

Effects of Fasting on Plasma Lipoprotein(a) in Cynomolgus Monkeys: Preliminary Experiments Results

Masataka Kusunoki^{1*}, Kazuhiko Tsutsumi², Daisuke Sato³, Tetsuro Miyata⁴, Takao Nakamura³

¹Department of Internal Medicine, Medical Clinic, Aichi Medical University, Nagoya, Japan

²Okinaka Memorial Institute for Medical Research, Tokyo, Japan

³Department of Biomedical Information Engineering, Graduate School of Medical Science, Yamagata University, Yamagata, Japan

⁴Vascular Center, Sanno Medical Center, Tokyo, Japan

Email: [*info@tonyo.jp](mailto:info@tonyo.jp)

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Abstract

Plasma lipoprotein(a) (Lp(a)) is known to be a strong independent risk factor for ischemic heart disease. However, it is not easily modulated by drugs that are presently available. Lp(a) is not present in many experimental animals except for Old World monkeys. In this study, we examined whether cynomolgus monkeys are useful model for research of human plasma Lp(a), and observed that plasma Lp(a) in cynomolgus monkeys varied as much as humans. As a result of 4 day-fasting in cynomolgus monkeys, the plasma Lp(a) level decreased in a monkey with originally high Lp(a) level. The Lp(a) level continued to decrease even 3 days after banana feeding, but returned to the original level 3 days after monkey chow feeding. On the other hand, in a monkey with low Lp(a) level, fasting had no effect on the Lp(a) level. However, in the third monkey having originally high Lp(a) level, the Lp(a) was not affected by decreasing the amount of monkey chow feeding by 50%. In summary, we found that cynomolgus monkeys may be an useful model for studying the effects of food on plasma Lp(a) in place of humans, and that high Lp(a) level may be controllable by strict diet regulation.

Keywords

Lipoprotein(a), Fasting, Cynomolgus Monkey, Banana

*Corresponding author.

1. Introduction

Apolipoprotein(a) is a glycoprotein that attaches to apolipoprotein B (apoB) in plasma low-density lipoprotein (LDL) particles, forming lipoprotein(a) (Lp(a)) [1] [2]. Several studies have demonstrated a strong association between Lp(a) and cardiovascular disease. It has also been reported that there is a strong positive relationship between elevated plasma levels of Lp(a) and myocardial infarction as well as angiographically-defined coronary artery disease [1] [3]. The importance of decreasing elevated plasma Lp(a) levels depends on whether a reduction in plasma Lp(a) levels will be accompanied by a reduction in the risk of cardiovascular disease due to elevated Lp(a) levels. However, Lp(a) levels are not easily modulated by diet or by routinely employed medications [4].

Recently, it has been reported that dimyristoylphosphatidylcholine inhibits Lp(a) formation in transgenic mice [5] (Wang *et al.*, 2009), and that existing anti-rheumatic agents reduce serum Lp(a) levels in rheumatic patients [6] [7]. We hope to find a novel drug that reduces Lp(a) and determine a food treatment that regulates Lp(a) levels. However, Lp(a) is not present in many species of experimental animals for researching Lp(a) except for Old World monkeys (e.g., cynomolgus monkey and rhesus monkey) [8] [9] and Lp(a) transgenic animals [5] [10].

In this study, we preliminarily investigated that whether cynomolgus monkeys are possible to use for research of Lp(a) instead of humans, and determined the effects of fasting on plasma Lp(a) levels in cynomolgus monkeys.

2. Materials and Methods

2.1. Animals

Three Philippine male cynomolgus monkeys weighing approximately 5.8 kg and aged 5 to 8 years old were obtained from Kearsy Co., Ltd. (Wakayama, Japan). They were housed in stainless steel cages (100 W × 100 D × 165 H cm, CLEA Japan, Inc.) in conventional housing. The trays under the cages were changed once a day. Room temperature was maintained at 23°C ± 2°C with 55% ± 10% relative humidity. The artificial light/dark cycle was 12 hours with lights on at 7:00 am, and there was an average of 13 - 16 air changes per room per hour. Monkeys were fed 150 g of monkey chow (Monkey BIT, Nihon Nosan Kogyo Co., Ltd., Yokohama, Japan) once a day at 10:00 am. The monkey chow consisted of 9.0% water, 20.5% protein, 8.5% fat, 4.0% fiber, 7.4% ash and 50.6% carbohydrate. Metabolic energy was 303 kcal per 100 g of the monkey chow. All animals had access to tap water *ad libitum*.

All animal experiments were approved by the animal ethics committee of Aichi Medical University and conformed with the Guide for the Care and Use of Laboratory Animals issued by the National Institutes of Health.

2.2. Animal Experiments

Flow chart of this study is shown in **Figure 1**. After a standard monkey chow feeding, two monkeys (one with a high level of Lp(a) and one with a low level of Lp(a)) were placed on a zero diet for 4 days. After the fasting, both monkeys received one banana (approximately 100 g)/day for 3 days. And then, the monkeys received 150 g/day of the monkey chow for 3 days.

The third monkey having a high level of Lp(a) was placed on a diet consisting of half the amount of monkey chow fed per day (75 g) to determine whether mild affects Lp(a) levels.

Blood samples were obtained from an antebachial vein before the start of the experiment, and 4, 7 and 10 days after the start of experiment at 9:00 am for determination of plasma lipids using a heparinized syringe.

3. Results

Plasma lipid levels are shown in **Table 1**. Regarding pre-fasting Lp(a) levels, monkey no. 3 had the highest level of Lp(a), monkey no. 1 also had a high Lp(a) value, but was lower than monkey no. 3, monkey no. 2 had the lowest level. Plasma Lp(a) levels varied among the monkeys.

In monkey no. 1 with an originally high Lp(a) level, the Lp(a) level decreased 26% after fasting for 4 days.

This reduction reached 40% after 3 days of banana feeding. The Lp(a) level recovered to the original level at day 10, after 3 days of the monkey chow feeding. The TC and HDL-C levels were not affected by fasting for 4

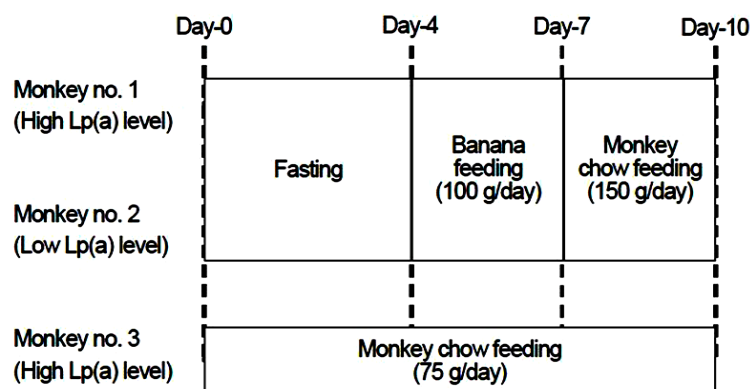


Figure 1. Schema of this study. Until start of the experiment period, all three monkeys were fed with a standard monkey chow (150 g/day).

Table 1. Plasmalipid levels in cynomolgus monkeys.

Animal Number		Before	4 days	7 days	10 days
1	Lp(a) (mg/dl)	31	23	19	32
	TC (mg/dl)	92	90	69	95
	HDL-C (mg/dl)	51	52	31	49
	TG (mg/dl)	35	56	60	37
	NEFA (μ Eq/l)	275	2530	324	288
2	Lp(a) (mg/dl)	14	15	13	16
	TC (mg/dl)	85	68	65	81
	HDL-C (mg/dl)	43	35	34	41
	TG (mg/dl)	48	62	67	50
	NEFA (μ Eq/l)	350	2730	386	325
3	Lp(a) (mg/dl)	42	45	43	41
	TC (mg/dl)	96	94	89	99
	HDL-C (mg/dl)	53	51	52	54
	TG (mg/dl)	45	48	45	46
	NEFA (μ Eq/l)	326	365	355	334

Lp(a), lipoprotein(a); TC, plasma total cholesterol; HDL-C, plasma high density lipoprotein cholesterol; TG, plasma triglyceride; NEFA, plasma nonesterified fatty acid.

days, but decreased 25% and 40%, respectively, after 3 days of banana feeding. The TC and HDL-C levels increased after 3 days of the monkey chow feeding. The TG level was increased by fasting, and further increased after banana feeding, but returned to the original level after the monkey chow feeding for 3 days. The NEFA level increased 9.2-fold after 4 days of fasting, respectively. However, NEFA levels decreased after banana and monkey chow feeding.

In monkey no. 2 with an originally low level of Lp(a), the Lp(a) level did not decrease after fasting. However, the TC and HDL-C levels decreased and the TG and the NEFA levels increased after fasting. The NEFA level returned to the original level after banana feeding. The TC, HDL-C and TG levels returned to near original values after the monkey chow feeding.

In monkey no. 3, halving the amount of monkey chow fed showed almost no effect on plasma lipid levels.

4. Discussion

Lp(a), which was originally discovered in 1963, has recently been confirmed to be atherogenic [11]. Plasma Lp(a) levels seem to be largely regulated by genetic factors [4] [8] [12]. There are large individual differences of Lp(a) levels in human. Accordingly, it is thought that these levels are not always affected by drug treatment or diet. In this study, we used cynomolgus monkeys which react with anti-human Lp(a) sera [13]. We found that plasma Lp(a) levels of cynomolgus monkeys showed large individual variation, similar to humans.

Recently, it has been reported that the novel apoB synthesis inhibitor, mipomersen, potentially reduced Lp(a) level in patients with familial hypercholesterolemia and coronary artery disease [14]. So far there has been many papers reporting the effects of lipid-lowering drugs on plasma Lp(a) in human. However, at least current lipid-lowering drugs other than nicotinic acid and its derivative did not have a reducing effect on plasma Lp(a) [8] [12].

There are also reports mentioning the effects of dietary fatty acid on plasma Lp(a) level. In a part of the reports, it is described that especially n-3 polyunsaturated fatty acid has a mild Lp(a)-reducing effect [4]. In contrast, Kooshki *et al.* reported that n-3 fatty acids did not affect serum Lp(a) level in human [11]. It seemed that consensus on the effects of dietary fatty acid on Lp(a) has not yet been formed. Furthermore, very few studies have dealt with the effects of fasting on plasma Lp(a).

We studied whether fasting had a reducing effect on plasma Lp(a) levels by using cynomolgus monkeys. In this study, the plasma Lp(a) level decreased after fasting for 4 days in a monkey with an originally high level of Lp(a), but not in a monkey with an originally low level of Lp(a). The plasma Lp(a) level of the latter at the beginning of the experiment was less than half the level of the former (Table 1). These results may indicate that in monkeys with originally high levels, fasting results in a decrease in Lp(a) levels, but not in monkeys with originally low levels of Lp(a). NEFA levels in monkey no. 1 and 2 were markedly increased. It is well known that fasting cause elevation of NEFA level in order to produce energy source through fatty acid oxidation.

Kostner *et al.* have reported that five obese patients were placed on a zero diet for 3 weeks, but Lp(a) levels did not change [15]. Mean Lp(a) levels of these obese patients were very low, with mean values around 6.3 mg/dl. We think that the difference in results between our data and the data of Kostner *et al.* may result from the difference in original Lp(a) levels, and not due to species difference.

The Lp(a) level in the monkey with an originally high level showed a decrease after 4 days of fasting, while a decrease in TC level was observed at Day 7, after 3 days of banana feeding. Thus, the Lp(a) and TC levels showed independent movement.

The decreased Lp(a) level caused by fasting continued to decrease even after banana feeding, but returned to the original level after the monkey chow feeding. The continuous decrease of Lp(a) even after banana feeding is very interesting. The bananas weighed about 100 g weight and had 87 kcal each. One banana consists of 87% water, 1.1% protein, 0.1% fat, 0.3% fiber, 0.9% ash and 22.6% carbohydrate [16]. The difference between monkey chow and banana can be found in the nutritional contents of each. These results indicate that the control of Lp(a) is dependent not only on the amount of food consumed, but also by the type of food. Plasma Lp(a) did not change after an egg diet [17], a lacto-ovo vegetarian diet [18], or a prudent diet [7]. However, a low-fat high-carbohydrate diet (15% protein, 20% fat, and 65% carbohydrate) leads to elevation of plasma Lp(a) level [19]. Hence, extreme suppression of protein and/or carbohydrate intake may cause a reduction in plasma Lp(a). We are now interested in knowing whether a change from an ordinary to a vegan diet has a decreasing effect similar to bananas on plasma Lp(a) levels.

The plasma Lp(a) level did not decrease after halving the amount of monkey chow fed even in a monkey with an originally high level of Lp(a). This indicates that Lp(a) may not be affected by mild fasting. However, in this preliminary study, statistical significance among the data acquired from three monkeys has not been clarified yet because number of monkeys was not enough for statistical analysis.

5. Conclusion

The present study showed that the plasma Lp(a) levels in cynomolgus monkeys show large individual differences similar to humans. Complete fasting for 4 days decreased the plasma Lp(a) level in a monkey with a high level of Lp(a), and this decrease continued after banana feeding. The Lp(a) level returned to the original level by feeding normal monkey chow to the monkey. On the other hand, a monkey with a low level of Lp(a) and a monkey being fed half the amount of monkey chow as the other two monkeys showed no effect on Lp(a) levels.

These results show that high levels of plasma Lp(a) may be decreased by fasting or feeding of certain foods. Therefore, diet may be used as a method of preventing and/or treating high Lp(a) levels. This study indicates that the cynomolgus monkey can be used as an animal model in place of humans for research of plasma Lp(a) metabolism. However, since we used only 3 monkeys, further studies need to be performed to clarify this.

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