Defective Receptor Binding of Low Density Lipoprotein from Pigs Possessing Mutant Apolipoprotein B Alleles*

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We previously identified a defect in the in vivo catabolism of low density lipoprotein (LDL) from hypercholesterolemic pigs carrying a mutant apolipoprotein B allele. In the present studies, we examined the in vitro metabolism of mutant LDL in cultured pig fibroblasts. A 3-fold higher concentration of mutant LDL (compared to control) was needed to displace 50% of control 125I-LDL binding. Mutant LDL had a 6-fold higher dissociation constant than control LDL. Scatchard plots of the binding data were concave upward, suggesting multiple classes of binding sites or negative cooperativity. The mutant LDL degradation rate was reduced by 40%; this decrease could be attributed to a dense LDL subspecies. Mutant and control buoyant LDL subspecies were degraded more slowly than the corresponding dense LDL subspecies. Together, these studies show that diminished LDL receptor binding can result from mutations in apolipoprotein B and from changes in the lipid composition of LDL particles.

Elevated plasma cholesterol levels are associated with an increased risk of atherosclerosis and coronary heart disease (1). The most common form of cholesterol elevation is characterized by increased plasma low density lipoprotein (LDL) levels. LDL is the predominant cholesterol transporter in the bloodstream of humans and several other species, including pigs. Plasma LDL levels are regulated, in part, by the action of cell surface receptors that recognize LDL. This interaction is important in maintaining cholesterol homeostasis (2-4).

The best characterized genetic disorders leading to hypercholesterolemia affect LDL receptor activity. Patients with familial hypercholesterolemia (FH), as well as a mutant rabbit strain (the Watanabe Heritable-Hyperlipidemic Rabbit), have defective or absent LDL receptors (5, 6). Consequently, they have a large plasma LDL elevation and develop premature atherosclerosis. Although FH is relatively common in humans, it is responsible for just 1-2% of all primary hypercholesterolemia cases. The molecular basis for the majority of non-FH hypercholesterolemia cases remains undefined.

Defects in apolipoprotein B100 (apoB), the major protein component of LDL and a ligand for the LDL receptor, may lead to hypercholesterolemia. ApoB mutations that affect binding to the LDL receptor could result in retarded particle clearance, leading to elevated plasma cholesterol levels. Although the apoB cDNA sequence was recently determined (7-10), whether genetic variability in apoB is a significant contributor to hypercholesterolemia in the human population is unknown.

There is considerable genetically induced variability in the apoB structure. In a series of alloimmunization experiments, pigs immunized with pig serum responded strongly to LDL-associated antigens (11). These antigens corresponded to epitopes on apoB (12). Eight apoB alleles were identified, one of which is strongly associated with elevations in LDL (11). Pigs homozygous for this allele have severe hypercholesterolemia and premature peripheral (13) and coronary (12) atherosclerosis. ApoB structural polymorphism has also been identified in humans using monoclonal antibodies raised against human apoB (14). The human apoB DNA sequence shows a large number of differences between individuals (15). Together, these observations imply that apoB is a highly polymorphic plasma protein.

Vega and Grundy (16) showed metabolic variability between LDLs from individuals with moderate primary hypercholesterolemia. Plasma LDL turnover experiments were carried out in 15 such patients; one-third possessed LDL that was less efficiently catabolized than the LDL of normal subjects. The LDL from one of the patients with retarded LDL turnover exhibited a decreased ability to bind to the LDL receptor on cultured fibroblasts (17); this was apparently due to a defective apoB. Several first-degree relatives of this patient also had defective LDL binding, suggesting a genetic basis for this disorder (17).

Our laboratory recently characterized a mutant strain of pigs with spontaneous hypercholesterolemia. Although the cholesterol phenotype of the mutant pigs is strikingly similar to humans with FH, these pigs had normal LDL receptor activity (12). In normal pigs, mutant pig LDL was catabolized more slowly than normal LDL (18). Although the animals were homozygous for their apoB allele, we detected structural heterogeneity of LDL particles (18). In normal pigs, we observed several different LDL subspecies differing in size and density. In the mutant pigs, we detected an additional subspecies, possessing a larger particle diameter and a significantly lower density.

The present experiments were performed to determine whether the defective mutant LDL catabolism in vivo is paralleled by defective LDL receptor binding in vitro. Additionally, we tested the possibility that LDL particle structural heterogeneity results in metabolic heterogeneity, leading to differences in the ability of each LDL subspecies to bind to the LDL receptor. One of the difficulties in studying human...
apoB is the identification of subjects who are homozygotes for apoB. As a result of inbreeding and selection for defined apoB alloantigens, the animals used in our experiments were homozygous for particular apoB alleles.

EXPERIMENTAL PROCEDURES AND RESULTS

Our studies demonstrate that LDL from an inbred hypercholesterolemic mutant pig strain is a defective ligand for the pig fibroblast LDL receptor. Mutant LDL had a decreased ability to compete for binding to the LDL receptor when compared to LDL from normal pigs; the affinity of mutant LDL was 2-6-fold lower than the affinity of control LDL. Because of the receptor binding defect, mutant LDL was degraded by cultured pig fibroblasts at a slower rate than control LDL. The receptor binding defect observed for mutant LDL in vitro accounts for the decreased catabolism of mutant LDL observed in our previous in vivo studies (18). However, when cells were incubated with mutant or control LDL under conditions where binding was not rate-limiting for cellular uptake (saturating concentrations), the degradation rates were the same. We suggest that mutant LDL does not differ in its ability to be metabolized by the LDL receptor pathway in fibroblasts after binding to the LDL receptor. Only a binding defect in mutant LDL can account for our observations.

Interestingly, the mutant LDL binding defect did not result in decreased cholesterol esterification relative to control LDL when equivalent masses of mutant and control LDL protein were compared. This was apparently due to the increased cholesterol content of mutant LDL. When comparisons were based on cholesterol available to the cells, mutant LDL cholesterol was less efficiently incorporated into intracellular cholesterol ester. Thus, despite the receptor binding defect exhibited by mutant LDL, the increased cholesterol content of the particle allowed delivery of normal amounts of cholesterol to fibroblasts.

Previous studies showed that mutant pig LDL consists predominantly of a more buoyant subspecies, whereas LDL from normocholesterolemic animals consists predominantly of a more dense subspecies of LDL (18). Since the composition of the full LDL particle spectrum differed between mutant and control animals, binding differences to the LDL receptor could be due to differences in lipid composition rather than differences in the apoB structure. We tested this possibility by isolating LDL subfractions containing similar lipid compositions from control and mutant pigs. By directly comparing these subfractions, we minimized the effect of lipid composition on our results. Mutant dense LDL was degraded less efficiently by pig fibroblasts than its normal counterpart. This observation suggests that changes in apoB result in defective binding of mutant LDL to the LDL receptor. This conclusion is also supported by previous studies of Rapacz (11) showing that the segregation of the mutant apoB allotype follows the segregation of the hypercholesterolemic phenotype. Although it is apparent that the receptor binding defect in mutant LDL is due in part to a defect in apoB, the lipid composition also influences the binding of mutant and control pig LDL to the LDL receptor. First, the buoyant LDL subfractions from both mutant and control had a reduced degradation rate (and had a decreased affinity for the LDL receptor) than the corresponding dense LDL subfractions. Secondly, the differences between mutant and control LDL binding and catabolism in fibroblasts were diminished in the more buoyant LDL subspecies. Because these experiments utilized LDLs obtained from pigs that were apoB homozygotes, heterogeneity in the primary structure of apoB did not determine the metabolic differences between LDL subspecies from the same animal.

The simplest explanation for our results is that increased amounts of lipid lead to decreased catabolism of pig LDL in vitro. Studies comparing the turnover of the various pig LDL subfractions in vivo support this conclusion. Even in control animals the catabolism of the buoyant LDL subspecies is decreased. One mechanism by which increased amounts of lipid could reduce catabolism of buoyant LDL is by masking regions of apoB important for interaction with the LDL receptor. Alternatively, the abnormal composition or larger size of buoyant LDL may alter the tertiary structure of apoB, causing it to have a lower affinity for the LDL receptor.

Because the majority of the cholesterol elevation in mutant pigs was in the buoyant LDL subfraction (18), we expected that the metabolic differences observed between mutants and control pigs would be most pronounced in this subspecies. However, we found that the differences between the buoyant LDLs were attenuated compared to the differences between dense LDLs. If critical apoB domains responsible for LDL receptor binding are altered by the increased lipid content in buoyant LDL, it is conceivable that those altered regions in normal buoyant LDL include the ones that are mutated in the mutant pigs.

Differences in the metabolism of compositionally different LDL subspecies have been observed in other experimental systems. Calvert et al. (32) obtained results in normal pigs analogous to ours; dense LDL was catabolized more rapidly in vivo than a more buoyant LDL species. Witztum et al. (33) observed a decreased fractional turnover of LDL isolated from cholestyramine-treated guinea pigs. This LDL had an increased density relative to untreated guinea pig LDL. In humans, but not in pigs, buoyant LDL is a better ligand for the LDL receptor. Hammond and Fisher (34) first recognized LDL structural heterogeneity in patients with primary hypertriglyceridemia. In such patients, and in individuals with familial combined hyperlipidemia, the denser LDL subspecies of LDL would be most pronounced in this subspecies. However, we found that the differences between the buoyant LDLs were attenuated compared to the differences between dense LDLs. If critical apoB domains responsible for LDL receptor binding are altered by the increased lipid content in buoyant LDL, it is conceivable that those altered regions in normal buoyant LDL include the ones that are mutated in the mutant pigs.

Differences in LDL subspecies metabolism can also arise in apoB heterozygotes; different allelic apoB molecules could be preferentially associated with compositionally distinct LDL particles. In a recent study by Innerarity et al. (17), dense LDL from a patient with defective apoB had a lower affinity for the LDL receptor than a more buoyant subspecies. However, this patient was most likely an apoB heterozygote, and these workers concluded that the denser LDL contained the mutant form of apoB (17). Our studies used LDLs from apoB homozygotes; thus genetically nonhomogeneous apoB molecules in pig LDL cannot account for our observations.

2 Portions of this paper (including "Experimental Procedures," "Results," Tables 1 and 2, and Figs. 1-6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are included in the microfilm edition of the Journal that is available from Waverly Press.

3 W. J. Checovich, R. J. Aiello, and A. D. Attie, unpublished observations.
Defective Receptor Binding of Mutant Pig LDL

Pig LDL binding to cultured fibroblasts did not fit well to the common one-site model. A significantly improved data fit to the two-site model occurred for whole LDL, as well as both dense and buoyant LDL subspecies. Scatchard plots of the data from saturation binding experiments showed a concave upward curvature. Although pig LDL possesses at least two subspecies with different affinities for the LDL receptor, ligand heterogeneity cannot account for the concave upward Scatchard plot. As demonstrated by Mendel et al. (38), ligand heterogeneity yields a concave \textit{downward} Scatchard plot, which can only be detected when the affinity differences between the ligands are very large. The affinity differences between the LDL subspecies were too small to be detected in our analysis of the whole LDL fraction. Thus, the dissociation constants we report for whole LDL are weighted averages of the entire LDL particle spectrum (38).

A concave upward Scatchard plot reflects multiple binding sites or negative cooperativity (30, 39). When we fit the data to a two-site model, most of the binding at saturation was to a site for which mutant LDL had a significantly lower affinity. The second binding site displayed a much higher affinity for both LDLs. Receptor self-association was recently reported for the human and bovine LDL receptors (40). The LDL receptor might have different affinities for LDL depending on the state of receptor self-association. LDL receptor self-association might also provide a mechanism for negative cooperativity. Occupancy-dependent affinity has been observed for a number of cell surface receptors, including the insulin receptor (39). Such binding behavior is thought to occur through receptor-receptor interactions (39). If negative cooperativity exists in pig fibroblasts, the dissociation constants we report for the lower affinity “site” would correspond to the affinity of LDL at high receptor occupancy. The dissociation constant for the higher affinity “site” would represent the lower limit of the dissociation constant at very low receptor occupancy (41, 42).

We have identified a receptor binding defect in LDL from hypercholesterolemic pigs with a mutant apoB allele. In addition, compositional heterogeneity of pig LDL particles correlated with distinct metabolic characteristics. Changes in receptor binding affinity can therefore arise from apoB mutations or from alterations in the lipid composition of the LDL particle.

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REFERENCES


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Defective Receptor Binding of Mutant Pig LDL

Experimental Procedures

Antibodies: Mutant and control pigs were classified according to the presence of three genomically defined lipoprotein-associated markers. LDL, Lp(α), and Lp(a) (11). LDL corresponds to apoA1 (12), Lp(α) corresponds to apoB-48, and Lp(a) is an antigenically defined Lp(a) in an allele which has not been biochemically characterized (13). Mutant animals possessed the genotype LDL, Lp(α), Lp(a), whereas L was either allele 1 or 2, and where non-functional receptor-glycosylation control genotypes were LDL, Lp(a), L (14) and Lp(a), LDL, L (15) and were non-cholesterol containing (plasma cholesterol <40 mg/dl). All pigs were fed a diet containing a corn-oat meal mixture fed at 56 and 58 kcal.

Lipoproteins: Lipoproteins were isolated as described (16, 17), and further purified as follows: approximately 2-3 ml of LDL was layered beneath 0.6 M of sucrose solution (18-20 mg/ml) and centrifuged in a Beckman Ti 45 rotor (10 hours, 46,000 rpm, 15°C). The top of the tube was sucked and discarded. The lipoprotein-bound was collected and the density was determined by p = 1.064 g/ml with sodium. This fraction was centrifuged through a 1.0 M sucrose solution and was centrifuged in a Beckman Ti 50 rotor (10 hours, 46,000 rpm, 15°C). The lipoprotein was collected from the top of the tube. All LDL were dialyzed against phosphate-buffered saline (PBS: 0.15 M NaCl, 0.05 M potassium phosphate, 0.1% NaN3, pH 7.4). Finally, the LDL were filtered through 0.22 m filters (Centricron Acrodex) and were stored at 4°C. All LDL were used within 1-2 weeks.

Preparations of LDL were isolated by equilibrium density gradient ultracentrifugation (20). The LDL preparations were dialyzed to p = 1.026 g/ml against water and n = 0.01 was layered between equal volumes of p = 1.216 g/ml (n = 0.01) and water. At 10°C, the gradient was formed by ultracentrifugation in a Beckman Ti 50 rotor (40,000 rpm, 72 hours, 15°C), and 0.01 fractions were collected after sedimentation at a density gradient fractionator (ISCO, model 120). Fractions of each preparation were loaded on SDS-PAGE gels and used for production of antibodies.

Indices of apolipoprotein B-100 and its variants were isolated from a blank tube run in parallel. The LDL subfractions were pooled into two ranges: p = 1.019-1.024 g/ml and p = 1.026-1.029 g/ml. LDL was isolated as previously described (18). Each LDL preparation was analyzed for routine lipoprotein-immunodiagnostic and mobility and concentration was determined (18). The specific activities were between 200 and 400 mg ty. More than 95% of the radioactivity was precipitable by 3% TCA (trichloroacetic acid) (19). The radioactivity was determined by liquid scintillation (18). No significant differences in the mean density were observed between the two LDL preparations.

Lipid composition: Lipoprotein composition was determined by the methods of Brown and Goldstein (20). Aliquots (50 μl) of each LDL preparation were analyzed for routine lipoprotein-immunodiagnostic and mobility and concentration was determined (18). The specific activities were between 200 and 400 mg ty. More than 95% of the radioactivity was precipitable by 3% TCA (trichloroacetic acid) (19). The radioactivity was determined by liquid scintillation (18). No significant differences in the mean density were observed between the two LDL preparations.

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Table 1.

Composition of Pig Low Density Lipoproteins* (percent mass)

<table>
<thead>
<tr>
<th></th>
<th>Cholesterol</th>
<th>Triacylglycerol</th>
<th>Phospholipid</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cholesterol</strong></td>
<td>9.9 ± 1.0</td>
<td>46.7 ± 3.2</td>
<td>7.6 ± 4.2</td>
<td>17.7 ± 6.4</td>
</tr>
<tr>
<td><strong>Buoyant</strong></td>
<td>6.8</td>
<td>53.5</td>
<td>2.7</td>
<td>19.1</td>
</tr>
<tr>
<td><strong>Dense</strong></td>
<td>3.2</td>
<td>47.1</td>
<td>2.6</td>
<td>26.6</td>
</tr>
</tbody>
</table>

**Mutant**

|                | 7.3 ± 2.4   | 55.3 ± 4.3      | 5.4 ± 3.3    | 18.2 ± 1.7 | 19.7 ± 1.4 |
| **Buoyant**    | 4.6         | 63.6            | 2.0          | 16.4      | 13.5     |
| **Dense**      | 7.8         | 48.5            | 2.6          | 26.3      | 20.9     |

1. Mutant and control LDL composition according to density. The density classifications are as follows: Total = 1.019-1.043 g/ml; Buoyant = 1.019-1.038 g/ml; Dense = 1.038-1.043 g/ml.
2. Mean of three separate LDL preparations (standard deviation). All other values represent the mean of two LDL preparations.

Fig. 1. Polyacrylamide gel electrophoresis of mutant and control LDL. Equal amounts of delipidated LDL protein (30 μg) were run on 10% gels and the bands were visualized with Coomassie blue R-250. Lane A, whole mutant LDL; Lane B, whole control LDL (λ = 1.039 ± 0.063); Lane C, mutant buoyant LDL; Lane D, control buoyant LDL (λ = 1.019 ± 0.038); Lane E, mutant dense LDL; Lane F, control dense LDL (λ = 1.038 ± 0.063).

Competition studies in cultured pig fibroblasts.

In receptor binding studies at 4°C, mutant LDL competed less efficiently with 125I-labeled control LDL than did unlabeled control LDL (figure 2A). In a representative experiment (n=3) the concentration of unlabeled control LDL necessary to achieve 50% inhibition of 125I-LDL binding (at 1 μg/ml) was 5.1 μg/ml; for mutant LDL this concentration was 17.7 μg/ml. This apparent decrease in mutant LDL binding was also reflected in experiments measuring competition for degradation at 37°C (figure 2B). The concentration of unlabeled mutant LDL needed to achieve 50% inhibition of 125I-labeled control LDL (at 5 μg/ml) degradation was 18.0 ± 1.1 g/ml (n=4); compared to 7.4 ± 2.5 g/ml (n=4) for unlabeled control LDL. Similar results were obtained in experiments measuring competition for cell-associated LDL (figure 2C).

Fig. 2. Competitive displacement of 125I-labeled control LDL by mutant (O) or control (0) LDL to cultured pig fibroblasts. The ability of mutant LDL to compete with 125I-labeled LDL for (A) binding at 4°C, (B) proteolytic degradation at 37°C, and (C) cell-associated LDL at 37°C were determined. In the 4°C experiments, cells received 1 μg/ml 125I-labeled control LDL and the indicated concentrations of unlabeled mutant or control LDL. The maximal amount of binding was 73 ng LDL/mg cell protein. The mutant LDL displacement curve was significantly different from control (p=0.000001). In the 37°C experiments, the cells received 5 μg/ml 125I-labeled control LDL. The maximal amount of LDL degraded after 5 hours was 1000 ng/mg cell protein; the maximal amount of cell-associated LDL was 140 ng/mg cell protein. In both experiments, mutant LDL was significantly different from control LDL (p=0.01 and p=0.0035, respectively). Each data point represents the average of duplicate incubations.

Direct binding of LDLs at 4°C.

To directly determine binding constants for the two types of LDL, we carried out equilibrium binding studies at 4°C. Mutant LDL had diminished binding activity in cultured pig fibroblasts (figure 3A). A nonlinear least squares curve-fitting program allowed us to determine the binding parameters, including non-specific binding (B). A summary of our analysis is shown in Table 2. When we used the equation for a one-site model to fit the curve to the data points a high mean square was obtained, reflecting a poor fit. A highly significant (p<0.002) improvement in curve fitting resulted when we employed a model with two classes of binding sites.

Fig. 3. Direct binding of mutant (O) and control (0) LDL to cultured pig fibroblasts 4°C.

Cells received the indicated concentrations of 125I-labeled mutant or control LDL. After incubation for 4 hours at 4°C, the amount of LDL surface-bound LDL (ng LDL bound/mg cell protein) was determined. Scatchard plots of the data (R,C) using the two-site model (corrected for non-specific binding) are shown, with the lines indicating the individual binding components. The binding constants are shown in Table 2.
Defective Receptor Binding of Mutant Pig LDL

Using the one-site model, the calculated dissociation constants were in apparent contradiction to what would have been expected from the competition experiments: mutant LDL had a lower dissociation constant than control LDL. The data (corrected for non-specific binding) were not randomly distributed about the line of best fit (data not shown) (127,12). Although the experiments were conducted under conditions where the amount of ligand bound at saturation was identical for both ligands, forcing the model to be the same for mutant and control LDL resulted in a significant decrease in the goodness of fit (p<0.000001). However, data analysis using a two-site model distributed the data points randomly about the line of best fit. Moreover, forcing the k_1 to be identical for both mutant and control LDL did not result in a significantly worse fit of the data (p=0.37). Therefore, we rejected the one-site model. The values describing the fit to the two-site model are those obtained under this constraint (Table 1).

Backward representation of the binding data (corrected for non-specific binding) clearly concave upward appearance characteristic of multiple binding sites (figures 5B and D) (13). One class of binding sites bound 80-95% of the total amount of dextran sulfate-releasable LDL on pig fibroblasts. Mutant LDL had a significantly lower affinity for this site. The k_2 were 3.1±1.0 x 10^-5 M and 5.7±4.0 x 10^-5 M for control LDL (n=4). The second class of sites had a 20-120-fold higher affinity for both mutant and control pig LDL, but the affinity for the second class of sites was higher in control LDL (n=4). Although the two affinity sites were not equal, a linear fit of the data did not reveal significant differences between the binding affinities of mutant and control LDL for this site. This higher affinity site was detected in at least 10 replicate experiments using the A25471 pig fibroblast cell line. In the absence of cells, binding was negligible (data not shown). Thus, LDL binding to tissue culture dishes cannot account for our observations.

Relative degradation rates of control and mutant LDLs

To determine how the diminished receptor binding affinity might affect the kinetics of mutant LDL delivery to cells, we measured the LDL degradation rates in cultured pig fibroblasts. When cells were incubated with non-saturating LDL concentrations, mutant LDL was degraded at approximately 6% of the rate measured for control LDL (figure 4B). When cells were incubated with saturating LDL concentrations such that receptor binding was not rate-limiting for cellular uptake (5%), the degradation rates were identical (figure 4B), consistent with a defect in receptor-binding resulting in decreased catabolism rather than defective LDL internalization.

LDL-enhanced cholesterol esterification

Receptor-mediated endocytosis of LDL results in enhanced cholesterol esterification (4). In the absence of apo-B protein, both LDLs displayed similar ability to stimulate cholesterol esterification (figure 5A). Alternatively, when data were analyzed in terms of cholesterol available to the cells, n LDL was a poorer stimulator of cholesterol esterification (figure 5B). This may be explained by the large difference in liquid composition of particles: mutant LDL has approximately 5% more cholesterol per mg relative to protein than control LDL (table 1).

**Table 1.** Equilibrium Binding Constants for Control and Mutant LDL

| LDL | 
|-----|-----|-----|
| Control | Mutant | Control | Mutant |
| k_1 | 0.05 ± 0.09 | 0.15 ± 0.43 |
| k_2 | 0.03 ± 0.03 | 0.1 ± 0.07 |
| k_bound | 16.7 ± 2.9 | 23.9 ± 4.5 |
| K | 16.5 ± 1.4 | 16.3 ± 1.1 |
| n | 1.0 ± 0.0 |
| R | 9.7 ± 1.3 |
| R | 7.1 ± 1.7 |
| n | 6.9 ± 0.8 |
| r | 1.9 ± 0.9 |

**Fig. 5.** Enhancement of cholesterol esterification in cultured fibroblasts by mutant (O) and control (A) LDL in equi-particle-muLDL concentration. Cells were incubated with indicated concentrations of mutant or control LDL. After 48 hours incubation with 3H-cholesterol-labeled LDL, 3H-cholesterol esterified (cpm/mg protein) was measured. Data are expressed in terms of the cholesterol concentration available to the cells. The data point is the avg of duplicate measurements.
Defective Receptor Binding of Mutant Pig LDL

Degradation rates and direct binding of LDL subfractions.

Since LDL from normal pigs consists primarily of a dense subspecies, and LDL from mutant pigs consists of a more buoyant subspecies [18], the LDL particles that were compared differed in protein and lipid composition. To examine the effect of lipid composition on LDL metabolism, we isolated dense and buoyant LDL from mutant and control animals. The dense LDLs were relatively depleted in lipids, and the compositions did not differ between mutant and control (Table 1). Accordingly, comparing these subspecies minimized the effects of lipid composition on LDL metabolism, allowing us to investigate more directly whether the mutant LDL binding defect was due to an apo-B structural defect, or to the particle's abnormal lipid composition.

Mutant dense LDL was degraded at a much slower rate (45%) than control dense LDL (Figure 6A). Additionally, the buoyant LDL subspecies from both types of pigs were degraded at a slower rate than the corresponding dense LDL subspecies. Finally, the mutant LDL catabolic defect was much more pronounced in the dense LDL particles than it was in the buoyant particles (Figure 6A and 6B).

In direct binding studies at 4°C, the two-site model significantly improved the data fit for both the dense and buoyant LDL subspecies (p<0.00001 and p<0.000005, respectively). The dissociation constants for the predominant binding sites were 11 and 13 ng/ml for dense and buoyant mutant LDL; 1.8 and 5.1 ng/ml for dense and buoyant control LDL (data not shown). Thus, in analogy to the degradation experiments, the buoyant LDL subspecies had a lower affinity for the LDL receptor than the dense LDL subspecies. Similarly, the difference between the affinity of mutant and control dense LDL (4-fold) was much greater than the difference between mutant and control buoyant LDL (1.5-fold).

Fig. 6. Degradation rates of mutant (○) and control (■) LDL subspecies. Dense (○: p<0.00001) and buoyant (■: p<0.0001) LDL subspecies were isolated. Cells received 5 µg/ml of mutant or control (A) dense LDL or (B) buoyant LDL subfractions. The amount of proteolytic degradation (ng LDL degraded/mg cell protein) was determined. Each point is the average of duplicate determinations.