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Investigation of Elevated Ketone Bodies in Low Carbohydrate Diet (LCD)

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Abstract

Background: Discussion has been continued concerning Calorie Restriction (CR) and Low Carbohydrate Diet (LCD). For years, authors have investigated LCD associated with lipids, renal function and Ketone Bodies (KB) for clinical application of super LCD formular meal. In this study, 3-hydroxybutyric acid (3-OHBA) and acetoacetic acid (AcAc) was measured.

Subjects and Methods: The subjects were 105 patients with type 2 diabetes mellitus (T2DM), (M/F 47/58, 62.7 years. in average) they were admitted for treatment of T2DM. The protocol consists of 3 steps. 1. Calorie Restriction (CR) diet was given on day 1 and 2 with 60% of carbohydrates. 2. Low Carbohydrate Diet (LCD) was given after day 3 with 12% of carbohydrates which is super-LCD formula meal. 3. Total ketone bodies (T-KB), 3-OHBA and AcAc were measured, and investigated the value and ratio of these markers.

Results: Median T-KB was 349, 415, 486, 415, 445 μmol/L, in day 4-6, 7-9, 10-11, 12-15, 21-30, in 5 groups, respectively. There was significant correlation between value of 3-OHBA and ratio of 3-OHBA / T-KB ($p<0.01$, $r=0.72$). When 3-OHBA value was less or more than 1000 μmol/L, 3-OHBA ratio showed 65-89% or 90-94%, respectively.

Discussion and Conclusion: Hyperketonemia is due to continuation of LCD, which is physiological ketosis without clinically hazardous acidosis. As value of 3-OHBA increased, the ratio of 3-OHBA/T-KB increased. These results may become the fundamental data for clarifying the pathophysiological role of 3-OHBA and AcAc in hyperketonemia from carbohydrate restriction.

Keywords: Low carbohydrate diet; Type 2 diabetes mellitus; Ketone bodies; 3-hydroxybutyric acid; β-hydroxybutyrate; Acetoacetic acid

Introduction

For years, lots of discussion has been found concerning Calorie Restriction (CR) and Low Carbohydrate Diet (LCD). Atkins and Bernstein have originally begun LCD, which had been an new idea at that time [1,2]. After that, the efficacy of LCD has been known and reported in Western countries [3-6].

On contrast in Japan, the author and colleague researchers have firstly started LCD and continued clinical research [7,8]. Furthermore, we clarified the physiological important role of ketone bodies in the axis of fetus, placenta, newborn and mother, and associated changes of renal and lipid aspects [9-12]. Through our clinical research about LCD and ketone bodies, we have accumulated lots of experiences of treatment for type 2 diabetes mellitus (T2DM) by super LCD therapeutic meal [13,14].

In recent years, clinical significance of hyperketonemia has been in focus, because of the possibilities for various beneficial functions [15-17]. Previous data from compared results of animal species and biochemical results revealed that fetuses would be grown by using ketogenic energy throughout evolution [18]. 3-hydroxybutyric acid (β-hydrxybutyrte, 3-OHBA) seemed to be the energy source or basic engine that produces energy in all terrestrial species [19].

Combined these situations together, we investigated the values of total Ketone Bodies (KB), 3-OHBA and acetoacetic acid (AcAc) in the patients with T2DM during nutritional treatment of super LCD.

Subjects and Methods

The subjects enrolled in this study were 105 patients with T2DM (M/F 47/58, 62.7 ± 10.2 years old in average). Methods were summarized as follows. They were admitted for further evaluation and treatment of T2DM. The protocol of research has 3 steps with 1, 2 and 3.

1. CR diet was given on day 1 and 2, which had 60% carbohydrates, 25% lipids and 15% protein with 1400 kcal/day. As for CR, the content is along the guideline of Japan Diabetes Society, in which PFC ratio is 14.7%, 26.9%, 58.4%, respectively [20]. This ratio has been stable from 1985 to 2015 on the national survey in Japan [21].

2. LCD was given and continued after day 3, which had 12% carbohydrates, 64% lipids and 24% protein with 1400 kcal/day. This LCD has been so-called “super-LCD formula” in our clinical research

for LCD. It is one of the Very low-carbohydrate ketogenic diet (VLCKD) by the definitions of LCD, and was originated by Dr. Ebe who is one of the author in Japan [13,14].

3. We measured lots of biomarkers of glucose, lipids and renal function, as well as KB. According to the date that total ketone body (T-KB) was measured, subjects were classified into 5 groups (Table 1).

From those data obtained above, complete data including 3 components that are T-KB, 3-OHBA and AcAc were 66 samples. We investigated the correlation among these components (Table 2).

Variables	Group-1	Group-2	Group-3	Group-4	Group-5
Classification days on LCD	4-6	7-9	10-11	12-15	21-30
Subjects					
Number	17	23	25	19	21
M/F	9/8	13/10	11/14	5/14	9/12
Age average	64.1	63	61.8	62.1	62.6
SD	9.3	10.9	9.9	10.1	10.4
Total ketone body median μmol/L	349	415	486	415	445

Table 1: T-KB values on LCD due to LCD duration.

Variables	Age (YO)	T-KB (μmol/L)	3-OHBA (μmol/L)	AcAc (μmol/L)	3-OHBA (%)	AcAc (%)
Average	60.7	662	115	21.3	78.5	21.5
SD	9.3	650	72	6.9	7.2	6.9
Median	63.0	460	98	21.8	78.2	21.8
Quartile 25%	55.0	320	77	17.3	75.0	17.3
Quartile 75%	66.8	754	129	24.7	82.7	24.7
Minimum	34.0	99	63	31.0	62.4	5.5
Maximum	78.0	4000	3577	42.3	94.5	37.6
T-KB: Total ketone bodies; 3-OHBA: 3-hydroxybutyric acid; AcAc: Acetoacetic acid; SD-Standard deviation; YO-Years old						

Table 2: Correlation among T-KB, 3-OHBA and AcAc.

Statistical analyses

In this study, obtained data was represented as the mean ± standard deviation (SD) and also represented median, quartile of 25% and 75% in biomarkers. For statistical analyses, correlation coefficients were calculated using Pearson or Spearman test of the Microsoft excel analytical tool, which is four steps excel statistics 4th edition [22].

Intergroup comparisons were made using the Wilcoxon rank sum test or the Bonferroni multiple comparisons (Lambert method). A significance level of less than 5% obtained using a two-tailed test was considered to be statistically significant.

Ethical Considerations

Current study was conducted in compliance with the ethical principles of the declaration of Helsinki and Japan's act on the Protection of Personal Information along with the Ministerial Ordinance on Good Clinical Practice (GCP) for drug (Ordinance of Ministry of Health and Welfare No. 28 of March 27, 1997). No ethical committee meeting was held. Informed consent was obtained from the subjects. The study was registered with UMIN #R000031211.

Results

T-KB values during LCD:

The basal data of 105 patients enrolled were shown in Table 1. Median value of T-KB in 5 groups was 349, 415, 486, 415 and 445 μmol/L, respectively. T-KB values representing by boxplot were shown in (Figure 1).

Ratio of 3-OHBA/T-KB:

The results concerning the ratio among T-KB, 3-OHBA and AcAc were shown in Table 2. There was significant correlation between value of 3-OHBA and ratio of 3-OHBA/T-KB ($p<0.01$) (Figure 2). When 3-OHBA value is around less than 1000 μmol/L, 3-OHBA ratio ranges from 65% and 89%. When 3-OHBA value is more than 1000 μmol/L, 3-OHBA ratio is approximately 90-94%.

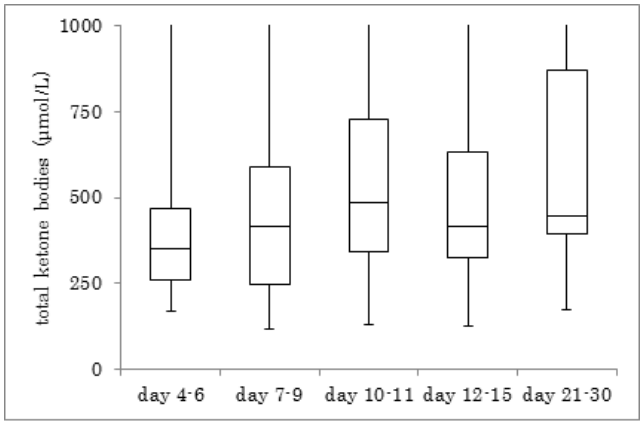

Figure 1: Total ketone bodies in 5 groups.

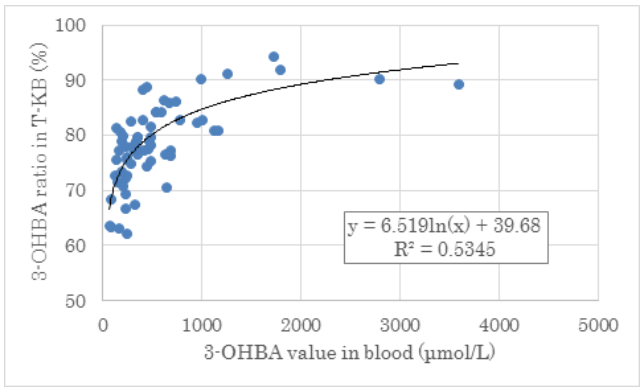

Figure 2: Correlation between 3-OHBA value and 3-OHBA ratio in T-KB.

Discussion

Authors have reported clinical remarkable improvement of hyperglycemia in this protocol using super LCD nutrition treatment so far [8,10,11]. From recent data classified 4 groups due to HbA1c (6,6, 7,4, 8,5, 9.5%)(n=93)[11], fasting glucose on day 2 and 14 were, 117 *vs.* 99 mg/dL, 147 *vs.* 110 mg/dL, 184 *vs.* 119 mg/dL, 227*vs.* 133 mg/dL, respectively. We have reported remarkable effect of super-LCD in short period.

In this study, we investigated and focused ketone bodies, which are T-KB, 3-OHBA and AcAc. On fasting situation, the metabolism of glycolysis is decreased, in which substrates flowing into the citric acid cycle is decreased and ketone production is started. Formerly, glucose metabolism of fasting people was studied for forty days [23,24]. As a result, 3-OHBA in starvation and AcAc are produced in the liver from long-chain fatty acids, suggesting 3-OHBA as the energy source in the brain and other tissues.

The reason of this would be the changed pathway. During the starvation, decreased insulin levels would easily make acyl-CoA enter the mitochondria, resulting much acetyl-CoA that is diverted into the synthesis of ketone bodies [25]. As to ketogenic pathway, mitochondrial 3-OH-3-methyglutaryl-CoA synthase and HMGCS2 are involved as rate limiting enzyme [26,27].

3-OHBA, AcAc and glucose seem to be used for energy sources for the brain of the people who has elevated ketone bodies without any symptoms for ketoacidosis [28].

The organs and tissue producing 3-OHBA seems to be liver, kidney and brain astrocytes [29]. On fasting or severe carbohydrate restriction, hepatocytes, renal tubular cells, intestinal epithelial cells and astrocytes can make ketogenesis. Probable ratio in 3 organs would be 40-60% in liver, 40% in kidney and up to 20% in astrocytes, in order to support to maintain brain function [29]. When certain amount of glucose is given by gluconeogenesis in liver and kidney, production of ketone bodies decreases.

As for the beneficial role of 3-OHBA, it seems to act as a ligand to the G-protein coupled receptor GPR109, leading to anti-inflammatory properties [30]. It would give positive effect for health by anti-inflammatory and anti-oxidant pathways protecting against brain impairment, myocardial problems and arteriosclerosis [30].

In the light of clinical situation, hyperketonemia has been usually classified into in 1) diabetic ketoacidosis (DKA) and 2) physiological ketosis. The former revealed that blood sugar is more than 300 mg/dL with acidosis, and ketone body is about 20-25 mmol/L [31,32]. In clinical report for DKA, average value were: Glucose 650 mg/dL, pH 7.13, T-KB 10.2 ± 1.8 mmol/L, Acetoacetic acid (AcAc) 2.7 ± 0.5 mmol/L, 3-OHBA 7.5 ± 1.3 mmol/L the ratio of 3-OHBA 74% [33].

On contrast, the latter revealed that blood glucose is normal and plasma ketone bodies would increase to 6-8 mM during a prolonged fast without clinically risky acidosis [33]. The cause would be from prolonged starvation and ketogenic diet, which was proposed by biochemist Crebs given Nobel Prize [34].

In current study, we applied super-LCD including 1400 kcal/day, 12% of carbohydrate and 42 gm of carbohydrate per day. T-KB value was up to 4000 μmol/L (4 mmol/L), and 3-OHBA value was up to 3.6 mmol/L. These data would be similar to previous reports.

Fasting starts to increase blood T-KB and 3-OHBA about 0.3～0.5 mmol/L in 24 hours, 1～2 mmol/L in 2-3 days, 3 mmol/L in 3-4 days and 4-5 mmol/L in 7-10 days [24]. In previous experiment, obese subjects continued fasting 40 days, in which they showed no acidosis and 3-OHBA were 6-8 mmol/L [35].

The value of 3-OHBA for carbohydrate restriction until 4 weeks seems to be compatible for previous reports, in which 0.36 mmol/L in fasting [24]. The ratio of 3-OHBA to T-KB was reported to be about 78% [24]. In our data, the ratio of 3-OHBA was increased as the value of 3-OHBA increased. This result may become the fundamental data for clarifying the pathophysiological role of 3-OHBA and AcAc in hyperketonemia due to carbohydrate restriction. Recently, the beneficial aspect of hyper ketosis and wellness has been focused and emphasized [36]. Consequently, further investigation among KB, LCD and glucose metabolism would be expected to develop from now on.

Conclusion

In this study, we investigated the value and ratio of 3-OHBA and AcAc in patients with T2DM on super LCD for nutritional treatment. Elevated value and ratio of 3-OHBA were observed, which may become the fundamental data for evaluation of pathophysiological role

of 3-OHBA in the future research concerning KB, LCD and glucose metabolism.

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