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Effects of Insulin on the Disposal of ^{14}C-Labelled Very Low Density Lipoprotein Triglycerides in Intact and Hepatectomized Rats

J. Argilés[1] and E. Herrera[2]

[1]Cátedra de Fisiología General, Facultad de Biología, University of Barcelona, and
[2]Departamento de Investigación, Centro 'Ramón y Cajal', Madrid, Spain

Summary. In sham-operated rats, intravenous administration of ^{14}C-very low density lipoprotein triglycerides (with labelled esterified fatty acids) caused an initial decrease and subsequent increase in plasma ^{14}C-lipids of both very low density lipoproteins (VLDL) (density < 1.006) and lipoproteins of density > 1.019. There was a similar change in ^{14}C-lipids in adipose tissue and heart whereas in kidney, spleen and liver, ^{14}C-lipids increased initially and then decreased. Insulin treatment in sham-operated animals decreased circulating ^{14}C-lipids in VLDL and in lipoproteins of density > 1.019, while intermediate density (1.006–1.019) lipoproteins increased. Insulin also enhanced the radioactivity retained in spleen. In functionally hepatectomized rats, ^{14}C-lipids progressively increased in heart. Insulin treatment in these rats enhanced the disappearance from circulation of ^{14}C-VLDL and of lipoproteins of density > 1.019, as well as the appearance of ^{14}C-intermediate density lipoproteins. The appearance of ^{14}C-lipids in white adipose tissue also was augmented, while it decreased in heart and lung. Thus, in sham-operated animals, insulin apparently stimulates the uptake of products of VLDL metabolism by cells in the reticuloendothelial system, while in functionally hepatectomized rats there is increased heart utilization of VLDL triglycerides, and insulin enhances the net extrahepatic catabolism of these lipoproteins.

Key words: Plasma lipoproteins, rat, insulin, spleen, hepatectomy.

Insulin administration is known to reduce the elevated levels of circulating triglycerides and very low density lipoprotein (VLDL)-triglycerides in diabetic subjects [1] and it is well established that insulin enhances the deposition of triacyglycerol fatty acids in adipose tissue by increasing its lipoprotein lipase activity [2–4]. There are, however, situations in which insulin excess is associated with augmented circulating triglyceride concentrations [5–7], and the effects of insulin on lipoprotein lipase activity in other extrahepatic tissues do not follow the changes found in the adipose tissue enzyme [8, 9]. As insulin also stimulates the secretion of newly synthesized VLDL-triglycerides by liver preparations [10, 11], changes in circulating lipoproteins 'in vivo' produced by insulin are the result of a combination of hepatic and extrahepatic effects. In the present study, disassociation of these effects was achieved by determining the changes produced by insulin on the disposal of prelabelled VLDL-triglycerides administered to functionally hepatectomized rats and to sham-operated controls, to determine the effects of the hormone 'in vivo' on extrahepatic VLDL-triglyceride catabolism.

Materials and Methods

Female Wistar rats weighing 160–190 g and fed standard rat chow were maintained in a temperature (22 ± 2 °C) and light cycle (light from 9.00 to 21.00 h) controlled room. Animals were subjected to functional hepatectomy under sodium pentobarbital anaesthesia (40 mg/kg, IP) between 10.00 and 12.00 h, following the method of Russell [12], in which blood vessels supplying the liver (the coeliac axis and portal vein just above the first division to the hepatic lobes) were tied. The medium and left lateral hepatic lobes were ligated [13] and the abdominal wall closed with wound clips. The liver was left in situ but proved to be entirely excluded from circulation as $< 0.09\%$ of injected isotope was recovered in liver 30 min after injection through the inferior vena cava of 1-^{14}C-palmitate bound to 8% bovine albumin, which is known to be taken up readily by the liver [14]. The entire surgical procedure took < 15 min. In sham-operated control rats studied in parallel, laparotomy and handling of the liver were performed as in experimental animals but without tying any vessel.

Immediately after surgery, animals were injected through the inferior vena cava with 1 IU/kg body weight Actrapid-monocomponent insulin (Novo Industri, Copenhagen, Denmark) or with 0.9% NaCl, followed 5 min later by 0.7 ml of rat ^{14}C-labelled VLDL-triglycerides containing 0.35 mg of triacyglycerols, prepared as described below. Animals were sacrificed 5, 15, or 30 min thereafter and blood, lumbar fat pads, kidneys, spleen, liver, heart, lungs, and skeletal muscle from one leg immediately collected. Aliquots of plasma samples were used for counting radioactivity and for purification of lipids [15].

Table 1. Plasma ^{14}C-lipids after the intravenous administration of ^{14}C-VLDL-triglycerides in the rat treated or not with insulin (1 IU/kg)

	Untreated animals			Treated with insulin		
	VLDL (density < 1.006)	IDL (density > 1.006 < 1.019)	LDL + HDL (density > 1.019)	VLDL (density < 1.006)	IDL (density > 1.006 < 1.019)	LDL + HDL (density > 1.019)
Sham-operated control rats						
5 min	7153 ± 1347	960 ± 113	4404 ± 1016	7415 ± 2054	1601 ± 499	4455 ± 1018
15 min	2963 ± 363[c]	1273 ± 192	1838 ± 256[c]	1264 ± 261[2a, c]	2069 ± 286[a]	1096 ± 291[c]
30 min	4045 ± 442	1030 ± 173	3877 ± 940	1215 ± 107[3a, c]	1066 ± 171	716 ± 114[a, 2c]
Hepatectomized rats						
5 min	8378 ± 819	1244 ± 150	4638 ± 713	7831 ± 760	1437 ± 269	4516 ± 790
15 min	6406 ± 696[2b]	1684 ± 233	4805 ± 662	4378 ± 918[b, c]	1843 ± 217[c]	1196 ± 333[2a, 2c]
30 min	4639 ± 475[2c]	1208 ± 114	3773 ± 297	3038 ± 270[a, 3b, 3c]	1899 ± 231[a, b]	2284 ± 208[a, 3b, c]

Results are expressed as disintegrations · min^{-1} · ml^{-1} (mean ± SEM, n = 5). Significance of difference values are as follows: [a]insulin treated versus untreated rats; [b]hepatectomized versus sham-operated control rats; [c]versus corresponding 5 min values. [a, b, c] $= p < 0.05$; [2a, 2b, 2c] $= p < 0.01$; [3a, 3b, 3c] $= p < 0.001$

Other plasma aliquots were used for lipoprotein fractionation by sequential flotation in a Beckman L5-75 ultracentrifuge (Beckman Instruments, Palo Alto, California, USA) as described previously [16].

Rat ^{14}C-labelled VLDL-triglycerides were obtained by IV injection into the tail of other female rats (185–190 g body weight) of 1-^{14}C-palmitate (100 µCi, 51.8 mCi/mmol, Radiochemical Centre, Amersham, Bucks, UK) as described elsewhere [17]. Animals were sacrificed 20 min later under light ether anaesthesia, and blood was collected from the aorta into a syringe containing 100 mg of Na_2-EDTA. Plasma was centrifuged under 0.15 mmol/l NaCl (d = 1.006) containing 1 mmol/l EDTA for 18 h at 143,000 g and 15 °C in a Beckman L5-75 ultracentrifuge. Supernatants were collected with a tube slicer and were purified further by dialysis against three changes of 0.9% NaCl (5l) for a total of 18 h at 4 °C. This preparation contained particles of diameter (150–600) Å when observed under electronmicroscopy [18] and was characterized as rat ^{14}C-VLDL-triglycerides, since it contained only pre-beta lipoproteins on agarose gel electrophoresis [16] and more than 91% of the label appeared as esterified fatty acids of neutral lipids (presumably triglycerides) on fractionation [19]. Radioactivity was measured in a PPO/POPOP scintillation cocktail dissolved in xylene and Triton X-100 and using a Nuclear-Chicago counter (Isocap 300) provided with an external standard device. Radioactive measurements were adjusted to 10^6 d.p.m. for the ^{14}C-labelled VLDL preparation injected into each animal. Statistical comparison between groups was performed with the Student's t-test.

Results

Values of ^{14}C-lipids in plasma lipoprotein fractions at different times after the IV administration of ^{14}C-VLDL-triglycerides are summarized in Table 1. In sham-operated control rats not receiving insulin, radioactivity in lipids of plasma VLDL ($d < 1.006$) and low density lipoproteins plus high density lipoproteins (LDL + HDL) ($d > 1.019$) fractions decreased from 5 to 15 min after tracer injection ($p < 0.05$ for all 15-min versus corresponding 5-min values) but at 30 min, values did not differ significantly from those at 5 min. In contrast, radioactivity in plasma as intermediate density lipoproteins (IDL) ($d > 1.006$, < 1.019) did not change after tracer injection. Prior insulin treatment did not modify the distribution of label in plasma lipoproteins at 5 min but later it reduced the radioactivity in lipids of both VLDL and LDL + HDL, the difference from untreated animals being statistically significant at 15 and 30 min for VLDL and at 30 min for LDL + HDL (Table 1). On the contrary, insulin treatment enchanced ^{14}C-IDL values at 15 min versus values at that time in untreated animals ($p < 0.05$), and this difference disappeared at 30 min (Table 1). When the same experiments were performed in hepatectomized animals not receiving insulin, values of plasma radioactive lipoprotein fractions did not differ from those in sham-operated rats at 5 min. Unlike sham-operated untreated rats, in the hepatectomized animals, there was no change in plasma radioactivity in lipoprotein fractions at 15 min, thus values of VLDL and LDL + HDL were significantly higher than those of sham-operated basal values at this time. These differences disappeared at 30 min due to the rise in values of sham-operated untreated rats and the very small changes in hepatectomized animals. Treatment of hepatectomized rats with insulin showed that radioactivity in lipids in VLDL at 30 min, and in LDL + HDL at 15 and 30 min was significantly reduced, while in IDL it was increased at 30 min compared with values in hepatectomized untreated animals.

At each of the times studied, animals were sacrificed and ^{14}C-lipids determined in organs (Table 2). Per unit of wet weight, the greatest radioactivity in lipids of organs from sham-operated untreated animals was found at 5 min in liver, followed by spleen, lung, heart, skeletal muscle, white adipose tissue, and kidney. In spleen, liver, and kidney, ^{14}C-lipids significantly increased from 5 to 15 min, this change being particularly manifest in the spleen ($p < 0.001$; Table 2). From 15 to 30 min, ^{14}C-lipids in these three organs decreased, values in liver falling to the 5 min value, while those in spleen and kidney remained significantly augmented. Variations of ^{14}C-lipids in adipose tissue, heart and lung with time were opposite to those in spleen, liver, and kidney, although they were statistically significant for heart only (Table 2).

In skeletal muscle, ^{14}C-lipids did not change at the times studied. Insulin treatment in these sham-operated rats enhanced the appearance of ^{14}C-lipids in adipose

Table 2. Tissue ^{14}C-lipids after the intravenous administration of ^{14}C-VLDL-triglycerides in sham-operated or hepatectomized rats treated or not with insulin (1 IU/kg)

	Adipose tissue	Kidney	Skeletal muscle	Spleen	Liver	Heart	Lung
Sham operated rats							
Untreated							
5 min	3056 ± 556	2254 ± 438	5200 ± 727	13226 ± 1970	31448 ± 3718	7093 ± 448	7809 ± 1259
15 min	2128 ± 335	5366 ± 412[3c]	5217 ± 718	40393 ± 4456[3c]	47327 ± 5341[c]	3571 ± 936[c]	6787 ± 1197
30 min	4307 ± 854	4256 ± 568[c]	4050 ± 662	22921 ± 1503[2c]	26881 ± 1637	4175 ± 574[2c]	7009 ± 1232
Insulin treated							
5 min	4878 ± 804	3381 ± 945	4581 ± 605	17236 ± 1785	36336 ± 3351	6139 ± 1273	8177 ± 1540
15 min	4643 ± 504[2a]	3738 ± 302[a]	4787 ± 617	42745 ± 1663[3c]	51478 ± 3038[2c]	3093 ± 231[c]	6130 ± 410
30 min	5018 ± 961	3699 ± 730	4647 ± 532	35460 ± 1621[3a, 3c]	29698 ± 2345	3452 ± 696	5343 ± 806
Hepatectomized rats							
Untreated							
5 min	3827 ± 709	2506 ± 705	7206 ± 1770	241 ± 122[3b]	242 ± 76[3b]	7204 ± 2390	9994 ± 2552
15 min	3202 ± 299[b]	4947 ± 1118	5215 ± 1502	219 ± 35[3b]	189 ± 42[3b]	8552 ± 1622	9869 ± 1642
30 min	2973 ± 433	5585 ± 780[2c]	5154 ± 1103	347 ± 42[3b]	517 ± 60[3b]	12408 ± 1433[3b, c]	11118 ± 888[b]
Insulin treated							
5 min	4052 ± 885	4028 ± 820	6594 ± 1854	390 ± 125[3b]	280 ± 51[3b]	6618 ± 1335	8325 ± 1581
15 min	3402 ± 318	4531 ± 603	4175 ± 1108	282 ± 68[3b]	479 ± 129[3b]	7775 ± 1739[b]	7085 ± 609
30 min	5222 ± 318[2a]	4521 ± 603	4226 ± 974	232 ± 59[3b]	335 ± 60[3b]	8490 ± 676[3b, a]	6396 ± 1696[a]

Results are expressed as disintegrations · min^{-1} · g wet weight^{-1} (mean ± SEM, n = 5). Significance of difference values are as follows: [a]insulin-treated versus untreated rats; [b]hepatectomized versus sham-operated control rats; [c]versus corresponding 5 min values. [a, b, c] = $p < 0.05$; [2a, 2b, 2c] = $p < 0.01$; [3a, 3b, 3c] = $p < 0.001$

tissue and reduced it in kidney at 15 min, while a marked effect was produced in spleen, where the disappearance of ^{14}C-lipids occurring between 15 and 30 min in untreated animals decreased (Table 2). In hepatectomized animals, ^{14}C-lipid values in adipose tissue, kidney and skeletal muscle were similar to those in sham-operated animals (Table 2). On the contrary, radioactivity in both spleen and liver lipids was always < 0.06%/g of the administered tracer, indicating the efficiency of hepatectomy (Table 2). Although ^{14}C-lipid values in heart and lung were the same in hepatectomized and sham-operated animals at 5 min, they rose subsequently in the former versus the latter group, the difference being statistically significant at 15 min for heart and at 30 min for both heart and lung (Table 2). At 30 min, insulin treatment significantly increased ^{14}C-lipids in white adipose tissue while decreasing them in heart and lung (Table 2).

Discussion

The appearance in plasma of radioactive IDL and lipoproteins of $d > 1.019$ (LDL + HDL) 5 min after intravenous administration of ^{14}C-VLDL-triglycerides in sham-operated rats corresponds to the known short (2–5 min) half-life of VLDL [20, 21]. The subsequent disappearance from circulation of both ^{14}C-VLDL and ^{14}C-(LDL + HDL) coincided with a great increase in radioactivity in spleen and liver, and these results may be due to reticuloendothelial tissue uptake of products of extrahepatic VLDL metabolism such as VLDL remnants and LDL. It has been shown recently that VLDL remnants are efficiently taken up by liver [21] and that macrophages and reticuloendothelial tissues contribute substantially to receptor-independent LDL catabolism [22, 23]. Retention of radioactivity by the spleen after insulin treatment coincided with a decrease in circulating ^{14}C-(LDL + HDL) demonstrating for the first time that insulin stimulates the uptake of VLDL metabolism products (presumably LDL) by cells of the reticuloendothelial system. This finding may be important in view of present evidence that hyperinsulinaemia is a risk factor for atherosclerosis [24].

In functionally hepatectomized rats, the appearance of ^{14}C-lipids in plasma IDL and LDL + HDL after administration of ^{14}C-VLDL-triglycerides demonstrates the capacity of the rat to degrade VLDL to lipoproteins of higher density without the intervention of the liver, in agreement with Suri et al. [25]. The conversion of VLDL to IDL is catalyzed by the action of extrahepatic lipoprotein lipase [26], releasing non-esterified fatty acid (NEFA) from the hydrolysis of VLDL-triglycerides which are taken up by the subjacent tissue. After functional hepatectomy in the rat, this process seems to be enhanced in heart, and less markedly so in lung, as shown by the rise in ^{14}C-lipids in these tissues after administration of ^{14}C-VLDL-triglycerides. Increased formation of glyceride glycerol from administered glycerol has been reported previously in the heart of hepatectomized-nephrectomized rats [27]. All these data indicate that hepatectomy enhances heart and lung lipoprotein lipase activities and that NEFA released from VLDL-triglyceride hydrolysis are subsequently taken

up by these tissues and re-esterified with α-glycerol phosphate formed from circulating glycerol or other substrates (i.e., glucose). This interpretation is in agreement with the observed insulin effect decreasing ^{14}C-lipid appearance in these tissues in hepatectomized rats, since this hormone is known to inhibit lipoprotein lipase activity in heart [8, 9] and has been shown to decrease the incorporation of circulating glycerol into lipids of both heart and lung in hepatectomized rats [28]. These negative effects of insulin on the uptake of fatty acids from circulating VLDL-triglycerides in heart and lung were overcompensated for by opposite effects in other extrahepatic tissues, as shown by the accelerated disappearance of ^{14}C-VLDL-triglycerides from the circulation and the enhanced levels ^{14}C-IDL in functionally hepatectomized rats. The insulin effect of increasing the appearance of ^{14}C-lipids in adipose tissue in these animals is in agreement with the hormonal effect of lipoprotein lipase enhancement in this tissue [2–4, 8, 29]. The latter, together with the relatively large mass of adipose tissue indicate that it is the main tissue responsible for the enhanced disappearance from circulation of VLDL-triglycerides produced by insulin in functionally hepatectomized rats.

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Professor E. Herrera
Departamento de Investigación
Centro Ramón y Cajal
Ctra. de Colmenar, KM 9
E-Madrid 34
Spain