Physiologic, metabolic, and toxicologic profile of 1,3-butanediol

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1,3-BD: 1,3-butanediol
βHB: β-hydroxybutyrate

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ABSTRACT

Ketone bodies are essential energy substrates in the absence of exogenous nutrients, and more recently, they have been suggested to prevent disease and improve longevity. β-hydroxybutyrate (βHB) is the most abundant ketone body. The secondary alcohol, 1,3-butanediol (1,3-BD), is commonly administered to raise βHB bioavailability in vivo and in the absence of nutrient deprivation. However, the concentration of 1,3-BD that yields a systemic concentration of βHB similar to that observed after a 24 h fast has yet to be determined. To evaluate this knowledge gap, we administered 5%, 10%, or 20% 1,3-BD via the drinking water to adult, male Wistar-Kyoto rats for four weeks. In addition to systemic and excreted βHB concentration, physiologic, metabolic, and toxicologic parameters were measured. We report that only 20% 1,3-BD significantly elevates the systemic and urinary concentrations of βHB. Rats treated with 20% 1,3-BD had a rapid and sustained reduction in body mass. All concentrations of 1,3-BD decreased food consumption, but only the 20% concentration decreased fluid consumption. Urine volume, red blood cell count, and hematocrit suggested dehydration in the 10% and 20% 1,3-BD treated rats. Finally, 20% 1,3-BD treated rats presented with indicators of metabolic acidosis and sinusoidal dilation, but no evidence of fatty liver or hepatotoxicity. In summary, we report that 20% 1,3-BD, but not 5% or 10%, produces a systemic concentration of βHB similar to that observed after a 24 h fast. However, this concentration is associated with deleterious side effects such as body mass loss, dehydration, metabolic acidosis, and sinusoidal dilation.

Significance statement: 1,3-Butanediol (1,3-BD) is often administered to stimulate the biosynthesis of the most abundant ketone body, β-hydroxybutyrate (βHB), and its purported salubrious effects. Here, we report that supra-pharmacological concentrations of 1,3-BD are
necessary to yield a systemic concentration of βHB similar to that observed after a 24 h fast, and this is associated with undesirable side effects. On the other hand, low concentrations of 1,3-BD was better tolerated and may improve health independent of its conversion into βHB.
INTRODUCTION

Ketogenic diets, comprised of high fat and low carbohydrate composition, and ketogenic interventions, such as intermittent fasting, are scientifically supported strategies for improved cardiometabolic health (Bueno et al., 2013; Varady et al., 2013). In the absence of carbohydrates and caloric restriction, ketone bodies become the primary energy source via free fatty acid oxidation. β-hydroxybutyrate (βHB) is the most abundant circulating ketone body and is predominantly synthesized in the liver to be transported to the peripheral tissues for conversion into energy (Newman and Verdin, 2014).

In order to study the long-term consequences of enhanced βHB bioavailability, the secondary alcohol, 1,3-butanediol (1,3-BD), is commonly administered in drinking water as a precursor. After consumption, 1,3-BD is catabolized by the liver into βHB (Tate et al., 1971). Most notably, exogenous 1,3-BD treatment provides an alternative method to elevate βHB in lieu of nutrient deprivation.

Previously, we have observed potent anti-hypertensive effects (Chakraborty et al., 2018), reduced renal damage (Chakraborty et al., 2018), and amelioration of endothelial dysfunction (McCarthy et al., 2021) in Dahl salt-sensitive (SS/Jr) and Dahl salt-resistant (SR/Jr) rats when 20% 1,3-BD was administered in conjunction with a high salt diet. However, these positive effects were also associated with stunted growth, metabolic acidosis, and hepatotoxicity (McCarthy et al., 2021). Therefore, there were two questions we aimed to answer from the current investigation: (1) what concentration of 1,3-BD most closely yields the systemic concentration of βHB after a 24 h fast? And (2) what concentration of 1,3-BD increases βHB bioavailability without deleterious side effects? To answer these objectives, we administered 5%,
10%, or 20% 1,3-BD via the drinking water for four weeks and analyzed physiologic, metabolic, and toxicologic parameters.

MATERIALS AND METHODS

Experimental animals

Inbred, 33-39 week old male Wistar-Kyoto (WKY) rats were used (384-496 g, n=35). Due to our laboratory’s long-time interest in hypertension research, we have maintained WKY rats in-house as a normotensive control for the spontaneously hypertensive rat (SHR) since 1985. Rats were bred and maintained on a low salt diet (0.3% NaCl; Teklad diet 7034, Envigo).

All breeding and experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Toledo College of Medicine and Life Sciences. Pups were weaned between 28-30 days. All rodents were maintained on a 12:12 h light-dark cycle and were allowed access to both chow and water ad libitum, unless specifically fasted (please see treatments section below). Euthanasia of rodents was performed by thoracotomy and exsanguination via cardiac puncture under isoflurane anesthesia administered via nose cone (5% in 100% O2), consistent with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (2013). All euthanasia and tissue harvesting were performed in the Department of Laboratory Animal Resources from 0900-1200 per experimental day.

The sample size per experiment (see figure panels and legends) is the number of independent rodents used, respective of treatment group. Previous work from our laboratory estimating a large effect size (Cohen’s d > 0.8), as well as power analysis (desired power of 0.80
to 0.85 with a probability of a Type I error of 0.05), has provided a basis for the projected number of rodents required per experimental group.

**Treatment**

1,3-BD (Millipore-Sigma) was diluted to 5% (0.56 mol/L), 10% (1.12 mol/L), or 20% (2.23 mol/L) concentrations with drinking water for 28 days; drinking water served as the vehicle control. All rats had free access to food throughout the investigation, although some vehicle-treated rats were fasted for the final 24 h before study termination to serve as a positive control. Therefore, our design comprised of five groups of seven rats, and no rats were euthanized for health concerns prior to 28 days.

**β-hydroxybutyrate measurement**

βHB was measured in serum and urine. For systemic βHB measurement, arterial blood was collected from the abdominal aorta in silicone-coated vacutainers specified for serum (BD Biosciences) prior to thoracotomy and exsanguination via cardiac puncture. Blood was left to clot at room temperature for ~20 min. After clotting, blood was centrifuged at 2000 g for 15 min at 4°C. Separated serum was collected, flash frozen in liquid nitrogen, and stored at -80°C until the time of measurement. For excreted βHB measurement, urine was collected from the metabolic cage study executed on day 26 of treatment.

Immediately prior to βHB measurement, both serum and urine samples were thawed and diluted 1:10 in assay buffer. βHB measurement was performed using a colorimetric assay according to the manufacturer’s instructions (Cayman Chemical).

**Food and fluid consumption**

In a subset of rats (n=4/group due to space limitations in the metabolic cage apparatus), excreted urine volume and food and treatment consumption were measured for 24 h on days 19
and 26. Rats were housed in individual metabolic cages (Lab Products Inc.) that prevented food and fecal contamination of urine samples. Food and fluid were available *ad libitum* and were weighed and measured before and after 24 h, respectively. Data presented are the average of the two 24 h periods.

**Blood chemistry analysis**

The CardioChek Plus Analyzer (PTS Diagnostics) was used to measure total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and glucose using freshly drawn, whole arterial blood. The VetScan HM5 Hematology Analyzer (Zoetis) was used to measure red blood cell (RBC) count and hematocrit, also from freshly drawn arterial blood. The VetScan VS2 Chemistry Analyzer (Zoetis) was used to measure indicators of liver profile and electrolytes using serum samples. Specifically, the VetScan Mammalian Liver Profile rotor was used to measure alkaline phosphatase (ALP), alanine aminotransferase (ALT), bile acids, bilirubin, albumin, and blood urea nitrogen (BUN), and the Electrolyte Plus rotor was used to measure chloride (Cl\(^-\)), sodium (Na\(^+\)), and total carbon dioxide (tCO\(_2\)). Metabolic acidosis was measured by calculating the anion gap using the following formula: \(\text{Anion gap} = \text{Na}^+ - (\text{Cl}^- + (\text{tCO}_2 - 1))\).

**Histology**

Oil red O staining was used to measure lipid droplet accumulation and sinusoidal dilation. Liver biopsies from the left anterior lobe were immediately embedded in tissue freezing medium and flash frozen in liquid nitrogen. Transverse cross-sections (8 µm) were processed by the University of Toledo College of Medicine and Life Sciences Histology Core following standard staining procedures. Stained cross-sections were viewed with a light microscope (Olympus VS120) using a 20x objective. Cross-sections of each biopsy were examined and
analyzed in quadruplicate for (1) the abundance and size of red lipid droplets and (2) intercellular space. Images were analyzed using ImageJ software.

**Statistical analysis**

The statistical procedures used included one-way and two-way analysis of variance (ANOVA). Dunnett’s *post-hoc* testing was used in all cases using a one-way ANOVA and Tukey’s *post-hoc* testing was used in all cases using a two-way ANOVA. All analyses were performed using data analysis software GraphPad Prism 9.2.0. Statistical significance was set at p<0.05. Data are presented as mean ± SEM.
RESULTS

Ketone body, βHB, serves as an energy source in times of nutrient deprivation, and 1,3-BD is an exogenous precursor that can be administered to raise βHB, independent of substrate availability (or lack thereof). Serum βHB measurement revealed a significant condition effect [F (4, 26) = 17.75, p<0.0001] as the 24 h fast (positive control) and 20% 1,3-BD groups had a 2.4-fold (p=0.0008) and 3.6-fold (p<0.0001) increase, respectively (Figure 1A). On the other hand, no increases in βHB were measured for the 5% (p=0.8527) and 10% (p=0.7946) concentrations of 1,3-BD. In the urine, a significant condition effect was also noted [F (4, 27) = 75.01, p<0.0001] as the 20% 1,3-BD increased βHB 10.6-fold (p<0.0001). In contrast, no increases in βHB were measured in the 24 h fast group (p>0.9999) or the 5% (p=0.7409) and 10% (p=0.2284) 1,3-BD groups (Figure 1B). Overall, these data reveal that only the 20% concentration of 1,3-BD significantly increases systemic βHB and yields a concentration similar to that observed after 24 h fast. However, in contrast to the fasted condition, a significant amount of βHB is excreted.

For the change in body mass after the commencement of treatment, we report significant time [F (2.268, 68.05) = 9.258, p=0.0002] and condition [F (3, 30) = 44.28, p<0.0001] effects, with no interaction [F (9, 90) = 1.233, p=0.2855]. Specifically, we observed that 20% 1,3-BD significantly decreased body mass as early as seven days after treatment (p<0.0001), and this loss was maintained for the full 28 days (p<0.0001) (Figure 2A-B). Supporting this loss of total body mass, we report significant condition effects for liver mass [F (4, 29) = 35.79, p<0.0001] (Figure 2C), epididymal fat mass [F (4, 25) = 13.20, p<0.0001] (Figure 2D), heart mass [F (4, 26) = 6.388, p=0.0010] (Figure 2E), and spleen mass [F (4, 30) = 14.50, p<0.0001] (Figure 2F). Post-hoc analysis revealed that the 20% 1,3-BD group had a significantly decreased mass of each of
these tissues (liver: p<0.0001, epididymal fat: p=0.0157, heart: p=0.0036, and spleen: p<0.0001). On the other hand, the 5% 1,3-BD group had increased liver mass (p=0.0026) (Figure 2C) and epididymal fat mass (p=0.0017) (Figure 2D). No differences were observed in any group for tibia length [F (4, 30) = 0.09420, p=0.9835] (Figure 2G). Overall, these data reveal that 20% 1,3-BD treatment causes rapid and sustained body mass loss, whereas 5% 1,3-BD increases adiposity.

To determine whether the decreased body mass was associated with a loss of appetite during 1,3-BD treatment, metabolic cage analysis was performed. Measurement of food consumption revealed a significant condition effect [F (3, 15) = 8.912, p=0.0012] as each 1,3-BD-treated group had significantly reduced chow intake (5%: p=0.0129, 10%: p=0.0175, and 20%: p=0.0008) (Figure 3A). While a significant condition effect was noted for fluid intake [F (3, 15) = 7.838, p=0.0022], it was only the 20% 1,3-BD group that was significantly reduced (p=0.0011) (Figure 3B). This reduced fluid intake was associated with a significant condition effect for urine volume [F (3, 15) = 3.584, p=0.0392] as the 20% 1,3-BD group excreted significantly less (p=0.0192) (Figure 3C). Reduced fluid consumption and urine volume suggested that the 20% 1,3-BD treatment was causing dehydration. Indeed, we observed significant condition effects for RBC count [F (4, 27) = 4.518, p=0.0063] (Figure 4A) and hematocrit [F (4, 27) = 4.209, p=0.0089] (Figure 4B). Post-hoc analysis revealed that both the 10% and 20% groups had significantly increased RBC count (10%: p=0.0290 and 20%: p=0.0135) and hematocrit (10%: p=0.0124 and 20%: p=0.0121), similar to 24 h fast group (RBC: p=0.0033 and hematocrit: p=0.0064). Overall, these data reveal that 1,3-BD treatment, regardless of concentration, reduces food consumption, and the 20% concentration causes dehydration due to decreased drinking behavior.
Previously, we have made the claim that 1,3-BD is a caloric restriction mimetic as it was able to decrease blood glucose, circulating triglycerides, and total cholesterol in young and developing Dahl rats (McCarthy et al., 2021). In our current investigation, while significant condition effects were measured for non-fasting blood glucose \( [F (4, 26) = 19.17, p<0.0001] \) (Figure 5A) and triglycerides \( [F (4, 25) = 17.54, p<0.0001] \) (Figure 5B), post-hoc analysis revealed that was only the 24 h fast group that reached statistical significance (glucose: \( p<0.0001 \) and triglycerides: \( p<0.0001 \)), and not any of the 1,3-BD treated groups. On the other hand, a significant condition effect was measured for total cholesterol \( [F (4, 28) = 6.901, p=0.0005] \) (Figure 5C) and HDL cholesterol \( [F (4, 28) = 11.19, p<0.0001] \) (Figure 5D) as the 20% 1,3-BD group had significant increases (total: \( p=0.0006 \) and HDL: \( p<0.0001 \)). No changes were observed for LDL cholesterol \( [F (4, 18) = 0.9834, p=0.4413] \) (Figure 5E). Overall, and in contrast to our previous publication, these data suggest that in mature rats, 1,3-BD is not a caloric restriction mimetic, and the 20% concentration actually causes hypercholesterolemia and hyperalphalipoproteinemia.

A major concern that arose from our previous work was that 20% 1,3-BD caused metabolic acidosis, as indicated by increased anion gap, and hepatotoxicity, as indicated by representative indicators of liver function, including circulating liver enzymes, bile acids, bilirubin, and albumin (McCarthy et al., 2021). In the current study, we similarly observed a significant condition effect for the anion gap \( [F (4, 20) = 3.895, p=0.0169] \) (Figure 6A), and post-hoc analysis revealed that it was significantly increased in the 24 h fast group and in each of the 1,3-BD treatment groups (fast: \( p=0.0183 \), 5%: \( p=0.0187 \), 10%: \( p=0.0305 \), and 20%: \( p=0.0125 \)). Interestingly, results from the current study indicated little hepatotoxicity. While significant condition effects were noted for ALP \( [F (4, 26) = 6.409, p=0.0010] \) (Figure 6B) and
ALT \( F(4, 25) = 5.362, p=0.0029 \) (Figure 6C), post-hoc analysis revealed that was only the 24 h fast group that reached statistical significance (ALP: \( p = 0.0003 \) and ALT: \( p = 0.0461 \)), and not any of the 1,3-BD treated groups. No changes in any of the groups were observed for bile acids \( F(4, 24) = 1.751, p=0.1718 \) (Figure 6D), bilirubin \( F(4, 26) = 0.7030, p=0.5970 \) (Figure 6E), albumin \( F(4, 26) = 0.3349, p=0.8519 \) (Figure 6F), or BUN \( F(4, 26) = 1.164, p=0.3495 \) (Figure 6G), suggesting that the treatment was better tolerated in mature rats. In terms of hepatic lipid droplet accumulation, while a significant condition effect was noted \( F(4, 14) = 8.240, p=0.0012 \) (Figure 6H), post-hoc analysis revealed that was only the 24 h fast group that reached statistical significance (p=0.0017), and not any of the 1,3-BD treated groups. Unexpectedly during our histological analysis, we observed increased intercellular space in liver biopsies from 1,3-BD treated rats \( F(4, 14) = 7.838, p=0.0016 \) (Figure 6I). Post-hoc analysis demonstrated that increased intercellular space, indicative of hepatic sinusoidal dilation, was statistically significant in the 20% 1,3-BD group (p=0.0024). Hepatic sinusoidal dilation can occur with or without venous outflow obstruction, with the latter being associated with several extrahepatic inflammatory conditions (e.g., cholecystitis, pancreatitis, or intestinal bowel disease) (Brancatelli et al., 2018). Overall, these data reinforce that 1,3-BD, even at low concentrations, can cause metabolic acidosis independent of ketogenesis, and high concentrations may also lead to hepatic sinusoidal dilation.
DISCUSSION

To summarize our findings, we observed that only 20% 1,3-BD administered with drinking water significantly elevated βHB and yielded a systemic concentration of βHB similar to that observed after 24 h fast. While elevated ketone bodies have been proposed to be health enhancing and longevity promoting in some contexts (Roberts et al., 2017), rats treated with 20% 1,3-BD also had rapid and unhealthy decreases in body and tissue masses, dehydration, and hepatic sinusoidal dilation. On the other hand, 5% and 10% concentrations of 1,3-BD did not increase βHB, decrease body mass, or decrease fluid consumption. Interestingly, all concentrations of 1,3-BD decreased food consumption and increased the anion gap, a clinically used indicator of metabolic acidosis. Overall, we conclude that the 20% concentration of 1,3-BD is necessary to significantly elevate systemic βHB bioavailability, but we advise caution when interpreting the physiological significance of this increase as it is associated with several contraindicating phenotypes in adult, male rats.

Classically, 1,3-BD is a βHB precursor due to its catabolic conversion in the liver after consumption (Tate et al., 1971). We recently suggested that 1,3-BD could also be considered a βHB mimetic as well, as both compounds can cause potent vasodilation of isolated arteries (McCarthy et al., 2021). This dual definition of 1,3-BD needs to be taken into consideration when designing investigations that aim to elevate βHB bioavailability (and whether vasodilation may influence data interpretation). For the purposes of the current study, we were interested in the physiologic, metabolic, and toxicologic effects of exogenous 1,3-BD to raise endogenous βHB bioavailability. Indeed, administering 1,3-BD is more appropriate than exogenous βHB for \textit{in vivo} and chronic studies as it can be solubilized in water. In contrast, βHB can only be prepared in an organic solvent, such as dimethyl sulfoxide or ethanol.
Interestingly, the toxicological profile and impact on metabolism and physiology of 1,3-BD have been well investigated. As early as 1967, it was reported that 1%, 3%, and 10% 1,3-BD fed to rats, and 0.5%, 1%, and 3% 1,3-BD fed to dogs, for two years demonstrated no adverse effects on survival, body mass, or tissue masses in either species (Scala and Paynter, 1967). Subsequently, it has been reported that 1,3-BD, at varying concentrations, is health enhancing, including vasodilation of resistance arteries (McCarthy et al., 2021), lowering blood pressure (Chakraborty et al., 2018; Frye et al., 1981; Ishimwe et al., 2020), suppressing the central nervous system (Frye et al., 1981), and protecting against hepatic necrosis (Price and Jollow, 1983) and cancer (Poff et al., 2014). In response to ischemia and hypoxia, 1,3-BD has contributed to increased survival (Kirsch et al., 1980), reduced neuronal damage (Marie et al., 1987), reduced neurologic deficit (Marie et al., 1987), enhanced motor performance (Combs and D'Alecy, 1987), and prevention of edema formation (Biros and Nordness, 1996; Gueldry et al., 1990). Finally, prepartum administration has been shown to improve the pre-weaning survival of neonates (Stahly et al., 1985; Stahly et al., 2014). On the other hand, however, 1,3-BD has also been reported to induce physical dependence (similar to ethanol) (Frye et al., 1981), stunt growth, cause metabolic acidosis and hepatotoxicity (McCarthy et al., 2021), exacerbate hepatotoxicity in response to haloalkanes (Hewitt et al., 1980; Pilon et al., 1986), decrease fertility (Hess et al., 1981), and delay the development of fetal skeletal tissue (Hess et al., 1981). Overall, the literature comprises of a disparate mixture of health-promoting and toxic effects of 1,3-BD.

In contrast to our previous study, we observed little hepatotoxicity at the 20% concentration (or any of the concentrations, for that matter). This difference could be attributed to (1) the length of the treatment (i.e., 4 weeks vs. 8 weeks), (2) the age of rats and the treatment
occurring during the mature phase of development, as opposed to the growth phase, or (3) the background of the rats used. In this study, we used WKY rats, as opposed to Dahl rats. WKY rats are from the Wistar background and are selected based on their propensity not to develop essential hypertension, unlike their genetic control, the SHR (Okamoto and Aoki, 1963). On the other hand, Dahl rats are selected for their sensitivity or resistance to hypertension due to a high salt diet. Therefore, unknown genetic factors between WKY and Dahl rats could also contribute to the differing effects we observed on hepatotoxicity between our two studies. Interestingly, WKY rats present a hormonal, behavioral, and physiological profile that mimics depression (Will et al., 2003). However, we cannot comment on how this predisposition potentially impacts the phenotypes measured in our current study.

One limitation of the current study is the use of only male rats. Our rationale for using only male rats was because it was recently reported that a 10 week treatment with 20% 1,3-BD in female Dahl S congenic rats caused only minimal body mass loss, no change in treatment consumption or urine volume, and no mention of metabolic acidosis or hepatotoxicity (Ishimwe et al., 2020). Therefore, for reasons that are still unknown, the severe negative phenotypes after 20% 1,3-BD treatment seem to be more pronounced in male rats during their developmental phase (McCarthy et al., 2021).

In summary, data from the current study suggest is that interventions that aim to upregulate βHB biosynthesis may be better served implementing an alternative approach as only a supra-pharmacological concentration of 1,3-BD was sufficient to significantly increase βHB, and this concentration was associated with rapid body mass loss, dehydration, and hepatic sinusoidal dilation. Therefore, interventions such as intermittent fasting/caloric restriction, a diet high in fat and low in carbohydrate composition, or a ketone monoester may enhance βHB
without the undesirable side effects of 1,3-BD. On the other hand, while low doses of 1,3-BD did not significantly increase βHB, our previous revelation that 1,3-BD can cause vasodilation of isolated resistance arteries at nanomolar concentrations (McCarthy et al., 2021), suggests that low-doses of 1,3-BD may be a health enhancing and longevity promoting independent of its metabolism into βHB. Indeed, it well known that alcohols can activate G-protein-coupled receptors (Neasta et al., 2020). Therefore, it is plausible that 1,3-BD could activate novel signaling pathways and investigation into these ketogenic-independent mechanisms are warranted.

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AUTHOR CONTRIBUTIONS

Participated in research design: McCarthy, Chakraborty, Wenceslau, and Joe

Conducted experiments: McCarthy, Waigi, Singh, Castaneda, and Mell

Performed data analysis: McCarthy

Wrote or contributed to the writing of the manuscript: McCarthy
REFERENCES


FOOTNOTES

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FIGURE LEGENDS

Figure 1. 20% 1,3-butanediol (1,3-BD) treatment increases systemic β-hydroxybutyrate (βHB) concentration to a level similar as a 24 h fast. βHB was measured in serum (A) and urine (B) at the conclusion of treatment. Mean ± SEM. n=5-7. One-way ANOVA: ***p<0.001, ****p<0.0001.

Figure 2. 20% 1,3-butanediol (1,3-BD) treatment dramatically decreases body mass. Pictured are WKY rats after treatment with vehicle or 20% 1,3-BD for four weeks (A). The change in body mass (Δ) was measured weekly throughout treatment (B). Tissues, including liver mass (C), epididymal fat mass (D), total heart mass (E), spleen mass (F), and tibia length (G) were measured at the conclusion of treatment. Mean ± SEM. n=5-13. Two-way ANOVA: *p<0.05, ****p<0.0001; one-way ANOVA: *p<0.05, **p<0.01, ****p<0.0001.

Figure 3. 1,3-Butanediol (1,3-BD) treatment decreases food consumption, and 20% concentration decreases fluid consumption. Food consumption (A), fluid consumption (B), and urine volume (C) were measured for 24 h on day 26 of treatment. Mean ± SEM. n=4-7. One-way ANOVA: *p<0.05, **p<0.01, ***p<0.001.

Figure 4. 10% and 20% 1,3-butanediol (1,3-BD) treatments cause dehydration. Red blood cell count (A) and hematocrit (B) were measured as indicators of dehydration at the conclusion of treatment. Mean ± SEM. n=6-7. One-way ANOVA: *p<0.05, **p<0.01.
**Figure 5.** 20% 1,3-butanediol treatment increases total cholesterol and HDL cholesterol without changing blood glucose or triglycerides. Blood glucose (A), triglycerides (B), total cholesterol (C), HDL cholesterol (D), and LDL cholesterol (E) were measured at the conclusion of treatment. Mean ± SEM. n=3-7. One-way ANOVA: ***p<0.001, ****p<0.0001.

**Figure 6.** All concentrations of 1,3-butanediol (1,3-BD) treatment cause metabolic acidosis, but not hepatotoxicity, and only 20% causes hepatic sinusoidal dilation. Circulating ion concentrations were measured, and the anion gap was calculated as a measure of acid-base balance (A). Liver enzymes and metabolites, including alkaline phosphatase (B), alanine aminotransferase (C), bile acids (D), bilirubin (E), albumin (F), and blood urea nitrogen (G) were measured at the conclusion of treatment. Histological analysis was performed for lipid droplets (H) and sinusoidal dilation (I) in liver biopsies. Left, densitometric analysis; right, representative images of histological micrographs. Mean ± SEM. n=3-7. One-way ANOVA: *p<0.05, **p<0.01, ***p<0.001.
Figure 1

(A) Serum

(B) Urine

βHB (mmol/L)

- Vehicle ○ non-fasted
- 5% 1,3-BD (non-fasted)
- 10% 1,3-BD (non-fasted)
- 20% 1,3-BD (non-fasted)
Figure 2

(A) Vehicle 1,3-BD

(B) Graph showing body mass (g) over weeks 1 to 4 for different treatment groups:
- Vehicle (non-fasted)
- 5% 1,3-BD (non-fasted)
- 10% 1,3-BD (non-fasted)
- 20% 1,3-BD (non-fasted)

Week 1: ****  
Week 2: ****  
Week 3: ****  
Week 4: ****

(C) Liver mass (g)  
(D) Epididymal fat mass (g)
(E) Total heart mass (g)
(F) Spleen mass (g)  
(G) Thymus length (mm)
Figure 5

(A) Glucose (mg/dL)

(B) Triglycerides (mg/dL)

(C) Cholesterol (mg/dL)

(D) HDL (mg/dL)

(E) LDL (mg/dL)

Legend:
- Vehicle
- non-fasted
- fasted
- 5% 1,3-BD (non-fasted)
- 10% 1,3-BD (non-fasted)
- 20% 1,3-BD (non-fasted)

Significance levels:
- **** p < 0.0001
- *** p < 0.001
- * p < 0.05