Corticosteroid therapy is a risk factor for invasive fungal infections and this vulnerability is attributed to the complex dysregulation of immunity caused by glucocorticoids. Increased growth rate under corticosteroid exposure was demonstrated for some molds such as Aspergillus fumigatus and Exserohilum rostratum. No such data exist for Mucorales. Therefore, we investigated the influence of GC exposure on the growth of Rhizopus arrhizus (syn. R. oryzae) in different culture media and in different atmospheres. We measured continuous spore growth using spectrophotometry and biomass variations using XTT assay. We did not observe enhanced growth or biomass variation with any of the GCs regardless of the medium or conditions. These results support the existence of fungus-specific differences in the effect of GCs on fungal biology.

Glucocorticoid (GC) use is a common risk factor for invasive fungal infections. This is attributed to the complex dysregulation of immunity caused by GCs. However, studies have demonstrated increased growth with GC exposure for some molds, such as Aspergillus fumigatus and Exserohilum rostratum. No such data exist for Mucorales. Therefore, we investigated the influence of GC exposure on the growth of Rhizopus arrhizus (syn. R. oryzae) in different culture media and in different atmospheres. We measured continuous spore growth using spectrophotometry and biomass variations using XTT assay. We did not observe enhanced growth or biomass variation with any of the GCs regardless of the medium or conditions. These results support the existence of fungus-specific differences in the effect of GCs on fungal biology.

Glucocorticosteroids do not impact directly growth rate and biomass of Rhizopus arrhizus (syn. R. oryzae) in vitro

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Briefly, XTT (Sigma-Aldrich, St. Louis, MO) was dissolved in normal saline at concentrations of 1 mg/ml. Menadione (Sigma-Aldrich, St. Louis, MO) was initially dissolved in acetone at a concentration of 10 mg/ml and subsequently added to the above-mentioned XTT solutions at concentrations of 125 μM for each solution. A 10⁴ spores/ml of *R. arrhizus* were incubated in RPMI 1640, Yeast Extract Medium (YAG), and Yeast Nitrogen Base Medium (YNB) at 37°C under 5% CO₂ atmosphere in 96-well flat-bottom microtitration plates at a volume of 200 μl per well. After 18 h of incubation, 50 μl of one of the above-mentioned XTT-menadione solutions was added to each well and the plate was incubated at 37°C under 5% CO₂ atmosphere for an additional 2 h. After 2 h of incubation, 200 μl from each test group were plate in a sterile 96-well U-bottom micro titration plate for analysis. The formazan absorbance in each well was read at 492 nm and 690 nm (plate absorbance) with the use of a micro plate spectrophotometer (Power wave Biotech Instruments, Winooski, VT). We obtained the XTT results by subtracting to the result the optical density (OD) of wells containing media alone (background). These additional assays investigating *R. arrhizus*’s biomass under higher CO₂ atmosphere in presence of GC did not show any difference upon GC exposure (Fig. 4).

For example, a previous report showing toxicity of steroid such as progesterone on *Rhizopus nigricans* suggested that the underlying mechanism was depending on G protein activation and cAMP signaling.9

In conclusion, corticosteroid did not impact directly *R. arrhizus*’s growth or biomass in vitro. These negative results support the hypothesis that the immune dysfunction induced by corticosteroid is the main reason why patients are more vulnerable to Mucormycosis. Moreover, when compared with other previous reports,²,³ these negative data support the notion that there might be some fungus-specific differences regarding the effect of corticosteroids on fungal biology. The difference of impact of GCs on *Aspergillus fumigatus* growth in comparison with Mucorales might explain, at least in part, the higher incidence of cases of invasive aspergillosis among this patient population compared to mucormycosis.
Supplemental Material

Supplemental data for this article can be accessed on the publisher’s website.

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Figure 3. Continuous growth measurement of R. arrhizus in the presence of 0 (control), 80, 160, 320 and 640 ng/ml for MPN under standard atmosphere in RPMI (A), YNB (B) and YAG (C). No promotion of growth was observed (P value > 0.05; t Student non parametric test).

Figure 4. R. arrhizus’s biomass assessment using XTT in the presence of 0 (control), 80, 160, 320 and 640 ng/ml for DEXA (A), HC (B), MPN (C) under 5% CO2 atmosphere in RPMI, YNB and YAG. No promotion of growth was observed (P value > 0.05, t Student non parametric test).