Differential Effects of Prebiotics on the Gut Mucosal Immune Response Within the Peyer's Patches Compared to the Lamina Propria of C57BL/6 Female Mice

Avery Snethen[1], Pelumi Adedigba[2], Bethany Hatter[1], Sanmi Alake[1], Proapa Islam[1], John Ice[1], Jerry Ritchey[3], Edralin A. Lucas[1], Brenda J. Smith[2,4]

[1]Nutritional Sciences Department, College of Education and Human Sciences, Oklahoma State University; [2]Indiana Center for Musculoskeletal Health, Indiana University School of Medicine; [3]Veterinary Pathobiology Department, College of Veterinary Medicine, Oklahoma State University; [4]Department of Obstetrics and Gynecology, Indiana University School of Medicine

DEPARTMENT OF NUTRITIONAL SCIENCES

ABSTRACT

This study was designed to examine the effects of two difference prebiotics (i.e., tart cherry and fructooligosaccharides) on cytokines and chemokines that regulate T cell homing, differentiation and activation within gut-associated lymphoid tissue of the small intestine. 8-wk-old female C57BL/6 mice were randomly assigned to treatments in a 2 x 3 factorial with antibiotics (+ or – ABX) and diet (control, tart cherry [TC] or fructooligosaccharides [FOS]) as factors. At the end of 10 wks of treatment, whole body dual-energy x-ray absorptiometry (DEXA) scans were performed, intestinal samples were collected. Fecal short chain fatty acid (SCFAs) were analyzed using gas chromatography techniques. RNA was extracted from Peyer's patches and genes of interest were assessed using RT-PCR. Data were analyzed using 2-way ANOVA followed by post hoc testing with significant main effect or interaction was detected. FOS supplementation and not TC increased whole body BMC and BMD. The benefits of FOS on bone were unaltered in presence of antibiotics. T regulatory cells were increased within the lamina propria of the ileum with FOS and this response was suppressed with antibiotics. No changes occurred in the pro-inflammatory, Th17 cells, with FOS. Fecal SCFAs were upregulated with both TC and FOS diets, but FOS had a greater effect. Antibiotics suppressed the increase in SCFA induced by the prebiotics. Neither prebiotic increased gene expression of CCR5 and CCR9 in the Peyer's patches, but antibiotics increased CCR9 expression. An unanticipated increase in IL-6 gene expression was noted with the TC and FOS, but the antibiotic treatment blocked this response. Although antibiotics suppressed IL-10 expression, neither TC nor FOS had an effect on this anti-inflammatory cytokine. However, both the TC and FOS suppressed the expression of the highly pro-inflammatory cytokine, IL-17. We conclude that alterations in gene expression in the Peyer's patches with FOS supports a decrease in the IL-17 and no change in IL-10, which differs from the alterations in Th17 and Treg cell populations in the lamina propria. Furthermore, our findings indicated that FOS's effects on bone may be mediated by some other mechanism than SCFAs' effects on T regulatory cells via the gut-bone axis.

INTRODUCTION

- Osteoporosis is a condition of low bone density and compromised bone microarchitecture that leads to painful and debilitating fractures (1). In the United States alone, 54 million adults currently have osteoporosis or are at high risk for this condition (1).
- Recent studies have revealed that the gut microbiota can affect bone mass and that manipulating the microbiota by feeding prebiotics and probiotics has favorable effects on bone mass and strength (4).
- Efforts to understand the link between the gut and bone have revealed that short chain fatty acid (SCFA; e.g., butyrate, propionate, acetate) produced by the fermentation of oligosaccharides may alter T cell populations and their activation (2).
- Naïve T cells home to gut-associated lymphoid tissues (GALT) such as the Peyer's patches and lamina propria where they can undergo differentiation into immunosuppressive T regulatory cells (Tregs) and pro-inflammatory T helper (Th17) cells (7). These processes are regulated by chemoattractant molecules (e.g., chemokine C-C motif receptor [CCR]5 and 9) and cytokines that regulate T cell differentiation (e.g., transforming growth factor [TGF]-β and interleukin [IL]-6) (3,8).
- The efflux of these T cells from the gut allows them to travel to the bone where Tregs which express IL-10 have the capacity to upregulate bone formation by osteoblast and Th17 cells which express IL-17 can accelerate bone resorption by osteoclast (5).
- Previously our lab has shown that supplementing the diet with tart cherries or their juice can restore bone in animals that have experienced age-related bone loss and reduce bone resorption in postmenopausal women (2,6).
- Tart cherries are known to be a good source of fructooligosaccharides (FOS) and phenolic compounds, both of which have beneficial effects on the bone. However, questions remain regarding their effects on T cell homing, differentiation, and activation.

PURPOSE

The purpose of this study was to examine the effects of two different sources of prebiotics (tart cherries or FOS) on cytokines and chemokines within the Peyer's patches of the gut that regulate T cell homing, differentiation (i.e., Treg vs Th17 cells) and activation.

METHODS

- Following a 2-wk acclimation period, 8-wk-old female C57BL/6 mice (Taconic Biosciences) were randomly assigned to treatments (**Figure 1**).
- At the end of 10 wks of treatment, mice were anesthetized for whole body dual-energy x-ray absorptiometry (DEXA) scans to assess the bone mineral density (BMD) and bone mineral content (BMC) and intestine samples were collected.
- Lymphocytes from the ileum were isolated and stained for florescence-activated cell sorting (FACS) to characterize Treg and Th17 cell populations.
- Fecal short chain fatty acid (SCFAs) were analyzed using gas chromatography techniques.
- qRT-PCR was performed on samples from the Peyer's patches to assess the relative abundance of mRNA for cytokines and chemokines involved in T cell homing, differentiation and activation. Cyclophilin b (Cyclo, or PPIB) was used as the invariant control.
- Data were analyzed using 2-way ANOVA with DIET and ABX as factors. The alpha was set at 0.05.

EXPERIMENTAL DESIGN

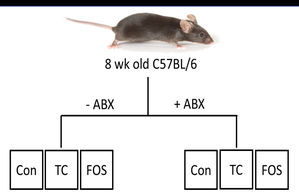

Figure 1: Female mice were randomly assigned to treatments in a 2 x 3 factorial design with DIET (Control, or Con diet supplemented with 10% Tart Cherry (TC) or 10% fructooligosaccharide (FOS) and ANTIBIOTIC (-- or +ABX) as factors. The ABX cocktail included metronidazole, ampicillin, vancomycin, neomycin and the anti-fungal, amphotercin B dissolved in drinking water. This cocktail was used to suppress the microbial populations and inhibit the effects of the prebiotics on the microbiota.

RESULTS

Table 1: Comparison of the Effects of Tart Cherry (TC) and Fructooligosaccharide (FOS) Supplementation With or Without Antibiotics on Bone Mineral Content (BMC) and Density (BMD)

	CON		TC		FOS		P-value		
	-ABX	+ABX	-ABX	+ABX	-ABX	+ABX	DIET	ABX	DIET*ABX
BMD (mg/cm²)	52.6±0.4^{b}	49.8±0.4^{c}	52.0±0.4^{b}	51.8±0.4^{b}	54.9±0.4$^{a\#}$	54.9±0.4$^{a\#}$	<.0001	0.0042	0.0018
BMC (mg)	484.1±9.0	446.7±9.0	499.2±8.7	442.8±8.7	516.1±8.4$^{\#}$	494.7±8.7$^{\#}$	<.0001	<.0001	0.1340

Table 2: Gut Lymphocytes Evaluated Using Florescence-activated Cell Sorting (FACS)

	CON		TC		FOS		P-value		
	-ABX	+ABX	-ABX	+ABX	-ABX	+ABX	DIET	ABX	DIET* ABX
CD4⁺	4443 ± 540^{b}	3984 ± 540^{b}	3188 ± 540^{b}	3554 ± 540^{b}	8882 ± 580$^{a\#}$	3673 ± 580$^{a\#}$	0.0001	0.0009	<0.0001
Tregs	431 ± 45^{b}	289 ± 42^{c}	234 ± 39$^{c\#}$	310 ± 39$^{c\#}$	872 ± 45$^{a\#}$	291 ± 39$^{c\#}$	0.0002	0.0006	<0.0001
Th17	143 ± 18	128 ± 18	94 ± 18	165 ± 21	165 ± 18	145 ± 19	0.3340	0.1901	0.0731

Abbreviations: CD4 positive (CD4+), T- regulatory cells (Tregs) and T helper 17 cells (Th17). Data were analyzed using 2-way ANOVA and are presented as LS mean ± SEM. Within a given row, groups that do not share the same superscript letter are significantly different from each other *(p<0.05)*. # denotes differences between a diet group compared to control. Values are expressed as absolute cell count.

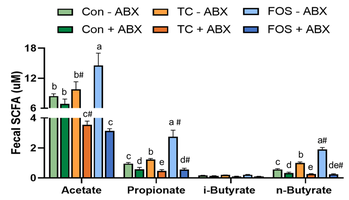

Figure 2. Fecal short chain fatty acids (SCFA) following 10 wks of dietary treatment (Control, tart cherry [TC] or fructooligosaccharides [FOS]) with or without antibiotics. Data analyzed using 2-way ANOVA. Bars represent the mean ± SEM. Bars that do not share the same superscript letter are significantly different (p<0.05)

Figure 3.A

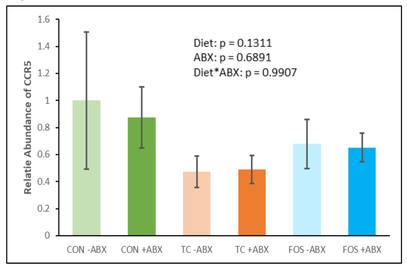

Figure 3.B

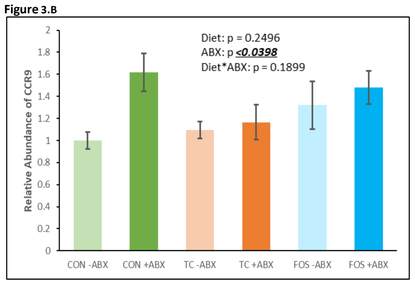

Figure 3. The effects of dietary supplementation of tart cherry (TC) or fructooligosaccharide (FOS) with or without antibiotics (ABX) on the relative abundance of chemokines in the Peyer's patches responsible for T cell homing to the gut, **A)** Chemokine C-C motif receptor 5 (CCR5) and **B)** Chemokine C-C motif receptor 9 (CCR9). Data were analyzed using 2-way ANOVA.

Figure 4.A

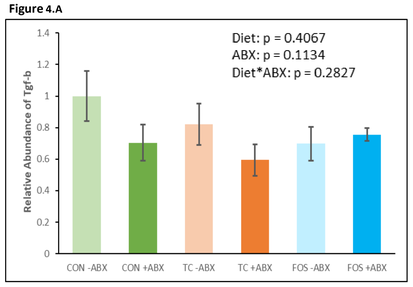

Figure 4.B

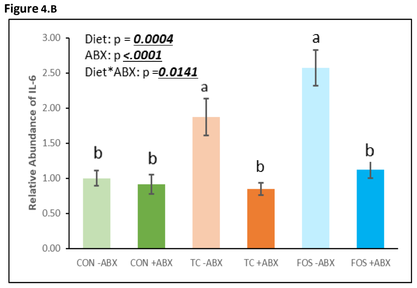

Figure 4. The effects of dietary supplementation of tart cherry (TC) or fructooligosaccharide (FOS) with or without antibiotics (ABX) on the relative abundance of cytokines in the Peyer's patches that regulate T cell differentiation, **A)** transformation growth factor – beta (Tgf-β) and **B) i**nterleukin 6 (IL-6). Data were analyzed using 2-way ANOVA. Bars that do not share the same superscript letters are significantly different (α=0.05).

RESULTS

Figure 5.A

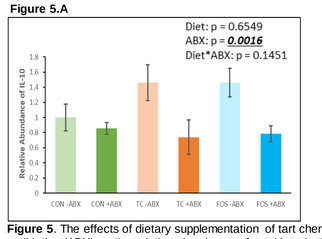

Figure 5.B

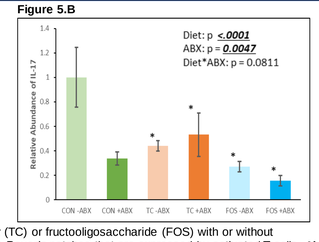

Figure 5. The effects of dietary supplementation of tart cherry (TC) or fructooligosaccharide (FOS) with or without antibiotics (ABX) on the relative abundance of cytokines in the Peyer's patches that are expressed by activated T cells, **A)** interleukin 10 (IL-10) by T regulatory cells or **B)** pro-inflammatory interleukin 17 (IL-17) expressed by Th17 cells. Data were analyzed using 2-way ANOVA. * denotes difference between TC or FOS diet groups compared to control.

Figure 6.A

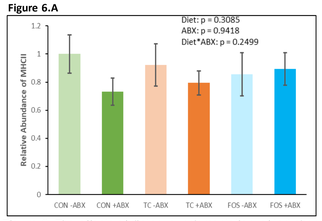

Figure 6.B

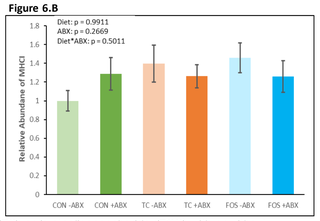

Figure 6. The effects of dietary supplementation of tart cherry (TC) or fructooligosaccharide (FOS) with or without antibiotics (ABX) on the relative abundance of major histocompatibility complex (MHC) classes I and II in Peyer's Patches, **A)** major histocompatibility complex class II (MHCII) and **B)** major histocompatibility complex class I (MHCI). Data were analyzed using 2-way ANOVA.

SUMMARY & CONCLUSION

- FOS supplementation, but not TC increased BMC and BMD. The benefits of FOS on BMD were not altered in presence of antibiotics.
- The immunosuppressive, T regulatory cells, were increased within the lamina propria of the ileum with FOS. This response was suppressed with antibiotics. No changes occurred in the pro-inflammatory Th17 cells with FOS. TC did not alter either T cell population.
- Fecal SCFAs were upregulated with both TC and FOS diets, but FOS had a greater effect. Antibiotics suppressed the increase in SCFA induced by the prebiotics' effect on the microbiota.
- Neither dietary supplementation with TC nor FOS increased gene expression of CCR5 and CCR9 in the Peyer's patches. Antibiotic treatment alone increased CCR9 expression.
- An unexpected increase in IL-6 was observed with the TC and FOS supplementation and the antibiotic treatment blocked this response.
- Antibiotics suppressed IL-10 expression, but neither TC or FOS had an effect.
- Both the TC and FOS suppressed the expression of the highly pro-inflammatory cytokine, IL-17.
- No changes were observed in the expression of Tgf-β, MHC-I or MHC-Ⅱ in response to diet or antibiotics.
- We concluded that alterations in gene expression in the Peyer's patches with FOS supports a decrease in the IL-17 and no change in IL-10, which differs from the alterations in Th17 and Treg cell populations in the lamina propria. Furthermore, FOS's effects on bone may be mediated by some other mechanism than SCFAs' effects on T regulatory cells.

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