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Indinavir acutely inhibits insulin-stimulated glucose disposal in humans: A randomized, placebo-controlled study

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Abstract

Background—Therapy with HIV protease inhibitors (PI) causes insulin resistance even in the absence of HIV infection, hyperlipidemia or changes in body composition. The mechanism of the effects on insulin action is unknown. *In vitro* studies suggest that PI selectively and rapidly inhibit the activity of the insulin-responsive glucose transporter GLUT-4. We hypothesized that a single dose of the PI indinavir resulting in therapeutic plasma concentrations would acutely decrease insulin-stimulated glucose disposal in healthy human volunteers.

Methods—Randomized, double-blind, cross-over study comparing the effect of 1200 mg of orally administered indinavir and placebo on insulin-stimulated glucose disposal during a 180-min euglycemic, hyperinsulinemic clamp. Six healthy HIV-seronegative adult male volunteers were studied twice with 7 to 10 days between studies.

Results—There were no significant differences in baseline fasting body weight, or plasma glucose, insulin, lipid and lipoprotein levels between placebo- and indinavir-treated subjects. During steady-state (t_{60-180} min) insulin reached comparable levels (394 ± 13 versus 390 ± 11 pmol/l) and glucose was clamped at approximately 4.4 mmol/l under both conditions. The average maximum concentration of indinavir was 9.4 ± 2.2 μ M and the 2-h area under the curve was 13.5 ± 3.1 μ M $\cdot$ h. Insulin-stimulated glucose disposal per unit of insulin (M/I) decreased in all subjects from 14.1 ± 1.2 to 9.2 ± 0.8 mg/kg $\cdot$ min per μ UI/ml (95% confidence interval for change, 3.7–6.1; $P < 0.001$) on indinavir (average decrease, $34.1 \pm 9.2\%$). The non-oxidative component of total glucose disposal (storage) decreased from 3.9 ± 1.8 to 1.9 ± 0.9 mg/kg $\cdot$ min ($P < 0.01$). Free fatty acid levels were not significantly different at baseline and were suppressed equally with insulin administration during both studies.

Conclusions—A single dose of indinavir acutely decreases total and non-oxidative insulin-stimulated glucose disposal during a euglycemic, hyperinsulinemic clamp. Our data are

compatible with the hypothesis that an acute effect of indinavir on glucose disposal in humans is mediated by a direct blockade of GLUT-4 transporters.

Keywords

HIV protease inhibitors; indinavir; insulin resistance; glucose transport; metabolic complications; diabetes; lipodystrophy; HIV; AIDS

Introduction

Treatment of HIV-infected patients with protease inhibitors (PI) has been associated with insulin resistance [1–3], hyperglycemia and development of diabetes mellitus [4]. Longitudinal studies in HIV-positive patients indicate that initiation of regimens including indinavir [5] or a variety of PI drugs [6] are associated with the onset of insulin resistance prior to any changes in body composition. Recently, using measurements of fasting insulin levels, oral glucose tolerance testing and a euglycemic hyperinsulinemic clamp, we found that indinavir induces significant insulin resistance in healthy HIV-seronegative volunteers [7]. Because insulin resistance occurred before any significant changes in lipids and lipoprotein levels or body composition and in the absence of HIV infection, the earliest metabolic effect of PI may be their effect on carbohydrate metabolism.

The mechanism by which indinavir or other PI cause insulin resistance is unknown. *In vitro* studies using preadipocyte cells suggest that PI, including indinavir, directly interfere with the transport function of the insulin-regulated glucose transporter, GLUT-4 [8]. This effect is observed at near-peak concentrations (10 μ M or 6140 ng/ml) of PI and is selectively specific for GLUT-4. More importantly, inhibition of glucose transport occurs within minutes, without any effects on intracellular signaling, which implies a direct effect of indinavir on the GLUT-4 transporters *per se*. Indinavir also decreases insulin- and contraction-stimulated glucose transport in isolated rat skeletal muscle *in vitro* [9], consistent with a selective and acute blockade of the GLUT-4 transporter.

GLUT-4 transporters are known to mediate glucose disposal and storage into insulin-responsive tissue in hyperinsulinemic states such as occurs post-prandially or during a euglycemic, hyperinsulinemic clamp procedure. Using this technique, we previously demonstrated a decrease in insulin-stimulated glucose disposal rate after 4 weeks of therapy with indinavir in healthy volunteers [7]. In this study, we now test the hypothesis that a single dose of indinavir sufficient to achieve therapeutic plasma concentrations in healthy volunteers would acutely decrease total and non-oxidative insulin-stimulated glucose during a euglycemic, hyperinsulinemic clamp.

Methods

Six healthy men were recruited from staff at the University of California, San Francisco (UCSF) and from the community. The subjects had no history of medical illnesses (including nephrolithiasis), showed no abnormalities on screening physical examination or routine hematology and chemistry tests, had stable weight over the preceding 6 months and a negative HIV-1 antibody test prior to the study. The study protocol was approved by the Committee on Human Research of UCSF and informed consent was obtained from each subject.

Exclusion criteria included body mass index > 27 kg/m², serum total cholesterol > 6.2 mmol/l, triglycerides > 3.8 mmol/l, fasting glucose > 7.0 mmol/l, serum aspartate or alanine aminotransferases > 50 U/l and creatinine > 124 μ M.

Study design

This was a randomized, double-blind, placebo-controlled, cross-over study. The subjects were instructed to abstain from vigorous exercise and to eat a diet containing at least 150 g of carbohydrates for 3 days prior to each study. During these 3-day periods, the subjects kept a diet journal, which was reviewed to assess dietary adherence. The subjects were admitted to the General Clinical Research Center (GCRC) at San Francisco General Hospital (SFGH) the evening prior to the study and began a 24-h urine collection. After an overnight (> 10 h) fast, blood was drawn for baseline studies at 0800 hours. The subjects randomly received either indinavir (Crixivan; Merck & Co., Rahway, New Jersey, USA) 1200 mg or placebo (kindly provided by Merck & Co.) at 0900 hours ($t = 0$), and underwent a euglycemic, hyperinsulinemic clamp procedure performed from 0900–1200 hours (t_{0-180}) by an investigator blinded to the study medication. The subjects completed a 24-h urine collection prior to discharge and returned to GCRC within 7–10 days at which time they were crossed over to the alternative treatment and the studies were repeated.

We chose a 1200 mg dose because indinavir plasma concentrations are highly variable, the time to peak concentration is less than 1 h and the half-life is only 1.8 h [10]. Because of these factors, we anticipated that in some subjects the administration of an 800 mg dose, as is typically used in three-times daily regimens, might not achieve and maintain plasma concentrations observed under steady-state conditions. Therefore, we surmised that a 1200 mg dose would assure plasma concentrations within the therapeutic range for the duration of the 3-h euglycemic hyperinsulinemic clamp procedure. It should be noted that 1200 mg is an indinavir dose that has been evaluated clinically in twice-daily regimens [11].

Euglycemic hyperinsulinemic clamp

The clamp was performed as described by DeFronzo *et al* [12]. Teflon cannulae were placed into an antecubital vein for infusion and into a vein in the dorsum of the contralateral hand, which was kept in a heated box at 50–55°C, for arterialized venous blood sampling. Subjects fasted overnight prior to the procedure. At $t = 0$ min, insulin (Humulin R; Eli Lilly, Indianapolis, Indiana, USA) was administered as a primed continuous intravenous infusion for 10 min, followed by a constant infusion at the rate of $40 \text{ mU/m}^2 \cdot \text{min}$ until $t = 180$ min. Whole blood glucose concentration was measured every 5 min after the start of the insulin infusion. A variable infusion of 20% dextrose was used to maintain the plasma glucose concentration at 4.5 mmol/l with a coefficient of variation < 5% based on the negative feedback principle. Blood samples were also collected for *post hoc* determination of plasma glucose and serum insulin concentrations.

Resting energy expenditure

O_2 consumption and CO_2 production were measured by indirect calorimetry (DeltaTrac metabolic monitor, Yorba Linda, California, USA). Non-protein respiratory quotient and substrate oxidation rates were calculated after correction for protein oxidation as estimated by urea nitrogen excretion measured in the 24-h urine collection [13]. The rate of non-oxidative glucose metabolism was calculated by subtracting the rate of carbohydrate oxidation from the rate of dextrose infusion during the clamp. At the insulin levels achieved during this procedure, hepatic glucose production is expected to be suppressed completely in healthy subjects.

Measurements

Fasting lipids and free fatty acids were measured by enzymatic colorimetric methods (Sigma Diagnostics, St. Louis, Missouri, USA and Wako Chemicals, Richmond, Virginia, USA, respectively). Whole blood and plasma glucose and lactate were measured using a glucose

analyzer (YSI 2300 STAT-Plus Glucose & Lactate Analyzer, YSI Inc., Yellow Springs, Ohio, USA). Serum insulin levels were determined by Coat-A-Count radioimmunoassay (Diagnostic Products Corp, Los Angeles, California, USA) with intra-assay coefficient of variation of 7.3%, lower detection limit of 9.3 pmol/l and 20% cross reactivity with proinsulin.

Indinavir levels were measured by liquid chromatography, tandem mass spectrometry within the Drug Research Unit, SFGH. The method has a lower detection limit of 5 nM, inter- and intra-assay coefficient of variations ranging from 1.6 to 2.6 and 2.4 to 5.3 % respectively [14]. The area under the concentration time curve (AUC) during steady state (t_{60-180} min) post-indinavir dosing was estimated using the linear-linear trapezoidal rule.

Statistical analyses

Data were analyzed using Sigma Stat v. 2.03 (SPSS, Inc. San Rafael, California, UDS). Paired *t* tests were used for normally distributed data. One-way analysis of variance (ANOVA) was used to test for the difference between treatments in repeated measurements of glucose during the clamp procedure. Non-parametric data were analyzed using Mann-Whitney or Wilcoxon Rank Sum test. Data are presented as mean $\pm$ SEM. *P*-values are two-tailed.

Results

The subjects ranged in age from 31 to 52 years (mean, 37.7 ± 7.5 years); four were Caucasian, one was Asian and one was African-American. Baseline body weight, fasting serum insulin, plasma glucose, lipids and lipoprotein levels did not differ prior to each study (Table 1). The plasma level of indinavir reached a concentration of 9.4 ± 2.2 μ M at t_{60} and remained > 4.5 μ M for the duration of the study (Fig. 1). The 2-h AUC (AUC_{60-180}) was 13.5 ± 3.1 μ M $\cdot$ h. During the euglycemic, hyperinsulinemic clamp, steady state (t_{60-180} min) insulin levels of approximately 400 pmol/l (394 ± 13 versus 390 ± 11 pmol/l) and glucose levels of approximately 4.4 mmol/l (4.3 ± 0.2 versus 4.4 ± 0.2 mmol/l) were achieved and maintained until the end of the study (Fig. 2a).

The rate of dextrose infusion required to maintain euglycemia during steady state was significantly lower in the study on indinavir compared to placebo ($P < 0.05$ by one-way ANOVA on repeated measures; Fig. 2b). Insulin-stimulated glucose disposal rate per unit of insulin (M/I) declined in all six subjects by an average of $34.1 \pm 9.2\%$ in the study on indinavir compared to placebo. Mean M/I decreased from 14.1 ± 1.2 to 9.2 ± 0.8 mg/kg $\cdot$ min per μ U/ml (95% confidence interval of the difference, 3.1–6.7; $P < 0.001$; Fig. 3a). The non-oxidative component of total glucose disposal decreased from 3.9 ± 1.8 to 1.9 ± 0.9 mg/kg $\cdot$ min ($P < 0.01$; Fig. 3b). Fasting free fatty acid levels were suppressed comparably with insulin administration in both studies (Fig. 4).

Discussion

Our results show that a single oral dose of the HIV PI indinavir induced insulin resistance in healthy HIV-negative volunteers. The onset of insulin resistance was rapid and occurred at plasma concentrations of indinavir approximating steady-state levels observed in HIV-infected patients maintained on standard clinical doses of this agent [15]. The magnitude of the decline in insulin-stimulated glucose disposal was approximately 34% and consistent in all subjects. Moreover, most of this reduction in glucose utilization was accounted for by a significant reduction in the rate of non-oxidative glucose disposal suggesting decreased glucose storage. The present finding, therefore, is compatible with the hypothesis that the

first event leading to the development of impaired glucose tolerance and type II diabetes in patients treated with the PI indinavir is insulin resistance in skeletal muscle and adipocytes.

Muscle cells and adipocytes are the primary sites for insulin-stimulated glucose disposal [16]. In response to insulin signaling, specialized glucose transporter vesicles are translocated from intracellular sites to the plasma membrane, thereby facilitating transport of glucose into the cells [17,18]. GLUT-4 is the main insulin-responsive glucose transporter in both muscle and adipose tissue. Intracellular transport of glucose by GLUT-4 is a rate-limiting step in insulin-stimulated glucose disposal and the abundance of GLUT-4 in different muscle types correlates roughly with the ability of those muscles to take up glucose [19].

In vitro studies of the effect of PI on glucose uptake in 3T3-L1 adipocytes and skeletal muscle have shown that various PI including indinavir, amprenavir, ritonavir [8] and nelfinavir [9] when added at close to peak therapeutic concentration of 10 μM or greater, cause inhibition of insulin-stimulated glucose uptake. For example, at this concentration, indinavir caused a 26% inhibition of glucose uptake within minutes of addition to the culture medium [8]. Moreover, indinavir at 5 μM decreased both insulin- and contraction-stimulated glucose transport by average of 40% in isolated rat skeletal muscle [9].

The effect on insulin-stimulated glucose transport into muscle is also rapid and detectable after a 4-h incubation in the presence of indinavir (shorter incubation times were not tested). The 40% inhibition at 5 μM in isolated muscle is comparable to the 34% decline in glucose disposal we observed in the present study after a 1200 mg oral dose, and the 20% decrease reported by us previously with a dose of 800 mg three times daily [7]. Our findings in humans, therefore, are consistent and compatible with the observations made *in vitro*.

The concentrations of indinavir at which we conducted our study (mean C_{max} 9.4 μM , AUC 13.5 $\mu\text{M} \cdot \text{h}$) closely resemble those observed in pharmacokinetic studies of healthy volunteers (C_{max} , 11.7 μM ; AUC, 23.15 $\mu\text{M} \cdot \text{h}$) [20]. Drug concentrations are important and may account for some of the discrepancies among published studies. For example, *in vitro* studies using indinavir at near-therapeutic concentrations of 10 μM or less for short incubations times (1 h) in both cultured cells [8] and isolated tissue [9] suggest that the mechanism of this effect is due directly to rapid blockade of the GLUT-4 transporters. At these concentrations, indinavir caused no defects in signaling pathways, particularly the phosphoinositide-3 kinase or protein kinase B phosphorylation in 3T3-L1 preadipocytes. A similar finding was reported by Caron *et al.* using 3T3-F442 preadipocytes, where incubation for up to 8 days in the presence of indinavir at 15 μM (10 $\mu\text{g/ml}$) did not alter tyrosine phosphorylation [21]. However, when used at supra-therapeutic concentrations of 100 μM for 48 h, indinavir impaired insulin signaling in HepG2 hepatoma cells [22]. Similarly another PI, nelfinavir, impaired insulin stimulation of protein kinase B phosphorylation [23] *in vitro* but only after 18 h of incubation at concentrations of $\geq 20 \mu\text{M}$, nearly fourfold its C_{max} of 5.2–5.6 μM in humans [24]. This effect was not observed at lower nelfinavir concentrations ($\leq 10 \mu\text{M}$) that are in the therapeutic range.

Our findings in human volunteers confirm that the metabolic defect caused by indinavir is rapid and detectable within minutes of achieving standard pharmacologic plasma concentrations of the drug. This strongly suggests a direct mechanism rather than a secondary effect on insulin signaling leading to an impaired ability to inhibit lipolysis. Consistent with this theory, we observed that free fatty acid levels were not acutely increased during euglycemic, hyperinsulinemic clamp and the suppressive effects of hyperinsulinemia on free fatty acid levels did not diminish following indinavir administration. This observation is further reinforced by our earlier published finding that

free fatty acid levels were not increased even after 4 weeks of indinavir therapy [7]. Finally, the bulk of the change in glucose disposal rate was due to a decline in the rate of non-oxidative glucose disposal, the latter reflecting an acute decrease in the rate of glucose storage in muscle and adipocytes. Our findings in humans, therefore, are compatible with the hypothesis that the mechanism by which indinavir decreases insulin-stimulated glucose disposal may be a direct block in the uptake of glucose through the GLUT-4 transporter. Clinically, decreases in this order of magnitude in the rate of muscle glycogen synthesis and insulin-stimulated glucose disposal have been shown to contribute significantly to the development and pathophysiology of type II diabetes mellitus [25].

Although the first metabolic defect caused by PI to appear is inhibition of insulin-stimulated glucose disposal, probably through a blockade of the GLUT-4 transporter, other metabolic effects cannot be ruled out. For example, long-term exposure (30 days) to indinavir at concentrations of 15 μ M inhibits preadipocyte differentiation presumably by altering the nuclear localization of the sterol regulatory element-binding protein-1 [22]. However, the effects of glucose deprivation due to long-term blockade of GLUT-4 by indinavir in these cell lines remains unclear. Thus, it is conceivable that with long-term exposure, additional metabolic effects beyond the effect on GLUT-4, such as hypertriglyceridemia, adipocyte de-differentiation and changes in body composition, may follow. Future studies are needed to determine the dose dependence and time-course of the effects of indinavir and other PI drugs in humans.

Recent insights in animal nutrition and fuel storage suggest that GLUT-4 may be more than a passive glucose transporter [26]. The data implicate a role for these transporters in the communication about nutritional status, fuel processing and storage between the muscle and adipose tissue. For example, selective knock-out of the GLUT-4 gene from fat cells results in a degree of insulin resistance similar to that seen with a muscle-specific knock-out [27]. Furthermore, the expression of this transporter is tissue specific and regulated by dietary intake [28]. This might explain how GLUT-4 could play an important role in regulating energy storage in adipose tissue. It is not known whether the longer term complications seen in HIV-infected patients (e.g. body fat redistribution) are manifestations of long-term GLUT-4 blockade or are due to other independent mechanisms. However, changes in body fat distribution have been reported in HIV-infected patients not on therapy with a PI, suggesting that blockade of GLUT-4 cannot be the sole mechanism accounting for these changes [29].

We have shown that the effect of indinavir on insulin-stimulated glucose disposal *in vivo* is rapid and of the same magnitude as that observed after 4 weeks. Recent data from animal studies suggest that this effect on glucose metabolism is also acutely reversible. Rats infused with indinavir developed insulin resistance, which reversed rapidly within 4 h of discontinuation of indinavir [30]. The rapid onset of the effects of indinavir and other PI have practical implications for studies of PI-induced insulin resistance. If the morning dose of a PI is omitted before measurement of fasting glucose and insulin, a lower effect may be seen, as PI levels will be in the trough range. For PI that are to be taken with meals (e.g. nelfinavir, zidovudine and zalcitabine) holding the drug before study may be common. Consistent dosing is needed to understand the comparative effects of PI and timing of PI ingestion may explain some differences between studies. The delay in peak plasma concentration may also contribute to more profound effects seen in certain metabolic studies such as oral glucose tolerance testing, where the peak plasma levels may coincide with the critical 2-h time point. In the present study, we did not assess the acute effects of a single dose of indinavir on more conventional measures of insulin resistance such as fasting glucose and insulin levels. However, we have previously reported induction of insulin resistance using fasting glucose and insulin after 4 weeks of treatment with indinavir at 800

mg three times daily, with dosing before measurements [7]. In that study, healthy HIV-negative volunteers had an average of 20% decrease in insulin-stimulated glucose disposal. The magnitude of decrease in insulin sensitivity using the clamp method coincided with an approximate 34% increase in fasting insulin levels and a 47% increase in insulin resistance index by homeostasis model assessment.

It should be noted that we have studied only one PI and our data may not be applicable to all drugs in this class. However, *in vitro* data using other PI suggest that this may represent a common mechanism. We studied men only; metabolic effects might be different in pre- and postmenopausal women, although there is no reason *a priori* to suspect that this will prove to be the case given the expression of the GLUT-4 gene.

In summary, our study suggests that the earliest and most direct effect of treatment with indinavir on glucose metabolism is a decrease in insulin-stimulated glucose utilization. The effect is rapid and can be detected at pharmacologic plasma concentrations of indinavir well within the therapeutic range commonly observed in patients. The bulk of the change in glucose disposal rate comes from an acute decrease in the rate of non-oxidative glucose disposal (i.e. storage rate). These findings are compatible with the hypothesis that a mechanism by which indinavir causes insulin resistance is by direct blockade of the glucose transporter GLUT-4. Insights from these studies may provide the basis for designing a new generation of PI drugs that do not perturb glucose metabolism. Future human studies are needed to assess whether these effects are common to other PI and how they might contribute to other observed metabolic and body composition changes reported in patients with HIV.

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Fig. 1.

Pharmacokinetics of indinavir during the euglycemic, hyperinsulinemic clamp. Indinavir, 1200 mg, was administered orally at $t = 0$. The vertical lines indicate the steady-state period (t_{60-180} min). The horizontal line at $10 \mu\text{M}$ denotes the concentration at which *in vitro* effects on glucose uptake have been observed. Data represent mean $\pm$ SEM.

Fig. 2.
 (a) Plasma insulin (diamonds) and glucose (circles) concentrations during euglycemic, hyperinsulinemic clamp with placebo (open symbols) and insulin (filled symbols). (b) Dextrose infusion rate required to maintain euglycemia during the study on placebo (open diamonds) and indinavir (filled diamonds). Vertical lines indicate steady state (t_{60-180} min). Data are means $\pm$ SEM. $P < 0.05$ for dextrose infusion rate by one-way ANOVA on repeated measures.

Fig. 3. (a) Glucose disposal rate (mg/kg per min) per unit of plasma insulin ($\mu\text{U/ml}$) (M/I) expressed as mean $\pm$ SEM during steady state on placebo and indinavir (IDV). (b) Pathways of glucose disposal rate (M) during steady state. Data are mean $\pm$ SEM. $**P < 0.01$ for the difference in non-oxidative component. Refer to Methods for calculation of non-oxidative component.

Fig. 4. Free fatty acid levels during euglycemic, hyperinsulinemic clamp on placebo (open circles) and indinavir (closed circles).

Table 1Baseline parameters (values are means $\pm$ SEM).

	Placebo	Indinavir	<i>P</i> ^a
FPG (mmol/l) ^b	4.5 $\pm$ 0.1	4.5 $\pm$ 0.2	n.s
FSI (pmol/l) ^c	33.0 $\pm$ 12.2	30.8 $\pm$ 8.6	n.s
Weight (kg)	70.6 $\pm$ 2.1	70.9 $\pm$ 2.2	n.s
Body mass index (kg/m ²)	22.7 $\pm$ 0.5	22.8 $\pm$ 0.6	n.s
Lactate (mmol/l) ^d	0.60 $\pm$ 0.07	0.62 $\pm$ 0.07	n.s
Total cholesterol (mmol/l) ^e	4.0 $\pm$ 0.2	3.7 $\pm$ 0.2	n.s
LDL cholesterol (mmol/l) ^e	2.5 $\pm$ 0.2	2.0 $\pm$ 0.1	n.s
HDL cholesterol (mmol/l) ^e	1.0 $\pm$ 0.1	1.0 $\pm$ 0.1	n.s
Triglycerides (mmol/l) ^f	1.4 $\pm$ 0.2	1.7 $\pm$ 0.4	n.s
Resting energy expenditure (kcal/day)			
Fasting	1777 $\pm$ 59	1813 $\pm$ 79	n.s
Energy expenditure (kcal/day)			
Clamp	1936 $\pm$ 73	1901 $\pm$ 96	n.s
Non-protein respiratory quotient			
Fasting	0.87 $\pm$ 0.02	0.87 $\pm$ 0.02	n.s
Clamp	0.92 $\pm$ 0.02	0.92 $\pm$ 0.02	n.s

^a All *P* values are by paired *t* test (*n* = 6).^b To convert glucose from mmol/l to mg/dl, multiply by 18.1.^c To convert insulin from pmol/l to μ U/ml, multiply by 0.14.^d To convert lactate from mmol/l to mg/dl, multiply by 9.01.^e To convert cholesterol from mmol/l to mg/dl, multiply by 38.7.^f To convert triglycerides from mmol/l to mg/dl, multiply by 88.6.

n.s., Not significant; FPG, fasting plasma glucose; FSI, fasting serum insulin; LDL, low density lipoprotein; HDL, high density lipoprotein.