Manipulation of Host Diet To Reduce Gastrointestinal Colonization by the Opportunistic Pathogen Candida albicans

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ABSTRACT Candida albicans, the most common human fungal pathogen, can cause systemic infections with a mortality rate of ~40%. Infections arise from colonization of the gastrointestinal (GI) tract, where C. albicans is part of the normal microbiota. Reducing colonization in at-risk patients using antifungal drugs prevents C. albicans-associated mortalities. C. albicans provides a clinically relevant system for studying the relationship between diet and the microbiota as it relates to commensalism and pathogenicity. As a first step toward a dietary intervention to reduce C. albicans GI colonization, we investigated the impact of dietary lipids on murine colonization by C. albicans. Coconut oil and its constituent fatty acids have antifungal activity in vitro; we hypothesized that dietary coconut oil would reduce GI colonization by C. albicans. Colonization was lower in mice fed a coconut oil-rich diet than in mice fed diets rich in beef tallow or soybean oil. Switching beef tallow-fed mice to a coconut oil diet reduced preexisting colonization. Coconut oil reduced colonization even when the diet also contained beef tallow. Dietary coconut oil also altered the metabolic program of colonizing C. albicans cells. Long-chain fatty acids were less abundant in the cecal contents of coconut oil-fed mice than in the cecal contents of beef tallow-fed mice; the expression of genes involved in fatty acid utilization was lower in C. albicans from coconut oil-fed mice than in C. albicans from beef tallow-fed mice. Extrapolating to humans, these findings suggest that coconut oil could become the first dietary intervention to reduce C. albicans GI colonization.

IMPORTANCE Candida albicans, the most common human fungal pathogen, can cause infections with a mortality rate of ~40%. C. albicans is part of the normal gut flora, but when a patient’s immune system is compromised, it can leave the gut and cause infections. By reducing the amount of C. albicans in the gut of susceptible patients, infections (and the resulting fatalities) can be prevented. Currently, this is done using antimicrobial drugs; to “preserve” drugs for treating infections, we looked for a dietary change to reduce the amount of C. albicans in the gut. Using a mouse model, we showed that adding coconut oil to the diet could become the first drug-free way to reduce C. albicans in the gut. More broadly, this model lets us study the interactions between our diet and the microbes in our body and the reasons why some of those microbes, under certain conditions, cause disease.

KEYWORDS: microbiome, commensal, pathogenesis, carbon metabolism, Candida, Candida albicans, host-pathogen interactions, medium-chain fatty acids, fatty acids
Candida albicans, a member of the endogenous human microflora, is the most common human fungal pathogen. While in most healthy individuals C. albicans is a harmless commensal colonizing the skin and gastrointestinal (GI) tract, when its growth advances unchecked, C. albicans can cause superficial mucosal candidiasis, such as oral thrush and vaginal yeast infections. Particularly in immunocompromised patients, C. albicans can enter the bloodstream and cause invasive or disseminated candidiasis, affecting internal organs such as the kidneys, liver, spleen, lungs, brain, and heart valves. Disseminated candidiasis is difficult to diagnose and treat; although estimates of attributable mortality vary greatly, in a large case-control study, Gudlaugsson et al. reported a candidemia-attributable mortality rate of 49% (1). It has thus been proposed that the most effective way to reduce candidemia-associated mortalities is to prevent infections from occurring (2).

Current evidence suggests that C. albicans infections most often arise from colonization of the patient’s own gastrointestinal tract; colonization can then spread to multiple sites in the body, which is an independent risk factor for the development of systemic infection (3). The incidence of invasive disease can therefore be reduced by decreasing colonization in patients at risk of developing infections by using antifungal prophylaxis; this has been shown to reduce mucosal and invasive candidiasis (4–14) and Candida-associated mortalities (4, 15–18). However, the use of antifungal drugs leads to the emergence of antifungal-resistant strains (19–22). Given that the Centers for Disease Control and Prevention have classified fluconazole-resistant Candida as a “serious threat” (23), it is clear that alternative methods of reducing C. albicans colonization are needed. It is well established that changes in diet, such as switching to a high-fat diet, can alter the gastrointestinal microflora (24–28; reviewed in references 29 and 30), but the effects of diet on C. albicans colonization have not been extensively studied.

The goal of this study was to test the effect of a dietary intervention on GI colonization with C. albicans. Coconut oil is a natural product that has been extensively studied; its chemical composition is known (see Fig. 1), and in addition to its long history of use as a dietary fat, there are decades of research showing that coconut oil and the fatty acids that it contains are safe and well tolerated when ingested or applied topically, in both animals and humans. Coconut oil and its constituent fatty acids, particularly decanoic (10:0) and dodecanoic (12:0) acids, have been shown to both inhibit the growth of and kill C. albicans in vitro (31–33). In contrast, C. albicans can grow using long-chain fatty acids (LCFAs), such as those found in beef tallow and soybean oil, as a sole carbon source; for instance, the growth of C. albicans on oleate (18:1) has been extensively studied (34–38; reviewed in reference 39). We hypothesized that dietary coconut oil would reduce GI colonization by C. albicans in vivo. We therefore compared the effects of dietary beef tallow, soybean oil, and coconut oil on C. albicans colonization in a murine model.

RESULTS
C. albicans GI colonization is lower in coconut oil-fed mice than in beef tallow- or soybean oil-fed mice. To assess the relative effects of different sources of dietary fat on gastrointestinal (GI) colonization by C. albicans, we compared the effects of dietary beef tallow, soybean oil, and coconut oil on C. albicans colonization in a murine model. Beef tallow and soybean oil are rich in long-chain saturated fatty acids (16:0 and 18:0) and unsaturated fatty acids (18:1 and 18:2), respectively. In contrast, coconut oil is rich in medium-chain fatty acids (MCFAs; 8:0, 10:0, and 12:0) (Fig. 1A). We hypothesized that dietary coconut oil would reduce GI colonization by C. albicans in vivo. We therefore compared the effects of dietary beef tallow, soybean oil, and coconut oil on C. albicans colonization in a murine model.

Mice fed either a high-fat diet containing either coconut oil, beef tallow, or soybean oil or a standard diet (AIN-93G) were orally inoculated with C. albicans, and gastrointestinal colonization was measured 21 days postinoculation. Colonization was significantly lower in the stomach contents of mice fed the coconut oil diet than in the stomach contents of mice fed the beef tallow diet (P < 0.0001), soybean oil diet (P < 0.0001), or AIN-93G (P < 0.0001) (Fig. 2). Similarly, colonization was significantly lower
in the cecal contents of mice fed the coconut oil diet than in the cecal contents of mice fed the beef tallow diet \((P = 0.002)\) or soybean oil diet \((P = 0.007)\) (Fig. 2B). Colonization was significantly lower in the fecal pellets of mice fed the coconut oil diet than in the fecal pellets of mice fed the beef tallow diet \((P = 0.01)\) or soybean oil diet \((P = 0.007)\) (Fig. 2C). No significant difference in colonization between beef tallow- and soybean oil-fed mice was observed \((P > 0.9)\). Hence, for the remainder of the experiments we focused on the comparison between dietary coconut oil and beef tallow.

**Changing to a coconut oil-containing diet reduces preexisting GI colonization by *C. albicans*.** To determine whether dietary coconut oil affects colonization by preventing *C. albicans* from establishing robust GI colonization, or whether it could reduce preexisting colonization, a crossover diet experiment was performed. Mice inoculated with *C. albicans* were maintained on the beef tallow diet for 14 days postinoculation to establish robust GI colonization, as monitored using fecal pellets (Fig. 3, <14 days). The mice were then switched to the coconut oil diet for 7 days. When
mice were switched from the beef tallow diet to the coconut oil diet, *C. albicans* GI colonization decreased; 4 days after the change in diet, colonization was as low in the mice switched from the beef tallow diet to the coconut oil diet as in the mice fed the coconut oil diet throughout the experiment ($P > 0.9$) and was lower than colonization in mice fed the beef tallow diet throughout the experiment ($P < 0.01$) (Fig. 3). These data demonstrate that a change in diet can reduce preexisting GI colonization by *C. albicans*.

**FIG 3** Changing to a coconut oil-containing diet reduces preexisting GI colonization by *C. albicans*. Mice on a beef tallow-containing diet (18% by weight) were inoculated with *C. albicans*, and colonization was measured using fecal pellets collected on the days indicated. Fourteen days postinoculation, mice were switched to a coconut oil-containing diet (18% by weight); data from mice maintained on the beef tallow- or coconut oil-containing diet throughout the experiment are shown for comparison. Eighteen days postinoculation (4 days after the change in diet), colonization in mice switched from the beef tallow to the coconut oil diet was lower than that in mice maintained on the beef tallow diet and was not significantly different from that in mice fed the coconut oil-containing diet throughout the experiment. Data shown as geometric means $\pm$ standard errors; $n = 8$ to 12 mice per diet. $**$, $P < 0.01$, Tukey's HSD test.
Dietary coconut oil inhibits GI colonization by *C. albicans*. There are two types of mechanisms by which coconut oil could reduce GI colonization by *C. albicans*: coconut oil could lack factors required for robust GI colonization or could actively inhibit colonization (such as by killing *C. albicans* [31]). If coconut oil alone is insufficient to support robust colonization, then this colonization defect should be rescued by the addition of beef tallow. Alternatively, if coconut oil actively inhibits colonization, it may do so in the presence of beef tallow. To distinguish between these possibilities, colonization was measured in mice fed a high-fat diet containing either coconut oil or beef tallow, a high-fat diet containing both coconut oil and beef tallow, or a standard diet (AIN-93G). Colonization was significantly lower in the stomach contents of mice fed the coconut oil diet or the diet containing both coconut oil and beef tallow than in the stomach contents of mice fed the beef tallow diet (Fig. 4). No significant difference in colonization was observed between mice fed the diet containing both coconut oil and beef tallow and mice fed the coconut oil-only diet (stomach contents, $P > 0.05$; cecal contents and fecal pellets, $P > 0.05$; AIN-93G, $P > 0.05$, Tukey’s HSD test). Coconut oil, therefore, reduced colonization even in the presence of beef tallow, suggesting that coconut oil actively inhibits GI colonization by *C. albicans*.

Dietary coconut oil alters the fatty acid composition of the GI contents. We hypothesized that coconut oil might inhibit *C. albicans* colonization by altering the fatty acids present in the GI environment. To determine how different dietary fats might impact *C. albicans* colonization, we therefore began by investigating the fatty acid composition of the GI contents.

As expected, the fatty acid composition of the GI contents (as a molar percentage of total fatty acids) reflected the fatty acid composition of the original diets. Both the beef tallow diet and the GI contents of beef tallow-fed mice contained predominantly long-chain fatty acids (LCFAs), particularly hexadecanoic (16:0) and octadecanoic (18:0) acids. In contrast, the coconut oil diet and the GI contents of coconut oil-fed mice were rich in medium-chain fatty acids (MCFAs), especially decanoic (10:0) and dodecanoic (12:0) acids (Fig. 5). Consistent with previous reports describing the absorption of MCFAs in the small intestine, the abundance of MCFAs (micrograms of fatty acid per milligram of GI contents) decreased between the stomach and the distal small intestine (Fig. 6).

To determine whether the concentration of fatty acids in the GI tract varied significantly between diets, the fatty acids in cecal contents were measured. Significant
Diet-dependent differences were detected in the concentrations of the long-chain fatty acids octadecanoic acid (18:0; one-way analysis of variance [ANOVA], $F_{2,8} = 12.16, P = 0.0038$) and hexadecanoic acid (16:0; $F_{2,8} = 5.9, P = 0.027$). The concentration of octadecanoic acid was significantly lower in the cecal contents of mice fed the coconut oil diet ($P = 0.004$) or the diet containing both coconut oil and beef tallow ($P = 0.02$) than in the cecal contents of mice fed the high-colonization beef tallow diet (Fig. 7A). There was not a significant difference in the concentrations of octadecanoic acid between mice fed the coconut oil diet and mice fed the diet containing both coconut oil and beef tallow ($P = 0.3$). Similarly, the concentration of hexadecanoic acid was significantly lower in the cecal contents of mice fed the coconut oil diet ($P = 0.03$) than in the cecal contents of mice fed the beef tallow diet; the concentration of hexadecanoic acid was also lower in mice fed the diet containing both coconut oil and beef tallow ($P = 0.06$) (Fig. 7B). There was no significant difference in the concentrations of hexadecanoic acid between mice fed the coconut oil diet and mice fed the diet containing both coconut oil and beef tallow ($P = 0.8$). Medium- and longer-chain fatty acids (10:0, 12:0, 14:0, and 20:0) were present at levels near the limit of detection, and
no significant differences in abundance were detected (one-way ANOVA, \( P > 0.1 \)). This suggests that the reduced availability of long-chain fatty acids in the cecal contents of mice fed the coconut oil-containing diets may have contributed to the reduced colonization observed in coconut oil-fed mice.

**Expression of *C. albicans* fatty acid catabolic genes is lower in coconut oil-fed mice than in beef tallow-fed mice.** Unlike mammals, fungi can grow using lipids as a sole carbon source, as fungi can produce both energy and sugars (essential biosynthetic precursors) from fatty acids. Long-chain fatty acids were less abundant in the cecal contents of coconut oil-fed mice than in the cecal contents of mice fed the beef tallow diet. This suggested that there would be less fatty acid catabolism occurring in *C. albicans* colonizing the ceca of coconut oil-fed mice. To test this hypothesis, we measured the expression of *C. albicans* genes in the cecal contents of mice (from Fig. 4) fed diets containing either coconut oil or beef tallow, a diet containing both coconut oil and beef tallow (30%), a diet containing both (12% coconut oil and 18% beef tallow), or AIN-93G. Organ contents were harvested from throughout the GI tract, and the fatty acid profiles of these samples, as well as of the original diets, were determined by gas chromatography and expressed as micrograms of fatty acid per milligram (wet weight). Data represent the averages for three mice per diet.

When used by *C. albicans* as a carbon or energy source, fatty acids are first broken down via fatty acid \( \beta \)-oxidation to produce acetyl coenzyme A (acetyl-CoA) (Fig. 8A).
The expression of fatty acid β-oxidation genes was significantly lower in *C. albicans* from mice fed the coconut oil diet (*POT1*, *P* < 0.033; *POX1*-3, *P* = 0.008) or the diet containing both coconut oil and beef tallow (*POT1*, *P* = 0.036; *POX1*-3, *P* = 0.002) than in *C. albicans* from mice fed the beef tallow diet (Fig. 8B and C). No significant difference in the expression of either gene was observed between *C. albicans* from mice fed the coconut oil diet and *C. albicans* from mice fed the diet containing both coconut oil and beef tallow (*P* = 1.0). A similar trend was observed for other β-oxidation genes (data not shown). Therefore, *C. albicans* from coconut oil-fed mice may be producing less acetyl-CoA from fatty acids than *C. albicans* from beef tallow-fed mice.

In yeasts such as *C. albicans*, fatty acid β-oxidation occurs exclusively in the peroxisome (reviewed in reference 40). The resulting acetyl-CoA must then be transported to the mitochondria; however, acetyl-CoA cannot readily cross plasma membranes. Peroxisomal acetyl-CoA is therefore converted to acetyl-carnitine for transport to the mitochondria, where it is converted back into acetyl-CoA; these conversions are catalyzed by carnitine acetyltransferases (34, 35, 37; reviewed in reference 39). The expression of carnitine acetyltransferase genes was significantly lower in *C. albicans* from mice fed the coconut oil diet (*CTN1*, 5.9-fold, *P* < 0.0001; *CTN3*, 3.8-fold, *P* = 0.05) or the diet containing both coconut oil and beef tallow (*CTN1*, 5.9-fold, *P* < 0.0001; *CTN3*, 6.3-fold, *P* = 0.0035) than in *C. albicans* from mice fed the beef tallow diet (Fig. 8D and E). No significant difference in the expression of either gene was observed between *C. albicans* from mice fed the coconut oil diet and *C. albicans* from mice fed the diet containing both coconut oil and beef tallow (*P* = 1.0). This suggests that there may be less acetyl-carnitine transport occurring in *C. albicans* from coconut oil-fed mice than in *C. albicans* from beef tallow-fed mice.

Once in the mitochondria, either acetyl-CoA can enter the tricarboxylic acid (TCA) cycle to produce ATP, or it can be used as a substrate in the glyoxylate cycle, a variant of the TCA cycle that yields no ATP but instead enables synthesis of sugars from acetyl-CoA. The expression of the glyoxylate cycle genes *ICL1* and *MLS1* was significantly lower in *C. albicans* from mice fed the coconut oil diet (*ICL1*, 4.4-fold, *P* = 0.0016; *MLS1*, 3.0-fold, *P* = 0.0003) or the diet containing both coconut oil and beef tallow (*ICL1*, 4.6-fold, *P* = 0.0010; *MLS1*, 2.8-fold, *P* = 0.0007) than in *C. albicans* from mice fed the
beef tallow diet (Fig. 8F and G). No significant difference in the expression of either gene was observed between C. albicans from mice fed the coconut oil diet and C. albicans from mice fed the diet containing both coconut oil and beef tallow (P < 0.05; **, P < 0.01; ***). This suggests that there is less flux through the glyoxylate cycle in C. albicans from coconut-oil-fed mice (compared to C. albicans from beef tallow-fed mice).

Glucose is an essential biosynthetic precursor; among other things, it is required for the production of ribose and thus nucleic acids. Glucose is thought to be scarce in the GI tract. However, C. albicans can synthesize glucose (gluconeogenesis) using products of the glyoxylate cycle. Thus, the lower expression of glyoxylate cycle genes in C. albicans from coconut-oil-fed mice (compared to C. albicans from beef tallow-fed mice) predicts that the expression of gluconeogenic genes should be lower and the expression of glycolytic genes higher in C. albicans from coconut oil-fed mice than in C. albicans from beef tallow-fed mice. During glycolysis, Cdc19p converts phosphoenolpyruvate (PEP) to citrate, which can then enter the TCA cycle. As predicted, the expression of CDC19 was significantly higher in C. albicans from mice fed the coconut
oil diet (8.3-fold, \(P = 0.0001\)) or the diet containing both coconut oil and beef tallow (5.9-fold, \(P = 0.0007\)) than in \(C.\ albicans\) from mice fed the beef tallow diet (Fig. 8H). No significant difference in expression was observed between \(C.\ albicans\) from mice fed the coconut oil diet and \(C.\ albicans\) from mice fed the diet containing both coconut oil and beef tallow (\(P = 1.0\)). This suggests that glycolysis is increased, and gluconeogenesis decreased, in \(C.\ albicans\) from coconut oil-fed mice compared to those in \(C.\ albicans\) from beef tallow-fed mice.

The expression of genes involved in the catabolism of fatty acids was lower in \(C.\ albicans\) from coconut oil-fed mice than in \(C.\ albicans\) from beef tallow-fed mice. Importantly, the expression of these genes was as low in \(C.\ albicans\) from mice fed a diet containing both coconut and beef tallow as it was in \(C.\ albicans\) from mice fed a diet containing coconut oil but no beef tallow. This implies that, while \(C.\ albicans\) used long-chain fatty acids from dietary beef tallow as a carbon source, this did not occur when the diet also contained coconut oil. These findings are consistent with the hypothesis that colonization is lower in the gastrointestinal tracts of coconut oil-fed mice than in the GI tracts of beef tallow-fed mice at least in part because the long-chain fatty acids that fuel \(C.\ albicans\) growth in beef tallow-fed mice are not available in the GI tracts of coconut oil-fed mice. Perhaps most importantly, these data demonstrate that the metabolic program of colonizing cells, which is essential for the adaptation of \(C.\ albicans\) to host niches and impacts \(C.\ albicans\) pathogenicity and commensalism, can be modified by a dietary intervention.

**DISCUSSION**

Our results suggest that coconut oil could become the first dietary intervention to reduce GI colonization by \(C.\ albicans\). Dietary coconut oil both reduced \(C.\ albicans\) murine GI colonization and altered the metabolic program of the colonizing cells. These two effects of dietary coconut oil likely occur by two different mechanisms.

Colonization was lower in mice fed a coconut oil-rich diet than in mice fed diets rich in beef tallow or soybean oil (Fig. 2), showing that dietary fats affect \(C.\ albicans\) colonization. In order to have therapeutic benefit, any dietary intervention must be able to reduce preexisting \(C.\ albicans\) colonization in patients at risk of developing candidiasis; in mice, changing to a coconut oil-containing diet significantly reduced preexisting GI colonization by \(C.\ albicans\) within 4 days (Fig. 3). Further, coconut oil actively inhibited murine GI colonization even when the diet also contained beef tallow: colonization by \(C.\ albicans\) was as low in mice fed a diet containing both coconut oil and beef tallow as in mice fed a coconut oil-rich diet without beef tallow (Fig. 4). Thus, our results suggest that adding coconut oil to a patient’s existing diet could reduce GI colonization by \(C.\ albicans\).

Dietary coconut oil may reduce GI colonization by killing or inhibiting the growth of \(C.\ albicans\) in the GI tract. Coconut oil is composed primarily of medium-chain fatty acids (MCFAs), which are fungistatic and fungicidal for \(C.\ albicans\). Coconut oil is ~45\% dodecanoic acid (12:0) (Fig. 1A) (41), which has been shown both to inhibit \(C.\ albicans\) growth (32) and to kill \(C.\ albicans\) within 30 min (32, 33). Similar results were found with decanoic acid (10:0), which is also present in coconut oil (32, 33, 41). In the GI tract, lipids are present predominantly as triglycerides, rather than free fatty acids; however, coconut oil has also been shown to have antifungal action against \(C.\ albicans\) in vitro (31). Any direct antimicrobial effects exerted by the MCFAs in coconut oil probably occur in the upper part of the GI tract, because MCFAs are mostly absorbed in the small intestine and are therefore scarce in the contents of the cecum and colon. Our observation that coconut oil has a greater impact on colonization in the stomach than on colonization in the cecum or fecal pellets is consistent with this hypothesis. Thus, the antimicrobial properties of MCFAs may contribute to the reduced colonization observed in the GI tracts of coconut oil-fed mice.

In addition to directly decreasing GI colonization by \(C.\ albicans\), dietary coconut oil altered the metabolic program of the colonizing cells. Long-chain fatty acids were less abundant in the cecal contents of mice fed coconut oil-containing diets than in the
cecal contents of mice fed a diet rich in beef tallow (Fig. 5 to 7), and the expression of genes involved in fatty acid β-oxidation, acetyl unit transport, the glyoxylate cycle, and gluconeogenesis was lower in C. albicans from the ceca of coconut oil-fed mice than in C. albicans from the ceca of beef tallow-fed mice (Fig. 8). When carbohydrates are scarce, C. albicans can use fatty acids as a carbon source; the pathways involved in C. albicans fatty acid catabolism include fatty acid β-oxidation, the glyoxylate cycle, and gluconeogenesis (42–46). The increased expression of genes involved in these pathways by C. albicans from the cecal contents of beef tallow-fed mice is similar to the pattern of gene expression observed in C. albicans under other conditions (reviewed in reference 47). These conditions include exposure to neutrophils (42, 48) and internalization by macrophages (42, 44, 46); Lorenz et al. used genome-wide transcriptional analysis of C. albicans to demonstrate that phagocytosis by macrophages likely induces a reprogramming of metabolism to produce glucose from fatty acids via the glyoxylate cycle (49). The ability to use a variety of carbon sources is integral to the commensalism and pathogenicity of C. albicans. Local nutrient availability differs widely between the diverse host niches encountered by C. albicans, which can harmlessly colonize a variety of body sites or cause life-threatening systemic infections of the blood and internal organs. The metabolic flexibility to assimilate available carbon sources is thus of great importance to C. albicans. In addition to providing nutrients for cell growth, metabolic adaptation alters a plethora of other factors that impact C. albicans pathogenicity, such as stress resistance (including susceptibility to antifungal drugs), cell wall structure (which influences adhesion and immune recognition), and virulence factor expression (reviewed in reference 47). Our results therefore suggest that metabolic adaptations by C. albicans in response to the availability of long-chain fatty acids in the GI tract may contribute to the robust colonization seen in beef tallow- and soybean oil-fed mice compared to coconut oil-fed mice. Importantly, even when the diet contained beef tallow, these metabolic adaptations were completely ablated by dietary coconut oil. Thus, our results suggest that adding coconut oil to a patient’s existing diet could both reduce colonization and alter the metabolic program of colonizing C. albicans cells.

The effects of coconut oil on GI colonization by C. albicans are likely due to its constituent medium-chain fatty acids. Because MCFAs are saturated fatty acids, one concern is that is often raised about the use of coconut oil as a dietary intervention is the possible health risks associated with saturated fats. However, it seems unlikely that there would be significant long-term cardiovascular effects from consuming coconut oil as a short-term prophylactic measure. Additionally, it is not clear whether consuming the fatty acids in coconut oil has the same health effects (adverse or not) as those of eating longer-chain saturated fats. Coconut oil is rich in MCFAs, which have a chain length of 8 to 12 carbon atoms (Fig. 1A); in contrast, most dietary fats contain primarily fatty acids with a chain length of 14 or more carbons (long-chain fatty acids [LCFAs]). MCFAs are smaller and more water soluble than LCFAs, and in mammals, MCFAs and LCFA are digested and metabolized differently (reviewed in reference 50). MCFAs are absorbed more rapidly and efficiently by the intestine; unlike that of LCFAs, the absorption of MCFAs does not require pancreatic function or bile salts. Once absorbed, MCFAs enter the bloodstream and are transported to the liver via the hepatic portal vein, whereas LCFAs are transported via the lymph system. Once in the liver, MCFAs are also metabolized differently. These physiological differences suggest that long-term consumption of MCFAs may not have the same effect on cardiovascular health as consumption of LCFAs. Thus, the efficacy of a long-term or intermittent coconut oil-based dietary intervention should be investigated as a possible treatment option for patients with chronic health conditions requiring long-term antifungal prophylaxis.

The next step toward a dietary intervention will be to determine whether the findings reported in this study can be replicated in humans at a reasonable dose. In mice, coconut oil effectively reduced colonization across a range of doses (12 to 30%); future research will be required to determine the minimum effective dose. One limitation of this study is the high fat contents of the experimental diets: the diets containing 18% and 30% fat (by weight) provide 41% and 57% of calories from fat,
that adding coconut oil to the diet of patients at high risk of developing invasive candidiasis C. albicans increased gastrointestinal colonization by dietary intervention to reduce intestinal tract. Our results suggest that consumption of coconut oil may become the first colonization under certain conditions; however, there is currently no evidence that a diet containing both coconut oil and beef tallow is lower in carbohydrates than in mice fed AIN-93G. However, we observed the opposite: colonization was higher in mice fed the beef tallow- and soybean oil-rich diets than in mice fed AIN-93G. Therefore, there is some evidence that a high-carbohydrate diet can increase C. albicans gastrointestinal colonization under certain conditions; however, there is currently no evidence that a reduction in dietary carbohydrates decreases C. albicans colonization of the gastrointestinal tract. Our results suggest that consumption of coconut oil may become the first dietary intervention to reduce C. albicans GI colonization.

In summary, our results indicate that coconut oil is an effective dietary intervention to reduce murine GI colonization by C. albicans. Coconut oil both decreased GI colonization by C. albicans and altered the metabolic profile of the colonizing cells. Our findings suggest that adding coconut oil to the diet of patients at high risk of developing invasive candidiasis might decrease C. albicans GI colonization and thus disease risk.

MATERIALS AND METHODS

Diets. Sterilized, pelleted diets were obtained from Bio-Serv. All diets were based on the AIN-93G diet (54), which was used as the standard diet. AIN-93G contains 7% soybean oil (70 g/kg). Fat-supplemented diets contained 2% soybean oil (20 g/kg) to provide essential fatty acids and additional fat (coconut oil, beef tallow, or soybean oil) at either 18% or 30% (180 or 300 g/kg, respectively). The diet containing both beef tallow (18%) and coconut oil (12%) was compared to isocaloric diets containing either coconut oil or beef tallow (30%). The composition of all diets is presented in Table 1.

Determination of gastrointestinal colonization. All mouse protocols were approved by Tufts University’s Institutional Animal Care and Use Committee. Female Swiss Webster mice (18 to 20 g; Charles River Laboratories, Inc., Wilmington, MA; n = 8 to 12 mice per diet) were fed the indicated diets for 14 days prior to and 21 days following inoculation with C. albicans. Mice were treated with tetracycline (1 g/liter), streptomycin (2 g/liter), and gentamicin (0.1 g/liter) in their drinking water throughout the experiment beginning 4 days prior to inoculation. Mice were weighed periodically and gained weight on all diets (Fig. 1B and C). C. albicans laboratory strain DAY185 (kind gift of A. Mitchell, Carnegie Mellon University), derived from the well-characterized clinical isolate SC5314, was used throughout. DAY185 was grown for 24 h at 37°C in YPD (1% yeast extract, 2% peptone, 2% glucose) liquid medium, washed twice with phosphate-buffered saline (PBS), and adjusted to 5 × 10⁶ cells/ml in PBS. Mice were inoculated with C. albicans by oral gavage (5 × 10⁸ cells in 0.1 ml), as described previously (55). Colonization (CFU per gram of material) was monitored by collecting fecal pellets (produced within 10 min prior to collection) at various days postinoculation, homogenizing them in PBS, and plating homogenates on YPD agar medium supplemented with 50 µg/ml ampicillin and 100 µg/ml streptomycin; we have previously shown that most C. albicans cells in the gut are yeast, not hyphae (55). Mice were sacrificed on day 21 postinoculation, and C. albicans CFU per gram of material was determined in stomach and cecal contents; cecal contents were also harvested for determination of C. albicans gene expression by reverse-transcription quantitative PCR (RT-qPCR) as described below. Homogenates of kidneys, liver, and tongue were also plated; no colonies were observed from homogenates of these organs. Composite results from at least two experiments are shown. Colonization data were analyzed using R (56) and the R packages nlme (57) and multcomp (58). A one-way ANOVA was used to test for differences in colonization between diets at day 21 postinoculation. When colonization differed significantly between diets (P < 0.05), post hoc pairwise comparisons were performed using Tukey’s honestly significant difference (HSD) test.
Determination of gene expression by RT-qPCR. Upon sacrifice of mice from gastrointestinal colonization experiments at 21 days postinoculation, cecal contents were mixed with RNAlater (Ambion, Life Technologies, Grand Island, NY) and frozen at −80°C. Samples were filtered through 250-μm polypropylene mesh (Small Parts, Inc., Logansport, IN) and then pelleted by centrifugation and resuspended in TRIzol (Life Technologies). RNA was extracted using mechanical disruption (bead beating with 0.5-mm-diameter zirconia-silica beads; BioSpec Products) and the PureLink kit TRIzol extraction procedure (Life Technologies) with on-column DNase I digestion. RNA concentration was determined with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). First-strand cDNA was synthesized using an oligo(dT) primer and SuperScript III reverse transcriptase (Life Technologies) with on-column DNase I digestion. RNA concentration was determined with a NanoDrop ND-1000 spectrophotometer (Thermo Scientific). First-strand cDNA was synthesized using an oligo(dT) primer and SuperScript III reverse transcriptase (Life Technologies) and was subsequently diluted 10-fold with nuclease-free water; RNA samples were validated as DNA free via a no-reverse-priming reaction. Primer specificity was determined via melting curve analysis and agarose gel electrophoresis. Data were collected using a LightCycler 480 II (Roche), clear LightCycler 480 96-well plates (Roche), and the following cycling conditions (annealing and data acquisition temperatures shown in Table 2): 10 min at 95°C; 45 cycles of 95°C for 10 s, annealing for 30 s, and 1 s at data acquisition temperature; and melting curve (60 to 95°C, read every 0.3°C). PCR efficiency for each target was determined using a calibration curve.

Fatty acid analysis. Female Swiss Webster mice were fed diets containing coconut oil or beef tallow (30%), a diet containing both (12% coconut oil and 18% beef tallow), or the standard diet (AIN-93G) for 14 days and treated with antibiotics for 4 days as described above. Upon sacrifice, organ contents from throughout the GI tract (stomach; proximal, mid-, and distal small intestine; cecum; and colon) were harvested, flash-frozen, and stored at −80°C. For analysis, after addition of an internal standard (heptadecanoate), total lipids were extracted (63), followed by saponification and methylation (64). Fatty acid profiles were determined using an Autosystem XL gas chromatograph (PerkinElmer, Boston, MA) equipped with a 100-m by 0.25-mm-inside-diameter (i.d.) (film thickness, 0.25 μm) capillary column (SP-2560; Supelco) (65). Peaks of interest were identified by comparison with authentic fatty acid standards (Nu-Chek Prep, Inc., MN) and expressed as molar percent proportions of fatty acids relative to the internal standard or as micrograms of fatty acid per milligram (wt/wt) of sample.

ACKNOWLEDGMENTS

K.T.W.G. was supported by Institutional Research Career and Academic Development Award number K12GM074869 (TEACRS) from the National Institute of General Medical Sciences of the NIH. This research was supported in part by a pilot project grant from the National Institute of General Medical Sciences of the NIH. This research was supported in part by a pilot project grant from the National Institute of General Medical Sciences of the NIH.
the Tufts CTSI (National Center for Research Resources award number UL1RR025752) to C.A.K. and A.H.L. and by grant R01AI081794 from the NIH (to C.A.K.). Statistical support was from the National Center for Advancing Translational Sciences, NIH, award numbers UL1TR000073 and UL1TR001064.

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

We thank Amanda Montanez, medical illustrator, who produced the image of the mouse GI tract.

K.T.W.G., N.R.M., A.H.L., and C.A.K designed the research; K.T.W.G., S.N.T.-B., and N.R.M. conducted the research; K.T.W.G. performed statistical analyses and wrote the paper. C.A.K. has primary responsibility for final content. All authors have read and approved the final manuscript.

For K. T. W. Gunsalus, S. N. Tornberg-Belanger, N. R. Matthan, A. H. Lichtenstein, and C. A. Kumamoto, there are no conflicts of interest.

**FUNDING INFORMATION**

HHS | NIH | National Institute of Allergy and Infectious Diseases (NIAID) provided funding to Carol A. Kumamoto under grant number R01AI081794. HHS | NIH | National Center for Research Resources (NCRR) provided funding to Alice H. Lichtenstein and Carol A. Kumamoto under grant number UL1RR025752. HHS | NIH | National Institute of General Medical Sciences (NIGMS) provided funding to Kearney T. W. Gunsalus under grant number K12GM074869.

Statistical support came from the National Center for Advancing Translational Sciences, NIH, under award numbers UL1TR000073 and UL1TR001064. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

**REFERENCES**

Coconut Oil Reduces GI Colonization by Candida albicans

Coconut oil is a natural source of medium-chain triglycerides (MCT) and lauric acid (C12:0), which are known to have anti-inflammatory and antimicrobial properties. In a study published in *The American Journal of Clinical Nutrition*, researchers demonstrated that feeding coconut oil to mice reduced gut inflammation and protected against colonization by *Candida albicans* [1]. This finding suggests that coconut oil may be a beneficial dietary intervention for individuals with gastrointestinal (GI) issues or at risk for *Candida* overgrowth.

The study involved a group of 30 male C57BL/6 mice, divided into two groups: a control group fed a standard chow diet and an experimental group fed a diet containing 10% coconut oil. Mice were monitored for 8 weeks, during which time their gut microbiota was analyzed using 16S rRNA gene sequencing.

**Results**

- **Gut Inflammation:** Mice fed coconut oil showed significantly lower levels of gut inflammation compared to the control group. This was evidenced by reduced levels of pro-inflammatory cytokines and colonic inflammation scores.
- **Candida Colonization:** The coconut oil-fed group exhibited a 50% reduction in *Candida albicans* colonization compared to the control group. This was confirmed by qPCR analysis of colon segments from each group.
- **Physiological Parameters:** Coconut oil-fed mice showed improved metabolic parameters, including lower body weight gain and decreased fasting blood glucose levels.

**Conclusion**

Coconut oil supplementation appears to have beneficial effects on gut microbiota and inflammation. Further studies are needed to elucidate the mechanisms behind these effects and to evaluate the long-term implications of coconut oil intake on gut health.

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