Non-medicinal excipients in infant vitamin D drops, fecal microbiota and their metabolites at 3 months of age

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Introduction

- Health Canada recommends exclusively breastfed, healthy, term infants be supplemented with vitamin D. Vitamin D liquid formulations contain non-medicinal excipients, such as glycerin (glycerol) or propylene glycol (PG).
- Digested glycerin and PG are fermentable by gut microbiota, producing metabolites likely influencing the host.
- In this respect, we sought to assess infant fecal samples for the presence of glycerin and PG levels in relation to vitamin D supplementation and their association with gut microbiota and metabolites.

Objective

To investigate the impact of non-medicinal ingredients of infant vitamin D drops (i.e. glycerol and PG) on fecal microbiota and metabolites of clinical relevance.

Methods

- Fecal samples and vitamin D supplementation and breastfeeding status information were obtained at 3-month age for 575 infants from the Canadian Healthy Infant Longitudinal Development (CHILD) Study.
- Fecal metabolites and microbiota were quantified using Nuclear Magnetic Resonance Spectroscopy and 16S rRNA sequencing, respectively.
- Multiple linear and logistic regression were used to determine the association between vitamin D supplementation with fecal levels of glycerol and PG, adjusting for covariates.
- Spearman analysis was applied to evaluate correlations between taxa, metabolites and the fecal glycerol and PG.

Results

Tab 1. Multiple linear regression for prediction of fecal PG, glycerol concentrations (μmol/g) from use of infant vitamin D drops

Predictor	Fecal PG[a]		Fecal glycerol[b]	
	Crude β	Adjusted β	Crude β	Adjusted β
Infant vitamin D drops				
No (ref.)				
Yes	1.1 (0.8, 1.4)	0.4 (0.0, 0.7)	-0.2 (-0.4, 0.0)	-0.23 (-0.4, 0.0)
Covariates				
Feeding modes				
Exclusive breastfeeding (ref.)				
Partial breastfeeding	-1.0 (-1.4, -0.7)	-0.9 (-1.3, -0.6)	-0.3 (-0.5, 0.0)	-0.4 (-0.6, -0.2)
Exclusive formula	-2.4 (-2.7, -2.0)	-2.2 (-2.7, -1.8)	-0.0 (-0.2, 0.2)	-0.3 (-0.5, 0.0)
Introduce solids at 3 months				
Yes (ref.)				
Not yet	1.0 (0.2, 1.8)	-0.1 (-0.8, 0.6)	-0.1 (-0.5, 0.3)	0.02 (-0.4, 0.5)
Age of stool collection	-0.2 (-0.3, -0.1)	-0.3 (-0.4, -0.2)	-0.02 (-0.1, 0.1)	
Mother's education				
Less than high school (ref.)				
High school	0.3 (-0.2, 0.8)		-0.1 (-0.4, 0.1)	-0.1 (-0.4, 0.1)
College or University	1.1 (0.6, 1.6)		-0.4 (-0.6, -0.1)	-0.31 (-0.6, -0.1)
Birth modes				
Vaginal no IAP (ref.)				
Vaginal with IAP	0.1 (-0.4, 0.5)		-0.01 (-0.2, 0.2)	
Elective CS	-0.1 (-0.7, 0.5)		-0.2 (-0.5, 0.2)	
Emergency CS	-0.3 (-0.8, 0.2)		-0.2 (-0.4, 0.1)	
Maternal pre-pregnancy weight				
Underweight (ref.)				
Normal	-0.8 (-1.9, 0.4)		0.5 (-0.1, 1.1)	
Overweight	-0.8 (-1.9, 0.4)		0.2 (-0.4, 0.8)	
Obese	-1.4 (-2.5, -0.2)		0.4 (-0.2, 1.0)	
Maternal postnatal vitamins				
No (ref.)				
Yes	0.1 (-0.5, 0.8)		-0.2 (-0.5, 0.2)	
Intercept		1.4 (0.5, 2.4)		0.8 (0.3, 1.3)
R^2, adjusted		0.3		0.03

[a] Fecal PG was transformed using the formula, ln (PG + 0.030667) to reduce data skewness. The range of original PG in analytical samples were 0.00 to 30.36 (μmol/g).
[b] Fecal glycerol in the model was transformed using the formula, ln (Glycerol + 0.4052815) to reduce data skewness. The range of original glycerol in analytical samples were 0.00 to 19.68 (μmol/g).

Fig.1 Correlation analysis of fecal PG, glycerol and microbiota

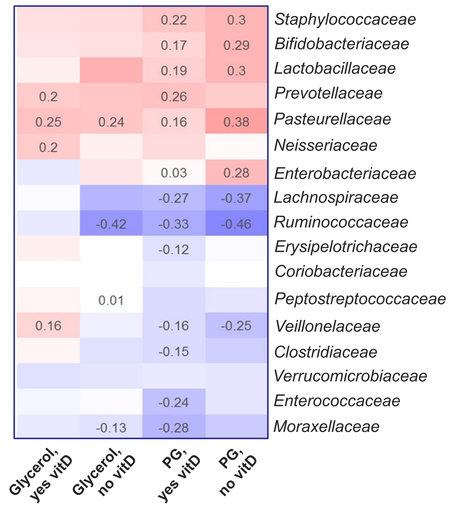


Fig.2 Correlation analysis of fecal PG, glycerol and SCFAs

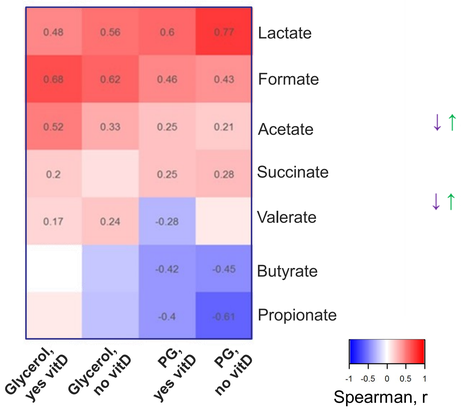
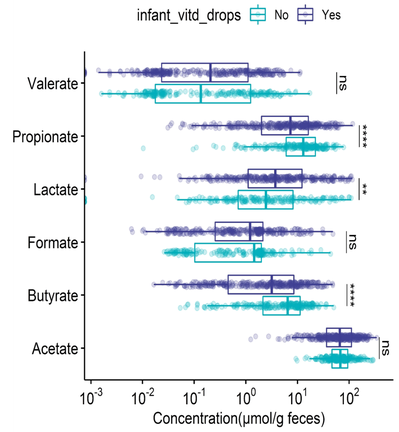
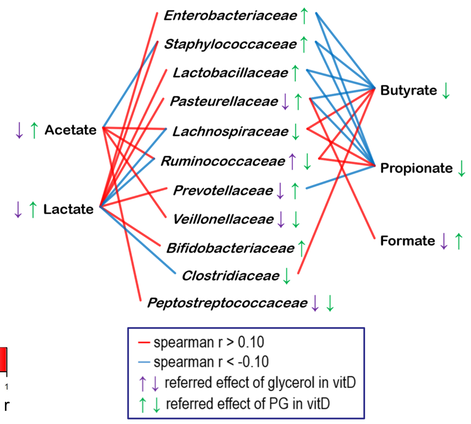


Fig.3 Comparison of fecal SCFAs by vitamin D drops groups



**** p.adj < 10^-5; ** p.adj <10^-3 ;
ns. Not significant. Wilcox test (BH-FDR).

Fig.4 Relationship between dietary glycerol, PG of vitamin D and microbiota and SCFAs



Conclusions

- Infants using vitamin D drops may have higher fecal PG, but lower fecal glycerol on average at 3 months, adjusting for feeding modes, solid foods at 3 months, age and maternal education.
- Vitamin D drops use may influence microbial metabolism (synthesis and hydrolysis) of glycerol and PG in the infant gut, resulting in increased lactate, but reduced propionate and butyrate.
- Dietary PG may be associated with changes in abundance of probiotic bacteria, such as *Bifidobacteriaceae.*

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