Independent Regulation of Chylomicron Lipolysis and Particle Removal Rates: Effects of Insulin and Thyroid Hormones on the Metabolism of Artificial Chylomicrons

Celina V. Zerbinatti, Helena C.F. Oliveira, Sergio Wechesler, and Eder C.R. Quintao

The processes of chylomicron lipolysis and removal from plasma were investigated by the intra-arterial infusion of doubly labeled artificial chylomicrons in rats. The rate of lipolysis was measured as a delipidation index (DI), which is the glyceryl-tri 9,10(*N*)-^{3}H oleate (^{3}H-TO) fraction removed from the particle as fatty acids, whereas the cholesteryl(1-^{14}C) oleate (^{14}C-CO) plasma disappearance rate measures the splanchnic organ particle uptake. In the alloxan-diabetic rats, despite a normal DI, the ^{14}C-CO plasma residence time (RT) was longer than in control animals and remained longer after stimulation of the lipoprotein lipase by heparin. DI and ^{14}C-CO removal rate were not significantly altered by insulin administration to glucose-supplemented control rats. Lipolysis was remarkable in propylthiouracil (PTU)-induced hypothyroidism, and yet the ^{14}C-CO removal rate was retarded. In hypothyroidism, heparin enhanced the ^{14}C-CO removal more than in the control group; however, after heparin, the ^{14}C-CO RT still remained higher in the hypotyroid animals as compared with the control group. Hyperthyroidism lowered the DI; nevertheless, the ^{14}C-CO disappearance rate was faster than in controls. In summary, lack or excess of thyroid hormone influences both the chylomicron lipolysis and removal systems, whereas lack of insulin impairs mostly the particle removal from plasma, and excess of insulin has no effect on the chylomicron metabolism.

Diabetes mellitus and hypothyroidism are frequent causes of secondary hyperlipidemia.[1-4] Several experimental studies in rats suggest that there is an impairment in the metabolism of chylomicrons in these secondary hyperlipidemias. In the blood stream, chylomicrons are transformed into remnants soon after losing some of their fatty acid content due to hydrolysis by the endothelial lipoprotein lipase.[5] These remnants are rapidly removed from blood by apolipoprotein E-dependent specific receptors in the liver.[6,7]

Brown[8] and Redgrave and Snibson[9] showed that the chylomicron removal rate slows down in diabetic rats, a finding frequently attributed to a low adipose tissue lipoprotein lipase activity.[8,10-12] On the other hand, in some studies, a direct correlation between the decrease of this lipolytic activity and the increase in the plasma triglyceride level was not found.[12-14] Therefore, other factors may intervene to explain the extent of this hyperlipidemia, such as nonenzymatic apolipoprotein glycosylation[15-18] or modification in the composition of the apolipoprotein cast.[19,20]

Peripheral chylomicron lipolysis seems undisturbed in cholesterol-fed experimental hypothyroid rats, and yet the remnants formed seemingly were removed slowly.[9] Nevertheless, in this experimental group, Florén and Nilsson[21] demonstrated that "in vitro" made remnants were taken up slowly by the perfused livers, and that hypothyroidism alone did not impair the liver chylomicron remnant uptake.

Metabolically, protein-free emulsions behave identically to lymph chylomicrons, as previously described by Redgrave et al[22,23]: they are depleted of triglycerides by the action of lipoprotein lipase and their remnants taken up by the rat liver.[24] Similarly to the natural particles, artificial chylomicrons accumulate in the rat plasma after blockade of hydrolysis by the intravenous (IV) administration of Triton WR-1339; furthermore, heparin infusion leads to faster rates of lipolysis and particle removal.[25]

In the present work, we used artificial chylomicrons in rats to identify the causes of the chylomicron metabolic disturbances, namely whether the major defect is in the peripheral delipidation or in the tissue chylomicron uptake, in opposing metabolic conditions, such as diabetes and hyperinsulinemia, or hypothyroidism and hyperthyroidism.

MATERIAL AND METHODS

Experimental Groups

Male Wistar rats weighing 250 to 300 g were maintained on commercial chow diet. Diabetes was induced by a single intraperitoneal injection of alloxan (140 mg/kg body weight) 2 days before the study. Hyperinsulinemia was obtained according to Kobayashi and Olefsky,[26] by administering to normal rats increasing amounts of NPH insulin (0.5 to 6.0 U/d) divided into two daily doses over 14 days. To prevent hypoglycemia, hyperinsulinemic animals were paired to a special control group supplemented with glucose: they had free access to glucose as tablets and as a 5% water solution.

Propylthiouracil (PTU)-induced hypothyroid rats received 0.1% PTU solution as the sole water source. Hyperthyroidism was achieved by daily subcutaneous injections of L-thyroxine (0.02 mg in 0.2 mL of 0.01N NaOH) over 10 days to normal rats.[27]

Control, diabetic, and hypothyroid rats were also investigated in another set of experiments aimed stimulating the enzyme lipoprotein lipase through heparin intra-arterial injections (250 U/kg body weight) 10 minutes before the infusion of the experimental artificial chylomicrons.[25]

Artificial Chylomicrons

Cholesterol, cholesteryl-oleate, and triolein were obtained from NuCheck Prep (Elysian, MN) and lecithin from Lipid Products (Surrey, UK). They were more than 99% pure as shown by thin-layer chromatography (TLC). Lipid mixtures (cholesterol 2%,

From the Lipid Laboratory, Medical School, University of Sao Paulo; and the Institute of Mathematics and Statistics, University of Sao Paulo, Sao Paulo, Brazil.

Supported by grants from Sao Paulo State Research Foundation (FAPESP 84/2147-4) and FINEP (4.3.84.0683.00).

Address reprint requests to Celina V. Zerbinatti, MsC, Lab Lipides (LIM 10), Hospital das Clínicas-FMUSP. Av. Dr. Arnaldo 455, CEP 01246, São Paulo, SP, Brazil.

0026-0495/91/4011-0002$03.00/0

lecithin 23%, cholesteryl-oleate 6%, and triolein 69% by weight) were prepared in scintillation vials together with glyceryl-tri 9,10(*N*)-^{3}H oleate (^{3}H-TO) and cholesteryl(1-^{14}C) oleate (^{14}C-CO) (Amersham International, UK). This mixture was sonified together with a sodium chloride water solution (1.101 g/mL) using a Branson Cell Disruptor Sonifier, model B-30 (Danbury, CT), with a 1-cm probe, submitting to 70 to 80 W for 30 minutes under nitrogen flow, and inside a temperature-controlled water bath.

Chylomicrons were obtained after a discontinuous gradient ultracentrifugation as previously described.[25] The artificial chylomicron composition achieved was cholesterol, 1.8%; cholesteryl-oleate, 8.9%; lecithin, 9.3%; and triolein, 80% by weight.

Plasma Chylomicron Removal Protocol

The right carotid artery of nonfasted rats was cannulated with a PE-50 polyethylene cathether (Clay Adams, NJ) after light ethyl-ether anesthesia and the animals rested in restriction-type Bollman cages for 1 hour before the injection of the chylomicrons. Emulsions (0.5 mL) containing approximately 3 mg of lipids and the radioactive moieties were pulse-infused through the cannula. Blood samples (0.4 mL) drawn at 2-minute intervals, over a 10-minute period, into previously heparinized tubes were used for measurement of the triacylglycerols and cholesteryl ester residence time. Plasma aliquots (0.1 mL) were extracted with chloroform: methanol (2:1 vol/vol), and lipids separated on TLC silica gel with the solvent system hexane/ethyl-ether/acetic acid (70:30:1 vol/vol/vol).[28] Radioactivity was measured in the triglyceride and cholesteryl-ester eluted bands using a Beckman LS-100 beta-scintillation counter (Irvine, CA).

Chemical Analyses

Chylomicrons. Triglycerides were measured by the method of Soloni[29] and phospholipids as described by Zilversmit and Davis.[30] Free and esterified cholesterol were determined by the method of Zlatkis and Zak[31] after alkaline hydrolysis of the TLC-separated fractions previously extracted with chloroform:methanol (2:1 vol/vol).

Plasma. A commercially available enzymatic procedure was used for triglycerides (Bio Diagnostica, PR, Brazil). Glucose was determined by the ortho-toluidine method according to Dubowski,[32] whereas the hormones triiodothyronine (T_3) and thyroxine (T_4) were measured by a radioimmunoassay kit (Travenol-Genentech, MA).

Calculations and Statistics

Plasma residence time (RT) of both ^{3}H-TO and ^{14}C-CO was obtained from their monoexponential curves estimated by the minimum square method, and represents the reciprocal of the slope of the plasma radioactivity curve expressed as radioactivity (cpm)/mL. A DI was calculated as proposed by Redgrave and Zech[33]; it stems on the assumption that the slope of the curve of radioactivity in plasma represents two interdependent processes: (1) ^{14}C-CO estimates the particle removal rate because radioactivity remains in the chylomicron during its course of lipolysis in plasma; (2) ^{3}H-TO plasma slope results from the combined process of the particle removal and the lipolysis of fatty acids. Therefore, the DI is the fraction of triglycerides that are shed off of the particle as fatty acids before the chylomicron internalization by the splanchnic organs. It is calculated as:

$$DI = 1 - \frac{^{3}\text{H-TO RT}}{^{14}\text{C-CO RT}}.$$

The Student's *t* test was used for statistical comparison of the data among all experimental groups. Data concerning the changes in heparin-treated groups were analyzed by ANOVA. A .05 level of confidence was considered significant.

RESULTS

Blood chemical analyses performed before the infusion of the artificial chylomicrons showed that diabetic rats were hyperglycemic (1,129 ± 549 mg/dL *v* 199 ± 66 mg/dL in control group) and hypertriglyceridemic (227 ± 53 mg/dL *v* 103 ± 27 mg/dL in control rats). Insulin administration together with glucose feeding had no influence on plasma triglycerides. In hypothyroid rats, T_3 was absent and T_4 was much lower in the plasma than in control rats (respectively, 1,100 ± 200 ng/dL *v* 3,600 ± 700 ng/dL); triglyceride plasma level was higher than in the control group (188 ± 66 mg/dL *v* 103 ± 27 mg/dL). As compared with the control rats, hyperthyroid animals had higher T_3 values (74 ± 39 ng/dL *v* 26 ± 13 ng/dL) and reduced triglyceride plasma levels (69 ± 27 mg/dL *v* 103 ± 27 mg/dL). Serum cholesterol concentration almost doubled in hypothyroid animals.

The disappearance curves and plasma RT of ^{3}H-TO and of ^{14}C-CO in all experimental groups are presented in Table 1 and Fig 1. In diabetic animals, the metabolism of artificial chylomicrons was severely impaired, since the RTs of ^{3}H-TO and of ^{14}C-CO were about three times longer than in control animals. Despite this, the DI in the diabetic group was not statistically different from that in the control animals. On the other hand, as compared with the glucose-supplemented group, experimental hyperinsulinemia diminished the RT of ^{3}H-TO, but did not alter either the RT of ^{14}C-CO or the DI to a statistically significant degree.

Hypothyroidism clearly slowed down the ^{14}C-CO RT without disturbing the removal rate of ^{3}H-TO. In other words, enhancement of the DI occurred simultaneously to an impairment in the plasma chylomicron removal rate. In hyperthyroid rats, RT of ^{3}H-TO was similar to that of ^{14}C-CO, a finding that can only be explained by a much quicker splanchnic uptake of the chylomicrons.

Table 1. Plasma RT of Artificial Chylomicron ^{3}H-TO and ^{14}C-CO and Particle DI in All Experimental Groups

Group	Plasma RT (min)		DI
	^{3}H-TO	^{14}C-CO	
Control (n = 8)	5.09 ± 0.97	9.38 ± 2.35	0.44 ± 0.11
Diabetic (n = 8)	15.83 ± 5.91*	22.63 ± 5.78*	0.29 ± 0.19
Glucose-supplemented (n = 7)	6.86 ± 2.13	10.55 ± 2.09	0.36 ± 0.13
Hyperinsulinemic (n = 9)	5.09 ± 1.05†	9.19 ± 2.51	0.43 ± 0.12
Hypothyroid (n = 9)	5.98 ± 1.60	21.88 ± 4.83*	0.71 ± 0.13*
Hyperthyroid (n = 10)	4.59 ± 1.66	5.56 ± 1.85*	0.18 ± 0.06*

NOTE. Calculation of the RT was based on the experimental plasma radioactivity curve (cpm/mL) in samples drawn every 2 minutes over a 10-minute period after the chylomicron injection. Values are shown as means ± SD.

Student's *t* statistical comparison of the groups: *significantly different from the control group; †significantly different from the glucose-supplemented group.

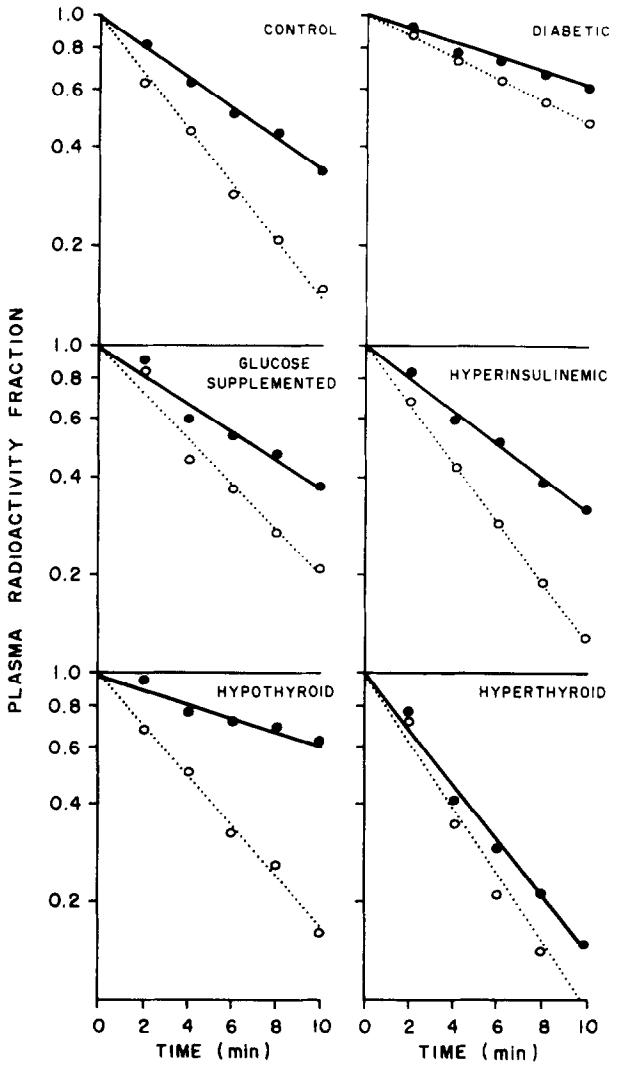

Fig 1. Plasma disappearance curves of intra-arterially pulse-injected artificial chylomicrons labeled with ^{3}H-TO and ^{14}C-CO in all experimental groups. Each point represents the geometrical mean of all animals values.

In all rat groups analyzed, heparin stimulated the activity of lipoprotein lipase to such an extent that the chylomicron lipolysis rate was exceedingly fast: ^{3}H-TO RT could not be measured (Fig 2). In the diabetic rats, some ^{3}H-TO radioactivity was still present in plasma after 10 minutes and must have been inherent to residual chylomicrons.

Heparin stimulated ^{14}C-CO removal from plasma in control, diabetic, and hypothyroid groups (Table 2, Fig 2). However, by ANOVA, despite the heparin enhancement of the particle removal in diabetes and in controls, ^{14}C-CO RT still remained much longer in the former as compared with the latter group of animals. The effect of heparin in the particle removal rate was greater in hypothyroid as compared with control rats: heparin lowered ^{14}C-CO RT 2.9 times in hypothyroidism and 2.2 times in the control group. Nonetheless, in hypothyroidism ^{14}C-CO RT never quite reached the low value found in control animals treated with heparin.

DISCUSSION

The RT of ^{14}C-CO in plasma measures the artificial chylomicron removal rate, since the ^{14}C-CO remains in the particle while it sheds off fatty acids in plasma. ^{14}C-CO is taken up mainly by the liver, probably due to specific lipoprotein receptors.[6,7] The fraction of ^{3}H-TO that leaves the plasma particle by a pathway independent from the removal of ^{14}C-CO represents the lipolytic process, namely, conversion of triolein into free fatty acids. Thus, the lipolysis rate is defined as a DI.

There was only a trend for a slow lipolytic rate in diabetic rats, although the plasma disappearance rates of ^{14}C-CO and of ^{3}H-TO were greatly impaired (Table 1, Fig 1). Previous studies in diabetic rats showed that the lipoprotein lipase activity decreased in the adipose tissue, but was not impaired in the muscle tissue.[10-12] Furthermore, a lack of an inverse correlation between the degree of impairment of the lipoprotein lipase activity and the severity of the hypertriglyceridemia suggests that other factors may explain the diabetic hypertriglyceridemia besides the diminished enzyme activity.[10,13,14] An efficient lipolysis rate after heparin administration to diabetic rats was also suggested by the work of Brown and Olivecrona[34] and recently by the results of Redgrave and Callow.[35] In fact, present data show that the ^{14}C-CO RT in heparinized diabetic rats still was greater than the ^{14}C-CO RT in control animals with or without heparin infusion. This result indicates that in diabetes there was an impairment in the removal by the splanchnic tissue receptors of the chylomicron remnants formed after heparin injection. This fact could theoretically be attributed to (1) a diminished recognition of the lipoproteins by the liver receptors due to nonenzymatic glycosylation of their apolipoproteins, similar to what happens to low-density lipoproteins (LDL)[15-18]; (2) a diminished remnant receptor number directly ascribed to the absence of insulin[36]; or (3) a low apolipoprotein E content, which has been reported in chylomicrons[19] and very-low-density lipoprotein (VLDL)[20] in diabetes.

There was a faster disappearance rate from plasma of ^{3}H-TO in experimental hyperinsulinemic rats (Table 1, Fig 1), a result that should be attributed to an enhanced adipose tissue lipoprotein lipase production elicited by insulin.[37,38] Nevertheless, this enhancement in the ^{3}H-TO removal rate was not sufficient to speed up the remnant uptake by the splanchnic tissues. These results support the conclusion that alterations in the insulin-related lipoprotein lipase activity are not critical in the chylomicron metabolism in the diabetic rat.

The remarkable increase in chylomicron lipolysis observed in hypothroidism in the present work (Table 1, Fig 1) is in agreement with previous reports of increased adipose tissue lipoprotein lipase activity.[9,39,40] Nonetheless, the particle removal rate was considerably slow in hypothyroid rats, as shown by the disappearance rate of ^{14}C-CO from plasma. This result supports the studies of Florén and Nilsson[21] and of Redgrave and Snibson[9] using natural chylomicrons in hypothyroid rats, although their animals had been fed a cholesterol-rich diet. Cholesterol feeding is known to greatly alter the composition of VLDL secreted by the liver,[41-45]

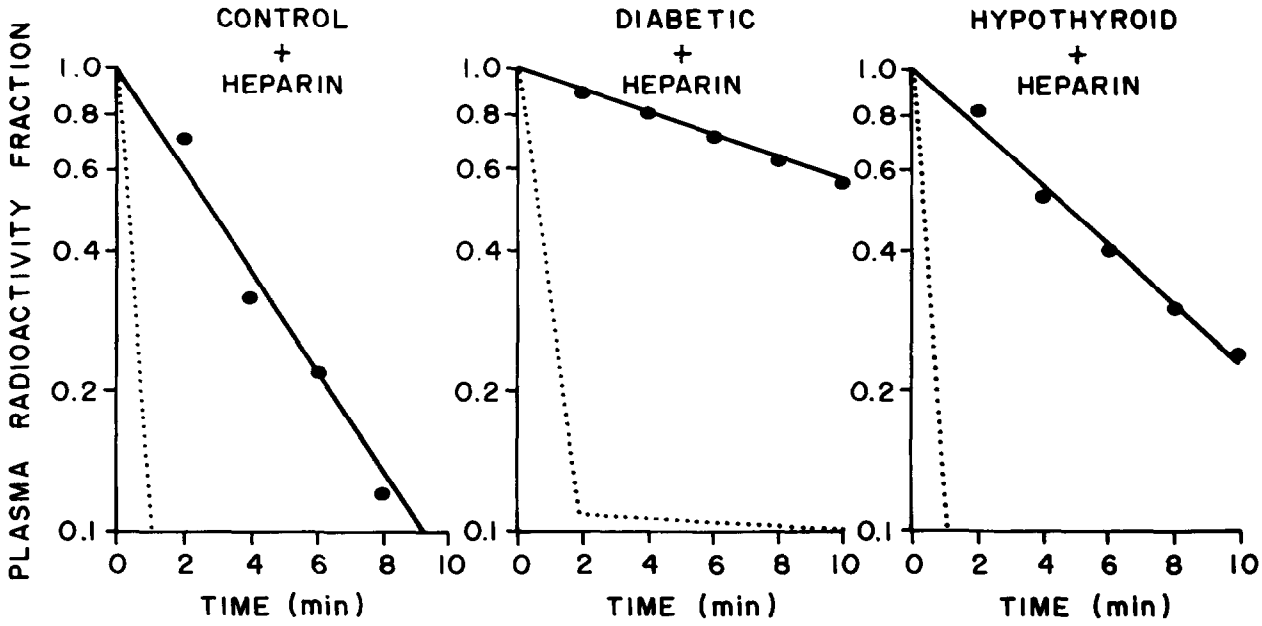

Fig 2. Plasma disappearance curves of intra-arterially pulse-injected artificial chylomicrons labeled with ^{3}H-TO and ^{14}C-CO in control, diabetic, and hypothyroid groups after heparin injection (250 U/kg body weight). Each point represents the geometrical mean of all animals values. *^{3}H-TO removal rates were exceedingly fast for reliable measurements.

whereas in hypothyroidism alone, a remarkable decrease of hepatic lipase activity[27] leads to an enhanced intermediate-density lipoprotein (IDL) accumulation in plasma.[46] This increase in plasma IDL was also shown after the inhibition of the hepatic lipoprotein lipase by a specific antibody.[47] Such an accumulation occurs because the enzyme is necessary for the hydrolysis of the chylomicron triglycerides, which is critical in the production and uptake of remnants by the liver.[48,49]

In the present study, the artificial chylomicron DI in hypothyroid rats was remarkably higher than in controls and could be explained by a prolonged exposure of the chylomicron to the action of the peripheral lipoprotein lipase. The effect of heparin infusion on the chylomicron plasma removal in hypothyroid animals was greater than that observed in the control rats. Nonetheless, after heparin administration, ^{14}C-CO RT still remained higher in hypothyroid as compared with control rats. This result must be secondary to a diminished number of hepatic remnant-recognizing receptors in hypothyroidism, since there are fewer B-E receptors in the hypothyroid liver.[50,51] These receptors are equally responsible for recognizing both the apolipoprotein B-E–containing (LDL) and the apolipoprotein E-rich lipoproteins.[52,53] Furthermore, in hypothyroidism accumulation of IDL derived from VLDL may compete with chylomicron remnants for common apolipoprotein E-recognizing receptors.

Hyperthyroid rats took up plasma chylomicrons at an unusually fast speed and with little loss of the particle fatty acid content (Table 1, Fig 1). This must result from the fact that thyroid hormones greatly increase the number of LDL receptors in the liver.[54] Furthermore, these hormones stimulate the production of hepatic lipase,[55] leading to a faster remnant formation, and consequently to their immediate uptake by the hepatocytes.

Table 2. Plasma RT of the Chylomicron Particle ^{14}C-CO After Heparin Administration (250 U/kg body weight) 10 Minutes Before Emulsion Injection

Group	Plasma RT (min) of ^{14}C-CO
Control + heparin (n = 10)	4.24 ± 1.18†
Diabetic + heparin (n = 6)	19.21 ± 6.50*†‡
Hypothyroid + heparin (n = 8)	7.66 ± 3.07*§

NOTE. Calculation of the RT was based on the exponential plasma radioactivity curve (cpm/mL) in samples drawn every 2 minutes over a 10-minute period after the chylomicron injection. Values are shown as means ± SD.

ANOVA statistical comparison of the groups: *significantly different from the control + heparin group; †significantly different from the control group; ‡significantly different from the diabetic group; §significantly different from the hypothyroid group.

ACKNOWLEDGMENT

The authors are grateful for the excellent technical assistance of R.A. Roela.

REFERENCES

1. Bagdade JD, Porte D Jr, Bierman EL: Diabetic lipemia: A form of acquired fat-induced lipemia. N Engl J Med 276:427-433, 1967

2. Chait A, Robertson HT, Brunzell JD: Chylomicronemia syndrome in diabetes mellitus. Diabetes Care 4:343-348, 1981

3. Abrams JJ, Grundy SM, Gisnberg H: Metabolism of plasma triglyceride in hypothyroidism in man. J Lipid Res 22:307-322, 1981

4. Valdemarsson S: Plasma lipoprotein alterations in thyroid dysfunction. Roles of lipoprotein lipase, hepatic lipase and LCAT. Acta Endocrinol 103:1-52, 1983 (suppl 255)

5. Redgrave TG: Formation and metabolism of chylomicrons. Int Rev Physiol 28:103-130, 1983

6. Lakshmanan MR, Muesing RH, LaRosa JC: Regulation of cholesterol biosynthesis and 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by chylomicron remnants in isolated hepatocytes and perfused liver. J Biol Chem 256:3037-3043, 1981

7. Nilsson A, Zilversmit DB: Distribution of chylomicron cholesteryl ester between parenchymal and Kuppfer cells of rat liver. Biochim Biophys Acta 248:137-142, 1971

8. Brown DF: Triglyceride metabolism in the alloxan-diabetic rat. Diabetes 16:90-95, 1967

9. Redgrave TG, Snibson DA: Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. Metabolism 26:493-503, 1977

10. Schnatz JD, Williams RH: The effect of acute insulin deficiency in the rat on adipose tissue lipolytic activity and plasma lipids. Diabetes 12:174-178, 1963

11. Borensztajn J, Samols DR, Rubenstein AH: Effects of insulin on lipoprotein lipase activity in the rat heart and adipose tissue. Am J Physiol 223:1271-1275, 1972

12. Chen Y-DI, Risser TR, Cully M, et al: Is the hypertriglyceridemia associated with insulin deficiency caused by decreased lipoprotein lipase activity? Diabetes 28:893-898, 1979

13. Chen Y-DI, Howard J, Huang V, et al: Dissociation between plasma triglyceride concentration and tissue lipoprotein lipase deficiency in insulin-deficient rats. Diabetes 29:643-647, 1980

14. Bar-On H, Chen Y-DI, Reaven GM: Evidence for a new cause of defective plasma removal of very low density lipoprotein in insulin-deficient rats. Diabetes 30:496-499, 1981

15. Witztum JL, Mahoney EM, Branks MJ, et al: Nonenzymatic glucosylation of low-density lipoprotein alters its biologic activity. Diabetes 31:283-291, 1982

16. Steinbrecher UP, Witztum JL: Glucosylation of low-density lipoproteins to an extent comparable to that seen in diabetes slows their catabolism. Diabetes 33:130-134, 1984

17. Yamamoto M, Ranganathan S, Kottke BA: Metabolism of glycosylated very-low-density lipoproteins in human skin fibroblasts. Biochim Biophys Acta 875:410-413, 1986

18. Triau JE, Arbetter J, Schaefer EJ: Impaired hepatocyte binding, uptake and degradation of glycosylated low-density-lipoproteins. Biochim Biophys Acta 877:359-365, 1986

19. Levy E, Shafrir E, Ziv E, et al: Composition, removal and metabolic fate of chylomicrons derived from diabetic rats. Biochim Biophys Acta 834:376-385, 1985

20. O'Looney P, Irwin D, Briscoe P, et al: Lipoprotein composition as a component in the lipoprotein clearance defect in experimental diabetes. J Biol Chem 260:428-432, 1985

21. Florén C-H, Nilsson A: Effects of hypothyroidism and cholesterol feeding on the clearance of chylomicron remnants in vivo and by hepatocyte monolayers. Eur J Clin Invest 11:11-18, 1981

22. Redgrave TG, Maranhão RC: Metabolism of protein-free emulsion models of chylomicrons in rats. Biochim Biophys Acta 835:104-112, 1985

23. Maranhão RC, Tercyak AM, Redgrave TG: Effects of cholesterol content on the metabolism of protein-free emulsion models of lipoproteins. Biochim Biophys Acta 875:247-255, 1986

24. Oliveira HCF, Hirata MH, Redgrave TG, et al: Competition between chylomicrons and their remnants for plasma removal: A study with artificial emulsion models of chylomicrons. Biochim Biophys Acta 958:211-217, 1988

25. Hirata MH, Oliveira HCF, Quintão ECR, et al: The effects of Triton WR 1339, protamine sulfate and heparin on the plasma removal of emulsion models of chylomicrons and remnants in rats. Biochim Biophys Acta 917:344-347, 1987

26. Kobayashi M, Olefsky JM: Effect of experimental hyperinsulinemia on insulin binding and glucose transport in isolated rat adipocytes. Am J Physiol 235:E53-E62, 1978

27. Murase T, Uchimura HG: A selective decline of post heparin plasma hepatic triglyceride lipase in hypothyroid rats. Metabolism 29:797-801, 1980

28. Kobayashi Y, Mandsley DV: Biological Application of Liquid Scintillation Counting. San Diego, CA, Academic, 1974, p 51

29. Soloni FG: Simplified manual micromethod for determination of serum triglycerides. Clin Chem 17:529-533, 1971

30. Zilversmit DB, Davis AK: Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. J Lab Clin Med 31:155-160, 1950

31. Zlatkis A, Zak B: Study of a new cholesterol reagent. Anal Biochem 29:143-148, 1969

32. Dubowski KM: An *o*-toluidine method for body-fluid glucose determination. Clin Chem 8:215-235, 1965

33. Redgrave TG, Zech LA: A kinetic model of chylomicron core lipid metabolism in rats: The effect of a single meal. J Lipid Res 28:473-482, 1987

34. Brown DF, Olivecrona T: The effect of glucose availability and utilization on chylomicron metabolism in the rat. Acta Physiol Scand 66:9-18, 1966

35. Redgrave TG, Callow MJ: The effect of insulin deficiency on the metabolism of lipid emulsion models of triacylglycerol-rich lipoprotein in rats. Metabolism 39:1-10, 1990

36. Jensen E, Florén C-H, Nilsson A: Insulin stimulates the uptake of chylomicron remnants in cultured rat hepatocytes. Eur J Clin Invest 18:226-232, 1988

37. Eckel RH, Prasad JE, Kern PA, et al: Insulin regulation of lipoprotein lipase in cultured isolated rat adipocytes. Endocrinology 114:1665-1671, 1984

38. Ashby P, Robinson DS: Effects of insulin, glucocorticoids and adrenaline on the activity of rat adipose-tissue lipoprotein lipase. Biochem J 188:185-192, 1980

39. Hansson P, Nordin G, Nilsson-Ehle P: Influence of nutritional state on lipoprotein lipase activities in the hypothyroid rat. Biochim Biophys Acta 753:364-371, 1983

40. Gavin LA, McMahon F, Moeller M: Modulation of adipose lipoprotein lipase by thyroid hormone and diabetes. The significance of the low T_3 state. Diabetes 34:1266-1271, 1985

41. DeLamatre JG, Roheim PS: Effect of cholesterol feeding on apo B and apo E concentrations and distributions in euthyroid and hypothyroid rats. J Lipid Res 22:297-306, 1981

42. Krul ES, Dolphin PJ: Secretion of nascent lipoproteins by isolated hepatocytes from hypothyroid and hypothyroid, hypercholesterolemic rats. Biochim Biophys Acta 713:609-621, 1982

43. Cole TG, Kuisk I, Patsch W, et al: Effects of high cholesterol diets on rat plasma lipoproteins and lipoprotein-cell interaction. J Lipid Res 25:593-603, 1984

44. Cooper AD, Kris-Etherton PM: Studies on etiology of the hyperlipemia in rats fed an atherogenic diet. J Lipid Res 21:435-442, 1980

45. Keyes WG, Wilcox HG, Heimberg M: Formation of very low density lipoprotein and metabolism of 1-^{14}C-oleate by perfused livers from rats treated with triiodothyronine or propylthiouracil. Metabolism 30:135-146, 1981

46. Dory L, Roheim PS: Rat plasma lipoproteins and apolipoproteins in experimental hypothyroidism. J Lipid Res 22:287-296, 1981

47. Murase T, Itakura H: Accumulation of intermediate density lipoprotein in plasma after intravenous administration of hepatic triglyceride lipase antibody in rats. Atherosclerosis 39:293-300, 1981

48. Rubinstein A, Gibson JC, Paternit JR Jr, et al: Effect of heparin-induced lipolysis on the distribution of apolipoprotein E among lipoprotein subclasses. Studies with patients deficient in hepatic triglyceride lipase and lipoprotein lipase. J Clin Invest 75:710-721, 1975

49. Nozaki S, Kubo M, Sudo H, et al: The role of hepatic triglyceride lipase in the metabolism of intermediate-density lipoprotein—Postheparin lipolytic activities determined by a sensitive, nonradioisotopic method in the hyperlipidemic patients and normals. Metabolism 35:53-58, 1986

50. Scarabottolo L, Trezzi E, Roma P, et al: Experimental hypothyroidism modulates the expression of the low density lipoprotein receptor by the liver. Atherosclerosis 59:329-333, 1986

51. Gross G, Sykes M, Arellano R, et al: HDL clearance and receptor-mediated catabolism of LDL are reduced in hypothyroid rats. Atherosclerosis 66:269-275, 1987

52. Howard BV, Egusa G, Beltz WF, et al: Compensatory mechanisms governing the concentration of plasma low density lipoproteins. J Lipid Res 27:11-20, 1986

53. Garg A, Grundy SM: Lovastatin for lowering cholesterol levels in non–insulin-dependent diabetes mellitus. N Engl J Med 318:81-86, 1988

54. Chait A, Bierman EL, Albers JJ: Regulatory role of triiodothyronine in the degradation of low density lipoprotein by cultures human skin fibroblasts. J Clin Endocrinol 48:887-889, 1979

55. Hansson P, Jensen E, Florén C-H, et al: Thyroid hormones stimulate the release of hepatic lipase from cultured rat hepatocytes. Horm Metab Res 18:107-109, 1986