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Low density lipoprotein receptor-binding activity in human tissues: Quantitative importance of hepatic receptors and evidence for regulation of their expression *in vivo*

(cholesterol/cholestyramine/low density lipoproteins/liver/metabolic regulation)

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ABSTRACT **The heparin-sensitive binding of ^{125}I-labeled low-density lipoprotein (LDL) to homogenates from 18 different normal human tissues and some solid tumors was determined. The binding to adrenal and liver homogenates fulfilled criteria established for the binding of LDL to its receptor—namely, (*i*) saturability, (*ii*) sensitivity to proteolytic destruction, (*iii*) inhibition by EDTA, and (*iv*) heat sensitivity. When the binding of ^{125}I-labeled LDL was assayed at a constant concentration (50 μg/ml), the adrenal gland and the ovary had the highest binding of normal tissues. The highest binding per g of tissue overall was obtained in homogenates of a gastric carcinoma and a parotid adenoma. When the weights of the parenchymatous organs were considered, the major amount of LDL receptors was contained in the liver. To study the possible regulation of hepatic LDL-receptor expression, 11 patients were pretreated with cholestyramine (8 g twice a day for 3 weeks). Increased binding activity (+105%, $P < 0.001$) was obtained in homogenates from liver biopsies from the cholestyramine-treated patients as compared with 12 untreated controls. It is concluded that the liver is the most important organ for LDL catabolism in humans and that the receptor activity in this organ can be regulated upon pharmacologic intervention. Further studies are needed to confirm the possibility that certain solid tumors can exhibit high numbers of LDL receptors.**

Elevated levels of plasma cholesterol are associated with an enhanced risk for the development of coronary heart disease (1). The major fraction of cholesterol in human plasma is contained within low density lipoproteins (LDL), and the reduction of this lipoprotein fraction by pharmacologic intervention has been shown to decrease the incidence of coronary events (2). The concentration of LDL in plasma is determined by the balance between the synthesis (occurring from the metabolism of very low density lipoproteins) and the catabolism of the lipoprotein particle. Of particular importance for the clearance of LDL is the presence of specific, high-affinity cell-surface receptors for these lipoproteins, the LDL receptors (3, 4).

The LDL receptor, originally described by Goldstein and Brown (5) in cultured human fibroblasts, has been demonstrated in a variety of cell types (3). After binding to its receptor, the LDL particle is internalized by endocytosis and transported to lysosomes, where it is degraded to free cholesterol and amino acids. The number of LDL receptors is regulated according to the cellular demand for cholesterol (3, 4). Thus, cells synthesizing steroid hormones and rapidly growing cells have high numbers of LDL receptors (3). An increased expression of LDL receptors has also been described in certain forms of leukemia (6, 7). A specific metabolic abnormality exists in the genetic disease familial hypercholesterolemia, where functional LDL receptors are absent (homozygotes) or are produced in reduced numbers (heterozygotes) (8).

Animal studies have indicated that the adrenal cortex and corpus luteum express the highest LDL-receptor activities (9). From a quantitative view, the liver appears to be the major site of LDL catabolism (10–13), and the degree of expression of hepatic LDL receptors seems to regulate the plasma LDL concentrations in several species (4, 14, 15). Virtually no information is available on the distribution of LDL receptors in adult humans. A series of attempts to measure the amount of LDL receptors in human liver has been made (16–22), but the data are somewhat inconclusive. Particularly, it has been difficult to establish whether or not regulation of hepatic LDL-receptor expression may occur in humans. Such knowledge is of major importance, not only for our understanding of lipoprotein and cholesterol metabolism, but also for our possibilities of influencing plasma LDL levels and thus interfere with the process of atherosclerosis.

In the present study, we have used a specific assay for the binding of ^{125}I-LDL to homogenate particulates (23) to study LDL-receptor expression in various adult human tissues. Particularly, we asked the questions: (*i*) What is the *in vivo* distribution of LDL receptors in various organs? and (*ii*) Can the expression of hepatic LDL receptors be regulated by interference with cholesterol metabolism? In this report, we present evidence that the expression of LDL receptors is high in human adrenal cortex and in premenopausal ovaries, that the liver contains a major fraction of LDL receptors in human *in vivo*, and that this amount of receptors can be regulated. Finally, we present preliminary evidence that some forms of human solid tumors may express an increased amount of LDL receptors.

MATERIALS AND METHODS

Patients and Tissue Sampling. Tissues were obtained from 58 patients undergoing elective surgery. One adrenal gland was removed at nephrectomy because of renal carcinoma. Ovaries were obtained at hysterectomy of a 58-yr-old postmenopausal woman with myoma of the uterus and at oophorectomy of a 48-yr-old premenopausal female with breast cancer. Testes were from two males (71 and 80 yr) with prostatic cancer undergoing orchidectomy. Liver samples were obtained from the left liver lobe of 23 patients (25–76 yr) operated for uncomplicated cholesterol gallstones (cf. ref. 24). All of these patients were normolipidemic and of normal weight (relative body weight <115%); 11 had been pretreated with cholestyramine (Questran, 8 g twice daily for 3 weeks).

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Abbreviations: LDL, low density lipoproteins; ^{125}I-LDL, ^{125}I-labeled LDL.

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Biopsies from adipose tissue, skeletal muscle, and skin were also obtained at cholecystectomy. Samples of normal small intestine (three jejunum and one ileum) were obtained at surgery because of inflammatory bowel disease. Normal splenic tissue was from a 59-yr-old male with idiopathic thrombocytopenia. Normal brain tissue was obtained from patients operated for brain tumor disease. Benign and malignant tumors and adjacent normal tissues were obtained from operated patients who were otherwise in good health.

The studies were approved by the Ethics Committee of the Karolinska Institute, and informed consent was obtained from each patient before surgery.

Materials. Na ^{125}I (carrier-free, pH 7–11) was obtained from The Radiochemical Centre, Amersham, U.K. Bovine serum albumin (fraction V) and EDTA were purchased from Sigma. Heparin, 10,000 international units/ml, was from AB Kabi, Stockholm. Durapore membrane filters were obtained from Millipore, Bedford, MA. Pronase from *Streptomyceus griseus* (77,000 international units/g, lot 202867) was purchased from Calbiochem–Behring. Newborn calf serum was from GIBCO–Biocult Ltd., Paisley, Scotland. All chemicals were of analytical grade.

Lipoprotein Preparation. LDL (density, 1.019–1.063 g/ml) were isolated from serum of healthy blood donors by ultracentrifugation, as described (25). LDL were quantified as protein by the method of Lowry *et al.* (26) with bovine serum albumin as standard. LDL were labeled with ^{125}I as described by Langer *et al.* (27), the specific activity ranging from 250 to 650 cpm/ng. The labeled LDL were handled and stored as described (25).

Preparation of Tissue Homogenates. The removed tissues were immediately placed in ice-cold phosphate-buffered saline (PBS; 140 mM NaCl/2.7 mM KCl/9.5 mM phosphate buffer, pH 7.4). The tissues were homogenized on ice in buffer A (50 mM NaCl/20 mM Tris·HCl/1.0 mM $CaCl_2$, pH 7.5) within 4 hr after excision, as described in detail elsewhere (28). In brief, 0.1–0.6 g of tissue (wet weight) was homogenized in 1.2–2.4 ml of buffer A. The homogenates were pooled and filtered through a nylon net (28) and thereafter stored at −20°C until assayed. The protein content of the homogenates was determined as described for LDL.

Binding Reaction with ^{125}I-Labeled LDL (^{125}I-LDL). Homogenates were thawed, and binding assays were performed in an albumin-containing Tris·HCl buffer, as described (28), with the indicated amounts of homogenate (protein) and ^{125}I-LDL. Binding reactions were started by the addition of ^{125}I-LDL, and the mixtures were incubated for 1 hr on ice. In some experiments, unlabeled LDL or EDTA were also included in the mixtures.

Washing of Tissue Homogenate Particulates and Release of ^{125}I-LDL by Heparin. Aliquots from the binding-reaction mixtures were applied on filters, washed, and subsequently incubated in the presence or absence of heparin, as described elsewhere (23). ^{125}I radioactivity was determined in a Packard Autogamma model 800 C γ counter. Blank values (<0.04% of the radioactivity added to filters) were obtained from parallel incubations without homogenate and have been subtracted from the results presented.

The heparin-sensitive binding of ^{125}I-LDL was calculated by subtracting the radioactivity of filters on which homogenate particulates had been incubated with heparin (heparin-resistant binding) from the radioactivity of filters on which homogenate particulates had been incubated without heparin (total binding). The LDL-displaceable binding of ^{125}I-LDL was calculated by subtracting the radioactivity of filters where binding reactions had been performed with unlabeled LDL in excess (LDL-resistant binding) from the radioactivity of filters where the binding reactions had been performed in the absence of unlabeled LDL (total binding).

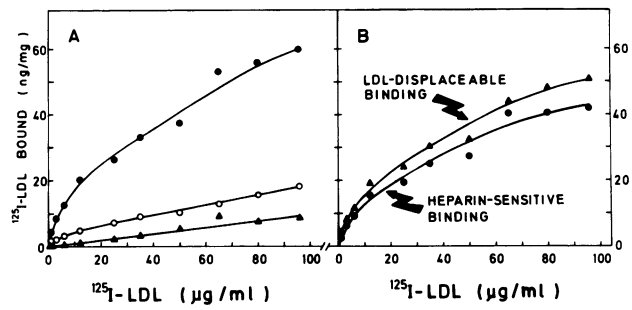

FIG. 1. Binding of ^{125}I-LDL to homogenate particulates from adrenal cortex as a function of ^{125}I-LDL. Aliquots from adrenal cortex homogenate (final concentration 3.3 mg of protein/ml) were incubated with ^{125}I-LDL, as indicated, on ice. (*A*) After 1-hr incubation, 60-μl aliquots (200 μg) were placed on filters for wash and subsequent determination of total (●) and heparin-resistant (○) binding, as described. ▲, Binding of ^{125}I-LDL when incubations were performed in 20-fold excess of unlabeled LDL (990 μg/ml). (*B*) Calculated heparin-sensitive (●) and LDL-displaceable binding (▲) of ^{125}I-LDL. Values for *A* and *B* are from single determinations.

RESULTS

To determine the binding characteristics of ^{125}I-LDL to homogenate particulates from human tissues, the first series of experiments was made on a homogenate from the adrenal cortex because this tissue was expected to contain high concentrations of LDL receptors (9, 28). When adrenal cortex homogenates were incubated with progressively increased higher concentrations of ^{125}I-LDL, both the heparin-sensitive binding and the LDL-displaceable binding of ^{125}I-LDL showed saturability (Fig. 1). Scatchard analysis (29) of the heparin-sensitive binding was compatible with the presence of high- and low-affinity binding sites (data not shown). K_d for the high-affinity site was 9 μg/ml, and B_{max} was 27 ng/mg of protein; saturation of binding at this site was present at 50 μg/ml. The receptor binding of LDL is inhibited by EDTA (3, 5, 9). To determine the influence of EDTA on the binding of ^{125}I-LDL, aliquots from the adrenal cortex homogenate were incubated with ^{125}I-LDL at progressively increased higher concentrations of EDTA in the presence and absence of an excess of unlabeled LDL (Fig. 2). At 25 mM of EDTA, 90% of the initial heparin-sensitive binding was abolished, whereas 65% of the LDL-displaceable binding of ^{125}I-LDL remained.

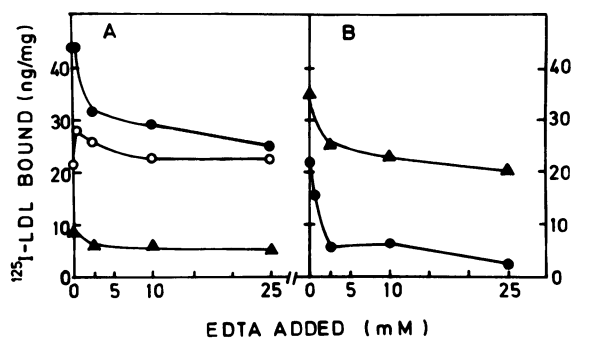

FIG. 2. Influence of EDTA on binding of ^{125}I-LDL to homogenate particulates from adrenal cortex. Aliquots from adrenal cortex homogenate (final concentration, 1.7 mg of protein/ml) were incubated for 1 hr on ice with 50 μg/ml of ^{125}I-LDL and EDTA, as indicated. (*A*) Sixty-microliter aliquots from binding-reaction mixtures (100 μg) were finally added to filters for wash and determination of total (●) and heparin-resistant (○) ^{125}I-LDL binding, as described. ▲, Binding of ^{125}I-LDL when incubations occurred in 20-fold excess of unlabeled LDL (990 μg/ml). (*B*) Calculated heparin-sensitive binding (●) and LDL-displaceable binding (▲). Values in *A* and *B* are from single determinations.

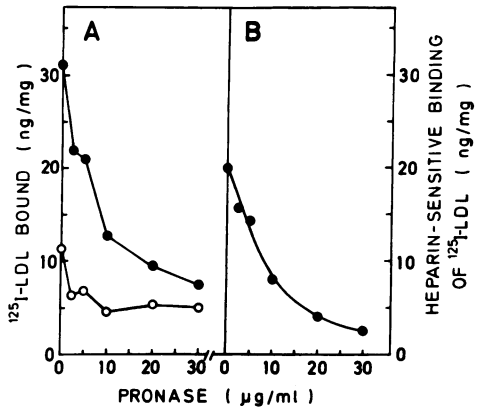

FIG. 3. Effect of proteolytic digestion of adrenal cortex homogenate before the binding reaction with ^{125}I-LDL. Aliquots from adrenal cortex homogenate (final concentration, 6.6 mg of protein/ml) were incubated for 10 min at 37°C in a shaking water bath with the indicated concentrations of Pronase in buffer A. Incubations were terminated by chilling the tubes in an ice-water bath. ^{125}I-LDL was thereafter added (50 μg/ml). (*A*) After incubating the homogenates for 1 hr on ice, 60-μl aliquots (150 μg) were applied on filters for wash and determination of total (●) and heparin-resistant (○) binding of ^{125}I-LDL. (*B*) Calculated heparin-sensitive binding of ^{125}I-LDL. Values for *A* and *B* are from duplicate determinations.

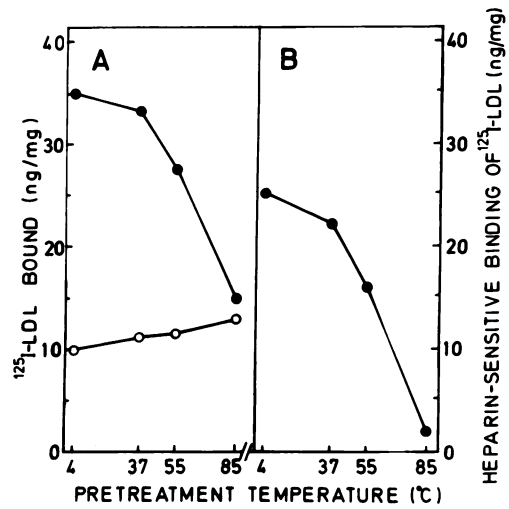

FIG. 4. Effect of pretreating aliquots from adrenal cortex homogenate at various temperatures before the binding reaction with ^{125}I-LDL. Aliquots (440 μg) were incubated for 10 min at the indicated temperatures in a shaking water bath. Incubations were stopped by putting the tubes in an ice-water bath. The binding reaction with ^{125}I-LDL was then started by adding ^{125}I-LDL at 50 μg/ml. (*A*) After incubating homogenates for 1 hr on ice, 60-μl aliquots (170 μg) were added to filters for wash and determination of total (●) and heparin-resistant (○) binding of ^{125}I-LDL. (*B*) Calculated heparin-sensitive binding of ^{125}I-LDL. Values for *A* and *B* are from duplicate determinations.

Fig. 3 shows the influence of incubating aliquots of the adrenal cortex homogenate with progressively increased higher concentrations of the proteolytic enzyme Pronase for 10 min at 37°C before the binding reaction with ^{125}I-LDL. The heparin-sensitive binding of ^{125}I-LDL already decreased when Pronase concentration was 2.5 μg/ml (Fig. 3*B*); at 30 μg/ml, 90% of the initial heparin-sensitive binding was abolished. The effect of heat pretreatment of the adrenal cortex homogenate before the binding reaction with ^{125}I-LDL is shown in Fig. 4. Pretreatment of homogenates already at 37°C for 10 min decreased the heparin-sensitive binding by 10% (Fig. 4*B*). When homogenates were pretreated at 55°C and 85°C, the heparin-sensitive binding was reduced by 40 and 90%, respectively.

Studies using homogenates from normal liver revealed similar properties of the heparin-sensitive binding of ^{125}I-LDL (Fig. 5). Analysis of the binding curves was also compatible with a high-affinity binding site with K_d of 15 μg/ml and B_{max} of 4.9 ng/mg of protein. In contrast to what has been reported for binding of LDL to human liver membranes (18, 19), the heparin-sensitive binding was reduced when liver homogenates were preincubated for 15 min at 37°C (data not shown).

To determine the distribution of LDL receptors in the human body, the heparin-sensitive binding of ^{125}I-LDL was assayed on homogenates from various human tissues by using a constant concentration (50 μg/ml) of radiolabeled LDL (Fig. 6). The highest binding activities of the normal tissues were found in homogenates from the cortex of the adrenal gland and from the ovary of a premenopausal female. Relatively high binding was seen in the kidney, small bowel, liver, gallbladder, spleen, parathyroid, the ovary from a postmenopausal female, and salivary glands, whereas the binding to skeletal muscle, brain, skin, adipose tissue, colon, thyroid gland, testis, and prostate was low.

When binding was expressed as μg of ^{125}I-LDL bound per g (wet weight) of tissue, <0.1 μg of binding per g was present in brain, adipose, skeletal muscle, skin, prostate, and colon tissue. Testis, gallbladder, thyroid, and postmenopausal

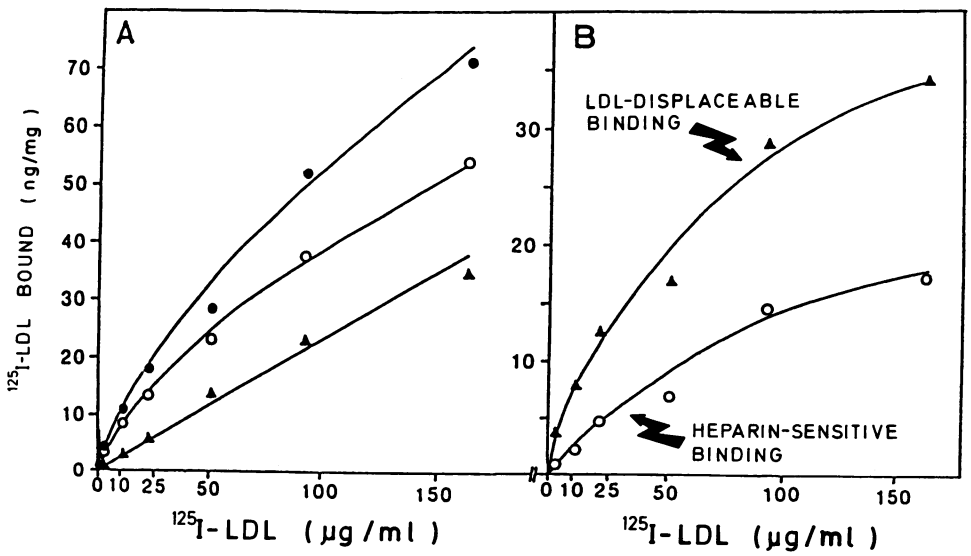

FIG. 5. Binding of ^{125}I-LDL to normal liver homogenate as a function of ^{125}I-LDL in the binding-reaction mixture. Aliquots from liver homogenate (final concentration 3000 μg/ml) were incubated with ^{125}I-LDL, as indicated. (*A*) After incubation, 240 μg of homogenate were placed on filters for wash and subsequent determination of total (●) and heparin-resistant (○) binding, as described. ▲, Binding of ^{125}I-LDL when binding reactions were done with excess unlabeled LDL. (*B*) Calculated heparin-sensitive (○) and LDL-displaceable binding (▲) of ^{125}I-LDL. Values in *A* and *B* are from single determinations.

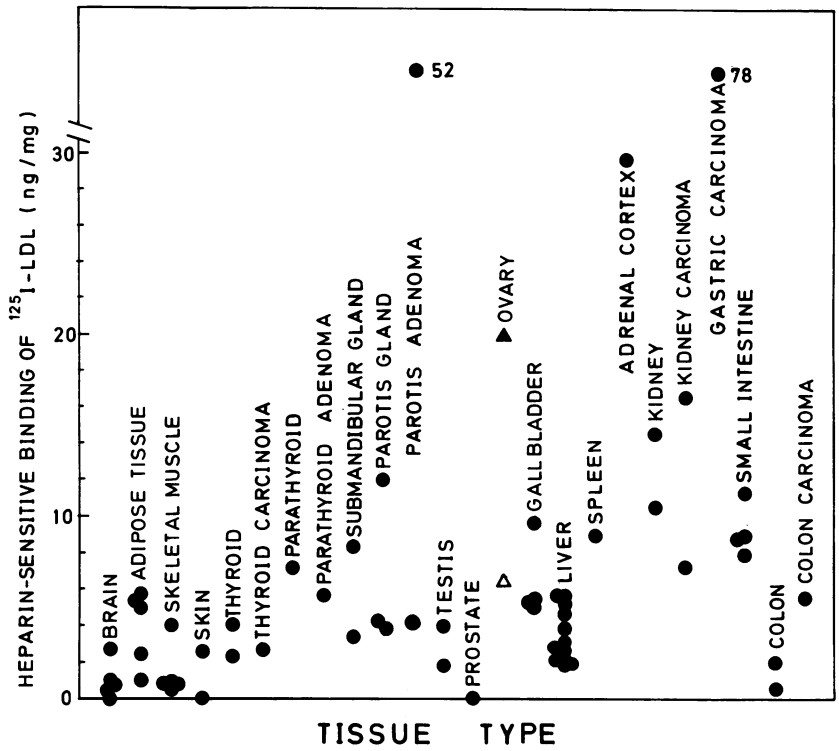

FIG. 6. Heparin-sensitive binding of ^{125}I-LDL to homogenate particulates of various human tissues. Tissue homogenates were prepared and incubated (final concentration, 1.0–3.0 mg of protein per ml) with ^{125}I-LDL at 50 μg/ml, as described. Aliquots from incubations (80–240 μg of protein) were then added to filters for wash and determination of the heparin-sensitive binding of ^{125}I-LDL, as described. For every tissue type, each point is the average from triplicate determinations from a separate individual. ▲, Tissue obtained from a 48-year-old menstruating woman; △, tissue obtained from a 57-year-old postmenopausal woman. Off-scale values are indicated.

ovary displayed binding values of 0.1–0.3 μg/g. Submandibular, parotid, and parathyroid glands bound 0.4, 0.5, and 0.7 μg/g, respectively. Binding values expressed as μg per g of tissue were highest in adrenal cortex (2.8), followed by premenopausal ovary (1.2), kidney (1.1), spleen (1.1), liver (0.8), and small intestine (0.5).

To determine whether metabolic regulation of LDL receptor expression may occur in humans, we studied the heparin-sensitive binding of ^{125}I-LDL to liver homogenates from 11 patients treated with cholestyramine for 3 weeks before cholecystectomy. This treatment, which interrupts the enterohepatic circulation of bile acids, was expected to increase the hepatic demand for cholesterol. Table 1 shows that cholestyramine treatment of gallstone patients was associated with a 2-fold increase in hepatic LDL-receptor-binding activity as compared with controls ($P < 0.001$).

Tumor tissues showed considerable variability (Fig. 6): homogenates from a gastric carcinoma and one parotid pleomorphic adenoma displayed very high binding [7.2 and 2.5 μg/g (wet weight), respectively], whereas no difference between normal and tumor tissue could be demonstrated in the kidney, thyroid, or parathyroid gland. One case of colon cancer had higher binding than normal colon tissue.

DISCUSSION

The heparin-sensitive binding of ^{125}I-LDL in homogenates prepared from fresh human adrenal cortex tissue and liver shares important features established for LDL-receptor binding (3, 9)—namely, (*i*) saturability, (*ii*) sensitivity to proteolytic digestion, (*iii*) heat sensitivity, and (*iv*) inhibition by EDTA. These results indicate that this binding represents LDL-receptor-bound ^{125}I-LDL, as has been shown for homogenates from cultured cells (23) and bovine tissues (28). At a ^{125}I-LDL concentration of 50 μg/ml, 80–90% of the heparin-sensitive binding fulfills stringent criteria for specific LDL-receptor function; for LDL-displaceable binding this is clearly not the case. The use of tissue homogenates instead of membranes for the binding assay should have minimized the problems related to receptor recovery (9).

When homogenates from normal human tissues were assayed for the heparin-sensitive binding of ^{125}I-LDL, the highest binding was seen in homogenates from the cortex of the adrenal gland and the ovary from a premenopausal woman. Relatively high binding was found in the small bowel, kidney, spleen, liver, postmenopausal ovary, parathyroid, parotid, and submandibular gland, whereas the binding to homogenates from skeletal muscle, brain, skin, adipose tissue, colon, thyroid gland, testis, and prostate was low. The findings are overall in agreement with previous results on the binding of LDL to purified membrane preparations (9) and crude homogenates (28) from fresh bovine tissues.

Table 1. Binding of ^{125}I-LDL (heparin-sensitive binding) to homogenates prepared from liver biopsies from 23 patients with uncomplicated gallstone disease

Control		Cholestyramine	
Sex/age, yr	^{125}I-LDL bound, ng/mg of protein	Sex/age, yr	^{125}I-LDL bound, ng/mg of protein
F/72	5.3	F/76	7.7
F/66	3.9	F/65	5.8
F/65	1.6	F/63	7.1
F/63	4.7	F/58	6.9
F/58	2.6	F/55	9.1
F/52	5.7	F/55	9.4
F/51	3.3	F/46	6.1
M/49	5.7	M/43	4.9
F/42	2.8	F/37	10.1
F/39	1.8	F/37	5.2
F/32	1.5	F/25	4.6
F/32	2.0		
Mean 52 ± 4 (SEM)	3.4 ±0.5	51 ±5	7.0* ±0.6

Twelve patients were untreated controls, and 11 patients had been treated with cholestyramine (Questran) before operation, as described. The heparin-sensitive binding of ^{125}I-LDL (ng/mg of protein) was determined after adding 0.24 mg of tissue (protein) to filters from incubations where homogenates (3.0 mg/ml) had been incubated with ^{125}I-LDL at 50 μg/ml. M, male; F, female.
*Significantly different from controls, $P < 0.001$ (Wilcoxon rank sum test).

Although homogenates from the liver did not exhibit the highest binding per g of tissue (Fig. 5), this organ had the highest total content of LDL receptors when total weight of the parenchymatous organs was considered. When the binding data were related to wet weight of tissue, the liver bound ≈0.8 μg of ^{125}I-LDL per g. Because these values are lower than those reported by several other investigators (17–19, 22), it is relevant to consider the possible physiological role of the measured (highly specific) heparin-sensitive binding of LDL to liver tissue. If the recirculation time for the LDL receptors of ≈6 min, as calculated by Goldstein *et al.* (30) from experiments on cultured fibroblasts, is also valid *in vivo*, it can be estimated that a normal 1500-g liver would have a daily receptor-mediated uptake of ≈300 mg of LDL protein. This would be consistent with the notion that ≈40% of the catabolism of LDL occurs by means of hepatic receptors because ≈700 mg of LDL protein is cleared from plasma each day (3). Although such calculations are obviously of limited validity, from our data the organs of major quantitative importance for LDL elimination *in vivo* would be the liver, small intestine (≈120 mg/day), and the kidney (≈100 mg/day); this is in good agreement with *in vivo* estimations of relative LDL-receptor tissue distribution in several other species (12, 13, 31).

Of particular importance was the evidence of regulation of hepatic LDL receptors. Thus, patients treated with cholestyramine, an agent that interferes with bile acid enterohepatic circulation (32), displayed a 2-fold increase in the binding of ^{125}I-LDL in their liver homogenates. This therapy, which increases bile acid production considerably (32), is known to enhance receptor-mediated clearance of plasma LDL in humans (33) and to lower LDL plasma levels. A stimulation of LDL fractional catabolic rate of ≈30% has been seen in hypercholesterolemic patients given cholestyramine (33). This result would agree well with the ≈100% stimulation of hepatic receptors observed in our work. Preliminary studies have indicated that cholestyramine treatment concomitantly leads to a 6-fold increase in the hepatic synthesis of cholesterol (34), a response that probably counteracts the more profound expression of hepatic LDL receptor. Further studies of the regulation of hepatic synthesis of cholesterol and LDL receptor expression will be of great therapeutic interest.

In the preliminary studies reported here, the highest binding overall was found in tumors from the stomach and the parotid gland. In contrast, tumors from the kidney, thyroid, or parathyroid did not show higher binding compared with their normal counterparts. A more detailed knowledge of LDL-receptor activity in human solid tumors is of interest for several reasons. (*i*) It may establish the cause–effect relationship between hypocholesterolemia and cancer. (*ii*) The number of LDL receptors in tumors may provide prognostic information, as recently indicated for breast cancer (35). (*iii*) This knowledge may be of value in developing treatment for cancer patients because specific cholesterol-synthesis blocking agents, such as lovastatin, and LDL-bound toxic drugs kill cells that depend on cellular synthesis of cholesterol or have high numbers of LDL receptors, respectively (25, 36–39).

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