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Review Article

Wellness Fasting and Intestinal Microbiota in Chronomedicine

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Abstract

Since ancient times, fasting has been performed for religious reasons or as a cure of illnesses. However, a suitable fasting method for healthy longevity or daily wellness has not been established as of yet.

The possibility of BHB absorption from intestinal lumen like butyrate is not known. Therefore, we tried to clarify the occurrence of ketogenesis during fasting by measuring concurrent changes in glucose, ketone bodies, fatty metabolism and various hormonal changes in both blood and urine in relation to the intestinal microbiota.

Keywords: Microbiota; Fasting; Ketone; Glucose; Macrobiotics

Introduction

The importance of ketone body metabolism in diabetic patients has been well known since the 1970s, but recently, β-hydroxybutyrate (BHB) has been identified as a key component of a metabolic signaling pathway [1-9].

Recently the relationship between fasting and circadian rhythm has been reported [10]. The bidirectional relationship between circadian disruption and metabolic abnormalities is found in diabetic patients.

The circadian rhythm in food intake should be strongly affected by fasting. When glucose function is altered, circadian behavior should be altered.

Mitsuo Koda [11] developed fasting dietary therapy and confirmed beneficial effects for many patients with intractable diseases. About 900-1000 kcal/day by unpolished brown rice, green vegetable paste and *tofu* constitute the basic regimen of Koda's therapy. We found a successful patient who had recovered from spino-cerebellar degeneration at a young age by Koda's dietary therapy [12,13]. She had been living on only one glass of fresh vegetable juice per day for 18 years since her acute episode at age 20. Her ketone bodies, especially BHB in the blood, were more than 3 mM, so the main energy should come from ketone bodies.

Her biochemical changes coincided with the metabolic adaptation to yield BHB, as shown by elevated aspartate aminotransferase (AST) and creatine kinase (CK). High aspartate was a reflection of above metabolic change. Increased BHB was observed in two participants among five, and both of them had *Bifidobacteriacae* in thier fecal bacteria [14] Some Bacteroides had xylanase or cellulose activities, so these species may play an important role in fiber degradation in a strict vegetarian. Hayashi., *et al* [15]. had previously analyzed the fecal bacteria of Mori 15 years ago. They found that *Clostridium* and *Bacteroides* were the dominant groups. They also found many *Bifidobacterium* by direct culture.

Our previous study in wellness fasting and BHB production by Pass analysis showed that the decreased BMR and insulin significantly contributed to the increase in BHB and AcAc [12]. From our experience of the Mori case, we considered different gut microbiome should influence the production of BHB.

Subjective and physical change by wellness fasting

We have practiced Kushi macrobiotics for more than 10 years and developed the teaching and practicing system of a 4-day "Wellness-fasting". In this paper I would like to focus on the changes of intestinal microbiota during wellness fasting.

In ARSOA Wellness Fasting program, participants received a combination of a very low-energy vegetarian diet, physical exercise, meditation, and lectures about healthy lifestyles [12,16] (Figure 1). Peripheral blood was collected on days 1 and 4 for routine biochemical analyses and for the determination of serum levels of insulin, glucagon and other hormones. Glucose and BHB were measured every morning by finger-tip blood. The stools were collected in the pre-, mid- and post-fasting stage, and profiles of microbiota were analyzed by the metanalytic cytotechnicon [17-21].

Figure 1: Schedule of Wellness Fasting.

Body weight decreased from 55 kg to 53.5 kg, and it continued at least one month later. Waist circumference decreased from 85 cm to 82 cm at day 4, and it also continued for one month. Body weight reduction was 2.6 kg in males and 1.7 kg in females. Body fat rate decreased 1.3% and 0.7% in males and females, respectively, and muscular weight decreased 1.4 kg in males and 0.8 kg in females. Systolic blood pressure decreased 12 mmHg in both males and females, and 5 mmHg and 6 mmHg in diastolic pressure in males and females, respectively. On the contrary, the median pulse rate increased 10/min in males and 7.5/min in females. Body temperature decreased 0.2 – 0.3°C in both sexes. By correlation analysis BHB difference only correlated to the body temperature and pulse rate, and glucose decrease correlated with lowered diastolic pressure.

Although headaches, hunger, GI tract distress, emesis, depression and cold feeling occurred toward the 2nd day of fasting, these symptoms dramatically disappeared at the 3rd day, and active and vivid feeling increased on the 4th day and after (Figure2). Headache was common in females but improvement was better than males. Skin condition also improved by fasting.

Figure 2: Changes of subjective and physical condition during 4-day fasting Males (blue line), females (red line).

BMR decreased from 1107+/-101 kcal/day at 1st day to 1087+/-96 kcal/day on the 3rd day.

Biochemical data showed decrease of triacylglycerol (TG) and marked increase of free fatty acids (FFA) (Table 1). Glucose and insulin became lower and glucagon increased. AST increased one third, while ALT and γ-GTP did not change significantly. Growth hormone lowered, but changes of thyroid hormone did not occur. Urinary catecholamins were variable, but all catecholamins, such as adrenalin, noradrenalin and dopamine increased in the high BHB producer. Creatinin was slightly increased and estimated glomerular filtration rate (eGFR) lowered about 10% in all groups.

Fasting and hyperketosis

Decreased gluconeogenesis less than 4.5 mM seemed to stimulate ketogenesis (Figure 3).

BHB in the blood increased from 0.3±0.2 mM at the day 1 to 2.0±1.2 mM at the day 4 of fasting, while the glucose level decreased from 5.6±1.6 mM to 3.9±1.3 mM at 4th day. According to the increase of blood ketone bodies, urinary excretion of median acetylacetate increased to 40.9 mM (7.76-159.2) (min-max), BHB 25.1 mM (2.72-597.0) and total ketone bodies 66.1 mM (10.5-756.5) on the 4th day. These values returned to 0.16 mM (0-10.1) on 14th day (10 days after fasting).

Changes of serum biochemical markers on day 1 and 4th day of Fasting				
	day 1	day 4	dif	
n = 53	Mean sd	Mean sd	Mean sd	
TG	99.8 ±60.2	56.0 ± 20.9	-45.5 ± 58.0	***
FFA	736.2 ± 344.8	1900.2 ± 535.5	1181.4 ±607.3	***
glucose	5.0 ± 1.2	3.9 ±1.2	1.1 ±1.0	***
Insulin	6.2 ± 5.3	2.8 ±1.7	-3.5 ±5.2	***
glucagon	153.7±33.6	183.1 ± 60.0	26.0 ± 63.7	***
AST	21.4± 5.2	31.5 ±8.9	10.0 ±6.5	***
ALT	14.3 ± 6.8	16.1±7.5	1.8 ±3.3	
y_GTP	28.3 ± 32.1	27.1 ±28.3	-1.6 ± 5.4	0.113
Cre	0.6 ±0.1	0.7 ±0.1	0.1 ± 0.1	
eGFR	84.4 ±14.8	74.0 t 15.0	-10.1 ± 10.3	
AcAc	0.0± 0.0	0.3 ± 0.2	-0.3 ± 0.2	***
BNB	0.1± 0.1	1.8 ± 1.1	-1.7 ± 1.1	***
total ketol	0.0± 0.0	2.2 ± 1.3	-2.1 ± 1.3	***
TO; tryglyceride, FFA; free fatty acids, AST; ALT y_GTP, Cr, creatinine, eGFR; estimated glomelular filtration rae, AcAc: acetoacetate, BHB: $_{B}$ -hydroxybutyrate				

Table 1: Changes of serum biochemical markers on day 1 and 4th day of fasting.

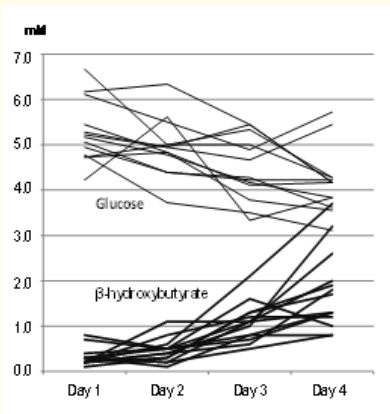

Figure 3: Decrease and increase of glucose and β- hydroxybutyrate.

Various factors to correlate with increase of BHB was analyzed by AMOS (Figure 4).

Figure 4: Correlation analysis by AMOS.
Changes of glucose, insulin, BMR, and body weight affected the BHB increase. Acetoacetate had positive correlation with BHB and negative correlation with body weight and insulin change.

Changes of microbiota by fasting

More than 600 microbiotas were determined by our system [17-21]. Rejected hits were 29.6% and undetermined rate was 9.4%. These were summarized to 60 genus, 29 families, 14 orders, 11 classes and 9 phyla. Most common bacteria in phylum was *Firmicutes* (36-40%), next *Bacteroidetes* (24%), *Actinobacteria* (11-16%), and then *Proteobacteria* (0.7-1.9) and *Verrucomicrobia* (0.602%.). When compared bacterial profiles in 3 different times, pre-, mid- and post-fasting period, *Actinobacteria, Proteobacteria* and *Verrucomicrobia* showed significant changes by fasting (Table 2).

The number of species in the prefasting stool was 201 specimens, which decrease to 140 specimen at the mid fasting, and increased to 221 specimens the post fasting stool. Long term stability of human gut microbiota was reported [22,23], but rapid change was also reported by David., *et al* [24]. like our cases. Diversity increased about 10% after fasting.

Time	Prefasting			Midfasting			Postfasting			ANOVA
phyllum	Mean	sd	median	mean	sd	median	mean	sd	median	p
Firmicutes	40.31	8.12	40.57	36.88	11.03	38.61	41.09	9.38	40.71	0.057
Bacteroidetes	24.06	10.93	23.19	24.11	11.17	24.80	24.05	7.99	23.86	0.999
Actinobacteria	11.70	7.59	10.04	16.63	12.52	13.85	11.87	10.73	8.73	0.024*
Proteobacteria	6.65	6.51	3.94	3.90	4.77	1.58	7.04	7.74	3.61	0.026*
Verrucomicrobia	0.69	1.71	0.02	1.93	3.88	0.01	0.70	1.84	0.01	0.024*
Fusobacteria	0.38	1.46	0.00	0.31	1.16	0.00	0.18	0.69	0.00	0.68
Lentisphaerae	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	nd
Spirochaetes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	nd
Synergistetes	0.00	0.01	0.00	0.01	0.03	0.00	0.00	0.02	0.00	nd
Rejected hit	16.20	7.76	15.80	16.24	9.70	14.36	15.06	6.69	15.43	0.699

Table 2: Comparison of microbiotic profile at phylum level by pre-, mid-, and post fasting period.

Fasting caused quick disappearance of *Bacteroides, Blautia* and *Akkermansia*. They returned to the prefasting state in post fasting stool with increased diversity of microbiota (Table 3).

Correlation of BHB levels and microbiota

Microbiota profile at family level in the different fasting stage divided by BHB levels by the 4th day.

Table 3: Increased diversity of microbiota by pre-, mid- and post fasting. Yellow shows Firmicutes, blue Bacteroides, pink. Actinobacteria, brown Proteobacteria and circled is Akkermansia

BHB concentration at the 4th fasting day showed variety, so it was categorized to less than 1 mM, 1-2 mM, 2-3 mM and more than 3 mM. If we examined various relationship, significant correlation was found between highest BHB group and *Enterobacteriacea* family in the pre-fasting (Figure 5).

Figure 5: Microbiota profile at family level in the different fasting stage divided by BHB level at 4th day

Significant correlation was found between highest group of BHB and Enterobacteriaceae (red) in the pre-fasting stool.

Syntrophic and suppressive changes of microbiota by ketone production

Further analysis of bacterial profile at the species level clarified that 15 species were directly or indirectly related to BHB level, such as *Providencia vermicola, P. rustigianii, P. sneebia, Morganella morganii, M. psychrotolerans, Proteus hauseri, Butyricimonas virosa*, etc. Five cases with very low *Enterobacter* presence showed lowest plasma BHB increase. Only one case with high BHB level did not show any attributable bacterium. Plasma concentration of BHB was related to Enterobacteriaceae directly or indirectly. The latter suggested syntrophic effects on Enterobacteriaceae. Known butyrate producing bacteria seemed to suppress by competitive

Seven species directly related to BHB level, such as *Providencia vermicola, P. rustigianii, P. sneebia, Morganella morganii, M. psychrotolerans, Proteus hauseri,* and *Butyricimonas virosa* (Table 4). They showed syntrophic growth among them. *Butyricimonas virosa* coexisted with *Providencia rustigianii* (CC = 1.000***) and *Morganella morganii* (CC = 0.763***). *Bacteroides finegoldii* and *Para-*

Table 4: Correlation between microbiota with significant association with BHB concentration.

bacteroides distasonis in *Bacteroidaceae* and some *Firmicutes*, such as *Clostridium bolteae, C. lavense, Enterococcus avium, Megamonas repellensis, Allisonella histaminiformans* and *Flavonifractor plautii* were indirectly associated with *Enterobacter families*, except for *Flavonifractor plauti* (Table 4).

Roseburia faecis and *Blautia faecis* were negatively associated with BHB concentration, but they showed syntrophic correlation with *Providencia rettgeri, Hafnia paralvei,* and other enterobacters. Five cases with very low Enterobacter profile showed lowest BHB level. Only one case with high BHB level showed no positive association to proteobacteriae, but broad association was present, such as *Megamonas funiformis, Prevotella copri* and P. *stercorea, Bifidobacterium adolescentis, Faecalibacterium prausnizii*, etc.

These associations were confirmed by individual level. *Enterobactereciae* species were recognized in 20/22 high BHB producers, 13/17 moderate BHB producers and 15/16 low producers. Two in high BHB, 4 in middle BHB, and 1 in low BHB did not have *enterobacteriacea* species, and in these cases, *Clostridium bolteae, C. lavalense, Enterococcus avium, Hunatella hathewayi, Megamonas rupellensis* and *Ruminococcus bromii* seemed to be related to BHB production by coexistence with *Enterobacteriaceae*.

Cooperation (syntrophic) or suppression (competitive) of bacilli was suggested by correlation network among individual species (Table 4). Generally, BHB producing bacteria seemed to suppress butyrate producing bacteria. Summary relationship between BHB level and network of microbiota is shown in table 5.

	Primary correlation	CC with BHB	phylum	Secondary relationship	CC with left column
Direct correlation	*Providencia vermicola*	.418**	P		
	Providencia rustigianii	.400**	P		
	Providencia sneebia	.399**	P		
	Morganella morganii	.332*	P		
	Morganella psychrotolerans	.399**	P		
	Proteus hauseri	.281*	P		
Indirect correlation	*Butyricimonas virosa*	.400**	B	*Providencia rustigianii*	1.000***
	Clostridium lavalense	.399**	F	*E. coli/shigela*	.385**
	Enterococcus avium	.399**	F	*P. sneebia, P.vermicola, Morganella*	1.000***
	Parabacteroides distasonis	.360**	B	*Providencia*	.990***
	Bacteroides finegoldii	.318*	B	*Providencia, Butyricomonas*	.442***
	Megamonas rupellensis	.293*	F	*Kluyvera, citrobacter*	.390***
	Allisonella histaminiformans	.285*	F	*Proteus, Hafnia*	.994**
	Clostridium bolteae	.282*	F	*E. coli/shigela*	.385**
	Flavonifractor plautii	.272*	F	many	.600***
Negative correlation	*Roseburia faecis*	-.288*	F	*Enterobacter, Hafnia, Klebsiella*	.405***
	Blautia faecis	-.350**	F	many	.400-.500***
	Rejected hit	-.285*			

Table 5. Microbiota with direct and indirect correlation with serum BHB level.

Relationship to butyrate producing bacteria

Cahill studied the glucose metabolism of people who fasted for 40 days, and found that in the starving human adult, BHB and aceto-acetate were produced in the liver from long-chain fatty acids and BHB could be the energy source in the brain and other tissues. A rise of BHB blood concentration to approximately 6 mM was characteristic. Approximately all of the lactate, pyruvate, glycerol, and amino acid carbons which are removed by the liver and kidney are converted into glucose, as evidenced by substrate balances across these organs to keep the basic level [25]. Increased AST is a reflection of metabolic change. BHB is considered to be produced in the liver, kidney and astrocytes in the brain, but our case report suggested the involvement of intestinal microbiota [14]. Fasting caused hyperketonemia, but the degree was different from individual to individual.

Butyrate-producing bacteria may represent a functional group, rather than a coherent phylogenetic group, within the microbial community of the human large intestine. Butyrate formation can play a special role in bacterial energy metabolism [26-29] and this implies that certain features of energy metabolism and microbial ecology may be shared between phylogenetically distinct groups of butyrate-producing bacteria. Numerically, two of the most important groups appear to be *Faecalibacterium prausnitzii*, which belongs to the *Clostridium leptum* cluster, and *Eubacterium rectale/ Roseburia* spp., which belong to the *Clostridium coccoides* cluster of firmicute bacteria. After reduction to butyryl-CoA, butyrate can be formed either with the enzymes phosphotransbutyrylase and butyrate-kinase via butyry-l-phosphate or with the enzyme butyryl-CoA: acetate CoA-transferase, which utilizes acetate as a co-substrate and generates acetyl-CoA (Figure 6).

Figure 6: Synthesis and degradation of ketone bodies. Reference pathway and bacteria with key enzyme.

Could synthesis of BHB come from butyrate? The current data suggested Enterobactericeae mainly correlated to the BHB production, but several other species in Clostridium and Bacteroides that had correlation with BHB were indirectly related to Enterobactericeae. Enterobacter seemed to suppress Firmicutes, so butyrate producing bacteria would be suppressed in the colon. The possibility of metabolism by intestinal microbiota from butyrate to BHB in the body seemed to be unlikely.

In addition, there are many bacteria in the soil that can synthesize poly (3-hydroxy butyrate-co-3 hydroxyvalerate) oly-beta hydroxyl butyrate [30-32].

It was suggested that the metabolic pathway of butyrate synthesis and BHB production seemed to be done by different microbiota. Absorption of BHB from intestinal microbiota could occur like a BHB supplement use, so dietary effects should be clarified more. The possible absorption of BHB through intestinal wall could be accepted independently from butyrate production.

Conclusion

Four days of fasting induced hyperketosis without concurrent clinical symptom. The magnitude of changes in ketone body concentrations correlates with the levels of insulin, glucose, free fatty acids and several hormones. Very stable glucose levels were noteworthy under metabolic change of fasting. Enhanced ketone body production occurred when the blood glucose level was less than 4.5 mM (90 mg/dl). The urinary excretion of BHB/acetylacetate (AcAc) ratio increased from 0.56 among low ketone concentration, and 3.36 among high ketone concentratuin. The highest excretion of ketone bodies into the urine was 756 mmole/g creatinine. Further studies are needed to clarify the hormonal effects and to establish whether high urine excretion rates of ketone bodies could affect the renal function.

Wellness fasting caused metabolic and physiological changes, and induced hyperketonemia which showed correlation with pre-fasting microbiota profile.

High BHB level showed association with family *Enterobacteriaceae* directly or indirectly.

The dominance of *Enterobacteriaceae* seemed to suppress butyrate producing bacteria. So, the BHB production seemed to be independent from butyrate pathway in the gut. The rout of BHB synthesis would be multiple by syntrophic and/or competitive growth of bacteria. Composition of Intestinal microbiota influenced the level of plasma BHB. Prefasting Enterobacteriaceae increased BHB level. Suppression of butyrate producing bacteria was noticed. Direct and indirect relationship to produce BHB levels was present.

As the legacy effects of wellness fasting, improved lifestyles, normalized blood pressure, increased diversity of intestinal microbiota. Dietary intervention is effective for continuation of a good healthy state.

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Conflict of Interest

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