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## Infusion of Lipoproteins into Volunteers Enhances the Growth of *Candida albicans*

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Infusion of reconstituted high-density lipoproteins (rHDL) is being studied in clinical trials as an adjunctive therapy for gram-negative sepsis. Since no data are available on its possible effects in systemic candidiasis, we investigated the effect of rHDL infusion into volunteers on the growth of *Candida albicans*. *C. albicans* growth was 10- to 100-fold higher in the plasma of volunteers infused with 80 or 100 mg/kg rHDL than in plasma collected before infusion; administration of 60 mg/kg rHDL had marginal effects. *In vitro*, the isolated lipoprotein subfractions had a growth-promoting effect on *C. albicans*. These data suggest potential adverse effects of rHDL if infused into patients with systemic candidiasis. Thus, rHDL infusion into patients with sepsis caused by an unknown microorganism may be contraindicated.

Despite the availability of new and potent antibiotics, mortality from gram-negative bacterial sepsis has remained constant during the last 30–40 years [1]. The lipopolysaccharide (LPS) component of the gram-negative bacterial cell wall mediates most of the inflammatory actions induced by these microorganisms, and conceptually, its neutralization could have beneficial effects on the outcome. Recent studies have applied reconstituted high-density lipoproteins (rHDL) for binding and neutralization of LPS. It has been shown that rHDL administered to animals protects against lethal endotoxemia [2] and gram-negative sepsis [3]. In addition, its infusion into humans downmodulates the inflammatory reaction induced by LPS [4]. Because of these effects, it has been suggested to use rHDL infusion as an adjunctive therapy in gram-negative sepsis. However, sepsis can be caused by a variety of microorganisms, including gram-negative bacteria, gram-positive bacteria, and fungi, and on clinical grounds it is often impossible to distinguish between these causes. Therefore, it is important to know whether rHDL infusion would affect the course of sepsis due to microorganisms other than gram-negative bacteria.

Acute disseminated candidiasis is a serious infection, of which the incidence has increased at least 10-fold during the last decade [5]. We have recently shown that hypercholesterolemia in low-density lipoprotein (LDL) receptor-deficient mice increases the susceptibility of these animals to systemic candi-

diasis [6]. In addition, earlier studies have demonstrated that *Candida albicans* grows better in lipid-containing emulsions used for parenteral feeding than it does in standard preparations [7–9]. These data suggest that a lipid-rich environment promotes *C. albicans* growth, and this could be a serious drawback for empirical rHDL treatment. For the present study, we asked whether rHDL infusion into human volunteers leads to increased candidal growth. In addition, we investigated whether freshly isolated human lipoproteins promote the growth of *C. albicans* *in vitro* and whether *C. albicans* grows better in plasma of hyperlipoproteinemic mice than in control plasma.

### Materials and Methods

**Infusion of rHDL into human volunteers.** Three groups of four healthy volunteers each were infused with rHDL at various doses (60, 80, or 100 mg/kg) over a 4-hour period. Blood samples were collected just before the infusion (time 0) and 4, 12, and 24 hours after the start of the infusion. Plasma samples from subjects receiving the same dose were pooled at each time point and were provided by Dr. Jan Eva Doran and Dr. Alphonse Hubsch (ZLB Central Laboratory, Bern, Switzerland).

**Isolation of the lipoprotein subclasses.** LPS-free very-low-density lipoprotein (VLDL; final cholesterol concentration, 0.9 mmol/L), LDL (2.0 mmol/L cholesterol), and HDL (0.5 mmol/L cholesterol) subclasses, as well as lipoprotein-depleted plasma (LPDP) (<0.1 mmol/L cholesterol), were isolated by sequential ultracentrifugation from fresh EDTA-treated plasma of healthy volunteers who did not receive rHDL infusion. The methods for isolation of lipoprotein subclasses have been described earlier [10]. Lipoproteins were dialyzed for 24 hours against 0.05 mM phosphate buffer, pH 7.4, containing 5 mM EDTA/L, with one exchange of the buffer. Lipoprotein subfractions isolated from six volunteers were studied in triplicate.

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Informed consent was obtained from all participants, and the guidelines for human experimentation of the participating institutions were followed.

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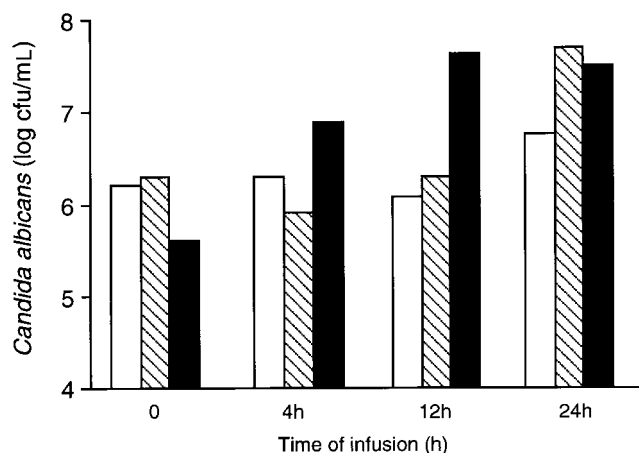
**Animals.** Homozygous C57BL/6J mice lacking LDL receptors (LDLR<sup>-/-</sup>) and wild-type LDLR<sup>+/+</sup> controls were obtained from Jackson Laboratories (Bar Harbor, ME) as mating pairs and bred in our local facility. LDL concentrations  $\pm$  SD in the LDLR<sup>-/-</sup> mice were  $4.92 \pm 0.53$  mmol/L, compared with  $0.35 \pm 0.11$  mmol/L in control LDLR<sup>+/+</sup> mice [11]. Blood was collected on EDTA from 6- to 8-week-old mice, and plasma was obtained for the use in *C. albicans* growth experiments. LPDP from LDLR<sup>-/-</sup> and control animals was prepared by ultracentrifugation, as described above. Plasma isolated from five LDLR<sup>-/-</sup> and five LDLR<sup>+/+</sup> control mice was studied in three separate experiments.

***C. albicans* growth in vitro.** *C. albicans* (American Type Culture Collection 10231;  $10^4$  cfu/mL) was grown in the pooled plasma of rHDL-infused volunteers, as well as in the various lipoprotein subfractions or lipoprotein-depleted plasma from untreated volunteers, after 1:1 dilution in Sabouraud broth with 2% glucose. Similarly, *C. albicans* ( $10^4$  cfu/mL) was grown in a 1:1 mixture of Sabouraud broth with murine lipoprotein subfractions or lipoprotein-depleted murine plasma. After 24 and 48 hours of incubation at 37°C, aliquots of 40  $\mu$ L were plated (Wasp spiral plater; DW Scientific, Shipley, UK) on Sabouraud dextrose agar. After another 24 hours of incubation at 37°C, the colonies were counted and the growth was expressed as the logarithm of colony-forming units per milliliter. Comparison between groups was done by use of the Mann-Whitney test. The Kruskal-Wallis test was used to compare the growth curves of *C. albicans* in depleted and nondepleted murine plasma.

## Results

**Growth of *C. albicans* in plasma of volunteers infused with rHDL.** The total cholesterol and HDL concentrations in the group receiving 60 mg/kg rHDL increased from a basal level of 3.87 and 0.95 mmol/L, respectively, to 4.63 and 1.03 mmol/L at the end of the 4-hour infusion interval and to 4.72 and 1.32 mmol/L at 12 hours and 4.30 and 1.58 mmol/L at 24 hours after the start of the infusion. The total cholesterol and HDL concentrations in the group receiving 100 mg/kg rHDL increased from 3.58 and 1.12 mmol/L, respectively, to 4.38 and 1.30 mmol/L at the end of the 4-hour infusion interval and to 5.00 and 1.60 mmol/L at 12 hours and 4.50 and 2.33 mmol/L at 24 hours after the start of the infusion.

The control growth is represented by the growth of *C. albicans* in plasma obtained from volunteers before the start of rHDL infusion (time 0; figure 1). Infusion of 60 mg/kg rHDL had little effect on the growth of *C. albicans*, but *C. albicans* grew better in plasma obtained after infusion of 80 or 100 mg/kg rHDL (figure 1). The number of *C. albicans* colony-forming units grown in plasma obtained 24 hours after rHDL infusion was  $\sim$ 100-fold greater than that grown in control plasma. Similarly, infusion of 100 mg/kg rHDL at various

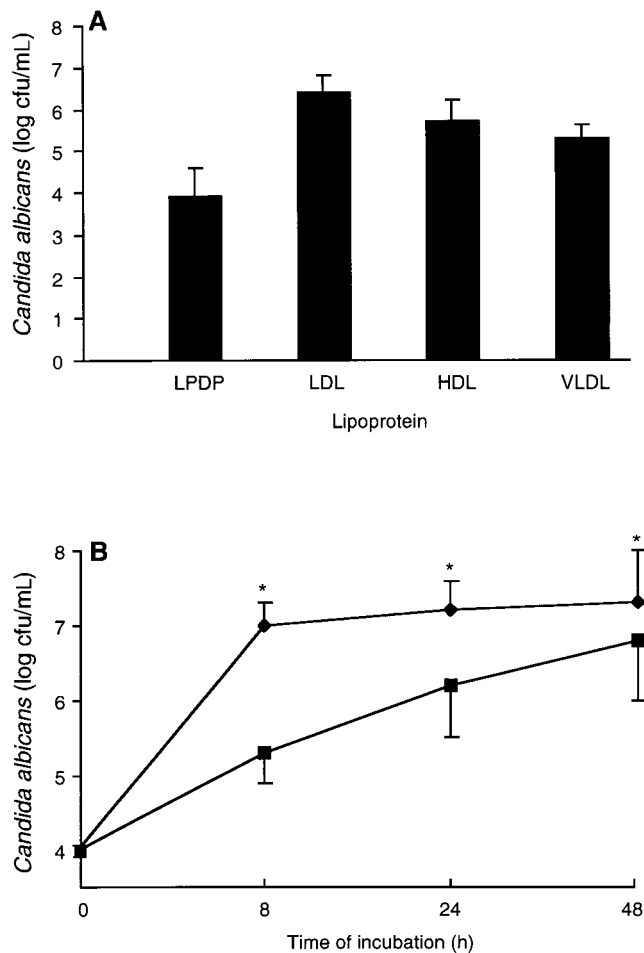


**Figure 1.** Growth of *Candida albicans* in plasma of volunteers before and after a 4-hour infusion with reconstituted high-density lipoproteins (rHDL). Plasma samples were collected from volunteers before (0 hours) and 4, 12, or 24 hours (h) after infusion with 60 mg/kg (□), 80 mg/kg (▨), or 100 mg/kg (■) rHDL. After 48 hours of incubation, *C. albicans* grew better in the plasma of volunteers who had received 80 or 100 mg/kg rHDL.

infusion rates (4–12 hours) resulted in a significantly increased outgrowth of *C. albicans*, compared with the outgrowth of the yeast in plasma obtained before infusion ( $P < .05$ , Kruskal-Wallis test; data not shown).

**Growth of *C. albicans* in freshly isolated human lipoprotein subclasses.** As shown in figure 2A, *C. albicans* grew better in the lipoprotein emulsions from normal human donors than in lipoprotein-depleted plasma, suggesting that all lipoprotein subfractions are able to either enhance *C. albicans* growth or to inactivate fungistatic factors present in plasma. To further investigate these two possibilities, the growth of *C. albicans* was investigated in plasma of LDLR<sup>-/-</sup> mice.

**Growth of *C. albicans* in plasma from hyperlipoproteinemic LDLR<sup>-/-</sup> mice.** In contrast to plasma collected from human volunteers, murine plasma inhibited the growth of *C. albicans* in vitro. However, this growth inhibition was significantly reduced in plasma from LDLR<sup>-/-</sup> mice [6]. After 12 hours of incubation of  $10^4$  cfu of *C. albicans* in LDLR<sup>-/-</sup> plasma, the log colony count  $\pm$  SD was  $3.7 \pm 1.1$ , versus  $3.2 \pm 0.9$  in control plasma, and after 24 hours of incubation, it was  $3.4 \pm 1.3$ , versus  $3.0 \pm 0.7$  in control plasma ( $P < .05$ ). To discern whether this effect is due to the use of lipoproteins as nutrients or to the binding and neutralization of a candidacidal factor by lipoproteins, we compared the growth curves of *C. albicans* in lipoprotein-depleted plasma from hyperlipoproteinemic and normal mice. *C. albicans* grew significantly better in lipoprotein-depleted plasma from LDLR<sup>-/-</sup> mice than in lipoprotein-depleted plasma from the LDLR<sup>+/+</sup> controls, although both subfractions contained similar amounts of lipoproteins ( $<0.01$  mmol/L cholesterol) after the depletion procedure (figure 2B). This suggests that a candidacidal factor may be bound to lipo-



**Figure 2.** In vitro growth of *Candida albicans* in the presence of lipoproteins. *A*, *C. albicans* grows better in plasma containing freshly isolated very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), or high-density lipoproteins (HDL), diluted 1:1 in Sabouraud broth, than in lipoprotein-depleted plasma (LPDP). *B*, *C. albicans* grows significantly better in LPDP from mice lacking LDL receptors (♦) than in that from control mice (■). Each point represents mean  $\pm$  SD of 5 experiments. \*  $P < .05$  (Kruskal-Wallis test).

proteins and extracted together with lipoproteins during the lipoprotein depletion procedure.

## Discussion

The results of the present study show that rHDL infusion (at  $\geq 80$  mg/kg) increases the growth of *C. albicans* in the plasma of human volunteers by 10- to 100-fold, compared with the basal growth curve in their plasma collected before rHDL administration. In contrast, there was no significant difference in the growth of *C. albicans* in plasma before and after infusion of a moderate amount of rHDL (60 mg/kg). Similarly, *C. albicans* growth was increased in the presence of freshly isolated human lipopro-

tein subfractions and in hyperlipoproteinemic plasma from LDLR $^{-/-}$  mice. These data are in agreement with our previous findings in hyperlipoproteinemic LDLR $^{-/-}$  mice, which were more susceptible to disseminated candidiasis because of an increased fungal outgrowth in their organs [6]. Although lipid profiles differ between mice and humans, the results of both studies suggest that hyperlipidemia can have deleterious effects by stimulating *C. albicans* growth in both species.

At least two different mechanisms seem to be responsible for the observed effects. First, in agreement with other reports showing increased growth of *C. albicans* in lipid emulsions used for parenteral feeding [7, 8], lipids themselves seem to promote the growth of *Candida*. Cholesterol may act as a nutrient for *C. albicans*, as has been demonstrated for other microorganisms, such as *Staphylococcus aureus* [12]. After infusion of rHDL, natural HDL has to be formed through extraction of cholesterol from cell membranes and other lipoprotein subfractions, since rHDL consists of phospholipids and recombinant apolipoprotein A-1 without cholesterol [2]. This process requires several hours, and this explains the increasing effect of rHDL on *C. albicans* growth at later time points after infusion. Indeed, the total cholesterol and HDL concentrations were found to be higher 12 and 24 hours after the start of the infusion than at the end of the 4-hour infusion interval.

Second, since the growth of *C. albicans* was enhanced in lipoprotein-depleted plasma of hyperlipoproteinemic mice, binding and neutralization of candidacidal factors, such as sphingosine [13], platelet microbicidal protein [14], or the calprotectin complex [15], by lipoproteins may contribute to the observed effect. Although no lipoproteins were present in lipoprotein-depleted plasma isolated from either LDLR $^{-/-}$  or normal mice, *C. albicans* growth was significantly increased in lipoprotein-depleted plasma from LDLR $^{-/-}$  mice, from which five-fold more lipoproteins had been extracted. This suggests that a candidacidal factor associates with lipoproteins, and because of this binding, the candidacidal effects of plasma may decrease. Indeed, it has been reported that cholesterol is able to inhibit the candidastatic capacity of sphingosine [13]. Association of an antimicrobial factor with HDL has been reported for *S. aureus* [16], and this may also explain the differences observed between HDL and VLDL effects on *C. albicans* growth, despite the higher cholesterol content of the VLDL subfraction (figure 2A).

Infusion of rHDL as adjunctive therapy for presumed gram-negative infections is currently under investigation in clinical trials. Although infusion with a moderate rHDL regimen (60 mg/kg) seems to have no significant effects on *C. albicans* growth, higher quantities of rHDL strongly potentiate growth of these organisms. This suggests that rHDL may have significant adverse effects when infused into patients with systemic candidiasis, and infusion with rHDL into patients with sepsis caused by an unknown microorganism may be contraindicated. In addition, it has been suggested that a lipid-rich environment po-

tentiates also the growth of other microorganisms, such as *S. aureus* [7–9], and the clinical implication of this phenomenon for rHDL infusion warrants further investigation.

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