A Pharmacokinetic Comparison of Three Butyrate Products

Original Research

Michael B. La Monica[1], Trevor Kirby [2], Shelley L. Hartshorn[1], Ashley Gustat[1], Jodi Grdic[1], Jennifer Sandrock[1]

[1]*The Center for Applied Health Sciences, Canfield, OH, USA*
[2]*Department of Pharmacotherapy, College of Pharmacy and Pharmaceutical Sciences, Washington State University, Spokane, WA, USA*

Open Access

Published: January 20, 2025

Journal of Exercise and Nutrition: *2025, Volume 8 (Issue 1): 4*

ISSN: 2640-2572

Abstract

Introduction: The aim of this study was to compare the pharmacokinetic (PK) parameters of lysine butyrate (LysB) to sodium (NaB) and tributyrin (TB).

Methods: Ten men (29.1 ± 10.4yr) completed this randomized, three-arm, crossover clinical trial (#NCT06700785) over four visits (a screening and three testing visits). Serum butyrate and indices of affect (well-being, calm/relaxed, stressed/anxious, mood, motivation to perform tasks, alertness, and concentration) were measured prior to product ingestion, and 20-, 45-, 90-, 150-, and 210-min post-ingestion. Each butyrate product delivered a total amount of 786 mg of butyric acid.

Results: There was a trend for an interaction (p=0.095) for serum butyrate concentrations, however there were no post hoc differences over time or between treatments. NaB (144±214µg/mL/min, p=0.042, d=0.75) and LysB (189±306µg/mL/min, p=0.023, d=0.86) had a significantly greater $AUC_{0\text{-}210}$ than TB (108±190µg/mL/min). NaB (2.51±4.13µg/mL, p<0.001, d=1.66) and LysB (4.53±7.56µg/mL, p=0.007, d=1.11) had a significantly greater Cmax than TB (0.91±1.65µg/mL). NaB (22.5±7.91min, p=0.008, d=1.21) and LysB (20.0±0.0min, p=0.004, d=1.45) had a significantly lower Tmax than TB (51.5±21.7min). There was a main effect of time for well-being (p=0.005), calm and relaxed (p=0.013), mood (p=0.002), motivation to perform tasks (p=0.040), alertness (p=0.035), and a treatment trend for concentration (p=0.063) while there were no differences between treatments over time for stressed and anxious (p>0.10).

Conclusions: This study is among the first to simultaneously evaluate three commercially available butyrate formulations in a controlled setting, which may help inform formulation-specific therapeutic strategies in the future. This PK study demonstrates that LysB and NaB exhibit greater bioavailability and more rapid systemic appearance compared to TB.

Key Words: short chain fatty acids; pharmacokinetics; gut-brain axis; lysine; tributyrin.

Corresponding author: Michael La Monica, ml@appliedhealthsciences.org

Introduction

Short chain fatty acids (SCFAs) are a subset of fatty acids with six or fewer carbon atoms that are predominantly formed in the lower gut through the microbial fermentation of dietary fibers. Within the human colon, acetate, propionate, and butyrate are the most abundant SCFAs [1]. Butyrate, a four-carbon SCFA, plays a crucial role in maintaining gut health and exerts various systematic effects on metabolism, immune function, and possibly neurological health. Butyrate plays several therapeutic roles including cancer suppression (prevents proliferation and induces apoptosis of colorectal cancer cells), inflammation reduction (reduction of NF-κβ), intestinal barrier regulation (stimulates the formation of mucin, antimicrobial peptides, and tight junction proteins), oxidative stress reduction (stimulates glutathione and decreases uric acid), antidiarrheal (stimulates reabsorption of water and sodium) [2,3], brain

health and cognitive function [may reduce neuroinflammation and promote neuroprotective gene expression, increase brain-derived neurotrophic factor (BDNF) levels, support neurogenesis] [4,5], and metabolic health (improve insulin sensitivity, reduce insulin resistance, serves as a substrate for mitochondrial energy production, promotes the expression of genes associated with fatty acid oxidation) [6]. Moreover, in the large intestine butyrate is mainly taken up by colonic epithelial cells and is thereby the preferred energy source for colonocytes [3]. Together, the various health benefits of butyrate make butyrate supplementation an attractive strategy for promoting human health and wellness.

Butyrate is produced by the gut microbiota from acetyl-CoA, lysine, glutarate, or succinate pathways in the colon [7]. Various species in the gut microbiota, such as *Faecalibacterium prausnitzii* and *Clostridium spp*., help synthesize butyrate from fermentable substrates [7]. Butyrate is rapidly absorbed by host epithelial cells through passive nonionic diffusion or active carrier-mediated transport [7]. Various transporters such as proton-coupled monocarboxylate transporter 1 (MCT1), sodium-coupled monocarboxylate transporter 1 (SMCT1), and solute carrier family 5 member 8 (SLC5A8) help carry the ionized form of butyrate into colonocytes [7]. The absorbed butyrate is metabolized by the intestinal epithelial cells where butyrate is converted to acetyl-CoA and enters the Krebs cycle in the mitochondria to produce ATP for colonocytes, while ~2% of butyrate enters the portal circulation arriving at the liver where butyrate is again metabolized into acetyl-CoA to become a substrate for fatty acids, cholesterol, and ketone bodies by hepatocytes, and any remaining butyrate is excreted through the lungs and urine [7,8].

Butyrate acts as a signaling molecule by binding to G-coupled receptors, such as GPR41 and GPR43 (also known as free fatty acid receptors FFA3/FFA2), which are presented on the intestines, liver, adipose tissue, bone marrow, and peripheral blood mononuclear cells [7–10] or as an inhibitor of histone deacetylase activity (HDACs) to modulate immune function/response [11]. Inhibition of HDAC modulates gene expression relevant to metabolic pathways, inflammation, and oxidative stress, making butyrate ideal for metabolic syndrome management, neuroprotection, and inflammatory diseases of the gut [12,13]. Butyrate has been shown to increase glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) secretion, particularly post prandial GLP-1 and PYY concentrations [14], via FFA3/FFA2 on the surface of L-cells providing potential benefits for metabolism and glucose regulation [15,16] and appetite suppression [17,18].

Several butyrate supplements exist commercially. These supplements typically come in the form of salts with minerals, like sodium butyrate (NaB), but also can include salts with other organic molecules, like lysine butyrate (LysB). While these types of butyrate supplements exist on the market, there are practical concerns about their usage or providing an unpleasant odor and flavor which presents adherence challenges for oral ingestion. Specifically, even a small amount of NaB provides a high sodium intake which can be problematic for individuals at risk for hypertension, those who are following a low sodium diet, and/or those at high risk of cardiovascular disease. On the other hand, preclinical experiments examining the organoleptic profiles of various butyrate supplement products rated LysB as the most palatable and provided a far more pleasant smell and taste than every other product [namely NaB and tributyrin (TB)]. Clinical/therapeutic implications for butyrate supplementation may depend on its deliverable form (i.e., free form or bound to triglycerides). For example, in murine models, butyrate covalently bound to triglyceride (e.g., butyrate esters) has an extended breakdown because it needs to be cleaved by gastric and pancreatic enzymes (i.e., lipase) and thus reaches further distally into the intestines/colon to support gut function [13,19]. More clinical research is needed to better understand and characterize the metabolism of butyrate esters and their final disposition in humans. Alternatively, the free form does not need to be broken down and thus can be absorbed earlier in the GI tract, possibly leading to enhanced circulating levels in the systemic vasculature, leaving at least some butyrate susceptible to reaching metabolically active organs and subsequently supporting metabolic regulation [10,20]. In this regard, it is worth noting that at orally ingested NaB also appears to survive the upper gastrointestinal tract in humans [21–23].

Tributyrin (propane-1,2,3-triyl tributanoate) is a pre-butyrate compound which is comprised of three separate butyric acid molecules esterified to a glycerol backbone. Because tributyrin is a precursor molecule, it is theoretically more resistant to gastric and pancreatic enzymes. Theoretically, this enhanced enzymatic resistance suggests that tributyrin may reach the large intestine intact or partially intact (e.g., as dibutyrin or monobutyrin) where it may exert beneficial effects. However, because of its low molecular weight and melting point, standard tributyrin is a liquid at room temperature. Pharmacokinetic studies on tributyrin have been done previously but explored in the context of cancer biology [24,25] or in mice and rat models [26]. These reports confirm that tributyrin has a significantly lower plasma appearance than butyrate salts.

Although NaB is well-studied, direct comparative data on the pharmacokinetics of different butyrate formulations is sparse. This study is among the first to simultaneously evaluate sodium, lysine, and tributyrin formulations in a

controlled setting which may eventually inform formulation-specific therapeutic strategies. By elucidating differences in absorption parameters including peak concentration and duration, this study seeks to identify which butyrate formulation might best suit clinical needs, such as immediate gut health support versus systemic anti-inflammatory applications. Lysine butyrate may offer improved palatability, tolerance, and therapeutic efficacy over NaB and TB, which could expand clinical applications for butyrate supplementation. Thus, this clinical trial sought to compare pharmacokinetic (PK) parameters of these analogues to understand their bioavailability and potential efficacy.

Methods

Protocol

This was a randomized, single-blinded, three-arm, crossover trial in which participants visited the laboratory on four occasions (one screening visit and three testing visits). This study was conducted according to the guidelines outlined in the Declaration of Helsinki of 1975, and all procedures involving human subjects were approved by the Advarra IRB on 4/29/23 (Pro00078858). Written informed consent was obtained from all subjects prior to enrollment. The study was retrospectively registered on clinicaltrials.gov (#NCT06700785). This study was conducted at The Center for Applied Health Sciences, a contract research organization located in Northeast Ohio. During the initial screening visit each participant's medical history and blood work [Complete Blood Count (CBC), Comprehensive Metabolic Panel (CMP), and lipid panel] were assessed to ensure they were within acceptable clinical ranges, body composition, and their 24-hr dietary recall was evaluated. During the testing visits (visits 2, 3, and 4 which were spaced at least 7 days apart), participants completed all baseline assessments before consuming one of three active study products. A Latin Square design was used for randomization. Assessments included serum butyrate levels at baseline (prior to product ingestion), and 20-, 45-, 90-, 150- 210-min post ingestion, as well as subjective feelings of affect (well-being, calm and relaxed, stressed and anxious, mood, motivation to perform tasks, alertness, and concentration), and vital signs (prior to product ingestion), and 45-, 90-, 150-, and 210-min post-ingestion.

Participants

Given the novelty of this investigation, a small pilot of 10 healthy men participated and completed all testing visits (See Table 1 for participant characteristics). Potential participants were deemed eligible if they were in good health as determined by medical history and safety screening blood work (CBC, CMP, and lipid panel), between the ages of 25 and 45 years, had a body mass index (BMI) of 18.5-25.9 $kg \bullet m^{-2}$, weighed a minimum of 110 lbs (50 kg), did not exhibit moderate-to-severe hypertension (i.e., resting SBP ≤140 mm Hg and DBP ≤90 mm Hg), possessed a resting heart rate ≤90 bpm. Prior to participation, all participants indicated their willingness to comply with all aspects of the experimental and supplement protocol. Participants were excluded if they: (a) had a history of diabetes or pre-diabetes or any endocrine disorder, hepatorenal, musculoskeletal, autoimmune, or neurologic disease; (b) had a history of malignancy in the previous 5 years except for non-melanoma skin cancer (basal cell cancer or squamous cell cancer of the skin); (c) had prior gastrointestinal bypass surgery; (d) had medical diagnoses of gastrointestinal or metabolic diseases that might impact nutrient absorption or metabolism (e.g. short bowel syndrome, diarrheal illnesses, history of colon resection, gastroparesis, Inborn-Errors-of-Metabolism); (e) had medically-diagnosed chronic inflammatory conditions or diseases (e.g., rheumatoid arthritis, Crohn's Disease, ulcerative colitis, lupus, HIV/AIDS); (f) had previous medical diagnoses of asthma, gout, or fibromyalgia; (g) had history of unstable or new onset cardiovascular, liver, renal, or thyroid disease or current use of thyroid, hyperlipidemic, hypoglycemic, anti-hypertensive, or anti-coagulant medication/s; (h) history of using butyrate or tributyrin-containing dietary supplements within the past seven days. (i) were current smokers, nicotine users, or discontinued smoking within one month of enrollment, (j) had a known allergy to any of the ingredients in the study products; (k) had currently been participating in another research study with an investigational product or have been in another research study in the past 30 days; (l) used corticosteroids or testosterone replacement therapy (ingestion, injection, or transdermal); (m) possessed a history of or recent treatment for alcohol ingestion or history of drug/alcohol dependence/abuse; (n) were excessive consumers of alcohol (>2 drinks per day or >10 drinks per week); (o) possessed fasting blood sugar >125 mg/dL; (p) had any other diseases or conditions that, in the opinion of the medical staff, could confound primary endpoints or place the participant at increased risk of harm if they were to participate; or (q) did not demonstrate a verbal understanding of the informed consent document.

Participants were instructed to follow their normal dietary and activity patterns throughout their period of enrollment in the study. Participants were required to complete a 24-hour diet record prior to arriving at the laboratory for their initial screening visit. Participants were given a copy of this dietary record and instructed to duplicate their food and liquid intake 24 hours prior to each subsequent laboratory visit. Prior to each subsequent visit, participants were asked to verbally confirm their previous day's 24-hour diet adherence. In addition, the participants were required to refrain

from exercise, caffeine, and alcohol for 24 hours and arrive after a 10-hour fast. All compliance with these requirements was verbally confirmed by a questionnaire at the beginning of each study visit.

Serum Butyrate

An intravenous catheter was inserted into each subject at the beginning of their testing visit for blood samples collected over the 3.5hr testing period. Serum blood samples were collected at 0 minutes (baseline) prior to the administration of the study product, then at 20 min, 45 min, 90 min, 150 min, and 210 min following ingestion of the study product. Samples were sent out for third-party testing (Creative Proteomics, Shirley, NY, USA). The quantification of free Short Chain Fatty Acids in serum was performed using GC-MS and due to its precision and accuracy samples were run in singlet. 100 μL serum was thawed and diluted with isotopically labelled internal standards. Free short-chain fatty acids were derivatized using methyl chloroformate in 1-propanol yielding propyl esters before subsequent liquid-liquid extraction into hexane and analysis on an Agilent 6890GC coupled to an Agilent 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA, USA). Separation was performed on an Agilent HP-5ms (30 x 0,25 x 1,0μm) column and quantification was performed using GC-EI-MS in SIM-mode against a 5-point calibration curve. Any values less than the LOQ of 0.029 were treated as missing data and handled as such.

Visual-Analog Scales (VAS)

100-mm anchored VAS were completed prior to the administration of the study product (0 min), and 45-, 90-, 150-, and 210-min post product ingestion on testing visits 2, 3, and 4. VAS assessed subjective ratings of state of well-being, calm and relaxed, stressed and anxious, mood, motivation to perform tasks, level of alertness, and ability to concentrate and were anchored with "Worst possible", "Lowest possible", "Strongly Disagree", or "Best Possible", "Highest Possible", "Strongly Agree". The validity and reliability of VAS in assessing similar subjective constructs have been previously established [27] and reported [28,29].

Study Product

Participants randomly received one of the butyrate products in a randomized order [Sodium butyrate (NaB), Lysine butyrate (LysB, as BIOMEnd™), or Tributyrin (TB, as CoreBiome®)]. Each product was administered in 3 capsules and delivered a total amount of 786 mg of butyric acid.

Statistical Analysis

Normality was assessed using Q-Q plot and Shapiro-Wilks test. Severe non-normal measures were normalized using log (ln) transformation. Serum butyrate concentrations, subjective ratings of affect, and vitals over time between treatments were analyzed using a mixed effects factorial ANOVA. Tukey post hocs were applied if significant main effects/interactions were observed. Separate paired samples t-tests were used to compare Cmax and $AUC_{0\text{-}210}$ (trapezoid method) between treatments whereas Wilcoxon rank test was used to compare Tmax between treatments because the latter data were not normally distributed. Change scores (i.e., deltas) were also computed for each timepoint relative to 0 min (i.e., 20 – 0 min, 45 – 0 min, 90 – 0 min, 150 – 0 min, and 210 – 0 min). Change scores were also analyzed using a mixed effects factorial ANOVA. P-values ≤ 0.05 were considered significant and p-values ≤ 0.10 were considered trends indicating a possible difference between treatments or over time. Effect sizes are reported as Cohen's d (with 0.2 considered a small effect, 0.5 considered a medium effect, and 0.8 considered a large effect). All analyses were conducted in GraphPad Prism v.10.4.0.

Results

Demographic & Baseline Characteristics

Table 1. Describes the characteristics of the 10 men that participated and completed all testing visits.

Table 1. Participant characteristics (N=10).

Age (yr)	29.1 ± 10.4
SBP (mmHg)	120.3 ± 13.6
DBP (mmHg)	72.6 ± 8.2
HR (BPM)	61.0 ± 8.6
BMI (kg/m^2)	24.3 ± 1.2
Weight (kg)	80.3 ± 5.8
Height (cm)	181.6 ± 3.6
Body Fat (%)	11.7 ± 4.3

Data are Means ± SD. SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; HR: Heart Rate; BMI: Body Mass Index.

Serum Butyrate

For serum butyrate levels there was a trend for an interaction (p=0.095) and time (p=0.100), but not for treatment (p=0.103). However, there were no post hoc differences. For delta butyrate there was a trend for interaction (p=0.059), time (p=0.063), and treatment (p=0.071). However, there were no post hoc differences. Regarding $AUC_{0\text{-}210}$, LysB did not differ from NaB (t=0.454, p=0.660, d=0.14 "small effect"), but NaB (t=2.37, p=0.042, d=0.75 "medium effect") and LysB (t=2.73, p=0.023, d=0.86 "large effect") were significantly greater than TB. Regarding Cmax, LysB did not differ from NaB (t=1.01, p=0.340, d=0.32 "small effect"), but NaB (t=5.25, p<0.001, d=1.66 "large effect") and LysB (t=3.51, p=0.007, d=1.11 "large effect") were significantly greater than TB. Regarding Tmax, LysB did not differ from NaB (sum of signed ranks=-1.0, p>0.999, d=0.32 "small effect"), but NaB (sum of signed ranks=36, p=0.008, d=1.21 "large effect") and LysB (sum of signed ranks=45, p=0.004, d=1.45 "large effect") were significantly less than TB.

Figure 1. Butyrate values over time for each treatment.

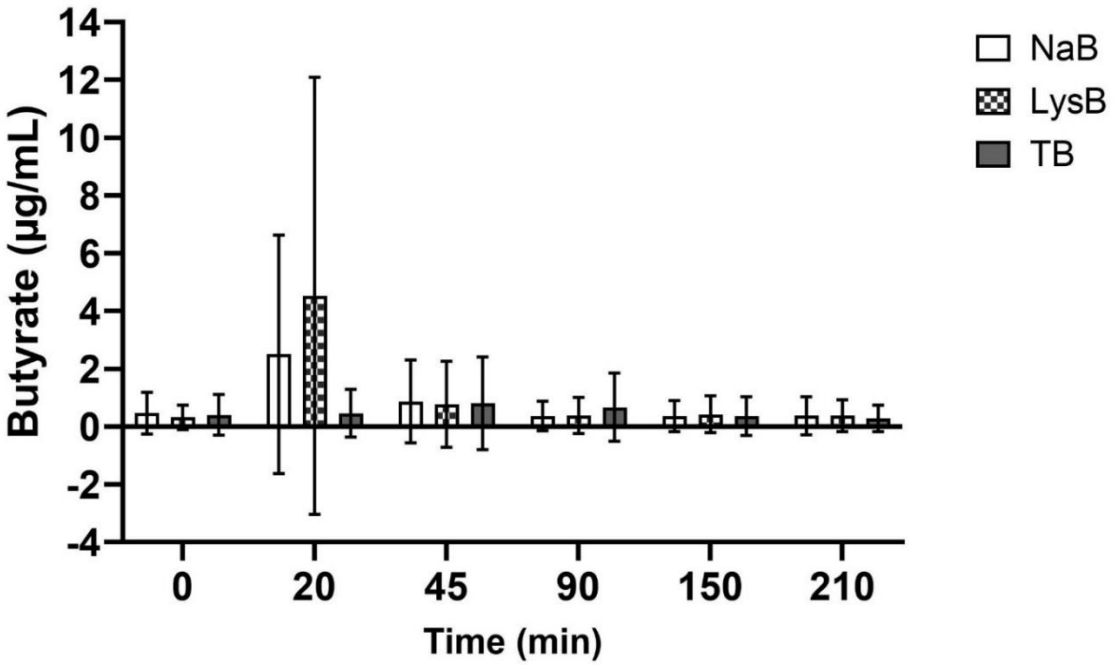

Figure 1 illustrates Means ± SD. Note. NaB=Sodium butyrate; LysB = Lysine butyrate; TB=Tributyrin

Visual-Analog Scales (VAS)

There was a main effect of time for well-being (p=0.005), calm and relaxed (p=0.013), mood (p=0.002), motivation to perform tasks (p=0.040), alertness (p=0.035), and a treatment trend for concentration (p=0.063). There were no differences between treatments over time for stressed and anxious (p>0.10).

Post hoc analysis for well-being showed a significantly greater sense of well-being for LysB at 210 min post ingestion as compared to 0 min (p=0.039) and 90 min (p=0.049) and possibly compared to 45 min (p=0.099) and 150 min (p=0.061). Similarly, TB had a significantly greater sense of well-being at 210 min post ingestion as compared to 0 min (p=0.018) and 90 min (p=0.038). There was no difference between treatments within well-being AUC (p=0.489). Post hoc analysis for calm and relaxed showed a possible greater feeling of calm and relaxed for NaB at 150 min as compared to 45 min (p=0.096) while LysB had a possible higher feeling at 210 min as compared to 0 min (p=0.052), 45 min (p=0.098), and 150 min (p=0.080), and TB had a significantly lower feeling at 45 min as compared to 90 min (p=0.047) and 210 min (p=0.048). There was no difference between treatments within calm and relaxed AUC (p=0.735). Post hoc analysis for mood showed a better overall mood for LysB at 210 min vs. 0 min (p=0.050) while TB possibly had

Table 2. Serum butyrate values over time.

	Treatment	0 min	20 min	45 min	90 min	150 min	210 min	AUC_{0-210}	Cmax	Tmax (min)
Serum Butyrate (μg/mL)	NaB	0.47 ± 0.73	2.50 ± 4.13	0.87 ± 1.44	0.37 ± 0.51	0.36 ± 0.53	0.38 ± 0.66	144 ± 214*	2.51 ± 4.13*	22.5 ± 7.91*
	LysB	0.32 ± 0.43	4.53 ± 7.56	0.77 ± 1.49	0.39 ± 0.62	0.43 ± 0.64	0.37 ± 0.55	189 ± 306*	4.53 ± 7.56*	20.0 ± 0.0*
	TB	0.41 ± 0.71	0.47 ± 0.83	0.80 ± 1.61	0.67 ± 1.18	0.36 ± 0.66	0.28 ± 0.46	108 ± 190	0.91 ± 1.65	51.5 ± 21.7

Data are Means ± SD. NaB=Sodium butyrate; LysB = Lysine butyrate; TB=Tributyrin; AUC=Area under the curve; Cmax=Concentration maximum; Tmax=Time to concentration maximum. * Significantly different vs. TB ($p \leq 0.05$). AUC_{0-210} and Cmax were transformed for analysis, but raw untransformed data are shown.

a better mood at 210 min as compared to 0 min (p=0.063), 45 min (p=0.058), and 90 min (p=0.061). There was no difference between treatments within mood AUC (p=0.205). Post hoc analysis for motivation to perform tasks showed a possibly higher motivation to perform tasks at 90 min (p=0.086) and 210 min (p=0.074) vs. 0 min for NaB. There was no difference between treatments within motivation to perform tasks AUC (p=0.461). Post hoc analysis for alertness showed a possibly higher level of alertness at 150 min as compared to 0 min (p=0.059) for LysB and a significantly higher alertness at 210 min vs. 90 min (p=0.033) and possibly 150 min (p=0.078) for TB. There was no difference between treatments within alertness AUC (p=0.169). Post hoc analysis for concentration showed that LysB was possibly greater than NaB at 90 min (p=0.081) and that TB was significantly greater than NaB at 150 min (p=0.025). There was a trend (p=0.051) for treatment differences within concentration AUC, however there were no post hoc differences. There was no difference between treatments or over time (Time: p=0.139; Treatment: p=0.439; Treatment x Time: p=0.711) for stressed and anxious as well as AUC (p=0.774).

There was a significant main effect of time for delta well-being (p=0.015), calm and relaxed (p=0.006), mood (p=0.014), and a time trend for stressed and anxious (p=0.058) while there were no differences between treatments or over time for delta motivation to perform tasks, alertness, and concentration (all p>0.10).

Post hoc analysis for delta well-being showed that 210-0 min was significantly greater than 150-0 min (p=0.043) and 90-0 min (p=0.034) while possibly greater than 45-0 min (p=0.070) for LysB while 210-0 min was significantly greater than 90-0 min (p=0.026) for TB. Post hoc analysis for delta calm and relaxed showed that 150-0 min had a positive change that was possibly different than the negative change at 45-0 min (p=0.068) for NaB, 210-0 min was possibly larger than 45-0 min (p=0.070) and 150-0 min (p=0.057) for LysB, and 45-0 min had a reduction that was significantly different than the positive change from 90-0 min (p=0.033) and 210-0 min (p=0.033) for TB. Also, for TB, 210-0 min had a positive change that was possibly different than the reduction from 150-0 min (p=0.079). Post hoc analysis for mood showed that 150-0 min was possibly greater than 45-0 min (p=0.095) for NaB and that 210-0 min was significantly greater than 45-0 min (p=0.041), 90-0 min (p=0.043), and possibly 150-0 min (p=0.077) for TB. Post hoc analysis for stressed and anxious showed that the reduction (i.e., positive change) from 150-0 min was significantly different than the increase (i.e., negative change) from 90-0 min (p=0.046) for LysB.

Vitals

There was a significant main effect of time (p=0.050) and treatment (p=0.041) for SBP. Post hoc analyses showed that TB had a significantly higher SBP than NaB at 0 min (p=0.007) and at 45 min (p=0.046). There was a time trend (p=0.090) for HR, however there were no post hoc differences. There were no differences between treatments over time for DBP (p>0.10).

AEs

All treatments were well tolerated and there are no adverse events to report in this study.

Table 3. Visual Analogue Scales values over time.

	Treatment	0 min	45 min	90 min	150 min	210 min	AUC_{0-210}
Well-being (cm)	NaB	7.0 ± 1.2	7.2 ± 1.2	7.3 ± 1.2	7.5 ± 1.1	7.6 ± 1.3	1543 ± 242
	LysB	7.3 ± 0.9*	7.6 ± 0.9#	7.6 ± 1.1*	7.7 ± 1.0#	8.0 ± 0.9	1610 ± 195
	TB	7.4 ± 1.2*	7.6 ± 1.1	7.6 ± 1.2*	7.5 ± 1.1	7.8 ± 1.2	1588 ± 234
Calm and Relaxed (cm)	NaB	7.6 ± 0.9	7.3 ± 1.2	7.6 ± 1.0	7.9 ± 1.1¥	7.9 ± 1.2	1608 ± 216
	LysB	7.5 ± 0.8#	7.7 ± 0.9#	7.6 ± 1.2	7.6 ± 1.1#	8.0 ± 0.9	1612 ± 207
	TB	7.5 ± 1.2	7.2 ± 1.4ǂ*	7.5 ± 1.3	7.5 ± 1.2	7.9 ± 1.2	1571 ± 245
Stressed and Anxious (cm)	NaB	2.4 ± 1.4	2.3 ± 1.5	2.3 ± 1.6	2.1 ± 1.8	2.0 ± 1.8	466 ± 339
	LysB	2.0 ± 1.4	2.1 ± 1.6	2.3 ± 1.7	1.8 ± 1.3	2.0 ± 1.3	431 ± 288
	TB	2.7 ± 2.0	2.7 ± 2.2	2.4 ± 1.9	2.4 ± 2.0	2.3 ± 2.2	518 ± 408
Mood (cm)	NaB	7.1 ± 1.2	7.1 ± 1.1	7.4 ± 1.2	7.7 ± 1.0	7.8 ± 1.2	1558 ± 215
	LysB	7.7 ± 0.9*	7.9 ± 0.7	7.6 ± 1.1	8.0 ± 0.7	8.0 ± 0.8	1651 ± 171
	TB	7.1 ± 1.5#	7.5 ± 1.1#	7.6 ± 1.0#	7.7 ± 1.0	8.0 ± 1.0	1600 ± 214
Perform Tasks (cm)	NaB	6.5 ± 1.4£ #	6.8 ± 1.6	7.1 ± 1.3	6.9 ± 1.7	7.1 ± 1.5	1452 ± 297
	LysB	6.9 ± 1.7	7.1 ± 1.3	7.4 ± 1.1	7.6 ± 1.3	7.6 ± 1.3	1547 ± 262
	TB	6.9 ± 1.6	7.2 ± 1.3	7.1 ± 1.7	7.2 ± 1.8	7.3 ± 1.9	1505 ± 331
Alertness (cm)	NaB	6.4 ± 1.2	6.5 ± 1.6	6.8 ± 1.3	6.7 ± 1.6	7.1 ± 1.8	1411 ± 293
	LysB	6.9 ± 1.5€	7.2 ± 1.3	7.4 ± 1.2	7.5 ± 1.2	7.5 ± 1.1	1544 ± 249
	TB	6.7 ± 1.7	7.0 ± 1.5	6.9 ± 1.4*	7.1 ± 1.6#	7.5 ± 1.5	1481 ± 293
Concentration (cm)	NaB	6.4 ± 1.2	6.4 ± 1.7	6.6 ± 1.5	6.5 ± 1.9	6.9 ± 2.1	1371 ± 343
	LysB	7.1 ± 1.3	7.1 ± 1.4	7.5 ± 1.3β	7.4 ± 1.1	7.3 ± 1.6	1532 ± 241
	TB	6.9 ± 1.7	7.1 ± 1.6	6.9 ± 1.6	7.4 ± 1.4α	7.5 ± 1.6	1504 ± 315

Data are Means ± SD. NaB=Sodium butyrate; LysB = Lysine butyrate; TB=Tributyrin; AUC=Area under the curve; * Significantly different vs. 210 min ($p \leq 0.05$). # Trend vs. 210 min ($p \leq 0.10$). ǂ Significantly different vs. 90 min ($p \leq 0.05$). ¥ Trend vs. 45 min ($p \leq 0.10$). £ Trend vs. 90 min ($p \leq 0.10$). € Trend vs. 150 min ($p \leq 0.10$). β Trend vs. NaB ($p \leq 0.10$). α Significantly different vs NaB ($p \leq 0.05$).

Discussion

This pharmacokinetic study of three butyrate products showed significantly higher overall responses for butyrate (AUC_{0-210} and Cmax values) along with a quicker time to peak concentrations (lower Tmax values) for NaB and LysB vs. TB. This illustrates that NaB and LysB appear to be more bio-accessible within a quicker timeframe for systemic circulation than TB. Regarding feelings of affect, LysB and TB appeared to improve the sense of well-being 210 min

post ingestion, LysB may have promoted a greater sense of calm and relaxation and improved mood 210 min post ingestion while TB may have improved mood 210 min post ingestion, NaB may have increased motivation to perform tasks 90- and 210 min post ingestion, LysB may have increased alertness at 150 min post ingestion, and concentration may have been greater in LysB than NaB at 90 min while TB was greater than NaB at 150 min. Lastly all treatments were well tolerated with no adverse events or impact on vital signs.

The pharmacokinetics results were consistent with previous literature both for the butyrate salts as well as for TB [25,30]. Here, we demonstrate that the plasma appearance of TB was significantly lower when compared to NaB and LysB. This is likely due to the requirement for the butyrate molecules in TB to undergo enzymatic cleavage, which delays or significantly reduces the release of butyrate from the prodrug. Cmax was also significantly lower for TB when compared to NaB and LysB. While some release of butyrate was expected from TB during digestion, the release of butyrate did not significantly increase plasma concentrations from baseline. Both NaB and LysB reached Cmax at the observed 20 min time point which is also consistent with previous data for butyrate salts [30]. NaB and LysB are ionic compounds that easily dissociate into water and thus rapidly absorb as shown by a quicker Tmax within the plasma. Increased systemic availability of butyrate may enhance its uptake by metabolically active tissues and organs, such as adipose tissue, brain, muscle, liver, and pancreas. This uptake can lead to beneficial metabolic adaptations, including improved lipid and glucose homeostasis in the liver, reduced lipid accumulation in brown adipose tissue, liver, and muscle, enhanced mitochondrial function in skeletal muscle and liver, improved insulin sensitivity, and activation of GLP-1 receptor genes. Additionally, butyrate may help mitigate inflammation by reducing lipopolysaccharide-induced cytokine production in dendritic cells and decreasing joint inflammation, as observed in gout models [1,32]. Since lysine is a key molecule in the metabolism of butyrate, there may be additional benefits to LysB from a formulation perspective beyond the potential enhancement in plasma appearance. Although speculative, various disease states may be prevented or improved through the administration of LysB such as solid tumor cancers, blood disorders, epilepsy, type 2 diabetes, cardiovascular disease, arthritis, obesity, depression and anxiety, Alzheimer's disease, and/or Parkinson's Disease. More research is needed to fully understand the role lysine from LysB plays in the butyrate metabolism. On the other hand, in some animal models, TB has been shown to have the chemical stability to reach the large intestines releasing butyrate slowly over time, which matches the smaller and smoother curve in butyrate levels we observed. It is possible that the low appearance in plasma for TB suggests that it may have reached the large intestines. Delivery to the distal gut may aid in preserving intestinal barrier function, reducing the severity of colitis, protecting against severe allergic food reactions [13] and suppress tumor activity in colon cells [19]. Continued research on the various forms of butyrate supplementation is warranted to further understand what form is ideal for a targeted therapeutic benefit.

Butyrate's uptake and influence in the brain may be the reason why we observed transient fluctuations in feelings of affect. For example, a whole-body PK study in baboons showed that butyrate was rapidly metabolized and distributed to the spleen, pancreas, and in low concentrations within the brain (over 90 min), suggesting that high doses are necessary for potential therapeutic interventions in memory and learning [32]. Further, isotope tracing suggests that butyrate clearly is distributed to peripheral tissues (small and large intestines, brain, brown and white adipose tissue) where it may impart various beneficial effects. Butyrate has also been reported to impact brain function through the gut-brain neural circuit (i.e., subdiaphragmatic vagus nerve) [17] as suggested through metabolism in the Krebs cycle [33]. These data likely result from the rapid absorption and subsequent distribution of butyrate only possible by the butyrate salts (like NaB or LysB). TB, conversely, has a slower absorption and distribution and is thus thought to only confer clinical effects in the colon where most of the gut microbiota is located. While there is promise for oral butyrate for colorectal cancer [21,34], the rapid utilization of butyrate before reaching the colon results in poor oral bioavailability and is a valid pharmaceutical concern leading others to formulate butyrate prodrugs [35,36].

This study is one of the first to evaluate and compare three distinct butyrate formulations in a controlled setting, offering crucial insights for developing formulation-specific therapeutic strategies. Butyrate's suitability for clinical use suggests these formulations may operate under different therapeutic needs based on their PK profiles (gut health vs. systemic inflammatory and neurological effects). Further studies on serum leukocytes are warranted to determine if the butyrate salts can increase cellular concentrations of butyrate or enhance immune function. We acknowledge the novelty of this study and since this is the first step in a series of follow-up investigations there are some limitations to acknowledge, such as the absence of fecal or urine butyrate levels to examine the metabolism (by colonocytes and/or hepatocytes) and excretion of butyrate, the truncated timeline for serum measurements (<4hr), interindividual variability [as a few participants had hyper responses (i.e., elevated plasma butyrate concentrations)] compared to the majority of participants for each formulation), and a relatively small sample size. Future studies should include more

definitive measurements of bioavailability to assess absorption, distribution, metabolism, and excretion and metagenomic analyses to see if colonic delivery of butyrate would create meaningful changes in gut microbiota communities. Pharmacogenomics would be meaningful to explore as well to potentially explain metabolic differences between hyper-responders and non-responders. In addition, future studies should explore beneficial clinical outcomes comparing rapid release or delayed release butyrate in the plasma, formulation modifications, or investigate specific populations.

Informed Consent Statement:
Informed consent was obtained from all subjects involved in the study.

Funding:
This research was funded in part by The American Institute of Gastrointestinal Health.

Conflicts of Interest:
The authors declare no conflict of interest. The funders had no role in the collection, analyses, interpretation of data, or in the writing of the manuscript. This study was conducted at The Center for Applied Health Sciences in Ohio, where Tim N. Ziegenfuss, PhD, is a principal owner. While Tim Ziegenfuss had no direct involvement in the execution (which was conducted and led by the PI, Michael La Monica, PhD) of this study (including recruiting, data collection, statistical analyses, or writing of the manuscript), he is a co-inventor of a patent on lysine butyrate. He is disclosing his intellectual property interests to maintain research integrity and transparency. In addition, none of the co-inventors of the patent had any direct contact with study participants or study personnel.

References

1. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes*. 2016;7(3):189-200. doi:10.1080/19490976.2015.1134082
2. Leonel AJ, Alvarez-Leite JI. Butyrate: implications for intestinal function. *Current Opinion in Clinical Nutrition & Metabolic Care*. 2012;15(5):474-479.
3. Rivière A, Selak M, Lantin D, Leroy F, De Vuyst L. Bifidobacteria and butyrate-producing colon bacteria: importance and strategies for their stimulation in the human gut. *Frontiers in microbiology*. 2016;7:979.
4. Bourassa MW, Alim I, Bultman SJ, Ratan RR. Butyrate, neuroepigenetics and the gut microbiome: Can a high fiber diet improve brain health? *Neuroscience Letters*. 2016;625:56-63. doi:10.1016/j.neulet.2016.02.009
5. Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan TG, Cryan JF. The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis? *Neurochemistry International*. 2016;99:110-132. doi:10.1016/j.neuint.2016.06.011
6. Gao Z, Yin J, Zhang J, et al. Butyrate Improves Insulin Sensitivity and Increases Energy Expenditure in Mice. *Diabetes*. 2009;58(7):1509-1517. doi:10.2337/db08-1637
7. Recharla N, Geesala R, Shi XZ. Gut Microbial Metabolite Butyrate and Its Therapeutic Role in Inflammatory Bowel Disease: A Literature Review. *Nutrients*. 2023;15(10):2275. doi:10.3390/nu15102275
8. Guilloteau P, Martin L, Eeckhaut V, Ducatelle R, Zabielski R, Immerseel FV. From the gut to the peripheral tissues: the multiple effects of butyrate. *Nutrition Research Reviews*. 2010;23(2):366-384. doi:10.1017/S0954422410000247
9. Kimura I, Inoue D, Maeda T, et al. Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proceedings of the National Academy of Sciences*. 2011;108(19):8030-8035. doi:10.1073/pnas.1016088108
10. Van Deuren T, Smolders L, Hartog A, et al. Butyrate and hexanoate-enriched triglycerides increase postprandrial systemic butyrate and hexanoate in men with overweight/obesity: A double-blind placebo-controlled randomized crossover trial. *Frontiers in nutrition*. 2023;9:1066950. doi:10.3389/fnut.2022.1066950
11. Davie JR. Inhibition of Histone Deacetylase Activity by Butyrate. *The Journal of Nutrition*. 2003;133(7):2485S-2493S. doi:10.1093/jn/133.7.2485S
12. Donohoe DR, Garge N, Zhang X, et al. The Microbiome and Butyrate Regulate Energy Metabolism and Autophagy in the Mammalian Colon. *Cell Metabolism*. 2011;13(5):517-526. doi:10.1016/j.cmet.2011.02.018
13. Wang R, Cao S, Bashir MEH, et al. Treatment of peanut allergy and colitis in mice via the intestinal release of butyrate from polymeric micelles. *Nat Biomed Eng*. 2023;7(1):38-55. doi:10.1038/s41551-022-00972-5
14. Canfora EE, van der Beek CM, Jocken JWE, et al. Colonic infusions of short-chain fatty acid mixtures promote energy metabolism in overweight/obese men: a randomized crossover trial. *Sci Rep*. 2017;7(1):2360. doi:10.1038/s41598-017-02546-x

15. Tolhurst G, Heffron H, Lam YS, et al. Short-Chain Fatty Acids Stimulate Glucagon-Like Peptide-1 Secretion via the G-Protein–Coupled Receptor FFAR2. *Diabetes*. 2012;61(2):364-371. doi:10.2337/db11-1019
16. Yadav H, Lee JH, Lloyd J, Walter P, Rane SG. Beneficial Metabolic Effects of a Probiotic via Butyrate-induced GLP-1 Hormone Secretion. *Journal of Biological Chemistry*. 2013;288(35):25088-25097. doi:10.1074/jbc.M113.452516
17. Li Z, Yi CX, Katiraei S, et al. Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. *Gut*. 2018;67(7):1269-1279. doi:10.1136/gutjnl-2017-314050
18. Zhang L, Liu C, Jiang Q, Yin Y. Butyrate in Energy Metabolism: There Is Still More to Learn. *Trends in Endocrinology & Metabolism*. 2021;32(3):159-169. doi:10.1016/j.tem.2020.12.003
19. Wong JMW, de Souza R, Kendall CWC, Emam A, Jenkins DJA. Colonic Health: Fermentation and Short Chain Fatty Acids. *Journal of Clinical Gastroenterology*. 2006;40(3):235.
20. Canfora EE, Hermes GDA, Müller M, et al. Fiber mixture-specific effect on distal colonic fermentation and metabolic health in lean but not in prediabetic men. *Gut Microbes*. 2022;14(1):2009297. doi:10.1080/19490976.2021.2009297
21. Kaźmierczak-Siedlecka K, Marano L, Merola E, Roviello F, Połom K. Sodium butyrate in both prevention and supportive treatment of colorectal cancer. *Front Cell Infect Microbiol*. 2022;12. doi:10.3389/fcimb.2022.1023806
22. Vieira ELM, Leonel AJ, Sad AP, et al. Oral administration of sodium butyrate attenuates inflammation and mucosal lesion in experimental acute ulcerative colitis. *The Journal of Nutritional Biochemistry*. 2012;23(5):430-436. doi:10.1016/j.jnutbio.2011.01.007
23. Zhang M, Wang Y, Zhao X, Liu C, Wang B, Zhou J. Mechanistic basis and preliminary practice of butyric acid and butyrate sodium to mitigate gut inflammatory diseases: a comprehensive review. *Nutrition Research*. 2021;95:1-18. doi:10.1016/j.nutres.2021.08.007
24. Conley BA, Egorin MJ, Tait N, et al. Phase I study of the orally administered butyrate prodrug, tributyrin, in patients with solid tumors. *Clinical Cancer Research*. 1998;4(3):629-634.
25. Edelman MJ, Bauer K, Khanwani S, et al. Clinical and pharmacologic study of tributyrin: an oral butyrate prodrug. *Cancer Chemother Pharmacol*. 2003;51(5):439-444. doi:10.1007/s00280-003-0580-5
26. Egorin MJ, Yuan ZM, Sentz DL, Plaisance K, Eiseman JL. Plasma pharmacokinetics of butyrate after intravenous administration of sodium butyrate or oral administration of tributyrin or sodium butyrate to mice and rats. *Cancer Chemotherapy and Pharmacology*. 1999;43(6):445-453. doi:10.1007/s002800050922
27. Lee KA, Hicks G, Nino-Murcia G. Validity and reliability of a scale to assess fatigue. *Psychiatry Research*. 1991;36(3):291-298. doi:10.1016/0165-1781(91)90027-M
28. Lopez HL, Cesareo KR, Raub B, et al. Effects of Hemp Extract on Markers of Wellness, Stress Resilience, Recovery and Clinical Biomarkers of Safety in Overweight, But Otherwise Healthy Subjects. *Journal of Dietary Supplements*. 2020;17(5):561-586. doi:10.1080/19390211.2020.1765941
29. Ziegenfuss TN, Kedia AW, Sandrock JE, Raub BJ, Kerksick CM, Lopez HL. Effects of an Aqueous Extract of Withania somnifera on Strength Training Adaptations and Recovery: The STAR Trial. *Nutrients*. 2018;10(11):1807. doi:10.3390/nu10111807
30. Daniel P, Brazier M, Cerutti I, et al. Pharmacokinetic study of butyric acid administered in vivo as sodium and arginine butyrate salts. *Clinica Chimica Acta*. 1989;181(3):255-263. doi:10.1016/0009-8981(89)90231-3
31. Mayorga-Ramos A, Barba-Ostria C, Simancas-Racines D, Guamán LP. Protective role of butyrate in obesity and diabetes: New insights. *Frontiers in Nutrition*. 2022;9:1067647. doi:10.3389/fnut.2022.1067647
32. Kim SW, Hooker JM, Otto N, et al. Whole-body pharmacokinetics of HDAC inhibitor drugs, butyric acid, valproic acid and 4-phenylbutyric acid measured with carbon-11 labeled analogs by PET. *Nuclear Medicine and Biology*. 2013;40(7):912-918. doi:10.1016/j.nucmedbio.2013.06.007
33. Li B, Li L, Li M, et al. Microbiota Depletion Impairs Thermogenesis of Brown Adipose Tissue and Browning of White Adipose Tissue. *Cell Reports*. 2019;26(10):2720-2737.e5. doi:10.1016/j.celrep.2019.02.015
34. Sun J, Chen S, Zang D, Sun H, Sun Y, Chen J. Butyrate as a promising therapeutic target in cancer: From pathogenesis to clinic (Review). *International Journal of Oncology*. 2024;64(4):1-16. doi:10.3892/ijo.2024.5632
35. Cao S, Budina E, Raczy MM, et al. A serine-conjugated butyrate prodrug with high oral bioavailability suppresses autoimmune arthritis and neuroinflammation in mice. *Nat Biomed Eng*. 2024;8(5):611-627. doi:10.1038/s41551-024-01190-x
36. Russo R, Santarcangelo C, Badolati N, et al. *In vivo* bioavailability and *in vitro* toxicological evaluation of the new butyric acid releaser N-(1-carbamoyl-2-phenyl-ethyl) butyramide. *Biomedicine & Pharmacotherapy*. 2021;137:111385. doi:10.1016/j.biopha.2021.111385