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The Consumption of Low Animal Food with Low n-6/n-3 Ratio Reduce LDL Cholesterol in Humans

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Abstract: The purpose of this study was to examine the effect on lowering LDL-C in a adult human by applying low animal foods with a lower n-6/n-3 ratio. It was found that the n-6/n-3 ratio was significantly lowered in the low Hanwoo beef, the low pork, the low chicken, the low egg and the low milk to 4:1 or below, compared with commercial animal foods ($p<0.05$). Oleic acid was significantly increased in the low Hanwoo beef, the low chicken, the low egg and the low milk by 11.49, 8.16, 11.25, 2.69 and 24.12%, respectively, compared with commercial animal foods ($p<0.05$). Compared with the common Hanwoo beef and the low Hanwoo beef, the imported beef showed that the n-6/n-3 ratio was higher by 8.70 and 15.46 times, respectively while oleic acid was significantly decreased by 5.61 and 17.75%, respectively ($p<0.05$). When commercial animal foods were taken, the blood LDL-C level related with an increase or a decrease of cholesterol was not changed only in the common egg but it was significantly increased in the imported beef, the common Hanwoo beef, the common pork, the common chicken and the common milk by 27,02, 7.85, 25.81, 5.77 and 8.97%, respectively ($p<0.05$). When low animal foods with lower n-6/n-3 ratio were taken, it was significantly decreased in the low Hanwoo beef, the low pork, the low chicken and the low milk by 22.05, 22.89, 27.55, 12.38 and 14.78%, respectively compared with the imported beef and commercial animal foods ($p<0.05$).

Key words: n-6/n-3, LDL-C, low animal foods, oleic acid, chicken, beef

INTRODUCTION

The second place and the third place in the mortality rates of Koreans are the cerebrovascular disease and the cardiovascular disease caused by an increase of LDL-C (Low Density Lipoprotein Cholesterol) in the blood so it is important to take animal foods good for health.

Recently, the high level of omega-6 to omega-3 fatty acid (n-6/n-3) ratio in human diets has become a matter of concern about health in the point that it may increase the mortality rate of the Cardiovascular Disease (CVD) by increasing LDL-C in the blood (Burghardt *et al*., 2010; Henauw *et al*., 2007; Simopoulos, 1998; Leaf and Weber, 1988). Omega-6 and omega-3 fatty acids (n-6 and n-3 fatty acids) are the essential fatty acids required for human health which must be taken from diets. Even if n-6 and n-3 are a little different in the molecular structure, they are much different in the bio-metabolic mechanism and bio-activation. N-6 and n-3 fatty acids act competitively on the same enzyme system in the bio-metabolic pathway and they cannot be conversely converted into the other one. Also, they perform the essential functions by synthesizing eicosanoid in the body. While omega-6 fatty acid promotes inflammation, blood clotting and tumor growth, omega-3 fatty acid acts completely in reverse.

However, these two dispositions act for each other to improve health so it is important to keep a balance between omega-6 and omega 3 fatty acids in foods.

The proper balance of essential fatty acids maintains and improves health but an unbalance, i.e., a high level of n-6/n-3 ratio causes various metabolic diseases (Daley *et al*., 2010; Gogus and Smith, 2010; Simopoulos, 2004; 2002a, b). The unbalanced n-6/n-3 ratio causes coronary heart diseases, inflammation, blood clotting, cancer, obesity, diabetes (type 2) and autoimmune diseases and has an important effect on health including bones (Williams *et al*., 2011; Xia *et al*., 2005; Watkins *et al*., 2000; Simopoulos, 1991). While the n-3/n-6 ratio of Western diets resulting from current dietary habits is 12-30:1, it was evaluated at 1-2:1 in the ancestors' and pre-agricultural diets. The high n-3/n-6 ratio in Western diets results from the corn-oriented animal feeds with a high n-6 fatty acid and the excessive intake of fat such as margarine and salad oil, etc., made from vegetable oil extracted from corn or sunflower seeds which are used for

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human nutrition. Researchers believe that the ideal n-3/n-6 ratio containing diets useful for health is 4:1 or below (Simopoulos, 2001, 2000). Salem (1989) reported that the n-3/n-6 ratio of grass-leaf was 0.09-0.12 and such grass leaves could be taken by many cattle so the n-3/n-6 ratio of beef from cattle fed completely with grass was evaluated at 1:1 or below. As the lipid taken by animals is stored in the body and affects directly the lipid form of the tissue fat, the freely grazed animals taking grass may have a lower n-6/n-3 ratio, compared with the feedlot animals taking cereal grains. The people taking animal foods produced from the freely grazed animals has a lower n-6/n-3 ratio in their tissue and can enjoy healthier living, compared with the people taking animal foods produced from the feedlot animals fattened upon cereal grains. The animal foods with enhanced omega-3 fatty acid, i.e., egg (Farrell, 1998), pork (Coates *et al*., 2009), chicken (Lopez-Ferrer *et al*., 2001), beef (McNiven *et al*., 2011) and milk (Gulati *et al*., 2002), etc. were produced but nothing is almost known about any clinical experiment report on intake of animal foods with a lower n-6/n-3 ratio (Legrand *et al*., 2010). The n-6/n-3 ratio in corns is 46:1 and the ratio in the animal foods from the animals fed upon cereal grains based on corns is very high at 6-15:1 (Kris-Etherton *et al*., 2000; Simopoulos, 2000). The n-6/n-3 ratio in the animal foods produced by Korean livestock industry feeding animals on cereal grains made mainly with corns and soybean meal shows a similar level to that of Westerners and production of a novel animal food with a lower n-6/n-3 ratio is greatly needed for health against cardiovascular diseases. However, nothing is known about any animal food or any clinical experiment report related thereto.

This study was performed to examine an effect of intake of low animal foods with a lower n-6/n-3 ratio as produced using seeds with plenty of n-3 fatty acid on a decrease of blood LDL cholesterol in a adult human.

MATERIALS AND METHODS

Low animal foods with the n-6/n-3 ratio lowered to 4:1 or below was produced by feeding livestock with the experimental feed as a mixed feed based on corns and soybean meal which was manufactured by mixing linseed, perilla seeds, chia seeds and full-fat soybeans additionally and appropriately so that it may meet the nutrient requirement for animals (NRC, 1994).

In order to examine the fatty acid composition of the low animal foods and perform a clinical experiment on them, beef loin and pork belly were collected from 10 heads by each 1 kg per head and whole chicken meat, eggs and milk were used as experimental materials. Some materials except samples for the clinical experiment were crushed by a household mixer and testing materials for three times-repetitive analysis of fatty acids were collected from one experimental material by selecting three parts randomly from the crushed experimental material. The lipid was separated by the method used by Folch as follows the mixed organic solvent (chloroform/ Methanol = 2:1, v/v) containing BHT (Butylated Hydroxyl Tolune) as an antioxidant was added to a certain amount of testing material at the rate of 20 to 1 (vol./wt) and then it was intensely stirred and filtered 3 times for 10 sec each time at 2,500 rpm with the Ultra Turrax T25 homogenizer (Janke and Kunkel, IKA Labortechnik) and then a certain amount of distilled water was added to and mixed with it and the lipid layer was primarily separated from the solution by centrifugation at 3,000 rpm for 15 min. The lipid was extracted by repeating this process three times and finally concentrated by using nitrogen gas. The methylation process of lipid was performed by modification of the method used by Morrison and Smith which is briefly described as follows: about 10 mg of the concentrated lipid fraction was put into a reaction vessel for saponification; 1 mL of the newly-prepared 0.5 N methanolic NaOH (2 g $NaOH^{-1}$ 100 mL methanol) was added thereto; it was heated for 15 min and it was cooled. After it was cooled, 2 mL of BF_3-methanol (Boron trifluoride) as a methylation reagent was added thereto and then it was heated again for 15 min. After it was cooled sufficiently to the room temperature, 1 mL of heptane and 2 mL of NaCl saturated solution were added thereto and they were mixed for 1 min and then it was left as it was for 30 min at the room temperature. About 1 uL of the supernatant was taken from it and injected into GLC for analysis of fatty acid (ACEM 6000 Model, Youngin Science, Korea) and then the fatty acid was analyzed. It was repeatedly measured three times per each sample of each treatment group. As the standard solution, the product of Supelco, USA (37 component FAME Mix, Sigama-Aldrich Co., ST, Louis, MO) was used and as the internal standard substance, nonadecanoic acid (19:0) was used. SP™-2560 Capillary GC Column (L x I.D. 100 m × 0.25 mm, df 0.20 um Omegawax 320 capillary column, USA) was used and the initial temperature was programed at 150°C for 8 min and then the temperature was increased by 2°C per min up to 190°C and the final temperature was fixed at 190°C. Helium was used as a carrier gas and its flow rate was adjusted at 40 mL^{-1} min. The split ratio was set at 100:1. The injection temperature was adjusted at 250°C and the detector temperature was adjusted at 265°C.

For the clinical experiment on low fatty animal foods, 12 volunteers of normal adult male and female college students were selected. They were instructed in the purpose, the necessity and the cooperative requirements of the experiment and they were allowed to keep their usual dietary tastes and habits. However, they were not allowed to take commercial animal foods, cola, coffee, alcohol, marine algae food, instant foods and/or fast foods in addition to the provided low fatty animal food. They were instructed to observe the foresaid requirements from 3 days before measuring the fasting blood lipid. Each low fatty animal food was taken during each different period and the intake of each animal food was repeated three times. They were provided with each 200 g per person of beef and pork belly during each different period and instructed to take it after roasting over a hot grill. Chickens were provided after cooking enough in the boiled form and two eggs were provided to both the control group and the experimental group in the form which they were fried with the grape seed oil every day for 1 week during each different period and milk was provided everyday three times at breakfast, lunch and dinner by each 200 mL for 1 week during each different period. In order to measure the lipid level before an intake of the experimental products, they remained fasting from 12 pm of the day before intake of the experimental products until 10 pm of the following day and their fasting blood lipid was measured using Cholestech LDX® System (USA). In order to measure the blood lipid level after the intake of the experimental products, they were instructed to take the experimental products for a given period from the day of measuring the fasting blood and then their fasting blood was measured at 10 am of the following day after the last intake in the same manner as it had been measured before the intake. In the control group, they were provided with commercial animal foods and they were examined in the same manner as in the experimental group and as a result of measuring it in the case that any difference was shown in the decrease rate and the increase rate in 70% of subjects or more such decrease and increase data were recognized as an effective result so that they were compared each other.

Statistical analysis: Statistical analysis of the analyzed data was made by analysis of variance using GLM procedure of SAS (2004) and the statistical significance ($p<0.05$) between the analyzed mean values was tested at the level of 95% by Paired t-test.

RESULTS AND DISCUSSION

The change of the fatty acid composition examined in low animal foods with the lower n-6/n-3 ratio and commercial animal foods are as shown in Table 1. It could be identified that the n-6/n-3 ratio was significantly lowered in the low Hanwoo beef, the low pork, the low chicken, the low egg and the low milk to 4:1 or below, compared with commercial animal foods ($p<0.05$). The saturated fatty acid was significantly decreased in the low Hanwoo beef, the low pork, the low chicken, the low egg and the low milk by 11.56, 18.28, 43.03, 14.68 and 7.55%, respectively, compared with commercial animal foods ($p<0.05$). However, on the contrary, the unsaturated fatty acid was significantly increased in the low Hanwoo beef, the low pork, the low chicken, the low egg and the low milk by 9.12, 13.39, 29.07, 7.81 and 12.68%, respectively compared with commercial animal foods ($p<0.05$). Oleic acid among unsaturated fatty acids was significantly increased in the low Hanwoo beef, the low chicken, the low egg and the low milk by 11.49, 8.16, 11.25, 2.69 and 24.12%, respectively, compared with commercial animal foods ($p<0.05$). It could be identified that compared with

Table 1: Changes in fatty acid composition of low animal foods with low n-6/n-3 ratio (percentage of total fatty acids)

Items	Common Hanwoo beef	Imported beef	Low Hanwoo beef	Common pork	Low pork	Common chicken	Low chicken	Common egg	Low egg	Common milk	Low milk
8:0	0.01^{b}	0.08^{a}	0.07^{a}	-	-	-	-	-	-	0.75^{a}	0.15^{b}
10:0	0.06^{b}	0.21^{a}	0.18^{a}	0.15^{a}	0.09^{b}	-	-	0.01^{a}	0.001^{b}	1.06^{a}	0.65^{b}
12:0	0.16	0.17	0.15	0.22^{a}	0.11^{b}	0.07^{a}	0.001^{b}	0.03	-	2.07^{a}	1.11^{b}
14:0	4.30^{a}	3.87^{b}	3.30^{b}	2.16^{a}	1.44^{b}	1.30^{a}	0.790^{b}	0.53	0.450	3.92	3.02
16:0	27.72^{a}	28.16^{a}	25.60^{b}	25.08^{a}	20.54^{b}	30.73^{a}	15.840^{b}	26.05^{a}	22.290^{b}	19.85^{a}	16.38^{b}
16:1n-9	5.84	5.42	5.72	2.61	2.49	6.92^{a}	2.660^{b}	3.33^{b}	4.060^{a}	2.74^{a}	1.64^{b}
18:0	11.80^{b}	13.76^{a}	9.81^{c}	14.10^{a}	11.49^{b}	7.82^{a}	5.230^{b}	8.03^{a}	6.690^{b}	8.19^{a}	7.81^{b}
18:1n-9	47.59^{b}	45.06^{c}	53.06^{a}	38.70^{b}	41.86^{a}	42.83^{a}	47.650^{b}	44.97^{b}	46.180^{a}	29.85^{b}	37.05^{a}
18:2n-6	2.28^{b}	3.15^{a}	1.57^{c}	15.28^{b}	16.85^{a}	9.37^{b}	18.060^{a}	16.49^{a}	14.360^{b}	3.88^{a}	2.05^{b}
18:3n-3	0.09^{b}	0.07^{b}	0.54^{a}	1.14^{b}	4.25^{a}	0.57^{b}	8.500^{a}	0.47^{b}	5.760^{a}	0.37^{b}	0.77^{a}
20:0	0.10	0.01	-	0.52	0.49	-	0.390	-	-	0.01	0.01
22:0	0.03	0.03	-	-	-	0.36^{b}	0.560^{a}	0.08	-	-	-
22:1	0.02	-	-	0.05^{b}	0.40^{a}	0.04^{b}	0.160^{a}	-	0.110	-	-
24:0	-	0.01	-	-	-	-	-	-	0.100	-	-
SFA	44.20^{b}	46.30^{a}	39.11^{c}	42.28^{a}	34.55^{b}	40.32^{a}	22.970^{b}	34.74^{a}	29.640^{b}	63.16^{a}	58.39^{b}
UFA	55.80^{b}	53.70^{c}	60.89^{a}	57.72^{b}	65.45^{a}	59.68^{b}	77.030^{a}	65.26^{b}	70.360^{a}	36.84^{b}	41.51^{a}
n-6/n-3	25.33^{b}	45.00^{a}	2.91^{c}	13.40^{a}	3.96^{b}	16.44^{a}	2.120^{b}	35.09^{a}	2.490^{b}	10.49^{a}	2.66^{b}

$^{a-c}$Mean values with different superscripts differ in each foods significantly ($p<0.05$)

the common Hanwoo beef and the low Hanwoo beef, the imported beef showed that the n-6/n-3 ratio was higher by 8.70 and 15.46 times, respectively and the saturated fatty acid was increased by 4.53 and 15.52%, respectively but the unsaturated fatty acid was significantly decreased by 3.91 and 13.39%, respectively and oleic acid was significantly decreased by 5.61 and 17.75%, respectively ($p<0.05$).

It can be thought that the n-6/n-3 ratio was higher in commercial animal foods because the intake of the n-6 fatty acid from the commercial mixed feed based on corns was high while the intake of the n-3 fatty acid from it was low (Simopoulos, 2002b; Kris-Etherton *et al*., 2000). It can be also assumed that the imported beef which was a livestock product produced on the basis of corn-oriented feed had the n-6 fatty acid higher than the n-3 fatty acid when it was shipped and it is thought that the n-6/n-3 ratio was lowered owing to the long import channel and distribution process. Oleic acid is known as a fatty acid to contribute to production of high quality meat by enhancing the beef marbling as well as improving the taste and flavor of livestock products and further to lower the blood LDL-C (Park and Yoo, 1994; May *et al*., 1993). Park and Yoo (1994) reported that compared with the imported American beef and the imported Australian and New Zealand beef from cattle fed on grass, Hanwoo beef (beef from Korean native cattle) was more excellent in quality because oleic acid was significantly higher in Hanwoo beef and they explained that this result was attributed to the longer import channel and distribution period for the imported beef. The fatty tissue of cattle synthesizes oleic acid from steric acid (18:0) through desaturating action of Δ-9 desaturase by the stearyl CoA desaturase gene and accumulates the oleic acid in loin and thereby enhances marbling. As a result, it can produce high quality meat (Smith *et al*., 2006).

Figure 1 shows the result of the clinical experiment on an decrease of blood LDL-C in normal persons who took low animal foods with the lower n-6/n-3 ratio and commercial animal foods, respectively. When commercial animal foods were taken, only in the case of taking common eggs, the blood LDL-C level had no change related with an increase or a decrease of cholesterol. However, it was identified that in the case of taking the imported beef, the common Hanwoo beef, the common pork, the common chicken and the common milk respectively, it was significantly increased by 27.02,7.85, 25.81, 5.77 and 8.97%, respectively ($p<0.05$). It could be also identified that when low animal foods with a lower n-6/n-3 ratio were taken in the case of taking the low Hanwoo beef, the low pork, the low chicken and the low milk respectively, it was significantly decreased by 22.05, 22.89, 27.55, 12.38 and 14.78%, respectively, compared with the case of taking the imported beef and commercial animal foods respectively ($p<0.05$).

In Fig. 2 it is known that an increase of LDL-C in the blood resulting from a high n-6/n-3 ratio in a diet increases a mortality rate due to cardiovascular diseases such as myocardial infarction vascular sclerosis, etc. and an increase of HDL-C (High Density Lipoprotein-Cholesterol) helps to prevent such diseases (Burghardt *et al*., 2010 Henauw *et al*., 2007). LDL is the most important lipid carrier for accumulating cholesterol in arteries, transporting cholesterol ester from the liver to the blood and peripheral tissue cells of various regions in the body while HDL has the reverse transport function to transport

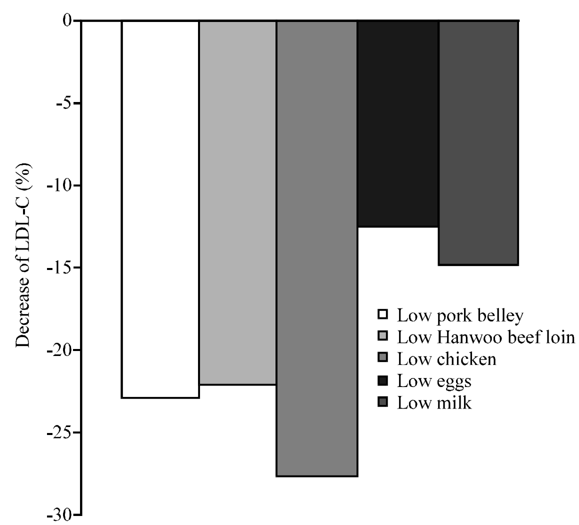

Fig. 1: Changes in decrease of LDL-C in volunteers

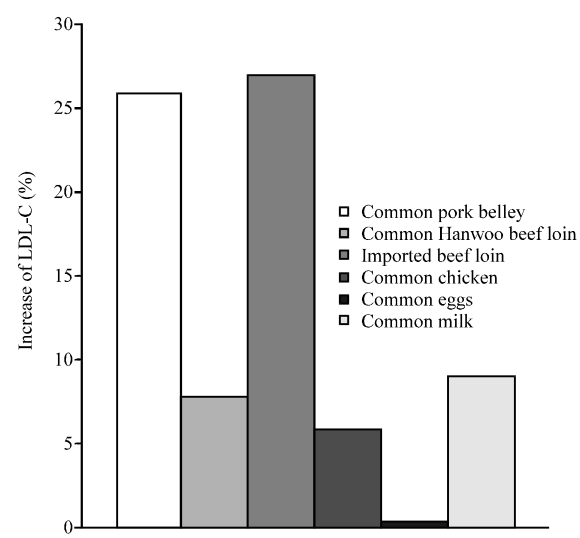

Fig. 2: Changes in increase of LDL-C in volunteers

cholesterol from arteries to the liver. The increase of blood HDL-C as lipoprotein eventually enhances the capability to transport cholesterol from the blood and tissues to the liver so that it performs a function to remove the blood cholesterol. Thus, LDL-C is called as bad cholesterol because excessive LDL-C is harmful to health while HDL-C is called as good cholesterol (Grundy, 1986). Frequent intake of animal foods with high cholesterol and a high n-6/n-3 ratio in the human diets increases LDL-C, the harmful cholesterol in the blood so that it may increase a risk rate of causing various adult diseases including cardiovascular diseases (Daley *et al*., 2010; Simopoulos, 2004; 2002a, b; Renaud and Longeril, 1994).

CONCLUSION

As a result of conducting this study, it could be identified that the intake of low animal foods with the lower n-6/n-3 acid ratio lowered significantly LDL-C, bad cholesterol in the blood. It suggests that low animal foods may meet the well-being oriented consumer tastes, help to reduce medical expenses and cope with the imported livestock products. Also, it is thought that more clinical experiments should be conducted in patients with a metabolic disease.

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