Appearance of Circulating and Tissue ^{14}C-Lipids After Oral ^{14}C-Tripalmitate Administration in the Late Pregnant Rat

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Studies were performed to determine whether and/or how dietary lipids participate in maternal hypertriglyceridemia during late gestation in the rat. After oral administration of glycerol-tri(1-^{14}C)-palmitate, total radioactivity in plasma increased more rapidly in 20-day pregnant rats than in either 19-day pregnant rats or virgin controls. At the peak of plasma radioactivity, four hours after the tracer was administered, most of the plasma label corresponded to ^{14}C-lipids in triglyceride-rich lipoproteins (d < 1.006), and when expressed per micromol of triglyceride, values were higher in pregnant than in virgin rats. The difference was less after 24 hours, although at this time the level of ^{14}C-lipids in d < 1.006 lipoproteins was still higher in 20-day pregnant rats than in virgins. Tissue ^{14}C-lipids, as expressed per gram of fresh weight, were similar in pregnant and virgin rats, but the values in mammary glands were much higher in the former group. Estimated recovery of administered radioactivity four hours after tracer in total white adipose tissue, mammary glands, and plasma lipids was higher in pregnant than in virgin rats. No difference was found between 20-day pregnant and virgin rats either in the label retained in the gastrointestinal tract or in that exhaled as ^{14}C-CO_2 during the first four hours following oral administration of ^{14}C-tripalmitate. These findings plus the known maternal hyperphagia, indicate that in the rat at late pregnancy triglyceride intestinal absorption is unchanged or even enhanced and that dietary lipids actively contribute to both maternal hypertriglyceridemia and lipid uptake by the mammary gland.

PREGNANT RATS develop progressive hypertriglyceridemia, which corresponds to increased concentrations of both chylomicron and very-low-density lipoprotein (VLDL) triglycerides.[1-3] It has been suggested that hypertriglyceridemia during pregnancy results from the increased production of endogenous triglycerides[4-7] and/or decreased triglyceride uptake by extrahepatic tissues (primarily adipose tissue because of diminished lipoprotein lipase activity).[8-10] The modified intestinal absorption of dietary lipids may also contribute to hypertriglyceridemia during pregnancy. This possibility has been indirectly evaluated but has not as yet been demonstrated. Childs et al[11] described a mild increase in circulating triglyceride-chylomicron concentration in fat-fed pregnant versus nonpregnant rats. Otway and Robinson reported that hypertriglyceridemia was similar in pregnant rats fed a fat-containing diet and rats fed a high carbohydrate fat free diet from day 9 of gestation through term,[8] indicating that there is an endogenous basis for hypertriglyceridemia with negligible dietary lipid contribution. To study this point further, the present work measures levels of labeled lipids in the gastrointestinal tract, plasma, tissues, and also the exhaled ^{14}C-CO_2 after oral administration of ^{14}C-tripalmitate to feed late pregnant and virgin rats. Results indicate that in the rat at late pregnancy triglyceride intestinal absorption is unchanged or even enhanced and that the mammary glands of pregnant rats retain a higher proportion of absorbed triglycerides than those of nonpregnant rats. We also propose that dietary lipids actively contribute to maternal hypertriglyceridemia during late gestation.

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MATERIALS AND METHODS

Female Wistar rats from our own colony were mated when weighing 160 to 180 g, fed Purina Chow, and maintained in a controlled environment (23°C, 12-hour light-dark cycles). Age- and sex-matched virgin animals were kept under the same conditions and used as controls. At day 19 or 20 of gestation (estimated by counting day 0 as the morning when spermatozoids appeared in the daily vaginal smears), ad libitum fed pregnant rats and their controls were given an oral ^{14}C-tripalmitate emulsion, prepared as described below, by means of a plastic tube connected to a syringe. After this treatment animals had access to drinking water but not to food. The emulsion was prepared in a tube, immersed in an ice bath by sonication in an Ultrasonics Ltd. sonifier (set at 12 μm, 1 min × 5), and it consisted of a mixture of 4 mg phosphatidylcholine, 20 mg tripalmitine, 50 μCi glycerol tri(1-^{14}C) palmitate (57 Ci/mol, from The Radiochemical Centre, Amersham, UK), and 5 mL saline (NaCl 0.9% w/v). At 0, 0.5, 1, 2, 4, 6, 8, and 24 hours after administration of 1 mL of the emulsion to each rat, blood samples were collected from the tip of the tail into receptacles containing EDTA. Plasma aliquots were used to count total radioactivity, for lipid extraction[12] to determine ^{14}C-total lipids, and for triglyceride evaluation.[13] Some animals from each group were guillotined at 4 or 24 hours after treatment. The blood from these rats was collected from the neck wound, and plasma was used for lipid extraction and lipoprotein fractionation and analysis by sequential flotation in a Beckman L5-75 ultracentrifuge as previously described.[3,14] Tissues and whole fetuses were rapidly excised, weighed, and used for lipid extraction.[14] After the oral ^{14}C-palmitate treatment another group of 20-day pregnant rats and nonpregnant controls were immediately placed in individual glass cages, through which air was pumped at a constant rate of 200 mL/min, attached to individual ^{14}C-CO_2 trapping devices. Exhaled CO_2 was collected for four hours in soda lime, after which time the animals were decapitated. After acidification of the soda lime with 2N HCl, evolved $^{14}CO_2$ was collected in a ethanolamine-methanol mixture (20:80 by vol) for counting.[15] The gastrointestinal tract of each animal with its content was rapidly dissected and pooled in 50 mL of 2N NaOH along with the feces

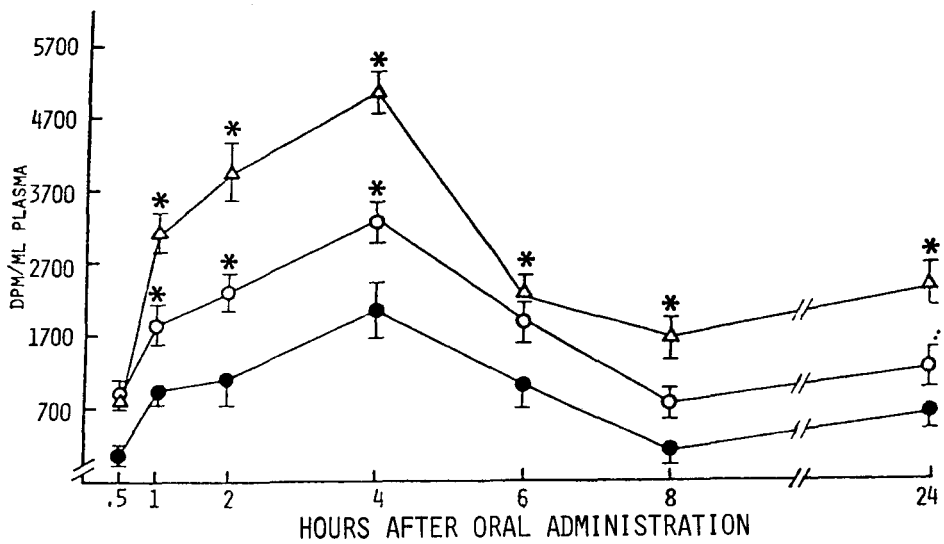

Fig 1. Total radioactivity in plasma in pregnant and virgin rats after oral ^{14}C-tripalmitate. Pregnant rats at either the 20th (△) or the 19th day of gestation (○) and age- and sex-matched virgin animals (●) were intragastrically given 10 μCi of glycerol-tri(1-^{14}C) palmitine containing 4 mg tripalmitine and 0.8 mg of phosphatydilcholine. Blood was collected from the tail at different times after tracer administration to determine its total radioactivity. Values correspond to mean ± SEM, and statistical comparisons *v* virgin animals are shown by asterisks: *$P < .05$; n = 6 to 8 rats/group.

excreted during the four-hour period. After digestion for one hour at 100°C, lipids were extracted in 5 mL of hexane from 5-mL aliquots of the digest and used for counting. The recovery of the label from the ^{14}C-tripalmitine, which had been added to the gastrointestinal tract samples from untreated rats and subjected to the same lipidic extraction procedure, was always above 78.7% in the hexane phase. Radioactive measurements were assessed in a PPO/POPOP based scintillation cocktail in Xylene and Triton X-100 and the samples were counted in a Nuclear-Chicago scintillation counter (Isocap 300) with an external standard device. To allow intergroup comparisons and to unify the amount of radioactivity given to each animal, all radioactive values were corrected as per million of DPM in the ^{14}C-tripalmitate emulsion administered to each rat. Statistical comparisons between groups were performed with the Wilcoxon two-sample rank sum nonparametric test, and results are expressed as mean ± SEM.

RESULTS

As shown in Fig 1, plasma radioactivity 30 minutes after oral administration of the same amount of ^{14}C-tripalmitate to pregnant rats after the 19 or 20 days' gestation was slightly, but not significantly, higher than in the virgin controls. Plasma radioactivity values increased progressively in all three groups up to the fourth hour and then decreased. The rise was consistently higher in pregnant than in virgin rats, the difference between these groups being statistically significant at 1, 2, and 4 hours. When compared with their virgin controls, values in pregnant rats continued to be augmented between 4 and 24 hours, the difference being significant at 6, 8, and 24 hours in 20-day pregnant rats and at 24 hours in 19-day pregnant animals (Fig 1). More than 87.7% of the plasma radioactivity recovered in plasma always corresponded to ^{14}C-lipids in all groups at all the times studied. As shown in Table 1, under basal conditions (time 0), plasma triglyceride concentration was significantly higher in 19-day and 20-day pregnant rats than in virgins, and values remained stable after tracer administration in all rats at each of the times studied. Distribution of plasma ^{14}C-lipids in the lipoprotein fractions per micromole of triglycerides 4 and 24 hours after oral administration of the tracer is shown in Table 2, where it may be seen that at 4 hours, the triglyceride-rich lipoproteins ($d < 1.006$, corresponding to chylomicrons plus very low density lipoproteins, [VLDL]) contained the highest amount of radioactivity. This level was higher in the 20-day than in the 19-day pregnant rats and in both cases was higher than in virgin controls. At four hours the lowest amount of radioactivity appeared in the intermediate density lipoproteins ($1.006 > d < 1.019$). These levels were higher in 19-day pregnant than in virgin rats, whereas there were no significant group differences in the values of higher density lipoproteins ($d > 1.019$). These relationships were altered 24 hours after tracer administration when ^{14}C-lipid levels in triglycer-

Table 1. Plasma Triglyceride Concentration Under Basal Conditions and After ^{14}C-Tripalmitate Intragastric Administration

	Hours After ^{14}C-tripalmitate							
	0	0.5	1	2	4	6	8	24
19-day pregnant rats	248 ± 13*	268 ± 22*	254 ± 20*	257 ± 12*	255 ± 23*	219 ± 15*	258 ± 22*	259 ± 25*
20-day pregnant rats	290 ± 18*	284 ± 18*	287 ± 24*	284 ± 14*	321 ± 25*	311 ± 19*	304 ± 13*	284 ± 16*
Virgin controls	157 ± 14	149 ± 23	149 ± 19	144 ± 17	174 ± 17	154 ± 20	159 ± 19	145 ± 22

Pregnant rats at 19- and 20-days gestation and age- and sex-matched virgin controls were given orally a ^{14}C-tripalmitate emulsion containing 10 μCi of glycerol-tri(1-^{14}C) palmitine, 0.8 mg of phosphatydilcholine and 4 mg of tripalmitine per rat. Blood was collected from the tip of the tail before and at different times after treatment for triglyceride evaluation. Values correspond to mean ± SEM.

N = 6 to 8 rats per group.

*Statistical comparison *v* virgin animals, $P < .05$.

Table 2. Plasma ^{14}C-Lipids in Lipoprotein Fractions After ^{14}C-Tripalmitate Intragastric Administration

	Lipoproteins of Density < 1.006 (VLDL + chylomicrons)	Lipoproteins of Density > 1.006 <1.019 (IDL)	Lipoproteins of Density > 1.019 (LDL + HDL)
4 hours after the tracer			
19-day pregnant rats	707 ± 81*	114 ± 8*	316 ± 61
20-day pregnant rats	1,145 ± 246*	74 ± 19	268 ± 69
Virgin controls	569 ± 99	98 ± 12	236 ± 35
24 hours after the tracer			
19-day pregnant rats	114 ± 18	7 ± 5	242 ± 53
20-day pregnant rats	417 ± 59*	25 ± 17	351 ± 11
Virgin controls	117 ± 28	17 ± 9	240 ± 59

Pregnant rats at 19- and 20-days' gestation and age- and sex-matched virgin controls were treated as indicated in Fig 1 and Table 1. Animals were killed 4 or 24 hours later and plasma aliquots were used for lipoprotein fraction and analysis by sequential flotation and lipid extraction and ^{14}C-lipid determination. Means ± SEM of dpm/μmol triglyceride/mL of plasma are shown. N = 4 to 6 rats/group.

*$P < .05$.

ide-rich lipoproteins (d < 1.006) per μmol triglyceride were no longer higher than in lipoproteins of density >1.019 and only the values of ^{14}C-lipids in d < 1.006 lipoproteins were higher in 20-day pregnant rats than in nonpregnant animals (Table 2).

Because variations in plasma radioactivity could be a secondary consequence of the different amount of label taken up by the tissues, the ^{14}C-lipid tissue content was also estimated 4 and 24 hours after tracer administration. As shown in Table 3, ^{14}C-lipid values per gram of fresh weight in tissues from pregnant and virgin animals were greater at 4 than at 24 hours. Tissue ^{14}C-lipids did not differ in pregnant rats and virgins, except in the mammary glands where values were much higher in pregnant rats at both 4 and 24 hours after administration (Table 3). Placentas also contained a substantial amount of radioactive ^{14}C-lipids at these two times and, while fetuses showed a low proportion of label at 4 hours, this value was increased at 24 hours (Table 3). Because all radioactivity in fetal lipids corresponded to ^{14}C-fatty acids, present data indicate their transfer from the mother.

Recovery of administered radioactivity in tissue and plasma lipids at the peak of circulating radioactivity was obtained by correcting the four-hour values of Table 3 by whole tissue weights and plasma volume. Due to the estimated character of these values, statistical comparison between the groups was omitted. As shown in Table 4, recovery of administered radioactivity was higher in white adipose tissue, mammary gland, and plasma lipids of 19-day and 20-day pregnant rats than in virgin controls, with no difference in the other tissues studied. Total recovered radioactivity in tissue lipids was also higher in pregnant than in virgin rats (Table 4).

To determine whether these results were affected by differences in the retention of ^{14}C-lipids in the gastrointestinal tract or in their endogenous oxidation, another set of 20-day pregnant rats and virgin controls received the same oral ^{14}C-tripalmitate treatment as in the preceding experiments. Exhaled ^{14}C-CO_2 and ^{14}C-lipids present in the gastrointestinal tract were measured together with the feces accumulated in the four hours following treatment. As shown in Table 5, exhaled ^{14}C-CO_2 was lower in pregnant than in virgin animals, although not significantly, and both the ^{14}C-lipid levels in the gastrointestinal tract plus feces and the

Table 3. Tissue ^{14}C-Lipids After ^{14}C-Tripalmitate Intragastric Administration

	19-Day Pregnant Rats		20-Day Pregnant Rats		Virgin Control Rats	
	4 H	24 H	4 H	24 H	4 H	24 H
Brown adipose tissue	34,810 ± 5,054	20,557 ± 3,893	29,953 ± 2,693	18,356 ± 1,728	25,718 ± 3,170	18,068 ± 3,429
White adipose tissue	8,227 ± 2,254	6,415 ± 1,221	7,123 ± 2,517	4,224 ± 604	4,428 ± 977	3,640 ± 1,177
Kidneys	2,559 ± 404	2,819 ± 598	2,582 ± 254	2,227 ± 576	2,406 ± 434	2,295 ± 542
Striated muscle	826 ± 55	422 ± 64	852 ± 215	431 ± 65	841 ± 75	576 ± 158
Spleen	3,028 ± 1,154	1,730 ± 513	3,219 ± 1,120	1,682 ± 331	4,298 ± 849	2,119 ± 603
Liver	24,133 ± 8,407	6,786 ± 746	20,993 ± 5,286	7,665 ± 892	31,250 ± 3,185	10,711 ± 4,150
Heart	10,388 ± 1,177	4,846 ± 771	8,601 ± 1,694	4,617 ± 898	10,221 ± 1,730	5,400 ± 1,147
Lungs	11,397 ± 3,505	3,433 ± 640	10,593 ± 2,042	3,385 ± 612	14,369 ± 2,240	7,165 ± 1,674
Mammary glands	5,835 ± 1,178*	6,631 ± 1,315*	8,433 ± 1,208*	7,861 ± 731*	730 ± 236	896 ± 143
Placenta	7,613 ± 921	8,258 ± 1,114	9,612 ± 762	9,532 ± 699	—	—
Whole fetus	621 ± 68	2,235 ± 293	890 ± 188	2,590 ± 771	—	—
Fetal liver	1,592 ± 242	2,789 ± 508	2,254 ± 921	3,150 ± 623	—	—

Experimental procedure and expression of the results as indicated in Tables 1 and 2. Tissues were excised immediately after death for lipid extraction. 4 H, four hours after the tracer; 24 H, 24 hours after the tracer. Values are expressed as dpm/g of fresh tissue.

Table 4. Recovery of Administered Radioactivity in Tissue and Plasma Lipids 4 Hours After ^{14}C-Tripalmitate Intragastric Administration

	% of Administered Radioactivity/Total Tissue		
	19-Day Pregnant Rats	20-Day Pregnant Rats	Virgin Control Rats
White adipose tissue	1.05 ± 0.13	1.14 ± 0.10	0.40 ± 0.05
Kidneys	0.43 ± 0.08	0.36 ± 0.06	0.35 ± 0.05
Striated muscle	8.09 ± 0.94	8.78 ± 0.85	6.36 ± 1.66
Spleen	0.15 ± 0.04	0.22 ± 0.04	0.23 ± 0.05
Liver	27.0 ± 3.37	20.1 ± 3.55	29.25 ± 3.37
Heart	0.75 ± 0.22	0.69 ± 0.12	0.64 ± 0.07
Lungs	1.35 ± 0.16	1.14 ± 0.07	1.55 ± 0.23
Mammary glands	5.14 ± 1.04	9.49 ± 1.36	0.11 ± 0.04
Placenta	3.69 ± 0.43	4.90 ± 1.99	—
Whole fetus	1.54 ± 0.53	4.15 ± 0.81	—
Plasma	5.37 ± 0.26	8.14 ± 0.39	1.66 ± 0.16
Total recovery	55.5 ± 5.82	59.3 ± 5.1	40.6 ± 4.7

Experimental procedure and expression of the results as in Tables 1 and 2. Total mass of striated muscle and whole plasma volume were estimated as function of body weight by using previously reported values.[29,20]

gastrointestinal tract weight were similar in the two groups (Table 5).

DISCUSSION

Present results show that the amount of ^{14}C-lipids in plasma after oral administration of ^{14}C-tripalmitate is higher in late pregnant than in virgin rats, and although radioactivity exhaled as $^{14}C\text{-}CO_2$ up to the time of peak values of plasma ^{14}C-lipids is slightly reduced, radioactivity remaining in the gastrointestinal tract does not differ in either group. These findings, together with the enhanced specific activity of circulating triglycerides in $d < 1.006$ lipoproteins four hours after the tracer and the augmented recovery of administered radioactivity in tissue lipids, indicate that the rate of intestinal triglyceride absorption is either unchanged or augmented in the late pregnant rat. This conclusion contrasts with the slower gastrointestinal transit reported in both rats[16] and women[17] at late gestation, which would indicate a delayed absorption activity. Our findings are, however, consistent with those of Hornnes et al[18] which showed that following oral ingestion of triglycerides the rise in both circulating triglycerides and gastrointestinal hormones was similar in women at late gestation and at postpartum, indicating that gut absorption of triglycerides is not affected by pregnancy. The level of ^{14}C-lipids was higher in pregnant than in virgin rats in plasma at four hours after tracer administration and in the mammary glands at both four and 24 hours. Even considering the presence of ^{14}C-lipids in conceptus structures and the greater blood volume[19,20] and tissue sizes[21] of pregnant rats, it is seen that the ^{14}C-lipids retained in these animals are clearly greater than those in virgins. These findings, together with the enhanced endogenous pool of cold triglycerides, indicate that in this condition there is an enhancement in the entrance of triglycerides from dietary lipids and not a delay in the removal of circulating triglycerides. Because no direct measurements of such removal was made, the conclusion is hypothetical but is also supported by the increase in ^{14}C-triglycerides specific activity found in circulating lipoproteins of density less than 1.006 four hours after tracer administration and is in agreement with a similar conclusion reached by Humphrey et al,[19] using a different experimental model, consisting in measurements of ^{14}C-chylomicron triglyceride kinetics after an IV bolus in late rat gestation. Although no attempts were made to differentiate chylomicrons from VLDL, present findings indicate that, even in conditions of unmodified gut absorption, pregnant animals synthesize a higher proportion of triacylglycerol-rich lipoproteins from dietary lipids. This greater efficiency in lipoprotein triglyceride formation from dietary fat coincides with reported increases in food intake,[22,23] endogenous VLDL formation,[8] and the availability of free fatty acids mobilized from adipose tissue,[24,25] all of which contribute to maternal hypertriglyceridemia. The similar level of radioactivity found in the adipose tissue of pregnant and nonpregnant rats after oral ^{14}C-tripalmitate administration is compatible with the reduced lipoprotein lipase activity present in this tissue at late pregnancy.[1,8-10] Plasma circulating lipoprotein triglyceride concentration is known to modulate adipose tissue lipoprotein lipase catalytic efficiency due to its high Km value.[26] The high concentration of triglyceride-rich lipoproteins in the mother's circulation may therefore compensate for reduced enzyme activity, producing stable uptake in adipose tissue. The increased uptake of ^{14}C-lipids absorbed from the diet by the mammary glands in pregnant rats parallels the progressive increase of lipoprotein lipase activity found in these organs around parturition,[10,27] supporting the hypothesis that this activity facilitates the use of circulating triglycerides in preparing the mammary gland for lactation immediately before delivery when lipogenesis has not yet been enhanced.[28]

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Table 5. Exhaled $^{14}C\text{-}CO_2$ and Gastrointestinal (GI) ^{14}C-Lipids 4 Hours Following ^{14}C-Tripalmitate Intragastric Administration

	Total $^{14}C\text{-}CO_2$ Exhaled (dpm)[2]	^{14}C-Lipids in GI Tract + Feces (dpm/g)	GI Tract Weight (g)
20-day pregnant rats (n = 4)	119,233 ± 22,489	6,055 ± 1,138	17.1 ± 1.7
Virgin controls (n = 5)	186,669 ± 23,537	5,776 ± 476	17.0 ± 1.1

Pregnant rats at 20-days' gestation and age- and sex-matched virgin controls were treated as indicated in Table 1 and placed in closed individual glass cages attached to a CO_2 trapping device. Animals were killed 4 hours later and their GI tract and excreted faces were pooled for ^{14}C-lipid extraction. Values are mean ± SEM. There were no significant differences at $P > .05$.

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