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Changes in Body Weight and Lipid Profile of Mice Treated with Sodium Butyrate and Metformin

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ABSTRACT

Previous animal studies have shown that butyrate (sodium butyrate, NaB) administration can protect against diet-induced obesity. We investigated the effect of oral (1g/kg body weight/day) and intraperitoneal (0.5/kg body weight/day) administration of NaB on body weight and lipid profile of mice. Oral (400mg/kg body weight/day) and intraperitoneal (200mg/kg body weight/day) metformin was similarly administered as positive control. Placebo was the drug vehicle (phosphate saline buffer). Fifty seven mice (15 -18 weeks old) were divided into three groups and administered freshly prepared NaB (n=19), metformin (n=16) and placebo (n=16), respectively for 16 days. Within each group half the mice were given the drug orally and half intraperitoneally. Weights were recorded daily and serum lipids were measured from blood samples obtained at the end of the study. Both oral ($p=0.001$) and intraperitoneal ($p<0.001$) metformin caused significant weight loss (7.56±2.47% and 8.72±2.75%, respectively) compared to placebo. Oral NaB also caused significant weight loss (4.77±3.49%) in mice as compared to placebo (1.81±2.89%) at $p=0.04$. However, there was no significant difference in weight change between intraperitoneal NaB (4.08±2.65%) and placebo (4.78±2.23%) at $p=0.85$. Additionally serum lipids were raised in all groups with significant weight loss. The most prominent change was elevated triglycerides (214.42±81.96 mg/dL) in NaB treated mice (p-0.01) and raised cholesterol (189.01±39.89 mg/dL at $p=0.04$) in metformin treated mice as compared to placebo. To conclude NaB, causes significant weight loss in mice along with raised lipids. However oral NaB seems to be more effective than intraperitoneal butyrate.

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Authors' Contribution

FM conceived and supervised the work. TAS designed the study, carried out studies on rats, analyzed the data and wrote the article. KIK helped in study designing and writing of manuscript. MA contributed to the animal dosing, sample collection and manuscript writing. FA performed the biochemical tests.

Key words

Butyrate, Metformin, Short chain fatty acids, Weight loss, Serum lipids.

INTRODUCTION

Butyrate is a short chain fatty acid produced by fermentation of indigestible fiber by microbiota of the colon (Brahe *et al*., 2013). Many researches have hinted towards a possible relationship between these gut microbiota and obesity (Raoult, 2016; Ridaura *et al*., 2013; Shen *et al*., 2013). It is now being hypothesized that butyrate may in fact mediate the beneficial effects of microbiota on host metabolism (Brahe *et al*., 2013). Animal studies show that butyrate administration can improve insulin sensitivity, increase energy expenditure (Gao *et al*., 2009) and protect against diet-induced obesity (Lin *et al*., 2012). Additionally, unlike other SCFAs, butyrate has also been shown to stimulate lipolysis in adipocytes (Berndt *et al*., 2012). Gao *et al*. (2009) have previously shown that a group of mice fed high fat diet mixed with butyrate did not gain significant weight as compared to mice fed high fat diet without butyrate. We sought to validate these findings by administering calculated doses of sodium butyrate (NaB). Moreover, we tested the effects of oral versus intraperitoneal administration which has not been done before. In addition to the placebo group we used metformin treated mice as positive controls, since effect of metformin on weight loss is well known (Brufani *et al*., 2013; Ravn *et al*., 2013; Sever *et al*., 2014; Yanovski and Yanovski, 2014).

METHODOLOGY

Animal housing and dosing

Fifty seven (57) Swiss Webster albino mice (15-18 weeks old) were housed in the animal facility of School of Biological Sciences, University of the Punjab with a 12 h light/dark cycle and constant temperature (22–24°C).They were all fed standard diet and water. Six mice were sacrificed before start of intervention, their organs were harvested and plasma sample obtained from blood drawn by cardiac puncture. The rest of the mice

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(n=51) were divided into six groups and given different treatments as shown in Table I. The mice that had already been living together in groups were allowed to remain in their respective groups in order to minimize stress.

Table I.- The treatment groups.

Group #	Treatment	Dose	N
1	Oral NaB	1g/kg body weight/day	11
2	i.p. NaB	500 mg/kg body weight/day	8
3	Oral Metformin	400mg/kg body weight/day	8
4	i.p. metformin	200mg/kg body weight/day	8
5	Oral Placebo (PBS)	100 µL/day	8
6	i.p. Placebo (PBS)	100 µL/day	8
Total			51

*i.p., intrapertitoneal

All animals were weighed daily in a plastic beaker placed on a top-loading balance. Fresh doses of oral and intraperitoneal NaB, metformin and placebo were prepared daily based on the body weights of the animals as shown in Table I. The drugs were dissolved in phosphate saline buffer (PBS) and sterilized by using syringe nano-filters. Placebo was 100 µl PBS buffer. The volume on administered dose varied between 70 and 120 µl for the drugs. In order to make oral doses more palatable and ensure accurate dosing approximately 150 mg of glucose was added to each 1000 to 1200 µl of drug preparation for all groups. The final glucose concentration varied between 0.15 to 0.12 mg/µL. Pure metformin powder was provided by Merck, Pakistan and sodium butyrate (NaB) of pharmaceutical grade was purchased from Santa Cruz Biotech. The chemicals were stored at -80 degree Celsius and their aliquots were stored at -20°C.

Animal dissection and acquiring samples

The mice were all sacrificed in fasting state after the 17th day. Animals were anesthetized using ketamine 100 mg/kg of body weight and Xylazine (about 80µL of intra-peritoneal injection for each mouse). After the mice were unconscious, blood was drawn via intra cardiac puncture. An average of 1 ml of blood was obtained from each mouse. Plasma was separated from blood after centrifugation at 4000 rpm for 4 min. The mice were decapitated before internal organs were dissected out and snap frozen for future genetic and protein expression studies.

Determining plasma lipids

Plasma total cholesterol and triglyceride levels were spectrophotometrically determined using commercially available kits (Analyticon Biotechnologies AG, 4046 and 5052, respectively). For the estimation of high-density lipoprotein cholesterol (HDL-C), other lipoprotein fractions were precipitated using HDL-C precipitation reagent (Analyticon Biotechnologies AG, 410). HDL-C was then estimated using aforementioned Analyticon kit (4046) for cholesterol estimation. For estimation of low-density lipoprotein cholesterol (LDL-C) we used recently described method by Martin *et al*. (2013).

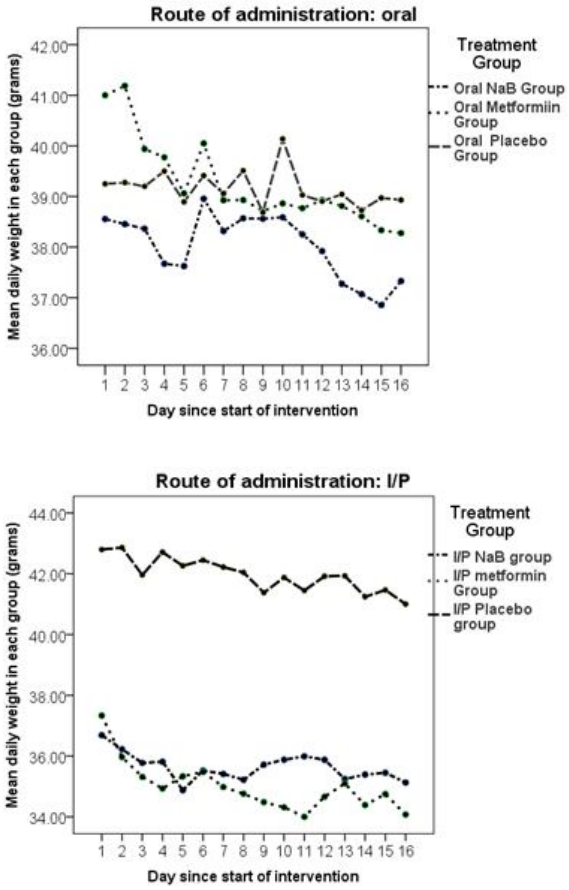

Fig. 1. Daily body weights of mice administered with sodium butyrate and metformin orally (top: NaB, 1g/kg/d for 16 days and metformin, 400mg/kg/d for 16 days) and intraperitoneally (bottom: NaB, 500mg/kg/d for 16 days and metformin, 200mg/kg/d for 16 days).

RESULTS

During the course of the sixteen (16) days of intervention all groups of mice lost weight. The two leanest mice from the intraperitoneal metformin group died after receiving 2 doses. The difference in absolute weight from day 0 to day 16 was statistically significant in all groups except oral placebo group (Fig. 1). Oral NaB

Table II.- Total body weight loss (%, Mean±SD) of Swiss Webstar albino mice after administration of sodium butyrate and metformin.

Treatment group		Butyrate	Metformin	Placebo	Adjusted R2
Oral administration		4.77±3.49 (n=11)	7.56±2.47 (n=8)	1.81±2.89 (n=8)	0.32
	P	0.04	0.001		
Intraperitoneal administration		4.08±2.65 (n=8)	8.72±2.75 (n=6)	4.78±2.23 (n=8)	0.34
	P	0.85	<0.001		
Oral and intraperitoneal		4.48±3.09 (n=19)	8.06±2.56 (n=14)	3.29±2.93 (n=16)	0.29
	P	0.19	<0.001		

*ANOVA with post hoc Dunnet test using relevant placebo group as control.

Table III.- Differences in weight loss adjusting for initial differences in weights (ANOVA).

	Statistics	Corrected model	Intercept	Effect of initial weight	Treatment effect
Oral treatment	F	8.54	12.56	7.53	11.05
	P value	0.001	0.002	**0.01**	**<0.001**
	Partial Eta Squared	0.53	0.35	0.25	0.49
Intraperitoneal treatment	F	4.24	0.18	0.32	6.36
	P value	0.02	0.67	0.58	**0.01**
	Partial Eta Squared	0.41	0.01	0.02	0.4

Group lost 1.79±1.15 g (p<0.001), intraperitoneal NaB group lost 1.53±0.99 g (p=0.003), oral metformin group lost 3.07±0.89 g (p<0.001), intraperitoneal metformin group lost 3.26±1.10 g (p=0.001) and intraperitoneal placebo group lost 2.04±0.99 g (p=0.001). Oral placebo group did not lose significant average weight (0.63±0.99 g at p=0.11). However, when compared against their respective placebo groups (Table II), it was evident that mice from intraperitoneal metformin group lost most weight (8.72±2.75% at p<0.001), followed by oral metformin group (7.56±2.47% at p<0.001) and finally oral NaB (4.77±3.49% at p=0.04) as shown in Table II. Weight loss in intraperitoneal NaB group was not statistically significant when compared against intraperitoneal placebo group at p=0.85.

The mean difference in weight loss in the groups of mice remained statistically significant even after adjusting for initial differences in weights for both oral treatment groups (P=0.001) and intraperitoneal groups, P=0.01 (Table III).

There were significant differences in serum lipid levels between treatment and placebo groups. As shown in Table IV, there is an overall trend of increased serum lipids in treatment groups as compared to placebo group. Serum triglycerides (p=0.01) and serum VLDL (p=0.03) were significantly raised in butyrate group. In addition serum cholesterol showed a statistically significant increase (p=0.04) in metformin treated mice, the significance being more marked in intraperitoneal group (p=0.04).

DISCUSSION

The most important and novel finding in our study is the significant weight loss in mice treated with oral butyrate (4.77±3.49%) as compared to oral placebo group (1.81±2.89%) at p=0.04. This effect, however, was less prominent than oral metformin (7.56±2.47% at p=0.001) whose role in weight loss is quite established (Brufani *et al*., 2013; Group, 2012; McDonagh *et al*., 2014; Ravn *et al*., 2013; Sever *et al*., 2014; Yanovski and Yanovski, 2014). A pervious study by Gao *et al*. (2009) showed that when high fat diet (HFD) was supplemented with butyrate the mice tended to gain less weight than the ones given HFD alone. In their study the authors made a rough estimation of butyrate intake based on average daily intake of chow by mice. Our study validates this anti-obesity effect of butyrate in non-obese mice using calculated doses given by oral gavage and intraperitoneal route.

Table IV.- Comparison of serum lipid profile of mice after oral administration of NaB at 1g/kg/d for 16 days and metformin at 400mg/kg/d for 16 days, and intraperitoneal administration of NaB at 500mg/kg/d for 16 days and metformin at 200mg/kg/d for 16 days.

Treatment group		Butyrate	Metformin	Placebo
Triglycerides (mg/dL)				
Oral administration		199.72±51.97 (n=9)	159.41±56.12 (n=8)	129.98±20.50 (n=8)
	P*	0.01	0.18	
Intraperitoneal administration		230.96±108.05 (n=8)	178.89±73.91 (n=5)	174.87±68.50 (n=7)
	P*	0.20	0.64	
Oral and intraperitoneal		214.42±81.96 (n=17)	166.90±61.28 (n=13)	150.93±52.52 (n=15)
	P*	0.01	0.40	
Cholesterol (mg/dL)				
Oral administration		148.98±22.71 (n=9)	194.02±46.84 (n=8)	152.27±46.79 (n=8)
	P*	0.98	0.09	
Intraperitoneal administration		168.89±25.54 (n=8)	181.01±28.39 (n=5)	149.55±21.24 (n=8)
	P*	0.12	0.04	
Oral and intraperitoneal		158.38±25.39 (n=17)	189.01±39.89 (n=13)	150.9±35.13 (n=16)
	P*	0.40	0.04	
LDL (mg/dL)				
Oral administration		117.51±21.07 (n=9)	115.79±55.49 (n=8)	127.63±44.14 (n=5)
	P*	0.83	0.85	
Intraperitoneal administration		102.36±30.82 (n=8)	101.66±53.46 (n=5)	98.34±43.90 (n=7)
	P*	0.60	0.62	
Oral and intraperitoneal		110.38±26.43 (n=17)	110.36±52.91 (n=13)	113.96±45.05 (n=15)
	P*	0.78	0.75	
VLDL (mg/dL)				
Oral administration		21.47±5.59 (n=9)	27.31±7.20 (n=8)	24.63±3.65 (n=8)
	P*	0.02	0.28	
Intraperitoneal administration		32.29±9.10 (n=8)	27.19±7.49 (n=5)	28.40±8.73 (n=7)
	P*	0.31	0.76	
Oral and intraperitoneal		31.85±7.21 (n=17)	22.26±6.98 (n=13)	26.39±6.56 (n=15)
	P*	0.03	0.53	
HDL (mg/dL)				
Oral administration			101.84±24.92 (n=4)	
Intraperitoneal administration		91.31±3.52 (n=3)	86.93±11.19 (n=3)	64.75±34.88 (n=3)
	P*	0.29	0.39	
Oral and intraperitoneal		91.31±3.52 (n=3)	95.45±20.39 (n=7)	64.75±34.88 (n=3)
	P*	0.28	0.12	

*ANOVA with post hoc Dunnet' test using relevant placebo group as control.

There are several mechanisms by which butyrate may reduce weight. Butyrate has been reported to cause activation of AMPK (Gao *et al*., 2009) much like metformin (Zheng *et al.*, 2013). AMPK activation leads to lipolysis (Dagon *et al.*, 2006). It has been reported that butyrate may induce lipolysis by mechanisms independent of AMPK activity, possibly through HDAC inhibition (Rumberger *et al.*, 2014). Thus weight loss observed with butyrate treatment in our group of mice may be due to lipolysis leading to raised serum lipids as it occurs in starvation (Kartin *et al*., 1944). This, however, requires further investigations particularly genetic expression studies on enzymes of lipid metabolism in our frozen tissue samples.

The current study further shows a general pattern of raised serum lipids in butyrate and metformin groups as compared to placebo. However owing to limited sample size only triglycerides, VLDL and cholesterol showed statistically significant differences after 2 weeks of intervention. Serum cholesterol was raised in intraperitoneal metformin group (181.01±28.39 mg/dL) versus i.p. placebo group (149.55±21.24 mg/dL) at

p=0.04. Increase in serum cholesterol was also observed in oral metformin group (195±46.84 mg/dL) compared to oral placebo group (152.27±46.79 mg/dL) but it was not statistically significant (P=0.09). Our findings are similar to Martin-Montalvo *et al*. (2013) who showed that long term chronic oral metformin administration in mice was associated weight loss along with raised triglycerides and cholesterol.

Triglyceride changes were observed in NaB group. Oral NaB treatment significantly raised serum triglycerides in (199±51.97 mg/dL) compared to placebo (129±20.50 mg/dL) at p=0.01. However the intra-peritoneal group did not show such a trend (230.96±108.05 mg/dL versus 174.87±68.50 mg/dL in placebo at p=0.64). In the current study we observed different effects of intra-peritoneal and oral routes of NaB on profile and weight loss. This gives a hint towards diverse mechanisms and metabolic effects of NaB when administered orally as compared to systemically and merits further research. It is known that intra-peritoneal dose increases bioavailability of an administered drug. The peritoneal dose was thus kept half of the oral dose. Predictably, even with a 50% reduced dose, the percentage weight loss in intra-peritoneal metformin group (8.7±2.8%) was greater than oral metformin group (7.6±2.5%) when compared with their respective placebo groups (weight losses of 4.8±2.2 in intraperitoneal and 1.8±2.9% in oral placebo group respectively). This difference may even have been underestimated since the two leanest mice of the intra-peritoneal metformin group expired on the 2nd and the 4th day. Oral NaB however produced a significantly greater weight loss than intra peritoneal group. The oral NaB mice lost 4.6±3.5% weight versus the oral placebo group (1.8±2.9%) at p=0.057 as compared to intraperitoneal NaB in which there was no significant weight loss (4.1±2.7%) compared to its placebo group (4.8±2.2%) at p=0.85. A recent study (Berndt *et al*., 2012) has shown a conspicuously different responses of oral versus intraperitoneal butyrate on chemically induced colitis in mice. Oral butyrate exacerbated the colitis and up-regulated IL23 expression by colonic dendritic cells whereas intraperitoneal administration attenuated the colitis. Although the context is different, this finding does underline the complexity of butyrate's mechanism of action. Further studies are required to look into the mechanism of action of oral versus systemically introduced butyrate. In our case we noticed weight loss in case of oral but not in case of intraperitoneal NaB.

There are several aspects to consider when looking at effects produced by oral and intraperitoneal butyrate. The beneficial metabolic effects of orally administered butyrate may occur by several mechanisms. First it may alter gut microbiota as hinted by some animal studies (Castillo *et al*., 2006; Galfi and Bokori, 1990; Van Immerseel *et al*., 2004). Second oral NaB may improve the intestinal barrier function by nourishing the colonocytes (Meijer *et al*., 2010), increasing the expression of epithelial tight junctions (Ma *et al*., 2012; Peng *et al*., 2009) and mucins (Gaudier *et al*., 2004) in the colon. This enhanced intestinal barrier is likely to reduce metabolic endotoxemia which is linked to obesity and metabolic syndrome (Moreno-Navarrete *et al*., 2012).Third, it may reduce intestinal secretion of chylomicron and very low-density lipoproteins (Marcil *et al*., 2002, 2003). Finally the differences may arrive from actions of NaB via the liver versus direct actions on peripheral tissues. Orally administered butyrate is transported directly to the liver where it may exert its major metabolic effects. Very little appears in the systemic circulation (van der Beek *et al*., 2015). In contrast intraperitoneal route is more likely to give NaB more access to peripheral adipose tissue, skeletal muscles and relatively less exposure to the liver.

CONCLUSION

Oral NaB butyrate given for 16 days caused significant weight loss in mice along with raised serum lipids. Such a trend was not observed with intraperitoneal route of administration. In comparison metformin therapy produced weight loss and raised lipids when given orally and intraperitoneally.

Statement of conflict of interest

The authors have no conflicts of interest to declare.

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