review

Adipose tissue and cholesterol metabolism

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Abstract Adipose tissue in man is a major site for cholesterol storage. In obesity over half of total body cholesterol may reside within this tissue; however, relatively little attention has been directed toward understanding the cholesterol metabolism and its relationship to whole body cholesterol homeostasis in this tissue. In this review the factors which influence cholesterol storage are discussed, with particular emphasis on the effects of diet and drug treatment in both animals and man. The uptake, synthesis, and mobilization of adipose tissue cholesterol appears to be mediated and/or regulated, as in other tissues, by the plasma lipoproteins, and these processes are examined with regard to both normal and pathologic states.—**Krause, B. R., and A. D. Hartman.** Adipose tissue and cholesterol metabolism. *J. Lipid Res.* 1984. **25:** 97–110.

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I. INTRODUCTION

Adipose tissue is widely recognized for its efficient storage of excess calories in the form of triglyceride. In this context, interest has been primarily directed toward processes involving this lipid such as uptake of plasma triglyceride (via lipoprotein lipase), triglyceride synthesis (lipogenesis), and triglyceride mobilization (lipolysis). Comparatively little attention has been directed to the involvement of adipose tissue in processes other than those directly related to triglyceride metabolism. Early clinical observations implied, however, that adipose tissue might be involved in the metabolism of cholesterol since significant correlations could be described between plasma cholesterol and various indices of obesity (1). Others reported that plasma cholesterol rose and fell as body weight was lost and gained, respectively (2). Furthermore, a positive correlation was described between excess body weight and cholesterol production (3). Direct chemical analysis of rat adipose tissue subsequently showed that this tissue actually contains more cholesterol than liver, muscle, or kidney when expressed on a per mg of protein basis and more than all other organs except skeletal muscle when expressed on a whole organ basis (4).

These past observations must be considered in the light of recent knowledge concerning lipid/lipoprotein/apolipoprotein interactions. Recent discoveries of peripheral cellular lipoprotein receptors (5) have focused attention once again on the role of peripheral tissues in cholesterol homeostasis. The present overview is intended to re-evaluate the potential role of adipose tissue in whole body cholesterol and lipoprotein metabolism.

II. EXPRESSION OF DATA AND METHODOLOGY

Adipose tissue in man and animals contains from 1–2 mg cholesterol per g wet weight, the majority in most studies being in the free, unesterified form (4, 6–10). This is approximately equal to the amount of cholesterol in skeletal muscle when expressed in the same units (11, 12) but is probably somewhat less than muscle on a dry weight, calorie, or whole organ basis (4, 9, 13). The technique for the preparation of isolated adipocytes developed by Rodbell (14) allowed for the eventual expression of data on the basis of cell number. It is now generally accepted that cholesterol content of adipose tissue is best expressed with consideration for the cellularity of the tissue either as μg/cell or $\mu g/10^6$ cells. Cholesterol concentration, on the other hand, usually refers to the amount of cholesterol in relation to the amount of stored tri-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; ACAT, acyl-CoA cholesterol acyltransferase; LPL, lipoprotein lipase; FH, familial hypercholesterolemia; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins.

glyceride (μg/mg triglyceride). If no significant changes are anticipated between control and experimental groups with respect to either adipocyte size or the proportion of other cell types in the tissue, such as in age or body weight matched groups, or if the methodology for cell isolation and sizing is not available, expression of data per mg protein or per mg DNA may provide reliable comparative data for cholesterol content (15). Expressing cholesterol content on the basis of wet weight probably provides the crudest estimate of cholesterol concentration and is inappropriate when body weights and adipose cellularity differ among treatment groups. Such arguments have been presented previously in relation to the expression of lipolytic data in adipose tissue (16, 17) and have subsequently been used for expressing lipoprotein lipase activity in isolated fat cells (18, 19).

The relative contribution of free versus esterified cholesterol to the total adipose cholesterol pool in rats has been a subject of controversy. Values of 2% (20), 70–75% (4, 10), and 90% (21) have been reported for the percentage of free cholesterol in normal animals. In man, however, all values reported have been over 90% (4, 22). These data suggest that some of the variability may be attributable to methodological problems which may not allow quantitative recovery of sterols, or there may exist distinct differences between species or even among rat strains. In some instances, the large amounts of triglyceride present in total lipid extracts may interfere with either quantitation or separation of free and esterified forms. For example, Farkas, Angel, and Avigan (4) have reported the incomplete precipitation of free cholesterol by digitonin due to interference by triglyceride. The most commonly used technique of thin-layer chromatography has also provided variable recovery data in some laboratories. These problems led to the development of a method using lipophilic dextran gel column chromatography (gel filtration using Sephadex LH-20) (10). This method, previously used by others for plasma (23), separates free and esterified cholesterol in the presence of excess triglyceride. As for the determination of cholesterol in adipose tissue, most methodologies employ the ferric chloride-sulfuric acid reaction which may be affected by the large amount of triglyceride present. Gas–liquid chromatography provides a more specific and sensitive method (15, 24).

Changes in the cholesterol content of whole rat adipose tissue are largely due to changes that occur within the adipocyte fraction since the amount of cholesterol in other tissue constituents (e.g., stromal-vascular elements) is comparatively minor, especially in adult animals (4). Also in the adult, most of the cellular cholesterol is localized within the central oil droplet, not in cellular membranes (4). This subcellular localization and the fact that the excess, non-membrane associated cholesterol is stored primarily in the unesterified form illustrate the uniqueness of adipose tissue storage compared to other cell types.

Finally, cholesterol in adipose tissue is considered to be one of a number of tissues contained within a slowly-turning-over pool of body cholesterol as defined by isotopic compartmental analysis (3, 22, 25, 26). After intravenous injection of labeled cholesterol, the half-life of adipose tissue cholesterol in the rat was found to be 27 days (4). In the rat isotopic equilibration between adipose and plasma specific activities required 7–8 days (4), while in man isotopic equilibration occurs after 1–1.5 months (22, 27). The fact that this pool of cholesterol turns over so slowly suggests that adipocyte cholesterol data based on changes in cellular content derived from short-term studies may underestimate the participation of adipose tissue resulting from a given dietary and/or drug intervention. This and the other foregoing considerations concerning the importance of data expression and chemical analysis should be, as in this overview, integrated into any interpretation of experiments dealing with adipose tissue cholesterol metabolism.

III. FACTORS INFLUENCING CHOLESTEROL STORAGE IN ADIPOSE TISSUE

A. Body weight, age, and cell size

The application of the cell isolation technique of Rodbell (14) to studies on cholesterol storage led to the finding that cell size and fat cell cholesterol content are positively correlated (15, 21, 22, 28, 29). Thus, the larger the fat cells, the more cholesterol they contain. This conclusion was reported under at least three different experimental conditions: *1*) determining adipocyte cholesterol content in cells of increasing size resulting from sucrose feeding (21); *2*) determining cholesterol in adipocytes derived from rats of increasing age, and therefore, increasing body weight and cell size (15, 28); and *3*) by obtaining cells of increasing size from the same rat by differential flotation of fat cells in buffer-filled dialysis tubing (29). Although somewhat cumbersome, the latter approach provides the most unequivocal evidence for the overriding influence of cell size on adipocyte cholesterol storage since differences in plasma cholesterol (21) or rat age (15, 28) can be ruled out as contributing factors. The aging process per se in the absence of cellular hypertrophy or hypercholesterolemia probably does not alter the extent of adipocyte cholesterol storage since adipocyte cholesterol content does not change with age in a strain of rat (Fischer 344) in which such "complications" of aging do not occur (30).

B. Experimental models of hypercholesterolemia

The concentration of cholesterol in the plasma can serve as a potential source of adipocyte cholesterol and thus influence the extent of adipose tissue cholesterol storage. It has been recognized for many years that plasma cholesterol is in dynamic equilibrium with tissues, including adipose tissue (31, 32). In long-term turnover experiments in man, the size of the most slowly-turning-over pool of cholesterol was significantly correlated with the degree of adiposity and with serum cholesterol concentration (25–27), observations that indirectly suggest that the amount of cholesterol in adipose tissue could rise with elevations of circulating cholesterol. Direct evidence illustrating the potential importance of plasma cholesterol in determining adipose tissue cholesterol storage has been obtained using two types of nutritional perturbations: cholesterol feeding and the feeding of cholesterol-free, purified diets.[1]

In cholesterol-fed rabbits, it has been reported that the concentration of cholesterol/g adipose tissue is determined both by the degree of hypercholesterolemia and the duration of the experiment or "exposure time" (33, 34). In cholesterol-fed squirrel monkeys, the concentration (mg/g wet weight) of cholesterol in adipose tissue is significantly higher compared to normocholesterolemic monkeys (35). In one rat experiment in which adipocyte size differences among dietary groups were known to be minimal, a direct correlation between dietary cholesterol level and adipocyte cholesterol content has been demonstrated (15).

Adipose tissue cholesterol content and plasma cholesterol concentration can also be increased in animals fed purified diets containing no cholesterol (15, 30, 36). It is therefore pertinent to consider the effects of such experimental diets not only in the rat but also in other species. It has long been known that such diets are atherogenic (37) and hypercholesterolemic (38) in rabbits. No single dietary component is wholly responsible for this type of experimental hypercholesterolemia (39), which occurs within 1 day of feeding (40). In the guinea pig, a cholesterol-free, purified diet increases all major plasma lipids for a period of 3 weeks, after which time lipid concentrations decrease back to control levels (41). Qualitatively, it appears that for the protein, carbohydrate, fat, and fiber components a combination of casein, starch, saturated fat, and cellulose, respectively, results in the most elevated plasma cholesterol concentrations in cholesterol-free, purified diets (42–44). In rabbits (45), low density lipoprotein (LDL) cholesterol is primarily elevated, but in rats (46, 47) very low density lipoprotein (VLDL), as well as plasma triglycerides, increase in concentration to the greatest extent among the lipoproteins. Thus, the rat fed a purified diet has been used as a model of hypertriglyceridemia and the rabbit fed a purified diet provides a model in which to study cholesterol and LDL metabolism. Recently it has been shown that the hyperbetalipoproteinemia in rabbits fed cholesterol-free, purified diets is associated with a decreased number of hepatic LDL receptors (48), but unlike cholesterol-fed animals, fecal steroid excretion is decreased (49). This may be due to the twofold higher liver cholesterol concentrations in cholesterol-fed rabbits (45) which would increase the availability of cholesterol for bile acid secretion (50). In the rat purified diets tend to decrease fecal steroid excretion and increase the total body content of cholesterol (51). The latter finding is in agreement with the experiments demonstrating enhanced adipose tissue cholesterol storage in rats fed cholesterol-free, purified diets (15, 30). The overall picture represents a possible situation in which adipose tissue cholesterol content is increased, possibly via uptake of triglyceride-rich lipoproteins (see below), even though whole body synthesis and hepatic HMG-CoA reductase values are decreased.

It is important to note that the enhanced cholesterol storage in adipocytes from rats fed cholesterol-free, purified diets may be associated with both an absolute and a percentage increase in cholesteryl ester content (30, 36) and that the plasma cholesterol response to purified diets containing various amounts of cholesterol is time-dependent. Distinct differences in plasma cholesterol exist for approximately 2 months in rats receiving 0–5% (w/w) dietary cholesterol, but after this time plasma cholesterol concentrations are all uniformly elevated regardless of the dietary intake (15, 30).

In conclusion, hypercholesterolemia produced either by cholesterol feeding or by cholesterol-free, purified diets ("endogenous" hypercholesterolemia) results in the accumulation of cholesterol in adipose tissue. Furthermore, the lipoproteins mediating these responses are likely to be different in various animal species for either of these dietary perturbations. Undoubtedly these animal models will continue to provide interesting insights into lipid and lipoprotein homeostasis.

C. Reductions in plasma cholesterol: unsaturated fat diets and drug effects

The above observations suggest that the extent of cholesterol storage in adipose tissue can be influenced by both exogenous and endogenous hypercholesterolemia in some species. But elevation of adipocyte cholesterol

[1] The designation "purified" is used to apply to diets composed primarily of refined ingredients, and is used instead of "semipurified" or "semisynthetic," as recommended by the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies (*J. Nutr.* 1977. **107:** 1340–1348).

has also been reported in conditions in which plasma cholesterol is either not altered or actually lower compared to control. In the experiments of Angel and Farkas (15), the highest level of adipose tissue cholesterol content was found in rats with the lowest plasma cholesterol value but receiving the highest level of dietary cholesterol. This occurred in both short (2 months) and long-term (5 months) experiments. Thus, the level of dietary cholesterol, not plasma cholesterol, appeared to be directly correlated with adipose tissue cholesterol storage. In humans consuming high cholesterol diets, it has also been observed indirectly by sterol balance methods that cholesterol accumulates in body pools even though plasma cholesterol does not change (52). Enhancement of adipose tissue cholesterol storage in the face of unchanged or decreased plasma cholesterol concentrations has also been observed, directly or indirectly, in response to two general perturbations discussed in more detail below: unsaturated fat feeding and drug administration. These and other studies have led to the intriguing hypothesis that adipose tissue may act to "buffer" the plasma against hypercholesterolemia (4, 52).

1. *Unsaturated fat feeding*

Attempts to elucidate the mechanism(s) responsible for the hypocholesterolemic effect of polyunsaturated fat feeding in man (53, 54), nonhuman primates (55), and rabbits (56) have in part led to the hypothesis that unsaturated fats cause a redistribution of cholesterol between plasma and tissue pools. Increases in liver cholesterol concentrations (57–62) and adipose tissue cholesterol (63, 64) have been reported in rats fed unsaturated fat with minimal changes in plasma cholesterol. Based on one early report (65), it appears that liver cholesterol in humans is not altered by unsaturated fat feeding. No data are as yet available on human adipose tissue. However, the fact that human fibroblasts grown in medium supplemented with linoleic acid degrade more LDL than cells grown in the presence of palmitic acid (66) implies that peripheral cells such as adipocytes, which also bind and degrade LDL (see below), may store increased amounts of cholesterol during unsaturated fat feeding. This hypothesis is certainly worth testing and should be performed with careful attention to changes in tissue cellularity among dietary groups.

It should be noted in this discussion that the hypocholesterolemic effect of unsaturated fat so often reported in man and other species does not consistently occur in the rat. In some experiments, plasma cholesterol was increased in young rats by 34% but in older rats by only 13% using the same 10% corn oil supplement in a purified diet (67). In another study, plasma cholesterol concentrations were higher with soybean oil than with tallow when the purified diets used contained a high fiber content (63). In preliminary experiments, when non-purified diets were supplemented with either corn oil or olive oil, however, plasma cholesterol concentrations were not increased compared to lard, but liver cholesterol and plasma apoB concentrations were higher with the unsaturated fat.[2] From these studies it can be concluded that the plasma cholesterol response to unsaturated fat feeding in the rat may vary depending upon *1*) the age of the animal or *2*) the type of basal diet. Neither of these variables has been systematically tested. The latter is especially important since hepatic HMG-CoA reductase activity (68), as well as total body cholesterol synthesis (51), is lower in rats fed purified compared with non-purified diets and, as mentioned above, rats fed purified diets are mildly hyperlipidemic and provide an entirely different metabolic baseline. Despite these differences, however, most recent experiments have utilized well-defined purified diets to study the effects of fat saturation. From such experiments it is now fairly certain that, in rats, unsaturated fat compared to saturated fat increases hepatic ACAT activity (69), but decreases hepatic cholesterol 7α-hydroxylase activity (70). Whether these differences in enzyme activities are related to greater cholesterol absorption with unsaturated fat (71) or to the size and/or composition of chylomicron remnants reaching the liver (i.e., factors affecting cholesterol availability in the liver) remains to be established, as does the effect of dietary fat saturation on the assimilation of intestinally-derived particles by adipose tissue.

As stated in recent reviews (72, 73), determination of cholesterol absorption, synthesis, and excretion have not adequately and consistently explained the hypocholesterolemic effect of unsaturated fats in man. Since the plasma lipoproteins and their metabolism are probably more important in determining plasma cholesterol levels than changes in the absorption, synthesis, or excretion of body cholesterol (74), it is perhaps not surprising that the mechanism(s) of action of polyunsaturated fat feeding have not been consistently defined when these parameters alone are considered. Studies on the effects of altered cellular membrane lipids (fluidity) on lipoprotein receptor interactions and metabolism may be required to understand to what extent various tissues, including adipose tissue, contribute to the proposed redistribution phenomenon in various species. The fact that polyunsaturated fats in man decrease the concentration of both cholesterol and apoB in LDL (75, 76) suggests an effect on the ca-

[2] Krause, B. R., C. Hoffmeier, and P. S. Roheim. 1982. Type of dietary fat alters serum lipoproteins and apolipoproteins in the rat. *Federation Proc.* **41:** 1622 (Abstract).

tabolism of the entire particle, not only by the liver but by many other tissues, including adipose tissue (77).

2. *Drug effects*

Many pharmacologic agents known to lower plasma lipid concentrations apparently also influence the levels of tissue cholesterol. The increased excretion of fecal steroids reported with some drugs in man cannot be explained by changes in the plasma cholesterol pool. Therefore mobilization of tissue cholesterol, rather than sequestration as postulated for unsaturated fat diets, has been invoked as a contributing mechanism of drug action (78). In other clinical investigations, clofibrate and nicotinic acid both caused an elevation or flattening of the plasma cholesterol specific activity-time curve following intravenous labelling with [^{14}C]cholesterol. These results have been interpreted as representing mobilization of cholesterol from tissue storage sites with a higher specific activity than that of plasma (79–81). It has been postulated that mobilization of tissue cholesterol due to clofibrate and nicotinic acid is secondary to a fall in plasma cholesterol concentrations, and that the mobilized cholesterol is rapidly removed from plasma and excreted as biliary cholesterol (80, 82). In contrast to these drugs, cholestyramine has been shown to increase the size of tissue pools of cholesterol in man despite its hypocholesterolemic action (83).

The above clinical trials did not include the direct determination of cholesterol mass in tissues. In experimental animals this is obviously more feasible. In hypercholesterolemic swine, clofibrate did not alter the concentration of cholesterol in adipose tissue compared to controls in trials lasting only 3–4 weeks (84). In short-term experiments in rats, clofibrate increased lung and colon cholesterol concentration (85) and decreased the amount of cholesterol in kidneys and testes (86). Unfortunately, these studies did not include adipose tissue or skeletal muscle where most body cholesterol is stored (4). In one short-term study where these tissues were examined, clofibrate decreased liver cholesterol concentration but did not alter the concentration of cholesterol in adipose tissue (87).

In a more recent attempt to examine the possibility of cholesterol mobilization from adipocytes independent of triglyceride mobilization, oxandrolone or a combination of cholestyramine/clofibrate was administered to cholesterol-fed Fischer 344 rats in a long-term study (88). It was established earlier that such rats have an expanded adipose tissue cholesterol pool size (30). Furthermore, since this strain of rat ceases to grow after 1 year of age, there were also no alterations in adipocyte size which, as pointed out above, could have influenced cholesterol storage. The rationale for combined cholestyramine/clofibrate treatment was similar to that reported by others in animals, namely, that clofibrate would offset the increased cholesterol synthesis produced by cholestyramine (89). Presumably, this type of drug regimen would also increase the hepatic requirement for exogenous plasma cholesterol (90), producing a situation conducive to mobilization from peripheral storage sites. Oxandrolone, an anabolic steroid, has been shown to be hypocholesterolemic in retired breeder rats (91). This experiment, the duration of which was 6 months, demonstrated that cholesterol storage in adipocytes is actually enhanced by such long-term pharmacologic interventions, and that such increments are associated with decreases in the relative amounts of apoA-I and apoA-IV in the plasma lipoproteins ($d < 1.21$ g/ml fraction). These results lend experimental support to the clinical observation that cholestyramine increases the size of pool B (83), a theoretical pool comprising adipose tissue (27). The conditions under which adipose tissue cholesterol sequestration occurs must be addressed since other slowly-turning-over pools of cholesterol (e.g., arterial tissue) may behave in a similar fashion. It will also be of interest to determine the effects of drugs that increase rather than decrease plasma HDL and apoA-I concentrations (e.g., gemfibrozil, Lopid®) (92–94).

D. De novo cholesterogenesis

Theoretically, in situ synthesis of cholesterol could contribute to the cholesterol pool(s) in adipose tissue. Early turnover experiments in man demonstrated positive correlations between the production rate of cholesterol and excess body weight (3) or adipose cellularity (27), suggesting that adipose tissue might be the site of increased cholesterol synthesis in obesity. However, in vitro data thus far have failed to provide direct evidence to support adipose tissue as the source of the excess cholesterol production in obese man (22). In other species as well, adipose tissue does not appear to contribute appreciably to whole body cholesterol production. In squirrel monkeys (95) and rats (96, 97) low and unresponsive rates of [^{14}C]acetate incorporation into cholesterol have been reported in slices of adipose tissue. In addition, the rate of incorporation of ^3H_2O in vitro or in vivo (87, 98, 99) in adipose tissue is very low or not detectable, and [^{14}C]octanoate incorporation into cholesterol in rabbit adipose tissue slices is also minimal (100) despite the fact that overall extrahepatic cholesterogenesis probably exceeds hepatic cholesterogenesis in this species, as well as in the guinea pig (99, 100) and goat (101). However, it is possible that optimal conditions have not been obtained in studies examining adipose tissue and that differences in permeability or accessibility of substrate differ among tissues derived from the same or different species (99).

Rates of cholesterol synthesis are usually several-fold higher in whole tissue compared to isolated cells (102), suggesting that the low rates of cholesterogenesis may be due to the presence of other cell types in whole tissue. Furthermore, if cells are isolated after the tissue is incubated with labeled precursor, less than half of the radioactivity is found in the cells and more than half in vascular cells (21). It is also of interest that adipose tissue obtained from rats with negligible concentrations of plasma lipoproteins incorporates significantly more [^{14}C]acetate into cholesterol than tissue from controls (97). These data suggest that regulation of the low rates of cholesterogenesis in adipose tissue may be due to down-regulation by circulating lipoproteins under normal conditions.

Attempts to assess cholesterogenesis in adipose tissue have resulted in three intriguing observations. First, radioactivity from labeled precursors is incorporated readily into squalene and methyl sterols (102–105). Secondly, unlike other organs, adipose tissue may preferentially synthesize cholesterol and other nonsaponifiable lipids from leucine (21, 102, 106). Thirdly, in patients with homozygous familial hypercholesterolemia adipose tissue cholesterol synthesis is increased 15-fold above control values (107). These observations taken together suggest that adipose tissue has the potential to contribute substantially to overall cholesterol production directly or indirectly, and that cholesterol biosynthesis may normally be down-regulated. The comprehensive experiments of Tilvis, Miettinen, and co-workers (103, 104, 108, 109) point to the possibility that squalene and methyl sterols in adipose tissue, derived from de novo synthesis, are localized intracellularly in microsomal membranes and the lipid droplet, and that it is the rapidly-turning-over membrane pool which is involved directly in cholesterol biosynthesis. These recent data may explain why precursor incorporation in vitro is minimal in adipose tissue since radioactivity in these precursor molecules would be trapped and diluted by the large pools of squalene and methyl sterols, perhaps resulting in significant underestimation of the rate of cholesterol synthesis. In addition, in vitro studies indicate that labeled squalene synthesized in human adipocytes is not transferred to plasma lipoproteins when these are added to prelabeled adipocytes (103, 109). These results indicate that adipose tissue does not mobilize newly synthesized squalene for completion of cholesterol synthesis in another tissue site. It is possible that in obesity, release of squalene from the lipid droplet and conversion to cholesterol by microsomes occurs at a faster rate or is unregulated, thus accounting for the increased production of cholesterol (3, 27) as well as increased adipocyte cholesterol concentration (102, 105). Overall, these major findings suggest that adipose tissue is a unique and dynamic organ involved in cholesterol biosynthesis, but the exact extent of this involvement remains to be more precisely defined, especially in disease states.

IV. ENZYMATIC DISPOSITION OF CHOLESTEROL IN ADIPOSE TISSUE

Given the facts that adipose tissue cholesterol is in dynamic equilibrium with plasma cholesterol, and that adipose tissue contains both free and esterified forms of cholesterol, it is tempting to speculate that uptake of free cholesterol from plasma in excess of metabolic or structural requirements is associated with ester formation for storage in the fat droplet and that mobilization of stored esters or uptake of circulating esters is associated with hydrolysis. Such speculation has led to attempts to detect enzyme activities in vitro associated with such events. Arnaud and Boyer (110) detected a neutral cholesterol esterase in human adipocytes. Others have found that in rat tissue this activity is stimulated by conditions which activate hormone-sensitive lipase (111). In fact, hormone-sensitive lipase and neutral cholesteryl ester hydrolase activities may both be associated with the same enzyme protein (112). The fact that the activated esterase can hydrolyze [^{3}H]cholesterol ester in lipoproteins in vitro provides indirect evidence that the enzyme may play a physiologic role in degradation of lipoprotein-derived cholesteryl esters taken up by adipocytes (113). Although changes in enzyme activity have not been directly correlated with changes in either the form of stored cholesterol or content within adipocytes, the relative constancy of the cholesteryl ester pool during starvation despite mobilization of free cholesterol and triglyceride (21, 114) would seem to provide evidence for the hypothesis that this esterase may not function in the hydrolysis of the stored cholesterol pool under conditions in which its activity should be increased.

When esterase activity from rat (115) and pigeon (116) adipose tissue is characterized in the presence of glycerol-dispersed cholesteryl [^{14}C]oleate, a distinct optimum at pH 5 as well as high activity at pH 6–8 is detectable. This observation allows integration of the potential cellular events in adipose tissue with those already documented for liver and certain extrahepatic tissues in which degradation of lipoproteins involves lysosomal processes (77). The decrease in acid cholesterol esterase activity in fat tissue from thyroidectomized rats (115) suggests hormonal regulation of intracellular lipoprotein processing in adipose tissue. As far as the formation of cholesteryl esters is concerned, no activity ascribed specifically to ACAT activity has been described for adipose tissue, although the capability to esterify cholesterol in vitro has been demonstrated (117, 118). Again, it is possible that esters

of cholesterol do not accumulate unless the rate of accumulation exceeds the rates of their hydrolysis. This may occur in certain forms of experimental hypercholesterolemia in which enhanced ester content of adipocytes has been reported (30, 36).

V. CHOLESTEROL STORAGE AND LIPOPROTEINS/APOLIPOPROTEINS

A. Uptake of cholesterol

Recently, the possibility that LPL may be involved in the uptake of cholesterol in both adipose tissue (119) and heart (120) has been rekindled by exciting results with cell cultures. Uptake of a nondegradable analog of cholesteryl ester in chylomicrons exceeds that of ^{125}I-labeled chylomicron protein in cultured heart cells and is correlated with LPL activity (121). In human skin fibroblasts and bovine endothelial cells (cells devoid of endogenous LPL activity), uptake of the analog is increased dramatically by addition of iodinated bovine milk LPL (122). In many cell types, including preadipocytes, uptake of the analog is dependent on binding of LPL to the cell surface (123, 124) and can occur by a non-apolipoprotein B,E receptor-mediated transfer when the analog is presented in phospholipid liposomes (124). One may speculate from these results that in vivo, functional LPL at the capillary endothelium may play a role in the cellular uptake of the cholesteryl ester moiety of chylomicrons in addition to its role in triglyceride hydrolysis (122). Whether VLDL-cholesteryl ester could also enter cells via this mechanism is unknown, but extension of these findings to adipose tissue, a tissue rich in LPL activity, appears logical. Another potential problem is related to the mechanism of transfer of cholesteryl ester from the endothelial cells to adipocytes and whether this can be accomplished without hydrolysis. In vivo evidence may have to be obtained under conditions in which plasma HDL concentrations are low, since HDL apolipoproteins may inhibit the LPL-mediated uptake of esters (124). In rat experiments where drugs known to stimulate LPL and lower HDL result in accumulation of adipose tissue cholesterol (88), the first mechanism may occur but direct proof is lacking.

In addition to the potential role of LPL in uptake of cholesteryl ester from triglyceride-rich lipoproteins (119–124) via the capillary endothelium, specific saturable receptors for VLDL which are hormonally responsive have been described on isolated adipocytes and also on adipocyte membranes (125, 126). Whether this binding results in internalization of the lipoprotein or is important physiologically is currently unknown, but the proposed role of apoC as the ligand for VLDL binding is intriguing. This putative "VLDL-receptor" may mediate the enhanced storage of cholesterol reported to occur in adipocytes from patients with carbohydrate-induced hypertriglyceridemia (127). The attractiveness of the VLDL receptor mechanism is enhanced by the fact that VLDL-sized particles are found in biological fluid presumed to be analogous to interstitial fluid (i.e., peripheral lymph) (128).

The importance of the LDL pathway is emphasized by in vivo experiments in swine in which the uptake of [^{14}C]sucrose-labeled LDL catabolized by adipose tissue exceeds that of all tissues except small intestine and liver (77). In rats and rabbits the uptake by adipose tissue is less, amounting to 4–6% of the injected dose (129, 130). In the rat it can be calculated that approximately 25% of LDL uptake is receptor-mediated (129). This observation is consistent with results in the rat in which administration of the drug 4-aminopyrazolo (3,4-d)pyrimidine, which lowers the concentrations of all lipoproteins in the circulation, results in an increase in adipose tissue cholesterol synthesis (100). These results indicate that LDL plays a role in regulation of cholesterogenesis in the rat. Interestingly, recent studies in control and Watanabe rabbits, which lack the LDL receptor, suggest that, in this model of FH, adipose tissue synthesizes essentially all of the sterol it requires for daily turnover and so takes up little or no cholesterol (131). Furthermore, in homozygous FH patients, the enhancement of cholesterol synthesis in adipocytes is undoubtedly a reflection of the failure of circulating LDL to interact with the tissue and thereby down-regulate cellular cholesterol metabolism (107). The uptake and degradation of LDL by adipocytes is stimulated by norepinephrine and dibutyryl cyclic AMP and possibly linked functionally to the cAMP-dependent hydrolysis of the cellular triglyceride moiety (132–134). One major goal of future research must be to correlate receptor- and enzyme-mediated uptake with changes in intracellular events such as alterations in free/ester pool sizes, cholesterogenesis, and ACAT activity.

B. Cholesterol mobilization

It has been postulated that both chronic caloric restriction in obese humans (135, 136) and total starvation in rabbits (137) result in mobilization of cholesterol stored in adipose tissue. In obese subjects undergoing weight reduction, a physiologic role of adipose tissue cholesterol could be to prevent a decrease in the saturation of bile (136). In starved rabbits, however, the mobilized cholesterol is not cleared from plasma and therefore hypercholesterolemia develops (138). Direct measurement of adipocyte total cholesterol content before and after fasting suggests mobilization of cholesterol at rates different from triglyceride mobilization (15). If a time-course of these events is studied in the rat, it can be observed that free

cholesterol content decreases rapidly after only 24 hr and then decreases more slowly for up to 144 hr (114). The content of cholesteryl ester is not changed by fasting over this time period. In addition, adipocyte triglyceride content was not changed after 24 hr of food deprivation. These results suggest that two separate pools of free cholesterol may exist, one that is readily mobilized (membrane cholesterol?) and one that is mobilized more slowly (core cholesterol?). Furthermore, during the first 24 hr, cholesterol is mobilized independently of triglyceride, possibly because the core lipid is not involved at this early time point during starvation. It is of interest that the existence of two pools of squalene in fat cells has also been postulated (108).

In fasting rats, plasma HDL concentrations are increased (139). As mentioned above, this appears to be associated with cholesterol mobilization from adipose tissue. Conversely, when plasma HDL and/or its apolipoproteins are decreased in concentration either by drug treatment (88) or by polyunsaturated fat feeding (76, 140), it has also been reported that the cholesterol content of adipose and other tissues increases (63, 67, 88, 141–143). If HDL is responsible or at least principally involved in reverse cholesterol transport (144), it is possible that there exists a cause and effect relationship between the cholesterol storage alterations in adipose tissue and changes in HDL or apolipoprotein A-I concentrations observed during starvation, caloric restriction, or drug/diet interventions. Indeed, based solely on kinetic analysis of specific activity time-curves in man, it has already been concluded that "HDL is the principle acceptor of adipocyte cholesterol" (145).

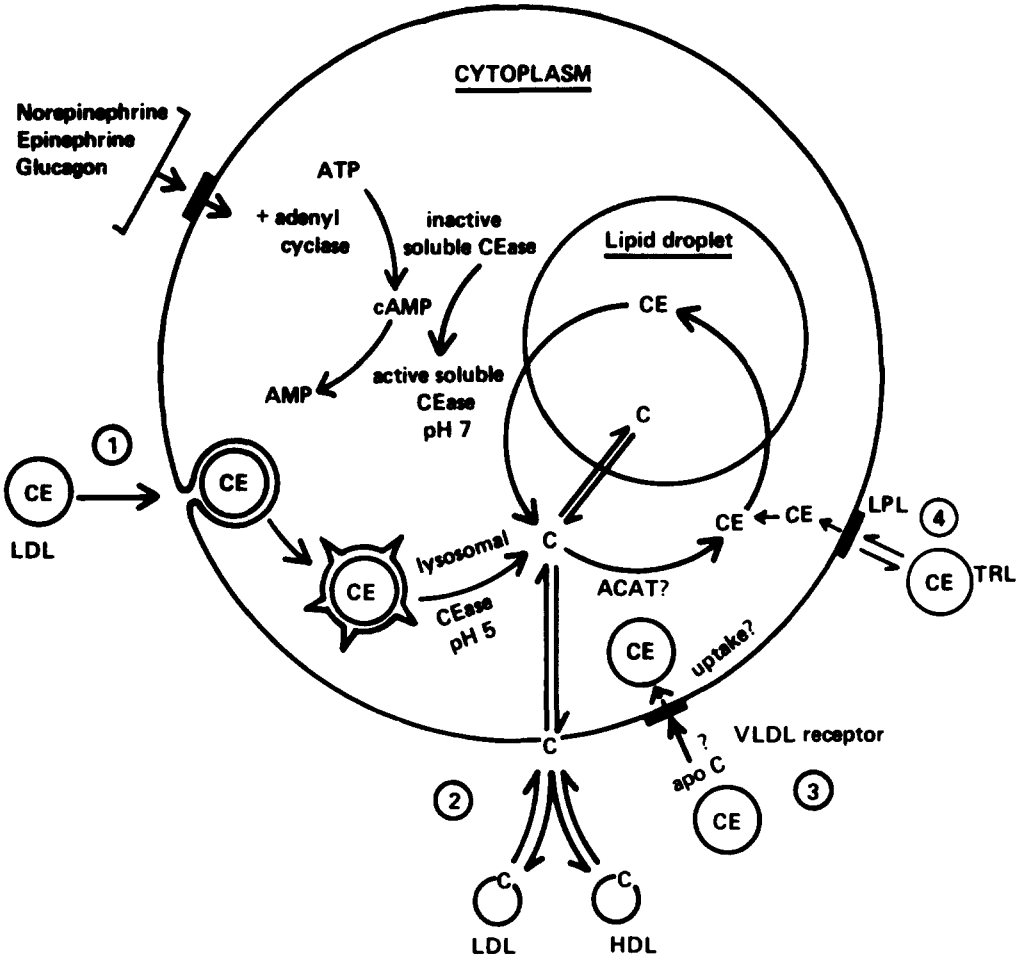
VI. RELATIONSHIP TO VASCULAR DISEASE

It has been stated that "major variations can exist . . . in the amount of cholesterol contained in the major pools of the body without having any necessary relationship to the level of cholesterol in the plasma" (3). Furthermore, it is now generally agreed that "serum cholesterol is not a suitable measure of the changes in cholesterol concentrations in the animal as a whole" (67). This knowledge combined with the recent evidence implicating HDL as "protective" against coronary heart disease (146–148) should center attention towards understanding the mechanisms by which cholesterol transport between plasma and tissues is regulated and related to disease states. Since adipose tissue and arterial tissue both appear to exhibit active sterol metabolism (receptors, LPL activity, ester hydrolase activity, etc.) and both tissues behave kinetically in identical fashion after pulse-labeling (i.e., both are members of the slowly-turning-over pool of cholesterol), it is possible that the further study of adipose tissue, which is easily biopsied, can lead to a clearer understanding of the atherosclerotic process. Furthermore, the efficacy of hypolipidemic drug therapy could be monitored using adipose tissue as a model of arterial metabolism. The determination of skin cholesterol content has been used to this advantage already in man and in rats (149). As more is learned about cellular lipid metabolism and the process of reverse cholesterol transport, the potential "buffering" capacity of adipose tissue in preventing hypercholesterolemia can be systematically explored. It may be that adipose tissue which is "the single major site for cholesterol storage in man" (127) can store increasing amounts of cholesterol which would not be detrimental but rather represent a process which could be exploited through drug or diet intervention. These and other questions remain to be solved in the future.

VI. CONCLUSIONS

The adipocyte can be viewed as a dynamic system in which interaction with lipoproteins may be at least partially coupled to the intracellular concentration of cAMP (**Fig. 1**). Although speculative at the present time, evidence derived from both human and animal studies supports the concept that cholesterol can gain access to the adipocyte by at least four distinct, but not necessarily unrelated, mechanisms: *1*) via the LDL receptor; *2*) via receptor-independent uptake of cholesterol (passive cholesterol exchange; *3*) via the VLDL receptor; and *4*) via hydrolysis of triglyceride-rich lipoproteins (TRL). Binding of both LDL and VLDL varies directly with the level of intracellular cAMP (126, 132). In the presence of insulin (decreased cAMP), cellular triglyceride stores expand and lipoprotein binding is diminished. In the presence of catecholamines (increased cAMP), cellular triglyceride is diminished and lipoprotein binding is enhanced. Thus, the expression of receptor binding appears to be linked functionally to the availability of cellular lipids by cAMP-dependent processes. Other mechanisms of cholesterol uptake include possible incorporation of free cholesterol into cell membranes by lateral diffusion during the hydrolysis of triglyceride-rich lipoproteins at the capillary endothelium (150), and uptake of free cholesterol from lipoproteins by exchange without net movement of sterol.

In the scheme depicted in Fig. 1, an enzyme activity designated as "cholesteryl ester hydrolase" (CEase) is proposed to be involved in both triglyceride and cholesteryl ester hydrolysis. At present, the physiologic importance of acid (lysosomal) esterase, which is dependent upon the thyroid status (115), and neutral (extra-lysosomal) esterase, which is cAMP-dependent (111–113), is unclear and the latter may represent the same enzyme protein as hormone-sensitive lipase. Regardless, the dual



Possible Pathways for Adipocyte Cholesterol Metabolism

1. LDL binding and uptake
2. Cholesterol exchange between plasma membrane and surface layer of LDL and HDL
3. Binding of VLDL to surface (apo C?) receptor; followed by uptake?
4. LPL associated uptake of Chylomicron (and VLDL?) cholesterol ester

Fig. 1. Cholesterol metabolism in adipose tissue.

specificity of this enzyme appears to be desirable in a system in which uptake of cholesteryl ester from both triglyceride-rich (VLDL) and cholesterol-rich (LDL) lipoproteins occurs via the same effector (cAMP) (132). Thus, binding and uptake of cholesteryl ester from these lipoproteins would be coupled with a mechanism for their intracellular hydrolysis. In cells with increased storage of cholesterol (e.g., cholesterol feeding), rates of ester hydrolysis may be exceeded due to the large increase of apoE- and apoB-containing lipoprotein particles in the plasma, and cholesteryl esters may accumulate. Furthermore, in such conditions decreases in plasma HDL may limit reverse cholesterol transport and result in adipose tissue cholesterol accumulation. In starvation, on the other hand, loss of cellular triglyceride (lipolysis) occurs despite attempts by the cell to bind and internalize more lipoprotein cholesterol, the latter preventing an appreciable change in stored cholesteryl esters but not triglyceride.

It has been demonstrated that adipocyte cholesterol can accumulate in the ester form without changes in cell

size (cellular triglyceride) (30). Thus, under some conditions, the uptake of cholesteryl esters may occur from these lipoprotein fractions independently of changes in either cAMP or lipolysis. It remains to be determined how and under what other circumstances this lack of coordinated activation occurs. With regard to the efflux of cellular cholesterol, very little is known. LDL may bind and act to remove rather than deliver cholesterol, depending possibly upon the apolipoprotein composition of the lipoprotein and ultimately, upon the species examined. Additionally, an HDL particle in the interstitium, possibly by virtue of its unique apoprotein content, may serve as a cholesterol acceptor as postulated using cultured fibroblast and smooth muscle cells (151–153) and in studies of dog (154) or human peripheral lymph (155). Further studies are required to assess the relative significance of these theoretical pathways in the hormonal regulation of adipocyte-lipoprotein interactions in health and disease.

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