Influence of δ-Amino levulinic acid on body fat, body weight, and blood property in diet induced obese dogs

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Abstract : δ-Amino levulinic acid (δALA or δ-ALA) is the first compound in the porphyrin synthesis pathway, which leads to heme, which can promote mitochondrial enzyme activity by functioning as a coenzyme in the mitochondrial electron transport chain to produce ATP. ALA has been reported to have potentials to improve energy metabolism and has been shown to inhibit accumulation of intra abdominal fat in rats. As such, we sought to determine whether or not ALA supplementation can exhibit the same fat preventative/reductive capacity in diet induced obese dogs. Body weight, body fat and fecal fatty acid composition were measured, plasma biochemistry analysis were performed. After 26 and 36 days of ALA supplementation, the rate of increasing weight in diet induced obese animals was 50% less than that of controlled animals which were being fed a high fat diet, and exhibited significantly lower (p<0.05) amounts of body fat % after 26 and 36 days. The amount of fecal saturated fatty acid which recovered from the ALA group, at the end of the ALA supplement period, was 15% higher than that of the control group (62.9% versus 56.1% of total fatty acid). This would indirectly suggest that ALA supplementation led to decreasing of fat-digestion-rate in diet. In addition, based on the results observed in our study and the data of pre-study by using rats, we hypothesize that ALA supplementation increased baseline metabolic levels in diet obese dogs like rats, and increases the rate of fatty acid beta-oxidation due to the availability of increased disposable levels of mitochondrial produced ATP.

Key word : δ-Amino levulinic acid, body weight, body fat, energy metabolism, obese dog

Introduction

The lifespan of companion animals, such as cats and dogs, has been extended and improved because of continued progress in animal medical care and breeding environments. Currently in Japan, the number of small sized dogs has been increasing resulting in humans and dogs living together in common space, sharing a similar environment. As such, certain types of lifestyle related diseases afflicting humans are beginning to rise in incidence with companion animals. For example, the incidence of human metabolic
related diseases such as obesity and diabetes are also increasing in companion animals, such as cats and dogs [5]. One cause of obesity is excess caloric intake by overfeeding. The diet which can prevent obesity is needed since obesity increases the risk of diabetes development which leads to a reduction in life quality. There are currently many low caloric dog food diets commercially available, which aim at weight control/loss in obese dogs. However, the bigger concern is excess caloric intake by excessive dog snack feeding or feeding of human food. Therefore, a dog food formulation which can inhibit or prevent obesity by increasing fat metabolism is needed.

δ-Amino levulinic acid (δALA or δ-ALA) is the first compound in the porphyrin synthesis pathway, the pathway that leads to heme in mammals and chlorophyll in plants which can act as a coenzyme of cytochrome or catalase. Heme can promote mitochondrial enzyme activities by functioning as a coenzyme in the mitochondrial electron transport to produce ATP. Some types of fatty acids absorbed by feeding are beta-oxidized and metabolized using ATP. Therefore, we hypothesized that ALA may be able to result in higher ATP production thereby providing a primary substrate (ATP) required for an increase in metabolism in general. It has been already demonstrated that ALA has the potential to improve energy metabolism and has been shown to inhibit accumulation of intra abdominal fat in rats[10]. We wanted to determine whether or not ALA supplementation can exhibit the same fat reductive capacity in dogs. Therefore, we investigated the potential of ALA diet-supplementation for improving the body condition of diet induced obese dogs.

Materials and Methods

Animals

Eight non-neutered/spayed beagles (2 males and 6 females) residing in the Fuji Animal Research Farm of Nippon Veterinary and Life Science University (Yamanashi Prefecture, Japan) were used in our study. The mean age and body weight of the animals were 7.25 years old (4-10 years old) and 13.81kg (13.00-15.15kg), respectively, at the start of the feeding period in our study.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Analyzed composition of regular diet and high fat diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>regular diet</td>
</tr>
<tr>
<td>Moisture (%)</td>
<td>9.00</td>
</tr>
<tr>
<td>Crude protein (%)</td>
<td>27.67</td>
</tr>
<tr>
<td>Crude fat (%)</td>
<td>7.01</td>
</tr>
<tr>
<td>Nitrogen free extracts (%)</td>
<td>45.15</td>
</tr>
<tr>
<td>Crude fiber (%)</td>
<td>3.78</td>
</tr>
<tr>
<td>Crude ash (%)</td>
<td>7.39</td>
</tr>
<tr>
<td>Gross energy (kcal/100g)</td>
<td>314.5</td>
</tr>
</tbody>
</table>

Diet induced obesity model

Dogs were fed on a “cod and rice” mix diet (referred to as regular food hereafter) made by the Royal Canin Company before we started our study. Since the dogs were needed to become obese from diet, a high calorie diet formulation was needed. If dogs are to become obese by excess caloric intake by consuming more regular food, they would need to overfeed and take in a great deal of food, which might induce gastric dilatation[4]. Common symptoms include abdomen intumescence and vomiting [7]. Therefore, we prepared a high fat diet which would prevent gastric dilatation for this experiment by commission of Oriental Yeast Inc. Because we thought it was untoward to over feed of common comprehensive diet for dogs to make obesity artificially, we used high energy diet containing 24.3% fat. It is shown by AAFCO that common comprehensive nutritional diet for dogs necessary to contain at least 5% of fat. All animals were fed on this high fat diet, containing 3.5x or 250% fat to regular commercially available dog food diets, for 36 days. The analytical composition of the high fat diet and the commercial diet are shown in Table 1. On Table 1, regular diet means the food which these animals had eaten as daily diet before this test.

Analysis of diet composition

Diet composition (moisture, crude protein, crude fat, Nitrogen free extracts, crude fiber, and crude ash) were analyzed using a standard method for feed analysis[11]. Specifically, crude fat amount was determined by the extraction method using hydrochloric acid and ether.

ALA diet supplementation

After inducing obese status in dogs by high fat diet,
obese dogs were split into 2 groups, with each group consisting of 1 male and 3 females. One group was designated as “control” whereas the other group was deemed to be “ALA supplement group.”

The control group continued to feed on the high fat diet with no ALA supplementation, whereas the ALA supplement group received 72mg/kg ALA mixed with their high fat diet. The appropriate amount of ALA supplementation for dogs was based on a previous study investigating the influence of ALA on rats and livestock [10].

All dogs were fed twice daily (AM6:00 and PM2:00) and were given water ad libitum.

**Analytical methods performed**

**a) Body measurements**

Body weight (BW), waist circumference, chest circumference, body height and leg length measurements were taken 7 times within a 12 week period (once prior to the start of the high fat diet, and subsequently every 2 weeks thereafter. Body fat % (BF) was calculated using the following body measurements:[2].

\[
\text{Male BF} = -1.4 \times \text{leg length} \\
+ 0.77 \times \text{waist circumference} + 4
\]

\[
\text{Female BF} = -1.7 \times \text{leg length} \\
+ 0.93 \times \text{waist circumference} + 5
\]

BW were measured using DP6000 Body Weight Scale (Yamato-Seiko Incorporation, Hyogo).

**b) Fatty acid analysis of Feces**

Feces were collected from each animal at 7 time points within a 12 week period (once prior to the start of the high fat diet, and subsequently every 2 weeks thereafter). The pH (DPA-1 Digital pH meter, Atago, Tokyo), moisture (feces were dried for 24 hours by 60 °C in a dryer) and crude fat (Soxtest SER148/6, Velp Scientifica, Italy) were measured. Fatty acid composition was measured as follows: 10mg of sample was put in a glass beaker in which contained 3ml of ethanol including 5% of hydro chronic. After that, the mixture was heated for 3 hours in water bath (100°C) and made it to a methyl ester and allowed to cool, 3ml of hexane was added and shaken it. The mixture was allowed to stand for 24 hours for layer separation, and the upper layer was moved to vial. It was analyzed by using GC (GC-2010 Gas Chromatography unit, Shimadzu Corporation, Kyoto). Specifically, fatty acid composition of the high fat diet with or without ALA supplement was assessed and compared between each other and subsequently against the fatty acid composition of collected feces as an indirect way to assess the fat-digestion-rate and the level of beta-oxidation.

**c) Plasma biochemical analysis**

Blood collection was done in 3 different periods in our experimental study (prior to the start of the high fat diet feeding, prior to the start of ALA supplementation feeding, and at the end of the ALA feeding period by using 7180 Clinical Analyzer as measurement instrument (Hitachi High-Technologies Corporation, Tokyo, Japan). Blood samples were collected after 8 hours fasting.

Total cholesterol (T-cho), Triacylglycerol (TG), glucose, cholesterol subtypes (chylomicron triglyceride; CMtg, very low-density lipoprotein triglyceride; VLDLtg, low-density lipoprotein cholesterol; LDLc, and high-density lipoprotein cholesterol; HDLc), AST (aminotransferase), ALT (transaminase) and BUN (blood urine nitrogen) were analyzed by using commercial enzymatic assay kit. These enzymatic assay reagent were Cholestest®CHO, Cholestest®TG, Pureauto®S AST, Pureauto®S ALT (above mentioned SEKISUI MEDICAL CO., LTD., Tokyo, Japan). IatrolQ-GLU and IatrolQ-BUN (MITSUBISHI SCIENCE MEDIENCE Corporation, Tokyo, Japan).

Lipoprotein profiles analyzed by the dual-detection GP-HPLC method for lipoprotein profile analysis described previously[6,9,13]. This method was performed by Skylite Biotech Corporation in their laboratory (Akita, Japan).

**Statistical Analysis**

Data are presented as mean±SE. Statistical analysis was performed by using t-test in a completely randomized block design. Significant difference was determined at p<0.05 and a tendency was determined at 0.05≤p<0.10.
Results and Discussion

Body weight and body fat

The effect of ALA supplementation with a high fat diet over a 72 day period on BWgain and BFgain is shown in Figure 1 and 2. In control group, they had only eaten a high fat diet for 72 days. On the other hand, ALA supplementation group had eaten a high fat diet for the first 36 days, after that, eaten ALA supplementation diet for 36 days (from 0 day in Figure 1 and 2). Overall, after 36 days of ALA supplementation, the rate of the increasing weight of those animals was 50% less than that of control animals which were being fed a high fat diet (4.58kg vs 3.23kg). The BWgain of the ALA supplementation group was significantly lower after 26 and 36 days of ALA supplementation as compared to control group (Figure 1, p<0.05).

The effect of ALA supplementation over a 6 week period on BF is shown in Figure 2. Similar to the pattern observed with BW, BF was significantly lower after 26 and 36 days of ALA supplementation (Figure 2, p<0.05). Moreover, cumulative increase in BF was 50% lower in the ALA supplementation group as compared to the control group at the end of the 36 day ALA supplementation period.

ALA supplementation can enhance fat metabolism to prevent or reduce the BF deposition. We speculate that ALA supplementation increased the rate of fatty acid beta-oxidation leading to more robust level of fat metabolism and decreased the rate of BF deposition.

Fatty acid composition of feces

Fecal analysis demonstrated no significant differences in moisture, crude fat and pH between control and ALA supplemented groups (Table 2). ALA supplementation did not appear to negatively affect digestion of dog food components.

Comparing crude fats of -1st day with 36th day in each group, in control, -1st day was 3.67±0.73 and 36th day was 4.08±1.68. The gap between them was 0.41. On the other

<table>
<thead>
<tr>
<th></th>
<th>control group</th>
<th>ALA supplement group</th>
</tr>
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<tbody>
<tr>
<td>moisture (%)</td>
<td>-1 77.8±1.8</td>
<td>73.8±3.5</td>
</tr>
<tr>
<td></td>
<td>13 69.2±0.1</td>
<td>74.5±4.7 ns</td>
</tr>
<tr>
<td></td>
<td>26 75.0±1.9</td>
<td>72.8±3.5</td>
</tr>
<tr>
<td></td>
<td>36 74.2±1.8</td>
<td>70.2±4.6 ns</td>
</tr>
</tbody>
</table>

| crude fat (%)    | -1 3.7±0.7    | 2.8±2.0              |
|                  | 13 3.3±0.4    | 3.6±0.1 ns           |
|                  | 26 2.6±1.4    | 1.6±1.8              |
|                  | 36 4.1±1.7    | 4.2±1.0 ns           |

| pH               | -1 5.8±0.2    | 5.3±0.1              |
|                  | 13 5.3±0.2    | 5.5±0.1 ns           |
|                  | 26 5.5±0.3    | 5.8±0.2              |
|                  | 36 5.6±0.3    | 5.9±0.3              |

mean±SE, "ns" means not significant difference between -1 and 13, 26, 36 in a group.
hand, in ALA supplemented group, crude fat of the -1st was 2.81±1.96 and the 36th was 4.17±1.00. The gap between them was 1.36. There was no significant difference in each group, but the gap of ALA supplemented group was three times as much as that of control. So we thought the rate of digestion of fat had a tendency to decrease in ALA supplemented group by supplementation of ALA.

Fatty acid composition analysis in feces is shown in Figure 3a. Feces in ALA supplemented group contained significantly higher C16:0 (p<0.05), whereas C18:0 level tended (0.05<p<0.10) to be higher than that of obese control group. Moreover, C18:1 and C18:2 also tended to be lower (0.05<p<0.10) than those of obese control group.

The composition of total saturated fatty acids recovered in feces taken from the ALA group, at the end of the ALA supplementation period, was slightly higher than those of the control group (62.9% versus 56.1%, Figure 3b).

In the case of calves, low fat digestion shows high saturated fatty acid in feces[8]. Our results also suggested that the rate of digestion of fat was decreased by feeding ALA.

In addition, it is known that unsaturated fatty acids have a lower melting point and are therefore easier to melt and to be absorbed by the body[1].

**Plasma biochemical analysis**

Results of the plasma biochemical analysis are shown in Table 3, while cholesterol subfraction values (CMtg, VLDLtg, LDLc and HDLc) are shown in Table 4. These were all not significant.

No significant difference was observed in TG between control and ALA supplemented group. Both groups demonstrated an increase in T-Chol and plasma glucose following the start of the ALA supplementation period (p<0.05). Plasma glucose level increased (p<0.05) throughout the ALA supplementation period for ALA supplemented group. Dietary and exercise habits can affect plasma glucose level variation[12]. The increase in plasma glucose is mostly likely attributed to the high fat diet, especially, by constant ingestion of C18:1 and C18:2 [3].

In addition, no significant differences were observed with AST, ALT and BUN levels between control and ALA supplemented groups. The values were within normal range for dogs, therefore ALA supplementation did not appear to have any effect on liver function in dogs.

Overall, the plasma biochemistry results indicated that although ALA supplementation might be enhancing fat metabolism, there are no manifestations being demonstrated at the blood level.
\(\delta\)-Amino levulinic acid in obese dog

**Table 3** Blood data of control group and ALA supplement group

<table>
<thead>
<tr>
<th></th>
<th>control group before</th>
<th>ALA supplement group before</th>
<th>control group after</th>
<th>ALA supplement group after</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cho (mg/dL)</td>
<td>160.5±33.3</td>
<td>186.3±33.2 b</td>
<td>176.3±29.1 ca</td>
<td>214.5±37.6 db</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>56.7±33.5</td>
<td>51.3±19.2</td>
<td>42.8±18.1 a</td>
<td>53.3±22.6 b</td>
</tr>
<tr>
<td>GLU (mg/dL)</td>
<td>98.0±8.1</td>
<td>109.5±4.0</td>
<td>94.5±8.4 a</td>
<td>113.0±3.6 b</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>38.5±18.2</td>
<td>34.0±12.0</td>
<td>32.3±7.2</td>
<td>30.8±11.3</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>46.0±18.3</td>
<td>55.7±18.0</td>
<td>69.3±40.8</td>
<td>59.5±38.6</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>11.8±5.4</td>
<td>12.4±4.0</td>
<td>11.1±2.3</td>
<td>10.3±2.8</td>
</tr>
</tbody>
</table>

mean±SE With different superscripts in the same row are different significantly \(p<0.05\). "ns" means not significant difference between before and after in a group.

**Table 4** Proportion of CM, VLDL, LDL, HDL

<table>
<thead>
<tr>
<th></th>
<th>control group before</th>
<th>ALA supplement group before</th>
<th>control group after</th>
<th>ALA supplement group after</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM (mg/dL)</td>
<td>0.3±0.3</td>
<td>0.2±0.1 ns</td>
<td>0.1±0.0</td>
<td>0.2±0.1 ns</td>
</tr>
<tr>
<td>VLDL (mg/dL)</td>
<td>3.5±1.5</td>
<td>3.2±1.8 ns</td>
<td>2.1±0.4</td>
<td>2.1±1.3 ns</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>6.5±1.1</td>
<td>6.4±0.7 ns</td>
<td>6.1±1.8</td>
<td>6.4±1.9 ns</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>89.8±2.0</td>
<td>90.3±1.5 ns</td>
<td>91.7±1.9</td>
<td>91.3±1.6 ns</td>
</tr>
</tbody>
</table>

mean±SE, "ns" means not significant difference between before and after in a group.

**Conclusion**

The decreased body fat deposition in ALA supplemented group may be caused by promoting of beta-oxidation and by decreasing of fat digestion.

Based on the results observed in our study, we hypothesize that ALA supplementation can enhance fat metabolism to prevent or reduce the BF deposition and weight gain, under a high fat diet intake.

**Acknowledgement**

We appreciate provision of ALA from COSMO OIL Co., Ltd.

**References**


Determined by Carcass Composition Analysis, Deuterium Oxide Dilution, Subjective and Objective Morphometry and Bioelectrical Impedance (PhD Dissertation). pp357. Blacksburg, VA: Virginia Polytechnic Institute and State University.


δ-アミノレプチン醗給与が肥満犬の体重・体脂肪量
および血液性状に及ぼす影響

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要 約：δアミノレプチン酸（以下ALA）は、近年のラットでの研究で脂質代謝に影響を及ぼし、エネルギー代謝を亢進する可能性があることが報告されており、肥満のイスでも同様の影響が認められるかを検討した。8頭のブリ（雄2頭、雌6頭）を高脂肪フードで肥満状態にし、高脂肪フードにALAを72 mg/kg添加した群をALA給与群、添加しない群を対照群として4頭ずつに分け、36日間のALA給与試験を行った。試験中、約2週間毎に体重、体脂肪率、体尺を測定した。また、糞を採取し、水分含量、粗脂肪含量、pH、脂肪酸組成を測定した。体重及び体脂肪重増量は共に、ALA給与群で、ALA給与開始から26日目、36日目に有意に低い値を示した（p<0.05）。36日目に採取した糞中の飽和脂肪酸濃度は、C16:0はALA給与群で高（p<0.05）、C18:0ではALA給与群で高い傾向が見られた（0.05≤p<0.10）。これにより、ALA給与で脂肪の消化率が低下した可能性が示唆された。以上の結果、ALAの給与でエネルギー代謝の亢進と、脂肪の消化率低下の可能性が示唆され、体重と体脂肪率の増加が抑えられたものと考えられた。


キーワード：δ-Amino levulinic acid, body weight, body fat, energy metabolism, obese dog