Effect of insulin on low-density-lipoprotein metabolism in human lymphocytes *in vitro*

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The metabolism of low-density lipoproteins (LDL) *in vitro* in the presence of insulin was studied in freshly isolated human peripheral-blood lymphocytes. Insulin appeared to decrease the binding affinity of ^{125}I -LDL to its cell-surface receptor, without any change in apparent V_{max} or in the number of LDL receptors. As a consequence, the absolute amounts of ^{125}I -LDL internalized and degraded were lower in the presence of insulin than in its absence, although the fraction of internalized ^{125}I -LDL degraded in either instance was quite similar. 3-Hydroxy-3-methylglutaryl-CoA reductase activity, and hence cholesterol synthesis, were stimulated by insulin. This effect of insulin was independent of the inhibitory effect of LDL on cholesterol synthesis. At the same time, acid cholesterol esterase and acyl-CoA:cholesterol *O*-acetyltransferase activities were lower in cells incubated with insulin than in controls. The net effect of these metabolic alterations seems to be that cells accumulate greater quantities of free and esterified cholesterol when treated with insulin.

INTRODUCTION

Circulating human peripheral-blood lymphocytes possess specific receptors for low-density lipoproteins (LDL), by which they degrade LDL and derive cholesterol for membrane synthesis (Ho *et al.*, 1976). Freshly isolated lymphocytes acquire a large number of high-affinity LDL receptors when incubated *in vitro* in the absence of lipoproteins. The lymphocyte LDL pathway involves high-affinity surface binding, internalization by endocytosis and lysosomal degradation of LDL. Free cholesterol derived from LDL suppresses 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase) activity, stimulates acyl-CoA:cholesterol acyltransferase (ACAT) activity and regulates the LDL-receptor activity by a feedback mechanism (Goldstein & Brown, 1977; Ho *et al.*, 1976). The overall function of the LDL pathway is to transfer cholesterol from plasma LDL into the cell in a controlled manner. Certain hormones may play a role in the regulation of LDL-receptor activity. In cultured human skin fibroblasts, LDL degradation is stimulated by insulin (Chait *et al.*, 1979a) and tri-iodothyronine (Chait *et al.*, 1979b) and inhibited by glucocorticoids (Henze *et al.*, 1979). Though there is stimulation of LDL degradation by insulin in mononuclear cells *in vivo*, no such stimulation has been reported in monocytes *in vitro* (Mazzone *et al.*, 1984). We studied the effect of insulin on the receptor-mediated LDL pathway in peripheral-blood lymphocytes and describe here a sequence of metabolic changes in insulin-treated cells.

MATERIALS AND METHODS

Materials

Crystalline pig insulin was given by Eli Lilly and Co. (Indianapolis, IN, U.S.A.). RPMI 1640 culture medium

was purchased from GIBCO (Grand Island, NY, U.S.A.). Ficoll-Hypaque (Isolymp) was obtained from Gallard-Schlesinger Chemical Manufacturing Corp. (Mineola, N.Y., U.S.A.). Kryo EOB was given by Procter and Gamble Co. (Cincinnati, OH, U.S.A.). Penicillin/streptomycin mixture was purchased from GIBCO. DL-[methyl- ^3H]HMG-CoA (10.9 Ci/nmol), DL-[2- ^{14}C]mevalonolactone (47 mCi/mmol), [7- ^3H]cholesterol (16 Ci/mmol), cholesteryl [1- ^{14}C]oleate (56 mCi/mmol), [9,10- ^3H]oleic acid (16 Ci/mmol), [1- ^{14}C]oleoyl-CoA (58.1 mCi/mmol) and [2- ^{14}C]acetic acid (55 mCi/mmol; sodium salt) were obtained from New England Nuclear Corp. (Boston, MA., U.S.A.). Na ^{125}I (carrier-free, in 0.05 M-NaOH) was obtained from Research Products International Corp. (Mount Prospect, IL, U.S.A.). Bovine plasma γ -globulin was purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.).

Isolation of LDL and preparation of ^{125}I -LDL

LDL (density 1.019–1.063 g/ml) was isolated from plasma of normolipidaemic healthy subjects by sequential preparative ultracentrifugation in a Beckman L8-80 ultracentrifuge, by the method of Havel *et al.* (1955). LDL was dialysed exhaustively at 4 °C against 0.15 M-NaCl/350 μM -EDTA, pH 7.4. ^{125}I -LDL was prepared by the method of MacFarlane (1958) as modified by Bilheimer *et al.* (1972). Less than 4% of the radiolabel was found in lipid, as determined by chloroform/methanol (2:1, v/v) extraction, and 96% of radioactivity was recovered in the trichloroacetic acid precipitate. ^{125}I -LDL was stored at 4 °C, and it retained full biological activity for at least 4 weeks. For experimental purposes ^{125}I -LDL was diluted with native LDL to give the final specific radioactivity indicated in legends to figures. ^{125}I -LDL was passed through a 0.45 μm Millipore filter just before use.

Abbreviations used: LDL, low-density lipoprotein; HMG, 3-hydroxy-3-methylglutaryl; ACAT, acyl-CoA:cholesterol acyltransferase (EC 2.3.1.26); ACEH, acid cholesterol esterase (EC 3.1.1.13).

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Protein was measured by the method of Bradford (1976), with bovine plasma γ -globulin as standard.

Cells

Peripheral-blood lymphocytes were isolated from heparinized blood obtained from healthy individuals with no history of any metabolic disorder. Monocytes were depleted by controlled incubation of whole peripheral blood diluted 1:3 with RPMI 1640 medium in Petri dishes for 60 min (Whiteside & Rowlands, 1977). The non-adherent lymphocytes were gently washed off the solid surface and recovered. Lymphocytes were isolated by Ficoll-Hypaque gradient centrifugation by the method of Böyum (1968). The monocyte-depleted preparations contained 97–99% of lymphocytes as determined by immunofluorescence with monoclonal antibodies (Warnke & Levy, 1980). Cell viability was assessed as described previously (Sanghvi & Warty, 1985) by the method of Marcus *et al.* (1956). In each case at least 90% of the recovered cells were found to be viable.

Cholesterol synthesis by intact lymphocytes

The rate of incorporation of [2- 14 C]acetate into cholesterol in isolated lymphocytes was measured as previously described (Sanghvi *et al.*, 1981a). Cholesterol synthesis is expressed as pmol of [14 C]acetate converted into [14 C]cholesterol/h per mg of protein.

Assay of HMG-CoA reductase activity in cell-free extracts of lymphocytes

After cells were harvested, detergent (Kyro EOB)-solubilized cell extract was prepared, and HMG-CoA reductase activity was determined as previously described (Sanghvi *et al.*, 1981a). The enzyme activity is expressed as pmol of [3 H]mevalonate formed/min per mg of supernatant protein.

Assay of ACAT activity in cell-free extracts

ACAT activity was measured by the method of Goodman *et al.* (1964) as modified by Brown *et al.* (1975). The lymphocytes were washed three times with 100 mM-phosphate-buffered saline (pH 7.4). Cell-free extracts were prepared by sonication of the washed pellets suspended in 20 mM-potassium phosphate/2 mM-dithiothreitol, pH 7.4. Samples of the cell-free extracts were incubated for 60 min at 37 °C in a final volume of 0.2 ml containing 50 mM-potassium phosphate, pH 7.4, 2 mM-dithiothreitol, 1.2 mg of bovine serum albumin and 0.1 mM-[1- 14 C]oleoyl-CoA. The reaction was stopped by the addition of 4 ml of chloroform/methanol (2:1, v/v), and the cholesteryl [14 C]oleate was isolated by t.l.c. and quantified, by using cholesteryl [3 H]oleate as internal standard. ACAT activity is expressed as nmol of cholesteryl [14 C]oleate formed/h per mg of supernatant protein.

Measurement of ACEH activity

ACEH activity was measured by the method of Brecher *et al.* (1976) as modified by Haley *et al.* (1980), and is expressed as nmol of cholesteryl [14 C]oleate hydrolysed/min per mg of protein.

Measurement of cellular free and esterified cholesterol

The lymphocytes were washed three times with PBS. The cell suspension was sonicated and lipids were extracted with chloroform/methanol (2:1, v/v), after the

addition of cholestane as internal standard. A portion of the extract was used for determination of free cholesterol. The remainder was saponified with 0.625 M-KOH at 65 °C for 1 h and the unsaponifiable fraction was extracted with light petroleum (b.p. 40–60 °C) to quantify the total cholesterol. Cholesterol ester content was computed by difference. A g.l.c./mass-spectrometric procedure utilizing a Hewlett–Packard 5986A instrument was used to quantify cholesterol. The analyses were carried out in selected-ion-monitoring (SIM) mode, by using ratio response factors for cholesterol and 5 α -cholestane, as previously described (Sanghvi *et al.*, 1981b).

Measurement of LDL-receptor activity

125 I-LDL surface binding, cellular uptake and degradation were determined by the method essentially as described by Goldstein *et al.* (1976). After 22 h incubation in RPMI 1640 medium containing 1% bovine serum albumin, with and without insulin, the cells were harvested in growth medium at a concentration of 2×10^6 cells/ml. Then 1 ml samples of the cell suspension were placed in the wells and incubated for 4 h at 37 °C with different concentrations of 125 I-LDL ranging from 0 to 100 μ g/ml in the absence and in the presence of excess unlabelled LDL. The determination of heparin-releasable and heparin-resistant 125 I-LDL was carried out as described by Goldstein *et al.* (1976). To determine the amount of 125 I-LDL degraded by the cells in a 4 h period, a sample of the cell-free medium was treated with 10% (v/v) trichloroacetic acid to remove precipitable proteins. Free iodine was removed by treating the acid-soluble fraction with KI and H₂O₂, followed by extraction with chloroform. The aqueous phase was counted for 125 I radioactivity to get a measure of the amount of acid-soluble material released by the cells into the medium. Non-specific binding, internalization and degradation were less than 8% of the total.

RESULTS

The effect of insulin on cholesterol synthesis was evaluated by measuring the rate of incorporation of [2- 14 C]acetate into cholesterol in lymphocytes preincubated with 0, 1, 10 and 100 munits of insulin/ml of the incubation medium. In experiments involving short-term insulin treatment, lymphocytes were incubated in lipid-free medium for 22 h, followed by treatment with insulin for 15 min. In long-term incubation experiments, the cells were treated with insulin for 22 h during incubation in lipid-free medium. There was no change in the rate of cholesterol synthesis as a result of short-term incubation. On the other hand, long-term insulin treatment caused an increase in the rate of cholesterol synthesis, with maximal synthesis rate (33% increased compared with control; $P < 0.001$) at 10 munits of insulin/ml (Fig. 1). In all the subsequent experiments, long-term incubation with 10 munits of insulin/ml was carried out.

Regulation of cholesterol synthesis by LDL was documented by incubating the cells for 5 h with 0, 10, 25 and 50 μ g of LDL/ml, followed by pulse treatment with [2- 14 C]acetate. The cells had been preincubated with and without 10 munits of insulin/ml in lipid-free medium for 22 h, before incubation with LDL. Cholesterol synthesis was suppressed by LDL in both insulin-treated and

Fig. 1. Effect of insulin on lymphocyte cholesterol synthesis

Short-term effect of insulin (□) was evaluated by incubating the lymphocytes in lipid-free medium for 22 h, followed by treatment with insulin for 15 min at the indicated concentrations. The cells were then harvested and pulse-treated with [2-¹⁴C]acetate (5 μ Ci/well) for 3 h. The rate of incorporation of the radiolabel into cholesterol was determined. In experiments involving long-term incubation with insulin (▨), the cells were treated with insulin for 22 h during incubation in lipid-free medium. Values represent means $\pm$ S.E.M. of determinations carried out on five individuals.

Fig. 2. Effect of insulin on LDL-induced suppression of cholesterol synthesis and HMG-CoA reductase activity in lymphocytes

The cells were incubated for 5 h with indicated concentrations of LDL, after 22 h preincubation with (●, ▲) and without (○, △) 10 munits of insulin/ml in lipid-free medium. At the end of the 5 h period, the rate of cholesterol synthesis from [2-¹⁴C]acetate in intact cells (○, ●) and HMG-CoA reductase activity in cell-free extracts (△, ▲) were determined. Values represent means $\pm$ S.E.M. of determinations done on five individuals.

Fig. 3. Effect of insulin on LDL-induced stimulation of acyl-CoA:cholesterol *O*-acetyltransferase activity in lymphocytes

Cells were incubated for 5 h with LDL at the indicated concentrations, after 22 h preincubation with (●) and without (○) insulin (10 munits/ml) in lipid-free medium. At the end of 5 h, cell-free extracts were prepared for the assay of ACAT as described in the Materials and methods section. Values represent means $\pm$ S.E.M. of determinations carried out on six individuals.

untreated cells (Fig. 2). The magnitude of increase in the rate of cholesterol synthesis observed in the presence of insulin at 0 μ g of LDL/ml persisted at all concentrations of LDL studied. However, the pattern of suppression of cholesterol synthesis by LDL was similar in both the presence and the absence of insulin. Under similar experimental conditions, the HMG-CoA reductase activity was significantly higher in insulin-treated cells than in the controls (Fig. 2; $P < 0.05$ at 10 μ g of LDL/ml, $P < 0.01$ at 25 and 50 μ g of LDL/ml).

Fig. 3 shows the LDL-induced stimulation of ACAT activity in lymphocytes preincubated in lipid-free medium. The enzyme activity was negligible (0.013 nmol/h per mg of protein) at 0 μ g of LDL/ml. Increasing amounts of LDL in the medium stimulated the enzyme activity in both insulin-treated and untreated cells. However, insulin-treated cells had significantly ($P < 0.05$) lower ACAT activity compared with controls at all concentrations of LDL studied. The activity of lysosomal ACEH was also significantly lower in the presence of insulin than in its absence under identical experimental conditions (Fig. 4; $P < 0.01$).

The free and esterified cholesterol contents of the cells increased with increasing amounts of LDL in the medium (Fig. 5). However, this increase was significantly higher in insulin-treated cells than in controls ($P < 0.05$ at 10 μ g of LDL/ml; $P < 0.01$ at 50 μ g of LDL/ml). In addition, in insulin-treated cells, the magnitude of increase in esterified cholesterol content was more than that in free cholesterol content, the ratio of cholesterol ester to free cholesterol in insulin-treated and untreated cells being 0.245 and 0.167 at 0 μ g of LDL/ml, 0.350 and 0.166 at 10 μ g of LDL/ml, and 0.424 and 0.241 at 50 μ g of LDL/ml respectively. These differences were significant at $P < 0.01$ ($n = 6$).

Fig. 4. Effect of insulin on lymphocyte acid cholesterol esterase activity/time plot

At the end of 22 h incubation with (●) and without (○) insulin (10 munits/ml in lipid-free medium, cell-free extracts were prepared from lymphocytes and ACEH activity was determined as a function of time. Values represent means $\pm$ S.E.M. of determinations carried out on four subjects.

LDL-receptor activity was saturable in both insulin-treated and untreated cells, but the binding of ^{125}I -LDL to the cells was lower in the presence of insulin at all concentrations of LDL studied (Fig. 6). A double-reciprocal plot of the binding data indicated that the major effect of insulin was to produce a change in the apparent binding constants, k_d (24.3 $\mu\text{g}/\text{ml}$ in insulin-treated cells, 17.4 $\mu\text{g}/\text{ml}$ in controls; $P < 0.05$, $n = 4$), without any change in apparent $V_{\max}$ (96 ng/mg of protein in insulin-treated, cells 105 ng/mg of protein in controls). These results suggest that insulin decreases the affinity of LDL for its receptor, without any change in the receptor number. Consequently, the internalization and degradation of receptor-bound ^{125}I -LDL was less in

Fig. 6. Effect of insulin on ^{125}I -LDL binding, internalization and degradation in lymphocytes

Cells were treated with (●) and without (○) insulin (10 munits/ml) in lipid-free medium. At the end of 22 h, cells were incubated for 4 h at 37 C with ^{125}I -LDL (sp. radioactivity 156 cpm/ng) at the concentrations indicated. Binding, uptake and degradation assays were performed as described in the Materials and methods section. Values represent means $\pm$ S.E.M. of determinations carried out on four subjects.

Fig. 5. Free and esterified cholesterol content of lymphocytes

Cells were incubated with LDL at the indicated concentrations for 5 h, after 22 h preincubation with (▨) and without (□) insulin (10 munits/ml). Lipid was extracted and free and esterified cholesterol contents were quantified as described in the Materials and methods section. Values represent means $\pm$ S.E.M. of determinations carried out on six individuals.

Table 1. Cell-surface binding, internalization and degradation of ^{125}I -LDL in lymphocytes

Cells were incubated with and without 10 munits of insulin/ml in lipid-free medium. At the end of 22 h, the cells were treated with ^{125}I -LDL (sp. radioactivity 156 c.p.m./ng) for 4 h at 37 °C. Binding, internalization and degradation assays were performed as described in the Materials and methods section. Values represent means $\pm$ S.E.M. of determinations carried out with 50 μg of LDL/ml on four subjects: * $P < 0.05$ compared with controls.

Lymphocytes	LDL bound (ng/mg of protein) (A)	LDL internalized (ng/mg of protein) (B)	LDL degraded (ng/4 h per mg of protein) (C)	Internalization index $[(B+C)/A]$	Degradation of internalized LDL $[C/(B+C)]$ %
Control	82 $\pm$ 5.3	786 $\pm$ 65	1580 $\pm$ 155	28.85	66.78
Insulin-treated	66 $\pm$ 3.5*	615 $\pm$ 75	1370 $\pm$ 110	29.63	69.02

insulin-treated cells than in controls (Fig. 6). Internalization index and the fraction of internalized LDL degraded were calculated by using the data on the binding of ^{125}I -LDL to the cell-surface receptor and its uptake and degradation. Insulin treatment produced no change in these parameters, as shown in Table 1.

DISCUSSION

The observation that insulin increases the rate of incorporation of $[2\text{-}^{14}\text{C}]\text{acetate}$ into cholesterol in human peripheral-blood lymphocytes is consistent with the known effect of insulin in stimulating cholesterol synthesis in cultured cells (Bathena *et al.*, 1974). The hormone action is not immediate, as shown by the lack of change in cholesterol synthesis rate on short-term incubation of cells with insulin. An increase in cholesterol synthesis in long-term incubation paralleled an increase in the activity of HMG-CoA reductase. Krone & Greten (1984) have suggested that insulin-mediated induction of HMG-CoA reductase activity in mononuclear cells is dependent on the enzyme protein synthesis and that it is independent of newly synthesized RNA. Hence insulin may regulate HMG-CoA reductase at a post-transcriptional level.

Lymphocyte cholesterol synthesis declines in the presence of LDL in a predictable manner. However, the fact that the magnitude of insulin stimulation of cholesterol synthesis remains much the same in the presence of different concentrations of LDL indicates that stimulation of HMG-CoA reductase by insulin may be independent of the suppression of the enzyme activity by LDL.

The binding of ^{125}I -LDL to the cell-surface receptor is decreased in the presence of insulin (Table 1, Fig. 6). The apparent dissociation constant, k_d , obtained from the binding data is greater for insulin-treated cells than that for controls, signifying a decrease in binding affinity, without affecting V_{max} , and the receptor number.

There is apparent contradiction between our results and those of Chait *et al.* (1979a), who have shown that insulin increases LDL degradation in cultured human skin fibroblasts. Moreover, Henze & Chait (1981) and Mazzone *et al.* (1984) have demonstrated an increase in LDL degradation in mononuclear cells *in vivo*, as a result of insulin therapy. On the other hand, they have also demonstrated that insulin does not stimulate LDL degradation in monocytes *in vitro* (Mazzone & Chait, 1982; Mazzone *et al.*, 1984). In any case, one consequence

of decreased ^{125}I -LDL binding to its receptor in the insulin-treated cells is the lesser number of LDL particles internalized and degraded (Fig. 6), even though the differences between insulin-treated and untreated cells in this respect are statistically not significant (Table 1). Also, the internalization index and the fraction of internalized LDL degraded by cells in the presence and absence of insulin are similar, as shown in Table 1. These data suggest that insulin is without effect on events following the binding of LDL to its receptor and leading to the degradation of internalized LDL.

Insulin-induced decrease in LDL binding also appears to affect ACAT and ACEH activities in lymphocytes. The ability of LDL to stimulate ACAT activity is dependent on its ability to enter the cell via receptor-mediated endocytosis and its consequent degradation in the cell. Hence the assay of ACAT activity remains the most sensitive method for assessing the LDL-receptor activity. In the present study, the ACAT activity after incubation of cells with LDL was lower in insulin-treated cells than in controls, reflecting a lower LDL-receptor activity. The lower ACAT activity in insulin-treated cells may in turn be related to lower ACEH activity in these cells, resulting in a decreased availability of LDL-derived free cholesterol. ACAT activity is regulated by LDL-derived cholesterol, which is the preferred substrate for the enzyme (Goldstein & Brown, 1976). It may be that insulin has a direct effect on ACEH. Wolinsky *et al.* (1980) have shown that insulin deficiency in rats results in an increase in lysosomal ACEH activity in liver and kidney, and that the administration of insulin to these diabetic rats decreases ACEH activity to control values. Further, hyperinsulinaemia in rats and mice is reported to result in a significant decrease in ACEH activity in cardiac tissue (Severson & Fletcher, 1982). In this connection, it should be mentioned that Henze & Chait (1981) have shown an increase in ACEH activity in mononuclear cells *in vivo*, after insulin therapy. In contrast, more recently it has been observed by Finegold & Coates (1984) that, although ACEH activity in mononuclear cells in Type I diabetics was not different from that in controls before insulin therapy, there was a significant decrease after insulin treatment. Our findings are consistent with the latter observation.

The content of both the free and esterified cholesterol in insulin-treated cells is significantly higher than that in controls in the presence of various concentrations of LDL in the medium, as shown in Fig. 5. A direct effect of insulin on HMG-CoA reductase, and therefore on

cholesterol synthesis, may be responsible for the significantly higher contents of free cholesterol in these cells, and increases in esterified cholesterol may, in part, be related to the lower activity of ACEH.

It has been suggested (e.g. by Stout, 1981) that increased incidence of atherosclerotic complications in diabetics result from insulin excess, rather than from insulin deficiency. Our data suggests that cells incubated with insulin accumulate free cholesterol and cholesterol ester in the presence of LDL, even though the binding affinity of LDL to its receptor appears to be decreased. Whether this has relevance to the clinical development of the disease, of course, cannot be extrapolated from these data.

One possible consequence of the decreased LDL metabolism in the insulin-treated cell, if true *in vivo*, could be increased serum LDL. In conclusion, our data show a series of consistent findings related to the effect of insulin on LDL metabolism in lymphocytes, namely: (i) lowered binding of LDL to its receptor; (ii) consequent decrease in internalization and degradation of LDL; (iii) decrease in ACEH and ACAT activities; and (iv) stimulation of HMG-CoA reductase activity and cholesterol synthesis.

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REFERENCES

- Bathena, S. J., Avigan, J. & Schreiner, M. E. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2174–2178
- Bilheimer, D. W., Eisenberg, S. & Levy, R. I. (1972) *Biochim. Biophys. Acta* **260**, 212–221
- Böyum, A. (1968) *Scand. J. Clin. Invest.* **21**, Suppl. **97**, 51–76
- Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254
- Brecher, P., Chobanian, J., Small, D. M. & Chobanian, A. V. (1976) *J. Lipid Res.* **17**, 239–247
- Brown, M. S., Dana, S. E. & Goldstein, J. L. (1975) *J. Biol. Chem.* **250**, 4025–4027
- Chait, A., Bierman, E. L. & Albers, J. J. (1979a) *J. Clin. Invest.* **64**, 1309–1319
- Chait, A., Bierman, E. L. & Albers, J. J. (1979b) *J. Clin. Endocrinol. Metab.* **48**, 887–889
- Finegold, D. N. & Coates, P. M. (1984) *Metab. Clin. Exp.* **33**, 85–89
- Goldstein, J. L. & Brown, M. S. (1976) *Curr. Top. Cell. Regul.* **147**–181
- Goldstein, J. L. & Brown, M. S. (1977) *Annu. Rev. Biochem.* **46**, 897–930
- Goldstein, J. L., Basu, S. V., Brunschade, A. Y. & Brown, M. S. (1976) *Cell* **7**, 85–86
- Goodman, D. S., Deykin, S. & Shiratori, J. (1964) *J. Biol. Chem.* **239**, 1335–1345
- Haley, N. J., Fowler, A. & de Duve, C. (1980) *J. Lipid Res.* **21**, 961–969
- Havel, R. J., Eder, H. A. & Bragden, J. H. (1955) *J. Clin. Invest.* **63**, 1345–1353
- Henze, K. & Chait, A. (1981) *Diabetologia* **20**, 625–629
- Henze, K., Chait, A. & Albers, J. J. (1979) *Abstr. Int. Symp. Atherosclerosis 5th*, no. 456
- Ho, Y. K., Brown, M. S., Bilheimer, D. W. & Goldstein, J. L. (1976) *J. Clin. Invest.* **58**, 1465–1474
- Krone, W. & Greten, H. (1984) *Diabetologia* **26**, 366–369
- MacFarlane, A. S. (1958) *Nature (London)* **182**, 53
- Marcus, P. J., Cieciura, S. J. & Puck, T. T. (1956) *J. Exp. Med.* **104**, 615–627
- Mazzone, T. & Chait, A. (1982) *Arteriosclerosis* **2**, 487–492
- Mazzone, T., Foster, D. & Chait, A. (1984) *Diabetes* **33**, 333–338
- Sanghvi, A. & Warty, V. (1985) *Biochem. J.* **227**, 397–404
- Sanghvi, A., Wight, C. & Warty, V. (1981a) *Biochim. Biophys. Acta* **665**, 48–52
- Sanghvi, A., Grassi, E., Bartman, C., Lester, R., Kienle-Galli, M. & Galli, G. (1981b) *J. Lipid Res.* **22**, 720–724
- Severson, D. L. & Fletcher, T. (1982) *Biochim. Biophys. Acta* **718**, 144–150
- Stout, R. W. (1981) *Diabetes* **30**, 54–57
- Whiteside, T. L. & Rowlands, D. T. (1977) *Am. J. Pathol.* **88**, 754–790
- Wolinsky, H., Katz, D., Markle, R., Mills, J., Brem, S. & Wassertheil-Smaller, S. (1980) *Circ. Res.* **47**, 433–442
- Warnke, R. & Levy, R. (1980) *J. Histochem. Cytochem.* **28**, 771–776

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