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Original article

Turnover of [^{14}C] sucrose HDL and uptake by organs in the normal or genetically hypercholesterolemic (RICO) rat using a constant infusion method

C Lutton *, K Ouguerram, M Sauvage, T Magot

Laboratoire de Physiologie de la Nutrition, URA D.0646 CNRS - Bâtiment 447, Université de Paris-Sud, 91405 Orsay Cedex, France

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Summary — The turnover and tissular uptake of HDL (d 1.095–1.21) have been compared in normocholesterolemic or genetically hypercholesterolemic rats by a constant infusion method of [^{14}C] sucrose labelled HDL for 8 h. The HDL clearance rate was not significantly smaller in the RICO than in the normocholesterolemic animal (320 ± 22 μl•h^{-1} *versus* 366 ± 24 μl•h^{-1} per 100 g of rat). It was the same case for the fractional catabolic rate, respectively equal to 7.8 and 9.4 ± 0.6%•h^{-1}. For both strains, liver and skeletal muscle were the main catabolic sites for HDL. The HDL uptake rates in intestine or kidney were 3–4-fold smaller than those in the liver. In the RICO rat, intestine, testis and adrenals showed a lesser HDL uptake capacity (expressed per g of organ) than the normocholesterolemic rat.

[^{14}C] sucrose HDL / clearance rate / fractional clearance rate

Résumé — Renouvellement plasmatique et captage tissulaire des HDL marquées au ^{14}C saccharose chez le rat normocholestérolémique ou génétiquement hypercholestérolémique (RICO) par une méthode d'infusion continue. *Par une méthode d'infusion continue qui permet de maintenir pendant 8 h un équilibre isotopique dans le plasma de HDL marqués sur leurs apolipoprotéines par du [^{14}C]saccharose, nous avons comparé le renouvellement plasmatique et le captage tissulaire des HDL (d 1,095–1,21) chez le rat normocholestérolémique et chez le rat génétiquement hypercholestérolémique (RICO). La vitesse de clairance totale des HDL n'est pas significativement plus basse chez le RICO que chez le normocholestérolémique (320 ± 22 μl•h^{-1} contre 366 ± 24 μl•h^{-1} par 100 g de rat). Il en est de même du taux de catabolisme, respectivement égal à 7,8 et 9,4 ± 0,6%•h^{-1}. Chez les 2 souches de rats, le foie et le muscle squelettique représentent les principaux sites du catabolisme des HDL. La vitesse de captage des HDL par l'intestin grêle ou les reins est 3–4 fois plus faible que celle du foie. Chez le rat RICO, l'intestin grêle, les testicules et les surrénales présentent une moindre capacité de captage des HDL (exprimée par g d'organe) que le normocholestérolémique.*

HDL [^{14}C]saccharose / vitesse de clairance / taux de catabolisme

* Correspondence and reprints

INTRODUCTION

In the rat, most plasma cholesterol is carried by high density lipoproteins. In the genetically hypercholesterolemic rat (RICO), high plasma cholesterol concentration results from an increase in the cholesterol content of the d > 1.006 lipoproteins (Cardona-Sanclemente *et al,* 1988). Several approaches have been used in an attempt to determine the turnover and the quantitative importance of various tissues in the degradation of lipoproteins. The content of ^{125}I has been measured in various organs several h after the intravenous administration of ^{125}I-lipoproteins, but a rapid release of free ^{125}I can occur after tissue uptake of ^{125}I lipoproteins. The apolipoproteins have also been covalently linked to [^{14}C] sucrose or to ^{125}I-cellobiose which are not degraded by lysosomal enzymes and accumulate in the cell (Pittman *et al,* 1983). A simple constant infusion method has been described to measure plasma clearance rates of labeled lipoproteins and identify their catabolic tissue sites (Koelz *et al,* 1982). In this method, an initial bolus of [^{14}C] sucrose-lipoprotein, administered intravenously, was followed by a subsequently adjusted hourly constant infusion rate of [^{14}C] sucrose-lipoprotein necessary to achieve a constant level of radiolabeled lipoprotein in the plasma. We now report the results obtained with this last technique in the genetically hypercholesterolemic rat (RICO) and in the normocholesterolemic heterozygote (SW).

MATERIALS AND METHODS

Animals and diet

Male RICO and normocholesterolemic SW rats weighing 140-150 g came from the Ciba-Geigy breeding unit (Basel, Switzerland). As soon as they arrived the animals were kept at a constant temperature of 24 ± 2 °C with an 8 am–8 pm photoperiod. They ingested the laboratory semi-synthetic base diet containing 53% sucrose, 23% caseine, 9.2% lard, 5% mineral mix, 4% skim milk, 2.5 yeast, 2.5% vitamins, 0.8% walnut oil, 0.2% cystine (Cardona-Sanclemente *et al,* 1987) until they were 4 months old. The mean fatty acid (mol percentage) composition of the lard was: myristic < 5, palmitic 22–28, stearic 8–11, oleic 43–53, linoleic 12–13 and unsaturated C_{20-22} fatty acids ≤ 1; that of walnut oil was: palmitic 4.6, stearic 0.9, oleic 18, linoleic 73, linolenic 3.

Isolation and labelling of lipoproteins

High-density lipoproteins (HDL) in the density range of 1.095–1.21 g/ml were isolated by preparative ultracentrifugation (Sérougne *et al,* 1984) from the plasma of male adult SW or RICO rats. They were then dialyzed against 20 mM sodium phosphate buffer/1 mM EDTA/0.15 M NaCl (buffer A, pH 7.2). 62 μCi of U [^{14}C] sucrose (540 μCi/μmol, 1 Ci = $3.7.10^{10}$ Bq) (CEA) and 112.5 nmol of sucrose were activated by reaction with 2 molar equivalents of cyanuric chloride (1, 3, 5-trichloro 2, 4, 6 triazine, Aldrich) in aqueous acetone containing 3 equivalents of NaOH, as described by Pittman *et al* (1979) with slight modifications. After 15 s, the reaction was quenched by adding 4 equivalents of acetic acid. The resulting activated [^{14}C]sucrose dichlorotriazine adduct was then added to 5–6 mg of HDL protein in buffer A, pH 7.2. After 3 h at room temperature, [^{14}C] sucrose HDL were separated from the low molecular weight products by gel filtration (Sephadex G_{25}) and dialysis in buffer A pH 6.8 then buffer A, pH 7.2. The final preparation contained 1.5 μCi/mg protein.

Infusion method

RICO and SW rats were fitted with a jugular vein catheter the day before the experiment. Each animal received an initial bolus (500 000 – 1 000 000 dpm) intravenously of lipoprotein in an amount equal to approximately 10 times the subsequent hourly infusion rate which was 1 ml/h with a solution containing 77.1 mM

NaCl, 20.6 mM KCl, 10.3 mM KH_2PO_4, 550 mM glucose and [^{14}C] sucrose HDL (0.5–2 mg/ml), to give approximately 50 000–100 000 dpm of radioactivity per ml. The infusion was continued at a constant rate for 8–10 h or adjusted in order to ensure isotope equilibrium. Blood samples (≤ 200 µl) were regularly taken on the tail to check plasma radioactivity. The ratio of hourly infusion rate to initial bolus gave the fractional catabolic rate (FCR, % h^{-1}) of the labeled HDL (table I). Dividing the clearance rate by the theoretical plasma volume also allowed a close estimation of FCR. The animals were anesthetized by pentobarbital and blood was aspirated from the abdominal aorta. The circulatory system was extensively washed. Triplicate aliquots of plasma and triplicate samples of liver, kidney, adrenals, jejunum, spleen, testis, colon, caecum and skeletal muscle were then assayed for radioactivity. Preliminary assays showed that aliquots of the tissue and plasma samples subjected to lipid extraction gave results similar to those of non-delipidated samples. Consequently, the amount of ^{14}C in the tissue samples (50–150 mg) was determined after digestion in 1 ml of soluene 350, acidification with acetic acid and counting in a Kontron scintillation spectrometer. The HDL clearance rate from the plasma was calculated as the ratio of dpm infused into the animal per h/lipoprotein-bound dpm per µl plasma. This calculation gave the microliters of plasma cleared of HDL per h per animal or per 100 g of rat.

RESULTS AND DISCUSSION

Plasma cholesterol was 0.83 ± 0.04 and 1.73 ± 0.15 mg/ml respectively in SW and RICO rats, in agreement with a previous study (Cardona-Sanclemente *et al*, 1987). Similar HDL clearance rates were obtained for both groups of animals (table I).

Expressed per 100 g of body weight, our values obtained in adult male Wistar rats weighing 400–500 g represent only 60% of those published by Koelz *et al* (1982) in the young female Sprague–Dawley rat. Although the weight of the animals was not noted by these authors, it can be estimated from their data that the HDL clearance rate per animal was similar to the one obtained in this paper. The fractional catabolic rate evaluated by 2 different methods (see legend to table I) is slightly but not significantly smaller in RICO than in SW rats and has a range of 0.078–0.10 h^{-1}. Following the serum decay of ^{125}I HDL (d 1.050–1.21) in young male Wistar rats fed a sucrose-purified high-carbohydrate diet, Van Tol *et al* (1978) found a FCR of 0.12 h^{-1}–FCR unmodified by removal of the liver.

Table I. HDL clearance rates (CR, expressed as µl•h^{-1} per rat or per 100 g of rat) and fractional catabolic rates (FRC, %•h^{-1}) in normocholesterolemic (SW) and hypercholesterolemic (RICO) rats. Results are expressed as means ± SEM (N = 5; SW; N = 4; RICO) [a] FCR calculated as the ratio of constant hourly infusion rate to initial bolus; [b] FCR calculated as the ratio of clearance rate (µl•h•per rat) to theoretical plasma volume (µl per rat) of SW (3.9% of the body weight) and RICO rats (4.1% of the body weight). [c] 5 data of 10%•h^{-1}.

	Unit	*SW rats*	*RICO rats*
CR	(µl•h^{-1} per rat)	1 488 ± 125	1 557 ± 109
CR	(µl•h^{-1} per 100 g rat)	366 ± 24	320 ± 22
FCR[a]	(%•h^{-1})	10.0[c]	8.2 ± 0.5
FCR[b]	(%•h^{-1})	9.4 ± 0.6	7.8 ± 0.5

The HDL clearance rate for a given tissue was expressed in terms of the plasma volume that would contain the same amount of radiolabelled compound (HDL clearance rate per g organ or for a tissue, $\mu l \cdot h^{-1}$/organ = protein-bound dpm into the tissue per h/protein-bound dpm per µl plasma) (table II).

The most potent organs for degradation of HDL (HDL clearance rate for a tissue expressed per g) are the adrenals and spleen. Following these, the liver, kidney, small intestine or cæcum show a similar capacity to accumulate [^{14}C]sucrose HDL (11–27 $\mu l\ h^{-1}$/g).

Expressed per whole organ the liver, skeletal muscle, small intestine and kidney represent the main sites of HDL catabolism, but the spleen, testis or cæcum account for 5–15% of the HDL catabolic activity of the whole liver. Apart from the kidney, most of these results agree with those of Van Tol *et al* (1978), after iodine-labeled HDL or with Pittman and Steinberg (1986) using HDL labeled with ^{125}I tyramine cellobiose-apoA-I. It is noteworthy that after ^{125}I labelling HDL or Apo A-I, the kidney was considered to have higher HDL catabolic activity than the liver, which does not agree with our observation after [^{14}C]sucrose HDL in RICO or in normocholesterolemic animals.

Although the HDL clearance rate per g of skeletal muscle was very low (1.1–1.8

Table II. HDL Clearance rate for each tissue ($\mu l \cdot h^{-1}$) per organ, per unit weight of tissue or expressed as a percentage of the liver HDL clearance rate (in brackets) in normocholesterolemic (SW) and hypercholesterolemic (RICO) rats. Results are expressed as means ± SEM ($N = 5$, SW; $N = 4$, RICO). [a] : $P \leq 0.05$ RICO *vs* SW; [b] : quadriceps femoris muscle; [c] : calculated from an estimated muscular mass of 45% body weight (Donaldson, 1924).

	SW rats				*RICO rats*			
	$\mu l \cdot h^{-1}$/g•organ		$\mu l \cdot h^{-1}$per organ		$\mu l \cdot h^{-1}$/g•organ		$\mu l \cdot h^{-1}$/per organ	
Liver	19.8 ± 1.7	(100)	297.1 ± 18.2	(100)	16.0 ± 1.9	(100)	273.0 ± 19.0	(100)
Skeletal muscle[b]	1.8 ± 0.2	(9)	312.8[c] ± 49.5	(105)	1.1 ± 0.1	(7)	235.2 ± 27.7	(86)
Small intestine	14.2 ± 1.4	(72)	93.3 ± 11.2	(31)	7.7[a] ± 0.4	(48)	68.3 ± 1.4	(25)
Kidney	27.2 ± 1.7	(138)	72.9 ± 7.1	(28)	25.9 ± 2.4	(162)	64.9 ± 6.1	(24)
Spleen	49.1 ± 5.2	(248)	38.6 ± 8.4	(13)	58.8 ± 6.0	(368)	38.6 ± 2.7	(14)
Testis	5.9 ± 0.6	(30)	18.8 ± 1.5		3.9[a] ± 0.6	(24)	12.8[a] ± 1.6	(5)
Caecum	11.6 ± 2.9	(59)	10.5 ± 3.3	(4)	11.3 ± 3.3	(71)	11.6 ± 3.7	(4)
Colon	5.3 ± 1.3	(26)	5.2 ± 1.5	(2)	3.7 ± 0.6	(23)	5.6 ± 1.1	(2)
Adrenals	115.4 ± 4.3	(583)	5.2 ± 0.5	(2)	46.5[a] ± 4.4	(291)	1.9[a] ± 0.2	(1)

$\mu l/h^{-1}$), skeletal muscle, which represents 45% of body weight (Donaldson, 1924), contributed very efficiently to the whole HDL clearance in SW as in RICO rats. It is noteworthy that the tissular HDL clearance rate, expressed per organ or per g of tissue, although generally lower in RICO compared to SW rats, only appears significantly decreased in small intestine, testis and adrenals.

Let us underline, however that the sum of the HDL clearance rates for the tissues reported in table II takes into account only 46–57% of the measured HDL clearance rate for the plasma (about 1 500 µl, table I). This is due to the fact that FCR determined from short experiments (≤ 10 h) only takes into account HDL exchanges between the plasma and extravascular compartment and not tissular uptake and degradation.

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