Probiotic Yogurt Consumption is Associated With an Increase of CD4 Count Among People Living With HIV/AIDS

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Aim: To evaluate the long term effect of yogurt supplemented with *Lactobacillus rhamnosus* Fiti on the immune function (CD4 count) of people living with HIV/AIDS.

Background: Gastrointestinal infections and the leakage of microbial products from the gut have a profound impact on the deterioration of the immune system among people living with HIV/AIDS. Among persons not infected with the virus, probiotics can prevent gastrointestinal infections and restore an effective gut barrier, suggesting they might have a beneficial effect on the immune function of people living with HIV/AIDS.

Study: We carried out an observational retrospective study over a period of 3 years, with longitudinal comparison of the CD4 count within participants (n = 68) before and during probiotic yogurt consumption, and compared with a control group of participants not consuming the yogurt (n = 82).

Results: Among the yogurt consumers before use and the nonconsumers, an average increase in CD4 count was seen of 0.13 cells/μL/day (95% CI; 0.07-0.20, $P = < 0.001$). After commencing consumption, yogurt consumers experienced an additional increase of 0.28 cells/μL/day (95% CI; 0.10-0.46, $P = 0.003$). When adjusting for length of time using antiretroviral medication, the additional increase explained by yogurt consumption remained 0.17 cells/μL/day (95% CI; 0.01-0.34, $P = 0.04$). Treatment with antiretroviral medication was associated with an increase of 0.27 cells/μL/day (95% CI; 0.17-0.38, $P = < 0.001$).

Conclusion: The introduction of probiotic yogurt, made by local women in a low-income community in Tanzania, was significantly associated with an increase in CD4 count among consumers living with HIV.

Key Words: probiotics, HIV, AIDS, diarrhea, *Lactobacillus*

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Recent attention on HIV/AIDS care in Africa has focused on increasing access to antiretroviral (ARV) medication. Although this is important, efforts are also needed to provide safe and affordable interventions for those without access to ARVs or with CD4 counts too high to initiate ARV therapy, yet whose quality of life is diminished by micronutrient deficiencies, diarrhea, and other conditions associated with HIV infection. An increased micronutrient intake, most notably vitamin B-complex in combination with C and E, seems to be an effective intervention that has been associated with reduced mortality and increased CD4 count.[1–5] The WHO recommends that an "increased micronutrient intake can be best achieved through an adequate diet,"[6] favoring food-based interventions.

The gut is one of the most severely affected sites by HIV.[7,8] Inflammation results in damage to the epithelial barrier, leading to an increased leakage of microbial products into the bloodstream. Recently, it was theorized that this may be an ongoing source of systematic immune activation that fuels HIV,[9] although this association was less clear in an African population.[10] Capsule proteins of HIV may further facilitate viral replication by eliciting a profound Th-2 activation that inhibits an effective immune response against the virus.[11]

Probiotic bacteria, live microorganisms which when administered in adequate amounts confer a health benefit on the host,[12] can potentially restore an effective gut barrier,[13,14] thereby reducing systemic immune activation. Furthermore, probiotics have been shown to upregulate T-regulatory lymphocytes,[15,16] potentially skewing the immune system away from a Th-2-dominant state.[17] Probiotic usage has been shown to be safe among people living with HIV[18–20] and recent randomized trials in Brazil[21] and Nigeria[22] suggest that probiotic use can increase the CD4 count.

The most commonly used vehicle for supplying probiotics; yogurt, is a significant source of vitamin A, B-complex, zinc, and high-biologic-quality protein[23] and is therefore, an excellent food-based intervention to improve micronutrient intake among people living with HIV/AIDS. On the basis of these notions, a community kitchen in Mwanza, Tanzania, was established in 2004 to produce yogurt supplemented with *Lactobacillus rhamnosus* GR-1 (Fiti) to be distributed as an adjunct to the diet of people living with HIV. To evaluate the impact of probiotic yogurt supplementation on immune function (CD4 count), this observational study was undertaken.

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MATERIALS AND METHODS

Study Design

An observational retrospective study was designed with the collection of CD4 count measurements from 3 years before the date of interview. This allowed longitudinal comparison of the development in CD4 count within participants (before and during probiotic yogurt consumption) and between probiotic yogurt consumers and nonconsumers. Participants were informed about the study and gave their written or thumb printed consent before participation, and all procedures were carried out according to the Helsinki Declaration as revised in 2000. The study was conducted during April and May 2008.

Setting

Both probiotic yogurt consumers and nonconsumers were recruited from 3 probiotic yogurt distribution sites in the urban area of Mwanza city (Mabatini, Mahina, and Bwiru). At the Mabatini site, regular yogurt was produced and supplemented with *L. rhamnosus* Fiti which was grown from stock cultures stored and harvested at the laboratory of the National Institute for Medical Research, Mwanza. Yogurt was served in portions of 200 mL containing 10^9cfu/mL of viable *L. rhamnosus* Fiti.

Participants

The participants were invited to participate when they were able to show their clinical HIV record with at least one CD4 count measurement. All people consuming yogurt at least once a week were invited to participate, whereas they were visiting one of the three sites to receive their portion of probiotic yogurt. People living with HIV who were not consuming yogurt but were visiting one of the sites for another nutrition intervention (monthly distribution of maize and beans) were also invited to participate without further standardization of the control group. Local leaders or the hospitals had referred both yogurt consumers and nonconsumers to these nutrition programs because of the participant's lack of adequate nutrition.

Data Collection

During a structured interview, data were collected pertaining to demographics, socioeconomic status, dietary intake through a 24-hours recall, medication, and disease history. Furthermore, participants were interviewed about the number of days they experienced febrile symptoms, diarrhea, and cough and the average number of hours participants were able to work per day. Lastly, they were asked to rate the severity of their gastrointestinal symptoms (stomach pain, stomach gas, nausea, diarrhea) and its impact on daily life.

CD4 count measurements 3 years before the interview were recorded from the clinical file. The CD4 count measurements were determined blinded for probiotic yogurt consumption under the National AIDS Control Program at Sekou-Toure regional hospital and Weil Bugando university hospital in Mwanza using conventional flow-cytometry.

Analyses

Differences between yogurt consumers and nonconsumers were detected by comparing characteristics between groups using a χ^2 test, whereas micronutrient data were compared using a t test. Trends in CD4 count measurements were analyzed, allowing for repeated measurements, by using generalized estimating equation methods with an Identity link, Poisson variance, and an exchangeable correlation structure. To adjust for the length of time on ARV medication, a variable was created with the number of days as date of commencement of ARV treatment at the time of each CD4 count. This continuous variable was then used to adjust the overall trend in time of CD4 count (number of days since first recording in clinical file) the effect of yogurt consumption (number of days since start of consumption), length of time living with HIV (number of days since HIV diagnosis), and length of time using ARV treatment (number of days since initiation of ARV treatment).

We were interested in the effect of short-tem probiotic yogurt consumption (70 days) since a randomized controlled trial (Hummelen et al, submitted) had indicated different effects before and after this period. To allow for this, we created a spline variable. Wald test with robust standard errors was used to compare differences between groups. All tests were carried out 2-sided at the $\alpha = 0.05$ significance level with no adjustments made for multiple comparisons. Data were stored in a Microsoft Access database and analyzed using SPSS version 15.0.

RESULTS

A total of 68 yogurt consumers and 82 nonconsuming controls participated in the study. Demographics, socioeconomic status, and years living with HIV were similar between both groups (Table 1). Furthermore, the proportion of consumers being treated with ARV (52 of 68, 76%),

TABLE 1. Characteristics of Control Group and Probiotic Yogurt Consumers

Characteristics	Control % (n)	Yogurt % (n)	*P*
Gender			
Female	85 (70)	81 (55)	0.5
Male	15 (12)	19 (13)	
Marital status			
Widow/divorced	66 (54)	54 (37)	0.1
Single	13 (11)	9 (6)	
Married	21 (17)	37 (25)	
Water source			
Pipe	68 (56)	56 (38)	0.1
Well	32 (26)	44 (30)	
BMI			
< 18.5	9 (7)	10 (7)	0.7
18.5 +	92 (75)	90 (61)	
Age (y)*			
< 18	2 (2)	5 (3)	0.5
18-45	73 (60)	64 (43)	
45+	24 (20)	31 (21)	
Education*			
No schooling	21 (17)	9 (6)	0.1
Grade 1-7	69 (56)	79 (52)	
Form 1-4 or higher	10 (8)	12 (8)	
Living with HIV*			
< 1 y	24 (20)	13 (9)	0.1
1+ year	76 (62)	87 (58)	
Antiretroviral medication			
No	31 (25)	24 (16)	0.3
Yes	70 (57)	76 (52)	

*Numbers do not add up due to missing values.
BMI indicates body mass index.

and nonconsumers (57 of 82, 70%) was similar ($P = 0.3$). Among yogurt consumers, 196 CD4 counts were recorded before yogurt consumption and 97 measurements were recorded during yogurt consumption. Yogurt was consumed for a median (range) of 357 (3 to 1062) days. Among yogurt consumers 62 of 68 (91%) participants had at least one CD4 count before commencing yogurt intake. In the group of 82 participants who did not consume the yogurt, a total of 233 CD4 counts were recorded. The probiotic yogurt group had an average higher daily intake of calories (2097 kcal vs. 1887 kcal, $P = 0.01$), protein (55 g vs. 49 g, $P = 0.03$), vitamin B_1 (1.7 mg vs. 1.5 mg, $P = 0.002$), vitamin B_2 (0.9 vs. 0.7 mg, p = 0.03), calcium (490 vs. 421 mg, p = 0.04), and iron (18 vs. 15 mg, $P = 0.001$) the day before the interview. No differences in daily intake of vitamin B12, C, D, E, selenium, and zinc were detected.

Within Consumer Differences

Before starting consumption, the 68 yogurt consumers experienced an average increase in CD4 count of 0.16 cells/μL/day (95% CI; 0.06-0.27, $P = 0.002$). This increased an additional 0.23 cells/μL/day (95% CI; 0.02-0.44, $P = 0.04$) after the start of yogurt consumption. After adjusting for ARV use, which was associated with an increase of 0.30 cells/μL/day (95% CI; 0.07-0.16, $P \leq 0.001$), the increase observed before the start of yogurt consumption was fully explained, as no significant increase remained after adjusting ($P = 0.6$). Furthermore, after adjusting for ARV treatment no significant association remained of commencing yogurt consumption with an increase in CD4 count ($P = 0.3$). However, without the development of CD4 count over time as a variable, commencing yogurt consumption was again associated with an increase in CD4 count of 0.16 cells/μL/day (95% CI; 0.0-0.31, $P = 0.05$). This indicates significant colinearity between the variables threatening their individual contribution; hence a control group was included that did not consume yogurt at any point in time.

Within Consumer and Between Group Differences

Taking into account the correlated nature of the repeated CD4 count measures within individuals, no differences were noted of CD4 count levels before the start of yogurt consumption between the yogurt consumers (mean 376 cells/μL) and nonconsumers (mean 366 cells/μL, $P = 0.8$). Among the yogurt consumers before use and the nonconsumers an average increase in CD4 count of 0.13 cells/μL/day (95% CI; 0.07-0.20, $P \leq 0.001$) was observed. After commencing consumption, yogurt consumers experienced an additional increase of 0.28 cells/μL/day (95% CI; 0.10-0.46, $P = 0.003$). When adjusting for length of time using ARV medication, the additional increase explained by yogurt consumption remained 0.17 cells/μL/day (95% CI; 0.01-0.34, $P = 0.04$). The increase seen before yogurt consumption and among the control group was fully explained by ARV treatment, as no significant increase remained after adjusting ($P = 0.6$) (Fig. 1). Treatment with ARV medication was associated with an increase of 0.27 cells/μL/day (95% CI; 0.17-0.38, $P \leq 0.001$). Time living with HIV was not associated with CD4 count development ($P = 0.3$) nor was the medical center at which treatment was received ($P = 0.6$). This indicates that no differences in treatment existed between the centers that significantly affected CD4 count development. When allowing for a different CD4 count development before and after 70 days, we found that

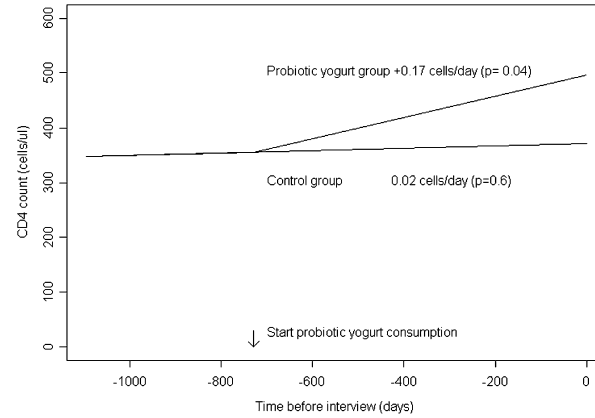

FIGURE 1. Increased CD4 count after commencing consumption of probiotic yogurt. Legend: Additional increase of 0.17 CD4 cells/μL/day (95% CI; 0.01-0.34, *P*=0.04) after start probiotic yogurt consumption when adjusting for antiretroviral treatment.

before the start of consumption and among controls, an increase of 0.13 CD4 cells/μL/day (95% CI; 0.06-0.20, $P \leq 0.001$) occurred. Among consumers, after commencing consumption an additional increase of 0.73 CD4 cells/μL/day (95% CI; −0.02-1.50, $P = 0.6$) during the first 70 days was seen. After this initial period, the CD4 count continued to rise an additional 0.2 CD4 cells/μL/day (95% CI; 0.03-0.4, $P = 0.02$). Using a Log link with a Poisson distribution provided similar inferences and using a generalized linear model.

General Health Questionnaire

All participants, except one consumer, completed the interview. Probiotic yogurt consumers reported the ability to work 8 hours a day, compared with 6 hours reported by nonconsumers ($P = 0.01$). Furthermore, consumers reported 1 day of fever a month compared with nonconsumers who reported a median of 2 days a month ($P = 0.01$). Regarding gastrointestinal symptoms, among those consuming yogurt, 56 of 67 (84%) participants did not report any diarrheal symptoms versus 57 of 82 (69%) participants among the nonconsuming group ($P = 0.05$). Lastly, 45 of 67 (52%) consumers did not report any impact (intermediate or severe) of gastrointestinal symptoms on everyday life compared with 24 of 82 (39%) among the nonconsumers ($P = 0.004$).

DISCUSSION

The study site is typical of many low-income communities in the developing world in which access to daily nutrition and medical care is limited, and HIV infection is highly prevalent.[24] The introduction of a locally produced highly nutritious food (yogurt) supplemented with a probiotic strain, not only provides an economic stimulus for farmers and those producing the yogurt, but may also improve the immune function among those living with HIV and their ability to work.

This study is, to our knowledge, the first to report the long-term effects of probiotic yogurt on CD4 count among people living with HIV/AIDS. The more pronounced increase of CD4 count during the first 70 days of

consumption corresponds to another study at this site, in which intake of *L. rhamnosus* GR-1 and *L. reuteri* RC-14 in capsule form increased CD4 count at 10 weeks more so than at 25 weeks (Hummelen et al submitted). The mechanism by which ingested lactobacilli can cause a boost in peripheral CD4 count is worthy of investigating and determining if the increase in CD4 count can be further stimulated by switching the type of lactobacilli used.

An important strength of this study is the long period (3 years) in which we assessed the development of the immune function, an asset that would have been difficult to achieve using a prospective study design. In addition the observational nature of the study is strength as we were able to assess the impact of probiotic yogurt at grassroots level among a wide range of patients, as opposed to the perfect conditions created for a homogenous group of participants to access and consume an intervention in a clinical trial setting.

Although selection bias may have occurred due to the unstandardized sampling of the groups, the similarities between the groups indicates that this bias was limited. We were able to adjust for potential confounders such as length of time living with HIV and ARV medication use, but not for co-trimoxazole use. When using the participant as his/ her own control, a significant increase in CD4 count was also found which validates the differences seen when including the control group in the analyses. As the intervention in this study, both included a probiotic strain and a nutritious carrier (micronutrients and high-quality protein) we were limited in distinguishing the individual contribution of each component. However, 2 randomized controlled trials have compared regular yogurt with a yogurt combined with probiotic strains and showed a significantly beneficial impact on the CD4 count when the yogurt was supplemented with additional probiotic strains.[21,22] Therefore, it is likely that the probiotic component of this intervention exerted beneficial affects on the immune system independently from its nutrient carrier.

Studies conducted in Africa have estimated the average annual increase in CD4 count of 90 cells/μL with ARV treatment[25] and an average decline of 20 to 50 cells/μL/year without ARV treatment.[26,27] In this study, a similar rate of increase was observed of 99 cells/μL with ARV treatment (0.27 cells/μL/d), whereas no significant decrease was observed without ARV treatment. The results of this study indicate that probiotic yogurt consumption is associated with an overall increase in CD4 count of 62 cells/μL/year (0.17 cells/μL/d). This could be due to an accelerated immune reconstitution after initiation of ARV treatment, thus shortening the time of severe immune deficiency, or may be due to an increase in CD4 count among those not yet eligible for ARV treatment, which may potentially delay the need for ARV medication. This hypothesis will require future assessment as our sample size did not allow for this subgroup analyses.

The differences in symptoms experienced between the groups suggest that probiotic yogurt might alleviate gastrointestinal symptoms among people living with HIV. However, due to the subjective nature of a questionnaire, these results need to be confirmed in a blinded setting. In conclusion; probiotic yogurt was significantly associated with beneficial long-term effects on the CD4 count and may provide an effective intervention to delay the deterioration of the immune system or potentiate ARV treatment among those living with HIV.

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