TESTING THE RATIONAL OF CANDIDA CLEANSE DIETS

A thesis presented to the faculty of the Graduate School of Western Carolina University in partial fulfillment of the requirements for the degree of Master of Science in Biology.

By

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ABSTRACT

Testing the Rationale of Candida Cleanse Diets

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Some practitioners of alternative medicine identify overgrowth of the fungus Candida as the cause of eczema, psoriasis, and conditions like fibromyalgia, depression, and chronic migraines. These naturopathic physicians may prescribe diets low in carbohydrates (Candida cleanse diets) to treat such conditions. This study examined the rationale of this diagnosis and treatment by testing for a relationship between the amount of carbohydrates people consume and the presence of Candida in their mouths. It is possible that nutrients other than carbohydrates also affect the growth of Candida. Therefore this study also tested for an association between the oral presence of Candida and the amounts of refined sugars, protein, fat, saturated fat, vitamin A, vitamin C, iron, and calcium consumed. Participants recorded the amounts of food and drink they consumed for three weeks. Daily average amount of each nutrient consumed was calculated for each participant each week. Participants sampled their mouths at the end of each week and inoculated CHROMagar plates (BD Diagnosis Systems) with the samples. The inoculated plates were incubated at 37°C for three days and then examined for Candida growth. The probability of Candida growth was negatively related to consumption of carbohydrate, protein, total fat, and saturated fat during week 1. No other
statistically significant associations were detected. These results refute the rationale behind *Candida* cleanse diets.
INTRODUCTION

Candida Hypersensitivity Syndrome (CHS) describes a variety of ailments/symptoms associated with Candida overgrowth (Crook 1983). According to Barrett (2005), advocates of CHS believe overgrowth of Candida can lead to hyphal penetration of protective mucosal linings of the gastrointestinal, oral, and vaginal cavities enabling a variety of foreign toxins and allergens to enter the blood stream where they can overwhelm the immune system (Truss 1981, Crook 1983, Murray 1997). Some practitioners of alternative medicine blame the overgrowth of C. albicans for superficial infections like vaginitis and oral thrush as well as systemic conditions such as fibromyalgia, depression, autism, and chronic migraine (Fasano 2003, Fife 2005, Edelson 2007). Candida survives by metabolizing sugars. This often prompts holistic physicians to prescribe diets low in or even absent of carbohydrates. These diets are known as Candida cleanse diets (CCD). Proponents of CCD reason that the many conditions they attribute to CHS can be relieved if Candida is reduced (Barrett 2005). They claim this dietary regimen is an effective treatment against the conditions linked to candidiasis and CHS.

The ever-increasing clinical threat presented by the growing immunocompromised population has brought Candida infections to the forefront of medical concern because they often lead to lengthened hospital stays and inflated medical costs (Barrett 2005). The growth of this high-risk group has been caused by a variety of factors including the continued spread of Human Immunodeficiency Virus (HIV) (Crowe et al. 1994, Cheng et al. 2005). As of 2010, an estimated 34 million people worldwide
were living with HIV/AIDS, a dramatic rise over the eight million documented cases in 1990 (Worldwide HIV and AIDS Statistics 2011). Overuse of antibiotics has also contributed to the increasing number of Candida infections (Wang et al. 2014). This is because along with eliminating harmful bacteria antibiotics often alter the balance of beneficial bacteria responsible for keeping Candida in check, particularly in the gastrointestinal tract and genitals. Members of the genera Lactobacillus and Bifidobacterium, many of which are widely used as probiotics, are most commonly associated with maintaining gastrointestinal conditions for proper nutrition (Lidbeck and Nord 1993, Isolauri et al. 2002, Isolauri et al. 2004). Immune compromising drugs are also blamed for increased cases of Candida infections (Louria et al. 1960, Singh 2001). These drugs include corticosteroids (commonly given to treat inflammation), antirejection medications (administered to organ transplant recipients), immuno-suppressants (used to treat autoimmune disorders such as systemic lupus erythematosus and Chrohn’s disease), and a variety of chemotherapeutics used to fight many cancers.

Most members of genus Candida are ubiquitous, having been cultured from soil and water. The most relevant clinical species, C. albicans, prefers conditions found in the human body (Blaschke-Hellmessen 1999, Shin 2001, Yamaguchi et al. 2007). This is primarily due to its affinity to grow at body temperature in a moist environment (Sullivan et al. 1983, Blaschke-Hellmessen 1999). Candida species are the most frequently recovered fungal pathogens that are isolated from clinical samples worldwide, and they are responsible for an estimated 10,000 deaths annually in the United States alone (Musial et al. 1988, Sudbery et al. 2004). Of the over 150 known species, fewer than 20 are pathogenic, the most common of these being C. albicans, followed by C. glabrata, C.
tropicalis, C. parapsilosis, and C. krusei (Pfaller et al. 2005, Lim et al. 2012). Although the incidence of infection by species other than C. albicans has dramatically risen over the past decade, C. albicans is still responsible for 50-66% of candidiasis cases (Sudbery et al. 2004, Lim et al. 2012).

The prevalence of C. albicans may be a result of its unique virulence factors with the most significant being its ability to morphologically switch between yeast and hyphal form (Sudbery et al. 2004). Candida exists in yeast form as single round or ovoid cells, a morphology well suited for dispersal. After dispersing through a person’s body, the fungus is able to switch to a filamentous form that allows it to penetrate protective mucosal linings and invade the tissues below leading to infection of organs (Sudbery et al. 2004). Such filaments may form as true hyphae or pseudohyphae. Other than the more recently classified C. dublineinsis, C. albicans is the only Candida species able to form true hyphae as opposed to just pseudohyphae (Ells et al. 2009). Constricted septa differentiate true hyphae from pseudohyphae (Sudbury et al. 2004). The change from yeast to filamentous form can be triggered by a variety of factors including an increase in temperature, lack of nutrients, a change in pH, and a reduction in O₂ levels (Uwamahor and Traven 2010).

Hazan et al. (2002) demonstrated that hyphae specific genes are activated under conditions typically found in the human body (i.e., 37°C and pH ≈ 7). Among the activated hyphae specific genes are ALS3 and ALS8, which encode for the production of adhesins. Adhesins allow C. albicans to stick to human cells and form biofilms (Ramage et al. 2005). The ability of Candida to form biofilms makes it particularly dangerous to people using catheters and respirators, a characteristic accounting for the dramatic

Traditionally, *Candida* related illnesses and infections have been managed by a class of drugs collectively known as antifungals. *Candida* infections are often challenging to treat because of their persistent nature. Just as antibiotics act by targeting the differences between mammalian and bacterial cells, antifungals act by exploiting the differences between mammalian and fungal cells. Antifungal design has been significantly more challenging than the design of antibiotics because fungi, like mammals, are composed of eukaryotic cells (Dixon and Walsh 1996). Host-pathogen similarity is primarily responsible for many of the adverse side effects brought on by the administration of traditional antifungals. The most common adverse reactions are renal toxicity, altered estrogen production, and several fatal drug interactions (Dixon and Walsh 1996, Dismukes 2000).

To date there are four main classes of antifungals each with their own distinct structure and mechanism of action: the azoles, the polyenes, the allylamines, and the echinocandins (Long 2003). The largest of these classes are the azoles, a group of 5-membered heterocyclic, nitrogen-containing compounds that act by inhibiting a fungal cytochrome P450 enzyme known as 14-α demethylase (MacCallum et al. 2010). The P450 protein is coded for by a gene superfamily known as CYR. The cytochrome P450 superfamily is primarily composed of enzymes able to catalyze the oxidation of organic molecules (Guengerich 1995, Bernhardt 2006). In the case of azoles, 14-α demethylase prevents the demethylation of lanosterol, a precursor of ergosterol, a necessary
component of fungal cell membranes (Andriole 1999). The azoles, polyenes, and allylamines all share a similar mode of action interrupting the synthesis of fungal cell membranes. Their activity is based on the inhibition of different enzymes required for the synthesis of ergosterol, a fundamental component of fungal cell membranes (Ryder 1987, Dixon and Walsh 1996, Hospenthal and Rinaldi 2008, MacCallum et al. 2010). This general mechanism of action prevents proper fungal cell membrane formation, thereby inhibiting Candida cell proliferation. Although effective, these drugs have been associated with dangerous side effects including many drug interactions as well as renal and hepatic toxicity (Ashley et al. 2006). In contrast, some drugs belonging to these classes such as Monistat, Gyne-Lotrimin, Lamisil, and Lotrimin Ultra are available over the counter. Butenafine, the most commonly used allylamine, is distinguished from other members of its class by superior activity against C. albicans (Ryder 1987). The increasing incidence of resistance to these antifungals suggests alternative treatments are needed (Pfaller 2012).

Unlike the previously mentioned drugs, echinocandins possess a novel mechanism of action and are therefore commonly referred to as the “penicillin of antifungals” (Long 2003). They act by noncompetitively inhibiting 1, 3-β-glucan synthase, an enzyme in the cell membrane responsible for producing the glucan polymers required for proper cell wall construction (Morris and Villmann 2006). This damage ultimately leads to fungal cell death. Another distinguishing characteristic of the echinocandins is their mode of administration. They are administered intravenously because they are poorly absorbed when taken orally. Currently approved echinocandins
include micafungin, anidulafungin, and caspofungin, the therapy of choice for treating systemic Candida infections (Morris and Villmann 2006).

**Details of Candida cleanse diets**

The recent increase in Candida infections has fueled interest in Candida cleanse diets (CCD), especially among corporations marketing naturopathic products. The rationale behind CCD is simple. Sugars are the primary energy source for Candida spp. Therefore, removing sugars from a patient’s diet should, in effect, “starve” the invading yeast and eventually end the symptoms brought on by its overgrowth (Crook 1983, Truss 1983, Murray 1997). There are a number of drawbacks to this approach with the most significant being how difficult the strict regimen is to maintain. Furthermore, there is little scientific evidence that it is effective.

CCDs stress that patients completely refrain from simple sugars. Simple sugars include both monosaccharides and disaccharides. Commonly ingested monosaccharides include glucose, fructose, and galactose. They are usually consumed as the disaccharides sucrose (glucose–fructose), lactose (glucose–galactose), and maltose (glucose–glucose). Although limiting these sugars is beneficial (Martin and Rona 2000), their complete restriction is highly impractical, requiring the elimination of breads, many fruits and vegetables, and all dairy products. Mold-containing foods and foods commonly thought to cause allergies are also prohibited by CCD (Crook 1983, Murray 1997, Martin and Rona 2000). In addition to refraining from foods containing refined sugar, people adhering to CCD must also be wary of white bread, alcohol, mushrooms, squash, yams, potatoes, parsnips, corn, peanuts, cashews, dairy products, figs, melons, honey, and raisins (Murray 1997). Length of diets vary, but are typically at least 60 days.
Proponents of CCD also advocate limiting the use of antibiotics, avoiding estrogen and progesterone based contraceptives, and consuming commercially available probiotics (Murray 1997, Donaldson 2004, Ignacio and Thai 2005). Less regimented plans are available (Alter 2010), however, as with the traditional *Candida* cleanse diet, their efficacy has yet to be demonstrated. There have been very few reliable experiments investigating the clinical value of CCD (Barrett 2005). Those that have been published have provided little real evidence that the *Candida* cleanse diet offers tangible relief from the conditions blamed on CHS (Barrett 2005). Nevertheless, increasing practice of holistic medicine has created renewed interest in CCD.

**Nutrition requirements of *Candida***

Like other organisms, *C. albicans* derives its energy from the breakdown of carbohydrates into their simpler constituent sugar molecules. Refined sugars are purified or nearly purified simple sugars (i.e., purified monosaccharides or purified disaccharides). Monosaccharides require no digestion, which means they are absorbed almost immediately by *Candida* or us, depending on which organisms’ absorptive surface contacts the sugar first. Disaccharides require only one chemical bond to be broken during digestion, so they too are absorbed almost immediately and easily metabolized. For this reason all variations of *Candida* cleanse diets include complete restriction of refined or simple sugars. Although carbohydrates are the focus of *Candida* cleanse diets, *Candida* requires other nutrients. These include protein and fat. Proteins provide the amino acids *Candida* uses to assemble proteins and nitrogen for other molecules. Fats provide fatty acids for energy and the lipid components *Candida* uses to build its own cell membranes and organelle membranes.
Vitamins A and C are both known to contribute to proper immune function and have both been shown to decrease growth of *C. albicans*. Large doses of vitamin A induce nonspecific resistance to *C. albicans* infection in mice (Cohen and Elin 1974). Likewise, vitamin A deficiency contributes to the presence and proliferation of *C. albicans* (McClary 1952). Vitamin C also discourages *C. albicans* overgrowth. This effect may be caused by vitamin C lowering stomach pH because alkalinity has been shown to encourage the hyphal switch responsible for *C. albicans* pathogenicity (Walther and Wendland 2008). These studies suggest that participants who consumed more vitamin A and vitamin C are less likely to test positive for *C. albicans*.

Iron is required for the proliferation of *C. albicans*, functioning as an obligate enzymatic cofactor in a number of biological processes including DNA replication and electron transport (Elin and Wilff 1973 and Askwith and Kaplan 1998). Individuals taking iron supplements to treat anemia and related disorders have an increased risk of developing *Candida* infections (Nevitt 2011). Furthermore, iron depletion makes the cell membranes of *Candida* more permeable to antifungal drugs (Prasad et al. 2006). Calcium plays a critical role in morphogenesis of *C. albicans* by inhibiting mycelial growth (Holmes et al. 1991). Studies suggest that this may be the result of a disruption in calcium-dependent directional growth (Brand et al. 2007). These studies suggest that vitamin A, vitamin C, iron, and calcium may also affect *C. albicans* proliferation.

**Study objectives**

This study examined the rationale of *Candida* cleanse diets by testing for an association between the amount of total carbohydrate, refined sugar, protein, fat, saturated fat, vitamin A, vitamin C, iron and calcium in people’s diets and the growth of
Candida albicans within the people. A positive association with total carbohydrate or refined sugar would indicate that the rationale of Candida cleanse diets is valid, whereas a negative association or no association would indicate that the rationale underlying this dietary treatment is incorrect. In addition, this study will test for an association between the amount of protein, fat, saturated fat, vitamin A, vitamin C, iron and calcium people consume and the growth of Candida albicans within them. Evidence demonstrating the efficacy of CCD may provide the medical community with a more effective, less toxic option for combating candidiasis, a particularly persistent and widespread condition. Additional nutrient associations may provide alternative avenues of treatment.
METHODS AND MATERIALS

Collection of data

Participants in the study were volunteers from a biology course at Western Carolina University during the 2011 fall semester (Western Carolina University IRB 2012-021). Each participant was asked to record what they consumed each day for three consecutive weeks. These records included estimated amounts of each item by volume or unit (e.g., 1 cup of cereal, ½ cup 2% milk, 1 stick of gum). Participants sampled the inside of their mouths with sterile cotton swabs once each week and then inoculated “CHROMagar Candida” plates (BD Diagnostic Systems) using aseptic technique. The growth medium contained in CHROMagar Candida plates exclusively selects for Candida species (e.g., C. albicans, C. glabrata, C. tropicalis, C. krusei) and prevents the growth of other organisms (Bouchara et al. 1996, Willinger and Manafi 1999). After sampling the inside of his or her mouth with a sterile cotton swab, each participant inoculated a CHROMagar plate using a zig-zag pattern. Plates were placed into a 37°C incubator within 15 minutes of inoculation. All plates were observed for growth every 24 hours for three days.

Each participant’s plates and corresponding dietary records were identified with a randomly assigned number. The total amount of carbohydrate, refined sugar, protein, fat, saturated fat, vitamin A, vitamin C, Fe and Ca consumed each day by each participant was determined from the USDA’s “MyPlate” website (choosemyplate.gov) and similar nutrition websites (See Appendix 1). In many cases nutritional data were readily available, particularly for food items available at commercial/chain restaurants (e.g.
McDonalds, Chik-fil-a, Starbucks, etc.). In these cases, nutrient quantity consumed was calculated by multiplying or dividing the amount of nutrient per serving by the number of servings or the fraction of a serving consumed. Determining the nutritional data for other items, specifically food prepared by the participants themselves, required more thorough investigation. This required breaking down certain items into their ingredients, locating the nutritional data for each ingredient, and then combining the individual data to calculate the items’ total nutrient content.

The amount of carbohydrate, refined sugar, protein, total fat, and saturated fat contained in a food item was consistently reported, recorded, and analyzed as mass using the metric unit milligram. Nutritional labels and websites reported values for vitamin A, vitamin C, Fe, and Ca as the percentage daily value (%DV) of each nutrient. Recommended daily allowances (RDA) or daily values (DV) of vitamins and minerals are set by the Food and Drug Administration (FDA) and are based on daily recommendations for a 2000 calorie per day diet. A nutrient’s %DV is the percentage of that nutrient’s RDA or DV a food or beverage contains. According to www.fda.gov, the RDA of vitamin C, vitamin A, Ca, and Fe is 60 mg, 5000 International Units (IU), 1000 mg, and 18 mg respectively. The nutritional supplement database, sponsored by the National Institute of Health (NIH), reports 1 IU of vitamin A is equal to approximately 0.33 µg meaning the %DV for vitamin A is approximately 15mg. Because vitamin A, vitamin C, Fe, and Ca data are consistently reported as %DV this study also recorded and analyzed each nutrient using %DV.

Each individual’s average daily nutrient intake was determined for weeks 1, 2, and 3 then analyzed for its association with Candida growth. Total carbohydrate, refined
sugar, protein, fat, saturated fat, vitamin A, vitamin C, Fe, and Ca intake was summed for each combination of individual, week, and day, which yielded each individual’s daily total intake of each measured nutrient. Next the mean of the daily total intake was determined generating the average daily total of each measured nutrient for each combination of individual and week.

Data analysis

Not all of the 116 participants kept dietary records of sufficient quality to be used. For example, too little information about ingredients and quantities in some records prevented accurate collection of nutritional information. Thus, sample size differed from week to week: 50 during week 1, 46 during week 2, 84 during week 3.

The effect of carbohydrate consumption on the presence of Candida was examined with logistic regression using the “glm” function in R version 3.0.1 (R Core Team 2013). Logistic regression yielded a measure of how likely the presence of Candida is for every 1g increase in carbohydrate consumption. Specifically, it quantified the log odds of Candida being present for every 1g increase in carbohydrate consumption. Odds is related to probability by the following function: odds = probability / (1–probability). The same approach was used to test for an association between presence of Candida and consumption of each measured nutrient, respectively: refined sugar, protein, total fat, saturated fat, vitamin A, vitamin C, Fe, and Ca. The “glm” function in R also calculated Wald’s z to determine p-values associated with coefficients for each logistic regression.

Scatterplots showing observed data (circles) in combination with results of the logistic regressions (regression curve and 95% confidence interval) were produced with
the “ggplot” package (Wickham 2009) in R version 3.0.1 (R Core Team 2013). The estimated curves and 95% confidence intervals in the scatterplots were produced with R’s “glm” smoothing function. Data from week 1 were examined independently of data from week 2 and data from week 3. Data from week 2 and data from week 3 were also examined independently from one another. This approach, rather than a repeated measures design, was taken because participants varied somewhat week to week and because each week represented a partly-independent replicate of the survey.
RESULTS

Association between *Candida* growth and carbohydrate consumption

The association between carbohydrate consumption and the presence of *Candida* differed from week to week. For example, the probability of *Candida* growth among participants declined as carbohydrate consumption increased (b = −0.02) during week 1 (Figure 1). Specifically, the result from week one shows that the log odds of *Candida* being present in a person decreases by 0.02 for every 1g increase in carbohydrate consumption. This result is opposite the pattern expected if the rationale of the *Candida* cleanse diet is valid.

There was practically no change in the log odds of *Candida* presence with carbohydrate consumption during week 2 among the experiment’s participants (b = 0.0001, Figure 1). Week 3’s results indicated a slight increase in the log odds of *Candida* presence with carbohydrate consumption (b = 0.002, Figure 1), but as with the result from week 2, the probability of detecting associations of b = 0.0001 (week 2) and b = 0.002 (week 3) among the experiment’s participants even if the association does not exist in the populations represented by the samples is quite large (p = 0.98 for week 2; p = 0.5 for week 3). In other words, these weak positive trends in week 2 and week 3 probably don’t exist in the population that was sampled. Statistically speaking, these trends are not significant.

The log odds of *Candida* growth declined with consumption of refined sugar during week 1 (Figure 2). Slightly positive associations were observed in week 2 (b = 0.005) and week 3 (b = 0.004). However, the p-values associated with these slight
positive associations are quite large (p = 0.6 and p = 0.4), indicating that one cannot confidently conclude the trends exist in the overall population from which the participants were sampled.

**Association between Candida growth and consumption of other nutrients**

The log odds of Candida growth were negatively associated with protein consumption all three weeks (Figure 3). The associations were quite weak (b = −0.04 and smaller), and only week 1’s trend can be confidently viewed as likely existing in the population represented by the sample (p = 0.03).

The log odds of Candida growth among participants slightly decreased with total fat consumption during weeks 1 and 2 (Figure 4; b = −0.05 and b = −0.02, respectively). A very slight positive association was detected among week 3’s participants (Figure 4), but the trend probably doesn’t exist in the population represented by the sample (p = 0.9).

The log odds of Candida growth among participants decreased with saturated fat consumption during weeks 1 and 2 (Figure 5, b = −0.09 and b = −0.01, respectively). A negligible positive association was detected among participants during week 3 (Figure 5), however, week 3’s trend and week 2’s trend probably do not exist in the population represented by the sample (p = 0.9 and p = 0.5, respectively).

The log odds of Candida growth was negatively associated with %DV Vitamin A consumption for all three weeks (Figure 6). The associations were negligible (Figure 6; b = −0.03, b = −0.02, and b = −0.005, respectively) and probably not true of the population being sampled (Figure 6; p = 0.1, p = 0.4, and p = 0.4, respectively).

There was very slight positive association observed between the log odds of Candida growth and consumption of vitamin C among study participants throughout
weeks 1, 2, or 3 of the study (Figure 7; b = 0.002, b = 0.005, and b = 0.002, respectively). These slight trends probably do not exist in the population represented by the sample (Figure 7; p = 0.3 or greater).

Results show a slight negative association between Ca consumption and the log odds of *Candida* growth among study participants during week 1 (Figure 8; b = –0.01). However the magnitude of the associated p-value suggests this association is not true for the population (Figure 8; p = 0.2). Slightly positive associations were detected in week 2 (b = 0.007) and week 3 (b = 0.001). However, these small trends detected in the sample probably are not indicative of trends in the population because the p-values related to the associations are very large (Figure 8; p = 0.6 and p = 0.9, respectively).

A weak negative association between the log odds of *Candida* growth and Fe intake was apparent during week 1, whereas a slight positive association was seen during week 2 (Figure 9; b = –0.02, b = 0.01). Practically no association was detected in week 3 of the study (b = 0.001). The associated p-values were too large to conclude that these trends exist in the sampled population (Figure 9; p = 0.2, p = 0.6, and p = 0.9, respectively).
Figure 1. Association between *Candida* growth and carbohydrate consumption. Observed growth of *Candida* (open circles) and estimated probability of *Candida* growth (dashed curve) as a function of mean daily carbohydrate consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of *Candida* growth. Regression coefficient is identified as “b.” It expresses change in the log odds of *Candida* growth per 1g increase in carbohydrate consumption.
Figure 2. Association between Candida growth and refined sugar consumption. Observed growth of Candida (open circles) and estimated probability of Candida growth (dashed curve) as a function of mean daily refined sugar consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of Candida growth. Regression coefficient is identified as “b.” It expresses change in the log odds of Candida growth per 1g increase in refined sugar consumption.
Figure 3. Association between *Candida* growth and protein consumption. Observed growth of *Candida* (open circles) and estimated probability of *Candida* growth (dashed curve) as a function of mean daily protein consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of *Candida* growth. Regression coefficient is identified as “b.” It expresses change in the log odds of *Candida* growth per 1g increase in protein consumption.
Figure 4. Association between *Candida* growth and total fat consumption. Observed growth of *Candida* (open circles) and estimated probability of *Candida* growth (dashed curve) as a function of mean daily total fat consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of *Candida* growth. Regression coefficient is identified as “b.” It expresses change in the log odds of *Candida* growth per 1g increase in total fat consumption.
Figure 5. Association between *Candida* growth and saturated fat consumption. Observed growth of *Candida* (open circles) and estimated probability of *Candida* growth (dashed curve) as a function of mean daily saturated fat consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of *Candida* growth. Regression coefficient is identified as “b.” It expresses change in the log odds of *Candida* growth per 1g increase in saturated fat consumption.
Figure 6. Association between Candida growth and %DV vitamin A consumption. Observed growth of Candida (open circles) and estimated probability of Candida growth (dashed curve) as a function of mean daily vitamin A consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of Candida growth. Three values have been omitted from the graphs (not the analyses) to better show the majority of observations. Three values have been omitted from the graphs (not the analyses) to better show the majority of observations. Week 1: (0, 224). Week 3: (0, 249) and (0, 294).
Figure 7. Association between *Candida* growth and %DV vitamin C consumption. Observed growth of *Candida* (open circles) and estimated probability of *Candida* growth (dashed curve) as a function of mean daily vitamin C consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of *Candida* growth. Two values have been omitted from the graphs (not the analyses) to better show the majority of observations. Week 3: (0, 987) and (1, 1061).
Figure 8. Association between *Candida* growth and calcium consumption. Observed growth of *Candida* (open circles) and estimated probability of *Candida* growth (dashed curve) as a function of mean daily calcium consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of *Candida* growth. One value has been omitted from the graphs (not the analyses) to better show the majority of observations.

Week 3: (0, 341).

\[
\begin{align*}
\text{week 1} & : & b = -0.01 & \quad z = -1.2 & \quad p = 0.2 \\
\text{week 2} & : & b = 0.007 & \quad z = 0.5 & \quad p = 0.6 \\
\text{week 3} & : & b = 0.001 & \quad z = 0.11 & \quad p = 0.9
\end{align*}
\]
Figure 9. Association between *Candida* growth and iron consumption. Observed growth of *Candida* (open circles) and estimated probability of *Candida* growth (dashed curve) as a function of mean daily iron consumption during three consecutive weeks of measurement. Shaded area in each graph is the 95% confidence interval associated with the estimated probability of *Candida* growth. Two values have been omitted from the graphs (not the analyses) to better show the majority of observations. Week 1: (0, 207). Week 3: (0, 142).
DISCUSSION

The goal of this study was to examine the rationale of the Candida cleanse diet (CCD) by testing if there is a positive association between carbohydrate intake and Candida growth as claimed by proponents of the diet. The results of this study showed no positive association between the amount of carbohydrates an individual consumes and the presence of Candida in their mouths, suggesting the rationale behind the Candida cleanse diet is flawed. A limited number of studies have reported varying and often conflicting results, providing little scientific evidence for the rationale of the CCD (Vargas et al. 1993, Russell and Jones 1973). Conversely, there is an abundance of anecdotal evidence claiming the Candida cleanse diet effective in spite of a lack of scientific evidence. The discussion that follows will offer a possible alternative explanation for reported success of the CCD, namely that people on the diet tend to eat healthy foods that boost their immunity and maintain a healthy community of symbiotic microorganisms. It will also examine the week-to-week variability in experimental participants with Candida growth. Finally, the discussion that follows will offer some explanation for the differing frequencies of Candida congeners detected among the experiment’s participants.

Could healthier food explain positive effects of Candida cleanse diets?

Regardless of whether Candida cleanse diets are effective, the rationale claimed by the diet’s proponents is apparently wrong. Proponents claim the diet reduces Candida’s growth and abundance by depriving Candida of carbohydrates, refined sugar in particular. Results of the experiment reported herein clearly refute that claim. Perhaps
the diet is effective simply because people on the diet eat healthier food. After all, reducing carbohydrates typically means reducing consumption of processed foods, many of which are rich in simple carbohydrates. Eliminating processed foods from one’s diet leaves one eating whole foods. Eating whole foods does not eliminate carbohydrates or even simple carbohydrates such as disaccharides and mono-saccharides from one’s diet, but the carbohydrates in whole fruits and whole vegetables are typically found in combination with lipids, proteins, and vitamins. The balance of these nutrients likely improves immune function, which is likely the reason *Candida* cleanse diets are reportedly effective. A healthy diet, as explained below, improves immune response and helps maintain an internal microbial community that is better able to competitively exclude pathogens such as *Candida*.

Foods containing refined ingredients are poor in micronutrients vital to proper immune development (Rowbotham 2008). Proper nutrition containing a sufficient supply of micronutrients is responsible for numerous and varied immune mechanisms necessary to successfully combat invading pathogens (Calder and Jackson 2000). The interaction of various micronutrients found in a diet of whole foods maintains immune cell activity such as ensuring proper phagocytic activity by macrophages (Calder and Jackson 2000). People on *Candida* cleanse diets probably get these micronutrients because they’re likely eating whole foods rather than processed foods.

A balanced diet includes a proper balance of all food groups including a variety of fruits and vegetables which contain phytochemicals. Phytochemicals are chemical compounds found in fresh fruits and vegetables most processed foods lack. These compounds (e.g., phenolics, carotenoids) act as antioxidants, reducing damage done by
reactive oxygen species (Esfahani et al. 2011). Reactive oxygen species are produced during the course of infection to combat invading pathogens but can also cause damage to host tissue in large quantities (Nathan and Shiloh 2000). Such tissue damage may allow *Candida* to penetrate the mucous membranes leading to systemic infection, therefore the antioxidant properties of phytochemicals play a vital role in a healthy immune system. CCD advocates claim penetration of organs underlying the intestinal mucosa may be responsible for many *Candida* associated symptoms (Truss 1981).

Rats administered antioxidant supplements and subsequently irradiated to induce intestinal tissue damage had less damage and healed more quickly than control rats not given the supplements (Anwar et al. 2013). Also using a murine model researchers found that grape seed oil, a source rich in flavonoids, a ubiquitous polyphenol, is effective at protecting the mucosal lining of the gastrointestinal system from reactive oxygen species generated by stress (Manashi et al. 1999). The antioxidants we get by eating fruits and vegetables may reduce tissue damage from oxidation, and thereby protect against *Candida* infection.

People with a diet rich in carotenoids, a type of phytochemical, are more resistant to infection and have a lower mortality than counterparts lacking adequate carotenoid consumption (Diplock et al. 1998). Carotenoids are phytochemicals primarily assembled from catabolized fat molecules. The carotenoid β-carotene is a precursor to vitamin A meaning their consumption should lead to increased vitamin A thereby boosting the immune system and controlling *Candida* growth.

Erikson et al. (2007) has extensively reviewed the positive relationship between a diet containing the correct balance of vitamins including A, B6, B12, C, and D, with
healthy immune function. For example, neutrophil development is modulated by vitamin A (Twining et al. 1997). Furthermore, vitamin A shortage leads to a defect in neutrophils’ phagocytic capabilities despite their increased numbers in the bloodstream. This defect renders the immune cells relatively useless in the case of microbial invasion (Ongsakul et al. 1985). Although this study found a consistent, weak negative association between vitamin A consumption and Candida growth other studies indicate vitamin A plays a substantial role in proper immune function.

Vitamin C also plays a role in immune function and thus our defense against pathogens such as Candida. Studies have linked Vitamin C consumption to the production of T lymphocytes as well as their phagocytic capabilities (Jariwalla and Harakeh 1996, Hemila 1997). Although my results show a slight positive association between vitamin C consumption and Candida growth during all three weeks of the study, the magnitude of the association does not show the strong positive trend suggested by the literature.

Experiments reviewed by Wichers (2009) show adequate protein intake is related to proper functioning of innate immunity in mice, particularly the activity of T-cells (Mainali and McMurray 1998). However, restoring protein shortage is able to return immune function to usual levels (Wichers 2009). Protein plays a variety of roles in protecting the body from Candida and other invaders. The mucous membrane lining of the intestinal tract is impaired by protein deprivation (Deitch et al. 1990) allowing for the dissemination of Candida throughout the body. In addition protein malnutrition is associated with impaired lymphoid organs and decreased circulating lymphocytes (Woodward and Miller 1991). Calprotectin, a protein found in the healthy immune cells
granulocytes, leukocytes, and macrophages, is effective against *C. albicans* as well as other *Candida* species (Müller et al. 1993, Sohle et al. 1991, Steinbakk et al. 1990).

Deficiency of minerals, including iron, calcium and zinc, are well documented to impair immune function in experimental animals, and to the extent studied, in humans as well (Keusch 2003). Iron is important to the function of both the adaptive and innate immune mechanisms. More specifically iron is required for the timely development of cytotoxic effector T lymphocytes (Santos and Falcao 1990). Iron deficiency has been associated with impaired phagocytic activity toward fungi, including *Candida* (Higgs and Wells 1972). Calcium is also involved in proper T-cell maturation. Stimulation of T-cell receptors triggers massive Ca\(^{2+}\) influx into T-cells activating calcineurin, a phosphatase which activates the expression of genes involved in T-cell development (Crabtree and Olson 2002). The negligible associations between *Candida* growth and both iron and calcium may indicate that consumption of these minerals by most participants exceeded the minimum required by *Candida*.

A possible connection among nutrition, inflammation, and symptoms reportedly treated by CCD suggests that alleviation of symptoms by *Candida* cleanse diets is merely the result of a better functioning immune system associated, not with reduced carbohydrate, per se, but instead with simply eating healthier foods that enable the body to better maintain a healthy balance of intestinal microbes.

**Week-to-week variability**

Most associations between *Candida* growth and each nutrient varied slightly from week to week (Figures 1-9). Some nutrients shifted from a positive to negative association or the reverse, changing from week to week, whereas others maintained a
consistent positive or negative association. Responses for both carbohydrates and refined sugars varied in a similar manner, which makes sense because refined sugars are carbohydrates. Both had a strong negative association with Candida growth during the first week, and both had a slight positive association with Candida growth in weeks 2 and 3 (Figures 1 and 2). Likewise, trends in total fat were similar to those for saturated fat. Both had negative associations with Candida growth during weeks 1 and 2 and slight positive associations with Candida growth during week 3 (Figures 4 and 5). Protein, vitamin A, and vitamin C were the only measured nutrients for which results were consistent week to week. Protein had a relatively strong negative association with Candida growth during week 1. The negative trend was small in week 2 and negligible in week 3 for the study’s participants. Vitamin A consumption was consistently negatively associated with growth of Candida (Figure 6). Although the result is valid only for the observed samples and probably not the populations from which they were drawn, this trend makes sense because vitamin A is associated with immune function. In contrast, vitamin C intake was positively associated with Candida growth for the duration of the study (Figure 7). These results for vitamin A and vitamin C suggest that vitamin A might play a marginal role in controlling Candida growth in the study’s participants, whereas vitamin C might slightly facilitate Candida growth in the study’s participants. Both Ca and Fe were negatively associated with Candida growth during week 1, but both were slightly positively associated with growth during weeks 2 and 3 (Figures 8 and 9).

One explanation for these week-to-week discrepancies may be that participants maintained more accurate dietary records as the study progressed. Perhaps week 1’s results are anomalies associated with participants learning to keep track of their diets.
Participants initially failed to consider all ingredients in foods that contained many ingredients. Participants also had to learn how to judge the amount of each food item they ate. Consequently, accuracy probably increased from week to week. It is also possible that participants became better at sampling their mouths and inoculating CHROMagar plates.

**Conclusion**

The results of this study suggest the rationale of *Candida* cleanse diets is flawed. CCD advocates claim that a range of physical and mental ailments can be cured by eliminating *Candida* from one’s system, and they claim elimination of *Candida* can be accomplished by reducing carbohydrate consumption, particularly the elimination of refined sugars from one’s diet. This rationale predicts a positive association between the growth of *Candida* and the amount of carbohydrate consumed. However, consumption of carbohydrates, including refined sugar, appear to have either a negative association or no association with growth of *Candida*. Only a strongly negative association and negligibly weak positive associations were detected in this study.

Studies addressing the effect of these nutrients on *Candida* growth are sparse. However, many personal accounts of symptom improvement exist, suggesting *Candida* cleanse diets effectively reduce growth of *Candida*. These anecdotal reports of CCD efficacy apparently reflect an effect unrelated to carbohydrate restriction. Reported improvements are possibly a consequence of better immune function caused by the consumption of a healthier diet, particularly one higher in vegetables and unprocessed foods. Such a diet provides the essential vitamins, phytochemicals, and minerals that are often lacking in an unbalance diet. All of these factors have been shown to contribute to
a fully functional immune system. These observations lead to the conclusion that although the rationale underlying *Candida* cleanse diets is flawed, the diet may be effective simply because people on the diet likely become more conscientious about their food, reduce their consumption of processed foods, and consequently eat healthier foods that strengthen their immune systems and help maintain a balanced microbial community that can competitively exclude *Candida*. 
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   Microbioloogy 43(12):5848–5859.


Appendix 1. These websites were used to determine the nutritional value of a variety of items, especially those not available from manufacturer website or ingredients used in homemade meals. The remaining sites were used for more than one variety or type of item.

http://www.caloriecount.about.com

http://www.nutritiondata.self.com

http://www.fatsecret.com

http://www.enutrition.sysco.com

http://www.sparkpeople.com

http://www.myfitnesspal.com/nutrition-facts-calories

http://www.pepsico.com

http://www.productnutrition.thecoca-colacompany.com

http://www.drpepper.com/text/products/drpepper/nutrition

http://www.chick-fil-a.com/food/meal


https://pandaexpress.com/NutritionCalculator

http://www.subway.com/nutrition/nutritionlist.aspx


http://order.papajohns.com/nutrition.html

http://www.tacobell.com/nutrition

http://arbys.com/build-a-meal

http://www.ryans.com/menus/nutritional-information
Appendix 1 (cont.)

https://order.dominos.com/en/pages/content/nutritional/nutrition.jsp

http://www.applebees.com/~/media/docs/Applebees_Nutritional_Info.pdf

http://www.zaxbys.com/menu_nutrition/nutritional_information.aspx

http://www.einsteinbros.com/nutrition/calculator

http://www.starbucks.com/menu/nutrition

http://www.javacity.com/beverages_nutritionals.php

http://www.freshens.com

Appendix 2. A selection of sources describing *Candida* cleanse diets.

**Websites describing *Candida* cleanse diets**

http://naturalcandidacleansing.com

http://www.thecandidadiet.com

http://www.doctoroz.com/blog/jacob-teitelbaum-md/candida-eliminating-yeastfungal overgrowth

http://www.everydiet.org/diet/candida-diet

http://www.candidaplan.com/new/the_plan (Dr. Jeff McCombs)

http://www.naturalcandidacleansing.com/candidadietgoodfoods.html

http://www.everydiet.org/diet/candida-diet (Mizpah Matus B.Hlth.Sc)

http://www.bodyecology.com


http://www.raysahelian.com/candida

http://www.candidacleanser.com

http://www.candida-cure-recipes.com

http://www.candidasupport.org

http://www.guidetobodycleansing.com/candidacleansingdiet

http://www.wholeapproach.com/diet
Appendix 2 (cont.)

**Books describing Candida cleanse diets**


Reese, Michael E. The *Candida* Cleanse Diet: How To Cure *Candida* 12 Weeks with a Natural *Candida* Diet.


Jackson, Natalie. The *Candida* Cure: A Simple, Easy to Follow 5 Step *Candida* Diet Solution Guide.


Appendix 2 (cont.)

Supplements recommended by Candida cleanse diets

10 Day Candida Cleanse. (Dherbs)

Candida Cleanse. (CandidaPro)

Candida Cleanse and Digestion Formula102. (Kyolic)

Candida Cleanse - 7 Day Program Kit. (Nature's Plus)

Candida Cleanse Complex - All-in-One Yeast Infection Treatment. (Candida Cleanse Complex)

Candida Support Complex. (Vitaana Health)

Candida Quick Cleanse. (Zand)

Candistroy. (Nature's Secret)

Now Foods Candida Support Formula (Now Foods)

Original Parasite Cleanse and Candida Cleanse Kit. (OneLifeUSA)

Renew Life CandiGONE (Renew Life). Ultimate Candida Cleanse Complex. (Natural Goal)

Yeast Cleanse. (Solaray)
Appendix 3. Dietary record of one of the 116 participants.

<table>
<thead>
<tr>
<th>Day</th>
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<th>Calories</th>
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