 ± 3.3 mg in lean and obese sham adrenalectomized animals

vs. 17.0 ± 1.2 and 16.8 ± 1.3 mg in obese and lean adrenalectomized animals) (Table 8). This was mainly due to the markedly smaller livers of adrenalectomized animals. When whole liver cholesterol was based on total body weight, however, this trend was reversed (Table 8). This probably reflects curtailment of the capacity of other cholesterol storage sites, and the disproportionately smaller livers of obese adrenalectomized animals. There was no difference in hepatic phospholipid content due to genotype or adrenalectomy. This indicates that genotype and adrenalectomy had no effect on hepatic membrane content per mg of wet wt..

2.5 FAT DEPOT WEIGHT, ADIPOCYTE SIZE, AND CHOLESTEROL CONTENT:

Tables 9 and 10 , show perimetrial and perirenal fat pad weights, adipocyte size, and cholesterol content. The fat pads of obese animals were markedly larger than those of lean control animals (4.0 ± 0.4 g in obese vs. 0.7 ± 1 g in lean control animals, in the perimetrial depot). Adrenalectomized animals of both genotypes had lower fat pad weights relative to their respective control groups. Obese adrenalectomized animals had fat pads similar in size to those of lean control animals.

Table 8

Whole liver cholesterol and whole liver cholesterol per 100 gram of body weight:
The effect of genotype and adrenalectomy

	WHOLE LIVER CHOLESTEROL (mg)	WHOLE LIVER CHOLESTEROL (mg) PER 100 GRAM OF BODY WEIGHT
LEAN n=	22.2 $\pm$ 3.3 8	12.0 $\pm$ 1.1 8
OBESSE n=	24.1 $\pm$ 1.0 ² 12	9.7 $\pm$ 0.3 12
LEAN/ADRX n=	16.8 $\pm$ 1.3 3	13.0 $\pm$ 0.4 3
OBESSE/ADRX n=	17.0 $\pm$ 1.2 11	11.0 $\pm$ 0.5 11
F Lean vs Obese	0.45, NS	5.0, $\alpha=.05$
F Sham vs Adrx	7.7, $\alpha=.01$	4.66, $\alpha=.05$
F Interaction	0.55, NS	0.03, NS

² = Sham vs Adrx of the same genotype, $\alpha=.05$, Student Newman Keuls

Values represent mean $\pm$ S.E.M.

Table 9

Perimetrial fat depot weight, average adipocyte size and cholesterol content:
The effect of genotype and adrenalectomy

	DEPOT WEIGHT (g)	AVERAGE CELL DIAMETER (μ m)	AVERAGE CELL VOLUME (pl)	ADIPOCYTE TG CONTENT (ng/cell)	CHOLESTEROL PER MILLION CELLS (μ g)
LEAN n=	0.7 + 0.1 ^{1,2} 19	41 + 2 ^{1,2} 11	42 + 6 ^{1,2,3} 11	38.0 + 6.0 ¹ 11	25.6 + 5.6 ^{1,2} 6
OBESE n=	4.0 + 0.4 ² 24	84 + 3 ² 10	344 + 45 ² 10	330.0 + 40.0 ² 11	204.6 + 30.5 ² 10
LEAN/ADRX n=	0.20 + 0.03 10	26 + 1 6	11 + 2 6	10.0 + 0.2 6	13.1 + 5.1 4
OBESE/ADRX n=	0.9 + 0.1 21	47 + 4 10	79 + 15 10	54.0 + 11.0 10	39.0 + 7.1 10
F Lean vs Obese	211.96, $\alpha=.01$	119.75, $\alpha=.01$	207.99, $\alpha=.01$	114.56, $\alpha=.01$	41.64, $\alpha=.01$
F Sham vs ADRX	162.62, $\alpha=.01$	68.11, $\alpha=.01$	94.10, $\alpha=.01$	84.45, $\alpha=.01$	24.50, $\alpha=.01$
F Interaction	0.98, NS	11.18, $\alpha=.01$	0.94, NS	3.87, NS	3.56, NS

¹= Lean vs Obese, $\alpha=.05$, Student Newman Keuls

²= Sham vs ADRX of the same genotype, $\alpha=.05$, Student Newman Keuls

³= Lean vs Obese ADRX, $\alpha=.05$, Student Newman Keuls

Values represent mean + S.E.M.

Table 10

Perirenal fat depot weight, average adipocyte size and cholesterol content:
The effect of genotype and adrenalectomy

	DEPOT WEIGHT (g)	AVERAGE CELL DIAMETER (μ m)	AVERAGE CELL VOLUME (μ l)	ADIPOCYTE TC CONTENT (ng/cell)	CHOLESTEROL/ MILLION CELLS (μ g)
LEAN n=	0.5 + 0.1 ^{1,2} 19	37 + 3 ¹ 11	37 + 8 ^{1,2} 11	34.0 + 1.9 ¹ 11	22.4 + 6.5 ¹ 7
OBESE n=	4.6 + 0.4 ² 24	77 + 5 ² 8	295 + 40 ² 8	250.0 + 4.0 ² 9	177.1 + 58.3 ² 8
LEAN/ADRX n=	0.10 + 0.01 10	29 + 2 6	16 + 4 6	14.8 + 0.3 16	9.1 + 1.5 5
OBESE/ADRX n=	0.9 + 0.1 21	42 + 2 10	56 + 7 10	41.0 + 1.0 11	29.5 + 4.2 9
F Lean vs Obese	101.5, $\alpha=.01$	76.51, $\alpha=.01$	84.72, $\alpha=.01$	61.65, $\alpha=.01$	34.76, $\alpha=.01$
F Sham vs Obese	66.04, $\alpha=.01$	42.99, $\alpha=.01$	41.74, $\alpha=.01$	44.63, $\alpha=.01$	18.69, $\alpha=.01$
F Interaction	4.59, $\alpha=.05$	15.90, $\alpha=.01$	5.15, $\alpha=.01$	7.76, $\alpha=.05$	2.59, NS

¹= Lean vs Obese, $\alpha=.05$, Student Newman Keuls

²= Sham vs ADRX of the same genotype, $\alpha=0.5$, Student Newman Keuls

Values represent mean $\pm$ S.E.M.

The mean adipocyte diameter of obese control animals was approximately twice that of lean control animals in both depots examined (84 ± 3 μ m in obese vs. 41 ± 2 μ m in lean control animals, in the perimetrial depot). The mean adipocyte diameter was significantly lower in adrenalectomized animals of both genotypes relative to control animals. These differences in diameter are amplified if adipocyte size is expressed as cell volume. Furthermore, the adipocyte volumes remained greater in obese adrenalectomized animals relative to lean control animals (Tables 9, 10, Fig 6).

Cholesterol content per million cells is markedly greater in obese animals relative to lean control animals (204.6 ± 30.5 micrograms /million cells in obese vs. 25.6 ± 5.6 micrograms/million cells in lean control animals, in the PM depot) (Fig 7). Adrenalectomized animals of both genotypes had lower adipocyte cholesterol content relative to control animals. Adipocyte cholesterol content in obese adrenalectomized animals tended to be greater relative to that of lean control animals, though not significantly so.

When the combined weight of perimetrial and perirenal fat pads was normalized to body weight, obese adrenalectomized rats had significantly more fat per 100 gram of body weight than lean control animals

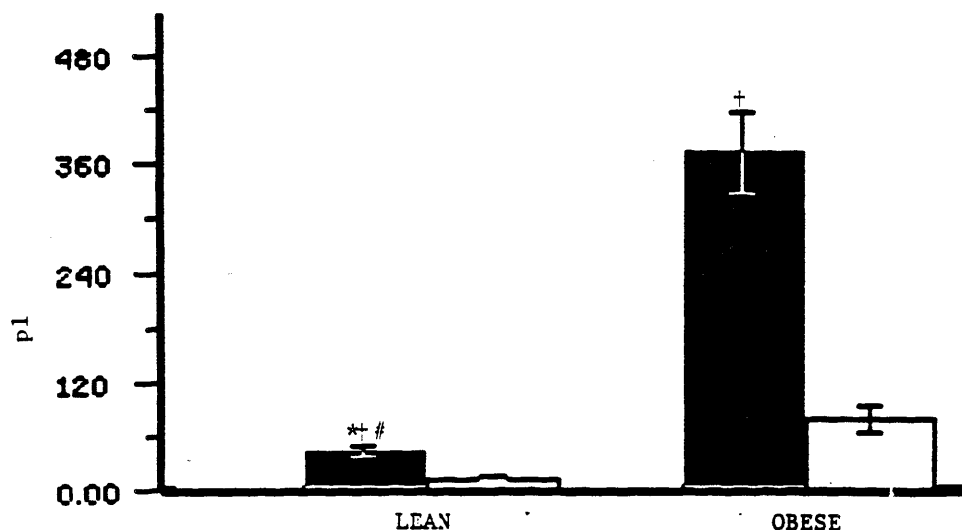


Fig. 6. Perimetrial adipocyte cell volume (pl): The effect of genotype and adrenalectomy. Closed bars represent Sham animals and open bars represent Adrx animals. *: significant, Lean vs Obese, †: significant, Sham vs. Adrx of the same genotype, #: significant, Lean vs. Obese Adrx, $\alpha = .05$, by Student Newman Keuls test. For details of 2-way ANOVA see table 9. Error bars represent S.E.M.

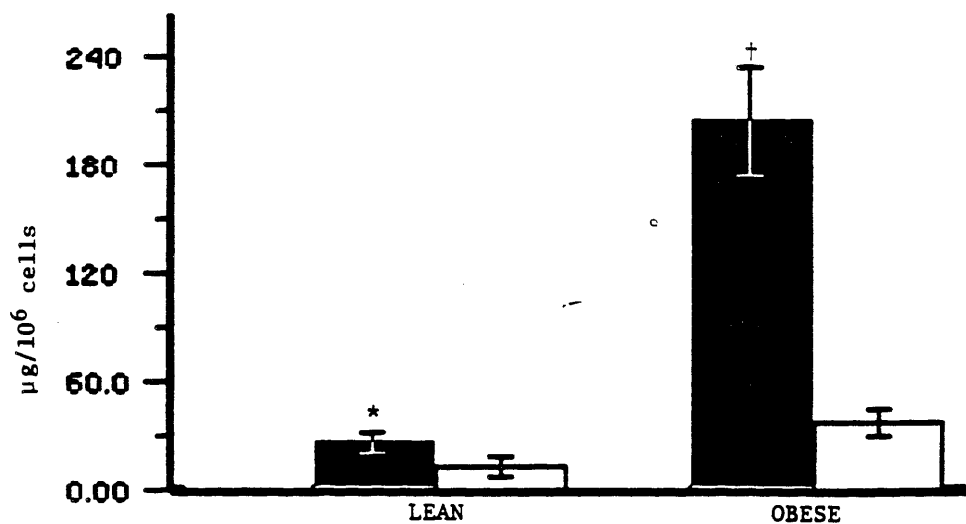


Fig. 7. Perimetrial adipocyte cholesterol ($\mu\text{g}/10^6$ cells): The effect of genotype and adrenalectomy. Closed bars represent Sham animals and open bars represent Adrx animals. *: significant, Lean vs. Obese, †: significant, Sham vs. Adrx of the same genotype, $\alpha = .05$, by Student Newman Keuls test. For details of 2-way ANOVA see table 9. Error bars represent S.E.M.

(Table 11). This observation, together with the persistent elevation of serum TG levels in obese adrenalectomized animals, indicated that lipid deposition and hepatic TG synthesis were not completely normalized by adrenalectomy in obese animals. Table 11 also shows that adrenalectomized animals exhibited significantly reduced fat deposition per 100 gram of body weight relative to control animals. (3.5 ± 0.2 and 0.6 ± 0.1 g/100 g in obese and lean controls vs. 1.1 ± 0.1 and 0.2 ± 0.04 g/100g in obese and lean adrenalectomized animals, respectively).

2.6 LIVER MICROSOMAL HMG-CO A REDUCTASE AND 7 ALPHA-HYDROXYLASE ACTIVITY:

To determine whether enhanced cholesterol synthesis contributed to the observed hypercholesterolemia in obese animals, or that observed due to adrenalectomy, HMG-Co A reductase activity was measured in livers from lean and obese control and from adrenalectomized animals. The total reductase activity was significantly greater in obese relative to lean animals (277 ± 34 pmol/min/mg prot in obese vs. 190 pmol/min/mg prot. in lean control animals) (Table 12) (Fig 8). Obese adrenalectomized animals had significantly lower reductase activity relative to obese control animals (Table 12, Fig 8). There was no

Table 11

Fat depots combined weight per 100 gram of body weight

TOTAL FAT PAD WEIGHT (PM+PR) (g) PER 100 GRAM
OF BODY WEIGHT

LEAN	$0.6 \pm 0.1^{1,2,3}$
n=	19
OBESSE	3.5 ± 0.2^2
n=	24
LEAN/ADRX	0.20 ± 0.04
n=	10
OBESSE/ADRX	1.1 ± 0.1
n=	21

F Lean vs Obese 289.91, $\alpha=.01$ F Sham vs Adrx 145.62, $\alpha=.01$

F Interaction 0.60, NS

¹= Lean vs Obese, $\alpha=.05$, Student Newman Keuls²= Sham vs Adrx of the same genotype, $\alpha=.05$, Student Newman Keuls³= Lean vs Obese Adrx, $\alpha=.05$, Student Newman KeulsValues represent mean $\pm$ S.E.M.

PM: Perimetrial depot

PR: Perirenal depot

Table 12

Liver microsomal HMG-CoA reductase and 7 α -hydroxylase activities:
The effect of genotype and adrenalectomy

	TOTAL HMG-CoA REDUCTASE ACTIVITY (p mole/min/mg prot.)	HMG-CoA REDUCTASE ACTIVITY + F1 (p mole/min/mg prot.)	(ACTIVITY + F1/TOTAL) x 100	7 α -HYDROXYLASE ACTIVITY (p mole/min/mg prot.)
LEAN n=	190 + 23 ¹ 14	41 + 5 15	25 + 4 14	13 + 3 3
OBESE n=	277 + 34 ² 22	53 + 7 ² 18	20 + 2 18	9 + 2 3
LEAN/ADRX n=	193 + 35 9	37 + 7 7	21 + 3 7	11 + 4 3
OBESE/ADRX n=	172 + 18 20	30 + 3 16	19 + 3 16	7 + 1 3
F Lean vs Obese	1.21, NS	0.06, NS	1.18, NS	0.54, NS
F Sham vs Adrx	4.13, $\alpha=.05$	6.48, $\alpha=.05$	0.51, NS	2.3, NS
F Interaction	2.59, NS	2.24, NS	0.32, NS	8.07, NS

¹ = Lean vs Obese, $\alpha=.05$, Student Newman Keuls

² = Sham vs Adrx of the same genotype, $\alpha=.05$, Student Newman Keuls

Values represent mean + S.E.M.

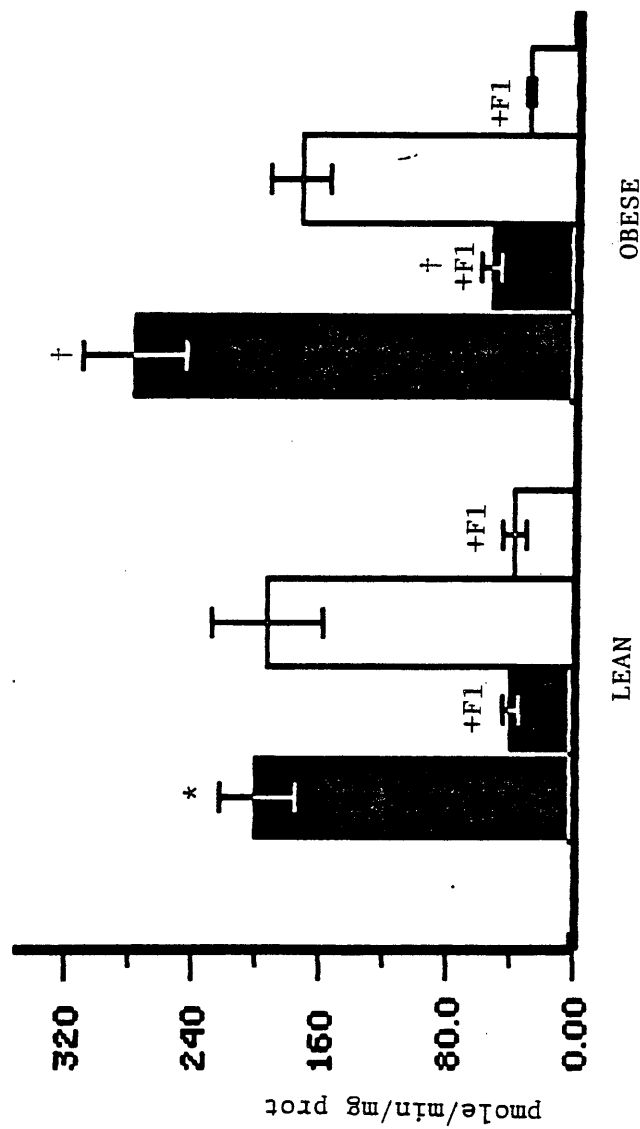


Fig. 8. Microsomal HMG-CoA reductase (p mole/min/mg prot.), in the presence and absence of KF1 (phosphatase inhibitor); The effect of genotype and adrenalectomy. Closed bars represent Sham animals and open bars represent Adrx animals. *: significant, Lean vs. Obese, †: significant, Sham vs Adrx of the same genotype, $\alpha=0.5$, by Student Newman Keuls test. For details of 2-way ANOVA see table 12. Error bars represent S.E.M.

effect due to adrenalectomy on the reductase activity in lean animals. The reductase activity in the presence of KFl (phosphatase inhibitor) followed the same pattern as that of total reductase activity. However, the genotype effect in control animals did not reach significance. Furthermore the ratio of the activity in the presence of KFl to the total activity was similar irrespective of genotype or adrenalectomy.

Cholesterol 7- α hydroxylase activity was measured to determine whether a decrease in cholesterol conversion to bile acids contributed to the hypercholesterolemia observed in obese animals and in adrenalectomized animals. However, there were no differences in 7 α -hydroxylase activity among the groups (Table 12).

2.7 MICROSOMAL PHOSPHOLIPID AND CHOLESTEROL CONCENTRATION:

Microsomal phospholipid and cholesterol concentrations are shown in table 13. These variables were measured to investigate whether the greater liver cholesterol content in lean control animals vs. obese control animals, and adrenalectomized animals vs. control animals reflected a similar trend at the microsomal level. The microsomal cholesterol pool is a

Table 13

Microsomal phospholipid and cholesterol concentrations:
The effect of genotype and adrenalectomy

	PHOSPHOLIPID ($\mu\text{g}/\text{mg}$ prot.)	FREE CHOLESTEROL ($\mu\text{g}/\text{mg}$ prot.)	(CHOLESTEROL/PHOSPHOLIPID) MOLAR RATIO X 100
LEAN n=	599 + 45 5	57 + 1 ¹ 7	19 + 2 ¹
OBESE n=	662 + 28 5	41 + 3 ² 9	14 + 1
LEAN/ADRX n=	539 + 55 3	62 + 6 4	22 + 1
OBESE/ADRX n=	616 + 62 4	66 + 4 12	19 + 2 ¹
F Lean vs Obese	2.12, NS	2.10, NS	7.94, $\alpha=.05$
F Sham vs ADRX	1.23, NS	13.81, $\alpha=.01$	8.4, $\alpha=.05$
F Interaction	0.02, NS	5.84, $\alpha=.05$	0.42, NS

¹ = Lean vs Obese, $\alpha=.05$, Student Newman Keuls

² = Sham vs ADRX of the same genotype, $\alpha=.05$, Student Newman Keuls

Values represent mean + S.E.M.

closer approximation of the pool that controls the activity of HMG-Co A reductase and 7-alpha hydroxylase activities. This was found to be true for the difference between lean and obese control animals; microsomal cholesterol content was significantly greater in lean relative to obese animals (41 ± 3 ug/mg prot. in obese vs. 57 ± 1 ug/mg prot. in lean control animals) (Fig 9). Additionally the F ratio of the adrenalectomy effect was significant. Post-hoc analysis revealed significance only in obese animals, however (41 ± 3 ug/mg prot. in obese control rats vs. 66 ug/mg prot. in obese adrenalectomized rats). Microsomal phospholipid concentration was similar across genotype and treatment.

There was a significant general effect (as determined by the F ratio) of adrenalectomy and genotype when the cholesterol/phospholipid ratio was determined. Lean animals and adrenalectomized animals had greater ratios than obese animals and sham-adrenalectomized animals. Post-hoc analysis showed a significant increase in lean control vs. obese control animals only, however.

These results show that microsomes from lean control animals were cholesterol enriched relative to obese control animals. They also indicate cholesterol enrichment of microsomal membranes due to adrenalectomy in both genotypes.

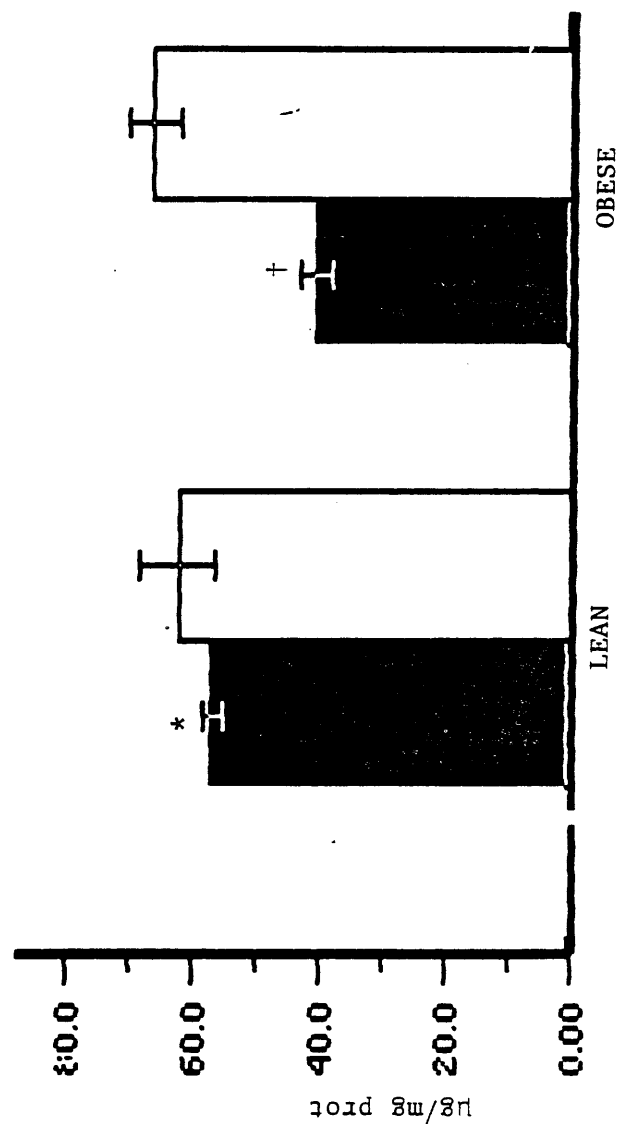


Fig. 9. Microsomal cholesterol concentration ($\mu\text{g}/\text{mg prot.}$): The effect of genotype and adrenalectomy. Closed bars represent Sham animals and open bars represent Adrx animals. *: significant, Lean vs. Obese, †: significant, Sham vs Adrx of the same genotype, $\alpha=.05$, by Student Newman Keuls test. For details of 2-way ANOVA see table 13. Error bars represent S.E.M.

2.8 CHOLESTEROL ABSORPTION:

To test the possibility that enhanced cholesterol absorption may contribute to the observed hypercholesterolemia, and the possible effect of the excess-glucocorticoid state on cholesterol absorption, cholesterol absorption was measured in lean and obese control and adrenalectomized animals. The percentage of ^{14}C -cholesterol dose absorbed was higher in obese animals across treatment (Table 14). Post-hoc analysis did not reveal any differences between individual groups, however. When cholesterol absorption was expressed as daily weight of cholesterol absorbed, significant differences due to genotype and treatment were evident (Table 14). These values were calculated based on daily food intake and diet cholesterol content. Post hoc analysis showed lower cholesterol absorption in lean relative to obese control animals (2.2 ± 0.1 mg/day in obese vs. 1.3 ± 0.1 mg/day in lean control animals). Additionally, obese adrenalectomized animals absorbed significantly less cholesterol per day than did obese control animals (2.2 ± 0.1 g in obese control vs. 1.3 ± 0.1 g in obese adrenalectomized animals). When the weight of cholesterol absorbed per day was normalized to body weight, no significant differences were observed (Table 14).

Table 14
Cholesterol absorption: The effect of genotype and adrenalectomy

	PERCENTAGE OF ^{14}C CHOLESTEROL ABSORBED	AVERAGE DAILY WEIGHT OF CHOLESTEROL ABSORBED (mg)	DAILY WEIGHT OF CHOLESTEROL ABSORBED PER GRAM OF BODY WEIGHT ($\mu\text{g/g}$)
LEAN n=	38 $\pm$ 1 3	1.30 $\pm$ 0.03 ¹ 3	6.9 $\pm$ 0.3 3
OBESE n=	47 $\pm$ 2 3	2.2 $\pm$ 0.1 ² 3	8.0 $\pm$ 0.3 3
LEAN/ADRX n=	34 $\pm$ 5 3	1.0 $\pm$ 0.2 3	7.5 $\pm$ 0.9 3
OBESE/ADRX n=	40 $\pm$ 2 4	1.3 $\pm$ 0.1 4	7.3 $\pm$ 0.5 4
F Lean vs Obese	6.48, $\alpha=.05$	35.27, $\alpha=.01$	1.16, NS
F Sham vs Adrx	3.35, NS	40.24, $\alpha=.01$	0.03, NS
F Interaction	0.24, NS	11.86, $\alpha=.01$	1.07, NS

¹ = Lean vs Obese, $\alpha=.05$, Student Newman Keuls

² = Sham vs Adrx of the same genotype, $\alpha=.05$, Student Newman Keuls

Values represent mean $\pm$ S.E.M.

2.9 CORRELATION ANALYSIS:

In pathological states in which insulin resistance and hyperinsulinemia are expressed, serum TG levels were found to correlate positively with serum insulin concentration. To investigate whether such a relationship exists in obese Zucker rats, linear regression analysis was performed on serum TG and insulin data. Additionally, a possible relationship between insulin levels and the degree of hypercholesterolemia was also examined by linear regression analysis. Because the obese rats are hyperglucocorticoidemic as well, linear regression analysis was also used to test whether a relationship exists between serum glucocorticoid levels and serum TG and cholesterol levels. Serum insulin was positively correlated with serum TG concentration (Corr. coef.= 0.82, α =.01) and serum free cholesterol concentration (Corr. Coef.= 0.49, α = .05) (Table 15). There was no significant correlation between serum insulin and serum total or esterified cholesterol, however (Corr. Coef.=.05, NS). There was also no significant correlation between serum corticosterone and serum TG concentrations and serum corticosterone and cholesterol concentrations (table 15).

Table 15

Linear regression analysis parameters: Hormones vs. serum lipids

VARIABLES (INDEPENDENT, DEPENDENT)	NO. OF DATA POINTS	Y-AXIS INTERCEPT	SLOPE	CORRELATION COEFFICIENT	SIGNIFICANCE
INSULIN, TC	13	-91.64	3.42	0.82	$\alpha = .01$
INSULIN, FREE CHOLESTEROL	24	22.27	0.09	0.49	$\alpha = .05$
INSULIN, ESTERIFIED CHOLESTEROL	13	53.63	-0.02	0.07	NS
INSULIN, TOTAL CHOLESTEROL	17	81.68	0.02	0.05	NS
CORTICOSTERONE, TC	12	252.2	-0.02	0.02	NS
CORTICOSTERONE, FREE CHOLESTEROL	14	33.57	-6.04×10^{-3}	0.10	NS
CORTICOSTERONE, ESTERIFIED CHOLESTEROL	13	48.32	0.01	0.16	NS
CORTICOSTERONE, TOTAL CHOLESTEROL	17	79.25	0.02	0.14	NS
SERUM TC, FREE CHOLESTEROL	11	22.71	0.03	0.63	$\alpha = .05$
SERUM TC, TOTAL CHOLESTEROL	10	83.1	-0.02	0.26	NS

To test for the presence of a positive relationship between serum TG (as an independent variable) and serum free ester and total cholesterol, linear regression analysis was performed on the data points from the obese control population (Table 15). Such a relationship would indicate the form of cholesterol carried by TG-rich lipoproteins (VLDL). There was no correlation in the case of serum total cholesterol (Corr. Coef.=0.26, NS), but there was a significant positive correlation with serum free cholesterol (Corr. Coef.= 0.63, α = 0.5).

Because free cholesterol is known to be a negative-feedback inhibitor of HMG-Co A reductase activity, a possible relationship between reductase activity and free cholesterol content was investigated by linear regression analysis (Table 16). Four groups of data were used: lean and obese control animals, control and adrenalectomized obese animals, sham-adrenalectomized and adrenalectomized lean animals, and the combination of all three groups. There was significant correlation in the lean and obese control group (Corr. Coef.=0.5, α = .05), the obese adrenalectomized and control group (Corr. Coef.=0.57, α =.05), and in the combined group (Corr. Coef.=0.44, α =.05). In the lean group, however, the correlation was not significant (Corr. Coef.=0.23, NS). The regression lines in all 4 groups had negative

Table 16

Linear regression analysis parameters: Hormones and microsomal free cholesterol concentration vs. HMG-CoA reductase activities

VARIABLES (INDEPENDENT, DEPENDENT)	NO. OF DATA POINTS	Y-AXIS INTERCEPT	SLOPE	CORRELATION COEFFICIENT	SIGNIFICANCE
MICROSOMAL FREE CHOLESTEROL, LOG HMG-CoA REDUCTASE ACTIVITY:					
GROUP 1, SHAM-ADRX LEAN AND OBESE	15	2.8	-0.01	0.50	$\alpha = .05$
GROUP 2, SHAM-ADRX AND ADRX LEAN	12	2.68	-6.14×10^{-3}	0.23	NS
GROUP 3, SHAM-ADRX AND ADRX OBESE	19	2.69	-7.32×10^{-3}	0.57	$\alpha = .05$
GROUP 4: GROUPS 1, 2, AND 3 COMBINED	31	2.69	-6.85×10^{-3}	0.44	$\alpha = .05$
INSULIN, HMG-CoA REDUCTASE ACTIVITY	22	312.16	-0.37	0.11	NS
INSULIN, + F1/TOTAL REDUCTASE ACTIVITY RATIO	18	0.19	3.28×10^{-5}	0.02	NS
CORTICOSTERONE, HMG-CoA REDUCTASE ACTIVITY	22	225.7	0.20	0.18	NS
CORTICOSTERONE, + F1/TOTAL REDUCTASE ACTIVITY RATIO	18	0.19	4.44×10^{-5}	0.09	NS

slopes which is consistent with an inhibitory effect of cholesterol on HMG-Co A reductase activity. (Table 16).

To test hormonal regulation of HMG-CoA reductase activity, the existence of positive correlation between the total enzymatic activity and +F1/total activity ratio data (dependent) and serum insulin and corticosterone concentrations (independent) was tested (table 16). There was no significant correlation between any of these variables.

2.10 SUMMARY OF RESULTS IN THE ADRENALECTOMY STUDY:

The obese control animals were insulin resistant in that they were hyperglycemic in the face of overt hyperinsulinemia. The obese animals were also hypertriglyceridemic, hyperlipoproteinemic, and hypercholesterolemic. They also had higher serum free to total cholesterol ratio. Additionally, serum TG levels correlated positively with serum free cholesterol, but not total or esterified cholesterol. All these observations indicate higher levels of serum VLDL in obese animals. Serum insulin levels may be related in a causative fashion with serum VLDL levels because they correlated positively with serum TG and free cholesterol concentration.

Obese animals also exhibited increased liver TG deposition, which probably reflected enhanced hepatic TG synthesis. Liver total cholesterol (mg/g wet wt.) and microsomal free cholesterol content (ug/ mg prot.) were lower in obese animals relative to lean control animals. The microsomal free cholesterol concentration correlated negatively with microsomal HMG-Co A reductase activity. This relationship may explain the greater HMG-Co A reductase activity observed in hepatic microsomes of obese control animals relative to lean control animals. Cholesterol 7- α hydroxylase activity was not different between lean and obese controls.

Obese control animals absorbed a higher percentage of the dose of ^{14}C -cholesterol ingested and more cholesterol per day (mg) than lean control animals. These differences were negated when cholesterol absorption was normalized to body weight, however.

Obese adrenalectomized animals were not hyperphagic relative to lean control animals. The enhanced lipid deposition was lowered but not completely prevented in obese adrenalectomized animals. Lipid deposition was markedly lower in lean adrenalectomized animals as well. In addition, the insulin resistance was apparently prevented by adrenalectomy in obese animals. The degree of

hypertriglyceridemia was markedly lowered in obese adrenalectomized animals relative to obese control animals, and they were normolipoproteinemic relative to lean controls. The adrenalectomy did not correct the hypercholesterolemia in obese animals, however. To the contrary, it appeared to exacerbate the hypercholesterolemia in obese animals, and to induce hypercholesterolemia in otherwise normocholesterolemic lean animals.

The hypercholesterolemia in adrenalectomized animals was accompanied by enhanced liver and liver microsomal free cholesterol storage (mg/g) and lower adipocyte cholesterol content (ug/million cells). These findings were evident regardless of genotype. Similar to the comparison between control groups, linear regression analysis of data suggested negative feedback inhibition by free cholesterol as basis for the lower HMG-Co A reductase activity in obese adrenalectomized rats. Microsomal cholesterol 7-alpha hydroxylase activity in adrenalectomized rats of both genotypes tended to be lower than that observed in control animals. These differences did not reach significance however.

Dietary cholesterol absorption was not enhanced by adrenalectomy as the hypercholesterolemia might indicate. To the contrary, daily cholesterol absorption

was lower in obese adrenalectomized animals relative to obese control animals and tended to be lower in lean animals due to adrenalectomy as well. The difference due to adrenalectomy in the lean group was not significant however.

Figure 10 summarizes the major differences found between lean and obese animals as well as those due to adrenalectomy in both genotypes.

Serum					
	Insulin (μ IU/ml)	Glucose (mM)	TG (mg/dl)	Cholesterol (total) (mg/dl)	Phospholipid (mg/dl)
Ob vs. Lean	††	†	††	†	††
Adrx vs. Sham	††	†	††	†?	††
Ob					
Lean	↔	↔	↔	††	↔
Liver					
	TG (mg/g wet wt.)	Cholesterol (total) (mg/g wet wt.)	Free Cholesterol/Phospholipid Molar ratio X 100		
Ob vs. Lean	††	†		↔	
Adrx vs. Sham	††	††		†?	
Ob					
Lean	↔	†?		†?	
Microsomes					
	Free Cholesterol (μ g/mg prot.)	Free Cholesterol/Phospholipid Molar ratio X 100	HMG-CoA Reductase (pmole/min/mg prot.)	7 α -hydroxylase (pmole/min/mg prot.)	
Ob vs. Lean	†	†	†	↔	↔
Adrx vs. Sham	††	†?	†	↔	↔
Ob					
Lean	†?	†?	↔	↔	↔
Adipose Tissue					
	Cell Diameter (μ m)	TG (μ g/cell)	Cholesterol (μ g/10 ⁶ cell)		
Ob vs. Lean	††	††		††	
Adrx vs. Sham	††	††		††	
Ob					
Lean	†	†?		†	

Fig. 10. Summary of major changes observed in the serum, liver, liver microsomes and adipose tissue.
† or ††, Moderately increased or decreased
†† or †††, Markedly increased or decreased
†?, ††?, Significant F ratio, but not significant by Student Newman Keuls test.
↔, No change

DISCUSSION

Findings in this study support the hypothesis that the excess-glucocorticoid state present in the obese female Zucker rat leads to the observed hypercholesterolemia. We conclude that excess-glucocorticoid-induced insulin resistance and hyperinsulinemia in obese rats (demonstrated in this and other studies) cause the hyperlipidemia (combined hypercholesterolemia and hypertriglyceridemia) by enhancing hepatic VLDL secretion. This conclusion is based on the following :

1. This study is the first to show that, in obese Zucker rats, similar to other insulin resistant states (endogenous hypertriglyceridemia, diabetes mellitus, and Cushing's syndrome), the degree of hyperinsulinemia is positively correlated with serum TG levels .
2. Evidence from this study and others suggest that the obese Zucker rat resembles patients with insulin resistant states in that serum levels of VLDL, VLDL-TG, and VLDL cholesterol are elevated.
3. We found that obese adrenalectomized animals (in which the development of insulin resistance and the hyperinsulinemia were prevented) displayed lower serum

TG and phospholipid concentrations relative to obese control rats suggesting lower levels of hepatic VLDL secretion.

We also found that obese Zucker rats had lower liver and liver microsomal free cholesterol levels compared to lean rats. The lowered microsomal free cholesterol levels were associated with greater microsomal HMG-Co A reductase (the rate limiting enzyme in cholesterol synthesis) activities. We suggest that the enhanced hepatic VLDL secretion imposed a "drainage" effect on hepatic free cholesterol which then lead to the lower cholesterol concentrations observed in obese rats. Accordingly, the enhanced hepatic VLDL secretion which caused hypercholesterolemia, stimulated hepatic cholesterol synthetic activities which also contributed to the hypercholesterolemia. Additionally, based on findings in this study we conclude that the hypercholesterolemia in obese rats is not caused by enhanced dietary input of cholesterol.

This study also showed that adrenalectomy during the rapid growth period in the rat lead to the development of hypercholesterolemia. We suggest that this adrenalectomy induced hypercholesterolemia was

caused by the curtailment of size and cholesterol storage capacity of adipose tissue.

We also found that the potent corticosterone synthesis inhibitor SKF-12185 failed to suppress corticosterone secretion chronically.

1. SKF-12185 STUDY:

There was no difference between serum corticosterone concentration of SKF-12185 treated rats and control animals. This indicated that at the time of sacrifice the drug was not effective in suppressing corticosterone secretion. Additionally, comparison of metabolic characteristics of SKF-12185 treated rats to those of adrenalectomized rats (as will be discussed below) also demonstrate that the drug was not effective in suppressing corticosterone secretion throughout the study period.

SKF-12185 treated rats displayed some but not all of the characteristics displayed by adrenalectomized animals. They shared with the adrenalectomized animals the lower body weight gain compared to control groups in both genotypes. The lower daily food intake of obese SKF-12185 treated animals compared to obese control rats was more drastic than that observed in obese adrenalectomized animals however. Additionally, unlike the adrenalectomy study, food intake was significantly lower in lean drug-treated rats compared to lean control rats. This indicated a possible effect of the drug (which was administered in the diet) on the palatability of the diet. As was seen with

adrenalectomy, the drug treatment normalized the hyperinsulinemia in obese animals.

The effect of drug treatment on fat deposition in lean animals was similar to that observed in lean adrenalectomized animals. In contradistinction to the results of adrenalectomy, the fat pads of obese treated animals remained significantly larger than those of lean control animals, and fat depot weight per gram of body weight was not different from that of obese control rats. These results are not compatible with partial suppression of corticosterone secretion. In the study of Freedman et al. (1986 a), fat deposition per 100 g of body weight and fat pad weight in adrenalectomized obese rats replaced with glucocorticoids were significantly lower than those in obese sham operated animals regardless of replacement dose.

The hepatic liver TG concentration was not affected in obese drug-treated animals. This observation, together with the lower food intake, imply little or no effect on hepatic TG synthesis in obese drug-treated animals. This is in contrast to the obese adrenalectomized animals, in which hepatic TG deposition was markedly lower despite a less marked effect on food intake. A decrease in hepatic fatty acid

synthesis and lipogenic activity in obese adrenalectomized rats has been demonstrated by other investigators (York and Godbole 1979).

Serum cholesterol levels were not affected by SKF-12185 treatment. Serum LCAT activity, however, was markedly lower in drug treated animals. Despite the lower serum LCAT activity, the serum free/total cholesterol ratio was not affected in lean animals but was lower in obese animals. This can only be explained on the basis that free cholesterol was more accessible to esterification by LCAT in obese drug treated animals.

The elevated liver total cholesterol concentration in treated animals compared to controls was accompanied by an increase in the esterified cholesterol fraction. This is in contrast to adrenalectomized animals, in which the difference was entirely due to greater free cholesterol content. One cannot deduce the cause of the greater liver cholesterol content with the available data. The increased accumulation of cholesterol could be due to the smaller adipose tissue depots and thus reduction of its cholesterol storage capacity. This explanation, while applicable in lean rats, in which adipose tissue weight and weight per gram of body weight was substantially lower than lean control rats,

does not apply in obese rats in which adipose tissue size was not significantly lower. A side effect of the drug on hepatic metabolism cannot be ruled out.

The adrenal hypertrophy observed in both lean and obese treated animals indicates that the drug had an inhibitory effect on corticosterone production and release. This is because corticosterone is a negative feedback inhibitor of adrenocorticotrophic hormone (ACTH) secretion. Over-secretion of ACTH, as in cases of lowered corticosterone production and release, leads to hyperplasia and hypertrophy of adrenocortical cells (Gill, 1976). This hypertrophic response may have allowed recovery of the adrenal gland's capacity to secrete corticosterone. Adrenal gland hypertrophy has also been observed in the study of Saunders et al. (1966), in which the authors report that in rats chronically treated with SKF-12185, the cold-stress-induced rise in corticosterone concentration is diminished, despite marked hypertrophy of the adrenal glands. However, the duration of their chronic study (1 week compared to 5 weeks in this study) may not be long enough for the adrenal to fully recover from the inhibitory effect of the drug.

In light of the above discussion, one is led to conclude that the inhibition of corticosterone

production by SKF-12185 was, at best, transient. Many of the effects observed in drug-treated animals can be ascribed to the decrease in food intake. Based on this conclusion, we decided to pursue examining the possible involvement of the excess-glucocorticoid state in the development of hypercholesterolemia in the obese Zucker rat by bilateral adrenalectomy.

2. THE ADRENALECTOMY STUDY:

Adrenalectomy is a radical procedure that abolishes corticosterone secretion or severely decreases circulating corticosterone levels rather than ameliorating the hypercorticosteronemia. It also removes the source of secretion of various other hormones. These include aldosterone, adrenal androgens, and epinephrine. Of these hormones the only one that is known to have notable effects on lipid metabolism is epinephrine. It is well established that epinephrine promotes lipolysis in adipose tissue and glycogenolysis in muscle. Acute administration of epinephrine has also been shown to increase HMG-Co A reductase activity (Edwards, 1973) and increase serum cholesterol levels in rats (Kunihara and Oshima, 1983) and dogs (Gans, 1965; Gans and Cater, 1969). However, it is unlikely that the effects of adrenalectomy on the aspects of lipid metabolism determined in this study were caused by epinephrine deficiency. This is because:

1. Some of the metabolic effects of epinephrine mentioned above are mimicked by norepinephrine, the availability of which is not affected by adrenalectomy (Gans and Cater, 1969; Silverberg et al., 1978; Edwards

et al., 1979; Devery et al., 1986; Devery and Tomkin, 1986).

2. Epinephrine is also secreted by chromaffin cells located at various parts in the body (most notably in major blood vessels and in sympathetic ganglia).

3. In hyperinsulinemic, hyperglycemic sand rats due to high caloric diet feeding, adrenal medullectomy does not prevent the development of hyperinsulinemia. Additionally, adrenal medullactomy does not enhance the blunted insulin inhibitory effect on the epinephrine induced rise in cyclic-AMP levels in adipose tissue. This indicates that adrenal medullectomy did not abolish the insulin insensitivity of adipose tissue in these rats (Kohler and Knospe, 1982).

4. In monkeys, four months after successful adrenal medullectomy, the epinephrine content of liver and brain is not affected. The content of epinephrine in heart is decreased. The change in the heart, however, is apparently accompanied by a compensatory increase in norepinephrine levels. Furthermore the urinary excretion of epinephrine was sharply reduced in the adrenal medullectomized animals but that of norepinephrine was markedly greater (Harrison and Seaton, 1966). Additionally, in adrenalectomized and

adrenalmedullactomized rats, the excretion of norepinephrine and its metabolic breakdown products is elevated (Utevski and Rasin, 1972).

The author is not aware of any report that suggest a significant effect of aldosterone or androgens on any of the aspects of lipid metabolism measured in this study.

2.1 COMPARISON OF LEAN AND OBESE CONTROL ZUCKER RATS:

2.11 SERUM INSULIN, CORTICOSTERONE AND GLUCOSE CONCENTRATIONS:

The obese control rats have elevated levels of serum corticosterone compared to lean control rats. The values measured in this study are morning levels of corticosterone, i.e. at the time of diurnal low. This observation is in agreement with the studies of Martin et al. (1978) and Fletcher et al. (1986). In these two studies the investigators report that the corticosterone levels in obese rats persist at the level of the diurnal high of lean rats throughout the day. In agreement with other studies the obese rats were hyperinsulinemic and hyperglycemic (Martin et al., 1978; Fletcher et al., 1986; Freedman et al., 1986 a).

The combined hyperinsulinemia and hyperglycemia indicates the presence of an insulin insensitive state. This conclusion is in agreement with many other studies (Zucker and Antoniades, 1972; Freedman et al., 1986 b). The observation in this and other studies (York and Godbole, 1979; Freedman et al., 1986 a and b) that adrenalectomy lowered the serum insulin and glucose concentrations to levels of lean rats suggest that the hyperinsulinemia and the insulin insensitivity are caused by the hypercorticosteronemia.

2.12 SERUM LIPIDS:

In agreement with other studies we found obese rats to be hypercholesterolemic compared to lean rats (Pector et al., 1981; Lin, 1985; McCune and Jurin, 1987). We also observed that obese rats had elevated serum phospholipid concentrations, which reflect general hyperlipoproteinemia. Furthermore, we observed that the hypercholesterolemia was caused by elevation of serum free cholesterol with no change in serum esterified cholesterol. This suggests that the hypercholesterolemia in obese rats is caused by the presence of excess free cholesterol carried mainly by very low density lipoproteins. This is because, cholesterol free/ester ratio is substantially greater

in very low density lipoproteins than in high density lipoproteins in the rat. Additionally, Lin (1985) has shown that in obese rats, most of the serum free cholesterol is in the VLDL fraction.

We also observed, in agreement with other studies, that the obese rat displayed fasting hypertriglyceridemia, which suggested that livers of obese rats over-secreted TG-enriched VLDL particles (Durstine et al., 1985). This conclusion is in agreement with other studies (Schonfeld and Pflieger, 1971; Witztum and Schonfeld, 1979). This over-secretion of VLDL particles may be derived by the higher levels of TG synthesis in livers of obese rats.

This study is the first to perform linear regression analysis on serum insulin and corticosterone vs. serum lipids in obese Zucker rats. This was done to investigate whether the hyperlipidemia was related to hyperinsulinemia and hypercorticosteronemia in a graded fashion.

We found that serum insulin was positively correlated with fasting levels of serum TG and free cholesterol in obese rats. This is in agreement with studies in hypertriglyceridemic patients (Reaven et al., 1967; Olefsky et al., 1974), in non-insulin

dependent diabetes mellitus patients (Laakso et al., 1987), and in healthy male volunteers (Capaldo et al., 1987) and in Cushing's syndrome patients (Taskinen et al., 1983). We found that serum TG levels were also positively correlated with serum free cholesterol concentrations. These two observations imply that the majority of serum TG and free cholesterol were carried by the same class of lipoproteins and that the degree of hyperinsulinemia correlates positively with levels of this class of lipoproteins in the serum. As discussed above, such a lipid composition resembles that of very low density lipoproteins. Thus, the degree of hyperinsulinemia correlated positively with the levels of VLDL in obese rats.

In light of the above discussion, the following conclusions can be drawn:

1. The hypercholesterolemia in obese rats is caused by the hypersecretion of VLDL particles from the liver.
2. The hyperinsulinemia is related in a positive fashion to the degree of hypercholesterolemia and hypertriglyceridemia.

We also found that the serum insulin concentration did not correlate with serum total or esterified cholesterol concentrations. This implies that the

degree of hyperinsulinemia does not influence serum HDL levels in obese rats. Because VLDL is a precursor of HDL, it seems contradictory that hyperinsulinemia influences VLDL levels and not HDL levels. However, obese female rats have been shown to have lower clearance rates of serum TG (Redgrave, 1977). This implies that the conversion of VLDL to HDL is deficient in obese rats. This perturbation may also contribute to the observed hyperlipidemia in obese rats.

There was no correlation between serum corticosterone concentration and serum TG or cholesterol concentration (total, ester, or free). This indicates that the observed hyperlipidemia is correlated with the degree of the insulin-insensitive state produced by the hyperglucocorticoidemia rather than the serum glucocorticoid concentration per se. In agreement with this finding, the VLDL turnover rate correlated positively with the degree of insulin-resistant (estimated by insulin response to an oral glucose load) but not serum cortisol levels in patients of Cushing's syndrome (Taskinen, 1983). Nevertheless, these results should be considered with caution because:

1. serum corticosterone concentration is subject to stress induced fluctuation.

2. Despite all precautions taken to minimize novelty stress, the animals may have been stressed.

There was no difference in serum LCAT activity between lean and obese animals despite the significantly greater free/total cholesterol ratio in obese animals. This is in agreement with our conclusion that the excess free cholesterol (which apparently caused the hypercholesterolemia) was carried by VLDL where it is less accessible to esterification by LCAT. This observation is in agreement with the study of Lin (1985).

2.13 ADIPOSE TISSUE WEIGHT, CELL SIZE AND NUMBER, AND CHOLESTEROL CONTENT:

In agreement with other studies adipose tissue depot weights were exceedingly higher in obese control animals compared to lean control animals (Zucker and Zucker, 1961; Zucker and Zucker, 1962; Bach et al., 1981). Additionally, in agreement with other studies, adipocyte size and TG content were 8-9 fold greater in obese animals (Freedman et al., 1986 a). We also found that the increase in adipocyte size and TG content were accompanied by a commensurate increase in cholesterol content.

In this study total fat cell number per pad was not determined. Adipocyte number however, was calculated indirectly by the method of Boshart and Pierre (1962). These authors demonstrated that fat pad weight and fat pad lipid content are strongly correlated above 0.5-1 g of fresh weight in Sherman male albino rats. Thus, total TG per pad was estimated by using their linear regression equation. Total cell number was then calculated by dividing the total fat pad TG content by the TG content per adipocyte (which was measured in this study, tables 9 and 10). Since all fat pad weights from the lean adrenalectomized group were under 0.5 g, this method was not applied to the data from this group. These calculations assume that the relationship of weight to TG content found in adipose tissue of Sherman rats holds for lean and obese Zucker rats. Thus, one should emphasize that the total cell number calculated in this manner serves only as a useful estimate.

Table 17 shows that there was no difference in fat cell number between lean and obese control animals in the perimetrial depot. The fat cell number was lower in the lean animals in the perirenal depot, however. Similar values were obtained in the study of Freedman et al. (1986 a), in which animals were adrenalectomized

at a similar age and maintained for 4 weeks post-adrenalectomy. However, these investigators, found no significant differences between lean and obese animals in either depot.

We calculated that the cholesterol content of fat depots from obese control Zucker rats was 9-11 fold greater than that of lean control rats. Furthermore, even when the total perimetrial plus perirenal fat pad cholesterol content was normalized to body weight it remained over 10 fold greater in obese rats than in lean rats. Thus it is clear that obese rats have an extensive cholesterol storage capacity in adipose tissue; and that notwithstanding this extensive storage capacity the obese rats remain hypercholesterolemic. This suggests a defect in the removal of serum cholesterol. In agreement with this conclusion, Redgrave (1977) reported lower levels of chylomicron cholesterol ester and TG clearance rates in obese female Zucker rats.

Table 17

Total fat pad cell number and cholesterol content

	TOTAL CELL NUMBER PER FAT PAD (PM) x10 ⁶	TOTAL CELL NUMBER PER FAT PAD (PR) x10 ⁶	TOTAL CHOLESTEROL PER FAT PAD (PM) (μg)	TOTAL CHOLESTEROL PER FAT PAD (PR) (μg)	TOTAL FAT PAD CHOLESTEROL (PM+PR) PER GRAM OF BODY WEIGHT (μg/g)
LEAN n=	18.2 ± 2.5 ³ 4	9.9 ± 1.1 ¹ 5	316 ± 56 ¹ 4	124 ± 23 ¹ 5	2.7 ± 0.5 ¹ 4
OBESE n=	12.3 ± 2.0 ² 6	15.1 ± 1.6 ² 4	2431 ± 503 ² 5	2403 ± 502 ² 5	22.2 ± 3.0 ² 3
LEAN/ADRX n=	7.0 ± 1.0 9	6.9 ± 1.6 7	272 ± 55 9	212 ± 48 7	3.5 ± 0.6 7

¹= Significant, Lean vs Obese, T test, α=.05²= Significant, Obese Sham vs Obese ADRX, T test, α=.05³= Significant, Lean Sham vs Obese ADRX, T test, α=.05

Values represent mean ± S.E.M.

PM: Perimetrial depot

PR: Perirenal depot

2.14 LIVER LIPIDS:

The most striking observation in this study is that hepatic total cholesterol concentration was higher in lean rats compared to obese rats. This elevated level of total cholesterol was reflected in an increase in both the free and the esterified fractions. This effect may be caused by the elevated hepatic VLDL secretion rates of obese animals which may impose a "draining effect" on hepatic free and esterified cholesterol content and thus lead to the observed lower concentration of hepatic cholesterol.

We also found that lean rats had a greater microsomal free cholesterol content (ug/mg prot.) The lean rats also had greater free cholesterol to phospholipid ratios. This implies cholesterol enrichment of microsomal membranes. The elevation of microsomal free cholesterol concentration may be the cause for the lower HMG-Co A reductase activity in lean control animals compared to that of obese control animals. This suggestion is based on the following :

1. Cholesterol is a negative feed-back inhibitor of HMG-Co A reductase. It accomplishes that chronically by decreasing the synthesis of the reductase protein, and acutely by affecting the microsomal membranous

environment (possibly via an effect on fluidity) (Mitropoulos et al., 1978).

2. In in vivo studies in which liver free cholesterol was increased and in vitro studies in which hepatocyte free cholesterol content is enriched, HMG-Co A reductase activity decreases and cholesterol 7-alpha hydroxylase activity increases (Raicht et al., 1975; Myant and Mitropoulos, 1977; Davis et al., 1983).

In accordance with this explanation we found a significant negative correlation between HMG-Co A reductase activity (dependent variable) and microsomal free cholesterol concentration (independent variable) when data points from lean and obese control animals were combined.

Inasmuch as obese subjects display increased VLDL secretion (Howard et al., 1987), it is tempting to speculate that the greater hepatic HMG-Co A reductase activity observed in obese individuals (Angelin et al., 1982) is caused by a mechanism similar to that suggested above.

Hepatic TG content (mg/g wet wt.) was greater in obese control animals compared to lean control animals. This reflects the greater hepatic TG synthetic activity in obese animals reported in other studies (Sullivan et

al., 1977; Godbole et al., 1978; Jamdar et al., 1978; Kannan et al., 1980; Azain et al., 1985).

2.15 HEPATIC MICROSOMAL HMG-CO A REDUCTASE AND CHOLESTEROL 7-ALPHA HYDROXYLASE ACTIVITIES:

Only morning levels of HMG-Co A reductase activity were determined in this study. HMG-Co A reductase activity displays a diurnal rhythm with the zenith being in the mid-dark cycle and the nadir in the mid-light cycle. The hepatic HMG-Co A reductase activity was lower in lean animals compared to obese animals. This is in agreement with the study of Lin (1985), in which she showed that HMG-Co A reductase activity was significantly lower in lean female rats compared to obese female rats when sacrificed in mid-light cycle but not mid dark cycle. As mentioned above, we observed significant negative correlation between microsomal free cholesterol concentration and HMG-Co A reductase activity, which indicates that the higher levels of microsomal free cholesterol may be the cause for the lower HMG-Co A reductase activity.

Cholesterol 7-alpha hydroxylase activity displays a diurnal rhythm similar to that of HMG-Co A reductase. In this study we found that morning levels of 7-alpha

hydroxylase in obese and lean control animals were not different, but the values tended to be lower in lean animals. This is in agreement with the study of Tang et. al. (1988), in which the activity was significantly lower in obese animals at the time of the diurnal zenith (mid-dark cycle), but not at the time of the diurnal nadir (mid-light cycle). Because in normal physiological conditions, microsomal free cholesterol enrichment enhances 7-alpha hydroxylase activity, the greater 7-alpha hydroxylase activity relative to that of obese rats observed in the study of Tang et al. (1988) is in accordance with our observation that microsomal membranes from lean animals are free cholesterol-enriched relative to obese rats.

Subbiah and Connely (1976) report greater excretion of bile acids in obese male Zucker rats relative to lean rats. This increase is probably due to the larger size of livers from obese rats and can not be used as indication of specific activity of 7-alpha hydroxylase. Furthermore, the total bile acid excretion must be related to body size before any conclusion can be made about its effect on cholesterol concentration in tissue storage pools.

Serum insulin concentration did not correlate with either total HMG-Co A reductase activity, or the

+F1/total activity ratio. In other words, insulin apparently did not influence the total HMG-Co A protein content nor the ratio of dephosphorylated protein to total protein. Insulin has been shown to increase HMG-Co A reductase in isolated hepatocytes (Ingebristen et al., 1979). This increase was due to an increase in the +F1/total ratio, i.e by increasing the amount of the enzyme that is in the dephosphorylated, active form. This is not necessarily in contradiction with the data in this study, however, because one study is on isolated hepatocytes and the other on intact animals. The author is not aware of any reports on the effect of experimentally induced hyperinsulinemia on HMG-Co A reductase activity. HMG-Co A reductase total activity and +F1/total activity ratio did not correlate with serum corticosterone concentration either. As mentioned above, corticosterone correlation data should be considered with caution, since the effect of novelty stress on serum corticosterone levels can not be excluded.

2.16 CHOLESTEROL ABSORPTION:

Analysis of the data on cholesterol absorption indicates that in obese animals a higher percentage of the total dose of ^{14}C -cholesterol administered was

absorbed. When the percentage of the dose absorbed was converted to daily weight of cholesterol absorbed based on daily food intake and cholesterol concentration in the diet, the difference between lean and obese rats was more prominent.

A possible criticism of the above data conversion would be that the cholesterol concentration in the intestinal tract might be modified by the cholesterol content of the secreted bile. Thus a difference between lean and obese animals in the rate of secretion of bile or the concentration of cholesterol in the bile or both may prejudice the value obtained by this conversion. However Subbiah and Connely (1978) reported higher rates of bile acid excretion in obese male rats compared to male lean rats. If this report is taken to mean that bile secretion is elevated in obese rats, then the end result would probably be enrichment of intestinal cholesterol content in obese rats. If this were to be the case then we would be in fact underestimating rather than overestimating the daily cholesterol absorption in obese rats.

Nevertheless, when we normalized daily cholesterol absorption to body weight, there was no difference between lean and obese animals, although absorption in obese animals tended to be greater. We suggest that

when the data are normalized to body weight, a more meaningful conclusion can be made as to the effect of the dietary influx of cholesterol on body storage pools. Based on this, we conclude that the greater daily dietary absorption of cholesterol does not contribute to the observed hypercholesterolemia in obese animals.

In contrast to the findings in this study, Bendjellit et al. (1984) found no significant difference in cholesterol absorption between lean and obese Zucker rats even though the values tended to be higher in obese animals. This disagreement may be due to methodological differences. In this study the fecal excretion method with sitosterol as internal standard was used, whereas in the study of Bendjellit et. al (1984) cholesterol absorption was determined by an isotope balance method. The isotope balance method is based on administering radioactive cholesterol for an extended length of time (4 weeks or more) and assuming that the administered cholesterol reaches equilibrium with cholesterol storage pools. This assumption is not necessarily valid (Grundy and Ahrens, 1969; Zilversmit and Wentworth, 1970) Furthermore, examination of the formulas they used to determine cholesterol absorption

reveal that they did not correct for non-absorptive loss of cholesterol.

2.2 COMPARISON OF ADRENALECTOMIZED AND SHAM-OPERATED (CONTROL) ANIMALS:

2.21 SERUM LIPIDS:

Our observation that obese adrenalectomized rats compared to obese control rats had markedly lower serum TG and phospholipid concentration probably reflects decreased hepatic VLDL-TG secretion and thus prevention of the hyperlipoproteinemia and elevated serum VLDL levels observed in obese control rats. This supports our conclusion that the glucocorticoid-induced insulin-resistant state leads to the hypercholesterolemia through enhanced hepatic secretion of VLDL; and that adrenalectomy reverses this perturbation, as well as the insulin-resistant state.

Serum phospholipid and TG levels were not affected by adrenalectomy in lean rats. This is probably because, in lean rats, hepatic VLDL secretion was not affected by adrenalectomy.

The startling observation, however, was that in adrenalectomized animals, serum cholesterol levels tended to be higher across genotype; and that this adrenalectomy-induced hypercholesterolemia was more pronounced in lean animals. In fact post-hoc analysis revealed that the higher serum cholesterol levels in adrenalectomized rats were significant only in lean animals. This observation strongly indicates that the hypercholesterolemia in obese adrenalectomized rats relative to lean control rats, was not a genotype effect but rather a side effect of the adrenalectomy. Thus the question as to whether the reduction of serum glucocorticoid levels leads to alleviation of the genotype-induced hypercholesterolemia can not be answered by direct interpretation of the serum cholesterol data.

The lower serum TG levels of obese adrenalectomized rats compared to those of obese control rats was accompanied by higher concentrations of total serum cholesterol and a lower free to total serum cholesterol ratio. This suggests a shift in the serum lipoprotein profile to lipoproteins of higher density. This is because high density lipoproteins are typically poor in TG and free cholesterol relative to very lower density lipoproteins. This indicates

preponderance of cholesterol-ester enriched HDL in sera of obese rats.

2.22 LIVER LIPIDS:

One of the most striking observations in this study was that both lean and obese adrenalectomized animals had higher levels of liver total cholesterol content (mg/g wet wt.). This effect was more pronounced in obese animals. The increase in total cholesterol content was almost exclusively caused by an increase in free cholesterol concentration. In normal physiological settings, the liver responds to an increased cholesterol storage demand by storing the excess in the form of esterified cholesterol (Mitropoulos et al., 1978). This response entails an increase in acyl-Co A cholesterol acyl transferase (ACAT), the enzyme that esterifies cholesterol in the liver. Thus the liver of adrenalectomized animals does not seem to be capable of invoking the usual response to increased cholesterol storage demand.

The greater hepatic free cholesterol content in adrenalectomized animals was also observed at the level of the microsomes. Similar to the finding in liver total cholesterol, this effect was more pronounced in

obese animals. Adrenalectomized animals also had greater microsomal cholesterol to phospholipid molar ratios which indicates free cholesterol enrichment of microsomal membranes. These observations provide an explanation for the observed decrease in hepatic HMG-Co A reductase activity due to adrenalectomy in obese animals. In support for this argument, microsomal free cholesterol concentration correlated significantly and negatively with microsomal HMG-Co A reductase activity in group 3 (data points from obese and obese adrenalectomized) and group 4 (data points from all four experimental groups). The lack of correlation in group 2 is probably due to the fact that in lean adrenalectomized rats, the reductase activity was not significantly lower and the microsomal free cholesterol content were not significantly greater than lean sham-adrenalectomized animals.

Our observation that the hepatic TG concentration was lower in obese adrenalectomized animals relative to that of obese control animals is probably the result of decreased food intake, TG absorption (Isselbacher, 1967), and de novo fatty acid synthesis (York and Godbole, 1979). However, TG synthesis in the liver or adipose tissue or both in adrenalectomized obese animals apparently remain elevated relative to lean

control animals. This is suggested by the higher serum TG levels as well as the higher perimetrial plus perirenal fat pad weight per gram of body weight in obese adrenalectomized animals relative to lean control. The lower hepatic TG synthetic activity in obese adrenalectomized rats probably lead to the depressed hepatic secretion of VLDL observed in these rats.

In lean animals hepatic TG levels were not affected by adrenalectomy. This is in agreement with the study of York and Godbole (1979), in which hepatic TG synthesis was not decreased by adrenalectomy in lean rats.

2.23 ADIPOSE TISSUE WEIGHT, CELL SIZE AND NUMBER, AND CHOLESTEROL CONTENT :

There was a marked decrease in adipose depot weight and adipocyte size in adrenalectomized animals. The decrease in hepatic fatty acid synthesis and TG release, the decrease in adipose tissue fatty acid synthesis, and the decrease in adipose tissue LPL activity, all likely led to the severe shrinkage of the adipocyte size and TG content observed in this study. There was also a sharp decrease in adipocyte

cholesterol content per million cells. In this study total fat cell number per pad was not measured and thus the absolute decrease in the adipocyte compartment cholesterol stores could not be calculated. However, adipocyte number was calculated indirectly by the method of Boshart and Pierre (1962). Based on this method, we found that adrenalectomy decreased total cell number in obese animals in both the perimetrial and perirenal depots. This is in agreement with the study of Freedman et al. (1986 b). The lowering of hepatic TG secretion provides one explanation for the decreased adipocyte number in obese animals. The lack of glucocorticoids, may also contribute directly to the decreased adipocyte number. Glucocorticoids have been shown to promote the differentiation of adipocytes (Ringold et al., 1986; Hauner et al., 1987; Schiwiek and Loffler, 1987).

2.24 HEPATIC MICROSOMAL HMG-Co A REDUCTASE AND 7-ALPHA HYDROXYLASE ACTIVITIES :

The data in this study, which showed no significant effect of adrenalectomy on mid-light cycle HMG-Co A reductase activity in lean rats, are consistent with the study of Mitropoulos and Blasubramaniam (1976) and Edwards (1973) in which a

significant decrease in the enzymatic activity was observed only when male Wistar rats were sacrificed at mid-dark cycle. It is likely that a significant decrease due to adrenalectomy in lean animals and a more pronounced decrease in obese animals due to adrenalectomy might have been observed if mid-dark cycle activity had been determined as well. Based on these observations, it is obvious that one can rule out an increase in HMG-Co A reductase activity as a cause for the observed hypercholesterolemia in adrenalectomized animals.

As mentioned earlier, in studies in which microsomal free cholesterol was increased both in vivo and in vitro, cholesterol 7-alpha hydroxylase activity increased (Raicht et al., 1975; Myant and Mitropoulos, 1977; Davis et al., 1983). This is not in agreement with the findings in this study. The 7-alpha hydroxylase activity tended to be lower in adrenalectomized animals of both genotypes despite the marked increase in hepatic and microsomal free cholesterol concentration. The apparent inability of the adrenalectomized animal to respond to the elevation of liver and liver microsomal free cholesterol content by enhancement of cholesterol 7-alpha hydroxylase

activity may be a key factor in the observed adrenalectomy-induced hypercholesterolemia.

Our findings in lean rats are in agreement with that reported by Mitropoulos and Blasubramaniam (1976). They found that adrenalectomized Wistar rats had lower levels of microsomal HMG-Co A reductase and cholesterol 7-alpha hydroxylase activity than intact rats only when rats were sacrificed at mid-dark cycle. Similar to our findings, they also showed that microsomal cholesterol content (ug/mg prot.) is greater in adrenalectomized animals. They also showed that the rate of conversion of cholesterol to cholic acid (a primary bile acid) and the cholic acid pool size was reduced in adrenalectomized animals. They concluded in their study that the accumulation of microsomal free cholesterol is caused by the lower 7-alpha hydroxylase activity, and that the accumulation of free cholesterol leads to the observed decrease in HMG-Co A reductase activity. They however, in contradistinction to our finding, reported that serum cholesterol level is not affected by adrenalectomy. This discrepancy is probably due to the fact that they looked into the effect of short term (1 week) adrenalectomy in mature rats, whereas in this study the rats were adrenalectomized shortly after weaning and maintained for 5 weeks post adrenalectomy.

This, as will be discussed below, affected the size of cholesterol storage pool.

2.25 CHOLESTEROL ABSORPTION:

In obese adrenalectomized animals, the total amount of cholesterol absorbed per day was reduced relative to that of obese control animals. This effect was produced directly by a decrease in the percentage of the cholesterol consumed that was absorbed, and indirectly through a decrease in food intake. However, when cholesterol absorption was based on body weight, no significant differences were observed, although the values in the obese animals tended to be higher. In other words the adrenalectomized obese animal took in the same amount of cholesterol per gram of body weight as obese sham operated animals. As such, these results do not support increased dietary input as a contributing factor to the hypercholesterolemia induced by adrenalectomy.

2.26 EFFECT OF ADRENALECTOMY ON TISSUE CHOLESTEROL STORAGE POOLS:

Examination of body cholesterol kinetics using radioactive cholesterol indicates the presence of 2 or

3 exchangeable pools. Pool A -the rapidly turning over pool- was thought to include the liver and plasma. Adipose tissue was thought to be in the slowest turning-over pool, i.e pool B in the 2-pool model or C in the 3-pool model. Muscle is thought to be in a slowly turning over pool as well (Krause and Hartman, 1984).

Several lines of evidence point to the unique status of adipose tissue as a storage depot not only for TG but for cholesterol as well. In rats, adipose tissue contains more cholesterol per mg of protein than any other tissue. Furthermore adipocytes store over 90% of their cholesterol content in the central lipid droplet, not plasma membranes as is the case in other body organs (Farkas et al., 1973). This suggests that this cholesterol does not serve any physiological need for the adipocyte. As the rats become older, adipose tissue cholesterol increases to exceed that in any other tissue with the exception of skeletal muscle. Skeletal muscle cholesterol content, however, reflects its mass (over 45% of total body weight compared to 7-8% of body weight for adipose tissue in normal rats) (Farkas et al., 1973) and its requirement as a component of plasma membranes. Adipose tissue's status as a depot for cholesterol storage is reflected in the

dependence of its cholesterol content on serum cholesterol concentration and age, and in the observation that it is readily mobilized when adipose tissue size is decreased as for instance by weight loss (Krause and Hartman, 1984). Additionally, under normal physiological conditions, cholesterol synthesis in adipose tissue does not contribute significantly to total body cholesterol synthesis (Krause and Hartman, 1984). This is probably due to down regulation by cholesterol influx from plasma (Kovanen et al., 1975). Cholesterol synthesis might be increased by obesity however, though this remains a controversial issue (Krause and Hartman, 1984).

Adrenalectomy decreased liver weight per gram of body weight. This effect was even more drastic in the case of adipose tissue. These two observation combined with the fact that no significant effect due to adrenalectomy was found when cholesterol absorption was expressed in per-gram-of-body-weight units implies that there is greater cholesterol influx per gram of these two compartments in adrenalectomized animals. Thus, one would expect these two depots of cholesterol storage, i.e the liver and adipose tissue, to end up glutted with cholesterol. This indeed is what was observed in the case of the liver. Despite the fact that whole

liver cholesterol content is decreased due to adrenalectomy in both genotypes, when the data were based on grams of body weight, the pattern was reversed. Thus in adrenalectomized animals the liver was storing more cholesterol per gram of body weight than in intact animals.

This is not the case, however, for adipose tissue. Adipose tissue stores cholesterol mostly in the central lipid droplet, consisting almost entirely of TG (Farkas et al., 1973). The severely reduced adipocyte size and TG content in adrenalectomized animals (compared to those of intact controls), in addition to the reduced number of fat cells led to severe curtailment of adipose tissue cholesterol storage capacity. Thus, whereas the liver could respond to the increased cholesterol influx by storing more cholesterol per gram, the adipose tissue could not respond similarly. In support of this analysis, table 17 shows that total perimetrial plus perirenal cholesterol per gram of body weight was severely decreased in obese animals due to adrenalectomy.

Muscle mass and muscle cholesterol content were not determined in this study. However, in the study of Freedman et al. (1986 a), the percentage of body weight accounted for by protein was not affected by

adrenalectomy. If the assumption is made that body protein content is indicative of its muscle content, then the muscle cholesterol content per gram of body weight might not be affected by adrenalectomy.

Accordingly, the severe curtailment of adipose tissue storage capacity, combined with no effect on the muscle pool, shifts the cholesterol into the liver and plasma. The liver and plasma are in rapid exchange with each other, as might be inferred from their inclusion in the same kinetic pool.

It is important to emphasize that adrenalectomy led to the underdevelopment of adipose tissue compared to intact rats and not a reduction from its pre-adrenalectomy size. This is especially true for obese adrenalectomized animals. Thus, it is highly unlikely that the hypercholesterolemia observed in adrenalectomized animals was due to mobilization of cholesterol stored in adipose tissue prior to adrenalectomy.

3. SYNTHESIS AND CONCLUSIONS:

The obese Zucker rat displays all of the hyperlipidemic and hormonal perturbations of patients with Cushing's syndrome, with additional complication due to obesity. These perturbations include the insulin resistant state and elevated hepatic VLDL secretion rates. In this study we showed that the degree of hyperinsulinemia is positively correlated with serum TG and free cholesterol concentration. This observation, as we discussed above, suggests a relationship between the insulin-resistant state and/or the hyperinsulinemia and the elevated serum levels of VLDL and thus the hypercholesterolemia. We suggest that the insulin resistant state promotes lipolysis, which in turn leads to the elevated serum FFA observed in obese rats (Martin et al., 1978). The elevated levels of serum FFA and the hyperglycemia provide substrate and thus promote the higher levels of hepatic TG synthesis which in turn promote VLDL secretion. The insulin resistant state is also manifested in the obese Zucker rat in that it displays lower metabolic clearance rates than lean rats (Redgrave, 1977). This reflects impaired response of tissue lipoprotein lipase activity to

insulin stimulatory action. This perturbation is also displayed by patients of Cushing's syndrome (Taskinen et al., 1983). On the other hand, the insulin resistant state does not necessarily imply that hepatic TG synthesis is resistant to insulin action. (See discussion on insulin effect on VLDL secretion, in the literature review.) Thus it is reasonable to assume that enhancement of hepatic TG synthesis by the hyperinsulinemia may contribute to the enhanced VLDL secretion

In support of the above argument, the lower serum TG and phospholipid concentrations of obese adrenalectomized rats compared to those of obese control rats show that the amelioration of the excess glucocorticoid state, the insulin-insensitive state, and the hyperinsulinemia, led to the suppression of hepatic over-secretion of VLDL.

This study revealed that liver and liver microsomal free cholesterol content were lower in obese rats compared to lean rats. We suggest that this was caused by the elevated VLDL secretion rates. Furthermore, the negative correlation between liver microsomal HMG-Co A reductase activity and microsomal free cholesterol content suggests that the elevated levels of HMG-Co A reductase activity in livers of

obese rats, compared to those of lean rats, is caused by the lower microsomal free cholesterol concentration in liver of obese rats. The greater HMG-Co A reductase activity in livers of obese rats may have contributed to the observed hypercholesterolemia. In this manner the elevated levels of hepatic VLDL secretion lead to the observed enhancement of HMG-Co A reductase activity. This mechanism has been suggested by other investigators (Ide and Ontko, 1981) in an attempt to explain the observations in the literature that HMG-Co A reductase activity and cholesterol synthesis rates are increased when hepatic VLDL secretion is stimulated (Goh and Heimberg, 1977, Ide and Ontko, 1981).

Our observation that microsomal free cholesterol content in livers of obese rats was lower than that in lean rats was in accordance with the lower microsomal cholesterol 7- α hydroxylase activity in obese rats compared to that of lean rats reported in the study of Tang et al. (1988). This implicates the lower microsomal free cholesterol content as the cause for the lower cholesterol 7- α hydroxylase activity. Lower cholesterol 7- α hydroxylase activity may decrease conversion of cholesterol to bile acids and thus decrease cholesterol excretion and contribute to the observed hypercholesterolemia.

The cholesterol absorption data indicate that enhanced dietary cholesterol influx does not contribute to the hypercholesterolemia of obese rats.

The adrenalectomy-induced hypercholesterolemia, on the other hand, seems to be caused by the following:

1. The underdevelopment of adipose tissue size; and the disproportional decrease in liver size which curtail the capacity of these two tissues to store cholesterol.
2. The failure of adrenalectomized animals to respond to the liver and liver microsomal free cholesterol accumulation by an enhancement of 7- α hydroxylase activity.
3. Adrenalectomy did not affect the weight of cholesterol absorbed per gram of body weight, which means that the dietary cholesterol influx was not affected despite the decrease in the size of the body cholesterol storage compartments.

Based on the above discussion, figures 11 and 12 outline mechanisms for the development of hypercholesterolemia in obese rats and adrenalectomized rats respectively.

In conclusion, the findings in this study support the hypothesis that hypercorticosteronemia in obese

rats leads to the observed hypertriglyceridemic-hypercholesterolemic state by promoting VLDL secretion. Furthermore findings in this study clearly show that the hypercholesterolemia observed in obese animals and that observed in adrenalectomized animals are distinct in nature and etiology. Additionally, this study is the first to show that hypercholesterolemia is induced by long-term adrenalectomy during the rapid growth period in the rat. The findings in adrenalectomized rats demonstrate the vital role of adipose tissue in maintaining cholesterol homeostasis. This study is also the first to describe the effect of adrenalectomy on cholesterol absorption.

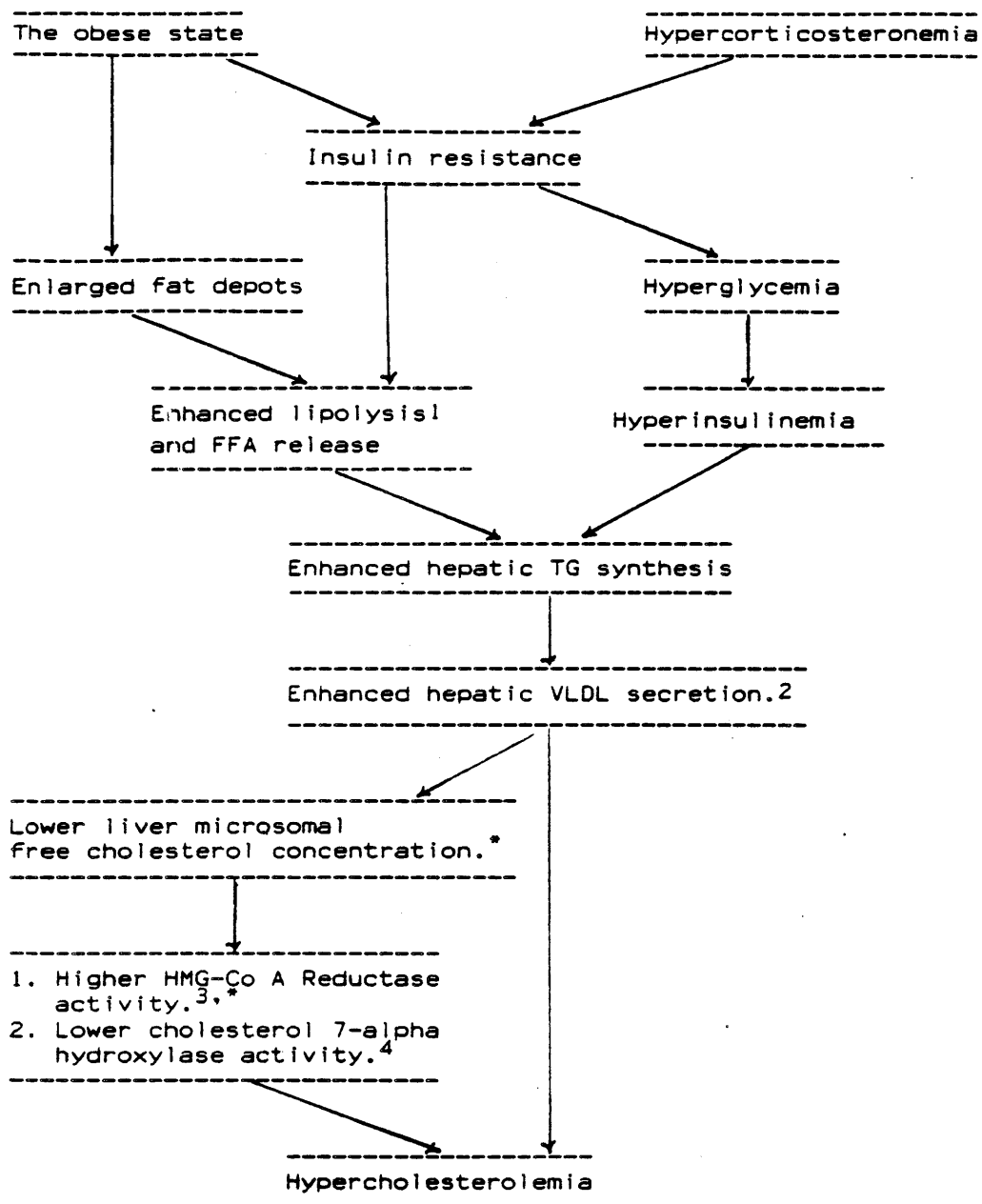


Fig 11. The development of hypercholesterolemia in obese animals.

*: Determined in this study. 1: York and Bray, 1973. 2: Schonfeld and Pflieger, 1971, Azain *et al.*, 1985. 3: Lin, 1985. 4: Tang *et. al*, 1988. Non-referenced observations are reported by many investigators, refer to literature review for details. Higher or lower refers to comparison with lean control rats.

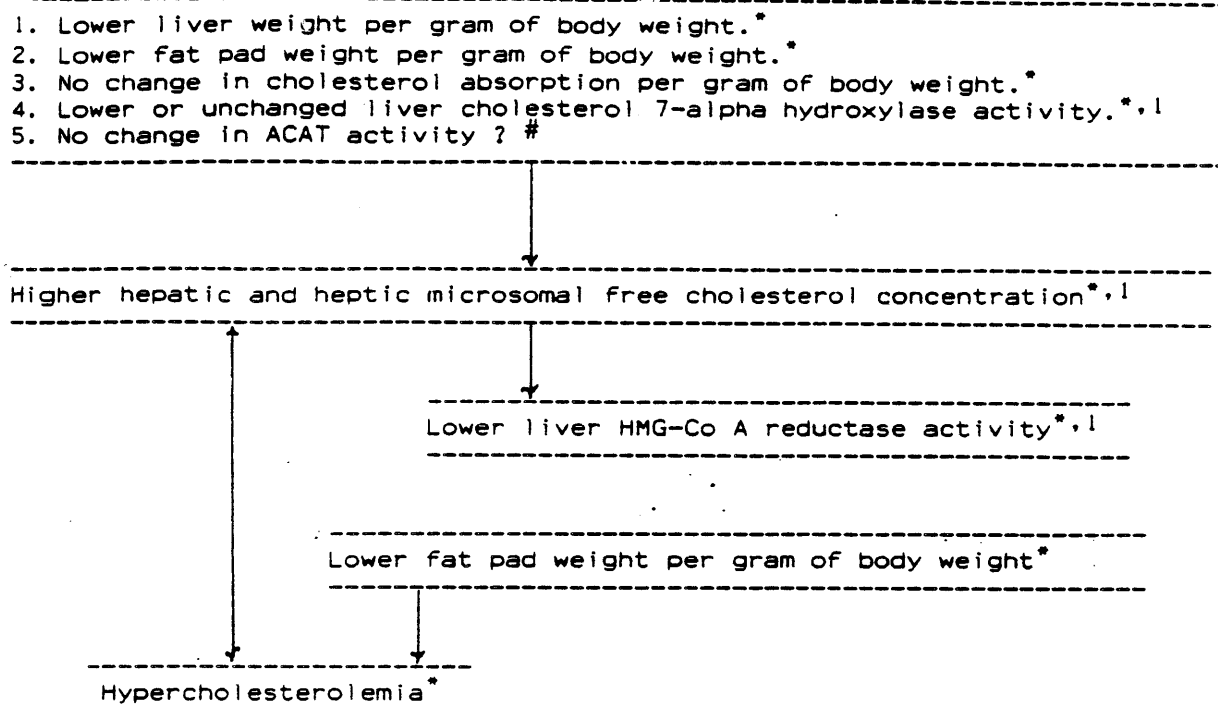


Fig 12. The development of hypercholesterolemia in adrenalectomized animals.
 *: Determined in this study. 1: Mirtopoulos and Blasubramaniam, (1976).
 #: Has not been examined. Higher or lower refers to comparison with sham-adrenalectomized rats.

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APPENDIX

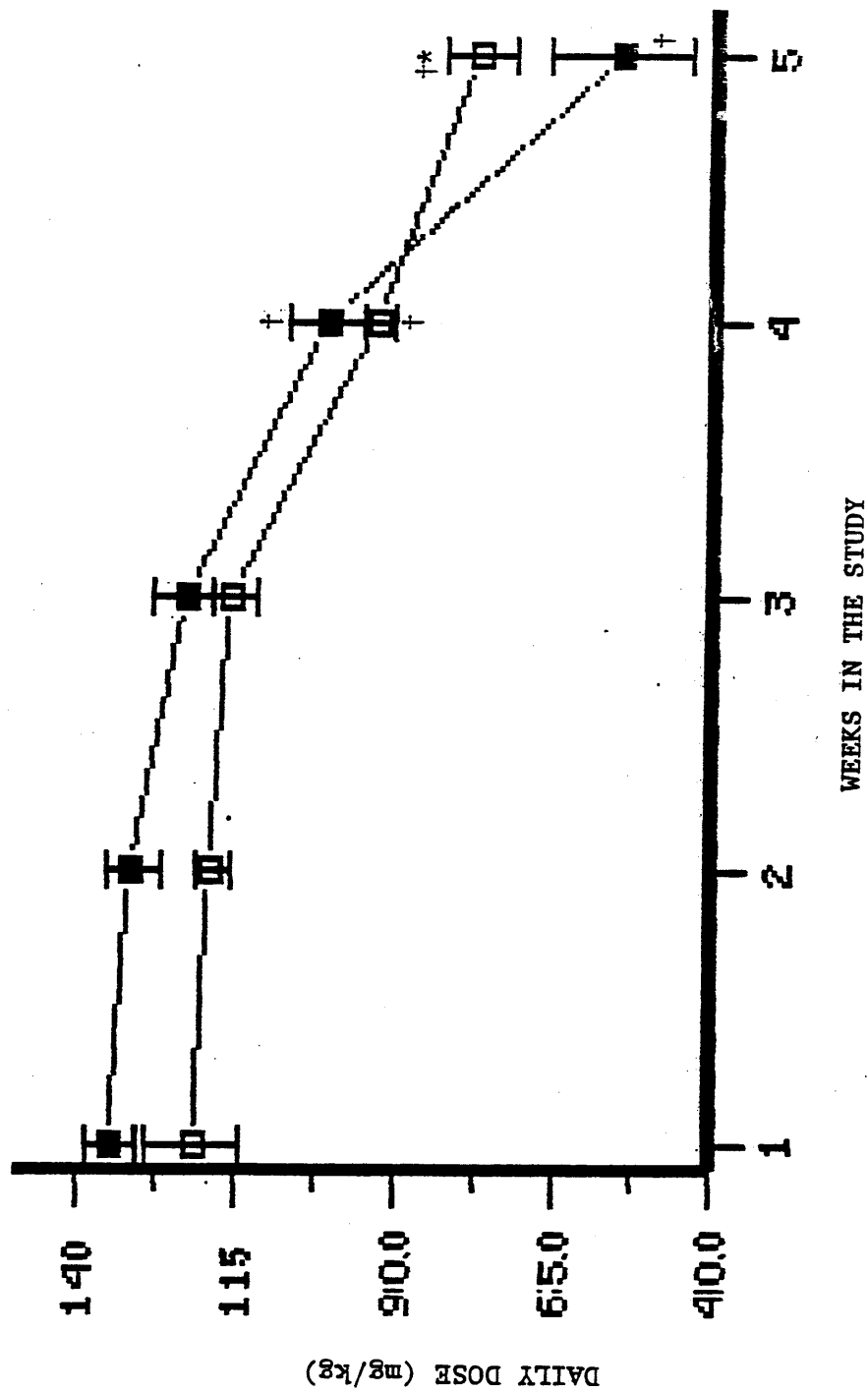


Fig. A. Average effective daily dose of SKF-12185 in lean and obese rats. Closed squares: Obese rats. Open squares: Lean rats. +: significant vs. dose at week 1 of the study. *: significant lean vs. obese, same study week. Student Newman Keuls, $\alpha = .05$

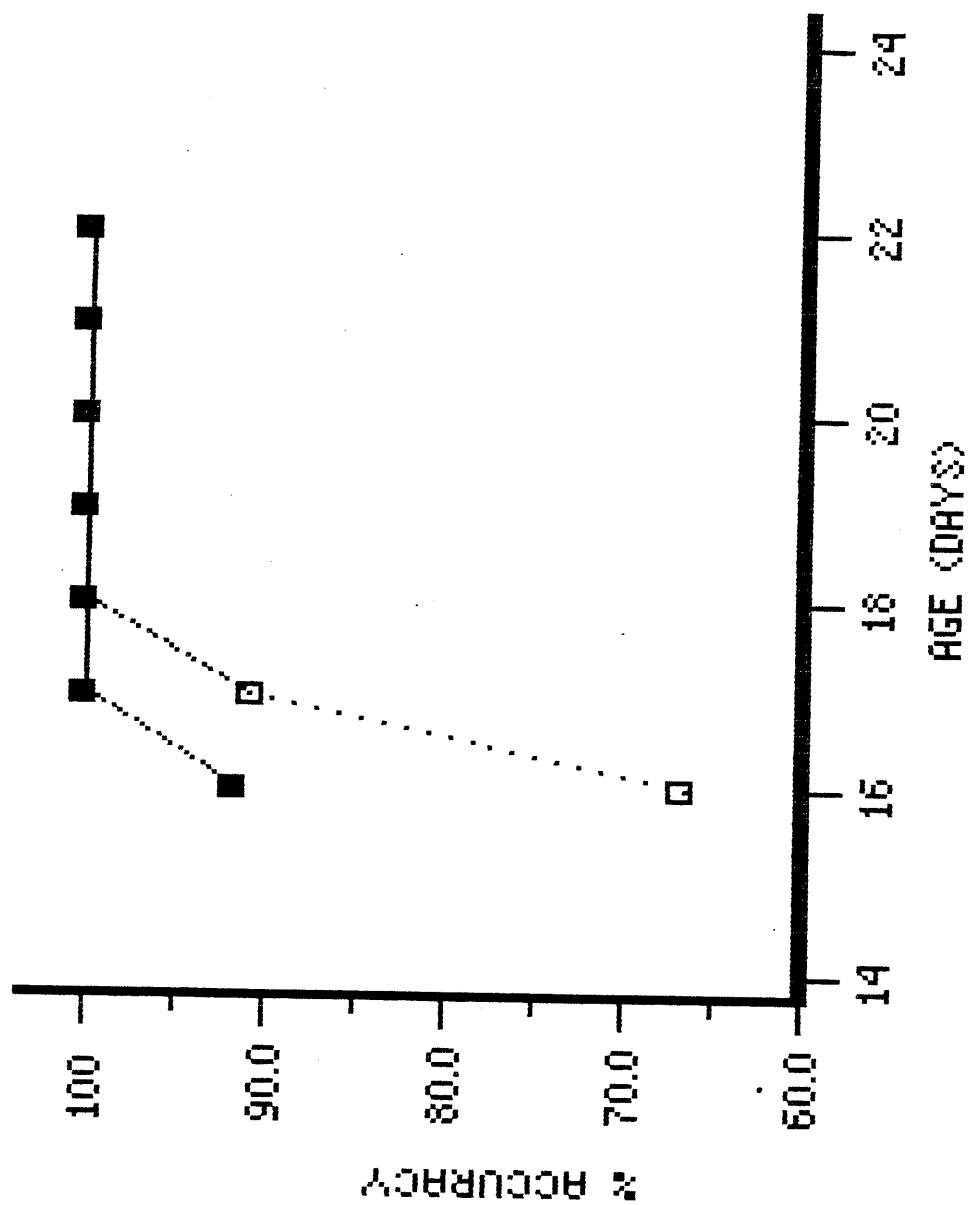


Fig. B. Percentage accuracy of genotype prediction. Closed square: Fat pad TG content method. Open square: Fat pad weight method.

CURRICULUM VITAE

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4. PORTER, J. R., F. ALARRAYED, C. CORLL, AND D. WHITE. The levels of various neurotransmitters in regional brain areas of intact and adrenalectomized fatty fa-fa rats. FASEB J., 2 (4) abstract 807, 1988.

ARTICLES:

1. ALARRAYED, F. AND J. R. PORTER. Cholesterol absorption in the obese Zucker rat: the effect of adrenalectomy. In preparation.
2. ALARRAYED, F., A. D. HARTMAN, AND J. R. PORTER. Cholesterol homeostasis in the obese Zucker rat: the effect of adrenalectomy. In preparation.

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