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Physiologic, Metabolic, and Toxicologic Profile of 1,3-Butanediol

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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-hour 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-hour 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. This article reports that suprapharmacological concentrations of 1,3-BD are necessary to yield a systemic concentration of βHB similar to that observed after a 24-hour fast, and this is associated with undesirable side effects. On the other hand, low concentrations of 1,3-BD were better tolerated and may improve health independent of its conversion into βHB.

Introduction

Ketogenic diets, composed 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).

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 antihypertensive 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 and Dahl salt-resistant 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-hour fast? 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

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ABBREVIATIONS: ALP, alkaline phosphatase; ALT, alanine aminotransferase; 1,3-BD, 1,3-butanediol; βHB, β-hydroxybutyrate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; RBC, red blood cell; WKY, Wistar-Kyoto.

laboratory's long-time interest in hypertension research, we have maintained WKY rats in-house as a normotensive control for the spontaneously hypertensive rat 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 and 30 days. All rodents were maintained on a 12:12 hour 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% O_2), 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 9:00 AM to 12:00 PM on experimental days.

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 was 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 hours before study termination to serve as a positive control. Therefore, our design comprised 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 minutes. After clotting, blood was centrifuged at 2000 g for 15 minutes 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 hours on days 19 and 26. Rats were housed in individual metabolic cages (Laboratory 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 hours, respectively. Data presented are the average of the two 24-hour 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, 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: Anion gap = $Na^+ - [Cl^- + (tCO_2 - 1)]$.

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 20× 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 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 means ± S.E.M.

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-hour 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 (Fig. 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-hour fast group ($P > 0.9999$) or the 5% ($P = 0.7409$) and 10% ($P = 0.2284$) 1,3-BD groups (Fig. 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-hour 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 ($P < 0.0001$) (Fig. 2, A and B). Supporting this loss of total body mass, we report significant condition effects for liver mass [$F(4, 29) = 35.79$, $P < 0.0001$] (Fig. 2C), epididymal fat mass [$F(4, 25) = 13.20$, $P < 0.0001$] (Fig. 2D), heart mass [$F(4, 26) = 6.388$, $P = 0.0010$] (Fig. 2E), and spleen mass [$F(4, 30) = 14.50$, $P < 0.0001$] (Fig. 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 ($P = 0.0026$) (Fig. 2C) and epididymal fat mass ($P = 0.0017$) (Fig. 2D). No differences were observed in any group for tibia length [$F(4, 30) = 0.09420$, $P = 0.9835$] (Fig. 2G). Overall,

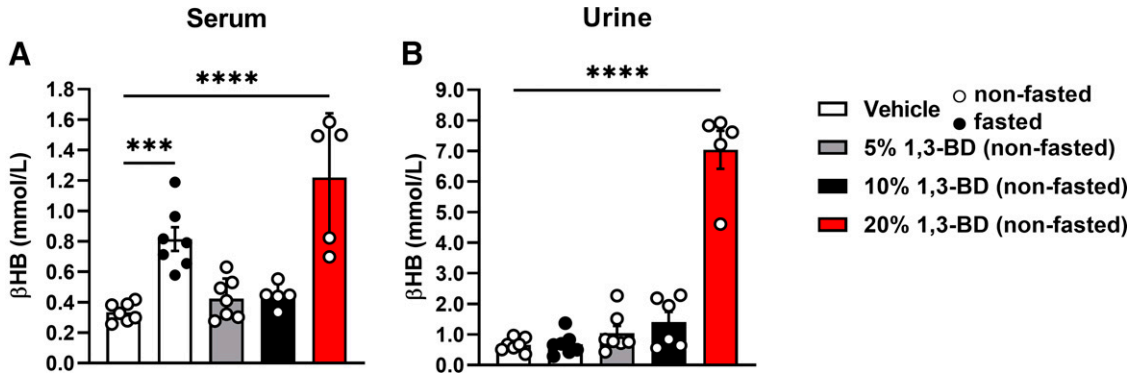

Fig. 1. Treatment with 20% 1,3-BD increases systemic βHB concentration to a level similar as a 24-hour fast. βHB was measured in serum (A) and urine (B) at the conclusion of treatment. Mean ± S.E.M. n = 5–7. One-way ANOVA: ***$P < 0.001$, ****$P < 0.0001$.

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) (Fig. 3A). Although 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) (Fig. 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) (Fig. 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] (Fig. 4A) and hematocrit [F (4, 27) = 4.209, P = 0.0089] (Fig. 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-hour 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, although significant condition effects were measured for

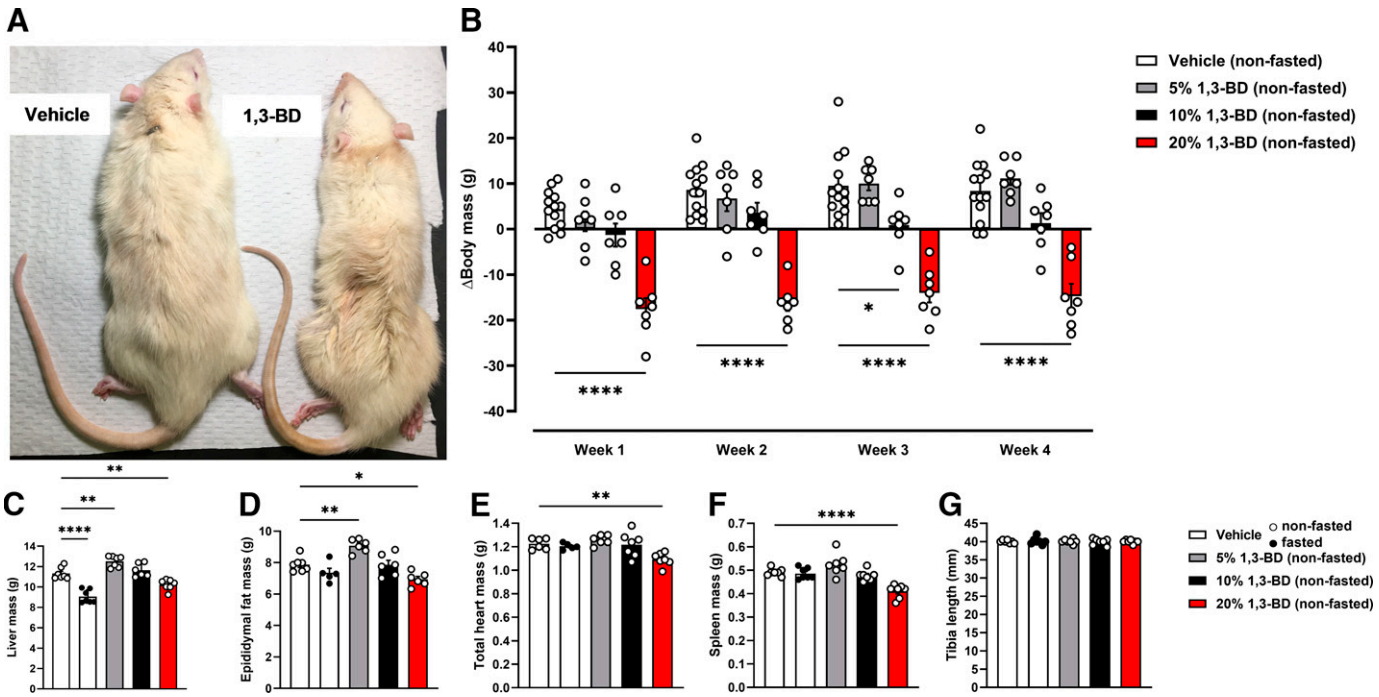

Fig. 2. Treatment with 20% 1,3-BD dramatically decreases body mass. (A) WKY rats after treatment with vehicle or 20% 1,3-BD for four weeks. (B) The change in body mass (Δ) was measured weekly throughout treatment. Tissues, including liver mass (C), epididymal fat mass (D), total heart mass (E), spleen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

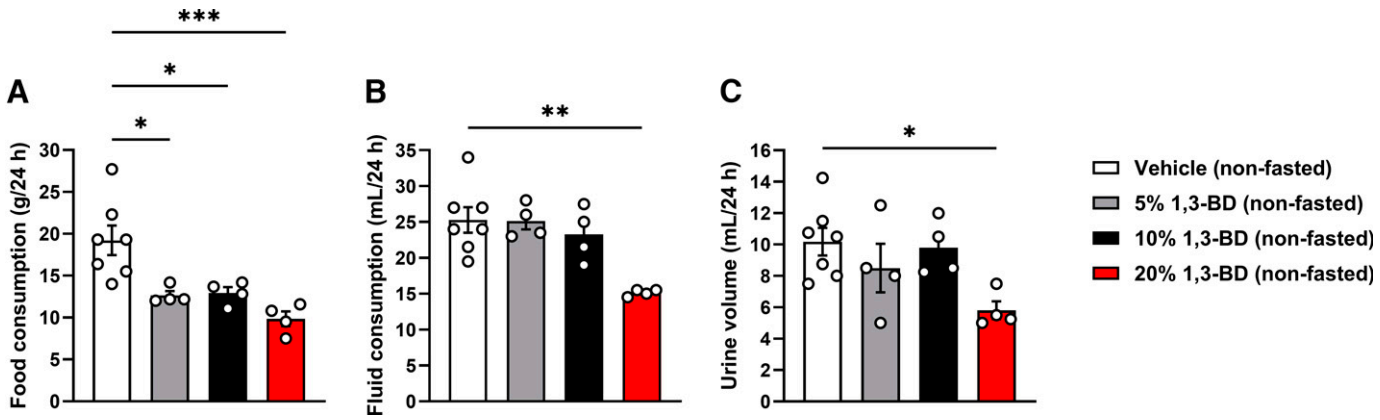

Fig. 3. Treatment with 1,3-BD decreases food consumption, and 20% concentration decreases fluid consumption. Food consumption (A), fluid consumption (B), and urine volume (C) were measured for 24 hours on day 26 of treatment. Mean ± S.E.M. n = 4–7. One-way ANOVA: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

nonfasting blood glucose [F (4, 26) = 19.17, $P < 0.0001$] (Fig. 5A) and triglycerides [F (4, 25) = 17.54, $P < 0.0001$] (Fig. 5B), post hoc analysis revealed that only the 24-hour 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, $P = 0.0005$] (Fig. 5C) and HDL cholesterol [F (4, 28) = 11.19, $P < 0.0001$] (Fig. 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, $P = 0.4413$] (Fig. 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, $P = 0.0169$] (Fig. 6A), and post hoc analysis revealed that it was significantly increased in the 24-hour 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. Although significant condition effects were noted for ALP [F (4, 26) = 6.409, $P = 0.0010$] (Fig. 6B) and ALT [F (4, 25) = 5.362, $P = 0.0029$] (Fig. 6C), post hoc analysis revealed that only the 24-hour 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, $P = 0.1718$] (Fig. 6D), bilirubin [F (4, 26) = 0.7030, $P = 0.5970$] (Fig. 6E), albumin [F (4, 26) = 0.3349, $P = 0.8519$] (Fig. 6F), or blood urea nitrogen [F (4, 26) = 1.164, $P = 0.3495$] (Fig. 6G), suggesting that the treatment was better tolerated in mature rats. In terms of hepatic lipid droplet accumulation, although a significant condition effect was noted [F (4, 14) = 8.240, $P = 0.0012$] (Fig. 6H), post hoc analysis revealed that only the 24-hour fast group that reached statistical significance ($P = 0.0017$) and not any of the 1,3-BD–treated groups. Unexpectedly during our histologic analysis, we observed increased intercellular space in liver biopsies from 1,3-BD–treated rats [F (4, 14) = 7.838, $P = 0.0016$] (Fig. 6I). Post hoc analysis demonstrated that increased intercellular space, indicative of hepatic sinusoidal dilation, was statistically significant in the

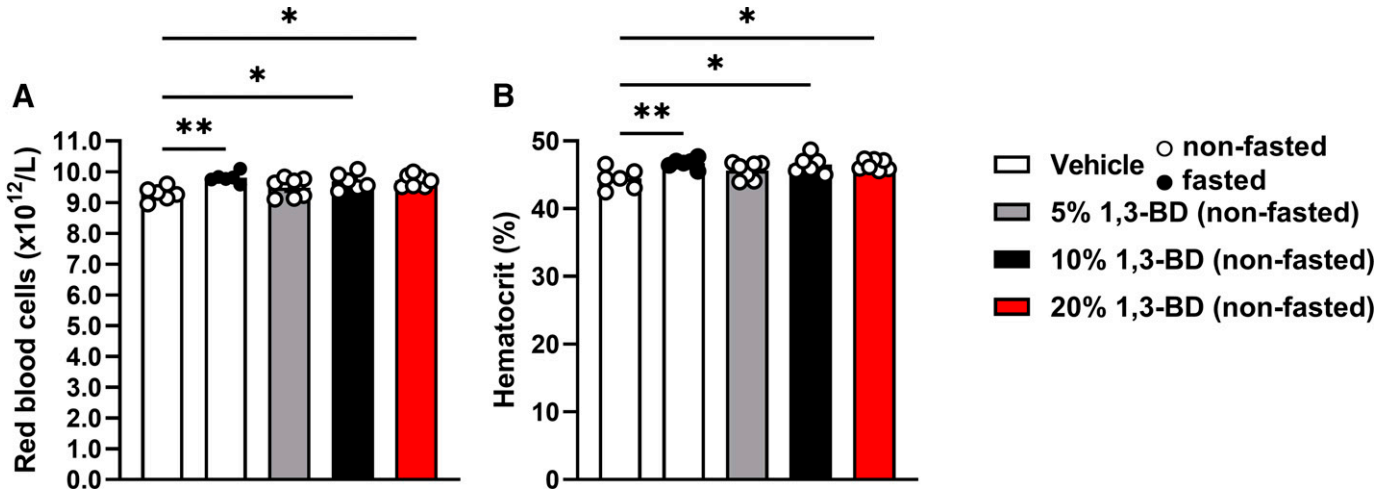

Fig. 4. Treatments with 10% and 20% 1,3-BD cause dehydration. Red blood cell count (A) and hematocrit (B) were measured as indicators of dehydration at the conclusion of treatment. Mean ± S.E.M. n = 6–7. One-way ANOVA: *$P < 0.05$, **$P < 0.01$.

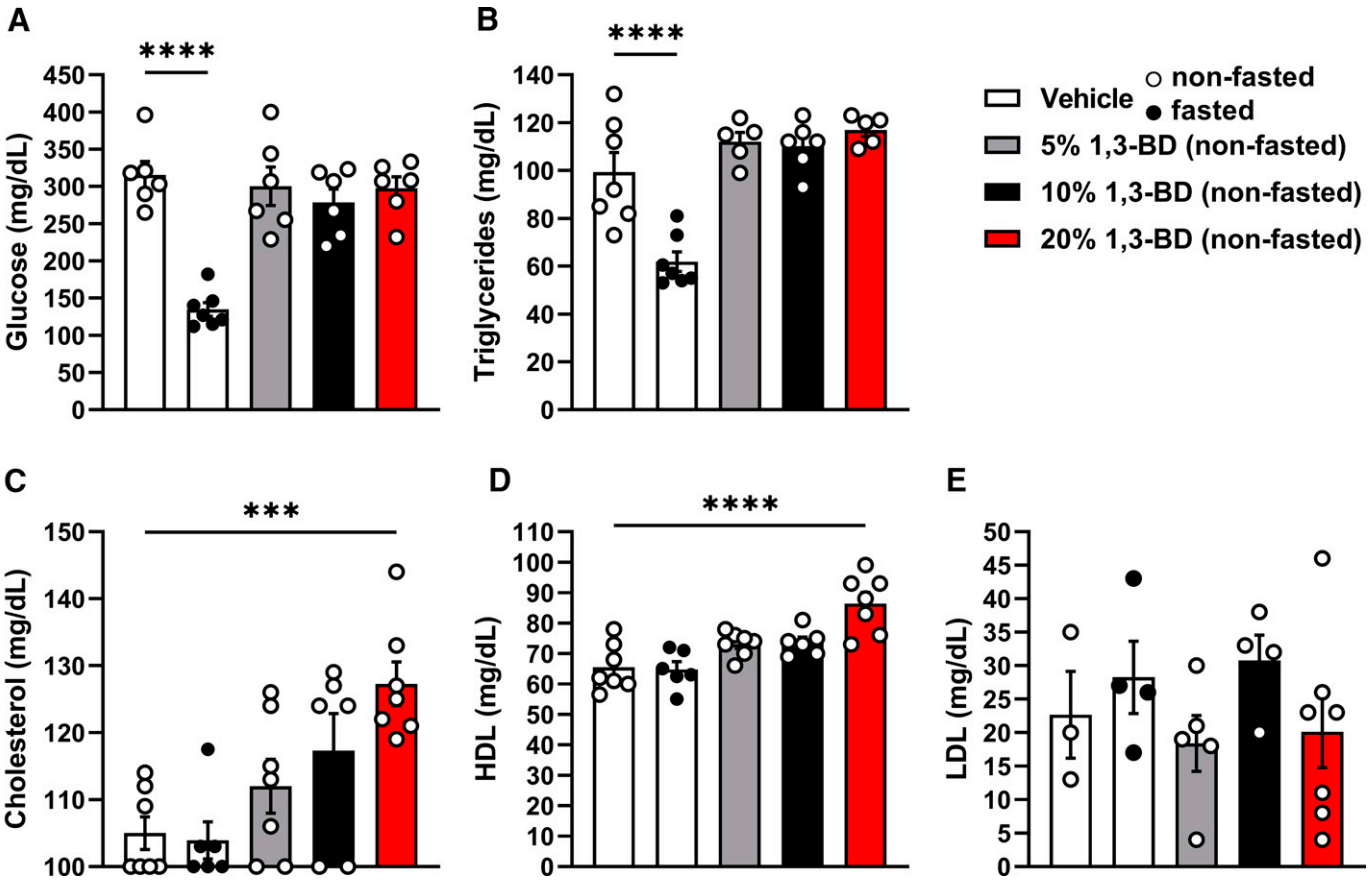

Fig. 5. Treatment with 20% 1,3-BD increases total cholesterol and HDL cholesterol without changing blood glucose or triglycerides. Blood glucose (A), triglycerides (B), total cholesterol (C), HDL cholesterol (D), and LDL cholesterol (E) were measured at the conclusion of treatment. Mean ± S.E.M. n = 3–7. One-way ANOVA: ***$P < 0.001$, ****$P < 0.0001$.

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-hour fast. Although 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 physiologic 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 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 toxicologic 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 of blood

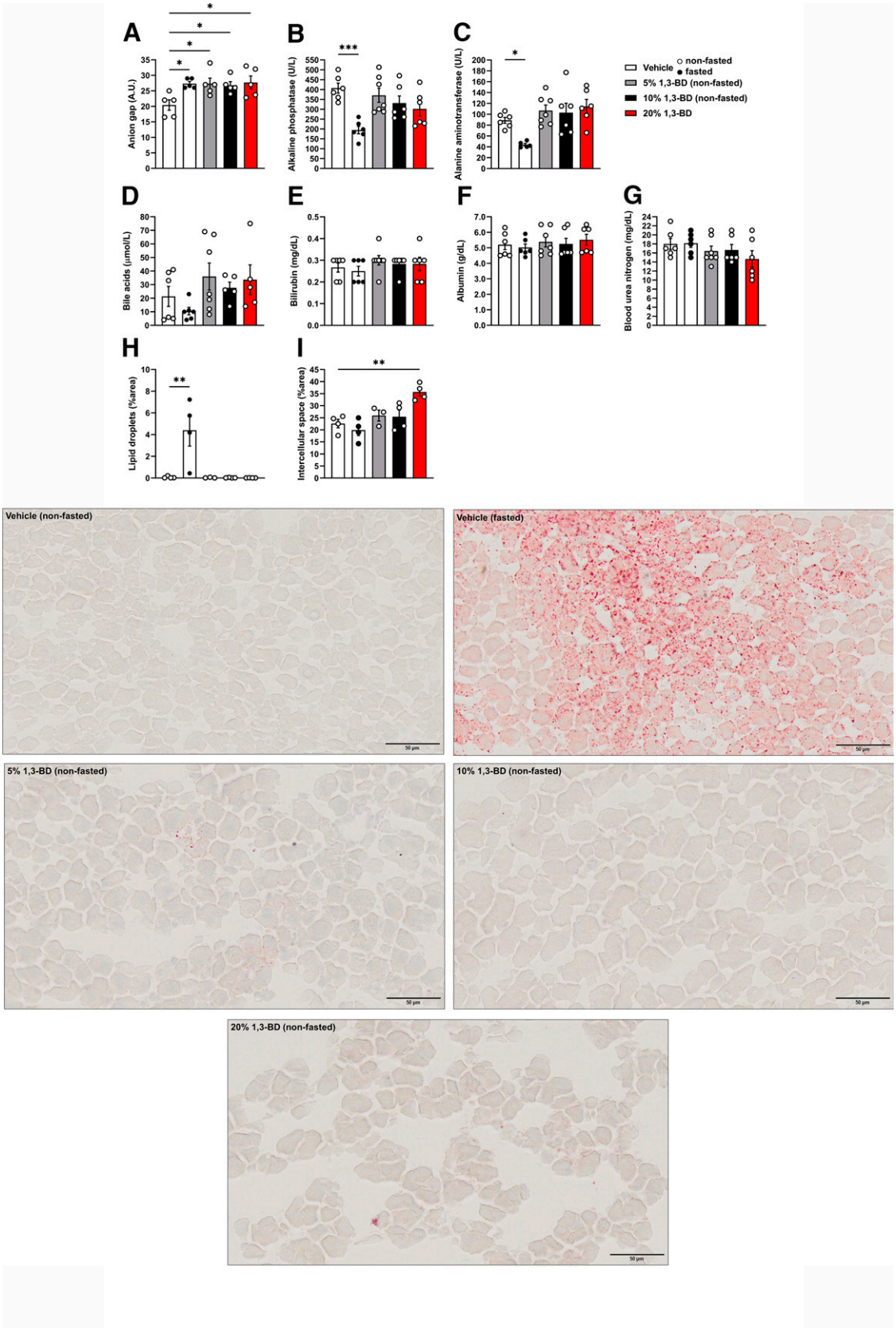

Fig. 6. All concentrations of 1,3-BD treatment cause metabolic acidosis, but not hepatotoxicity, and only 20% causes hepatic sinusoidal dilation. (A) Circulating ion concentrations were measured, and the anion gap was calculated as a measure of acid-base balance. Liver enzymes and metabolites, including 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. Histologic analysis was performed for lipid droplets (H) and sinusoidal dilation (I) in liver biopsies. Left, densitometric analysis; right, representative images of histologic micrographs. Mean ± S.E.M. n = 3–7. One-way ANOVA: *P < 0.05, **P < 0.01, ***P < 0.001.

pressure (Chakraborty et al., 2018; Frye et al., 1981; Ishimwe et al., 2020), suppression of the central nervous system (Frye et al., 1981), and protection 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 preweaning 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 versus 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 spontaneously hypertensive rat (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 physiologic 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 the recent report 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 that interventions that aim to upregulate βHB biosynthesis may be better served implementing an alternative approach, as only a suprapharmacological 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, although 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 is warranted.

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Authorship Contributions

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

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

Performed data analysis: McCarthy.

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

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