Interaction of aging and dietary fat in the regulation of low density lipoprotein transport in the hamster

David K. Spady and John M. Dietschy

Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75235-9030

Abstract These studies were undertaken to examine the effect of aging on low density lipoprotein (LDL) metabolism in the male hamster. When the hamsters were maintained on a low-cholesterol, low-triglyceride diet, rates of LDL transport in the various tissues of the body and plasma LDL-cholesterol concentrations remained constant over the entire life span (1–24 months) of the hamster. In contrast, rates of de novo cholesterol synthesis fell 50–97% in the various tissues of the body during the transition from rapid body growth in the young animal to the stable adult size. Thus, changes in tissue requirements for cholesterol over the life span of these animals were met by an appropriate adjustment in the rate of de novo synthesis rather than by alterations in LDL transport. When animals were fed a diet enriched in cholesterol and saturated triglycerides, rates of LDL production increased, total body LDL receptor activity was suppressed, and plasma LDL-cholesterol levels rose. Older animals, however, were not more susceptible than young animals to the detrimental effects of these dietary fats. These studies support the view that aging per se has no effect on LDL transport by the liver or other tissues. Rather, the progressive rise in plasma LDL-cholesterol levels seen in Western man is likely due to the consumption of a diet enriched in cholesterol and saturated triglyceride which increases the LDL-cholesterol production rate and suppresses receptor-dependent LDL transport.—**Spady, D. K., and J. M. Dietschy.** Interaction of aging and dietary fat in the regulation of low density lipoprotein transport in the hamster. *J. Lipid Res.* 1989. **30:** 559–569.

Supplementary key words liver • small intestine • receptor-dependent transport • cholesterol • triglycerides • cholesteryl esters • saturated fat

In Western societies plasma total cholesterol levels typically rise from values of 50–100 mg/dl at birth to levels of 200–300 mg/dl by middle age (1–5). Most of this increase in plasma sterols is due to a rise in cholesterol carried in the low density lipoprotein (LDL) fraction (2). Since this elevated concentration of LDL in plasma has been identified as a major risk factor in the development of atherosclerosis and coronary heart disease, it is critically important to understand why plasma LDL levels increase with age, and how best to prevent this rise.

The concentration of LDL in plasma is a function of the rate at which LDL enters the vascular space, i.e., the LDL production rate, and the rate at which LDL is removed from the plasma by receptor-dependent and receptor-independent pathways in the various tissues of the body (6–8). Little direct evidence is available regarding the effect of aging per se on any of these parameters of LDL metabolism. Nevertheless, it is generally accepted that plasma LDL levels rise with aging primarily because of a gradual loss of LDL receptors. For example, in one recent study an analysis was carried out of LDL apoB metabolism in healthy subjects, aged 20 to 70 years, that had been reported in 29 publications (9). This analysis showed that the fractional catabolic rate (FCR) of LDL decreased as a function of increasing age, suggesting that a fall in LDL receptor activity was responsible for the rise in plasma LDL levels. In a second study, the FCR of LDL was also found to decrease with age but, in addition, a simultaneous increase in the rate of LDL production was noted (10).

Thus, there seems to be little doubt that aging is associated with an increase in the plasma concentration of LDL-cholesterol and, further, that this increase is correlated with a reduction in the fraction of plasma LDL that is degraded per unit time. Unfortunately, it is now recognized that the FCR will necessarily decrease in any situation where the plasma LDL-cholesterol concentration increases, even if LDL receptor activity is constant (7). For example, an increase in LDL production will raise the plasma LDL-cholesterol concentration and decrease the FCR even when receptor-dependent LDL transport remains constant or actually increases slightly (7). Hence, it is unclear from these data whether aging has any effect on whole-body receptor activity in vivo.

There are other studies carried out in vitro, however, that do suggest that LDL receptor activity may decline with aging. For example, it has been reported that the binding, uptake, and degradation of LDL decreases with aging in cultured human diploid fibroblasts (11). In addition, LDL binding to crude liver membranes seems to disappear with

Abbreviations: LDL, low density lipoprotein(s); FCR, fractional catabolic rate; d, density.

aging in dogs and swine (12). It has never been shown, however, that such binding of LDL to hepatic membranes actually reflects the rate of receptor-dependent LDL transport by this organ under in vivo conditions. Indeed, in these studies the LDL-cholesterol level was apparently not elevated in the older animals, as would have been anticipated if all LDL receptor activity in the liver had been lost. Thus, although there is no doubt that plasma LDL levels rise with aging in Western societies, it is not entirely certain whether this is due to a gradual loss of LDL receptor activity, to a progressive increase in the rate of LDL formation, or to some other unrecognized event.

The situation is further complicated by the fact that it is not even clear whether these observed changes in LDL metabolism are due to aging per se or are the result of some dietary or other environmental factor. Indeed, it is now recognized that plasma total cholesterol and LDL-cholesterol levels remain low throughout life in several species, including humans, when maintained on a low-cholesterol, low-triglyceride diet (13–16). On the other hand, dietary cholesterol and saturated triglycerides are known to raise plasma LDL-cholesterol levels in all species, including humans (17–20).

The present studies were designed to specifically investigate whether aging alone has any effect on receptor-dependent LDL transport or whether the observed changes in LDL metabolism resulted primarily from the intake of certain dietary lipids. These studies were made possible because of access to a colony of hamsters that had been maintained on carefully controlled diets essentially their entire life span. Furthermore, we have previously found that the male hamster closely resembles man with respect to rates of hepatic cholesterol synthesis (21–23), the relative importance of receptor-dependent and receptor-independent LDL transport (6, 24), and the response of the plasma LDL-cholesterol to dietary additions (20, 25). Hence, direct in vivo measurements were made of the rates of cholesterol synthesis and LDL transport in the major organs of groups of these animals at ages that varied from 1 to 24 months. Furthermore, these measurements were carried out in animals that had been maintained on either a low-fat diet or on diets containing small amounts of cholesterol and saturated triglycerides.

MATERIALS AND METHODS

Animals and diets

Most of the studies were performed in male Golden Syrian hamsters of the F1B strain that were obtained from BioBreeders (Watertown, MA). When purchased from BioBreeders, the animals ranged in age from 2 months to 22 months. Mid-gestational females were also obtained and the male offspring were weaned onto control diet at about 3 weeks of age. In this manner it was possible to study animals that ranged in age from 1 month to 24 months, a period of time that essentially equaled the natural life span of this species. At BioBreeders the animals were maintained in colony cages with free access to Purina Rodent Laboratory Chow (Richmond, IN) and drinking water. This diet contained only 0.02% cholesterol and 4.5% triglyceride (w/w). The fatty acid profile of this diet, determined by gas–liquid chromatography of the methyl esters, was 13% 16:0, 6% 18:0, 27% 18:1, 45% 18:2, and 5% 18:3. Upon arrival at our facility the animals were kept in colony cages, given free access to the same diet, and subjected to light cycling (dark from 3 AM to 3 PM and light from 3 PM to 3 AM). All experiments were carried out during the mid-dark phase of the light cycle. In some studies animals were fed Purina rodent chow that had been enriched with 0.06% cholesterol and 20% saturated triglyceride (w/w). This diet was prepared by dissolving the cholesterol in warm hydrogenated coconut oil and then blending the mixture into the ground chow with a commercial food mixer. The fatty acid profile of the hydrogenated coconut oil was 15% 8:0 and 10:0, 46% 12:0, 20% 14:0, 9% 16:0, and 8% 18:0. Some studies were performed on male hamsters of various ages that had been obtained from other suppliers including Harlan Sprague-Dawley (Indianapolis, IN) and Charles River Laboratories (Wilmington, MA).

Determination of cholesterol synthesis rates in vivo

As previously described (22, 26) animals were killed 1 hr after the intravenous administration of [^{3}H]water (approximately 100 mCi). Aliquots of plasma were taken for the determination of body water specific activity, and samples of the various organs were taken for the isolation of digitonin-precipitable sterols. In the case of the small intestine and the remaining carcass, the entire tissue was saponified and brought to an exact volume. Aliquots were then taken for the isolation of digitonin-precipitable sterols. Rates of sterol synthesis are expressed as the nmol of [^{3}H]water incorporated into digitonin-precipitable sterols per hr per g of tissue (nmol/hr per g) or per whole tissue (nmol/hr per organ).

Determination of tissue LDL uptake rates in vivo

The hamster LDL used in these studies was isolated from the plasma of normocholesterolemic donors by preparative ultracentrifugation in the density range of 1.020–1.055 g/ml and labeled with either [^{125}I]tyramine cellobiose (27) or ^{131}I (28). The hamster LDL in this density range contained almost exclusively apoprotein B-100 on polyacrylamide gels. Lipoproteins were used within 48 hr of preparation and were filtered through a 0.45-μm Millipore filter immediately prior to use. As previously described (24, 29) rates of tissue LDL uptake were determined using a primed-continuous infusion of [^{125}I]tyramine cellobiose-labeled LDL. The radioactivity present in the priming dose, relative to the

radioactivity infused each hour, was adjusted so as to maintain a constant specific activity of LDL in plasma over the experimental period. The infusions of [^{125}I]tyramine cellobiose-labeled LDL were continued for 4 hr, at which time each animal was administered a bolus of ^{131}I-labeled LDL and then killed 10 min later by exsanguination through the abdominal aorta. Samples of various organs were quickly rinsed and weighed, and were assayed for radioactivity in a gamma counter along with aliquots of plasma. The amount of labeled LDL in each organ at 10 min (^{131}I dpm per g of tissue divided by the specific activity of ^{131}I in the plasma) and at 4 hr (the ^{125}I dpm per g of tissue divided by the specific activity of ^{125}I in the plasma) was then calculated and has the units of μg of LDL-cholesterol per g. The increase in the tissue content of LDL-cholesterol with time represented the rate of LDL uptake and has the units of μg of LDL-cholesterol per hr per g of tissue (μg/hr per g) or per whole organ (μg/hr per organ) (29).

Cholesterol content of liver, plasma, and bile

The hepatic content of free and esterified cholesterol was determined using silicic acid/celite columns as previously described (30). Plasma LDL-cholesterol concentrations were determined by simultaneously centrifuging plasma at densities of 1.020 and 1.063 g/ml. The cholesterol content of the top one-third of each tube, as well as the total plasma cholesterol concentration, was assayed colorimetrically as previously described (30). The cholesterol, phospholipid, and bile acid content of hepatic bile was also determined (21).

Calculations

Plasma LDL-cholesterol levels were greatly elevated in animals fed cholesterol and saturated triglycerides. In order to relate changes in tissue LDL-cholesterol uptake in the organs of these animals to changes in LDL receptor activity, the experimentally determined uptake rates were superimposed on kinetic curves defining rates of LDL uptake in the various tissues of the body as a function of the plasma LDL-cholesterol concentration (C_1) in control hamsters. These curves were constructed using appropriate values for $*K_m$, $*J^m$, and *P, where $*K_m$ and $*J^m$ equal the apparent Michaelis constant and maximal transport velocity, respectively, for the receptor-dependent process and *P equals the apparent uptake constant for receptor-independent LDL transport (6, 20). Using these curves, LDL uptake rates measured at any LDL-cholesterol concentration could be converted directly to $*J^m$ values (receptor activity) and these values are presented as percentages of the appropriate control values.

In order to define why the plasma LDL-cholesterol concentration (C_1) changed with aging and dietary manipulations, the curves defining the relationship between C_1 and the level of maximal receptor-dependent LDL-cholesterol transport ($*J^m$) and the rate of LDL-cholesterol production (J_t) in the whole animal were constructed using the following equation (see Fig. 7) (8, 31):

$$C_1 = \frac{(J_t - *J^m - *P*K_m) + \sqrt{(J_t - *J^m - *P*K_m)^2 + 4*K_m J_t}}{2*P}$$

In these calculations the appropriate values in the equation were determined directly in groups of control hamsters (as described in reference 6). These values equaled $*J^m$ of 7,560 μg/hr per kg body weight; $*K_m$ of 100 mg/dl; J_t of 1,650 μg/hr per kg; and *P of 10.8 μg/hr per kg per mg/dl. In Fig. 7 these values defining whole body LDL receptor activity ($*J^m$) and LDL-cholesterol production (J_t) in the control group were set equal to 100%, and changes induced by aging or diet are shown relative to these control values.

Where appropriate, mean values ± 1 SE for groups of data are shown. Statistical differences in mean values were calculated using one-way analysis of variance followed by Dunnett's test for comparing multiple groups to a control group.

RESULTS

Studies were first undertaken to examine the effect of aging on lipoprotein metabolism and de novo cholesterol synthesis in the individual organs and in the whole animal. In these studies animals were maintained on commercial rodent diet which is essentially devoid of saturated triglycerides and cholesterol, both of which are known to alter LDL receptor activity and sterol synthesis in the liver (20, 25). **Fig. 1** shows body weights and plasma cholesterol levels in male hamsters ranging in age from 1 month to 2 years. As illustrated in panel A, body weight increased from 38 g at 1 month to 147 g at 6 months and then very gradually declined to 125 g at 24 months of age. As shown in the bottom panel, plasma total cholesterol decreased slightly from 90 mg/dl at 1 month to 75 mg/dl at 6 months and thereafter remained essentially constant throughout life. Plasma cholesterol carried in the density fraction 1.020–1.063 g/ml (LDL) remained constant at about 26 mg/dl throughout life. Cholesterol in the d > 1.063 g/ml and d < 1.020 g/ml fractions also was relatively constant throughout life.

Rates of LDL transport were quantitated in vivo in these same animals and these data, expressed as the μg of LDL-cholesterol taken up per hr per g, are shown in **Table 1**. At 1 month of age rates of LDL transport per g of tissue were highest in the liver (23 ± 2 μg/hr per g) and adrenal gland (19 ± 2 μg/hr per g) and the transport rates in these two tissues remained essentially constant throughout life. At 1 month of age the small intestine, spleen, kidney, and lung transported LDL at somewhat lower rates (3.1 to 9.6 μg/hr per g) and again these rates remained essentially cons-

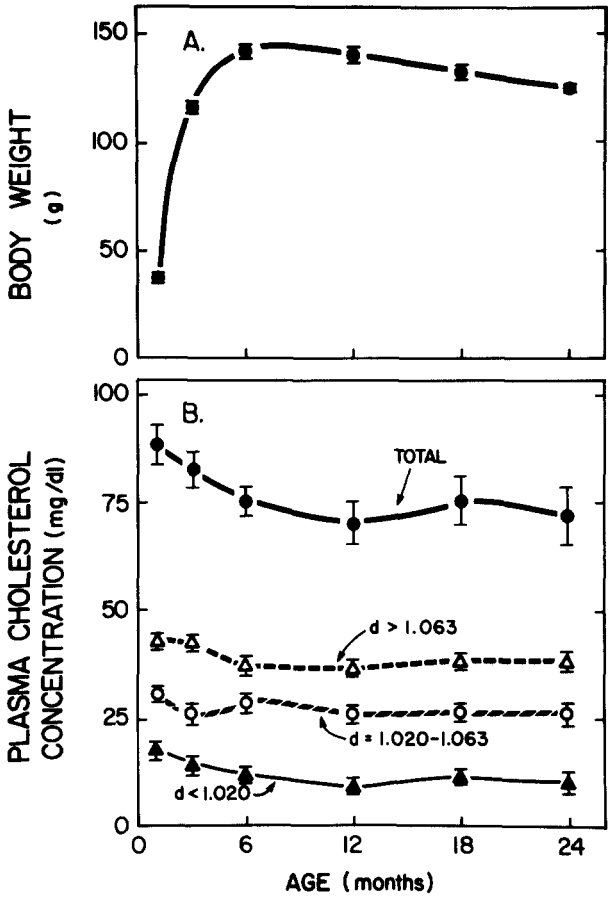

Fig. 1. Body weight and plasma cholesterol concentrations in hamsters of various ages. Animals were maintained throughout life on rodent diet. Body weight, total plasma cholesterol, and the distribution of plasma cholesterol among various density classes were determined at the different ages between 1 and 24 months. Each value represents the mean ± 1 SE for data obtained in six animals except for the 24-month-old animals where there were only four animals.

tant throughout life. Very low rates of LDL uptake were observed in the testes, skeletal muscle, adipose tissue, and brain (< 0.1 to 1.5 μg/hr per g) at 1 month. In the case of the testes, skeletal muscle, and adipose tissue, uptake rates were slightly higher at 1 month of age than at subsequent time points; however, transport rates in these tissues were so low as to be at the limits of detection by the method.

Rates of de novo cholesterol synthesis were quantitated in vivo in a second identically treated group of animals again ranging in age from 1 month to 24 months, and these data are shown in **Table 2**. The data are expressed as the nmol of [^{3}H]water incorporated in vivo into digitonin-precipitable sterols per hr per g of tissue. At 1 month, the highest rates of synthesis per g of tissue were seen in the adrenal gland (1,509 ± 275 nmol/hr per g) and liver (988 ± 174 nmol/hr per g). The brain and small intestine synthesized cholesterol at the somewhat lower rates of 367 ± 22 nmol/hr per g and 308 ± 17 nmol/hr per g, respectively, while synthesis rates of 27 to 148 nmol/hr per g were seen in the lungs, kidney, testes, skeletal muscle, adipose tissue, and remaining carcass. However, in contrast to the rates of LDL transport, which remained essentially constant throughout life, rates of sterol synthesis were 2- to 36-fold higher in the tissues of the young, rapidly growing animals compared to the adult animals of more constant size. Only in the small intestine, where rapid cell turnover continues into adult life, did rates of sterol synthesis continue essentially unchanged throughout the 2 years.

We have previously found that approximately 22 hydrogen atoms of the cholesterol molecule are derived from water during biosynthesis from acetyl-CoA (22, 32). Thus, these rates of [^{3}H]water incorporation into cholesterol could be converted to absolute rates of cholesterol synthesis (μg of cholesterol formed per hr) and compared directly to the

TABLE 1. Rates of LDL uptake per g of tissue in hamsters of various ages

Tissue	Age (Months)					
	1	3	6	12	18	24
	μg/hr per g					
Liver	23 ± 2	24 ± 2	24 ± 2	22 ± 1	21 ± 2	25 ± 3
Small intestine	9.6 ± 1	8.6 ± 1	7.8 ± 1	8.3 ± 1	6.2 ± 1	7.0 ± 1
Adrenal gland	19 ± 2	17 ± 1	18 ± 1	12 ± 1[a]	16 ± 2	14 ± 3
Testes	1.5 ± 0.2	0.4 ± 0.1[a]	0.5 ± 0.1[a]	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.4 ± 0.1[a]
Spleen	8.7 ± 1	9.1 ± 1	8.1 ± 1	7.8 ± 1	7.6 ± 1	8.9 ± 1
Kidney	3.1 ± 0.3	3.1 ± 0.3	2.8 ± 0.4	3.1 ± 0.3	3.2 ± 0.2	3.0 ± 0.5
Lung	6.5 ± 0.7	5.7 ± 0.8	5.3 ± 0.5	3.9 ± 0.4[a]	4.1 ± 0.3[a]	4.6 ± 0.4
Skeletal muscle	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Adipose tissue	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Brain	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1	< 0.4

Male hamsters were maintained throughout life on rodent diet. Rates of homologous LDL uptake were determined in vivo using a primed-continuous infusion of [^{125}I]tyramine cellobiose-labeled LDL as described in Materials and Methods. The data are expressed as the μg of LDL-cholesterol taken up per hr per g of tissue. Each value represents the mean ± 1 SE for data obtained in six animals except for the 24 month-old-animals where there were only four animals.

[a]Significantly different from the corresponding value for 1-month-old animals at the $P < 0.05$ level.

TABLE 2. Rates of cholesterol synthesis per g of tissue in hamsters of various ages

Tissue	Age (Months)					
	1	3	6	12	18	24
	nmol/hr per g					
Liver	988 ± 174	121 ± 13	87 ± 18	115 ± 44	39 ± 5	238 ± 71
Small intestine	308 ± 17	234 ± 17	288 ± 26	304 ± 30	269 ± 22	300 ± 31
Adrenal gland	1509 ± 275	761 ± 27	532 ± 43	407 ± 20	325 ± 66	396 ± 70
Testes	106 ± 16	32 ± 2	32 ± 3	28 ± 2	29 ± 1	33 ± 3
Spleen	66 ± 14	22 ± 6	24 ± 3	29 ± 4	16 ± 2	28 ± 3
Kidney	125 ± 8	18 ± 2	20 ± 3	18 ± 3	14 ± 1	27 ± 1
Lung	148 ± 23	42 ± 13	34 ± 4	36 ± 7	31 ± 2	28 ± 3
Skeletal muscle	32 ± 6	0.9 ± 0.1	0.8 ± 0.1	1.2 ± 0.4	0.7 ± 0.1	1.2 ± 0.3
Adipose tissue	27 ± 5	1.3 ± 0.3	1.0 ± 0.1	0.8 ± 0.3	0.4 ± 0.2	1.3 ± 0.3
Brain	367 ± 22	45 ± 2	30 ± 3	23 ± 1	20 ± 2	22 ± 2
Carcass	94 ± 6	36 ± 1	31 ± 2	24 ± 2	22 ± 2	22 ± 1

Male hamsters were maintained throughout life on rodent diet. Rates of sterol synthesis were determined in vivo using [^{3}H]water as described in Materials and Methods. The data are expressed as the nmol of [^{3}H]water incorporated into cholesterol per hr per g of tissue. Each value represents the mean ± 1 SE for data obtained in six animals except for the 24-month-old animals where there were only four animals. All values in the 3-24-month-old animals were significantly lower than the corresponding values in the 1-month-old animals at the $P < 0.05$ level except for the small intestine where the values in the 3-24-month-old animals did not differ significantly from the 1-month value.

mass of cholesterol derived from LDL uptake in each tissue. **Fig. 2** shows the rate of cholesterol acquisition, in μg of cholesterol per hr per g tissue weight, from de novo synthesis and LDL uptake in the liver, small intestine, and remaining carcass as a function of age. As shown in panel A, at 1 month of age the liver acquired more cholesterol from de novo synthesis (34 μg/hr per g) than from LDL uptake (23 μg/hr per g). Thereafter, hepatic LDL uptake remained constant while de novo cholesterol synthesis fell dramatically as the animals reached constant adult size so that, for the remaining 2 years, the liver derived only about 15% as much cholesterol from local synthesis as from LDL uptake. As shown in panel B, in the small intestine about equal amounts of cholesterol were derived from de novo synthesis and LDL uptake at all ages. Rates of cholesterol acquisition were very low in the remaining carcass (all tissues taken together except liver and small intestine) and most of this cholesterol was provided by de novo synthesis (panel C). It should be pointed out that cholesterol acquisition per g of tissue was very high in some tissues of the carcass such as the endocrine organs but, due to the small size of these organs, they contributed little to total body cholesterol synthesis or LDL turnover.

The contribution of de novo synthesis and LDL uptake to whole organ and whole body cholesterol balance throughout life is shown in **Fig. 3**. Rates of cholesterol synthesis and LDL uptake for the liver, small intestine, remaining carcass, and whole body (all normalized to 100 g body weight) are presented as a function of age. Again, rates of cholesterol synthesis fell markedly as body weight plateaued, especially in the liver and carcass. When normalized to 100 g body weight, LDL uptake rates also declined with aging in the liver, small intestine, and whole animal. These reductions, however, were due entirely to the fact that the relative weights of the liver and small intestine, the two most important sites for LDL catabolism, fell by about 50% between 1 month and 6 months of age. Since in the steady-state, the sum of these LDL-cholesterol uptake rates for the various tissues of the body (total body LDL-cholesterol uptake) equals the rate of LDL production, it is apparent that the rate of LDL-cholesterol production per 100 g body weight also decreased with aging. This figure also illustrates that the liver of the adult hamster accounts for 70-80% of total body LDL turnover, but for only about 10% of total body cholesterol synthesis.

The content of cholesterol in the liver and of cholesterol, phospholipid, and bile acid in gallbladder bile were also measured in animals used for the determination of LDL transport. As illustrated in **Fig. 4**, hepatic free cholesterol was unchanged with aging whereas the hepatic cholesteryl ester content increased slightly in animals 12 months of age and older (panel A). The molar percent of cholesterol in gallbladder bile remained essentially constant throughout life (panel B).

Since aging had no effect on LDL transport as long as the animals were raised on a diet essentially devoid of cholesterol and saturated fat, the next group of studies was undertaken to see whether old animals were more susceptible than young animals to the hypercholesterolemic effects of these two dietary lipids. As shown in **Fig. 5**, groups of animals ranging in age from 2 to 23 months were fed the standard rodent diet enriched with 0.06% cholesterol and 20% saturated triglyceride (hydrogenated coconut oil). After 1 month on these diets plasma LDL-cholesterol levels,

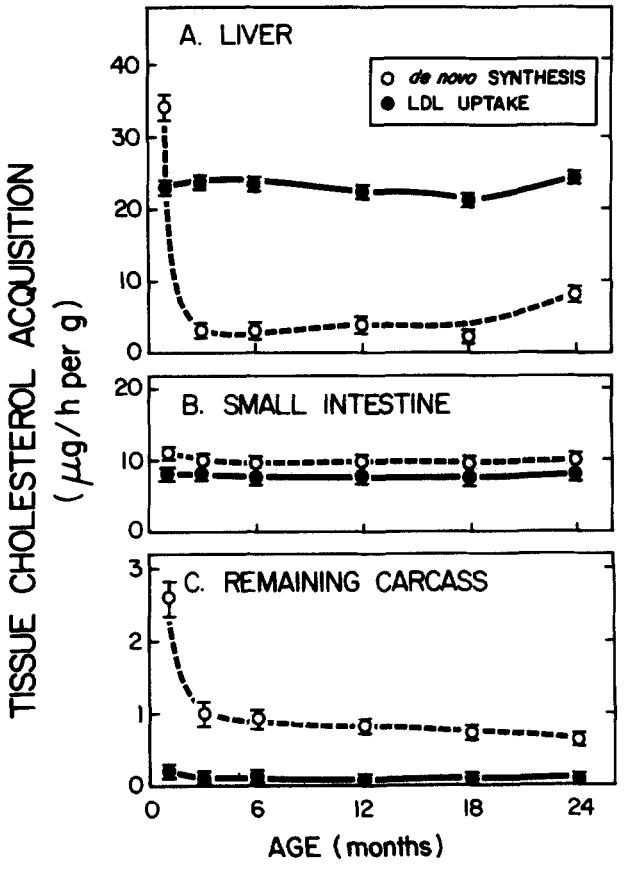

Fig. 2. Rates of tissue cholesterol acquisition from de novo synthesis and LDL uptake per g of tissue in hamsters of various ages. Animals were maintained throughout life on rodent diet, and rates of cholesterol synthesis and LDL transport were measured at various ages as described in Tables 2 and 3. The values for cholesterol synthesis (μg/h per g) were calculated from the absolute rates of [^{3}H]water incorporation into cholesterol assuming that 22 μg atoms of H were derived from body water during the biosynthesis of each μmol of cholesterol. The data in panel C includes all tissues other than the liver and small intestine. Each value represents the mean ± 1 SE for data obtained in six animals except for the 24-month-old animals where there were only four animals.

rates of LDL-cholesterol production, and rates of LDL catabolism were quantitated in vivo. As shown in panel A, plasma LDL-cholesterol levels in control animals remained constant at around 26 mg/dl throughout life. In contrast, plasma LDL-cholesterol levels equaled about 120 mg/dl in the 3-month-old animals that had been fed the cholesterol and saturated triglyceride diet for 1 month. The diet-induced rise in LDL-cholesterol levels was somewhat less in the 6- to 18-month-old animals (LDL-cholesterol concentrations of about 75 mg/dl) and even less in the 24-month-old animals (LDL-cholesterol concentration of about 70 mg/dl). As a corollary, suppression of total body LDL receptor activity (panel B) and stimulation of LDL-cholesterol production rates (panel C) were also greatest at 3 months of age.

Out-bred male Golden Syrian hamsters also were obtained from two other suppliers and maintained on rodent diet and drinking water ad libitum for varying periods of time up to 18 months of age. Data on hamsters obtained from Harlan Sprague-Dawley are summarized in **Fig. 6**. Again in these animals, as in the animals obtained from BioBreeders, plasma LDL-cholesterol concentrations and rates of LDL transport in the liver and other tissues remained constant between 3 months and 18 months of age. Groups of these animals were also weaned onto rodent diet enriched with 0.06% cholesterol and 20% hydrogenated coconut oil, and were maintained continuously on these diets for varying periods of time up to 18 months of age. As seen in the above studies, where animals of various ages were challenged with dietary cholesterol and saturated fat for 1 month, the highest level of plasma LDL-cholesterol and the greatest suppression of hepatic LDL transport were found in animals 2 to 4 months of age. Thus, whether challenged for only 1 month or maintained continuously on the

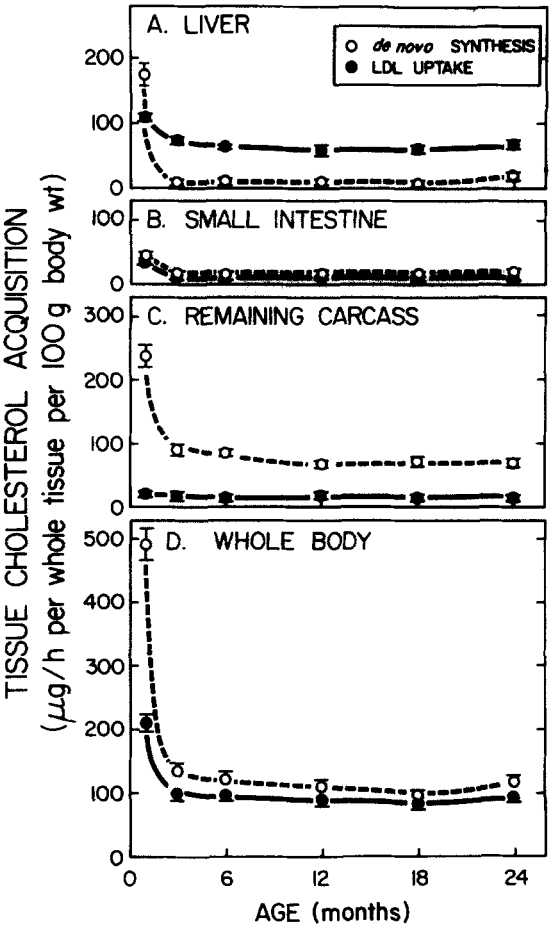

Fig. 3. Rates of tissue cholesterol acquisition from de novo synthesis and LDL uptake per whole organ and per whole body in hamsters of various ages. The values for cholesterol synthesis were calculated as described for Fig. 2 and were then multiplied by whole organ weights that had been normalized to 100 g of body weight. The data in panel C represent all tissues taken together except for the liver and small intestine. Each point represents the mean ± 1 SE for data obtained in six animals except for the 24-month-old animals where there were only four animals.

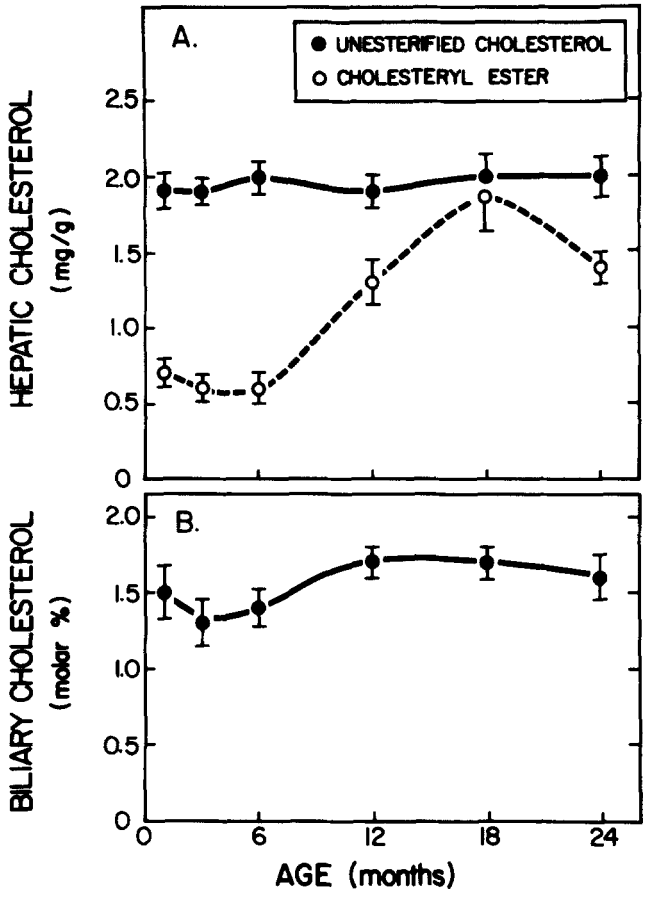

Fig. 4. The effect of age on hepatic and biliary cholesterol levels. Samples of liver and gallbladder bile were obtained from the animals used for the determination of LDL transport rates. Each point represents the mean ± 1 SE for data obtained in six animals except for the 24-month-old animals where there were only four animals.

high fat diet, the younger animals responded more dramatically to the dietary challenge with a more marked increase in LDL-cholesterol production rates and greater suppression of LDL receptor activity. The reason plasma LDL levels were highest at 2 to 4 months of age is probably due to the greatly increased food consumption (and thus cholesterol intake) per kg body weight in these rapidly growing animals. Indeed, body weight nearly quadrupled between 6 weeks and 6 months of age. Thereafter, body weight, as well as plasma LDL levels, remained relatively constant throughout the remainder of adult life (Figs. 1 and 5). In fact, plasma LDL levels might have continued to rise throughout adult life in animals fed the high fat diet if food consumption could have been increased to produce a progressive weight gain (as occurs in Western man).

DISCUSSION

These studies demonstrated that the rates of LDL uptake and catabolism in the various organs of the body and the plasma LDL-cholesterol concentration remained essentially constant over the entire life span of the male hamster when these animals were maintained on a low-cholesterol, low-triglyceride diet. In contrast, a diet enriched with saturated triglyceride and a small amount of cholesterol dramatically increased plasma LDL-cholesterol levels both by increasing the rate of LDL-cholesterol formation and by suppressing whole-animal receptor activity. Furthermore, when challenged with a diet enriched in cholesterol and saturated triglyceride for 1 month, older animals were no more susceptible to the detrimental effect of these lipids on LDL metabolism than were younger animals. In fact, when animals were weaned onto the diet containing cholesterol and saturated triglyceride and maintained on this diet for life, plasma LDL-cholesterol levels increased to about 125 mg/dl at 3–4 months of age and then gradually declined to about 80 mg/dl over the subsequent 12 to 15 months. Thus, in this model, aging per se had no adverse effect on LDL metabolism nor were aged animals more sensitive to

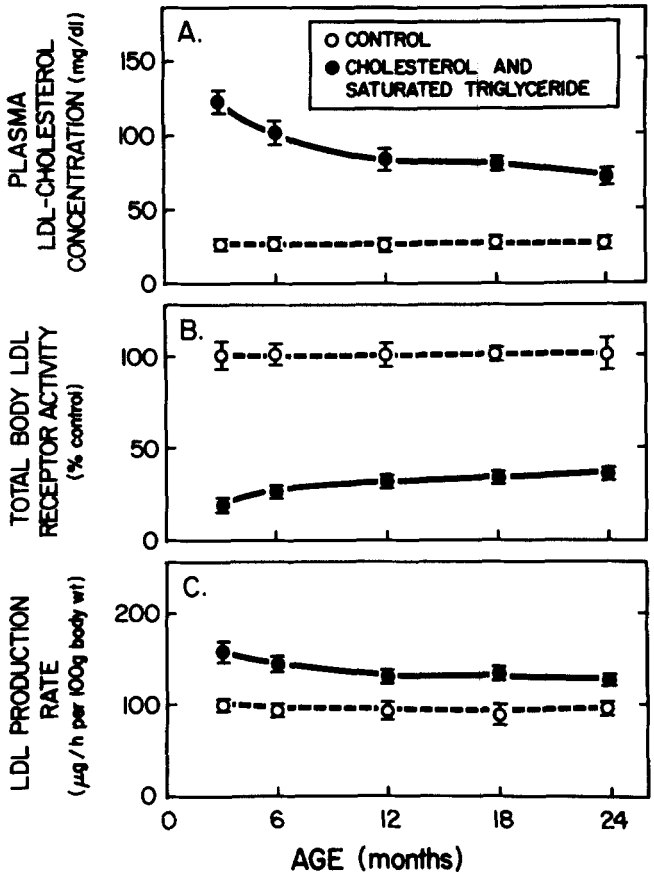

Fig. 5. Effect on LDL metabolism of challenging hamsters of various ages with dietary cholesterol and triglyceride for 1 month. Animals ranging in age from 2 to 23 months were fed rodent diet enriched with 0.06% cholesterol and 20% hydrogenated coconut oil for 1 month at which time plasma LDL-cholesterol concentrations, total body receptor-dependent LDL transport, and LDL production rates were quantitated in vivo. Each point represents the mean ± 1 SE for data obtained in six animals except for the 24-month-old animals where there were only four animals.

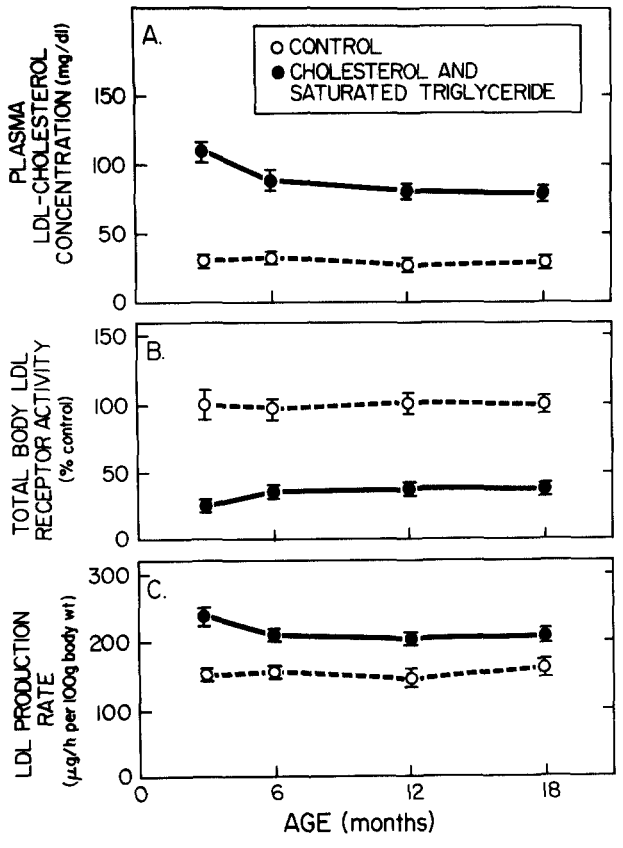

Fig. 6. Effect on LDL metabolism of raising hamsters on diets enriched with cholesterol and saturated triglyceride. In these studies male weanling hamsters were continuously fed rodent diet enriched with 0.06% cholesterol and 20% hydrogenated coconut oil for varying periods of time up to 18 months of age. Plasma LDL-cholesterol concentrations, total body receptor-dependent LDL transport, and LDL production rates were then determined as in Fig. 5. Each point represents the mean ± 1 SE for data obtained in six animals.

the detrimental effects of dietary cholesterol and saturated triglyceride.

In considering the effects of aging on whole-animal LDL metabolism it is important to take into account the age-related changes in relative organ weights. Thus, although rates of LDL transport per g of tissue remained constant throughout life in all organs (Table 1), the relative weights of the liver and small intestine, the two most important sites of receptor-dependent LDL catabolism, decreased by nearly 50% during the transition from rapid body growth in the young animal (1 month of age) to constant adult size (6 months of age). As a result, whole-animal LDL uptake per 100 g animal declined during this time period (Fig. 3); however, because of a corresponding decrease in the rate of LDL production per 100 g animal, plasma LDL-cholesterol concentrations remained constant. During adult life, relative organ size, whole-animal LDL uptake, and LDL production all remained essentially constant.

These results are similar to those reported in other species. For example, it was found that plasma LDL-cholesterol levels were essentially constant throughout life in monkeys maintained on low-cholesterol, low-triglyceride diets (13, 33). Similarly, the plasma LDL-cholesterol concentration was found to be identical in immature and adult dogs (12). On the other hand, several investigators have noted a definite increase in plasma LDL-cholesterol levels with aging in the rat, even when these animals were fed a low-lipid diet (34–36). The rat, however, is unique in that body weight continues to increase throughout life in most strains causing a significant decrease in the liver-to-body weight ratio. However, under conditions where body weight is constant, plasma cholesterol levels remain relatively constant, even in the rat (37, 38).

Unfortunately, in most studies of this type it has been difficult to compare the effect of aging on the plasma LDL-cholesterol concentration among different animal species either because the animal or human subjects were on diets containing different levels of lipids or because insufficient data were obtained to determine what specific event in LDL metabolism had been altered. Furthermore, only very recently has it become possible to measure the four major parameters of LDL metabolism that dictate steady-state LDL-cholesterol concentrations, and to understand the quantitative interaction of these parameters (7, 8).

Clearly the two most powerful determinants of circulating levels of plasma LDL-cholesterol are the rate of entry of LDL-cholesterol into the plasma space, i.e., the LDL production rate (J_t), and the level of whole-animal receptor-dependent transport capacity ($^*J^m$) for removing LDL-cholesterol from the plasma. The effect of varying each of these parameters independently on the plasma LDL-cholesterol concentration can be easily calculated and the results of such calculations in the hamster are illustrated by the solid lines in **Fig. 7**. For example, if receptor activity was progressively lost but the production rate was kept constant at 100%, then the plasma LDL-cholesterol concentration would increase along the lower curve until it reached approximately 160 mg/dl when there was no receptor-dependent LDL transport remaining. Conversely, if receptor activity was kept constant at 100% but the production rate was increased, the plasma LDL-cholesterol concentration would move directly upward in this graphic presentation.

Superimposed on these standard kinetic curves are the data from the present studies. As is apparent, the group of hamsters that had been maintained throughout their adult life on the low-fat, rodent diet manifested no change in any of these three parameters. However, when the animals were placed on the high-fat diet containing both cholesterol and saturated triglyceride, there was an increase in the production rate and a loss of receptor activity so that the plasma LDL-cholesterol concentration rose into the range of 75–120 mg/dl. The high fat diet used in these studies (rodent chow supplemented with 0.06% cholesterol and 20% hydrogenated coconut oil) was selected to study a specific problem, i.e., the effect of aging on sensitivity to the two

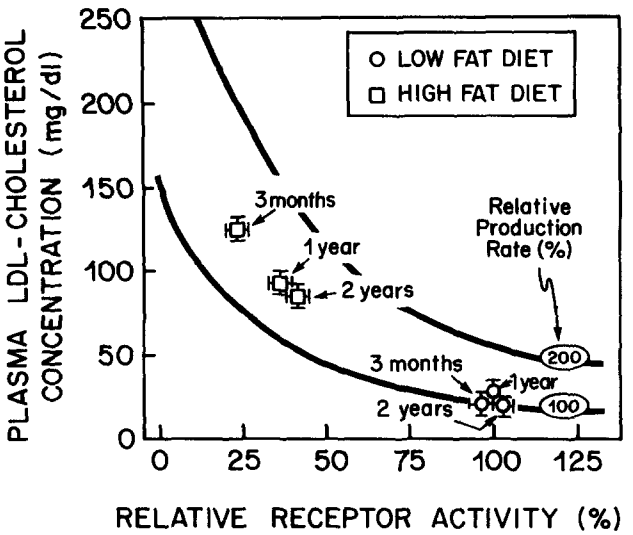

Fig. 7. The effect of aging on the plasma LDL-cholesterol concentration in the hamster. This diagram illustrates how the plasma cholesterol concentration changes under circumstances where there has been an alteration in either receptor-dependent LDL-cholesterol transport out of the plasma (horizontal axis) or a change in the rate of LDL-cholesterol production. The two solid curves show the manner in which the plasma LDL-cholesterol concentration would be expected to change if there were an alteration in total body receptor-dependent transport activity ($*J^m$) under circumstances where the production rate (J_i) was either normal or twice normal. In this diagram the normal values for $*J^m$ (7,560 μg/hr per kg) and J_i (1,650 μg/hr per kg) have been set at 100% of relative activity. The experimental values found in the animal groups of different ages are superimposed on these curves.

lipid components of the diet most active in elevating plasma LDL levels (cholesterol and saturated fatty acids). This diet, of course, is not completely analogous to Western diets in that Western diets contain a higher proportion of unsaturated fatty acids. Both saturated and unsaturated fatty acids appear to influence LDL metabolism independently in the hamster. For example, when cholesterol is present in the diet, the further addition of unsaturated fatty acids tends to increase LDL receptor activity while saturated fatty acids suppress receptor activity (25). On the other hand, both types of fatty acids increase the rate of LDL production. Thus, compared to the high fat diet used in these studies, a diet containing equal amounts of unsaturated and saturated fatty acids would produce a smaller rise in circulating LDL levels because of slightly less suppression of whole-body receptor activity.

The situation may be similar in humans. Although pooled data from human LDL turnover studies show that the FCR for LDL falls with aging, it is now apparent that this is largely the result of overproduction of LDL with saturation (and not down-regulation) of LDL receptor activity (7, 39–41). Since these human subjects were presumably on the high-fat diets typical of Western man, and since such dietary lipids are known to increase LDL-cholesterol production rates, even this increase in the entry of LDL-cholesterol into the plasma pool is probably diet-induced and not a manifestation of aging. It should be noted that when human subjects, such as the Tarahumara Indians of Mexico, live out their lives on a diet that is very low in both cholesterol (71–75 mg/day) and saturated triglyceride (2% of total calories), the mean plasma cholesterol level remains low and apparently does not signficantly increase with aging (14), as is true for the hamster fed a similar low-fat diet.

Thus, based on these many indirect studies in humans (39–41), monkeys (33), dogs (12), and some species of rat (37, 38), and on the direct measurements of LDL transport carried out in the rat (42) and in the hamster in the present study, it appears very likely that there is no direct effect of aging per se on receptor-dependent LDL transport in any species, including humans. Rather, the progressive increase in LDL-cholesterol levels that is seen is almost certainly the consequence of diet-induced changes in both LDL-cholesterol production rates and whole-body LDL receptor activity.

Finally, these studies also provide additional quantitative information on the importance of de novo cholesterol synthesis and LDL-cholesterol uptake as sources for tissue cholesterol. As anticipated from previous studies in the hamster and other species (23, 24, 29, 43), essentially 70–80% of plasma LDL-cholesterol is taken up by the liver. Hence, the sterol needs of the extrahepatic tissues of the body must be met almost entirely through local cholesterol synthesis (Fig. 3, panel C). All cells have an ongoing requirement for cholesterol that is used for the synthesis of membranes and, in some cases, steroid products. Cholesterol requirements for membrane synthesis are particularly high during periods of rapid growth. For example, the fetus of the rat synthesizes cholesterol at a rate that is 5-fold greater than the adult animal when these rates are expressed per kg of body weight (44). There are similar high rates of synthesis during the period of rapid growth after birth, as shown in the present studies, where the body weight of the hamster increased from 38 g at 1 month of age to 147 g at 6 months of age. This represents a net increase in total body cholesterol of approximately 200 mg, assuming that the average cholesterol content of tissues is 1.5–2 mg/g. Even in the absence of dietary cholesterol, this increment in total body cholesterol could easily be accounted for by the fact that the rate of total body cholesterol synthesis (per 100 g animal) was 4-fold higher at 1 month of age than at 6 months of age when body weight had plateaued (Fig. 3). This increased rate of sterol synthesis in the rapidly growing, young animal was even greater in specific tissues such as the liver (11-fold), brain (12-fold), fat (27-fold), and skeletal muscle (35-fold). In contrast, rates of sterol synthesis remained high throughout life in organs such as the small intestine, where rapid cell turnover occurs continuously, and in the endocrine organs, which require cholesterol for steroid hormone synthesis. Thus, changing tissue requirements for cholesterol over the life span of these animals were met entirely by appropriate

changes in the rates of local cholesterol synthesis while the rates of LDL-cholesterol uptake remained essentially constant.

This work was supported by U.S. Public Health Service research grants HL-09610, AM-19329, and AM-01221, and a grant-in-aid from the American Heart Association, and by a grant from the Moss Heart Fund. The authors wish to thank Janet Hainline and Mark Ventura for their excellent technical assistance and Imogene Robison for preparing this manuscript.

Manuscript received 8 July 1988 and in revised form 26 September 1988.

REFERENCES

1. Brody, S., and L. A. Carlson. 1962. Plasma lipid concentrations in the newborn with special reference to the distribution of the different lipid fractions. *Clin. Chim. Acta.* **7:** 694–699.
2. Abbott, R. D., R. J. Garrison, P. W. F. Wilson, F. H. Epstein, W. P. Castelli, M. Feinleib, and C. LaRue. 1983. Joint distribution of lipoprotein cholesterol classes. The Framingham Study. *Arteriosclerosis.* **3:** 260–272.
3. Kritchevsky, D. 1979. Diet, lipid metabolism, and aging. *Fed. Proc.* **38:** 2001–2006.
4. Lewis, L. A., F. Olmsted, I. H. Page, E. Y. Lawry, G. V. Mann, F. J. Stare, M. Hanig, M. A. Lauffer, T. Gordon, and F. E. Moore. 1957. Serum lipid levels in normal persons. Findings of a Cooperative Study of Lipoproteins and Atherosclerosis. *Circulation.* **XVI:** 227–245.
5. Carlson, L. A. 1960. Serum lipids in normal men. *Acta Med. Scand.* **167:** 377–397.
6. Spady, D. K., J. B. Meddings, and J. M. Dietschy. 1986. Kinetic constants for receptor-dependent and receptor-independent low density lipoprotein transport in the tissues of the rat and hamster. *J. Clin. Invest.* **77:** 1474–1481.
7. Meddings, J. B., and J. M. Dietschy. 1986. Regulation of plasma levels of low-density lipoprotein cholesterol: interpretation of data on low-density lipoprotein turnover in man. *Circulation.* **74:** 805–814.
8. Meddings, J. B., and J. M. Dietschy. 1987. Regulation of plasma low density lipoprotein levels: new strategies for drug design. *In* Progress in Clinical Biochemistry and Medicine. Volume 5. E. Baulieu et al., editors. Springer-Verlag, Berlin, West Germany. 3–24.
9. Miller, N. E. 1984. Why does plasma low density lipoprotein concentration in adults increase with age? *Lancet.* February **4:** 263–267.
10. Grundy, S. M., G. L. Vega, and D. W. Bilheimer. 1985. Kinetic mechanisms determining variability in low density lipoprotein levels and rise with age. *Arteriosclerosis.* **5:** 623–630.
11. Lee, H-C., M. A. Paz, and P. M. Gallop. 1982. Low density lipoprotein receptor binding in aging human diploid fibroblasts in culture. *J. Biol. Chem.* **15:** 8912–8918.
12. Mahley, R. W., D. Y. Hui, T. L. Innerarity, and K. H. Weisgraber. 1981. Two independent lipoprotein receptors on hepatic membranes of dog, swine, and man. *J. Clin. Invest.* **68:** 1197–1206.
13. Willcox, K. K., K. Y. Lei, and D. D. Williams. 1986. Aging and dietary influences on plasma lipoproteins and apolipoproteins and nonhuman primates. *Metabolism.* **35:** 1062–1069.
14. Connor, W. E., M. T. Cerqueira, R. W. Connor, R. B. Wallace, M. R. Malinow, and H. R. Casdorph. 1978. The plasma lipids, lipoproteins, and diet of the Tarahumara Indians of Mexico. *Am. J. Clin. Nutr.* **31:** 1131–1142.
15. Mendez, J., C. Tejada, and M. Flores. 1962. Serum lipid levels among rural Guatemalan Indians. *Am. J. Clin. Nutr.* **10:** 403–409.
16. Sinnett, P. F., and H. M. Whyte. 1973. Epidemiological studies in a total highland population, Tukisenta, New Guinea. Cardiovascular disease and relevant clinical, electrocardiographic, radiological and biochemical findings. *J. Chron. Dis.* **26:** 265–290.
17. Kinsell, L. W., J. Partridge, L. Boiling, S. Margen, and G. Michaels. 1952. Dietary modification of serum cholesterol anb phospholipid levels. *J. Clin. Endocrinol.* **12:** 909–913.
18. Keys, A., J. T. Anderson, and F. Grande. 1957. Serum-cholesterol response to dietary fat. *Lancet.* April **13:** 787.
19. Ahrens, E. H., W. Insull, R. Blomstrand, J. Hirsch, T. T. Tsaltas, and M. L. Peterson. 1957. The influence of dietary fats on serum-lipid levels in man. *Lancet.* May 11: 943–953.
20. Spady, D. K., and J. M. Dietschy. 1985. Dietary saturated triacylglycerols suppress hepatic low density lipoprotein receptor activity in the hamster. *Proc. Natl. Acad. Sci. USA.* **82:** 4526–4530.
21. Turley, S. D., D. K. Spady, and J. M. Dietschy. 1983. Alteration of the degree of biliary cholesterol saturation in the hamster and rat by manipulation of the pools of preformed and newly synthesized cholesterol. *Gastroenterology.* **84:** 253–264.
22. Spady, D. K., and J. M. Dietschy. 1983. Sterol synthesis in vivo in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. *J. Lipid Res.* **24:** 303–315.
23. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Rates of low density lipoprotein uptake and cholesterol synthesis are regulated independently in the liver. *J. Lipid Res.* **26:** 465–472.
24. Spady, D. K., D. W. Bilheimer, and J. M. Dietschy. 1983. Rates of receptor-dependent and -independent low density lipoprotein uptake in the hamster. *Proc. Natl. Acad. Sci. USA.* **80:** 3499–3503.
25. Spady, D. K., and J. M. Dietschy. 1988. Interaction of dietary cholesterol and triglycerides in the regulation of hepatic low density lipoprotein transport in the hamster. *J. Clin. Invest.* **81:** 300–309.
26. Jeske, D. J., and J. M. Dietschy. 1980. Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using [^{3}H]water. *J. Lipid Res.* **21:** 364–376.
27. Glass, C. K., R. C. Pittman, G. A. Keller, and D. Steinberg. 1983. Tissue sites of degradation of apoprotein A-I in the rat. *J. Biol. Chem.* **344:** 7161–7167.
28. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
29. Spady, D. K., S. D. Turley, and J. M. Dietschy. 1985. Receptor-independent low density lipoprotein transport in the rat in vivo. Quantitation, characterization, and metabolic consequences. *J. Clin. Invest.* **76:** 1113–1122.
30. Anderson, J. M., and J. M. Dietschy. 1978. Relative importance of high and low density lipoproteins in the regulation of cholesterol synthesis in the adrenal gland, ovary, and testis of the rat. *J. Biol. Chem.* **253:** 9024–9032.
31. Meddings, J. B., and J. M. Dietschy. 1987. Low density lipoproteins and atherogenesis: implications for modification through alterations in diet and new drug designs. *In* Contributions of Chemistry to Health. Vol. 2. H. Machleidt, editor. VCH Verlagsgesellschaft mbH, Frankfurt, West Germany. 269–282.

32. Dietschy, J. M., and D. K. Spady. 1984. Measurement of rates of cholesterol synthesis using tritiated water. *J. Lipid Res.* **25:** 1469–1476.
33. Lacko, A. G., and W. R. Hazzard. 1979. Plasma lipid, lipoproteins and lecithin:cholesterol acyltransferase (LCAT). *In* Aging in Nonhuman Primates. D. M. Bowden, editor. Van Nostrand Reinhold, New York. 247–280.
34. Carlson, L. A., S. O. Froberg, and E. R. Nye. 1968. Effect of age on blood and tissue lipid levels in the male rat. *Gerontologia.* **14:** 65–79.
35. Uchida, K., Y. Nomura, M. Kadowaki, H. Takase, K. Takano, and N. Takeuchi. 1978. Age-related changes in cholesterol and bile acid metabolism in rats. *J. Lipid Res.* **19:** 544–552.
36. Van Lenten, B. J., and P. S. Roheim. 1982. Changes in the concentrations and distributions of apolipoproteins of the aging rat. *J. Lipid Res.* **23:** 1187–1195.
37. Yamamoto, M., and Y. Yamamura. 1971. Changes of cholesterol metabolism in the ageing rat. *Atherosclerosis.* **13:** 365–374.
38. Liepa, G. U., E. J. Masoro, H. A. Bertrand, and B. P. Yu. 1980. Food restriction as a modulator of age-related changes in serum lipids. *Am. J. Physiol.* **238:** E253–257.
39. Bilheimer, D. W., N. J. Stone, and S. M. Grundy. 1979. Metabolic studies in familial hypercholesterolemia. Evidence for a gene-dosage effect in vivo. *J. Clin. Invest.* **64:** 524–533.
40. Kesaniemi, Y. A., and S. M. Grundy. 1982. Significance of low density lipoprotein production in the regulation of plasma cholesterol level in man. *J. Clin. Invest.* **70:** 13–22.
41. Grundy, S. M., and G. L. Vega. 1985. Influence of mevinolin on metabolism of low density lipoproteins in primary moderate hypercholesterolemia. *J. Lipid Res.* **26:** 1464–1475.
42. Stange, E. F., and J. M. Dietschy. 1984. Age-related decreases in tissue sterol acquisition are mediated by changes in cholesterol synthesis and not low density lipoprotein uptake in the rat. *J. Lipid Res.* **25:** 703–713.
43. Spady, D. K., M. Huettinger, D. W. Bilheimer, and J. M. Dietschy. 1987. Role of receptor-independent low density lipoprotein transport in the maintenance of tissue cholesterol balance in the normal and WHHL rabbit. *J. Lipid Res.* **28:** 32–41.
44. Belknap, W. M., and J. M. Dietschy. 1988. Sterol synthesis and low density lipoprotein clearance in vivo in the pregnant rat, placenta and fetus. Sources for tissue cholesterol during fetal development. *J. Clin. Invest.* **82:** 2077–2085.