Overproduction of a Buoyant Low Density Lipoprotein Subspecies in Spontaneously Hypercholesterolemic Mutant Pigs

William J. Checovich, Robert J. Aiello, and Alan D. Attie

We previously described the hypercholesterolemia of pigs with defined apolipoprotein B (apo B) alleles associated with reduced binding of low density lipoprotein (LDL) to its receptor in vitro and slow clearance from the circulation in vivo. The increased plasma LDL in the hypercholesterolemic pigs was confined to a buoyant LDL subspecies. Because of this qualitative change in the LDL subspecies profile, we studied the turnover of buoyant and dense LDL subspecies independently. Normal and mutant radioiodinated buoyant and dense LDLs were simultaneously injected into normal and mutant pigs, and the clearance rates, interconversion rates, and production rates were determined. The sevenfold increase in buoyant LDL levels in the mutant pigs was due to a fivefold increase in buoyant LDL production. Total mutant LDL production was increased approximately 25%, suggesting that part of the increase in buoyant LDL production is at the expense of dense LDL production. Conversion of dense LDL to buoyant LDL made a small contribution to the buoyant LDL increase. The turnover analysis showed that dense LDL, in both mutant and control pigs, is primarily derived from a source other than buoyant LDL. To test this more directly, [3H]leucine was intravenously injected, and the specific activity of the LDL subspecies was measured over 96 hours. There was a large discrepancy in the areas under the specific activity–versus–time curves, indicating that buoyant LDL cannot be the sole precursor of dense LDL and further supporting the conclusion that buoyant and dense LDL are, in part, metabolically independent particles. These results show that genetic variation in the apo B locus can affect the synthetic rate of LDL and the LDL subspecies distribution. (Arteriosclerosis and Thrombosis 1991;11:351–361)

Abnormalities in lipoprotein metabolism most often manifest themselves as changes in the number and/or composition of lipoprotein particles. In the case of genetically determined abnormalities, valuable insights into the fundamental mechanisms regulating plasma concentrations of lipoproteins and their chemical compositions can be obtained by identifying the causative genetic lesions. For instance, while low density lipoprotein (LDL) receptor mutations reduce the efficiency of plasma LDL catabolism, studies of an animal model with an LDL receptor defect, the Watanabe heritable hyperlipidemic rabbit, also showed that the receptor defect leads to overproduction of LDL. The latter phenomenon has been attributed to the prolonged circulation of intermediate density lipoprotein (IDL) particles and their consequent conversion to LDL.1

Plasma LDL comprises a spectrum of particles differing in size and in the amount of particle-borne lipid.2,3 Although an elevation in plasma LDL particle number places an individual at increased risk of coronary heart disease, Austin et al4 have also identified a correlation between a predominance of smaller, denser LDL particles and an increased risk of coronary heart disease. These authors concluded that the LDL particle distribution was genetically determined, yet the genes responsible for these differences in LDL distribution are unknown.

Apolipoprotein B (apo B) is the major protein constituent of LDL and a ligand for the LDL receptor. The connection between genetic variability in apo B structure and plasma cholesterol variation was first established by studies of defined apo B alleles in...
pigs. From these studies, LDL carrying an immunologically defined marker, termed Lpb5, was associated with hypercholesterolemia. That marker was subsequently shown to be an epitope of apo B. Recent studies have demonstrated that genetic variation in apo B also contributes to hypercholesterolemia in human subjects.

In vivo plasma turnover studies of LDL carrying the Lpb5 marker revealed an impairment in plasma clearance, suggesting that defective LDL clearance contributes to the hypercholesterolemia of the Lpb5 pigs. However, a second phenotype was also identified; the rise in plasma LDL concentration was almost entirely in a buoyant LDL subspecies. In the present study, we investigated the relation between defective clearance of LDL and the accumulation of buoyant LDL in the mutant pig.

Methods

Animals

Mutant pigs were originally obtained from Jan Rapacz and propagated by outbreeding and backcrossing to obtain affected animals. Originally, the pigs were immunologically identified by the presence of alloantigens, designated Lpb and Lpr. Lpb corresponds to apo B. Lpr corresponds to a 23-kd apo we have named apo R. Hypercholesterolemic animals were originally defined by an allotype designated Lpb5. We now identify these animals by probing genomic DNA for a restriction fragment length polymorphism (RFLP) created by a unique insertion at intron 28.1 The Lpb5 pigs exhibit two different phenotypes. One group has severe hypercholesterolemia (plasma cholesterol >190 mg/dl) and a predominance of a buoyant LDL subspecies. We call these pigs Lpb5.1. A second group of Lpb5 pigs has moderate hypercholesterolemia (plasma cholesterol 100–120 mg/dl) and are designated Lpb5.2. In the present study, all “mutant” pigs were Lpb5.1. The designation is based on the diagnostic RFLP, the extent of the hypercholesterolemia, and elevated levels of buoyant LDL. Because the molecular basis for Lpb5 heterogeneity is unknown, our Lpb5.1 and Lpb5.2 designations are phenotypes while Lpb5 is a genotype based on the RFLP at intron 28. Control pigs had a plasma cholesterol concentration of less than 112 mg/dl and did not exhibit an elevation in the buoyant LDL subspecies (Table 1).

Table 1. Genotypes of Pigs

<table>
<thead>
<tr>
<th>Animal</th>
<th>Lpb</th>
<th>Lpr</th>
<th>Plasma cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5/8 (5.2)</td>
<td>2/2</td>
<td>106</td>
</tr>
<tr>
<td>2</td>
<td>8/8</td>
<td>2/2</td>
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</tr>
<tr>
<td>3</td>
<td>8/8</td>
<td>1/1</td>
<td>82</td>
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<tr>
<td>4</td>
<td>Non-5</td>
<td>2/2</td>
<td>98</td>
</tr>
<tr>
<td>5</td>
<td>Non-5</td>
<td>2/2</td>
<td>109</td>
</tr>
<tr>
<td>6</td>
<td>ND</td>
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<td>48</td>
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<td>5/5 (5.1)</td>
<td>1/1</td>
<td>220</td>
</tr>
<tr>
<td>19</td>
<td>5/8 (5.1)</td>
<td>1/2</td>
<td>210</td>
</tr>
</tbody>
</table>

Lpr phenotype was determined by subjecting very low density lipoprotein (VLDL) samples to isoelectric focusing followed by immunoblot using a polyclonal antibody directed against apo protein R, a 23-kd protein in pig VLDL.

ND, not determined.

Lipoprotein Subfractionation

Very low density lipoproteins (VLDLs; d<1.006 g/ml), IDLs (d=1.006–1.019 g/ml), and LDLs (d=1.019–1.063 g/ml) were isolated, purified, and subfractionated as described previously. The LDL subfractions were pooled into two ranges: d=1.019–1.038 g/ml (buoyant LDL) and d=1.038–1.063 g/ml (dense LDL). The density cutoff at 1.038 g/ml was used for all animal LDL separations and represents the most common density at which a shoulder or depression in the mutant LDL density profile is present, compared with control LDL.

LDL subspecies were iodinated with 125I (Dupont, Boston, Mass.) or 31I (ICN, Cleveland, Ohio) as described previously, and each preparation was assayed for proper relative electrophoretic mobility and lack of bacterial contamination. Specific activities were 50–600 cpm/mg. Approximately one in every 30 apo B molecules was labeled with iodine, and more than 98% of the label appeared in apo B. Greater than 95% of the radioactivity was precipitable with trichloroacetic acid (TCA; final concentration, 10%), and less than 2% of the radioactivity was chloroform soluble. Apo E was not detected in the LDL preparations by overloading onto 10% sodium
was determined with a Packard gamma counter.

Bovine serum albumin (Sigma; final concentration, 1 mg/ml) was combined with an equal volume of a NaBr solution (d=1.065 g/ml) and layered between equal volumes of NaBr solutions. The samples were centrifuged in a Beckman Ti 50 rotor (24 hours, 50,000 rpm, 15°C) and dialyzed against 1 mM EDTA. The fractions with densities d=1.019-1.038 g/ml and d=1.038-1.063 g/ml were pooled. The specific activity of apo B in each fraction was determined by dividing the measured radioactivity (counts per minute per milliliter) by the apo B concentration (milligrams per milliliter) in each fraction.

In Vivo Endogenous Low Density Lipoprotein Production

[3H]leucine (5 mCi, Dupont) was intravenously injected into pigs. Samples were collected as described above for the iodine turnover experiments. The plasma samples were exhaustively dialyzed against a NaBr solution (d=1.006 g/ml, 1 mM EDTA) to remove free leucine. LDLs were isolated by sequential ultracentrifugation in a Beckman Ti 50 rotor (24 hours, 50,000 rpm, 15°C) and dialyzed against d=1.04 g/ml NaBr (1 mM EDTA). A 1.8-ml aliquot of each LDL was layered between equal volumes of d=1.026 g/ml (1 mM EDTA) and d=1.054 g/ml NaBr (1 mM EDTA) solutions and centrifuged in a Ti 50 rotor; fractions were collected as described above for the iodine turnover experiments. The fractions with densities d=1.019-1.038 g/ml and d=1.038-1.063 g/ml were pooled. The specific activity of apo B in both LDL subspecies was determined by the method of Egusa et al., with the following modifications: 1) sodium hydroxide-dispersed apo B samples were extracted once with 2 ml n-hexane to remove lipid before protein determination and 2) Hionic Fluor (Packard) was used as the scintillant.

In Vivo Turnover Studies

Iodinated LDLs (3 μg/kg body wt) were injected into nonanesthetized animals via vena caval puncture. The animals were fasted 12 hours before the start of each experiment and were fed approximately 3 hours after the injection. Thereafter, the animals were fed twice daily (12 hours apart). Ordinarily, 15 6-ml samples were collected from each animal by vena caval puncture into Vacutainer tubes (Becton Dickinson, Oxnard, Calif.) containing EDTA. The first sample was collected 10 minutes after injection and was considered time zero. Nine samples were collected during the initial 24 hours, after which a sample was collected every 12 hours for a total of 96 hours.

Each plasma sample was fractionated into buoyant and dense LDL, and both 125I and 131I radioactivities were determined. Thus, for the two injected tracers, four curves were generated. Each plasma sample (0.9 ml) was combined with an equal volume of a NaBr solution (d=1.065 g/ml) and layered between equal volumes of d=1.026 g/ml and d=1.054 g/ml NaBr solutions. The samples were centrifuged in a Beckman Ti SW 50.1 rotor (Beckman Instruments, Palo Alto, Calif.) (48 hours, 50,000 rpm, 15°C) and fractionated into 16 0.375-ml fractions. The density of each fraction was estimated from the refractive index of identical fractions isolated from a blank tube run in parallel. Less than 1% of total radioactivity was found in the d>1.063 g/ml fractions of plasma. Bovine serum albumin (Sigma; final concentration, 1%) was added to each fraction, and TCA (final concentration, 10% wt/vol)-precipitable radioactivity was determined with a Packard gamma counter. (Packard Instruments, Downers Grove, Ill.). LDL radioactivity in each fraction was pooled for the density ranges d=1.019-1.038 g/ml (buoyant LDL) and d=1.038-1.063 g/ml (dense LDL). Specific activity of the buoyant and dense fractions was calculated by dividing the measured radioactivity (counts per minute per milliliter) by the apo B concentration (milligrams per milliliter) in each fraction.

Kinetic Analysis

The fractional catabolic rates (FCRs) of coinjected mutant and control LDL subspecies (Table 2) were estimated using the nonlinear least-squares curve-fitting program KINETIC (as modified by G.A. McPherson, Elsevier-BIOSOFT, Cambridge, U.K.). This program uses a multienzyme algorithm to calculate the slopes and intercepts of each exponential component of the turnover curves. The biexponential fit was used to estimate the area under each curve.

The F test was used to compare each pair of mutant and control LDL turnover curves17,18 and to test the hypothesis that the two sets of turnover data could be better described by a single curve. In addition, the

<table>
<thead>
<tr>
<th>Animal</th>
<th>Control buoyant</th>
<th>Mutant buoyant</th>
<th>F test*</th>
<th>Control dense</th>
<th>Mutant dense</th>
<th>F test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.040±0.003</td>
<td>0.024±0.001</td>
<td>p&lt;0.001</td>
<td>0.056±0.007</td>
<td>0.044±0.006</td>
<td>p=0.006</td>
</tr>
<tr>
<td>2</td>
<td>0.038±0.007</td>
<td>0.023±0.006</td>
<td>p&lt;0.001</td>
<td>0.071±0.005</td>
<td>0.056±0.004</td>
<td>p=0.002</td>
</tr>
<tr>
<td>4</td>
<td>0.035±0.002</td>
<td>0.023±0.001</td>
<td>p&lt;0.001</td>
<td>0.059±0.011</td>
<td>0.049±0.005</td>
<td>p=0.008</td>
</tr>
<tr>
<td>5</td>
<td>0.072±0.007</td>
<td>0.055±0.009</td>
<td>p&lt;0.001</td>
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<td>0.056±0.004</td>
<td>p=0.002</td>
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<tr>
<td>6</td>
<td>0.083±0.016</td>
<td>0.053±0.028</td>
<td>p&lt;0.001</td>
<td>0.068±0.011</td>
<td>0.051±0.005</td>
<td>p&lt;0.01†</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>0.038±0.003</td>
<td>0.023±0.001</td>
<td>p&lt;0.01†</td>
<td>0.068±0.011</td>
<td>0.051±0.005</td>
<td>p&lt;0.01†</td>
</tr>
</tbody>
</table>

Control and mutant buoyant or dense low density lipoproteins (LDLs) were simultaneously injected into control pigs. Buoyant LDL, d=1.019-1.038 g/ml; dense LDL, d=1.038-1.063 g/ml.

*The turnover curves of each set of paired mutant and control LDL subspecies were compared using an F test.

†Each group of paired turnover values was analyzed using a paired t test.
FIGURE 1. Diagram of the two-accessible-pool/noncompartmental model used to analyze turnover data. In this model, pools 1 and 2 represent buoyant and dense low density lipoproteins (LDLs), respectively. These pools can exchange with each other as indicated by the arrows. Kinetics of the interactions of buoyant and dense LDL with nonaccessible pools are described by recirculation-exchange arrows. The difference between this model and a compartmental model is that in the latter, a compartmental structure is proposed to describe recirculation-exchange arrows. Radiolabeled buoyant and dense LDLs are injected as a bolus into their respective pools, and radioactivity associated with both labels is sampled from each pool, producing the four turnover curves reported. Transport parameters $F_i$ estimated using this model are indicated in the figure.

differences between control and mutant means were compared using paired or one-tailed $t$ tests.  

To interpret the turnover data, the two-accessible-pool/noncompartmental model originally proposed by Rescigno and Gurpide was used; in this case, the two accessible pools are plasma buoyant and dense LDL. This model is shown in Figure 1. In this model, the two LDL accessible pools numbered 1 and 2 are the plasma buoyant and dense LDL, respectively. Using this noncompartmental model approach, the interactions of these accessible pools with the nonaccessible pools in the system are taken into account.

This model permits the estimation of de novo production, $F_{10}$ and $F_{20}$, disposal, $F_{01}$ and $F_{02}$, and transport between buoyant and dense LDL, $F_{12}$ and $F_{21}$. Assuming the labeled material in the two accessible pools is kinetically homogeneous and assuming that the two pools are treated mathematically identically, this method of analysis will produce the same estimates for these transport rates as a compartmental model.

Each tracer sample was fractionated into buoyant and dense LDL. The program KINETIC was used to estimate the areas under the specific activity curves required by the model equations. Zero-time LDL doses were also measured for each injected LDL and for the corresponding contaminating spillover into the product density range. These four doses, together with the four corresponding areas under the specific activity–time courses, were used in the kinetic analysis, and unique values were calculated for each flux in the model.

Results

Turnover of Mutant and Control Low Density Lipoprotein Subspecies Coinjected Into Control Pigs

Mutant LDL subspecies are cleared more slowly than their control counterparts when compared directly in control pigs, consistent with a mutant LDL apo B defect. Representative turnover curves of mutant and control buoyant LDL simultaneously injected into control pigs are shown in Figure 2 (upper panel). In each experiment, the turnover curves are significantly different ($p<0.01$; Table 2). The FCR of mutant buoyant LDL was approximately 40% lower than the FCR of control buoyant LDL (0.023 versus 0.038 pools/hr). Similarly, a representative pair of turnover curves of mutant and control dense LDL injected into control pigs is shown in Figure 2 (lower panel). The FCR of mutant dense LDL was significantly lower ($p<0.01$) than that of control dense LDL (Table 2; 0.051 versus 0.068 pools/hr).

Comparison of these two sets of experiments also showed that buoyant LDL was cleared more slowly than dense LDL from both types of pigs. Both mutant and control buoyant LDLs were cleared 50% more slowly than their respective dense LDL particles (mutants: 0.023 versus 0.051 pools/hr; control: 0.038 versus 0.068 pools/hr).

Buoyant Low Density Lipoprotein Overproduction in Mutant Pigs

The concentration of buoyant LDL was sevenfold greater in the mutant pig than in control pigs (63.2 versus 8.6 mg/dl protein, $p<0.0001$; Table 3). The concentration of dense LDL was similar in the two pig types.

Autologous radiolabeled buoyant and dense LDLs were injected into control and mutant pigs, and the amount of tracer in buoyant and dense LDL was measured after isolation by density gradient ultracentrifugation at the indicated times (Figure 3). It is important to reemphasize that we measured the
Checwich et al  Altered LDL Subspecies Metabolism 355

Buoyant LDL Turnover

Dense LDL Turnover

FIGURE 2. Representative disappearance curves (%/hr) of buoyant (upper panel) and dense (lower panel) control (•) and mutant (○) low density lipoprotein (LDL) in a control pig including the line of best fit. LDL radioactivity was measured in the indicated subspecies density range and does not represent total plasma LDL radioactivity.

presence of tracer in each LDL subspecies and not whole plasma, allowing for the direct measurement of interconversion between the two LDL subspecies. The rates of production, interconversion, and catabolism of each LDL subspecies in mutant and control pigs, obtained from the kinetic analyses, are summarized in Table 3 and Figure 4.

Dense LDL in mutant pigs was cleared more slowly than dense LDL in control pigs (0.028 versus 0.043 pools/hr, p<0.05; Table 3), but there was no significant difference between the clearance rates of buoyant LDL in mutant and control pigs (0.011 versus 0.015 pools/hr; Table 3). The FCRs calculated from the LDL flux and pool size data (Table 3) are smaller than those in Table 2 because the turnover curves in Table 2 reflect both direct clearance along with net conversion to the other LDL subspecies and subsequent clearance, and therefore, they overestimate the true FCR.

Since the steady-state level of buoyant and dense LDL is a balance of input and output and since the FCR of mutant buoyant LDL is not less than that of control buoyant LDL, we conclude that overproduction is responsible for the increased mass of buoyant LDL.

Indeed, the major difference between mutant and control animals was the input rate of buoyant LDL (F10+F12). The mutant buoyant LDL input rate was fivefold greater than the input rate of control buoyant LDL (443 versus 89 μg/kg/hr). The most striking difference was the input into the buoyant LDL pool from outside the system, F10 (see Figure 1). This buoyant mutant LDL production was 23-fold greater than control buoyant LDL production from outside the system, which in many cases was undetectable.

While there was considerable interconversion of buoyant and dense LDL in both mutant and control pigs (approximately one third of each pool was converted to the other pool), conversion of dense LDL to buoyant LDL did not explain increased buoyant LDL concentrations in mutant pigs. While the percent of mutant dense LDL converted to buoyant LDL doubled (16% versus 31%), the amount of buoyant LDL derived from dense LDL was greatly reduced in mutant pigs compared with control pigs (26% versus 84%).

Dense Low Density Lipoprotein Production

The turnover experiments indicated that in both mutant and control pigs, dense LDL was produced from endogenous sources other than buoyant LDL. While the primary difference between mutant and control pigs is the input rate of buoyant LDL (F10), it was surprising that the mutant dense LDL input rate (F30) was reduced 44% compared with the control dense LDL input rate, undoubtedly resulting in a modest 25% increase in the total mutant LDL input from outside the system compared with control LDL production (F10+F30, 757 μg/kg/hr versus 463 μg/kg/hr). Although the total LDL production rate was only increased 25%, the plasma LDL concentration was increased 400% due to the shift of production from a relatively rapidly turning over LDL subspecies (dense LDL) to a much more slowly turning over subspecies (buoyant LDL).

While the direct input of mutant dense LDL was dramatically reduced, the steady-state concentration of mutant dense LDL was not smaller, due to the increased conversion of buoyant LDL to dense LDL (in control pigs, 7% of dense LDL was derived from buoyant LDL; in mutant pigs, 33% of dense LDL was derived from buoyant LDL) and the sluggish removal of mutant dense LDL from the circulation (control dense LDL, 0.043 pools/hr; mutant dense LDL, 0.028 pools/hr). To gain additional information concerning the potential precursor-product relations between buoyant and dense LDL, the time course of LDL production was followed after injection of [3H]leucine (Figure 5). [3H]labeled LDL was isolated and separated into two subtypes: buoyant, d=1.02–1.038 g/ml,
and dense, $d=1.038-1.063$ g/ml. In control pigs, the peak specific activity of dense LDL was not greater than the peak specific activity of buoyant LDL. In mutant pigs, the peak specific activity of the dense LDL was generally twofold greater than the peak specific activity of the buoyant LDL, demonstrating again that dense LDL could not be solely derived from buoyant LDL. If dense LDL were solely derived from buoyant LDL, the specific activity of dense LDL could never be greater than that of buoyant LDL. There must be another source of dense LDL with a specific activity greater than that of buoyant LDL.

In control pigs, the specific activity of buoyant [H]LDL peaked before dense LDL and increased more rapidly than dense LDL, suggesting input of buoyant LDL from outside the system. (Figure 5, pig Nos. 7 and 8). In contrast, the LDL turnover experiments employing exogenous tracers (which labeled LDL according to mass) indicated negligible production of control buoyant LDL from outside the system (Figure 4).

**Discussion**

Numerous studies have indicated the association of heterogeneous LDL in humans with the presence of hyperlipidemia or premature atherosclerosis. The genes and metabolic defects responsible for production of multiple LDL subspecies have not been identified; however, defects in hepatic apo B production or in VLDL, IDL, and LDL metabolism may all play a role. Fisher suggested that structural changes in apo B may be the determining factor in the formation of heterogeneous LDL. We have previously shown that pigs sharing a defined apo B allele, Lpb5, exhibit structural and chemical heterogeneity of LDL and defective LDL binding in vivo. The purpose of the current studies was to determine if the Lpb5 LDL would exhibit kinetic heterogeneity in vivo. Additionally, we sought to determine the mechanism by which buoyant LDL accumulates in the Lpb5.1 mutant pig.

Accumulation of buoyant LDL in mutant pigs could be due to overproduction of buoyant LDL from its metabolic precursors or to the conversion of dense LDL to buoyant LDL, as a result of its increased residence time in the circulation of mutant pigs. There is evidence for interconversion of dense to buoyant LDL in familial hypercholesterolemia. However, we found that conversion of dense LDL to buoyant LDL did not account for the predominance of buoyant LDL in mutant pigs.

**Overproduction of Mutant Low Density Lipoprotein**

Our studies show that the primary metabolic abnormality contributing to the elevation of buoyant LDL in the mutant pig is a fivefold increase in the input rate of buoyant LDL, leading to a sevenfold accretion in buoyant LDL levels. This result expands on our earlier work in which we identified only defective catabolism when using the FCR obtained after injecting whole-fraction LDL. In those studies, the production defect was not apparent because pig LDL is heterogeneous, and therefore, determining production rates from the FCR and the LDL pool size leads to an underestimation of the production rates as predicted by Berman.
We have also shown that mutant LDL is cleared more slowly than control LDL when compared in control animals. This result parallels our in vitro studies in cultured fibroblasts, which showed that mutant LDL binds to the LDL receptor with a lower affinity than does control LDL.13 The contribution of this defect in the present studies is unclear, but it could only exacerbate the pronounced hypercholesterolemia in the mutant pigs.

Another factor that may be of greater importance is downregulation of the LDL receptor in mutant pigs. In previous studies,10 we showed that mutant and control whole-fraction LDLs were cleared more slowly in mutant pigs than in control pigs, consistent with receptor downregulation. Experiments using glucosylated LDL to quantify the contribution of LDL receptor–independent catabolism allow us to speculate that virtually all LDL clearance in mutant pigs is non–receptor mediated (R.J. Aiello et al, unpublished observations). Despite downregulation of the LDL receptor pathway in mutant pigs, dense LDL was cleared more quickly than buoyant LDL, suggesting the surprising result that non–LDL-receptor-mediated clearance mechanisms (in addition to LDL-receptor-mediated clearance) discriminate between LDL subspecies.

Low Density Lipoprotein Metabolism in Pigs

While the primary conclusion of this study is that buoyant LDL production is dramatically increased in Lpb5.1 mutant pigs, our data suggest that dense LDL is not solely derived from buoyant LDL. This conclusion was supported by marked differences in the specific activities of buoyant and dense LDL in our experiments employing endogenous tracer. Dense LDL may arise from the VLDL cascade or from direct production. The work of others suggests that the majority of normal pig LDL is produced independently of the VLDL cascade. Huff and Telford10 observed that 80% of LDL in miniature pigs was derived from sources other than VLDL. Birchbauer et al13 estimated that 89% of LDL was derived from non-VLDL sources in normal pigs. In this regard, LDL metabolism in the pig is similar to that of the cynomolgus monkey,27 rat,33 and familial hypercholesterolemia patients,28 who appear to synthesize much of their LDL by VLDL-independent pathways. Huff and Telford34 also showed that in miniature pigs, dense LDL (d=1.040–1.063 g/ml) is primarily derived from direct synthesis and not from VLDL, while buoyant LDL (d=1.019–1.040 g/ml) was almost entirely derived from VLDL. These results, together with the present studies, suggest that buoyant LDL overproduction in mutant pigs is due to increased conversion of LDL precursors (VLDL or IDL-like particles) to buoyant LDL, but we cannot eliminate the possibility that mutant buoyant LDL is also produced directly by the liver.

One interesting observation from the experiments using the leucine tracer was that in contrast to our experiments using the exogenous tracer, there was some direct production of buoyant LDL, but not from dense LDL in control pigs. The most likely explanation, as proposed by Murthy et al,35 is that the endogenous tracer was incorporated into a small but rapidly turning over pool of buoyant LDL. This rapidly turning over pool of buoyant LDL could make a significant contribution to buoyant LDL production. Murthy et al35 also point out that when plasma lipoproteins are followed by exogenous labeled tracers, small, rapidly turning over pools might be undetected by traditional plasma lipoprotein turnover studies. Thus, one could underestimate the
Control pig
μg/kg/hr

Buoyant LDL
32 ± 26
75 ± 53
57 ± 27
406 ± 141

Dense LDL
449 ± 135

Mutant pig
μg/kg/hr

Buoyant LDL
326 ± 143
124 ± 60
117 ± 23
319 ± 151

Dense LDL
251 ± 117
259 ± 141

FIGURE 4. Diagram showing average production, interconversion, and catabolism fluxes of buoyant and dense low density lipoprotein (LDL) in control and mutant pigs. Fluxes for individual animals can be found in Table 3. The size of each box is proportional to the LDL pool size.

production rate of buoyant LDL determined with exogeneous tracers.

The elevation of buoyant LDL in the mutant pigs is primarily the result of overproduction of buoyant LDL. Although the mutant pig apo B defect does not appear to play a direct role in the buoyant LDL overproduction, these animals could have a concomitant defect, also associated with apo B, responsible for the overproduction of buoyant LDL. Whether the Lpb5.1 mutants have a second mutation, it is still conceivable that the apo B defect could lead to overproduction of buoyant LDL. For instance, apo B structural changes may affect its recruitment for VLDL or buoyant LDL production, or they may affect the proportion of VLDL or IDL converted to LDL.

The metabolic defects found in the Lpb5.1 pigs are similar to those of familial combined hyperlipidemia. Overproduction of LDL is common in these individuals, and many investigators have suggested that the increment in LDL is due to increased LDL production,27,36 a hypothesis we propose to explain the LDL elevation in the mutant pigs. Our results suggest, for the first time, that variability in the apo B locus can be associated with increased LDL subspecies production. This precedent makes apo B a viable candidate gene locus responsible for the increased LDL production observed in some individuals with familial combined hyperlipidemia.

Acknowledgments

We are grateful to Jan Rapacz for his help in creating our colony of mutant pigs. The authors wish to thank J. Bradach for his excellent technical assistance. The authors also wish to thank Drs. Barrett, Colas, Foster, Ginsburg, Kissebah, Le, and Marzetta for their advice and suggestions during the course of these studies.

Appendix: Equations of the Two-Accessible-Pool/Noncompartmental Model

Definitions

\[ D_j = \text{Amount of isotope in pool } j, \text{ a function of time.} \]
FIGURE 5. Line plots showing specific activity (dpm/μg) as a function of time (hr) after [3H]leucine was injected into control pigs (animals No. 7 and 8) and mutant pigs (animals No. 14, 16, 17, 18, and 19); specific activity of buoyant and dense low density lipoprotein (LDL) apolipoprotein B was determined.
\[A_i=\text{Specific activity in pool } i\text{ derived from bolus injection into pool } j, \text{ a function of time.}\]
\[F_j=\text{Flux from pool } j \text{ to } i, \text{ a constant.}\]
\[R_i^1=\text{Dose in pool } i \text{ derived from an injection into pool } j, \text{ at time zero.}\]
\[SA_i=\text{Area under specific activity-time course in pool } i \text{ from an injection into pool } j.\]

Considering a bolus injection, the amount of tracer in compartments 1 and 2 (see Figure 1) is described by

\[
\frac{dD_1}{dt}=-(F_{01}+F_{21})A_1+(F_{12}A_2)
\]
\[
\frac{dD_2}{dt}=(F_{21}A_1)-(F_{02}+F_{12})A_2
\]

The equations are simplified by the following substitutions.

\[F_{11}=F_{01}+F_{21}\]
\[F_{22}=F_{02}+F_{12}\]

Thus,

\[
\frac{dD_1}{dt}=-(F_{12}A_1)dt+F_{22}A_2dt
\]
\[
\frac{dD_2}{dt}=F_{21}A_1dt-F_{22}A_2dt
\]

Integrating from zero to infinity yields

\[D_1-R_1=-F_{11}A_1dt+F_{12}A_2dt\]
\[D_2-R_2=F_{21}A_1dt-F_{22}A_2dt\]

Since \(D_1=D_2=0\) and \(\int A dt\) is the area under the specific activity-time course

\[-R_1=F_{11}SA_1+F_{12}SA_2\]
\[-R_2=F_{21}SA_1-F_{22}SA_2\]

If the bolus injection of tracer is into pool 1, then the notation is

\[-R_1^1=-F_{11}SA_1+F_{12}SA_2\]
\[-R_1^2=F_{21}SA_1-F_{22}SA_2\]

where \(R_1^1\) is the injected dose and \(R_1^2\) is the zero-time contamination. \(SA_1^1\) and \(SA_1^2\) represent the areas under the specific activity-time courses in pools 1 and 2 as a result of a bolus injection into pool 1.

If compartment 2 is labeled in a similar way

\[-R_2^1=-F_{11}SA_1+F_{12}SA_2\]
\[-R_2^2=F_{21}SA_1-F_{22}SA_2\]

All four doses and four areas under the specific activity-time course were measured from the buoyant and dense LDL turnover experiments. Therefore, these four equations have four unknowns, \(F_{11}, F_{12}, F_{21},\) and \(F_{22}\).

We solved these equations by matrix analysis. The remaining fluxes were calculated as

\[F_{11}=F_{01}+F_{21}=F_{10}+F_{12}\]
\[F_{22}=F_{02}+F_{12}=F_{21}+F_{20}\]

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**KEY WORDS** • low density lipoproteins • hypercholesterolemia • apolipoprotein B
Overproduction of a buoyant low density lipoprotein subspecies in spontaneously hypercholesterolemic mutant pigs.
W J Checovich, R J Aiello and A D Attie

doi: 10.1161/01.ATV.11.2.351
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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