Limited effect of refined carbohydrate dietary supplementation on colonization of the gastrointestinal tract of healthy subjects by Candida albicans

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ABSTRACT
Background: Infections due to Candida albicans occur readily in situations in which ample glucose is available. In mice, dietary refined carbohydrate supplementation leads to higher rates of Candida growth in the gastrointestinal tract and favors mucosal invasion.

Objective: The modulating properties of dietary carbohydrate supplementation on colonization of the human gastrointestinal tract by C. albicans were evaluated.

Design: A 2-step study was conducted in 28 healthy volunteers. First, we determined the subjects’ habitual uptake of refined carbohydrates and correlated these data with the C. albicans conidia concentration in the mouth washes and feces of subjects. Second, we compared C. albicans counts in the specimens before, during, and after a high-sugar diet.

Results: No correlation between C. albicans counts in the specimens and the habitual uptake of refined carbohydrates was observed. A high-sugar diet did not increase the frequency of C. albicans-positive samples, the number of subjects positive for C. albicans in the mouth washes, or the concentration of candidal conidia in the samples of the 28 subjects. However, in selected subjects with elevated counts of oral C. albicans, we observed an increase in fecal C. albicans counts in response to the diet.


KEY WORDS Candida albicans, refined carbohydrate, colonization, concentration, gastrointestinal tract, glucose, modulation, sugar, yeast, humans

INTRODUCTION

The ascomycetous yeast Candida albicans is found frequently on the mucosal surfaces of the gastrointestinal tract in healthy people. In severely immunocompromised hosts, local gastrointestinal candidiasis and disseminated invasive infection are thought to arise from gastrointestinal colonization and overgrowth of C. albicans (1–6). Factors that increase the frequency and magnitude of colonization with C. albicans may influence pathogenicity. Although a definitive threshold surface concentration has not been identified, invasion of the gastrointestinal mucosal wall depends largely on the number of organisms in the gastrointestinal tract (6, 7). According to Krause et al (6), invasion of the gut and fungemia occurred in a healthy volunteer after ingestion of 10$^{12}$ C. albicans organisms. In addition, unbalanced C. albicans counts in the human gastrointestinal tract have been associated with a variety of clinical symptoms, such as diarrhea (8), relapsing vaginitis (9, 10), exacerbation of psoriasis and atopic- and seborrheic dermatitis (11, 12), and the speculative candidiasis hypersensitivity syndrome (13, 14).

Interestingly, candidiasis occurs more often in situations in which ample glucose is available, as in patients receiving parenteral nutrition or suffering from diabetes (15). A high local glucose concentration leads to an increased incidence of Candida paronychia in sugar cane workers (15) and rinsing with sucrose leads to Candida-induced stomatitis in humans (16). A propagating role of a carbohydrate-rich diet in relapsing Candida vulvovaginitis was proposed in a study involving 240 women (17). Carbohydrate-rich diets favor the oral carriage of C. albicans in different animal models (18, 19). In a neutropenic mouse model, Vargas et al (7) showed that dietary glucose supplementation led to higher rates of Candida growth in the gastrointestinal tract of mice and favors mucosal invasion. To reduce the gastrointestinal C. albicans burden, the avoidance of refined carbohydrates, including dextrose and fructose, was proposed as an “antifungal diet” (7). The rationale for this diet is that restriction of glucose as a major substrate of C. albicans might suppress gastrointestinal candidal growth. This procedure is widely propagated by the lay press (13). However, little scientific information is available concerning a modulating effect of refined carbohydrates on the multiplication of C. albicans organisms inhabiting the human gastrointestinal tract.

To evaluate the effect of refined carbohydrate intake on gastrointestinal C. albicans colonization, a 2-step study was performed...
healthy volunteers. In a first step, subjects were explored for their habitual uptake of refined carbohydrates with a food-frequency questionnaire. The data were correlated with the C. albicans blastoconidia concentration in the mouth washes and feces of subjects in whom there was no intervention. Subsequently, we compared the C. albicans counts in the mouth washes and feces of the subjects before, during, and after a high-sugar diet. During the diet period, the subjects ingested an excessive amount of refined carbohydrates.

SUBJECTS AND METHODS

Subjects
Twenty-eight healthy subjects (19 women and 9 men) with an average age of 26.3 y (range: 17–48 y) were randomly selected. None of the subjects had received antimycotic therapy in the 3 mo before the study. Body weight was determined for each participant. The study was conducted in accordance with the Helsinki Declaration.

Food-frequency questionnaire
The habitual mean daily frequency of sugar consumption (saccharose, glucose, and fructose) was evaluated with a food-frequency questionnaire. Subjects were asked what main sugar-containing foods and beverages they consumed, eg, baked goods, chocolate, candy, ice cream, fruit juice, or lemonade (20, 21). We asked the subjects to report their habitual diet rather than their diet during any specific period. We did not attempt to assess the role of total energy uptake or specific nutrients. Instead, we focused on the association of specific foods or food groups containing refined carbohydrates and colonization of the human gastrointestinal tract by C. albicans.

Diet and experimental protocol
The subjects were examined for 11 wk over 3 periods. In period 1 (baseline), the subjects continued their habitual diet, and samples were obtained over 6 wk without intervention. In period 2 (dying the high-sugar diet), we increased the total energy intake of the subjects by adding 110 g refined carbohydrates/d (10.8% glucose, 35.7% saccharose, 3.2% fructose, and 50.3% dextrin) to their habitual diet. The composition of the refined carbohydrates was confirmed in an authorized, reference chemical laboratory (Chemisches Laboratorium Ritz, Aachen, Germany). During the diet, the refined carbohydrates were delivered in 3 portions daily between regular eating times. In period 3 (follow-up), the subjects continued their habitual diet for another 4 wk without intervention.

Sampling
The fungal germ cell burden was determined in mouth washes and in homogenized fecal specimens. The samples were analyzed once a week during periods 1 and 3. In period 2, mouth washes and fecal samples were analyzed on days 3 and 7. Twelve stool samples and 12 mouth washes were obtained from each subject (n = 28). A total of 168 (6 samples/subject) fecal samples and 168 mouth washes were examined before the high-sugar diet began. During the high-sugar diet and shortly thereafter (periods 2 and 3), an additional 168 fecal samples and 168 mouth washes were analyzed. All 672 samples obtained were suitable for analysis.

Yeast quantification and differentiation
The oral cavity was rinsed with 10 mL sterile ampuwa (Fresenius, Bad Homburg, Germany) for 120 s and 100 μL of each mouth wash was streaked on Sabouraud glucose agar (Merck, Darmstadt, Germany). The mouth washes were diluted 10-fold in isotonic buffered saline and a serial dilution was prepared from every sample; 100 μL of each dilution was inoculated onto Sabouraud glucose agar. Stool samples were weighed and homogenized in sterile isotonic saline. The final homogenate for culture contained 100 g stool/L. The homogenized specimens were vortex mixed for 2 min and were diluted 10-fold in isotonic buffered saline; 100 μL of each dilution was inoculated onto Sabouraud glucose agar. Cultures were incubated at 32°C for 48 h. We attempted to differentiate all fungi by species. Yeasts were identified according to their ability to produce germ tubes and chlamydospores and on the basis of the Auxacolor system (Sano Diagnostics Pasteur, Marnes la Coquette, France) and the assimilation profile (22).

Statistical analysis
To evaluate the influence of the habitual frequency of refined carbohydrate uptake on colonization of the gastrointestinal tract by C. albicans, we formulated a multiple regression model. In this model we used the mean values of the logarithmic C. albicans counts in the specimens collected in period 1 (baseline) as the dependent variable. The habitual daily uptake of refined carbohydrates, age, sex, and body weight were used as independent variables. A Wilcoxon test for paired variables was performed to compare logarithmic yeast counts before, during, and after the high-sugar diet. STATISTICA for Windows (version 5.1; StatSoft Inc, Tulsa, OK) was used for the analyses.

RESULTS

Influence of habitual uptake of refined carbohydrates on C. albicans counts in the gastrointestinal tract
No significant correlation between the C. albicans counts in the mouth washes and the independent variables (habitual daily uptake of refined carbohydrates, age, sex, and body weight) of the multiple regression model were found. Likewise, the multiple regression analysis showed no correlation between the C. albicans counts in the feces and the independent variables.

Correlation of oral and fecal C. albicans blastoconidia concentrations
We added the oral C. albicans counts to the multiple regression model as an independent variable when evaluating C. albicans blastoconidia concentrations in the feces as the dependent variable. We found a highly significant correlation (P < 0.001) between the C. albicans concentration in the feces and the independent variables (C. albicans counts in the mouth washes, habitual daily uptake of refined carbohydrates, sex, age, and body weight). However, on the basis of β coefficients, this correlation was significant only between the C. albicans concentration in the feces and one independent variable—the C. albicans counts in the mouth washes. Thus, in the multiple regression model the degree of oral colonization with C. albicans was the only index that influenced the fecal C. albicans blastoconidia concentration.

Influence of a high-sugar diet on the species isolated from the samples and the frequency of C. albicans–positive samples
No significant change in the spectrum of candidal species isolated from the oral and fecal samples was observed in response
the high-sugar diet.

Period 1, before 3.58 4.54 5.95
Period 2, during 3.66 4.71 6.42
Period 3, after 3.65 4.69 6.12

Subjects whose baseline *C. albicans* counts in mouth washes were > 1.0 x 10⁴ CFU/L.

Significantly different from period 1, P < 0.05 (Wilcoxon matched-pairs test).

to the diet. *C. albicans* was the fungal organism most frequently identified in the specimen, detectable in 59.5% (100/168) of the fecal samples in period 1 and in 60.7% (102/168) of the fecal samples in periods 2 and 3. *Geotrichum candidum* was detectable in 13.7% (23/168), *Rhodotorula* spp. in 7.7% (13/168), *Saccharomyces cerevisiae* in 4.8% (8/168), and other non-*C. albicans* species in < 4% of the fecal samples examined during period 1. No significant increase in the isolation frequency of these organisms occurred during periods 2 and 3.

**Influence of a high-sugar diet on the number of subjects positive for *C. albicans***

During period 1, 78.6% of the subjects (22/28) had detectable amounts of *C. albicans* blastoconidia in the mouth washes and 71.4% of the subjects (20/28) had *C. albicans* in the fecal samples examined during this period. No increase in the number of positives with positive mouth washes was observed during periods 2 and 3. However, 78.6% of the subjects (22/28) had *C. albicans* in the feces during and shortly after the high-sugar diet ended.

**Influence of a high-sugar diet on the *C. albicans* blastoconidia concentration in the gastrointestinal tract**

Mean logarithmic *C. albicans* counts in the mouth washes and the feces of the 28 subjects were compared before, during, and after the high-sugar diet and are shown in Table 1. Mean counts in the mouth washes were as follows: 3.8 x 10⁴ colony-forming units (CFU)/L (3.58 log₁₀ CFU/L), 4.6 x 10⁵ CFU/kg (3.66 log₁₀ CFU/L), and 4.5 x 10⁷ CFU/kg (3.65 log₁₀ CFU/L) before, during, and after the high-sugar diet, respectively. Mean counts in the feces were as follows: 3.5 x 10⁴ CFU/kg (4.59 log₁₀ CFU/kg), 5.1 x 10⁴ CFU/kg (4.71 log₁₀ CFU/kg), and 4.9 x 10⁷ CFU/kg (4.69 log₁₀ CFU/kg) before, during, and after the high-sugar diet, respectively. There were no significant differences between periods (Wilcoxon matched-pairs test).

In a subgroup of subjects (n = 12) with > 1.0 x 10⁴ CFU *C. albicans* germ cells/L in the mouth washes collected in period 1, mean counts in the feces during and after the high-sugar diet were 8.9 x 10⁵ CFU/kg (5.95 log₁₀ CFU/kg), 2.6 x 10⁶ CFU/kg (6.42 log₁₀ CFU/kg), and 1.3 x 10⁷ CFU/kg (6.12 log₁₀ CFU/kg) before, during, and after the high-sugar diet, respectively. Mean logarithmic values are shown in Table 1. The mean fecal *C. albicans* count in this subgroup during the diet (period 2) was significantly greater than that at baseline (period 1). In contrast, there was no significant difference in *C. albicans* counts in the mouth washes as a result of the high-sugar diet.

**DISCUSSION**

Our study was the first to evaluate the influence of a high-sugar diet on *C. albicans* counts in the human gastrointestinal tract. Mouth washes and homogenized fecal samples were chosen as representatives of colonization of the gastrointestinal tract by *C. albicans*. We were able to confirm a correlation between *C. albicans* blastoconidia concentrations in the mouth washes and the stool samples of healthy subjects, indicating that both specimens may reflect the degree of candidal colonization in the gastrointestinal ecosystem. In contrast with our results, Kusne et al (23) found no correlation between the presence or absence of *Candida* spp. in either oral or rectal swabs and colonization at other anatomic sites in 30 randomly selected patients with advanced liver disease. This finding indicates that sampling plays a pivotal role in the assessment of *Candida* colonization.

Our study showed no correlation between oral or fecal *C. albicans* counts and habitual carbohydrate uptake in our subjects, as estimated with a food-frequency questionnaire. Frequent consumption of refined carbohydrates did not promote increased candidal concentrations in the gastrointestinal tract of healthy individuals. Age, sex, and body weight did not influence yeast counts in the gastrointestinal tract.

To evaluate the modulatory effect of excessive carbohydrate intakes on *C. albicans* growth in the human gastrointestinal tract, we dramatically increased the amount of refined carbohydrates consumed by healthy subjects over 7 consecutive days. Glucose is well metabolized by *C. albicans* and a dietary glucose-dependent effect has been shown in various animal models (7, 19). We supplemented the diet with refined carbohydrates, consisting predominantly of glucose and its short-branched oligomers. According to the National Food Consumption Survey (Nationale Verzehrsstudie), a study initiated by the German Federal Ministry of Research and Technology (Bundesministerium für Forschung und Technologie), which enrolled 24632 persons from 11141 randomly selected households (24), the average German citizen aged > 14 y consumes 37 g monosaccharides and 51 g disaccharides daily. On the basis of these data, we increased the subjects’ daily intakes of refined carbohydrates to 225% of their average intake.

There was no striking change in the ratio of candidal species isolated from the feces or from the mouth washes in response to the high-sugar diet. In an earlier study, Larmas et al (25) examined the effect of diets high in sucrose, fructose, and xylitol on human salivary yeast counts. They noticed increased salivary candidal concentrations in the fructose and sucrose groups, but the study failed to differentiate the yeasts by species. Because numerous yeast species contribute to the human orointestinal yeast flora, it is not clear whether Larmas et al’s results reflect an effect on *C. albicans* counts or whether multiplication of a species other than *C. albicans* occurred. A major strength of our study was that all yeasts were differentiated by species and that the dependent variable (*C. albicans* concentration in the human gastrointestinal tract) did not rely on recall or accuracy of a diagnosis, as in the case of *Candida* vulvovaginitis (17).

We observed no significant modulation in the frequency of *C. albicans*–positive fecal and oral samples and no alteration in the number of subjects positive for *C. albicans* in the mouth washes during or after the high-sugar diet. Furthermore, no significant change in the *C. albicans* germ cell concentration in the mouth washes and stool samples for the 28 subjects during or shortly after the diet were detected. These results suggest little modulating effect of dietary carbohydrates on colonization of the

| TABLE 1 |
| Mean logarithmic values of *Candida albicans* in the mouth washes and feces of the subjects before, during, and after a high-sugar diet |

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<th>All subjects</th>
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<td>Mouth washes (n = 28)</td>
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human gastrointestinal tract by *C. albicans*. Therefore, we conclude that healthy individuals do not develop increased *Candida* counts as a result of consuming high-sugar diets. Although glucose is a substrate for *C. albicans*, the complex interplay of the yeast germs with the host and the indigenous bacterial microflora might explain these results. In the gastrointestinal ecosystem, various mucosal defense mechanisms control *C. albicans* germ cell concentrations (26), and numerous bacterial properties and products are known to modulate candidal growth (27, 28).

Monosaccharides such as glucose and degradable oligo- and polysaccharides are absorbed in the small intestine. Therefore, it might be expected that dietary glucose does not affect yeast growth distal to this region. Hypothesizing that an effect of dietary glucose is restricted to yeasts inhabiting the proximal gastrointestinal tract, we speculated that the modulating effect of glucose is more likely to be observed in persons with an elevated candidal colonization of the upper gastrointestinal tract. Indeed, we did observe a significant, although moderate, increase in fecal *C. albicans* counts during the high-sugar-diet period in those subjects whose baseline mouth wash counts were $> 1.0 \times 10^5$ CFU/L. Furthermore, we observed an increased number of subjects with *C. albicans*-positive fecal samples in response to the high-sugar diet. Taken together, these data support the hypothesis that excessive dietary glucose leads to a multiplication of *C. albicans* proximal to the small intestine and that replicated yeasts reach the large intestine with the feces. However, it should be emphasized that the limited effects observed in our study apply to healthy individuals with intact control mechanisms to keep *Candida* counts low. The role of refined carbohydrates in patients with compromised defense mechanisms is still speculative.

Clinical follow-up studies should address the question of whether restriction of refined carbohydrates might decrease the number of *C. albicans* organisms colonizing the human gastrointestinal tract in specific patient groups, e.g., in persons receiving broad-spectrum antibiotics or in heavily colonized, immunosuppressed patients with a high risk of disseminated candidiasis. Because long-term restriction of food groups containing mono- and oligosaccharides seems nutritionally unbalanced, a clear definition of those patients who would benefit from such a diet is necessary.

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REFERENCES