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Fasting therapy, a medicine for human rescue: Live a life with fasting, yoga and brown rice.

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Abstract

Fasting therapy is an old folk therapy that has been used since ancient times. In this research, We, first, showed the series of changes of physical information before and after fasting therapy and second, classified the fasting therapies into calorie restriction, intermittent fasting and long-term fasting and explained them based upon the fundamental changes caused by fasting therapies and recent medical viewpoints.

In the seven-day fasting therapy, decreases of body weight and visceral fat, increases of values of hemoglobin and uric acid associated with dehydration, and increases of ketone bodies such as β-hydroxybutyric acid and acetoacetic acid were observed, and in relation to bone metabolism, the acceleration of bone absorption (the increase of bone-type tartrate-resistant acid phosphatase-5b and type-1 collagen cross-linked N-telopeptide) were observed. In relation to the glycation stress index, the increasing tendencies of 3-deoxyglucosone, acetaldehyde, glyoxal and methylglyoxal were observed; however, the fluorescence intensity of skin advanced glycation end products (AGEs) decreased by 13%. In the genetic analysis of gastric mucosa conducted in the past, the enhanced expression of von Hippel-Lindau tumor suppressor protein (VHL) was recognized, which suggested the possibility that it is related to the control of cancer growth.

Long-term fasting is fundamentally different from intermittent fasting and calorie restriction. Due to the long-term existence of extremely low blood glucose and hypoinsulinemia and ketosis, senescent and weakened cells and lesioned (genetic mutation and cancerous transformation) tissues are broken down, which makes it possible for them to self-replicate by recycling the broken-down parts. It can be said that fasting is the most appropriate simultaneous and multiple micro-surgery without using a scalpel.

***KEY WORDS*:** fasting therapy, genetic analysis, advanced glycation end products (AGEs), oxidative stress, von Hippel-Lindau tumor suppressor protein (VHL)

Introduction

Japan is a low birthrate and super-aging society. The most frequent cause of death of the elderly is malignant tumor (45%), followed by cardiac disease (13%), vascular brain disease (8%) and pneumonia (6%), and these diseases are caused by immune deficiency, arterial sclerosis, high-blood pressure, diabetes and inflammation. It has been proven that fasting therapy and a calorie restriction diet improves on these causes and leads to healthy life expectancy based on the research with humans and animals thus far. However, these therapies are not considered as important as taking medication and surgery. I am carrying out fasting therapy for seven days twice a year. In this report, I reported the changes of physical information at the time of fasting therapy and the measured results centering on glycolipid metabolism index relating to glycation stress, in particular, in the form of a case report, along with some literature reviews.

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Case

60 years old (as of 2011), male (born in 1951)

Back Ground

20 years old: My encounter with fasting, yoga and brown rice

After entering university, I lived in the house of my uncle in Kawaguchi, Saitama, Japan. This area was called "cupola (foundry) town" and there were many small and medium scale foundries. Affected by heavy smoke emission from these foundries, he developed bronchial asthma, and received pharmacotherapy and desensitization therapy; however, his symptoms did not improve. He tried various folk therapies but nothing went right. Finally, he tried raw food therapy, brown rice diet, fasting therapy as well as Yoga, and finally, he was able to recover from bronchial asthma without taking drugs.

There were many books including a complete collection of Indian philosophy and those of yoga and fasting therapy in his uncle's study. I read these books at random and I was very interested in yoga, in particular. As a result of yoga training, I acquired the yoga technique of Nauri (dent belly and move the muscles of both sides) in the course of a week, that of Sirsiasana (cross legs in the posture of handstand) in the course of a month and those of all Asanas of the instructional book [1] in the course of a year. When I was 20 years old (in the summer of my first year of university), I experienced fasting for the first time for two weeks in a fastinghall in Kofu, Yamanashi, Japan. I was relatively energetic during the first week and I had enough physical strength to take a walk with other participants. However, from the second week, suddenly my physical strength declined and I had a terrible experience where I could not sit still. Although I was reading a book to distract my mind, I could not concentrate in reading and various thoughts came to my mind one after another. My body weight decreased to 51 kg from 61 kg, 10 kg in 14 days (no change in my body height of 175 cm). Next year, I went to the National Kaivalyadhama Health and Yoga Research Center in Lonavla, Maharashtra, India for training with the assistance of my uncle. Since then, I have continued yoga and a brown rice diet.

Changes of Property of Stool: Actual Condition of Black Stool Impaction

Since 1980, I had studied abroad as an intern and resident. Because I was very busy during that time, I did not practice fasting therapy. I returned to Saitama National Hospital in 1988 and was assigned to the position of director of the hospital from 2010 to 2017. Since then, I have conducted fasting for one week during New Year holidays and summer vacation until now.

The change of property of stool caused by fasting therapy was as follows: 2-3 days after fasting, coal-black and sticky stool was excreted. This is called "stool impaction" and it had been said to be the mixture of sludge fastened to the intestinal wall and mucosa which peeled off because of fasting. However, looking at it carefully, I found that the color of stool impaction was deep green, which means that it was mainly the color of concentrated bile. During fasting, the gallbladder expands and concentrated bile is stored in it. Because the gallbladder shrinks due to the stimulus of returning to diet, concentrated bile is excreted, so that the stool becomes dark green in color.

In the Saitama National Hospital, fasting (very low calorie, 100 to 200 Kcal/day) therapy which is acknowledged by the Japan Fasting Therapy Society was conducted with patients interested in the therapy out of those with type 2 diabetes, obesity, high-blood pressure and metabolic syndrome. The achievements of fasting therapy were presented under the title of "Current Status of Fasting Therapy in Japan and Genetic Study of Gastrointestinal Mucosa" at the 48th Japan Digestive Disease Week (JDDW) Panel Discussion of "Merits and Demerits of Fasting (no eating and drinking) Therapy for the Patients with Digestive Diseases," held on October 13, 2006. A paper was presented concerning fasting (no eating and drinking) therapy and nutrition administration for patients with digestive diseases, which was published in a special edition "Gastrointestinal Medicine" [2]. Even now, fasting therapy (very low calorie) is conducted with patients who desire it in the Saitama National Hospital.

Current Status of Fasting Therapy

In the relevant literature shown in the journals of the West and America, the fasting therapies are classified into intermittent fasting, alternate-day fasting and long-term fasting based on fasting periods, with the patients with type 2 diabetes who are taking medicine and the sustained long-term effect into consideration. In Japan also, petit fasting, half-day fasting, one-day fasting, week-end fasting and three-days-or-more fasting are conducted. There are cases when water or low-calorie juice (300 to 400 Kcal/day) is given during fasting. Japanese words of *zesshoku and danjiki*, both expressing fasting, are often confused. *Danjiki* therapy or *danjiki* training places emphasis on the spirit more than the body, and *zesshoku* therapy places emphasis on the body more than the spirit. Both *danjiki* and *zesshoku* are translated as "fasting" in English.

In order to distinguish between the fasting as folk therapy and the one with religious aspects, the two related societies in Japan changed the names of the societies to the Japan Fasting Therapy Society and the Japan Fasting Study Group, respectively. It is truly regretful that Dr. Mitsuo Koda has passed away, who was leading the study group famous for "light eating fasting therapy" (died in 2008) [3]. Many great religious persons such as Buddha, Christ and Muhammad practiced fasting as a religious ascetic practice. In Japan also, thousand-day circumambulation and eating none of the five grains are practiced in Mt. Koyasan. The history of fasting therapy, which had so far been a religious practice and a folk therapy, started in 1930 as the medicine in Japan from the study by Hideo Takahira *et al*. of Department of Physiology, Keio University (Tokyo, Japan). After that, the Japan Fasting Therapy Society and the Japan Fasting Study Group were established. "Tohoku University method of fasting therapy" is covered by insurance as a psychosomatic therapy [3].

In the early days, fasting therapy was conducted for the purpose of conquering chronic and incurable diseases. However, recently, it is broadly applied for various purposes, such as improvements of metabolic syndrome including obesity, therapies for anti-aging, beauty, anti-cancer and spiritual exercise (***Table 1***) [4]. The period of fasting is often around a week as the typical, basic method. The period of fasting should be determined based on individuals' mental endurance and the purpose of fasting.

Table 1. The practical purpose of Fasting therapy (Rebuilding of body and mind).

Prevention and improvement of metabolic syndrome and weight reduction (obesity, diabetes, hypertension, dyslipidemia)
Overcome and treatment of chronic diseases, incurable disease and cancer
Health care and promotion
Constitutional improvement, rejuvenation, longevity and beauty (improvement of skin conditions)
Slow aging (no an anti-aging therapy)
Relieve of constipation and fecal impaction
Temperance (stop drinking) and off cigarettes (stop smoking)
Stress elimination and autonomous neural training
Religious training, spiritual exercises and comprehension
Enhancement in memory
Treatment and prevention of physical and mental diseases
Detoxication
Reflection of dietary behavior, improvement of unbalanced diet and promotion of appetite
Forced molting in chicken (restoration of egg production ability)

Table is quoted and modified from the reference "Fasting therapy for psychosomatic diseases" [4].

There are various periods of fasting therapies from very short-term fasting therapies of one day a week and one or two days a month to long-term fasting therapies of several weeks. Recently, long term fasting tends not to be conducted due to taking accidents and complications into consideration.

Fasting therapy is contraindicated for the following diseases: tuberculosis, non-obese diabetes, high emaciation, weakness, gastrointestinal ulcer, digestive tract stenosis and adhesion and more than moderate chronic kidney failure (blood urea nitrogen [BUN] is 40 mg/dL or more). Before you take fasting therapy for three days or more, you should receive a detailed doctor's examination.

In order to prevent accidents during this rigorous fasting therapy, a therapy that should be called "very low-calorie therapy" is devised by experts, and micro-diets, low-carb diets and ketogenic diets are incorporated into it.

Examination of Visceral Fat, Liver Stiffness and Various Blood Parameters Before and After Fasting Therapy

Purpose

Based upon the findings on fasting therapy until recently, and based upon the genetic study of gastrointestinal mucosa (2006) [2] and the experiment of this time (2017) including oxidative stress and glycation stress as accelerators of arteriosclerosis, malignant transformation and aging, we attempted to clarify the fundamental reaction of a living body to fasting therapy, including new aspects.

Method

A fasting therapy for seven days was conducted. Although at a previous time, fluid replenishment by intravenous drip by Tohoku University Method was applied, this time more than two liters of water were orally administered. Blood parameters on the starting day of fasting therapy, the 4th day, the 8th day and the 14th day were measured. As the indicators of glycation stress, the concentrations of 3-deoxyglucosone (3DG), acetaldehyde (AA), glyoxal (GO) and methylglyoxal (MGO) in plasma, and the fluorescence intensity of skin AGEs were measured. The fluorescence intensity of skin advanced glycation end-products (AGEs) was measured by RQ-1201J-SET (Sharp Life Science, Kobe, Hyogo, Japan) placed on the tip of the left middle finger [5]. 3DG, AA, GO, MGO are the substances with aldehyde group as functional group, and they were measured by HPLC-FL method using 2,3-diaminonaphtalen (DAN) indicator [6,7].

Visceral fat was measured using 320 columns CT (Toshiba; Minato-ku, Tokyo, Japan), liver fat content and liver stiffness were measured using liver stiffness measuring equipment (Fibro Scan 502; Echosens, Paris, France) on the starting day of fasting therapy and the 8th day. The gastric mucosa and large intestine mucosa of the previous therapy were biopsied and the results were histologically discussed and at the same time the results of the analysis of their gene expression analysis were discussed

Results

Body weight decreased by 8.0 kg and the rate of decrease was 8.4%. Body mass index (BMI) was 8.5% and abdominal girth decreased by 4.1%.

As a result of the measurement of visceral fat using CT, visceral fat area decreased to 85.9% and the ratio of visceral fat/subcutaneous fat area decreased to 83.5% (***Table 2***).

The time lapse of blood test is shown in ***Table 3***. In general biochemical examination of blood, deviation enzymes including AST (GOT), ALT (GPT) and creatine phosphokinase (CPK) increased by 20-30%, hemoglobin increased from 14.4 g/dL to 17.3 g/dL (20%), and uric acid increased from 7.0 mg/dL to 16.1 mg/dL. No gout attack was observed, so it was considered to be the variations due to dehydration. In glucose metabolism examination, fasting blood glucose shifted between 72 mg/dL and 82 mg/dL and HbA1c between 5.6% and 5.7%, which showed no large variations (***Fig. 1***). In lipid metabolism examination, low-density lipoprotein cholesterol (LDL-C), triglyceride (TG) and lipoprotein (a) (LP (a)) increased 1.4-1.8 times. In endocrine examination, insulin decreased by almost half from 13.1 μU/mL to 5.9 μU/mL (−45%), glucagon increased 1.8 times, growth hormone increased 1.5 times and insulin-like growth factor-I (IGF-I) remained almost unchanged.

Table 2. hanges of anthropometry.

	Hight cm	Body weight kg	BMI	Waist circumference cm	Total fat square cm^2	Visceral fat square cm^2	Subcutaneous fat square cm^2	Visceral ／ Subcutaneous square ratio
Before fasting (12/27)	175.0	89.2	29.1	96.7	385.7	236.3	149.3	1.58
Right after fasting (1/3)	175.0	81.7	26.7	92.9	356.8	202.9	153.7	1.32

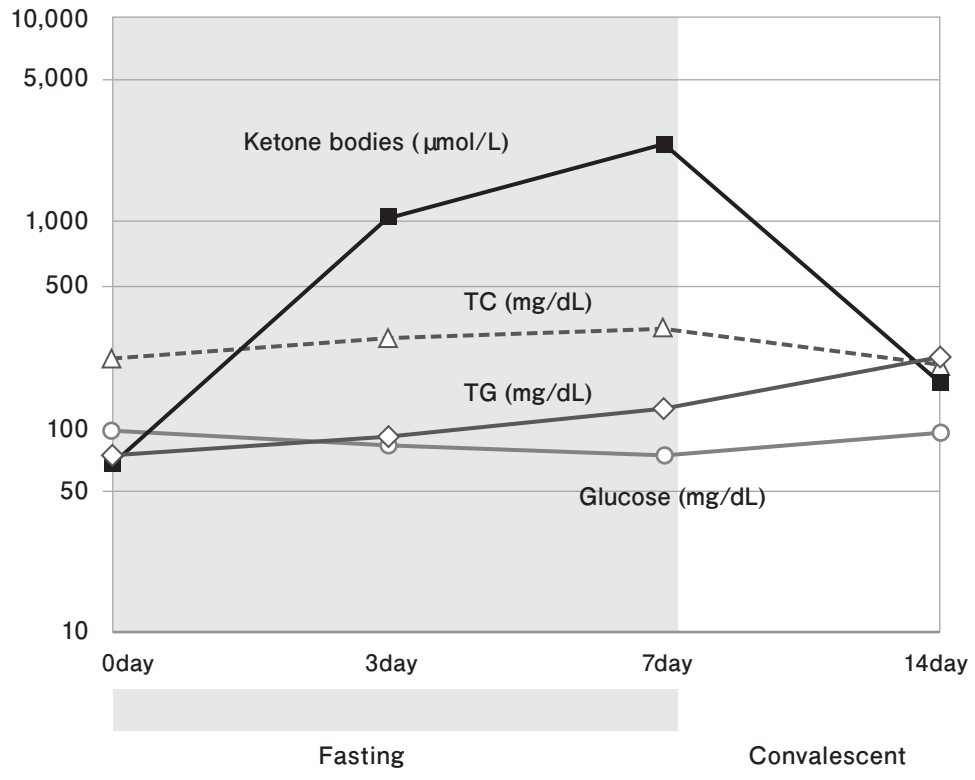

Fig. 1. **Glucose/lipid metabolism and fasting.**
TC, total cholesterol; TG, triglyceride.

Table 3. Blood biochemical examination.

		Before fasting 12/27	During fasting 12/30	Right after fasting 1/3	One week after fasting 1/10
【Blood chemistry】		—			
TP	g/dL	7.7	8.9	8.7	7.9
Albumin	g/dL	4.5	5.1	5.2	4.6
AST (GOT)	U/L	56	65	72	49
ALT (GPT)	U/L	60	81	80	64
ALP	U/L	142	161	162	147
γ-GTP	U/L	53	59	52	37
CPK	U/L	279	308	371	291
UA	mg/dL	7.0	10.9	16.1	9.3
BUN	mg/dL	17.4	16.0	26.7	18.4
Cre	mg/dL	0.91	1.21	1.55	1.06
Cystatin C	mg/L	0.9	1.1	1.3	NE
Na	mmol/L	141	140	136	142
K	mmol/L	4.6	4.3	4.7	4.7
Ca	mg/dL	8.8	9.7	9.9	8.8
CRP	mg/dL	0.31	0.07	0.09	0.34
【Peripheral blood examination】		—			
WBC	/μL	2,810	3,240	3,840	4,220
Lymphocyte %	%	51.6	55.2	50.5	57.1
Lymphocyte count	/μL	1,450	1,788	1,939	2,410
RBC	x10^4 /μL	456	528	537	430
Hb	g/dL	14.4	16.7	17.3	13.5
Plt	x10^4 /μL	18.4	23.2	25.3	20.6
【Lipid profile】		—			
TC	mg/dL	213	270	303	199
TG	mg/dL	71	88	122	217
LDL-C	mg/dL	123	174	217	108
Lipoprotein (a)	mg/dL	12.2	28.9	36.8	9.6
RLP-C	mg/dL	2.8	3.5	6.0	12.4
【Glucose metabolisim】		—			
Glucose	mg/dL	116	96	101	111
HbA1c	%	5.7	5.6	5.6	5.7
【Endocrinology】		—			
Insurin (IRI)	μU/mL	13.1	12.2	5.9	NE
Glucagon (IRG)	pg/mL	209	312	381	NE
Growth hormone	ng/mL	0.65	0.38	0.96	NE
IGF-I	ng/mL	129	145	139	NE
【Ketone bodies】		—			
Total ketone bodies	μmol/L	66	1,066	2,418	163
Acetoacetic acid	μmol/L	7	143	388	47
β-Hydroxybutyric acid	μmol/L	59	923	2,030	116
【Liver fibrosis marker】		—			
Hyaluronic acid	ng/mL	115.9	166.4	183.4	172.5
Type 4 collagen	ng/mL	143	105	83	164
【Bone metabolisim】		—			
TRCP-5b	mU/dL	226	307	319	322
Serum NTX	nMBCE/L	14.1	17.3	20.2	15.7
【Tumor markers】		—			
Pro-GRP	pg/mL	NE	38.8	53.7	78.2
Anti-p53 Ab	U/mL	<0.69	<0.69	<0.69	<0.69

NE, not examined; TP, total protein; AST (GOT), aspartate aminotransferase (glutamic oxaloacetic transaminase); ALT (GPT), alanine aminotransferase (glutamic pyruvate transaminase); ALP, alkaline phosphatase; γ-GTP, γ-glutamyl transpeptidase; CPK, creatine phosphokinase; UA, uric acid; BUN, blood urea nitrogen; Cre, creatinine; CRP, C-reactive protein; WBC, white blood cell; RBC, red blood cell; Hb, hemoglobin; Plt, platelet; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; RLP-C, remnant-like lipoprotein particles-cholesterol; IRI, immunoreactive insulin; IRG, immunoreactive glucagon; IGF-I, Insulin-like growth factor-I; TRCP-5b, tartrate-resistant acid phosphatase 5b; NTX, type I collagen cross-linked N-telopeptide; Pro-GRP, pro-gastrin-releasing peptide; Ab, antibody.

Due to the effects of starvation, ketone bodies increased 37 times, β-hydroxybutyric acid increased 34 times and acetoacetic acid increased 55 times, hyaluronic acid, which is a liver fibrosis marker, increased 1.6 times and type IV collagen decreased 40%.

Regarding bone metabolism, bone-type tartrate-resistant acid phosphatase-5b (TRACP-5b), which is a bone resorption marker, and type I collagen cross-linked N-telopeptide (NTx) increased 1.4 times. TRACP-5b is an enzyme existing in osteoclasts and leaks into the blood along with the acceleration of bone resorption. NTx is a degradation product of bone type I collagen and is excreted into urine, reflecting on bone resorption. As these findings indicate the acceleration of bone resorption, calcium was orally administered.

Regarding the intermediate aldehyde compounds, which are indicators of glycation stress measured a new in this research, 3-DG, AA and GO slightly increased 10% and MGO only increased nearly three times. In skin AGEs fluorescence intensity measurement, AGEs fluorescence intensity decreased 13% from 0.62 to 0.54 (***Table 4***).

Changes were observed in the property of stool. The stool two days after fasting was sticky and deep-green colored, almost black. This is the color of concentrated bile, as above mentioned. The stool excreted on the second day after the completion of fasting was noteworthy.

Discussion

Comparison with Previous Fasting Therapy

The results of the fasting therapy of this time and a previous one were discussed from the fundamental viewpoint of fasting therapy and the recent viewpoint of medicine,

Glycation stress indicators were measured in the previous report, too. As a result of previous therapy, pentosidine was nearly unchanged (***Fig. 2***). As a result of therapy of this research, 3-DG, AA and GO only slightly changed, MGO increased and the fluorescence intensity of skin AGEs decreased. 3DG, AA, GO and MGO are the indicators of early and interim periods in glycation stress reaction and skin AGEs is that of last period. It is certain that glycation stress indicators change in one fasting therapy: however, it is quite unknown about the variations in the periods from three to four months after fasting. How these variations affect living bodies is the task for the future.

In the previous observation, gene expression state was observed using biopsy specimens of gastric mucosa and large intestinal mucosa. As a result, enhanced expression of von Hippel-Lindau tumor suppressor protein (VHL) was recognized in gastric mucosa. VHL controls the sensing of oxygen concentration of peripheral tissues, and has the action of specifically combining with Hypoxia-inducible factor 1α (HIF-1α), a transcription factor important for hypoxia response, and collapsing it HIF has the function of promoting blood supply to cancer tissue, encouraging its growth [8-10], therefore, it can be expected that the collapse of HIF by VHL leads to controlling the growth of cancer tissue.

Gene expressions of various antioxidants including glutathione peroxidase, β2-microglobulin and ferritin and ribosomal protein having various functions were enforced in the large intestine.

Biochemical Characteristics of Fasting Therapy

The following are the fundamental aspects of the biochemistry of fasting therapy. The changes in living bodies by fasting therapy start from the dissolution of body fat and early dissolution of protein caused by stopping the energy supply. According to Voet's Biochemistry [11], because the storage of carbohydrates is used up in a day, after fasting for several days, oxaloacetic acid in liver is used up for gluconeogenesis, so that the metabolic capacity of acetyl-CoA by citric acid cycle has an extreme decrease. The secretion of insulin decreases and fatty acid is mobilized from fat tissue. Therefore, the liver converts acetyl-CoA to ketone body and releases it. The brain synthesizes necessary enzymes and gradually adapts to ketone body. After the three-day fasting, ketone bodies fill only a third of the energy demand of the brain; however, after around 40 days, they can

Table 4. Changes of intermediate aldehydes.

		Intermediate aldehydes in serum (ng/mL)			
		3-DG	Acetaldehyde	GO	MGO
Before fasting	12/27	97.5	37.3	6.2	8.8
During fasting	12/30	85.4	41.3	5.9	15.2
Right after fasting	1/3	111.8	45.8	6.7	22.6
One week after fasting	1/10	102.8	45.7	7.4	8.5

3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal.

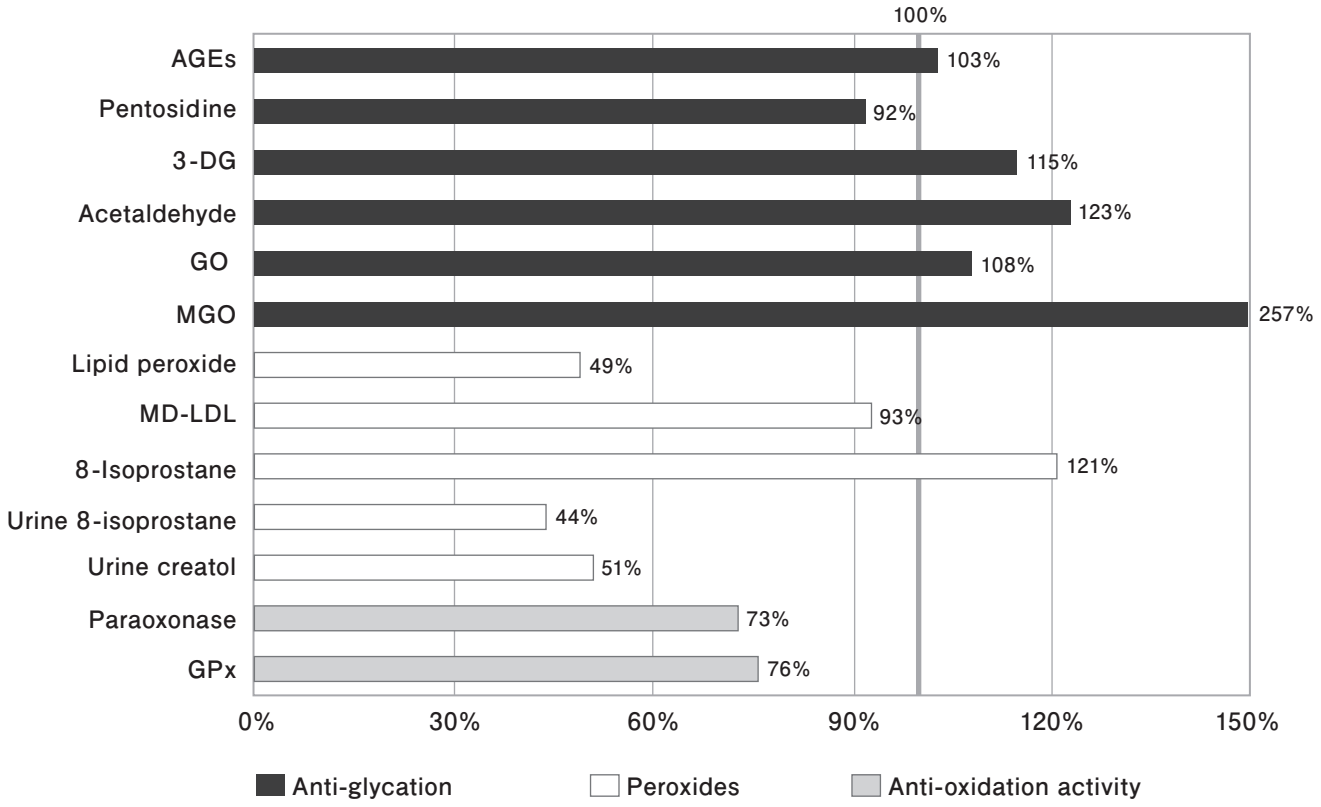

Fig. 2. **Previous results of fasting therapy showing percent change of data.**

AGEs, advanced glycation end products; 3-DG, 3-deoxyglucosone; GO, glyoxal; MGO, methylglyoxal; LDL, low-density lipoprotein; MD-LDL, malondialdehyde-modified LDL; GPx, glutathione peroxidase.

fill 75% of energy demand. The main constituents of ketone body are β-hydroxybutyric acid and acetoacetic acid, and their levels increase more than 70 times during fasting. The muscle break-down rate after long-term fasting is about 25% of that after fasting for several days.

In association with the decrease of blood glucose, the decrease of insulin and the increases of glucagon, ketone bodies, cortisol and norepinephrine are observed. The rise of the level of norepinephrine (adrenalin) prevents the lowering of metabolic rate. A moderate decrease in blood pressure is observed. Rapid increase of free fatty acid (FFA) and increases of TC, LDL-C and TG are recognized, which means that body fats are mobilized. Water dissolving vitamin B and C are led to a light-level deficiency condition due to fasting longer than a week. As vitamin A is stored in the liver for use for 5 to 10 months, it does not vary in a short period.

Regarding organs and systems of the body, the induction of AST and ALT in the liver, and the lowering of intestinal peristalsis and atrophy of the intestinal mucosa are recognized. A moderate decrease in high-sensitivity C-reactive protein (CRP) was observed. The increases of sex hormone binding globulin, IGF-I and IGF-II were observed. The markers of oxidative stress and inflammation also decreased. The symptom relating to asthma also decreased and improvements were found in some indicators of quality of life (QOL). AGEs causing glycation stress are said to worsen degenerative diseases including diabetes, atherosclerosis, chronic renal failure and Alzheimer dementia [12]. AGEs are also said to be the cause of diabetic vascular complications and accelerate cell damage caused by active oxygen and change the function of cells. AGEs derived from glyceraldehyde are the most poisonous [13].

Depending upon the recent medical findings, fasting therapies were classified into caloric restriction (CR), intermittent fasting (IF) and long-term fasting (LF) and compared (***Table 5***) [14,15]. The decreasing rates of fasting insulin and insulin resistance of both groups of LF and IF were moderate, and the decreasing amounts of LF and IF were larger than that of CR. The decreasing rate of body weight of LF was rapid in proportion to calorie restriction amount, and those of IF and CR were moderate. In the case of fasting for 24 hours, the growth hormone of female subjects increased 13 times and that of male subjects increased 20 times. The secretion of growth hormone increased more than twice during fasting for five days. A high level of growth hormone maintains muscle mass, lean tissue mass and bone density. The muscle degradation rate after a long-term fasting is about 25% of that of fasting for several days. Sirtuin gene, which is said to be a longevity gene and anti-aging gene, increases 2-4 times by fasting for 48 hours, and it increases from several times to 10 times by CR for seven weeks. Obese patients with asthma lose 8% weight and reduced oxidative stress and inflammatory markers by IF for eight weeks (20% calories at fasting time).

Table 5. Summary of recent medical finding of fasting therapy.

	LF	IF	CR
Body weight	↓ (right after ↓↓)	↓	↓
Visceral fat	↓	↓	↓
Lean body mass	～	～	↓
Energy metabolism	↓↓	↓↓	↓
Fasting plasma glucose	50～70	90～140	90～140
Ketone bodies	40 times	Slight elevation	～
Uric acid	↑↑↑	↑	～
Insulin	↓↓	↓↓	↓
Glucagon	↑	↑	～
Growth hormone	↑	↑	～
Noradrenaline	↑	↑	～
Cortisol	↑	～	～
Ghrelin	↓	～	↑
Sirtuin gene expression	2～4 times	NI	4～10 times
Skin gross and elasticity	↑↑↑	↑	～
High-sensitivity CRP	↓↓	↓	↓
B/T cell acute lymphoblastic leukemia	Inhibition	NI	～
Apoptosis	Elevation	NI	NI

LF, long-term fasting; IF, intermittent fasting; CR, caloric restriction; NI. No information; C-reactive protein.

Effect on Endoplasmic Reticulum

Endoplasmic reticulum (ER) is an important sub-cellular organelle controlling secretion within cells and quality control of membrane protein. The denatured protein formed by starvation, ischemia, infection and inflammation becomes abnormal protein with incomplete folding and is accumulated in ER and causes ER stress, and as a result, it becomes the cause of apoptosis (cell death). ER stress is confirmed in brain neuron cells of degenerative diseases such as Alzheimer's disease [16-18].

Oxidative stress and glycation stress cause abnormal holding of protein in ER and accelerate ER stress. If ER stress is enhanced, the generation of active oxygen by mitochondria increases and oxidative stress is further enhanced. Meanwhile, GRP78, which is one of the molecular chaperones as an indicator of the response to ER stress, which develops and advances in glucose starvation conditions [19, 20].

According to Professor Valter Long of the Longevity Institute of Southern California University (Los Angeles, USA), LF and IF decrease the enzyme inhibiting activation of stem cells (PKA) and decrease IGF-I increasing the risks of aging and cancer growth. Fasting can regenerate and protect the immune system damaged by aging and side effects of chemotherapy and promote its neogenesis by enhancing the regenerative powers of stem cells and hematopoietic stem cells. Seventy-two hours of fasting before chemotherapy can protect the immune system and increase the effect of chemotherapy up to 20 times. Hematopoietic stem cells associated with the generations of blood and immune system is activated by fasting, and as a result, new white blood cells are generated and the immune system is regenerated [21].

Effect on Cancer Cells

Fasting therapy is considered to exert some influence in cancer risk, too. Cancer cells metabolize glucose and amino acids, but not fatty acids or ketone [22]. In order to starve cancer cells, it is indispensable to restrict glucose and amino acid (glutamine). Ketogenic diet [23] was effective when it was combined with the period of intermittent diet, in particular. Ideal blood glucose concentration to weaken cancer cells is 60-70 mg/dL, and ketone level of 4-7 mM is recommended (***Table 6***). LF enhances Keap-Nrf2 system, decreases the production of heme oxygenase and activates cytotoxic T cells. It shows that fasting increased the expression of Nrf2 target gene via Nrf2- and SIRT1-dependent mechanisms. Fasting induces Nrf2 target gene by at least 1.5-5 times in the mouse liver.

GRP78 of ER stress response indicator is expressed and advanced in glucose starvation environment even in cancer cells [20]. The balance between ER stress response in normal cells and that in cancer cells at the time fasting should be considered; however, it has been clinically confirmed that cancer tissue shrinks by fasting [24-27]. It is simply the

Table 6. Comparison of energy use between normal cells and cancers cells.

	Normal food intake (non-fasting)		Fasting	
	Glucose	Ketone bodies	Glucose	Ketone bodies
Normal cells	○	×	△	◎
Cancer cells	◎	×	△	×

sophistication of the living body.

An enhanced expression of VHL in gastric mucosa was recognized in the previous experiment. VHL inhibits angiogenesis and cell growth by working as a ubiquitin ligase of HIF, specifically combining with HIF and disrupting it (***Fig. 3***).

Phospholipase C, which gene expression is inhibited in the large intestine, specifically acts on phosphatidylinositol diphosphate, lipids of the membrane, generates water-soluble inositol trisphosphate and increases the concentration of calcium in cell [28]. The inhibition of the expression of the phospholipase C gene is also possibly involved in the inactivation of protein kinase C having an effect on carcinogenesis.

Effect on Intestinal Flora

The number of genes of the whole intestinal flora is more than 1,000 times that of all human genes, so that intestinal flora is said to be a new organ and that it functions as a control tower of health maintenance. Due to the westernization of the diet (high-protein, high-lipid and low-fiber diet), the balance of the structure of intestinal flora, which is a very important partner for human beings, is being lost (dysbiosis). Even though the number of good bacteria is decreasing by aging, even the elderly can dramatically increase it by 24-hours of fasting [29]. Recently, it is said that intestinal flora is deeply involved in the working of brain and nerve system and it has effects on sleep, stress reaction, memory and cognitive ability [30-34]. There have been very few studies on fasting therapy affecting intestinal flora [35, 36], so that additional research in this respect is expected in the future. Finally, what we should not forget is the changes in psychological and spiritual aspects during fasting. The feelings of exhilaration and vitality and the awareness of changes in cognitive ability are deepened, which arise from the last part of the fasting period to the early part of fast-breaking period and are different from those which are thought to come from the increase of endogenous opioid.

Conclusion

We discussed the results of previous research and that of this current research, based on the fundamental changes caused by fasting therapy and recent medical viewpoint. The fundamental difference of long-term fasting from intermittent fasting and calorie restriction is considered to be caused by extremely low blood glucose, hypoinsulinemia and ketosis remaining for a long-term period. It is the process that long-term fasting causes the self-destruction of the cells aged and weakened with low blood glucose, hypoinsulinemia and ketosis and the tissues lesioned (by gene mutation and cancerous transformation), and make them regenerate by recycling broken-down parts, so that it can be said that " it is the most appropriate simultaneous and multiple micro-surgery without using a scalpel."

The cancers which age-adjusted death rate is increasing are pancreatic cancer for males and pancreatic cancer and breast cancer for females. Metastatic pancreatic cancer, having approximately 61 cancer-related mutations, was examined using the technique of molecular clock of evolutionary biology. It was found out that in order for an original cancer to generate in the pancreas, it takes more than 10 years after the first mutation and original cancer takes five years to have metastatic nature and it takes two additional years to lead to death. Fasting has the ability to make micro cancer disappear, reduce cancer and inhibit the migration and transplantation of cancer while metastasizing, by the induction of apoptosis and activation of hematopoietic stem cells, as well as conquer the immune depression caused by cancer chemotherapy [37].

For the longevity and cancer inhibition as the greatest purposes of anti-aging, the restriction of calorie intake is being positioned as an essential and fundamental therapy rather than indiscriminant replacement therapy. Calorie restriction optimizes the adaptation mechanism like this, and eventually it optimizes individual existence. Regarding the fasting therapy existing in the extreme position of calorie restriction, we would like to diffuse fasting therapy while continuing the research of the cases of fasting therapy, comparing with the results of animal experiments based upon the results of this research and clarifying the real mechanisms of inhibition of cancer, anti-atherogenic action, anti-inflammation action, immune improving action and spiritual trance condition.

The atrophy of intestinal tract caused by long-term fasting is observed and the breakdown of aged cells and cancerous tissues can be expected. Therefore, the direct absorption of nourishment through intestinal tract wall is not desirable because it stimulates the cells of the intestinal tract wall. Dehydration should be avoided in the elderly with advanced arterial sclerosis for the prevention of brain infarction, cardiac infarction and arrhythmia, so that the drip infusion of 500-1,000 mL/d including electrolytes recommended by fasting therapy society is necessary. In order to write this paper, I did fasting with water only for a week. Probably due to this, I excreted a beautiful straight stool 35 cm in length that I took a picture with a mobile

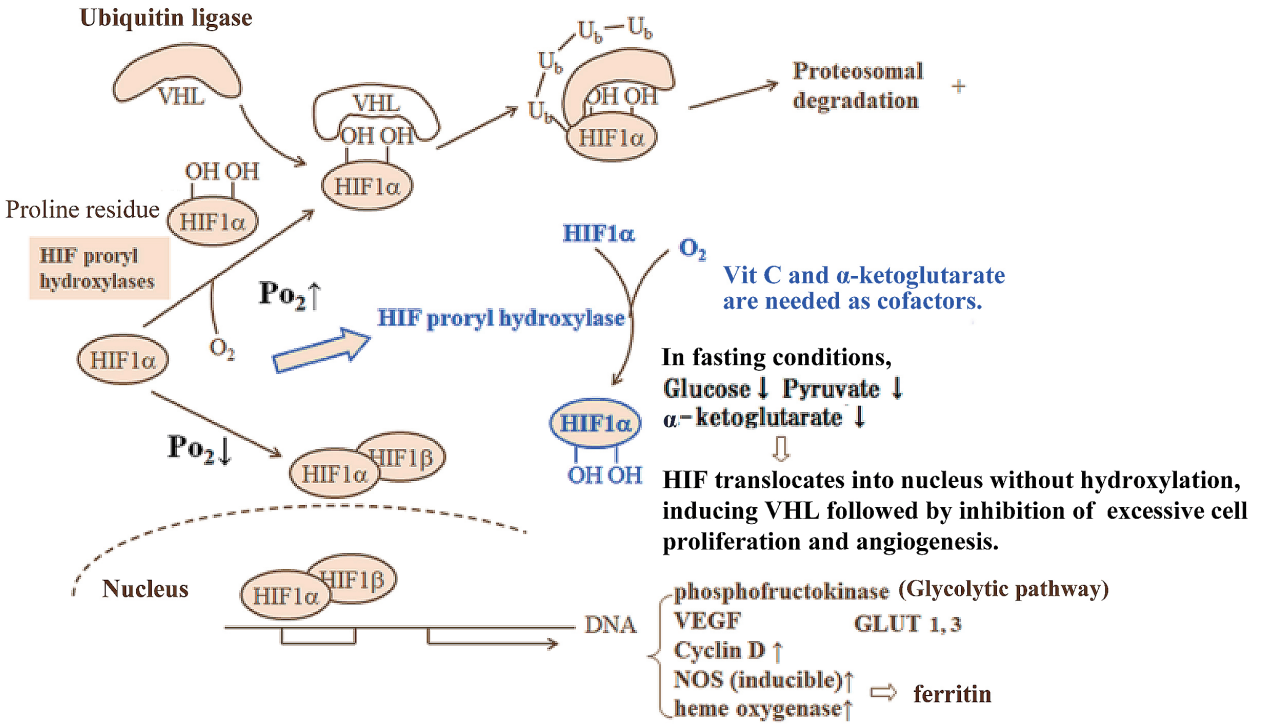

Fig. 3. **VHL/HIF pathway and cell proliferation.**

VHL, von Hippel-Lindau tumor suppressor protein; HIF, hypoxia-inducible factor; VEGF, vascular endothelial growth factor; GLUT, glucose transporter; NOS, nitric oxide synthase; PO_2, partial pressure of oxygen.

phone, because it was the first time in my life of 66 years. Long-term fasting (for around a week) is the best duration for me, not only from a physical aspect, but to look back at my life. As the fasting was proceeding, feelings of exhilaration and euphoria appeared. Various ideas were floating probably because of ketosis. It is dangerous to carry out the ideas immediately after returning to diet.

I wonder if I was reincarnated as a man with physically high efficiency. Because I gain weight consuming two meals a day, I am maintaining a diet of one meal a day. In short, "long-term fasting and daily calorie restriction" are necessary. When I do not have lunch with my colleagues, I take a walk in Meiji Shrine for an hour. I recommend everyday walking, a brown rice meal a day, yoga, bodily exercise, recitation of Heart Sutra (mantra in particular) and long fasting twice a year.

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Conflict of Interest Statement

The authors claim no conflict of interest in this study.

References

1) Yesudian S, Haich E. Yoshimura N (Ed). Mind and body health promotion method by yoga (Sport and yoga). Hakuyo-sha, Tokyo, 1986. (in Japanese)
2) Sekizuka E, Nakadate H, Hosoda Y, et al. Current status of fasting therapy in Japan and genetic study of gastrointestinal mucosa. Digestive Medicine. 2007; 45: 1-7. (in Japanese)
3) Koda M. Science of fasting therapy: Practical constitution remodeling. Shunju-sha, Tokyo, 2001. (in Japanese)
4) Japan Fasting Therapy Society (Ed). Fasting therapy for psychosomatic diseases. Van Medical, Tokyo, pp32-42, 1995. (in Japanese)
5) Yamanaka M, Matsumura T, Ohno R, et al. Non-invasive measurement of skin autofluorescence to evaluate diabetic complications. J Clin Biochem Nutr. 2016; 58: 135-140.

6) Steghens JP, van Kappel AL, Denis I, et al. Diaminonaphtalene, a new highly specific reagent for HPLC-UV measurement of total and free malondialdehyde in human plasma or serum. Free Radic Biol Med. 2001; 31: 242-249.
7) Kon M. Study for glycative stress evaluation by measurement of intermediate aldehydes. Doshisha University Graduation thesis, 2016. (in Japanese)
8) Miyake M, Fujimoto K, Anai S, et al. Heme oxygenase-1 promotes angiogenesis in urothelial carcinoma of the urinary bladder. Oncol Rep. 2011; 25: 653-660.
9) Isobe T, Aoyagi K, Koufuji K, et al. Clinicopathological significance of hypoxia-inducible factor-1 alpha (HIF-1α) expression in gastric cancer. Int J Clin Oncol. 2013; 18: 293-304.
10) Miyake S, Kitajima Y, Nakamura J, et al. HIF-1α is a crucial factor in the development of peritoneal dissemination via natural metastatic routes in scirrhous gastric cancer. Int J Oncol. 2013; 43: 1431-1440.
11) Voet D, Voet JG. Biochemistry, 4th Edition. Wiley, Hoboken, NJ, 2013.
12) Ichihashi M, Yagi M, Nomoto K, et al. Glycation stress and photo-aging in skin. Anti-Aging Med. 2011; 8: 23-29.
13) Takeuchi M, Takino J, Yamagishi S. Involvement of the toxic AGEs (TAGE)-RAGE system in the pathogenesis of diabetic vascular complications: A novel therapeutic strategy. Curr Drug Targets. 2010; 11: 1468-1482.
14) Moore J, Fung J. The complete guide to fasting: Heal your body through intermittent, alternate-day, and extended fasting. Victory Belt Publishing, Las Vegas, NV. pp39-261, 2016.
15) Lu Z, Xie J, Wu G, et al. Fasting selectively blocks development of acute lymphoblastic leukemia via leptin-receptor upregulation. Nat Med. 2017; 23: 79-90.
16) Kaneko M, Okuma Y, Nomura Y. Molecular approaches to the treatment, prophylaxis, and diagnosis of Alzheimer's disease: possible involvement of HRD1, a novel molecule related to endoplasmic reticulum stress, in Alzheimer's disease. J Pharmacol Sci. 2012; 118: 325-330.
17) Hosoi T, Ozawa K. Molecular approaches to the treatment, prophylaxis, and diagnosis of Alzheimer's disease: Endoplasmic reticulum stress and immunological stress in pathogenesis of Alzheimer's disease. J Pharmacol Sci. 2012; 118: 319-324.
18) Soejima N, Ohyagi Y, Nakamura N, et al. Intracellular accumulation of toxic turn amyloid-β is associated with endoplasmic reticulum stress in Alzheimer's disease. Curr Alzheimer Res. 2013; 10: 11-20.
19) Mote PL, Tillman JB, Spindler SR. Glucose regulation of GRP78 gene expression. Mech Ageing Dev. 1998; 104: 149-158.
20) Schönthal AH. Pharmacological targeting of endoplasmic reticulum stress signaling in cancer. Biochem Pharmacol. 2013; 85: 653-666.
21) Cheng CW, Adams GB, Perin L, et al. Prolonged fasting reduces IGF-1/PKA to promote hematopoietic-stem-cell-based regeneration and reverse immunosuppression. Cell Stem Cell. 2014; 14: 810-823.
22) Meidenbauer JJ, Mukherjee P, Seyfried TN. The glucose ketone index calculator: A simple tool to monitor therapeutic efficacy for metabolic management of brain cancer. Nutr Metab (Lond). 2015; 12: 12.
23) Khodadadi S, Sobhani N, Mirshekar S, et al. Tumor cells growth and survival time with the ketogenic diet in animal models: A systematic review. Int J Prev Med. 2017; 8: 35.
24) Park HR, Tomida A, Sato S, et al. Effect on tumor cells of blocking survival response to glucose deprivation. Natl Cancer Inst. 2004; 96: 1300-1310.
25) Brandhorst S, Longo VD. Fasting and caloric restriction in cancer prevention and treatment. Recent Results Cancer Res. 2016; 207: 241-266.
26) Sun P, Wang H, He Z, et al. Fasting inhibits colorectal cancer growth by reducing M2 polarization of tumor-associated macrophages. Oncotarget. 2017; 8: 74649-74660.
27) Buono R, Longo VD. Starvation, stress resistance, and cancer. Trends Endocrinol Metab. 2018; 29: 271-280.
28) Reddy BS, Simi B, Patel N, et al. Effect of amount and types of dietary fat on intestinal bacterial 7 alpha-dehydroxylase and phosphatidylinositol-specific phospholipase C and colonic mucosal diacylglycerol kinase and PKC activities during stages of colon tumor promotion. Cancer Res. 1996; 56: 2314-2320.
29) Crawford PA, Crowley JR, Sambandam N, et al. Regulation of myocardial ketone body metabolism by the gut microbiota during nutrient deprivation. Proc Natl Acad Sci U S A. 2009; 106: 11276-11281.
30) Daulatzai MA. Non-celiac gluten sensitivity triggers gut dysbiosis, neuroinflammation, gut-brain axis dysfunction, and vulnerability for dementia. CNS Neurol Disord Drug Targets. 2015; 14: 110-131.
31) Fröhlich EE, Farzi A, Mayerhofer R, et al. Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. Brain Behav Immun. 2016; 56: 140-155.
32) Gareau MG. Cognitive function and the microbiome. Int Rev Neurobiol. 2016; 131: 227-246.
33) Noble EE, Hsu TM, Kanoski SE. Gut to brain dysbiosis: Mechanisms linking Western diet consumption, the microbiome, and cognitive impairment. Front BehavNeurosci. 2017; 11: 9.
34) Anderson JR, Carroll I, Azcarate-Peril MA, et al. A preliminary examination of gut microbiota, sleep, and cognitive flexibility in healthy older adults. Sleep Med. 2017; 38: 104-107.
35) Michalsen A, Riegert M, Lüdtke R, et al. Mediterranean diet or extended fasting's influence on changing the intestinal microflora, immunoglobulin A secretion and clinical outcome in patients with rheumatoid arthritis and fibromyalgia: An observational study. BMC Complement Altern Med. 2005; 5: 22.
36) Rong ZH, Liang SC, Lu JQ, et al. Effect of intermittent fasting on physiology and gut microbiota in presenium rats. Nan Fang Yi Ke Da Xue Xue Bao. 2016; 37: 423-430. (in Chinese)
37) Rodwell VW, Bender D, Botham KM, et al. Harper's Illustrated Biochemistry 30th Edition. McGraw-Hill Education, New York, 2015.