Metabolic Syndrome

Chapter 3

History of Prolonged Dietary Deprivation in Human and a Novel Fasting Protocol with the Therapeutic Potential in Metabolic Syndrome

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1. Introduction

Calorie restriction (CR) has been first reported in extending lifespan since 1935 but it continues to be a major research area in biological gerontology [1]. The life-extending ability has been found to occur in both genders of many different mammalian species such as rat, hamsters and mouse strains, as well as nonmammalian species such as fish, flies, and water fleas [1]. Based on the results from rodents' studies, people estimated that if calorie restriction could apply in human, the lifespan would increase for at least 20 years. Therefore, investigation in the effects of CR on nonhuman primate has begun at 80's as the last animal model approaches before human practice among multi-center of rhesus monkey inside USA and yielded some significant markers improvement in disease risk and health [2]. However, the results from the rhesus monkey studies supplied insufficient support comparing with what from rodents' in regarding with increasing maximum lifespan. In fact, although CR monkeys were demonstrated an improved metabolic profile and less oxidative stress as indicated by plasma isoprostane levels, CR regimen implemented in young and older rhesus monkeys at the National Institute on Aging (NIA) has not demonstrated in significantly improving the survival effects [3]. Although the early reports from rhesus monkey center of Wisconsin-Madison showed a 30% increase in survival [4]. This improvement mainly related with the unhealthy diet of control food which led the heavier bodyweight [5]. Eventually the CR increased maximum lifespan in rhesus monkeys were not as impressive as the results from rodents' studies [5,6,7].

Both the non-human primate groups which initiated in young or older monkeys had demonstrated significantly improved health. However, survival curves were not significantly increased, which suggested by the authors that the effects of CR in long-lived animals are complex and likely dependent on a variety of environmental, nutritional, and genetic factors [3]. One critical difference in NIA CR study was that control monkeys were not truly fed AL, and the regulated portioning of food for the NIA control monkeys might be slightly restricted and thus, largely prevented obesity. The difference in NIA monkeys may experience survival benefits similar to the 10% restriction reported in the rodent data, and the difference might cause the diluted effect in longevity of CR [3]. Besides, although the differences in caloric intake were identified between control and CR monkeys for both research centers, old-onset NIA controls were lower than other groups of both centers [7]. In fact, an age-related decline in food intake and motivation in rhesus monkeys were observed that parallel with the findings in humans, and a long-term CR regimen were no more motivated for food comparing with the control [8]. Therefore, older CR monkeys were no longer consuming 30% less than the control, which might be a major cause of less CR benefit in longevity than shorter lived species [8].

Since both monkeys and humans are significantly impacted with age associated decline in normal food intake, it seems that the experience of a real cut off of calorie or energy supply might significantly impact on the health [3,7]. On the other side, current evidence have demonstrated that fasting could induce potent lifespan extension in E. coli, yeast, and C. elegans [9-12]. Under certain conditions, fasting regimens showed more effective and efficient to achieve health benefits than other types of dietary paradigms including dietary or calorie restriction (DR/CR) [13]. However, to date, fasting in human has only been extensively studied on short-term or intermittent schedules which was initially based on BUCHINGER's therapeutic fasting [14] or some very low calorie (200~500 kcal/day) fasting which lasted up to a year for weight management, disease prevention and chemotherapy facilitation [15,16]. Fasting paradigms have been proved to be more effective and efficient at achieving health benefits than are other types of dietary paradigms, including dietary or calorie restriction (DR/CR) [13].

Within recent years, for the safety and simple choice, fasting in higher species has mostly been studied as short-term or intermittent fasting schedules (IF) [15]. In the modern time, people choose easier way for the beneficial, and alternative fasting has turned to be the most popular choice of fasting practice. Alternative fasting has been developed in many choices such as one alternate between periods of eating and not eating, alternate day fasting, Eat Stop Eat Fasting practice. Alternate Day Fasting, which alternate between one day of eating what you like and either not eating, or eating restricted amounts the following day. In addition, the OMAD Diet stands for "one meal a day", which regarded as during every 24 period a person

can fast for 23 hours and then have a one hour eating period. Warrior Diet is one of the different types of fasting where you don't need to totally abstain from food for a period of time. Among various forms of IF, a recent report considered alternate day fasting (ADF), defined as strict 36-h periods without caloric intake ("fast days") followed by 12-h intervals with ad libitum food consumption ("feast days"), to be one of the most extreme dietary interventions [17]. In fact, there were more than choice of at lease a hundred alternative fasting paradigms in the market to fit most people's preference. However, all of these methods might have focused only on one concern: safety. Base on common knowledge and historical studies, people believed that prolonged fasting is unsafe. Therefore, we would like to review in detail on the historic reports of prolonged fasting and summary of some key points that people have ignored.

At the beginning of the current century, it has been reported that some individuals could survive continual no-food life up to 40 days [18, 19]. In fact, the earliest prolonged total fasting has been formally recorded since 1915, when a single volunteer was strictly observed in hospital for 31 days fasting experience. The most valuable data from this study was its body weight record [20]. Since then, the total fasting studies have been extensively performed after Bloom initiated in 1959 [21], and later the records of longest fasting time has been from 249days [22] to 382days which has been recorded as the longest fast in Guinness Book of 1971 [23]. The earlier historical book from China also recorded some examples of months to years of absolutely no food status (termed as bigu) as a way to attain longevity and spiritual distillation [24; 25]. The earliest formal bigu record came from Han Dynasty (206BC) in China when Prime Minister Zhang Liang had practiced "A life of Dao induced no grain intake, and ceased going out of door for more than ten years" due to his health issues [26]. However, there have been lack of systematic and detail studies of practical methodology of bigu on measurable health-related analysis. In the modern time, according to the historic description, bigu is further described as "taking in qi to avoid food," and is regarded as a special technique to achieve a long and healthy life. Research on mouse hybridoma cells has demonstrated longer survival without commonly acknowledged essential nutrients after receiving external qi treatments without serum or in phosphate-buffered saline buffer without further nutrient ingredients supplement [25; 27]. The qi practitioners of such society have tracked some individuals under absolutely no food but qi's life for years without publishing any formal research manuscript due to an attempt in protection of the qi related bigu practitioners [28].

To date, there have been three types of systematic studies related to prolong fasting: 1) those initiated during 1950 and 1970 that created Guinness World Records of total fasting for 250 to maximally 382 days [22,23]. A series of metabolism studies led by G.F. Cahill Jr. involving prolonged starvation that led to the establishment of ketone-body metabolism during fasting [29] a therapeutic fasting protocol established by O. Buchinger Jr. in 1952 that used fresh fruit or vegetable juice servings as alternative energy supplies, which led to an average

total calorie intake of 200–250 kcal per day to assist an up to 21-day incomplete fasting status [14]. Buchinger's practice have developed series intermittent or low calorie fasting schedules due to easier concept acceptation. There have been insufficient attempts to build up a proper, more tolerable prolonged total fasting regime as a health-improvement practice for society due to the following concerns: 1) safety issues – whether prolonged fasting is safe for practice in human society; 2) tolerance and discomfort during the entire total-fasting procedure. In fact, during the 60~80s of the last century, some safety concerns were reported regarding prolonged total fasting [30,31]. They were usually due to extensive application of dietary deprivation. Usually, if a total fasting was persisted for more than 40 days, negative consequences might start to happen [30,31].

The most advanced applications of alternative fasting schedules have been reported that used a very low-calorie diet (200~500 kcal/day) to substitute for complete fasting for 1 to 3 weeks or even a year for weight management, disease prevention, and chemotherapy facilitation [14,15]. The health improvement benefit of those paradigms was reported as satisfaction. However, an individual has to face months or even years of daily low-calorie dieting or hunger, which seemed to be discomfort, and inconvenient in life. Under the current schedules of either alternative fasting or CR, each individual has to face months or even years' life of suffering in low-calorie dieting, which might be negatively impact on the daily practice. Monkey's CR studies have demonstrated poor wound healing, immunological dysfunction and sexual ability in long term low calorie intake. On the other hand, although people believe that prolonged fasting paradigms might be lack of practical value and had been related with some accidents death in history, the reports on human to live without food for up to a year have supplied solid evidence that a human's ability in surviving without food might have no limitation. Some people also believe that prolonged fasting could lead to dehydration and even cause the body to kick into survival mode, where it starts storing more fat in response to extended periods of deprivation. However, if we develop some better paradigm to avoid these problems, it might lead the prolonged fasting turn to be more practical in resolving metabolic syndrome.

Based on the consideration on historical realities, we have designed a special natural form of prebiotic intervention, Flexible Abrosia (FA, with 113.4 KJ in each 10-g pack), which consists of food-grade polysaccharose, which might be mostly absorbed by bacteria rather than the human host [32,33]. It was hypothesized that long-term dietary deprivation (DD) leads gut microbiota to resort to host-secreted mucus glycoproteins as a nutrient source, which causes erosion of the colonic mucus barrier and hunger pangs [34]. We have applied a daily intake of 10 g FA at three mealtimes (3 x 113.4 KJ, which is less than 100 kcal per day) under a fasting paradigm. The calorie supply was not targeted at maintaining basic energy supply in human; rather, it was designed to reduce the status of starvation of intestinal flora, which might reduce

the injury of bacteria to the human digestive system. Compared with previously reported fasting-mimicking diet (FMD, 3000–4600 kJ per day) facilitated-fasting paradigms [35] and Buchinger's Periodic Fasting intervention, which contained fruit juice to maintain a minimum calorie intake of 200 kcal [14], our FA-facilitated continual dietary deprivation paradigm (FA-CDD, with less than 100 kcal non-human-absorbable daily calorie intake) would be close to a no-calorie intake protocol. Considering the life-threatening negative consequences that usually occur after more than 40 days' complete fasting [30,31,36], we have first suggested a practical fasting period of 1 week for the entire FA-CDD treatment. We realized that maintaining the real no food status would make the subject more efficient in overcoming the hunger sensation. Since then, the FA functional food supplement has assisted up to 1000 volunteers to achieve the FA-CDD paradigm with tolerable sensations of hunger for a continual 7 days for the purpose of weight control or chronic disease treatment [37]. Some of the volunteers voluntarily chose to extend to 14-d total fasting and experienced tolerable and favorable consequences [38].

Here, we collect 6 individual subjects with typical 7D-CDD experience and focus on reporting special phenomena of and scientific evidence on the 7D FA-CDD. We also included 3 subjects who voluntarily extended their CDD to 13~14 days. Our report supplies some preliminary results of this paradigm prior to applying more sophisticated clinical trials to further evaluate the potential health improvement value of its application in human society.

2. Materials and Methods

2.1 Study design and participants

A complete clinical trial registration has been deposited with the Chinese clinical trial registration organization (http://www.chictr.org.cn with registration # ChiCTR-OOC-17010377). Approval of the study protocol was given by the University of Henan Human Research Protection Program under the guidance of the China Association for Ethical Studies. The protocol was also recorded with the Medical Ethics Committee of Henan Medical Association of Henan Province, and the entire clinical study was under the supervision of the ethics board of The First Affiliated Hospital of Henan University. Before initiating the program, signed informed consent was obtained before everyone participated, and history, physical, electrocardiogram, laboratory, physical, and ultrasonic exams were also performed as pre-med checks. Inclusion criteria were as follows: (i) volunteers from the hospital staff, including doctors, nurses, lab and medical technicians, etc., and their relatives, (ii) age 21-65 years, (iii) absence of any exclusionary factor among the individuals participating, such as active medical or psychiatric problems, history of heart disease and potential heart problems such as heart failure, myocardial infarction, and cardiac arrhythmia, renal dysfunction, serious blood clots, intestinal obstruction or ulcer, or type-1 diabetes patients with islet dysfunction.

2.2 Procedures

Volunteers were recruited and introduced to the FA-CDD program. Before the trial started, the individuals received medical and laboratory examinations, including collections of serum, plasma, urine, and feces samples. FA-CDD involved daily oral application of a solid beverage of Flexible Abrosia (FA, Beijing Cloud Medical International Technology, Inc. China) 10 g/bag/person per treatment at three mealtimes every day on an outpatient basis during the fasting period. The ingredients of FA were designed to include dietary fiber and cordyceps polysaccharide, ganoderma lucidum polysaccharide, and hericium erinaceus polysaccharide [32], which were regarded as bacteria- but not human-consumed saccharides [36]. The National Food Inspection Center of China has reported the analyzed energy of 10 g FA as 113.4 KJ (27 kcal), which indicated that even if the calories from each treatment were completely absorbed by the human being, it would be less than 100 kcal daily in total, significantly less than recently reported low-calorie (500 kcal per day) intake in the treatment of cancer [13].

During the 7D-CDD period, individuals were advised to avoid any food intake, especially carbohydrates, and only to drink plenty of water and keep mineral electrolyte intake and vitamin supply constant. During the first 3~5 days, to overcome extreme hunger sensations and cravings for food, some individuals might consume a few pieces of fruits such as cucumber or tomato. Further, participants could also consume either 1 stick of 375 mg potassium/400 mg magnesium (DAS Gesunde Plus, Deutschland) or 1 pellet of 1.08 g (K=10 mEq) potassium citrate extended-release tablets (Dawnrays China) every day during extreme hunger periods in the day time, which could further ameliorate fasting-induced mineral loss and reduce peristaltic pangs of the smooth muscles of the gut.

According to the written informed consent forms, the individuals could quit the ongoing program at any time and at any step of the experiment without giving an explanation.

During the entire experimental fasting period, concomitant hunger sensations and tolerance limits categorized into different levels were recorded in a questionnaire supplied by the project administration. Physical and neurological examination, weight, blood and urine chemistries, electrocardiogram, bioelectrical impedance-directed body composition analysis, and ultrasound exam were checked at three time-points by the medical authorities of the hospital: 1) before the regimen was initiated (control baseline - ad libitum), 2) 7th day of fasting (7D-CDD), and 3) after recovery with food intake for 7-14 days or 4) after recovery with food intake for more than three months to as much as 6 months (**Figure 1**). The medical and basic laboratory exams were performed in The First Affiliated Hospital of Henan University. The extra serum and plasma samples were collected and stored under -800C for future molecular

and mechanistic analyses. The ELISA and metagenomics tests were performed at Beijing Institute of Radiation Medicine.

Bioelectrical impedance analysis (BEIA) was performed using the In Body 720 Body Composition Analyzer at the Nutritional Department of the First Affiliated Hospital of Henan University. In Body 720 has received the approval of the FDA to analyze impedance, reactance, and resistance. The instrument expresses the relationships of water, protein, muscle, mineral, and fat content, and more, rather than just measuring Body Mass Index (BMI). The device can determine the weight of lean muscle tissue in each limb, water content, percentage body fat, mineral content, protein content, and visceral fat levels. Measurements at five time points during the CDD experiment data were supplied via a full-color print-out and were analyzed by a professional analyst at the hospital.

2.3. Biological sample analysis

In addition to the routine clinical biochemical and blood/urine tests, we also performed molecular and biochemical tests on biological samples collected during the CDD experiment:

1. Plasma or serum preparations. Plasma or serum was centrifuged at 3000 rpm and analyzed according the protocol of the hospital.

2. Serum factor measurements. TNF-α (E-EL-H1205c) and Insulin-like growth factor 1 (E-EL-H0086c) levels in serum were detected with ELISA kits (Elabscience Biotechnology Co., Ltd., Wuhan, China http://www.elabscience.cn/). The optical density was read at 450 nm using a micro titer plate reader.

2.4. Statistical analysis

One-way ANOVA with repeated measures was applied to data from the individuals and plotted in GraphPad Prism 8.0.1 software (GraphPad Software, Inc). Maintaining prolonged fasting was quite a difficult task for all participants. To accurately reveal the effect of 7D-CDD and restrict the variation in individuals' control baselines, we used Dunnett's multiple comparisons test to evaluate the fasting and refeeding parameters by comparing them with the individual's own pre-fasting (0D-CDD) point as control. We also reported multiple comparisons between fasting and refeeding procedures. Among the eight subjects, there were two males and one female subject for whom the refeeding sample collections were achieved for more than 6 months. The individual parameters of different treatments are reported in **Table 1**.

3. Results

3.1 Physiological and Bioelectrical Impedance Analysis

Eight subjects successfully accomplished a 7D-CDD trial. Among them, DBX009 and 3DBPS003 voluntarily experienced 13D and 12D, and 3DPS23 experienced scheduled 14D total fasting under the assistance of FA and strict medical monitoring. During the experiment, the subject's physical experience was recorded in a diary table supplied by the project organizers, and their medical conditions were strictly monitored by hospital medical experts. Bioelectrical impedance analysis (BEIA) indicated that the body weight (BW) and body mass parameters reduced moderately during 7D-CDD (1~2 lbs. per day) and that the refeeding recovered these parameters at a reasonable rate (Table 1). Meanwhile, the basal metabolic rate (BMR) had reduced to a lower speed at 7D fasting and recovered after refeeding (**Table 1**). However, it seems that muscle-related parameters (skeletal muscle mass and trunk muscle mass) recovered faster than fat-related parameters (Body fats and Visceral fat area) during refeeding (Table 1). These results might imply that the human under fasting may utilize more fat than muscle after longer-term fasting, which might explain the slow drop in BW during total fasting (**Table 1**).

Table 1. Physical, BEIA and biochemical results under Flexible Abrosia facilitated Continual Dietary Deprivation

	DBB1006, Female, Age 43			DBB1044, Female, Age 39			DBB1020, Female, Age 48			DBB1027, Female, Age 34			3DPS23, Female, Age 56			
Female Subjects	Ctrl 0D	Fast 7D	Refeed 7D	Ctrl 0D	Fast 7D	Refeed 7D	Ctrl 0D	Fast 7D	Refeed 6D	Control 0D	Fasting 7D	Refeed 20D	Control 0D	Fasting 7D	Fast 14D	Refeed 7D
Weight (kg)	87.20	82.10	83.80	83.10	80.10	82.40	78.40	71.40	74.10	70.20	71.40	74.10	73.4	68.9	69.4	69.3
Basal Metabolic Rate (%)	1345.92	1160.42	1251.43	1285.51	1256.93	1341.54	1251.02	1203.02	1236.68	1301.02	1203.01	1234.66	1327.9	1246.7	1290.4	1291.3
Skeletal muscle mass (kg)	31.62	30.68	31.80	29.60	29.20	29.83	24.90	23.83	24.71	24.83	23.83	24.71	24.9	23.5	23.3	23.7
Body fats (kg)	28.70	25.80	24.70	19.80	18.60	18.40	21.00	20.80	22.40	21.00	20.80	22.40	20.6	20.4	26.5	26.2
Trunk muscle mass (kg)	18.26	16.66	16.10	20.90	19.23	20.23	20.12	18.83	20.00	20.12	18.83	20.00	21.33	19.23	19.93	19.99
Body mass parameters (kgm^{-2})	28.92	24.86	26.24	26.41	24.36	25.72	29.84	27.89	28.95	29.84	27.89	28.95	31.8	29.1	29.3	29.2
Uric Acid (μmol/L)	215.80	608.80	61.80	296.20	329.40	235.10	302.80	689.20	226.50	258.80	494.00	188.50	261.00	988.80	510.50	260.10
Creatine kinase U/L	68.00	121.80	92.80	86.00	96.00	87.80	87.00	97.80	96.00	123.80	142.06	121.80	84	108	84	68
Alanine aminotransferase (U/L)	13.20	17.20	14.80	20.40	21.00	41.80	21.70	143.90	22.10	25.00	30.50	18.18	41.20	112.80	25.40	23.8
Glutamic Oxaloacetic Transaminase (U/L)	24.00	32.00	26.80	20.40	27.70	30.80	29.20	139.90	22.40	23.10	35.00	20.20	18.50	45.50	19.00	20

	3DBP8008, Male, Age 30			3B03009, Male, Age 45, fast 13			3DBP8003, Male, Age 34, fast 12D in total				
Male Subjects	Ctrl 0D	Fast 7D	Refeed 14D	Ctrl 0D	Fast 12D	Refeed 6M	Ctrl 0D	Fast 7D	Fast 12D	Refeed 16D	Refeed 6M
Weight (kg)	87.20	81.00	82.10	82.00	78.00		104.40	98.10	96.00	97.10	
Basal Metabolic Rate (%)	1690.52	1654.12	1603.27	1414.2	1321.6		1812.16	1802.55	1714.27	1870.56	
Skeletal muscle mass (kg)	35.05	34.20	34.13	36.7	34.9		43.65	39.14	37.44	39.57	
Body fats (kg)	25.10	23.60	22.60	26.2	23.6		20.00	26.40	20.20	27.60	
Trunk muscle mass (kg)	27.65	27.06	26.72	31.53	30.71		32.36	36.51	29.44	30.61	
Body mass parameters (kgm^{-2})	29.48	28.06	27.78	32.6	28.8		32.22	36.28	29.63	29.97	
Uric Acid (μmol/L)	513.10	908.80	429.70	329.4	297.4	385.80	369.80	981.40	951.00	269.80	378.30
Creatine kinase U/L	201.80	198.80	187.80	165.80	185.00	120.00	289.80	297.00	264.00	191.80	203.00
Alanine aminotransferase (U/L)	145.40	176.90	85.60	19.00	28.50	18.40	57.40	48.90	43.60	65.10	52.70
Glutamic Oxaloacetic Transaminase (U/L)	74.00	87.00	34.70	26.10	37.10	28.90	35.80	37.10	33.30	38.60	25.10

Note: Collecting blood samples and BEIA tests were performed at different time scheduled by subject DBB1020 and 3DBP8003.
Therefore the timing points of biochemical examinations (in bold fonts) and BEIA were not the same.
Subject 3DBP8003 or DB03009 have automatically experienced 12D or 13D, and 3DPS23 has been scheduled 14D total fasting and accepted biochemical exam after 54D or 6 mos, and 3 mos refeeding recovery.

3.2 The Different Metabolic Patterns of Lipid and Protein during and after Fasting

Based on the different patterns of fat and protein indicated by BEIA, we further tested and analyzed blood biochemical lab results. At 7D fasting, there were increases in both total cholesterol and low-density lipoprotein (LDL-"bad") levels and decreases in triglyceride and high-density lipoprotein (HDL-"good"), which confirmed previous reports in cleansing cholesterol during fasting (**Figure 2A-D**, Total Cholesterol F=10.67, p=0.0054; LDL F=9.621) [40]. These results indicated the possibility of increased consumption of lipid-related energy supply (triglyceride and HDL) to support ketone bodies while enhancing side product clearance during autophagy of unhealthy tissue during starvation.

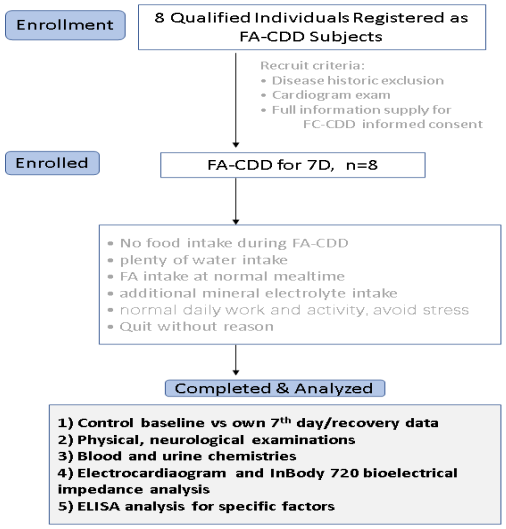

Figure 1: Flow chart of the clinical observation procedure.

Meanwhile, although the protein metabolism levels tested showed a relative increase at 7D fasting, they were either slightly decreased or stabilized after refeeding (Figure 2E-H, Creatine Kinase $F=6.27$, $p=0.021$). The pattern of action in albumin (Figure 2F, $p>0.05$), which free fatty acids attach and transport throughout the body for the alternative energy supply, might indicate activation of alternative energy supply from ketone metabolism. Accordingly, blood triglyceride levels, which involve fatty acid delivery via albumin, were decreased during fasting and quickly returned to control (**Figure 2A** $P<0.5$, limited sample size). Regarding carbohydrate metabolism, both glucose and insulin levels were down-regulated during 7D fasting as expected (**Figure 2I-L**). Prolonged fasting decreased insulin resistance (HOMA-IR), which was related to an increase in insulin sensitivity (**Figure 2I-K**, Glucose $F=8.015$, $p=0.0243$). Glycated hemoglobin (HbA1C), a form of hemoglobin used in clinic to identify the three-month average plasma glucose concentration, remained unaffected during fasting and even after more than 2 months of recovery (Figure 2L, $p>0.5$).

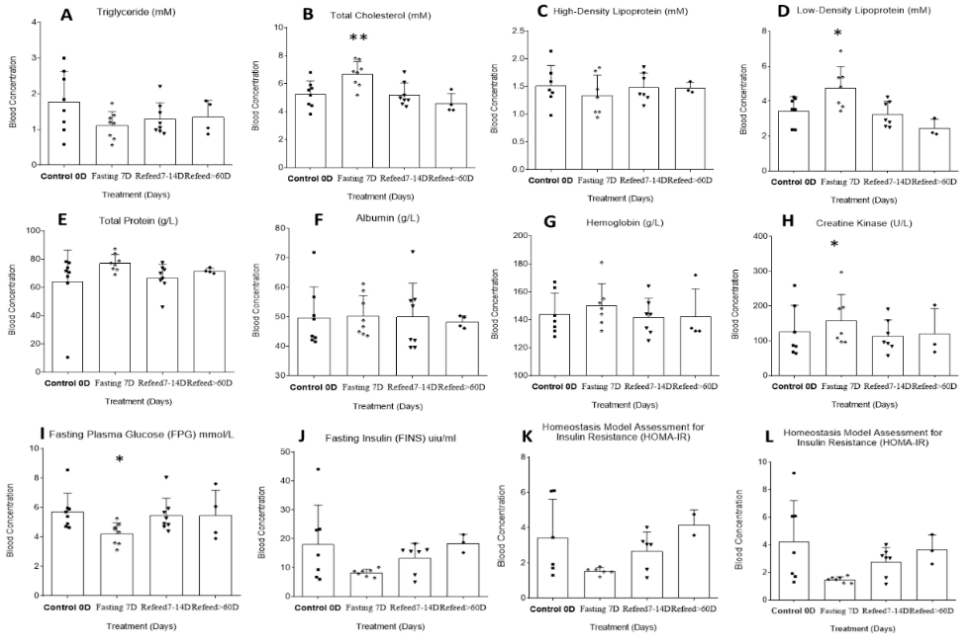

Figure 2: Differential analysis among 0D, 7D~14D fasting, and refeeding results from clinical laboratory tests.

All differences in 0D vs. 7D fasting and either 15D or more than 2~6 months' refeeding (three subjects donated biological samples over a longer recovery period) were analyzed. X-axes refer to the control 0D, fasting 7D, and refeeding 7-14D and >60D groups, respectively. Graph were created with GraphPad Prism 8.0.1 software using one-way ANOVA with repeated measures, and the dots around each column represent the actual values of group subjects on the specific treatments. The larger * symbol above the Fasting 7thD column indicates statistical significance (* $p<0.05$, ** $p<0.01$; the actual values are represented in the manuscript).

3.3. Tissues with Unhealthy Status Seem to be Preferentially Eliminated during Prolonged Fasting

While 7D-CDD induced a reduction in blood urea nitrogen (BUN), this prolonged fasting effectively increased both creatinine (Cr) and uric acid (UA) at 7D CDD (**Figure 3A-C**). Except for the markers of kidney function indicators, increase in daily Cr excretion, a breakdown byproduct in muscle metabolism, is usually related to high-protein diets, and a decrease in BUN may indicate the operation of protein recycling procedures [38]. Besides, long-term fasting efficiently enhanced both UA and Cr blood levels, even under limited sample size, and these returned to normal levels, as reported previously[13] (**Figure 3A-C**, BUN $p=0.085$; Creatinine $F=7.14$, $p=0.0093$; UA $F=28.62$, $p=0.0016$). In addition, both Alanine Aminotransferase (ALT) and Glutamic Oxalacetic Transaminase (GOT) showed a tendency to increase during fasting, as previously reported, but we found that the recovery from prolonged fasting led to a decrease in the tendency, though without statistical significance due to the limited sample size (**Figure 3G-H**). Correspondently, the results from creatine kinase (CK) also showed an increase during 7D-CDD (**Figure 2H**). CK is an enzyme found in the heart, liver, brain, and skeletal muscle. A higher level of CK usually indicates tissue damage, which releases it into circulation, and is regarded as a biomarker of heart failure, myocardial injury,

or liver damage in clinic. In the unhealthy subjects whose markers showed abnormal liver or heart function, 7D prolonged fasting caused a higher increase in enzyme activity. Longer-term recovery from 7D-CDD was related to lower than normal levels of the three factors in most of the subjects. The results indicated that, after the subject recovered from 7D fasting, the values of each unhealthy subject tended to return at a level that was lower than prefasting control (**Table 1**).

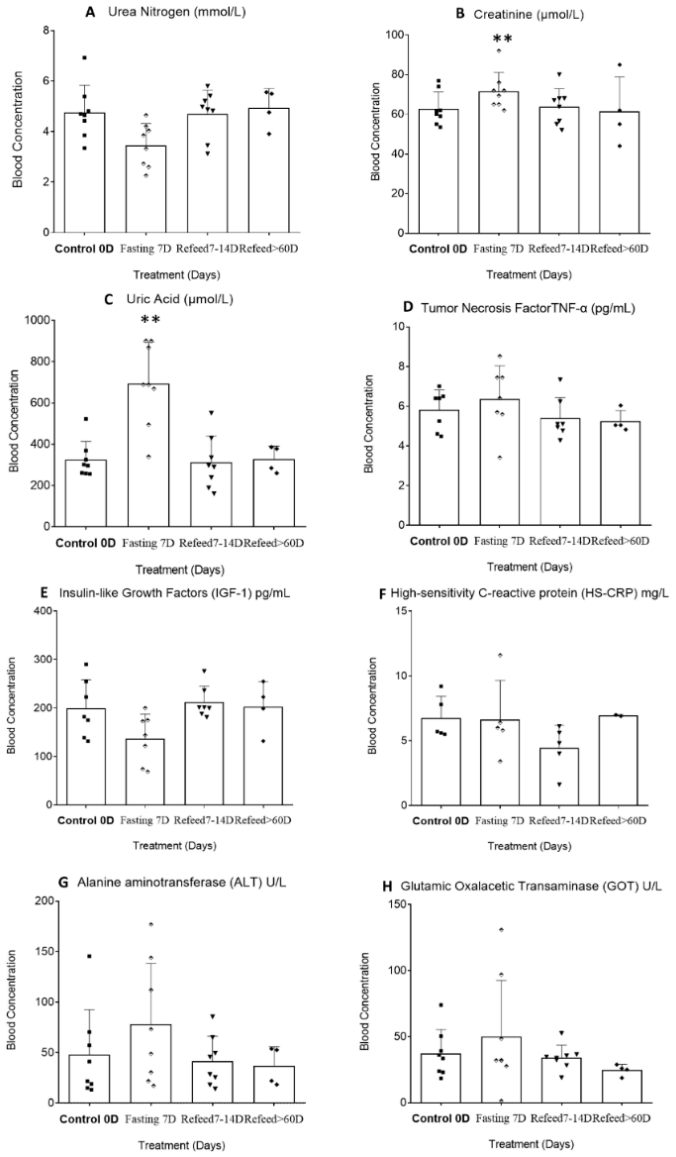

Figure 3: Differential analysis among 0D, 7~14D fasting, and refeeding results from either clinical laboratory tests or ELISA detection.

All differences in 0D vs. 7D fasting and either 15D or more than 2~6 months' refeeding (three subjects donated biological samples over a longer recovery period) were analyzed. X-axes refer to the control 0D, fasting 7D, and refeeding 7-14D and >60D groups, respectively. Graph were created with GraphPad Prism 8.0.1 software using one-way ANOVA with repeated measures, and the dots around each column represent the actual values of group subjects on the specific treatments. The larger * symbol above the Fasting 7thD column indicates statistical significance (* $p<0.05$, ** $p<0.01$; the actual values are represented in the manuscript).

Additionally, results from Insulin-like growth factor type I (IGF-I), tumor necrosis factor (TNF-α), and C-reactive protein (HS-CRP) showed significant changes during 7D fasting but

returned to normal after refeeding. However, there was a lack of significance due to the limited sample size (Figure 3D-F, $p>0.5$).

4. Discussion

Our 7D FA-CDD paradigm, which was applied by supplying limited amounts of polysaccharose which might be mostly absorbed by Intestinal flora of hungry human host at meal time, has been demonstrated to be a more tolerable and efficient regimen for long-term total fasting practice. The subjects' personal experience records indicated that, under the assistance of FA with proper mineral supply at every mealtime, subjects were more able to tolerate hunger sensations, with fewer pangs. Under the recommendation of drinking plenty of water to speed up the cleansing of metabolic wastes and sufficient mineral supply such as potassium and magnesium to release spasms of the smooth muscle of the digestive system, subjects experienced reasonable body weight decrease (about 1~2lb per day) plus moderate hunger sensations. In addition, the speed of body-weight reduction was moderate, which might be safely buffered and protected without showing dramatic dehydration, as in some current commercial body-weight reduction programs.

Of the factors tested, we found that triglyceride, HDL, glucose, insulin, BUN, and IGF-1 showed tendencies to decrease. On the other hand, cholesterol, LDL, total protein, hemoglobin, lactate dehydrogenase, creatinine, uric acid, TNF-α, Cr, CK, ALT, and GOT all showed tendencies to increase (Figures 2 and 3). The factors that were up-regulated during the long-term fasting seemed to be related to life-critical and beneficial survival nutrition, which the system would conserve sparingly. Those factors that were down-regulated during fasting were usually related with either alternative energy supply (such as total protein and hemoglobin), reserved system consumption (such as BUN, triglyceride, and IGF-1), or negative factors such as cholesterol, LDL, UA, TNF-α, ALT, GOT, and CK. It seems that the system automatically chose the beneficial and critical factors to reserve and selected the harmful elements to eliminate during total fasting, which might indicate that prolonged fasting-related autophagy is preferentially targeted to damaged or unhealthy tissue.

Fatty acid oxidation disorders (FAODs) has been reported to lead to deficient energy production and intermittent symptoms through increased β-oxidation, which may occur after 48 hours of fasting in adults [39]. Therefore, previous evidence assumed that 48-hour long-term fasting may cause injury to liver function. Choline-deficient diets were reported to cause hepatic dysfunction and steatosis. However, most of the previous so-called prolonged fasting studies were related only with short-term animal models (either alternate day fasting or a shorter fasting period of less than 3 days). There was a lack of evidence for liver injury being induced by real prolonged fasting for more than 5 days in human. In fact, other clinical studies indicated that prolonged fasting only modestly diminished plasma choline but was not associated with

signs of choline deficiency, such as perturbed lipoprotein secretion and liver damage [40]. Our results indicated that the changes in ALT, GOT, and CK after 7D or longer CDD might be quite beneficial, especially in our three subjects providing 6-month refeeding recovery data (Table 1). It seems that, after fat tissues have been fully metabolized to ketone body metabolism, it might start to clean up any potential harmful metabolites, creating a healthier environment for liver or myocardial function (unpublished results).

5. Conclusion

Our 7D FA-CDD paradigm has been demonstrated to be safe and more tolerable regimen in practicing prolonged total fasting. Meanwhile, we have limited fasting application length to less than 14D in order to avoid any potential risk during fasting performance. Ketogenic or very-low-carbohydrate diets favor mitochondrial respiration for energy metabolism by imitating the fasting process [41]. Wei tested the effects of a fasting-mimicking diet (FMD) associated with aging and age-related diseases and demonstrated that three FMD cycles could reduce BW and trunk and total body fat [25]. If we could apply some specific period of ketone-generating food such as a low-carbon diet in the refeeding session after our long-term CDD regimen, we could expect a significant reduction in gain of body weight in fat and resolve the critical concern of such a fasting regimen in longer-term practice. In that way, we would be able to apply such a paradigm for treating metabolic syndrome. Either the prolonged fasting could be more efficient in treating the chronic disease, or metabolic syndrome needs to be confirmed in more detail by larger, more strict clinical trials with longer-term analysis.

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