Fecal Short-Chain Fatty Acids in Protein-Supplementing Bodybuilders





University System AMU

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Introduction

The gut microbiota plays a crucial role in how diet affects human health, primarily through the production of short-chain fatty acids (SCFAs). The three primary SCFAs —acetate, propionate, and butyrate — are mainly produced by the fermentation of dietary fibers and serve as an important link between diet, microbial activity, and host health. Acetate regulates energy homeostasis in the gut and supports the growth of butyrateproducing bacteria. Propionate contributes to gluconeogenesis, a process that begins in the gut and supports systemic energy production. Butyrate maintains gut barrier integrity and serves as an energy source for colonocytes.

Protein is a key nutrient for muscle growth, and bodybuilders consume it in large quantities through diet and supplements to support resistance training. However, not all proteins are absorbed in the small intestine; some reach the colon, where they interact with the gut microbiota. Highprotein diets, combined with fiber intake and resistance training, may influence SCFA production by altering the microbial activity. While the significance of SCFA on gut health and various health benefits is well understood, limited studies have been conducted to examine SCFA production in the gut of bodybuilders, who often consume high-protein supplements.

Study Aim

The purpose of this study was to compare the levels of short-chain fatty acids in the fecal samples of bodybuilders based on their selfreported dietary fiber and protein supplement use.

Methods

Total of 39 fecal samples (26 males and 13 females) were collected and categorized into two groups : protein supplement (PS) and non-protein supplement (No PS)

Participant completed a 24-h food recall (ASA24) to obtain the nutrient data

Short-chain fatty acids were detected in fecal samples by

Table 1: Criteria used to select the participants

Inclusion	Exclusion	
>25 years	Prescription medications	
Actively building muscle	Laxatives in the last week	
Male or Female	Diarrhea inhibitors in the last week	
	Antibiotics in the last 3 months	
	Prebiotics in the last week	
	Probiotics in the last week	
	Cancer diagnoses	
	Irritable bowel disease	
	Crohn's disease	
	Currently tapering	



Fecal Analysis Metabolomics

LC-tims TOF-MS/MS

Results

Table 2. Participant characteristics.

	No PS (No Protein Supplement) (n = 17)	PS (Protein Supplement) (n = 22)	p- Value
Age (years)	33 ± 2	32 ± 1	0.84
Weight (lbs.)	176 ± 8	173 ± 7	0.79
Height (inches)	67 ± 1	67 ± 1	0.72
BMI (kg/m2)	27 ± 1	27 ± 1	0.99
Males	12	14	
Females	5	8	
Total Physical Activity (MET- minutes/week)	7040 ± 1282	12,081 ± 1870	0.03
Total Vigorous Activity (MET- minutes/week)	2535 ± 830	6331 ± 1284	0.02
Upper Body Resistance Exercise Volume (kg/week)	15, 743 ± 13,103	$31,067 \pm 49,323$	0.15
Lower Body Resistance Exercise Volume (kg/week)	16,694 ± 19,430	56,464 ± 127,594	0.16
Number of Supplements (count)	0.9 ± 1.5	1.4 ± 2.4	0.45

Table 3. Self-reported dietary intake from ASA24.

	No PS (n = 17)	PS (n = 22)	p- Value
Number of Foods	19 ± 2	21 ± 2	0.26
Energy (kcal)	2551 ± 429	2452 ± 199	0.84
Protein (g/ kg)	117.6 ± 11.8	169.3 ± 17.6	0.02
Carbohydrate (g)	228.3 ± 37.9	239.3 ± 25.9	0.81
Fiber (g)	18.9 ± 2.1	27.3 ± 3.1	0.03

Table 4. Short chain fatty acid values

	No PS (n = 17)	PS (n = 22)	p- Value
Acetic acid (m/z = 56)	791957 ± 74	573524 ± 47	0.02
Propionic acid (m/z= 57)	16.80 ± 6.15	19.22 ± 5.13	0.21
Butanoic acid (m/z = 71)	45.67 ± 11.92	44.85 ± 10.68	0.82

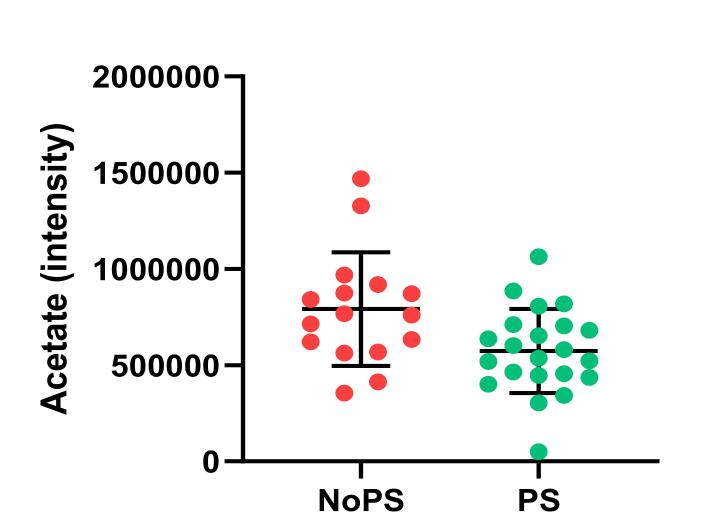


Figure 2: Acetate levels between NoPS and PS groups

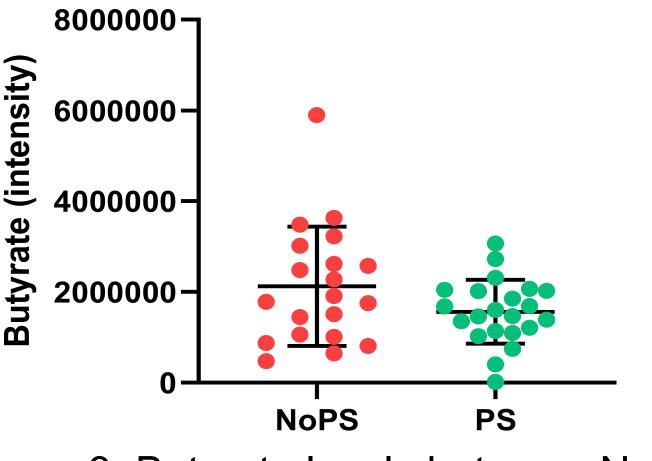


Figure 3: Butyrate levels between NoPS and PS groups

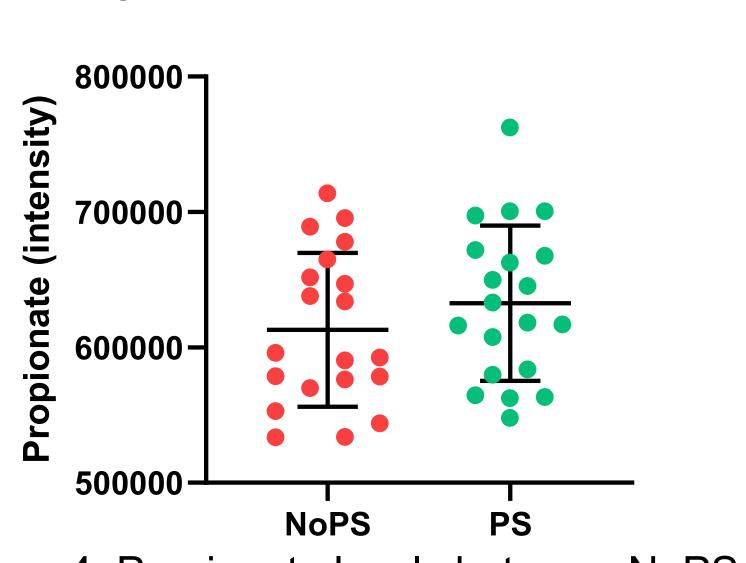


Figure 4: Propionate levels between NoPS and PS groups

Discussion

Undigested dietary fibers and other carbon chains, such as acetate, and deaminated amino acids, serve as sources for gut bacteria to produce SCFA. Previous studies have shown that a high-fiber diet increases butyrate levels by promoting the growth of butyrate-producing bacteria (Mukhopadhya & Louis, 2025). In contrast, a high-protein diet alters the proportions of SCFAs (Mann et al., 2024).

In this study, despite the PS group's higher fiber and protein intake, fecal acetate levels were significantly lower compared to the No PS group. This reduction may result from its consumption by butyrate-producing bacteria through cross-feeding, where acetate is used to produce butyrate (Louis & Flint, 2017). Although butyrate levels were higher than those of other SCFAs, the difference was not statistically significant.

The primary outcome of this study has been previously published (Byerley et al., 2022), the current findings present data on fecal SCFA in bodybuilders. There are limited peer-reviewed studies that specifically examine SCFA concentrations in this population. This analysis provides further insight into how diet, particularly protein and fiber, affects the production of SCFA in the gut.

Conclusions

- Protein and fiber consumption were higher for the PS group.
- Fecal butyrate was the SCFA in the highest abundance.
- Fecal butyrate and propionate were not significantly different between the groups.
- Fecal acetate levels were significantly lower for the PS group compared to the No PS group.
- Future studies are needed to identify the factors driving the lower acetate levels.

Acknowledgements

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References

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