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(RESEARCH ARTICLE)

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Adherence of *Candida albicans* to fibrin-clot matrices in a rat model for type 2 diabetes

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Abstract

Individuals with type 2 diabetes are at increased risk for the development of infective endocarditis and septic thrombosis. Adherence to fibrin clot matrices by *Candida albicans* is the initial step in initiation of the infective processes. Clots made from lean (Fa/fa) and obese (fa/fa) Zucker rat plasma were used to measure adherence of yeast grown at environmental temperature and human body temperature. Adherence to clots by *C. albicans* was influenced both by source of plasma and temperature of yeast growth. Amphotericin B was effective in diminishing the candidal binding to lean but not obese clots. The degree of fungal surface hydrophobicity did not correlate with adherence to clots. However, *Candida* adherence to clots from both lean and obese Zucker rats was inhibited by the pretreatment of cells with mannose. These findings indicate that treatment of thrombi-associated candidal infections with ergosterol-targeting drugs e.g., amphotericin B alone in hypercholesterolemic individuals may have decreased clinically efficacy.

Keywords: Diabetes; Cholesterol; *Candida*; Clot; Zucker rat; Adherence; Amphotericin B; Hydrophobicity; Fibrin; Mannose

1. Introduction

Rates of candidiasis have been increasing in frequency and severity nationwide. To date, candidal infections are the most prevalent fungal disease particularly in hospitalized severely compromised patients [1,2]. The mortality rate of candidal infections is ~40%, with the rate of candidal bloodstream infections having increased about 487% over last decade [2-5]. In the U.S.A. alone, *Candida* species are the fourth most common bloodstream pathogens, accounting for 8% of all hospital-acquired bloodstream infections. Of these infections, over a quarter of intensive care septicemias associated with central venous lines are the result of candidal infections. In addition, other biofilm-associated co-morbidities include valvular vegetations, and ascites formation [6,7]. A common risk factor is obesity, a global epidemic, which affects over 2 billion individuals worldwide and is increasing the mortality rate both alone, and in combination with other co-morbidities particularly type 2 diabetes [8-11]. The obesity epidemic, according to the World Health Organization, is particularly troubling since it generates a parallel upward swing in type 2 diabetes, wherein, every kilogram of weight gained increases the risk of diabetes of 4.5 to 9% [12,13]. The dysmetabolism associated with non-insulin dependent diabetes can be characterized by hyperglycemia, hyperinsulinemia and hyperlipidemia, which includes hypercholesterolemia and hypertriglyceridemia [14,15]. In addition, type-2 diabetics are at increased risk for infective endocarditis [16,17]. Although medical interventions have improved over the years, infective endocarditis including prosthetic valve endocarditis (PVE) still has a high associated mortality rate despite surgical interventions and extensive antimicrobial usage [18].

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The nutritional excess resulting in obesity-associated type II diabetes has an added impact, since it represents a patient population who is also at a high risk for the development of fungal disease [19,20]. Overall, fungi account for 2-4% of infective endocarditis [21]. In addition, one quarter of individuals who develop fungal infective endocarditis have as a co-morbidity heart and vascular disease [22-24]. Adherence of *C. albicans* to fibrin clot matrices, either as part of endothelial vegetations, vascular thrombi, or fibrin-coated abiologic implanted devices, e.g., shunts and venous catheters, is the precipitating event for initiation of fungal sepsis. Alterations in the levels of plasma components, such as is observed in obesity-associated type II diabetes, could affect candidal adherence [25-28]. In addition, patient management could be affected since individuals with type 2 diabetes display metabolic abnormalities which have been reported to alter the activity of antifungal agents for a number of fungal species [29,30].

The adult genetically obese Zucker rats are an accepted model for *Candida* endocarditis [31-34]. They exhibit many characteristics in common with human adult-onset (type II) diabetes, including many metabolic characteristics in common with human obesity and obesity-associated type II diabetes [35-37]. These characteristics are hyperlipidemia, hypercholesterolemia, hypertriglyceridemia, hyperinsulinemia and transient hyperglycemia [32,33]. In addition, obese Zucker rats have been shown to exhibit aortic endothelial and myofibroelastic changes indicative of early atherogenesis. The purpose of this study is to assess the ability of *C. albicans* to adhere to fibrin-platelet matrices made from the plasma of the obese Zucker rat, a model for obesity-associated type II (mature-onset) diabetes.

2. Materials and Methods

2.1. Fibrin clots

Pooled plasma, from various pooled lots of 6-month-old Zucker lean (*Fa/fa*; weight-494 $\pm$ 7 g; cholesterol- 117 $\pm$ 16 mg/dl) and obese (*fa/fa*; weight-932 $\pm$ 32 g; cholesterol-252 $\pm$ 29 mg/dl) rats, a generous gift provided by D. Paulson, was used. Animal procedures followed were in accordance with the ethical standards of the institutions' AALAC accredited facility, and approval was obtained from the institutional Animal Welfare Committee File#918. Plasma cholesterol concentrations were determined using kits purchased from Sigma Chemical Co. (St. Louis, MO; kit no. 339-20, 115-A, and 352-20, respectively) [36].

Fibrin clots were prepared from lean and obese plasma using a modification of the assay described by Maisch and Calderone [38]. Briefly, thrombin (500 U/ml, Sigma Chemical Co.) with 0.4 ml of 0.2 M calcium chloride was mixed with plasma (1 ml; 35- by -10-mm tissue culture dish). After incubation (37 °C; 30 min) to promote complete solidification, clots were stored at 4 °C for a maximum of 2 days after preparation.

2.2. *Candida* growth

The *C. albicans* strain used for this study was isolated from the kidneys of infected obese Zucker rats [36]. The organism was a low passage number (2) and maintained at -80 °C. For the experiments, the isolate was cultured on Sabouraud Dextrose (SD) agar. Prior to use, the organism was grown over night at 25 °C or 37 °C on a gyrorotary shaker in GYEP, (0.5% wt/vol glucose, 0.3% wt/vol yeast extract and 1 % wt/vol Bacto-peptone).

2.3. Adherence to fibrin clot matrices

Adherence of *Candida* was measured by two complementary methods, i.e., use of radiolabeled yeast and direct microscopic examination of clots with adherent cells. Radiolabeled yeast were prepared by transferring 0.2 ml of overnight culture to fresh GYEP (250 ml) with [U-^{14}C]glucose at 0.1µCi/ml (353 mCi/mmol;; Sigma). After overnight incubation, at either 25 °C or 37 °C, the cells were harvested by centrifugation (2,000 rpm, 4 °C), washed three times with cold phosphate-buffered saline (PBS) (pH 7.2), and resuspended at a concentration of 10^7 yeast/ml in PBS. This suspension (0.3 ml) was placed on the clots and incubated on a gyrorotary shaker (125 rpm) at 37 °C for 30 min. The clots were then washed extensively with PBS (5 times), then dissolved with 2 ml of trypsin (0.5 % wt/vol of normal saline, Sigma) at 25 °C for 2 hr. Radiolabeled yeast in dissolved clots (adherent cells) and cells in the washes (nonadherent cells) were counted after collection on glass fiber filters and digestion in tissue solubilizer. Loss of label was negligible (<< 1%) with this technique. All assays were done in triplicate and repeated once. Controls consisted of clots incubated on ice. *Candida* were added to clots and immediately washed off with both clot and effluent-associated radiolabel measured.

Adherence of germinated *C. albicans* was determined by direct microscopic examination of clots. Germ tube forms were obtained by suspending 10^5 yeast from the overnight GYEP culture in yeast nitrogen base (Difco) with 5 mmol proline (pH 7.1). After 3 hrs. incubation at 37 °C, >99 % of the yeast had germinated. This suspension was centrifuged (1500 rpm, 10 min), washed three times in PBS then suspended to 2 x 10^6 cells/ml PBS. To obtain a mixed suspension of yeast

and germ tubes, an unlabeled yeast suspension, prepared as described above, was adjusted to 2 x 10^6 and mixed 1:1 with the germ tube suspension. These suspensions (0.5 ml) were added to the clots. After the clots were washed as described above, 5 ml of formalin was added to each clot. Adherence of both the germ tube alone and mixed cell suspensions was measured by direct inverted phase microscopic enumeration of the numbers and types of cells in fifty random fields. All assays were done in triplicate and repeated once.

2.4. Amphotericin B and cell adherence

To determine if amphotericin B (AmB) incorporated into clots affects the binding of either yeast or germ tube forms of *C. albicans*, AmB (0.6 ug/ml clot; Sigma) was incorporated into clots derived from both lean and obese Zucker rats. AmB was dissolved in dimethylsulfoxide (DMSO), then diluted 1:1000 in homologous plasma immediately prior to use. Adherence to control clots containing DMSO alone was not significantly different from clots without DMSO ($p \leq 0.001$). The concentration of AmB used was previously determined to be cidal for this strain of *C. albicans* according to the method described by Hopfer, et al. [39]. All assays were done in triplicate and repeated once.

The effect of preincubation of yeast or germ tube forms in lean or obese Zucker rat serum, with or without AmB, on adherence was measured by suspending yeast (10^7 yeast/ml) in lean or obese serum with or without AmB or in AmB (0.6 µg/ml PBS) alone. After 3 min incubation at 37 °C, the cells were pelleted by centrifugation (2000 rpm, 4 °C, 5 min) and washed three times in PBS to remove any unbound AmB and/or serum components. The final washed cell suspensions were suspended to 10^7 cells/ml in PBS and used in adherence assays as described above. To confirm that the normal sera did not contain antibodies directed against *C. albicans*, yeast and germ tubes were dried onto slides, and exposed to lean or obese serum for 60 min in a humidified chamber. These smears were thoroughly washed, exposed to FITC-labeled Protein A and reincubated. Fluorescent microscopy examination of the cells revealed no bound antibody. Controls using anti-candidal serum were positive. All assays were done in triplicate and repeated once.

2.5. Influence of mannose on adherence to clots

Determination of the effect mannose has on adherence to clots was accomplished by suspending the yeast (10^7 yeast/ml) in PBS containing 1% mannose. The cell suspension was then placed on plasma clots as described above. The effect serum and mannose have in combination on adherence was determined by first preincubating the yeast in serum as described above. The pretreated-washed cells were then suspended in PBS containing 1% mannose at a concentration of 10^7 yeast/ml and placed on the clots. All assays were done in triplicate and repeated once.

2.6. Relative hydrophobicity of yeast and germ tubes

The surface hydrophobicity of yeast prior to and after preincubation (as described above) with serum and/or amphotericin B, or mannose was determined with a modification of the biphasic assay of Rosenberg et al. [40]. Briefly, washed yeast cells (sterile distilled water; 4^0C) were suspended in PUM buffer ($K_2HPO_4 \bullet 3H_20$, 22.2 g /L; KH_2PO_4, 7.26 g/L; urea, 1.8 g/L; $MgSO_4 \bullet 7H_20$, 0.2 g; Sigma-Aldrich; pH 7.1) to an absorbance of 0.400 A_{600nm}. This suspension (1.2 ml) was placed in acid-washed 13 by 75 mm glass tubes, then overlaid with 0.3 ml of xylene. After the hydrocarbon-yeast suspension was thoroughly vortexed (3 min) and the layers separated, the absorbance of the aqueous layer was measured. The absorbance of the untreated suspension served as the control. The relative hydrophobicity was determined according to the equation: (A_{600nm} treated cells /A_{600nm} original suspension) X 100. Each assay was performed in triplicate and repeated once.

2.7. Statistical analysis

Each assay was performed in triplicate and repeated once (total n=6). Data were analyzed (GraphPad Prism) by two-way ANOVA ($p<0.05$). Where appropriate, Tukey post-hoc tests were performed.

3. Results

3.1. Candidal growth temperature and clot adherence

Adherence levels of the yeast after growth at either 25 °C or 37 °C are shown in Figure 1. Organisms growing at 37 °C adhered to the clots made from lean plasma to a level significantly higher ($p<0.05$) at almost twice (1.8-fold) that observed for cells grown at 25 °C. However, the trend was reversed with respect to adherence to obese clots where adherence at 37 °C was 75% ($p<0.05$) that observed for lean clots. Level of binding of environmentally grown cells to obese plasma clots was significantly greater (3.7-fold; $p<0.05$) than adherence to lean plasma clots. While adherence of 37 °C grown cells to obese plasma clots was slightly, but significantly ($p<0.05$) greater (1.4-fold) than that associated with lean plasma clots. Incorporation of amphotericin B (AmB) into clots made with lean plasma resulted in a ~4-fold

(p<0.05) depression in adherent yeast cells, as compared to lean clot controls, regardless of yeast growth temperature. However, AmB had no significant effect on adherence to obese clots, regardless of cell growth temperature.

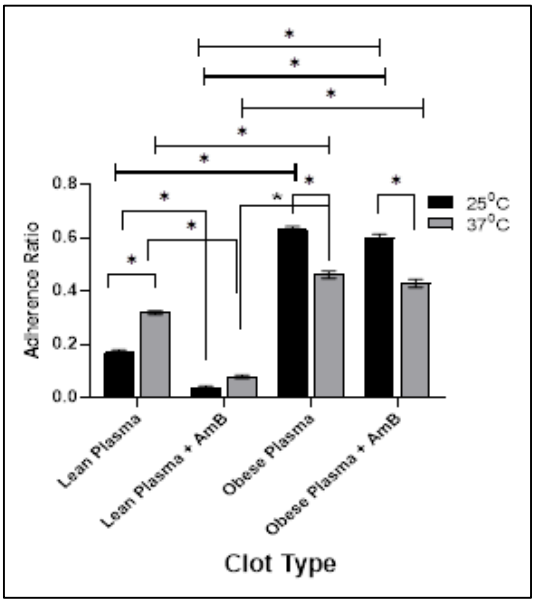

Figure 1 Adherence of *Candida albicans* blastospores grown at 25 °C and 37 °C to clots formed from lean or obese Zucker rat plasma. Adherence ratio was determined by comparison of test condition (clot + cells incubated at 37 °C or 25 °C; 30min) divided by that of control condition (clot on ice + cells then immediately washed) AmB = amphotericin B; *=p≤0.05

3.2. Pretreatment of *Candida*

The ability of cell pretreatment, with components from the serum, AmB, and a competitive inhibitor for candidal adherence (mannose) [41], to affect *C. albicans* adherence to clots is shown in Figure 2A and 2B. All pre-treatments, with the exception of AmB and mannose (25 °C and 37 °C; lean plasma clot), significantly (p<0.05) inhibited adherence, with obese rat serum in combination with mannose being the most effective in inhibiting adherence to lean plasma clots for yeast cells grown at both environmental and physiological temperatures (89% and 88% reduction, respectively). Pretreatment with serum from lean rats had no effect on *C. albicans* interaction with either lean or obese clots, regardless of yeast growth temperature. However, addition of either mannose, or AmB, to the lean serum, resulted in a slight, but significant (p<0.05), increase in adherence inhibition, as compared to lean serum alone; although, like lean serum alone, the level of inhibition was similar regardless of cell growth temperature. This indicates that the interaction of the organism with the clot may involve both hydrophobic and specific ligand-receptor interactions.

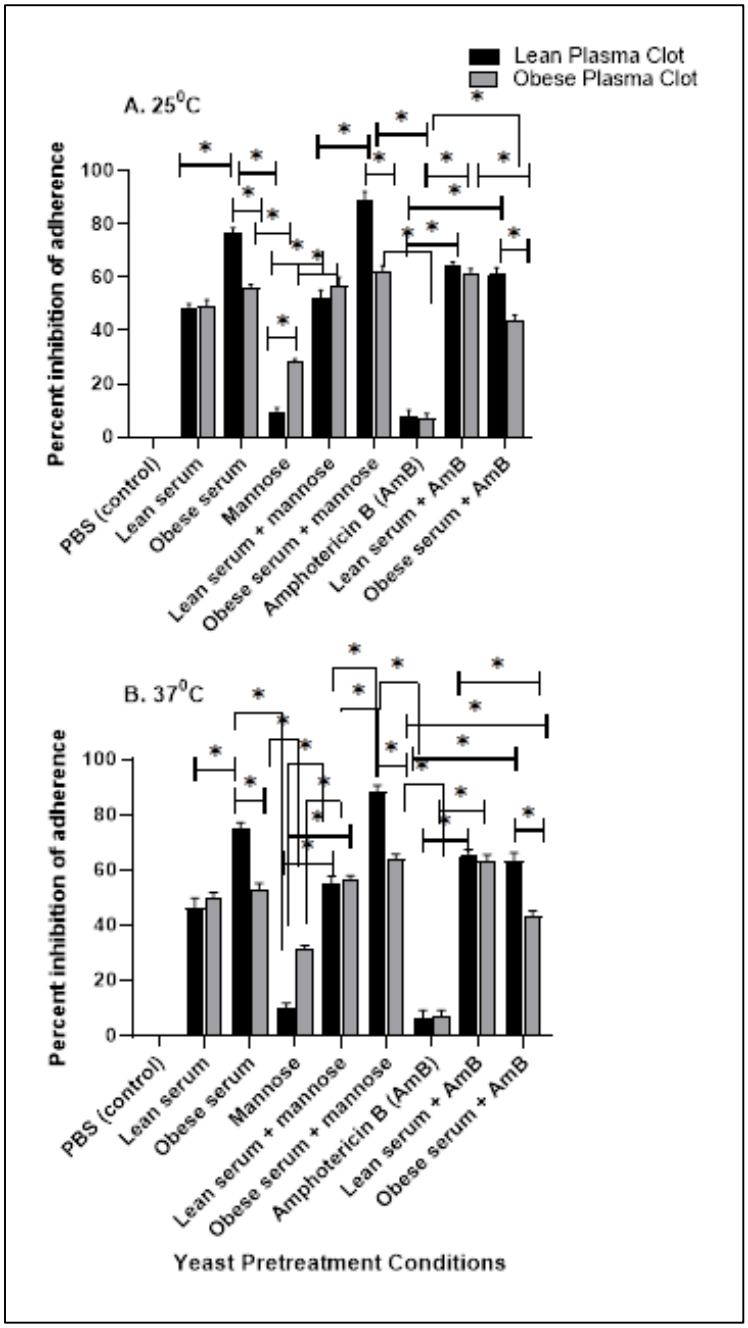

Figure 2 Effect of pretreatment of yeast cells grown at 25 °C (A) or 37 °C (B) with serum (lean or obese) with or without mannose or amphotericin B (AmB), and mannose and AMB alone. *=p<0.05

3.3. Effect of pretreatment on candidal hydrophobicity

All substances tested could presumably act by affecting the surface hydrophobicity of the organism, with the exception of mannose which specifically blocks adherence to a mannose-containing receptor in the clot [41-43]. Therefore, the relative hydrophobicity, as a measure of incorporation into the hydrophobic xylene layer after partitioning into aqueous buffer-xylene bilayers, of *C. albicans* after pretreatment is shown in Figure 3. Of the pretreatments, only exposure of 37 °C grown cells to obese serum, with and without AmB or mannose, significantly increased ($p< 0.05$), to a similar extent, the hydrophobicity of the yeast.

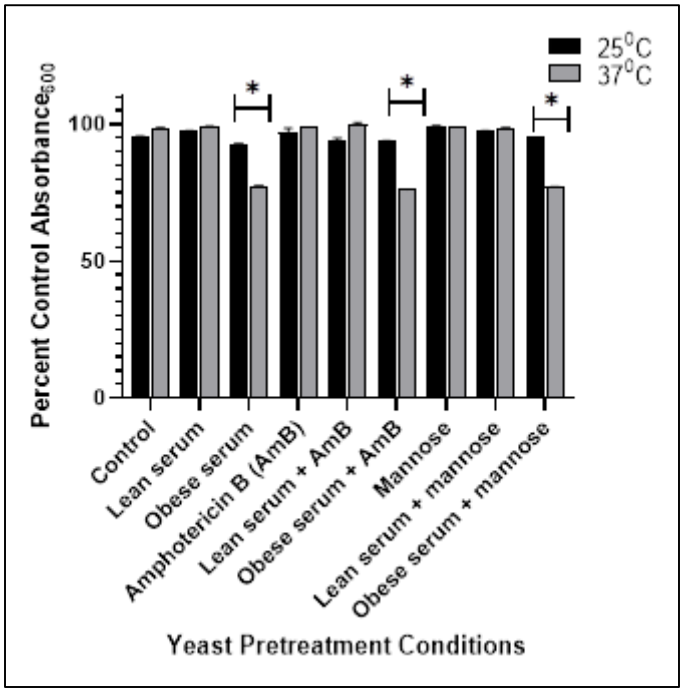

Figure 3 Relative hydrophobicity of yeast (25 °C and 37 °C growth temperature) after pretreatment with serum (lean or obese) with or without mannose or amphotericin B (AmB), and mannose and AMB alone. *=p<0.05.

3.4. Blastospore and germ tube adherence

Adherence of yeast blastospores to clots from lean plasma were twice that of germ tube (pseudohyphal) forms (Table 1). In contrast, adherence of blastospores to obese plasma clots was nearly 11-fold higher than that measured for germ tube forms. Germ tube forms were ~3 fold higher in their binding to lean clots than those made from obese plasma. Interestingly, the mixture (1:1) of yeast and germ tube forms adhered to a similar level as that measured for each separately, with respect to the germ tube forms, while yeast adherence in combination with germ tube forms was similar for both lean and obese Zucker rat plasma clots. The determination that the level of germ tube binding was similar regardless of the presence of yeast cells indicates that the two morphotypes interact differently from each other dependent on composition of the plasma.

Table 1 Adherence of *C. albicans* germ tube and yeast forms (37^0C) to clots formed from obese and lean Zucker rat plasma

	Lean Clots		**Obese Clots**	
	Germ tube Forms	**Yeast Cells**	**Germ tube Forms**	**Yeast Cells**
Cell Morphology				
Germ tube forms	46.7 ± 8.1[a]	----	14.7 ± 3.2	----
Germ tube -Yeast mixture	49.4 ± 7.6	101.5 ± 13.5	12.9 ± 2.1	141.1 ± 15.1
[a]Mean ± SEM				

4. Discussion

Initiation of endocarditis, and infective thrombophlebitis by *C. albicans* resides in its ability to colonize fibrin clots matrices [38,44,45]. In addition, candidal sepsis, as a result of biofilm formation, is linked to a variety of implanted medical products including vascular bypass grafts, abiotic heart valves, dental implants, vascular catheters, knee and hip joints, and cerebral shunts, all of which can function as sites for fibrin deposition, and subsequent substrates for biofilm growth [46-48]. In addition to fibrin deposition in the clot matrices, there will also be the presence of other host proteins and plasma components, including lipids, [49,50]. The concentrations of the additional plasma components, which can affect infectivity, would reflect the metabolic status of the host. For example, in the ApoE deficient mouse, a

model for atherosclerotic plaque formation due to accumulation of particles enriched with cholesterol esters in the circulation, both *in vivo* susceptibility to candidiasis and *in vitro* expression of virulence factors are increased, the latter being with cultivation of *Candida* in the presence of lipids [51-53]. Unfortunately, to date, the candidal interactions with host components that play a role in the initiation of septic infectious foci in obesity-associated metabolic dysfunction have not been defined. The Zucker rat system provides an opportunity to examine the effect various factors have on initiation of *C. albicans* biofilm formation in obesity-related dysmetabolism. For example, one such factor is the source of the infecting organism.

The source of organisms for initiation of fibrin matrix clot infections can be environmental, being spread via situational contamination, e.g., percutaneous introduction of fungi along vascular catheters, or via contaminated intravenous solutions [54-56]. This is particularly problematic for hospitalized cancer patients, and those diagnosed with diabetes, both of whom exhibit a high incidence of candidemia (71 per 100,000 and 28 per 100,000, respectively), with coincidently the near total presence of central venous catheters [1,57]. This hospital procedure accounts for candidemia's status as the third to fourth most common cause of a life-threatening nosocomial bloodstream infections, with a mortality rate of 38%, at its maximum reported level [46]. Alternatively, a body site, e.g., the gastrointestinal tract, could serve as primary source of fungemia since *Candida* can be a member of the host microbiome [58,59]. This variation in origin can affect the microbial surface-surface interactions, which serve as the prerequisite for adherence and subsequent colonization. This study shows that, overall, regardless of whether yeast was grown at environmental or human body temperature, their adherence to clots made from obese rat plasma was significantly greater ($p<0.05$) than that observed for clots made from lean rat (normophysiologic) plasma; however, the degree and pattern of binding to clots was dependent on the temperature at which the organisms were grown. Clots made from normal plasma were more permissive of adherence from yeast grown at human body temperature than yeast grown at environmental room temperature. This indicates that *Candida,* potentially originating from the host microbiome, have the advantage in colonization of fibrin clots in a normophysiologic host. However, yeast from the environment would adhere to a significantly greater extent to fibrin clots made using plasma from obese Zucker rats than those representing the normal flora. The ramifications of this are multifactorial, since although the environmentally grown yeast adherence to obese plasma clots was higher than those grown at body temperature, the overall extent of adherence to the obese plasma clots, regardless of growth temperature, was significantly greater than that measured for binding to lean plasma clots. This indicates that the dysmetabolism associated with obesity places individuals at increased risk of candidal colonization due to the altered components of the fibrin matrices to which *Candida* binds, a finding substantiated by the epidemiology of candidal infections [53,60]. Another potential factor affecting yeast binding to fibrin-clot matrices is the hydrophobic surface-surface interaction, since upon initiation of candidemia, the yeast cells are exposed to circulating plasma.

The mechanism of surface-surface interaction dependent on hydrophobic adherence is reliant on the relative level of cell surface hydrophobicity, which could alter the initiation events for endocarditis and thrombophlebitis [48,61]. Elevated levels of associated lipids, as a result of the accompanying hyperlipidemia, could be due to plasma preexposure to either obesity-associated hyperlipidemia, or conditions of temporary drug-induced dyslipidemia [31,62]. This exposure to dysmetabolic plasma components, prior to clot adherence, could affect binding even where the clots were formed from normoplasma. Interestingly, only pretreatment of yeast cells with serum from obese Zucker rats altered (increased) the level of yeast surface hydrophobicity, regardless of growth temperature, or the presence of other serum additives, i.e., amphotericin B and mannose. This indicates that surface hydrophobic interactions are not the most likely contributing cause in the differential adherence of yeast to obese plasma clots over that of lean plasma clots. Nor does the morphologic form of the *Candida* (yeast vs. pseudohyphal) appear to alter previously reported adherence patterns for *Candida* wherein, yeast forms exhibit higher levels of adherence to fibrin clots as compared to germ tube forms [63].

Another alternative hypothesis of what could contribute to candidal adherence, is the presence of different levels in lean vs. obese plasma clots of mannose-containing candidal receptor(s), or alternatively, the mannose-binding adhesin differentially expressed on *Candida,* dependent on yeast growth temperature [64,65]. The role of mannose in *Candida* adherence to fibrin matrices is supported by its ability to competitively inhibit the binding of pre-treated yeast to both lean and obese plasma clots. This indicates that mannose contributes to candidal binding to clots, but still leaves open the question of which component, or combination of components, is responsible for the preferential binding of *Candida* to obese plasma clots.

Of clinical importance in candidemia, particularly when dealing with biofilm-associated processes, is the ability to effectively treat the infection [20,66]. Effective treatment is particularly of concern in individuals with candidiasis, since they have high treatment failure rates [67]. Although there are a number of fungal drug resistance mechanisms, e.g., altered drug target and transporters, these do not account for phenotypic resistance attributable to the altered presence of plasma components in obesity-associated dysmetabolism [68]. To date, the alternative to surgical removal of the

infected clot from the vasculature, or indwelling device alone, is surgical removal of any affected implant together with antifungal and systemic anticoagulation therapy [69]. Unfortunately, removal and subsequent replacement of vascular catheters, together with possible surgical drainage, are not benign procedures. These actions don't address the underlying factors that play a role in the treatment failure. In addition, to surgical interventions, adequate treatment of endocarditis requires cidal levels of fungicidal drugs. Amphotericin B, which is the most efficient drug used in the treatment of systemic fungal disease, was incorporated at cidal levels into clots to determine if its activity is altered by clot composition, or growth temperature of the organism. When AmB was incorporated into lean clots, adherence was significantly inhibited ($p<0.05$) to a level approximately 24% of controls for cells grown at either 25 ℃ or 37 ℃. In contrast, incorporation of AmB into obese clots did not significantly affect adherence, as compared to amphotericin-free controls. This indicates that the source of the organism, i.e., whether it is from the environment (growing at room temperature), or from the individual's normal flora (growing at body temperature), may affect the level of initial clot infection, and thus, the rate of disease progression. This further indicates that AmB in clots from lean rats could partially block the binding of blastospores, thus the ability of yeast forms to subsequently bind to the clot would be reduced and the progress of infection may be slowed. These findings are not unanticipated since, lipid infusions, obese Zucker rat serum, as well as plasma proteins, affect amphotericin B activity [29,30,66,70,71]. Chemical analysis has demonstrated that competitive inhibition ergosterol-AmB by cholesterol occurs, and that the amount of amphotericin B incorporated in cholesterol lipid bilayers is the same as ergosterol, with mixtures of cholesterol/AmB showing a marked dependence on the mole fraction of the drug [72-74]. This is attributed to the chemical similarity of ergosterol and cholesterol (Fig. 4).

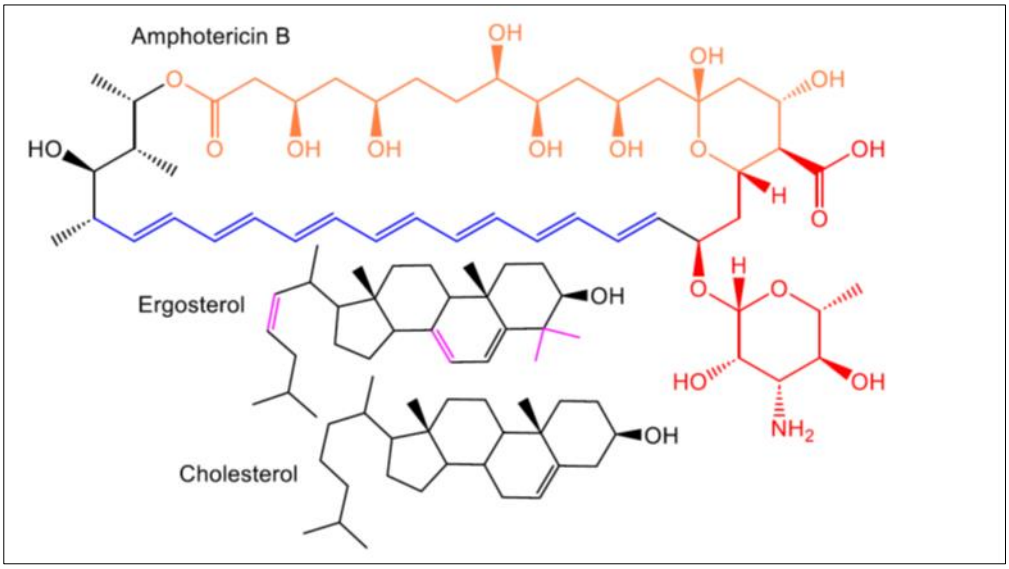

Figure 4 Amphotericin B and sterol binding. Amphotericin B's polar head is shown in red, hydrophilic polyol chain in orange, hydrophilic tail in black and the hydrophobic polyene chain in blue. The subtle structural differences between ergosterol and cholesterol are shown in fuchsia.

The similarity in the structures of ergosterol and cholesterol explains the affinity of AmB to both sterols, thus leading to hydrophilic pore formation in both fungal and mammalian cell membranes. While binding/pore formation to the fungal membrane is the main mechanism of action of AmB as an antifungal (disturbing of the cells' osmotic pressure), its binding/pore formation in mammalian cell membranes is the basis for the drug's toxicity (anemia, cardiotoxicity, nephrotoxicity) [75-77]. It is likely that in obese Zucker rat plasma, the sheer number of cholesterol molecules most likely bind to/overwhelm the binding capacity of amphotericin B molecules, at the therapeutic dosage, therefore, diminishing the ability of the drug to bind to the fungal membranes effectively.

5. Conclusions

C. albicans attached to obese plasma clots at a higher level than lean plasma clots, and the potential to initiate infection was enhanced by obese serum, as compared to lean serum. Further, serum components were observed to bind to the yeast and affect adherence to clots. Further work needs to be done in order to delineate the component(s) which mediate this effect and provide insight into the mechanism(s) underlying the epidemiology surrounding the higher level of fungal biofilm-associated infections in obese dysmetabolic individuals.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflicts of interest.

Author Contributions

Conceptualization, B.J.P.; methodology, B.J.P.; validation, B.J.P. and M.I.K.; formal analysis, B.J.P.; investigation, B.J.P.; resources, B.J.P.; data curation, B.J.P.; writing—original draft preparation, B.J.P.; writing—review and editing, M.I.K.; visualization, B.J.P.; supervision, B.J.P.; project administration, B.J.P.; funding acquisition, B.J.P. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data supporting reported results are available upon request.

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