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Complete down-regulation of low-density lipoprotein receptor activity in human liver parenchymal cells by β-very-low-density lipoprotein

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The effect of LDL and β-VLDL on the expression of the LDL receptor is studied in cultured human parenchymal cells. The high affinity binding of [^{125}I]LDL to cultured human parenchymal cells was down regulated to 37.3 ± 2.9% and 24.0 ± 2.6% of the control value, after preincubation with LDL or β-VLDL for 22 h, respectively. When LDL receptor synthesis was blocked at 22 h a residual receptor activity of 29% is noticed, indicating a half-life of LDL receptors in human parenchymal cells of 12 h. It is concluded that LDL receptor expression on human liver parenchymal cells is subject to complete down-regulation by β-VLDL, which may be held responsible for the cholesterol-rich diet induced down-regulation of LDL receptors, in vivo.

Low-density lipoprotein; β-very-low-density lipoprotein; LDL-receptor; Human parenchymal liver cell; Receptor-regulation

1. INTRODUCTION

Under physiological conditions two-thirds of the plasma LDL in man is cleared via the LDL receptor-mediated pathway [1]. The majority of the LDL receptor activity is localized in the liver, the only organ where cholesterol can be irreversibly removed from the body via secretion into the bile [2]. The major role of the liver in the removal of LDL cholesterol from the plasma was clearly illustrated by the finding that liver transplantation of a normal liver into a patient with homozygote familial hypercholesterolemia, which lacked the LDL receptor, resulted in an 81% decrease in plasma LDA cholesterol level [3].

Low-density lipoprotein receptors have been identified in the livers of a variety of animals like hamsters [1], rabbits [4] and rats [5]. In human liver, LDL receptor activity has been demonstrated in liver membrane preparations [6–8] and in cultured human parenchymal liver cells [9–11].

Since the liver is the decisive site for the regulation of blood cholesterol levels, we performed studies on the regulation of LDL receptor activity in human liver cells. Knowledge of the regulation of the low density lipoprotein receptor activity is mainly based on studies with fibroblasts [2,12] or hepatoma cell lines [13,14]. In human parenchymal liver cells it was observed that LDL receptors can only be down-regulated partially by preincubation of the cells with LDL [9–11]. It was speculated that the inability of LDL to block receptor synthesis completely, would allow the liver to take up LDL even at high circulating LDL levels [11]. Spady and Dietschy [15] however, showed in hamsters, that a diet rich in cholesterol and saturated triacylglycerols essentially abolished the receptor-dependent clearance of LDL from the blood. Under those conditions a specific VLDL fraction called β-VLDL is the major cholesterol(ester) carrying particle [16]. When radiolabelled β-VLDL is injected into rats the particles are rapidly taken up from the blood circulation by liver parenchymal cells [17,18].

In the present study we therefore investigated the regulation of LDL receptor activity in primary cultured human liver parenchymal cells by β-VLDL, in order to test to what extent β-VLDL can be responsible for the complete down-regulation of liver LDL receptors.

Abbreviations: LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; β-VLDL, cholesteryl ester-rich β-migrating very-low-density lipoprotein.

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2. MATERIALS AND METHODS

2.1. *Materials*

Fetal calf serum, penicillin and streptomycin were from Boehringer Mannheim (Mannheim, Germany). William's E medium and kanamycin were from flow laboratories (Irvine, Scotland, UK). Human serum albumin (fraction V) was from Sigma (St. Louis, MO, USA) and ^{125}I in NaOH was from Amersham (Buckinghamshire, UK).

2.2. *Isolation and culturing of human parenchymal cells*

Parts of human liver were obtained through the auxiliary liver transplantation program, which is carried out at the University

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Hospital Dijkzigt in Rotterdam, the Netherlands. Permission was given by the Medical Ethical Committee to use the remaining, not transplanted, part of the donor liver for scientific research. Human parenchymal liver cells were isolated as described before [19]. The parenchymal cells were cultured in 12-well plastic culture dishes (22-mm diameter) at 0.5×10^6 cells/well in Williams' E medium, supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 20 mU/ml insulin, 1 nM dexamethasone, 100 U penicillin, 100 μg/ml streptomycin and 50 μg/ml kanamycin at 37°C in a humidified 5% CO_2/95% air atmosphere. The medium was renewed 6 h after seeding and every 24 h thereafter. The viability of the cells directly after isolation was between 50–77%. Experiments with cultured parenchymal cells were performed between the second and the sixth day after seeding of the cells. At this stage the viability of the cells was more than 95%. The hepatocytes used in this study were non-dividing cells able to accumulate taurocholic acid intracellularly to the same extent as cultured rat hepatocytes [20]. Furthermore, the cells could synthesize and secrete VLDL, LDL, HDL (with VLDL as the major species, namely 68 ± 9%); apo B, apo A-I, apo A-II, apo E, apo-CII [21], albumin, and plasminogen activator inhibitor 1 [22]. Also, the transport and metabolism of thyroid hormones was qualitatively comparable to these processes in rat hepatocytes [23] and the cells do express a specific high-affinity site for HDL [24].

2.3. *Isolation and labeling of serum lipoproteins*

β-VLDL was obtained from fasted serum of male Wistar rats that were maintained on a cholesterol-rich diet (Hope Farms, Woerden, The Netherlands) that included 2% cholesterol, 5% olive oil and 0.5% cholic acid [17]. LDL and VLDL were obtained from fasted sera from healthy volunteers. Rat β-VLDL, human LDL and human VLDL were isolated according to Redgrave [25] followed by a second identical centrifugation. Cholesterol and cholesteryl content of LDL, VLDL and β-VLDL was determined enzymatically, using a CHOD-PAP kit (Boehringer Mannheim). LDL and β-VLDL were iodinated at pH 10 by the ICl method as modified by Bilheimer [26].

2.4. *Lipoprotein binding and association by cultured parenchymal cells*

Twenty-four hours before the start of the experiments, culture medium was removed and replaced by Williams' E medium containing 1% (w/v) human serum albumin. Preincubations for 22 h were performed with human LDL, human VLDL and rat β-VLDL in culture medium with 1% human serum albumin. At the end of the preincubations, cells were washed twice and incubated at 37°C for 10 min, washed again and incubated for 30 min in culture medium. This washing procedure was followed by incubations for either 3 h at 4°C or 3 h at 37°C with [^{125}I]LDL or with [^{125}I]β-VLDL with or without 300 μg/ml unlabeled lipoprotein as indicated in the figures. At the end of the incubation, the medium was removed and cells were washed five times with an ice-cold containing 0.15 M NaCl, 50 mM Tris-HCl and 2.5 mM $CaCl_2$ (pH 7.4) and 0.2% bovine serum albumin, followed by two washes with the same solution without bovine serum albumin. The cells were then dissolved in 0.1 N NaOH and cell-associated radioactivity and protein content were measured. Binding of [^{125}I]LDL was determined as cell-associated radioactivity after 3 h incubation at 4°C. Association (binding and uptake) of LDL was determined as cell-associated radioactivity after 3 h incubation at 37°C. Data were statistically analyzed with a two-tailed, unpaired Student's *t*-test.

3. RESULTS

The binding of [^{125}I]LDL to human parenchymal liver cells in the presence or absence of 300 μg/ml unlabeled LDL is shown in Fig. 1. High-affinity binding became saturated at 25 μg of LDL per ml and the maximal binding level of [^{125}I]LDL to human parenchymal liver cells was 75 ng LDL per mg cell protein. The maximal high-affinity binding level of [^{125}I]LDL

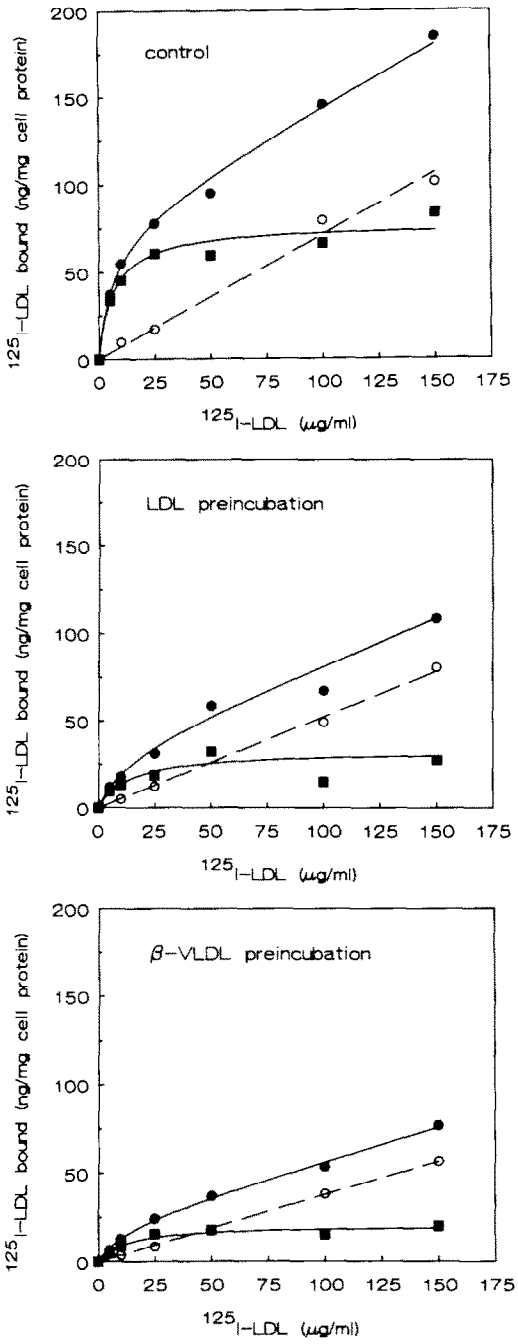

Fig. 1. The effect of the concentration of [^{125}I]LDL in the absence (•) or presence (○) of 300 μg/ml unlabeled LDL on the extent of binding to cultured human liver parenchymal cells. Cells were not preincubated (control) or preincubated for 22 h with 100 μg/ml LDL or 100 μg/ml β-VLDL, followed by an incubation for 3 h at 4°C with the indicated amounts of [^{125}I]LDL. High-affinity binding (■) was derived by subtracting non-specific binding, which was measured in the presence of 300 μg/ml unlabeled LDL, from total binding. Binding of [^{125}I]LDL to parenchymal cells is expressed as ng LDL per mg cell protein.

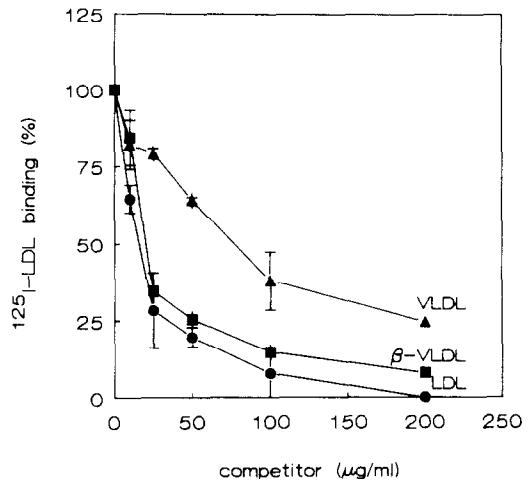

Fig. 2. Comparison of the ability of unlabeled lipoproteins to compete with the high-affinity cell binding of [^{125}I]LDL to human liver parenchymal cells. Cells were incubated for 3 h at 4°C with 10 μg/ml [^{125}I]LDL and with the indicated amounts of LDL (▲) or β-VLDL (■). High-affinity binding is expressed as precentage of the radioactivity obtained in the absence of competitor. When indicated the bars represent ± SD.

after preincubation with 100 μg/ml LDL was 31 ng LDL per mg cell protein, while after preincubation with 100 μg/ml β-VLDL the maximal binding level decreased to 19 ng LDL/mg cell protein. Competition experiments indicate that β-VLDL does compete with the binding of [^{125}I]LDL to human parenchymal cells with a similar effectivity as LDL while VLDL is clearly less effective (Fig. 2).

The effect of varying β-VLDL, VLDL and LDL concentrations during the 22 h preincubation on the expression of LDL receptors is shown in Fig. 3. Preincubations of human parenchymal cells with relatively low concentrations of LDL and β-VLDL resulted already in a marked decrease of the cell association of [^{125}I]LDL. Over the whole range of concentrations used, preincubation with β-VLDL lowered the LDL high-affinity association more than LDL. At concentrations higher than 10 μg/ml, this difference in down-regulation of [^{125}I]LDL association was significant ($P<0.05$). Preincubation of human parenchymal cells with increasing amounts of VLDL had little effect on the association of [^{125}I]LDL.

Because the regulation of LDL receptor expression is mediated by sterol regulation of the transcription of the gene encoding the LDL receptor [27], we determined the time course showing now the LDL receptor diminishes in activity when the synthesis is blocked by cycloheximide (Fig. 4). The relationship between the logarithm of the high-affinity binding of [^{125}I]LDL and incubation time was linear, indicating a half-life of the receptor of approx. 12 h. This indicates that with a 22 h preincubation time complete blockade of LDL receptor synthesis still results in the expression of 29% of the

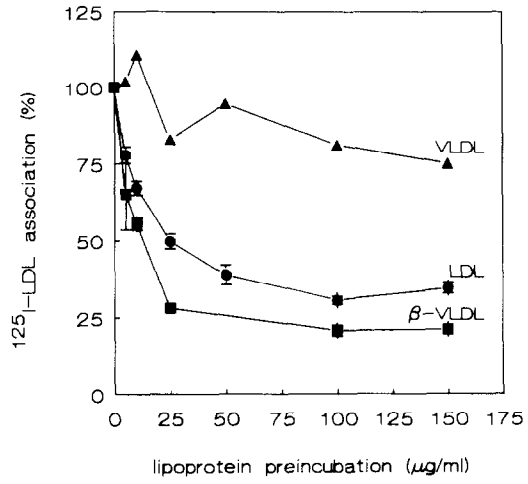

Fig. 3. The effect of preincubation with increasing amounts of LDL (•), VLDL (▲) or β-VLDL (■) on high-affinity association of [^{125}I]LDL with human liver parenchymal cells. Cells were preincubated for 22 h with the indicated amounts of LDL, VLDL or β-VLDL followed by an incubation for 3 h at 37°C with 10 μg/ml [^{125}I]LDL. High-affinity association of [^{125}I]LDL was determined by subtracting non-specific association which was measured in the presence of 300 μg/ml unlabeled LDL, from total association. High-affinity association of [^{125}I]LDL is expressed as the percentage of the radioactivity obtained in the absence of LDL, VLDL or β-VLDL in the preincubation. The 100% value for high-affinity association with human liver parenchymal cells is 115 ± 11 ng of LDL per mg of cell protein and for high-affinity degradation 34 ± 4 ng per mg of cell protein. When indicated the bars represent ± SE. ($n=3$).

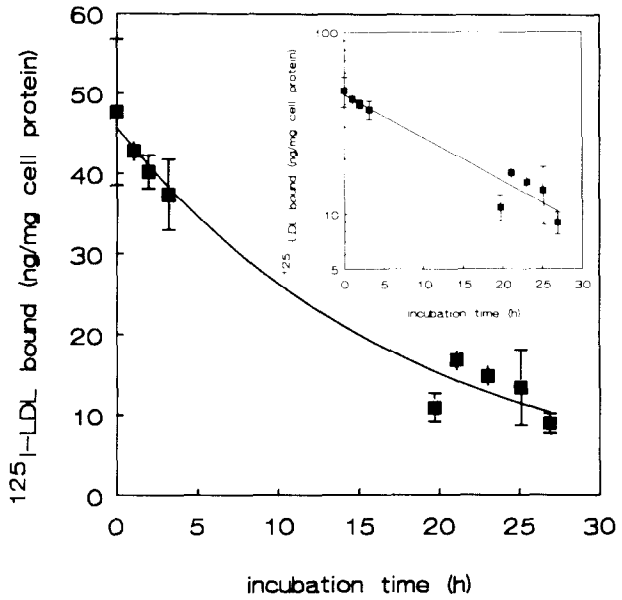

Fig. 4. Time course of the inhibition of high-affinity binding of [^{125}I]LDL to human parenchymal cells by cycloheximide. Cells were incubated with 5 μM cycloheximide for the indicated time followed by an incubation for 3 h at 4°C with [^{125}I]LDL. High-affinity binding was determined by subtracting non-specific binding, which was measured in the presence of 300 μg/ml unlabeled LDL, from total binding. Binding of [^{125}I]LDL to parenchymal cells is expressed as ng LDL per mg of cell protein. Insert: same data plotted semi logarithmically. Bars represent ± SD.

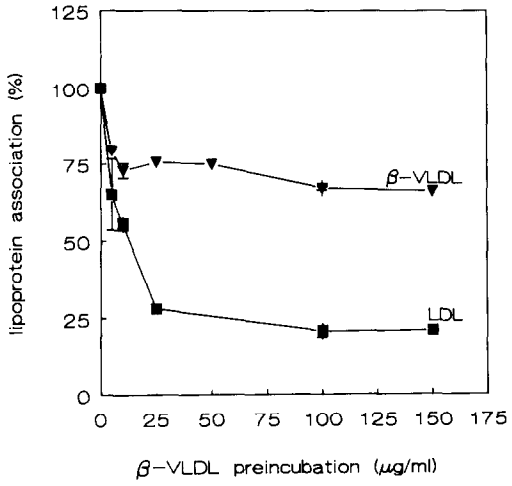

Fig. 5. β-VLDL induced regulation of [^{125}I]LDL of [^{125}I]β-VLDL high-affinity association with human liver parenchymal cells. Cells were preincubated for 22 h at 37°C with the indicated amount of β-VLDL, followed by an incubation for 3 h at 37°C with 10 μg/ml [^{125}I]LDL (■) or [^{125}I]β-VLDL (▾). High-affinity association of [^{125}I]LDL or [^{125}I]β-VLDL was determined by subtracting non-specific association, which was measured in the presence of 300 μg/ml unlabeled LDL or β-VLDL, from total association. The data are expressed as the percentage of radioactivity of the labeled lipoproteins obtained in the absence of β-VLDL in the preincubation. When indicated, bars represent ± SD.

initial amount of LDL receptors. The percentage of expression of LDL receptor at 22 h preincubation with β-VLDL under the same conditions is 24.0 ± 2.6%, thus indicating that a complete inhibition of the synthesis of new receptors is achieved by β-VLDL. Preincubation with LDL led to an inhibition of 37.3 ± 2.9% of the control (cf. also Fig. 1).

For reason that the effectivity of β-VLDL to compete with the binding of [^{125}I]LDL to the LDL receptor does not differ from that of LDL, we investigated whether an additional recognition system for β-VLDL on human parenchymal cells might explain its significantly higher down-regulatory potency. In Fig. 5 it is shown that the high-affinity association of β-VLDL to human parenchymal cells is only down-regulated to 68% of the control value while under identical conditions for LDL receptors this percentage is 21%. This might indicate that in addition to LDL receptors, an interaction of β-VLDL with a second recognition system for β-VLDL (remnant receptor or LDL receptor related protein) can lead to complete suppression of LDL receptors in human parenchymal cells.

4. DISCUSSION

The availability of human parenchymal cells enabled a study on the regulation of the human liver LDL receptor. Because most LDL receptor activity is localized in the liver, regulation of LDL receptor activity in human parenchymal liver cells is of major importance for cholesterol homeostasis in man.

We demonstrate here that a 22-h preincubation of cultured human liver parenchymal cells with LDL and β-VLDL, leads to a decrease in high-affinity binding or cell association of LDL. Preincubation of parenchymal cells with 100 μg/ml LDL led to a decrease in binding and association of [^{125}I]LDL, but this down-regulation of LDL receptor activity is less efficient as compared to down-regulation of LDL receptors found in extrahepatic cells. LDL receptor activity in fibroblasts is already down-regulated for 75% after preincubation with only 20 μg of LDL per ml [28]. Our finding, that down regulation of LDL receptor activity by LDL is less efficient in human parenchymal cells than in fibroblasts, is in accordance with previously reported results [9–11]. Maximal down-regulation of LDL receptors by LDL was reported to be between 35–63% [9,11]. However, it must be taken into account that at the commonly applied preincubation time (22 h) even under complete blockade of LDL receptors still a high-affinity [^{125}I]LDL binding of 29% of the control values will be found (as deduced from the $t_{\frac{1}{2}}$ for LDL receptors of 12 h in human parenchymal cells). When this finding is taken into account, our data still confirm that complete suppression of the synthesis of LDL receptors in human parenchymal cells cannot be achieved with LDL itself. This also agrees with animal studies where at physiological LDL concentrations, still an LDL receptor-mediated uptake of LDL is noticed [1]. However, such studies also indicated that a diet rich in cholesterol and saturated triacylglycerols led to a complete suppression of the LDL receptor-mediated clearance, at least in hamsters [15]. Our present data with the human parenchymal liver cells indicate that such a complete suppression of LDL receptor synthesis can be achieved by β-VLDL while normal VLDL is hardly effective.

The effectivity of β-VLDL to compete with [^{125}I]LDL binding to human parenchymal cells is not different from that of LDL. The greater effectivity of β-VLDL to suppress the synthesis of LDL receptors as compared to LDL is also not related to a difference in cholesterol content of the lipoproteins because replotting of the data from Fig. 3 (μg cholesterol instead of μg apolipoprotein) does not reveal a greater efficiency of β-VLDL cholesterol to suppress LDL receptors. The explanation for β-VLDL to be more effective than LDL may therefore be related to the presence of an additional recognition site on human parenchymal cells for β-VLDL. It appears that the high-affinity association of [^{125}I]β-VLDL to human parenchymal cells is only down-regulated to 68% of the control value by β-VLDL preincubation while the [^{125}I]LDL association under the same conditions is diminished to 21%. If this additional recognition site is the remnant receptor or the LDL

receptor-related protein [29] cannot be deduced from our experiments. It is, however, clear that this specific β-VLDL recognition site is less sensitive to down-regulation by β-VLDL than the LDL receptor.

In conclusion, our data demonstrate that the LDL receptor synthesis in human liver parenchymal cells is subject to complete down-regulation by β-VLDL. The complete down-regulation of LDL receptors in human liver parenchymal cells by β-VLDL suggest that the observed effect of a cholesterol-rich diet on LDL receptors in vivo may be mediated by β-VLDL and that β-VLDL is thus of decisive importance for regulating plasma LDL cholesterol levels.

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REFERENCES

[1] Dietschy, J.M. and Spady, D.K. (1986) in: Receptor-mediated Uptake (H. Greten, Windler, E. and U. Beisiegel, eds.) Springer, Heidelberg, pp. 56–65.
[2] Brown, M.S. and Goldstein, J.L. (1986) Science 232, 34–47.
[3] Bilheimer, D.W., Goldstein, J.L., Grundy, S.M., Starzl, T.E. and Brown, M.S. (1984) N. Engl. J. Med. 311, 1658–1664.
[4] Kovanen, P.T., Brown, M.S., Basu, S.K., Bilheimer, D.W. and Goldstein, J.L. (1981) Proc. Natl. Acad. Sci. USA 78, 1396–1400.
[5] Harkes, L. and Van Berkel, Th.J.C. (1982) Biochim. Biophys. Acta 712, 677–683.
[6] Harders-Sprengel, K., Wood, C.B., Thompson, G.R., Myant, N.B. and Soutar, A.K. (1982) Proc. Natl. Acad. Sci. USA 79, 6355–6359.
[7] Hoeg, J.M., Demosky, S.J., Schoefer, E.J., Starzl, T.E. and Brewer Jr., H.B. (1984) J. Clin. Invest. 73, 429–436.
[8] Havinga, J.R., Lohse, P. and Beisiegel, U. (1987) FEBS Lett. 216, 275–280.
[9] Kosykh, V.A., Preobrazhensky, S.N., Ivanov, V.O., Tsibulsky, V.P., Repin, V.S. and Smirnov, V.N. (1985) FEBS Lett. 183, 17–20.
[10] Edge, S.B., Hoeg, J.M., Triche, T., Schneider, P.D. and Brewer, Jr., H.B. (1986) J. Biol. Chem. 261, 3800–3806.
[11] Havekes, L.M., Verboom, H., De Wit, E., Yap, S.H. and Princen, H.M.G. (1986) Hepatology 6, 1356–1360.
[12] Goldstein, J.L. and Brown, M.S. (1977) Annu. Rev. Biochem. 46, 897–930.
[13] Krempler, F., Kostner, G.M., Friedl, W., Paulweber, B., Bauer, H. and Sandhofer, F. (1987) J. Clin. Invest. 80, 401–408.
[14] Havekes, L.M., Schouten, D., De Wit, E.C.M., Cohen, L.H., Griffioen, M., Van Hinsbergh, V.W.M. and Princen, H.M.G. (1986) Biochim. Biophys. Acta 876, 236–246.
[15] Spady, D.K. and Dietschy, J.M. (1985) Proc. Natl. Acad. Sci. USA 82, 4526–4530.
[16] Fainaru, M., Mahley, R.W., Hamilton, R.L. and Innerarity, T.L. (1982) J. Lipid Res. 23, 702–714.
[17] Harkes, L., Van Duijne, A. and Van Berkel, Th.J.C. (1989) Eur. J. Biochem. 180, 241–248.
[18] De Water, R., Hessels, E.M.A.J., Bakkeren, H.F. and Van Berkel, Th.J.C. (1990) Eur. J. Biochem. 192, 419–425.
[19] Princen, H.M.G., Huijsmans, C.M.G., Kuipers, F., Vonk, R.J. and Kempen, H.J.M. (1986) J. Clin. Invest. 78, 1064–1071.
[20] Kwekkeboom, J., Princen, H.M.G., Van Voorthuizen, E.M., Meyer, P. and Kempen, H.J.M. (1989) Biochem. Biophys. Res. Commun. 162, 619–625.
[21] Forte, T.M., Nordhausen, R.W. and Princen, H.M.G. (1989) Arteriosclerosis 9, 693A.
[22] Kooistra, T., Bosma, P.J., Tons, H.A.M., Van den Berg, A.P., Meijer, P. and Princen, H.M.G. (1989) Throm. Haemostas. 62, 723–728.
[23] Krenning, E.P., De Jong, M., Vos, R.A., Bernard, H.F., Docter, R. and Henneman, G. (1989) Endocrinology 122, T-36.
[24] Schouten, D., Kleinherenbrink-Stins, M.F., Brouwer, A., Knook, D.L., Kamps, J.A.A.M., Kuiper, J. and Van Berkel, Th.J.C. (1990) Arteriosclerosis 10, 1127–1135.
[25] Redgrave, T.G., Roberts, D.C.K. and West, E. (1975) Anal. Biochem. 65, 42–49.
[26] Bilheimer, D.W., Eisenberg, G. and Levy, R.I. (1972) Biochim. Biophys. Acta 260, 212–221.
[27] Südhof, T.C., Russell, D.W., Brown, M.S. and Goldstein, J.L. (1987) Cell 48, 1061–1069.
[28] Brown, M.S. and Goldstein, J.L. (1975) Cell 6, 307–316.
[29] Herz, J., Hamman, U., Rogne, S., Myklebost, O., Gausepohl, H. and Stanley, K.K. (1988) EMBO J. 7, 4119–4127.