

# Fish Intake, Independent of Apo(a) Size, Accounts for Lower Plasma Lipoprotein(a) Levels in Bantu Fishermen of Tanzania

## The Lugalawa Study

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**Abstract**—Plasma lipoprotein(a) [Lp(a)] levels are largely genetically determined by sequences linked to the gene encoding apolipoprotein(a) [apo(a)], the distinct protein component of Lp(a). Apo(a) is highly polymorphic in length due to variation in the numbers of a sequence encoding the apo(a) kringle 4 domain, and plasma levels of Lp(a) are inversely correlated with apo(a) size. In 2 racially homogeneous Bantu populations from Tanzania differing in their dietary habits, we found that median plasma levels of Lp(a) were 48% lower in those living on a fish diet than in those living on a vegetarian diet. Considering the relationship between apo(a) size and Lp(a) plasma concentration, we have extensively evaluated apo(a) isoform distribution in the 2 populations to determine the impact of apo(a) size in the determination of Lp(a) values. The majority of individuals (82% of the fishermen and 80% of the vegetarians) had 2 expressed apo(a) alleles. Additionally, the fishermen had a high frequency of large apo(a) isoforms, whereas a higher frequency of small isoforms was found in the vegetarians. When subjects from the 2 groups were matched for apo(a) phenotype, the median Lp(a) value was 40% lower in Bantus on the fish diet than in those on the vegetarian diet. A significant inverse relationship was also found between plasma n-3 polyunsaturated fatty acids and Lp(a) levels ( $r = -0.24$ ,  $P = 0.01$ ). The results of this study are consistent with the concept that a diet rich in n-3 polyunsaturated fatty acids, and not genetic differences, is responsible for the lower plasma levels of Lp(a) in the fish-eating Bantus and strongly suggest that a sustained fish-based diet is able to lower plasma levels of Lp(a). (*Arterioscler Thromb Vasc Biol.* 1999;19:1250-1256.)

**Key Words:** fish diet ■ lipoprotein(a) ■ apolipoprotein(a) isoforms ■ polyunsaturated fatty acids

Lipoprotein(a), or Lp(a), is a distinct class of serum lipoprotein particles very similar in lipid and protein composition to LDL but additionally containing apolipoprotein(a) [apo(a)], a highly glycosylated protein covalently linked to the apo B-100 component of LDL.<sup>1</sup> Apo(a) is formed by 3 different domains with >80% amino acid sequence identity with the corresponding kringle (K) 4, K5, and protease domains of plasminogen, a key component of the coagulation cascade.<sup>2</sup> Unlike plasminogen, which possesses a single copy of K4, 10 basic K4 types, designated type 1 through 10, are present in apo(a). These K4 types are all present as a single copy except K4 type 2, which is present in multiple copies. The number of K4 type 2 copies is genetically controlled, varies considerably within and among individuals, and accounts for the high degree of apo(a) size heterogeneity.<sup>3,4</sup> More than 34 apo(a) size isoforms have been detected in human plasma,<sup>5</sup> and their distribution significantly varies among ethnic groups.<sup>6,7</sup> The plasma concentra-

tion of Lp(a) also shows a high degree of heterogeneity in mean levels and in distribution across populations and is largely genetically controlled in both whites<sup>8</sup> and blacks.<sup>9</sup> A vast body of evidence has been presented on the relation of apo(a) size isoforms to Lp(a) concentrations in different ethnic groups.<sup>7,10-13</sup> A large number of clinical studies (for a review, see Reference 14) have provided strong evidence for an association between high Lp(a) levels and increased risk for coronary heart disease. Unlike the plasma level of LDL, which increases significantly on a high-fat diet, Lp(a) levels do not appear to be influenced significantly by dietary composition. However, there are conflicting results regarding the effect of changes in dietary fatty acid composition on Lp(a). Two supplementation studies with fish oil rich in n-3 polyunsaturated fatty acid (PUFA) resulted in a significant reduction in Lp(a) concentration,<sup>15,16</sup> whereas Lp(a) levels were not decreased in another supplementation study.<sup>17</sup> Additionally, no difference in Lp(a) levels was found in a small

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study comparing Eskimos, whose prevalent food intake is constituted by fish, and Danes.<sup>18</sup>

We have recently reported the results from the Lugalawa Study,<sup>19</sup> which are consistent with a 38% lower mean plasma Lp(a) level in people living on a freshwater fish diet compared with those living on a vegetarian diet (19.9 versus 32.3 mg/dL, respectively). This study was carried out in a population of Bantu fishermen of Tanzania consuming 300 to 500 g (3 to 5 g of n-3 PUFAs) of freshwater fish per day, similar to the amount of fish consumed by the Eskimos. An important strength of the Lugalawa study, in addition to the large number of subjects (1308), was that the 2 populations belonged to the same ethnic group and had similar life styles and caloric intakes. Hence, the 2 populations were much more genetically homogeneous than Eskimos and Danes. However, owing to the well-documented contribution of apo(a) phenotype to plasma Lp(a) concentration,<sup>10–13</sup> a direct evaluation of the distribution of apo(a) size polymorphs in the 2 populations was mandatory to define the role of the fish diet and the impact of genetic factors.

In the present study, we report on the apo(a) phenotype distribution between the 2 populations and the fact that differences in apo(a) size can only partly explain the observed difference in Lp(a) level. Our results strongly suggest that the fish diet plays a role in the determination of plasma Lp(a) levels.

## Methods

### Study Population

As previously reported, we compared 2 Bantu populations of Tanzania,<sup>19</sup> 1 living on freshwater fish (n=622) in a village on Nyasa Lake and the other living mainly on a vegetarian diet (n=686) in a nearby farming area. Both populations are far removed from the Western life style, processed food or beverages are not readily available, and smoking is nearly absent. In a time span of 2 weeks, blood samples were drawn from the antecubital vein of each subject and collected in vials containing 0.6% EDTA. After immediate centrifugation, multiple plasma aliquots from each subject were collected and immediately frozen at  $-20^{\circ}\text{C}$  to  $-25^{\circ}\text{C}$  with use of a portable, butane-charged freezer. Samples were then flown to Italy and the United States on dry ice and thereafter stored at  $-80^{\circ}\text{C}$  until use. Lp(a) levels were determined in Italy and apo(a) size isoforms in the United States. All of the analyses were completed within 10 months. Based on sample availability, apo(a) isoforms were determined in 618 subjects on the fish diet and 645 on the vegetarian diet. Plasma fatty acids were analyzed in 53 subjects of similar age and sex distribution from each population.

### Lp(a) and n-3 PUFA Levels

Lp(a) level was determined by a 1-step sandwich ELISA,<sup>20</sup> a commercially available method (Immunozygm Lp(a), Immuno). Lp(a) values were calculated as total Lp(a) mass from a standard curve constructed for each plate by using a commercially available Lp(a) reference standard (Immuno). The levels of n-3 PUFAs were measured by gas chromatography, as previously described.<sup>21</sup>

### Determination of Apo(a) Isoforms

The apo(a) size isoforms were determined by high-resolution SDS-agarose gel electrophoresis followed by immunoblotting as previously reported.<sup>5</sup> We have evaluated the relationship of the number of K4 domains, as determined by pulsed-field gel electrophoresis,<sup>22</sup> to the mobility of the isoforms on SDS-agarose gel electrophoresis<sup>5</sup> and found that the logarithm of the K4 number was highly correlated with the mobility of the isoforms on agarose gel.<sup>23</sup> Therefore, the apo(a) isoforms are designated by the relative number of K4 copies. This step is performed by scanning the Western blot with a

Hewlett-Packard ScanJet IICX with a transparency adapter. A computerized approach is then introduced to assign the K4 number by using Jandel Scientific Sigma Gel software in the molecular weight mode. The same approach is used to determine the amount of expression of each isoform in heterozygous subjects. In this case, the program is used in the spot measurement mode.

### Statistical Methods

Differences in isoform frequencies were assessed by the Mann-Whitney *U* test and the  $\chi^2$  test. At the tails of the distribution curve (number of K4 repeats  $<14$  and  $>31$ ) where only a small number of subjects was available (n=21 and n=19, respectively), cells were pooled before computing  $\chi^2$  to achieve a sufficient frequency to perform the analysis. To compare the Lp(a) levels in the 2 populations, subjects were grouped according to apo(a) isoform size, expressed in terms of the number of K4 repeats, and compared by Friedman's nonparametric rank test. To control for differences in apo(a) isoform frequencies between the 2 Bantu populations, subjects between the villages were matched on the basis of apo(a) phenotype. Heterozygous individuals were matched for both isoforms. Differences in Lp(a) levels of these matched pairs were assessed by the Wilcoxon matched-pairs test. The contribution of apo(a) size to the variance of Lp(a) levels was estimated by the  $R^2$  from the ANOVAs.

## Results

As previously reported,<sup>19</sup> we compared 2 Bantu populations from Tanzania highly differing in their dietary habits. Lp(a) and plasma lipid parameters of the 2 populations are summarized in Table 1. The unadjusted median difference in the plasma Lp(a) levels found between the 2 groups was not modified by adjusting for age and alcohol or after matching subjects for age, sex, and alcohol consumption. When analyzed by sex, the same difference in Lp(a) values was found between men and women from the 2 villages. On determination of apo(a) isoform size, the majority of individuals (82% in the fish-diet population and 80% in the vegetarian-diet population) had 2 expressed apo(a) alleles. As evidenced in Figure 1, the population living on the fish diet had a higher frequency of large apo(a) isoform sizes while a higher frequency of small isoforms was found in the population living on the vegetarian diet ( $P<0.001$ ). If one considers that an inverse correlation exists between apo(a) size and Lp(a) concentration,<sup>10</sup> the difference in Lp(a) values between the 2 Bantu groups could potentially be accounted for by the difference in apo(a) sizes. To evaluate this possibility, Lp(a) level was evaluated as a function of apo(a) size. As presented in Figure 2A, the respective number of K4 repeats was used for individuals expressing a single apo(a) allele while the heterozygous individuals were analyzed in 2 separate groups. In the majority of the heterozygous individuals in both villages, 1 of the 2 apo(a) isoforms was predominantly expressed. We define an isoform as predominantly expressed when it represents  $>85\%$  of the apo(a) as evaluated by scanning of the Western blot, as detailed in Methods. If one further considers that in this case the contribution of the second apo(a) isoform to Lp(a) value is very low, statistical analyses were then performed with only the predominantly expressed apo(a) size (Figure 2B). In the relatively small number of heterozygous individuals with equally expressed isoforms, we used the sum of the number of K4 repeats of the 2 isoforms (Figure 2C). Although both Bantu groups exhibited an inverse relationship between apo(a) size and Lp(a) concentration, as evidenced from the figure, the median Lp(a) for most of the apo(a) size groupings was significantly higher

TABLE 1. Plasma Lipids and Fatty Acids

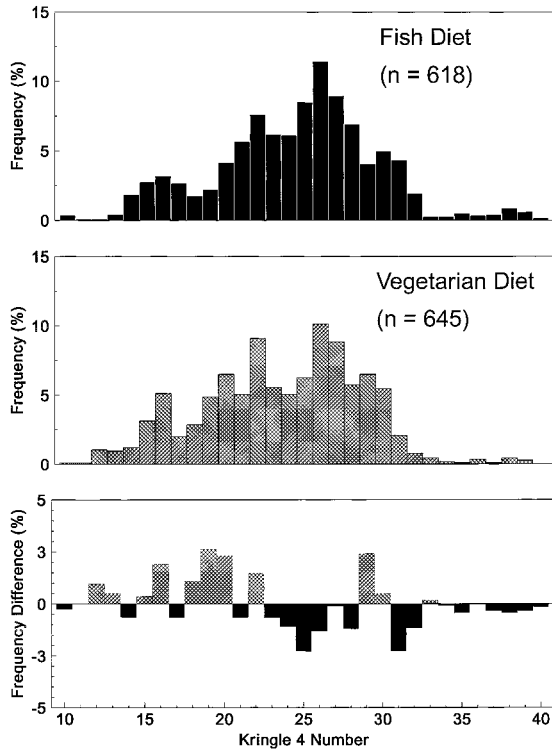
	Fish Diet	Vegetarian Diet	<i>P</i>
Lp(a), mg/dL			
Total population	14.0 (7.0–27.0) (n=618)	27.0 (17.0–43.0) (n=645)	<0.001
Women	15.0 (7.0–27.0) (n=368)	27.0 (16.0–46.2) (n=361)	<0.001
Men	14.0 (8.0–27.8) (n=250)	27.0 (17.0–41.0) (n=284)	<0.001
Plasma lipids, mg/dL			
Total cholesterol	134.1±37.6 (n=618)	160.4±40.6 (n=645)	<0.001
Triglycerides	79.5±38.3	116.4±64.8	<0.001
PUFAs as percentages of total plasma fatty acids*			
n-6	(n=53)	(n=53)	
18:2 (linoleic)	15.0±4.2	23.9±4.4	<0.001
20:4 (AA)	9.7±2.7	8.3±2.0	<0.005
Total n-6	25.8±4.8	33.1±5.5	<0.001
n-3			
20:5 (EPA)	2.3±1.3	0.7±0.2	<0.001
22:6 (DHA)	5.7±1.6	1.5±1.1	<0.001
Total n-3	9.7±2.9	3.5±1.2	<0.001
Ratios			
n-3/n-6 PUFAs	0.39±0.13	0.11±0.04	<0.001
EPA/AA	0.24±0.08	0.09±0.03	<0.001
DHA/AA	0.61±0.14	0.18±0.12	<0.001

Significance was determined by ANCOVA. Lp(a) values are reported as median (lower and upper quartiles); all other values are reported as mean±SD.

\*Sex and age matched; see Methods.

in the vegetarian population. The difference in median Lp(a) concentration between the 2 populations is particularly evident in Figure B. Owing to the large number of individuals in this group, the median Lp(a) values were significantly higher in the vegetarians in all of the apo(a) size groupings. Overall, the median Lp(a) value was 27 mg/dL in the vegetarian population and 14 mg/dL in the fish-diet population. This difference is highly significant ( $P<0.001$ ) and represents a 48% difference in median Lp(a) concentration between the 2 groups. We further evaluated the potential influence of the fish-based diet, independent of apo(a) size, by focusing our analysis on subjects from the 2 villages who could be matched according to the expressed apo(a) alleles. A total of 410 subjects for each village, corresponding to ≈65% of the study population, were matched. Of these, 96 pairs had a single expressed allele and 314 had 2 alleles. As presented in Table 2, median plasma Lp(a) levels were 40% lower in people living on the fish diet than in those living on a vegetarian diet (15.0 versus 25.0 mg/dL,  $P<0.001$ ). However, in heterozygous individuals, it cannot be excluded that the relative level of expression of each isoform could be different between the matched individuals, thus contributing differently to their Lp(a) level. Therefore, we analyzed separately the matched individuals with a single expressed allele and the matched heterozygotes. As presented in Table 2, the median Lp(a) level in heterozygotes was 33% lower in

individuals on the fish diet than in those on the vegetarian diet (17.0 versus 25.5 mg/dL,  $P<0.001$ ). In the group of individuals with a single expressed allele, those on the fish diet had a median Lp(a) value that was 47.8% lower than in vegetarians (12.0 versus 23.0 mg/dL,  $P<0.001$ ). Therefore, independently of the different approaches used for the evaluation, differences in apo(a) phenotypes explain only a minor part of the differences in Lp(a) concentration. On the whole, the analysis of  $R^2$  values for apo(a) size predicting Lp(a) concentrations showed that the apo(a) phenotype predicted 23.0% of plasma levels in the vegetarian population versus only 14.4% in the fishermen. If one considers that in heterozygous individuals the 2 alleles contribute differently to Lp(a) values, then the  $R^2$  values for apo(a) phenotype predicting Lp(a) concentration were separately calculated in homozygotes and heterozygotes to account for this possibility (Table 3). For heterozygous individuals with equally expressed isoforms, the sum of the number of K4 repeats of the 2 isoforms was used for analysis. As evident from Table 3, in each of the 3 groups, the contribution of apo(a) size to Lp(a) value was significantly higher in the vegetarians. Furthermore, in a sample representative of the 2 populations, a significant, inverse relationship was found between plasma levels of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and total n-3 PUFAs and plasma Lp(a) levels (Table 4). Plasma Lp(a) levels were also directly correlated with total

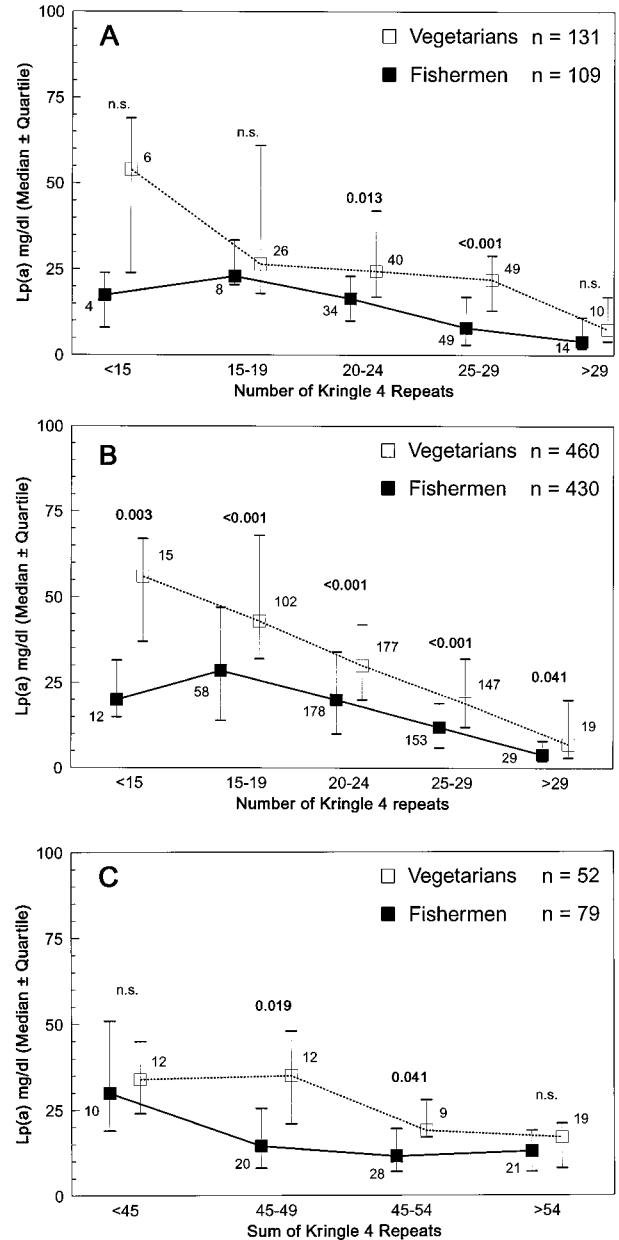


**Figure 1.** Apo(a) isoform frequencies in 2 Bantu populations. The difference in apo(a) isoform frequency (vegetarian-diet group minus fish-diet group) is shown in the bottom part of the figure. Isoforms with a higher frequency in the vegetarian population are shown in the cross-hatched bars, and isoforms with a higher frequency in the fishermen population are shown in the solid bars.

n-6 PUFAs ( $r=0.207$ ,  $P=0.026$ ) and indirectly with arachidonic acid (AA;  $r=0.199$ ,  $P=0.033$ ). However, the whole impact of n-6 PUFAs and AA on Lp(a) level seems to be weak, because the ratios n-3/n-6 PUFAs, DHA/AA, EPA/AA, and n-3/AA were inversely related to plasma Lp(a) values (Table 4). As expected, total cholesterol levels were directly correlated with Lp(a) plasma values in both populations (vegetarian diet,  $r=0.256$ ,  $P<0.0001$ ,  $n=654$ ; fish diet,  $r=0.213$ ,  $P<0.0001$ ,  $n=618$ ), whereas plasma triglycerides were directly correlated with Lp(a) plasma levels in the fish diet population ( $r=0.103$ ,  $P=0.011$ ,  $n=618$ ) but not in the vegetarian population ( $r=0.062$ ,  $P=0.117$ ,  $n=645$ ).

### Discussion

This is the largest population study on the dietary influence on Lp(a) levels thus far conducted and the only in which the contribution of apo(a) size to Lp(a) value differences has been rigorously and extensively evaluated. Although it is generally recognized that plasma Lp(a) levels are uninfluenced by common lipid-lowering therapy and only marginally affected by sex and age, some lowering effect has been achieved in 2 supplementation studies with n-3 PUFAs.<sup>15,16</sup> On the other hand, a recent controlled trial<sup>17</sup> did not show any change in Lp(a) levels. The discordant results provided by these studies may be explained by differences in the subjects' genetic backgrounds, in the degree of supplementation compliance, and by the possibility that a long-term consumption of n-3 fatty acid-rich food is required to achieve an effect on Lp(a) concentration. Additionally, in any of these studies, the



**Figure 2.** Relationship between median Lp(a) concentration and apo(a) size in 2 Bantu groups. Bantus on the fish diet are represented by solid squares and those on the vegetarian diet by open squares. The number close to the solid or open squares indicates the number of subjects for each isoform group, whereas the relative degree of significance is shown on top. A, Relationship between Lp(a) values and apo(a) size, expressed by the number of respective K4 repeats, in individuals with a single expressed apo(a) isoform. B, For heterozygous individuals with 1 of the 2 isoforms accounting for >85% of the apo(a) particles, the relationship between Lp(a) levels and apo(a) size was evaluated by using the number of K4 repeats of the predominantly expressed isoform. C, In individuals with equally expressed isoforms, the sum of the number of K4 repeats of the 2 isoforms was used for analysis.

biological variability of Lp(a), which has been reported to vary widely among individuals,<sup>24</sup> has been taken under consideration. In this view, population studies comparing people living on a fish diet with an appropriate reference group would represent a sound approach to test whether or not the n-3 PUFAs are able to reduce plasma Lp(a) levels. The Lugalawa Study<sup>19</sup> has provided a very good opportunity



TABLE 2. Lp(a) Concentrations Between Matched Apo(a) Phenotype Pairs

	Pairs, n	Median	Quartile		% Difference, Median	
			Lower	Upper		
Total population						
Fish diet	410	15.0	8.0	25.0	-40.0	] P<0.001
Vegetarian diet		25.0	17.0	38.0		
Single expressed apo(a) isoform size						
Fish diet	96	12.0	4.0	20.0	-47.8	] P<0.001
Vegetarian diet		23.0	14.0	35.0		
Heterozygous for apo(a) isoform size						
Fish diet	314	17.0	8.0	28.0	-33.3	] P<0.001
Vegetarian diet		25.5	17.0	38.0		

P determined by Wilcoxon matched-pairs test. Lp(a) concentrations are mg/dL.

for studying the impact of a fish-based diet on several risk factors, including Lp(a), because of the homogeneity in race, age, and sex of the 2 populations studied. Additionally, the 2 populations are exposed to the same climate and share the same life style. Although it is very difficult, even in Western populations, to accurately assess physical activity, its level should not be substantially different in the 2 villages. In both environments, electricity and mechanical equipment are lacking, and both farmers and fishermen perform intense manual work. This lack of a significant difference in the level of physical activity between the 2 populations is particularly important because it has been previously shown that Lp(a) lowering with n-3 fatty acids was mostly effective in patients on an exercise program.<sup>25</sup> Considering that apo(a) size plays an important role in the determination of Lp(a) levels, we conducted an extensive analysis of apo(a) isoform polymorphism. Our data elucidate the relative weight of genetic and dietary factors in the determination of Lp(a) levels. The

strikingly lower Lp(a) level found in Bantus living on a fish diet compared with the Bantus living on a vegetarian diet persisted after adjusting/matching for age, sex, and alcohol consumption, the only epidemiological differences found between the 2 population groups. On the other hand, even though the 2 populations are racially homogeneous, we have found a significant difference in apo(a) isoform size distribution between the inhabitants of the 2 villages, probably due to differences between the founders of the 2 villages. Because of the inverse relationship between apo(a) size and Lp(a) levels in plasma,<sup>10-13</sup> the significant prevalence of large Lp(a) molecules in the population living on the fish diet might have represented a valid explanation for the lower Lp(a) values found in the fishermen. Nevertheless, even after matching the subjects for apo(a) phenotypes, median Lp(a) values were still 40% lower in the fishermen than in the reference population. When the evaluation was performed again but only in matched individuals with a single expressed apo(a) size polymorphism, we found that the median plasma Lp(a) value in vegetarians was almost double that in individuals consuming a fish diet (23.0 versus 12.0 mg/dL). This striking 47.8% difference in median Lp(a) values between these 2 groups in which, unlike in the matched heterozygotes, we can exclude any confounding effect of possible differences in the degree of expression of the apo(a) alleles, further strengthens our conclusion that the fish diet represents an independent

TABLE 3. R<sup>2</sup> Values for Apo(a) Phenotype Predicting Lp(a) Concentration

	r	%	
Apparent homozygous			
Total population (n=240)	-0.45	20.0	] P=0.031
Fish diet (n=109)	-0.35	12.1	
Vegetarian diet (n=131)	-0.48	22.9	] P=0.008
Heterozygous (predominantly expressed isoform)			
Total population (n=890)	-0.43	18.7	] P=0.008
Fish diet (n=430)	-0.38	14.5	
Vegetarian diet (n=460)	-0.48	23.0	] P=0.008
Heterozygous (equally expressed isoforms)			
Total population (n=131)	-0.47	22.0	] P=0.008
Fish diet (n=79)	-0.38	14.7	
Vegetarian diet (n=52)	-0.59	34.8	] P=0.008

R<sup>2</sup> and adjusted r statistics from regression analysis.

TABLE 4. Coefficients of Correlation Between Plasma Lp(a) and Plasma n-3 PUFA Levels in a Representative Sample of the 2 Populations (n=106)

	r	P
DHA	-0.254	0.006
EPA	-0.184	0.048
Total n-3 PUFAs	-0.238	0.010
n-3/n-6 PUFAs	-0.248	0.007
DHA/AA	-0.255	0.006
EPA/AA	-0.167	0.073
n-3/AA	-0.217	0.019

Pearson correlation matrix and Bonferroni post hoc test.

factor for lowering Lp(a) levels. Moreover, the distribution of apo(a) size in individuals expressing 1 or 2 isoforms accounted for 23.0% of plasma Lp(a) levels in the vegetarian Bantus, quite the same magnitude found in African Americans<sup>26</sup> and in a Sudanese population.<sup>10</sup> On the contrary, the apo(a) polymorphism explained only 12.1% or 14.5% in individuals expressing 1 or 2 apo(a) isoforms, respectively, of Lp(a) levels in people living on the fish diet, suggesting that environmental factor(s) may play a role. However, it should be taken into consideration that, in addition to apo(a) size, other yet-to-be-identified sequence differences linked to the apo(a) locus could contribute to differences in Lp(a) values,<sup>8</sup> even though it is highly unlikely that they can entirely account for the striking difference in plasma Lp(a) concentration found between these 2 racially homogeneous populations. Recently, Kraft et al<sup>27</sup> have reported that a C→T transition in the 5' region of the apo(a) gene was associated with a significant reduction in Lp(a) levels in 2 groups of blacks from Africa. However, this polymorphism is relatively rare, and it is highly unlikely that a significant proportion of fishermen are carriers of the C/T polymorphism. Another possibility to consider is that apo(a) alleles expressing low Lp(a) concentrations have been selectively transmitted in the fishermen families. However, this hypothesis is unlikely because intervillage marriage is very common. In intervillage marriages, traditionally the women leave their village to join the husband's family. As evidenced in Table 1, no difference in Lp(a) values between men and women was found in the 2 villages. This suggests that a common environmental factor and not genetic differences is responsible for the lower Lp(a) values in the Bantu fishermen.

The inverse relationship found between plasma n-3 PUFAs and Lp(a) levels further endorses the view that a fish diet rich in n-3 PUFAs is the environmental factor responsible for the lower Lp(a) levels observed in our populations. On the other hand, the association between high intakes of both n-3 PUFAs and AA with the fish diet explains why there was also a weak inverse correlation between plasma AA levels and Lp(a). It is very likely, however, that AA does not affect Lp(a) levels, because the n-3/n-6 PUFA, DHA/AA, EPA/AA, and n-3 PUFA/AA ratios were all negatively correlated with Lp(a). In addition, it has recently been shown that a high intake of AA (1.7 g/d) by normal healthy subjects over a 50-day period does not modify lipoprotein distribution and apoprotein levels.<sup>28</sup> Finally, it is of interest to note that in the fish-diet population, but not in the vegetarians, a direct relationship exists between plasma Lp(a) and triglyceride levels, which is likely to be due to the lowering effect of n-3 PUFAs on both parameters. The mechanisms linking high fish intake and lower Lp(a) levels in plasma are unclear, because it is unknown at this time whether n-3 PUFAs affect apo(a) synthesis or metabolism. It has been suggested<sup>29</sup> that an appreciable proportion of n-3 fatty acids may directly enter the portal vein system, producing profound effects on liver function, such as a marked reduction in serum triglycerides. It can be speculated that the n-3 fatty acids may lower the rate of apo(a) synthesis and/or secretion from the liver. Additionally, it has been hypothesized that high levels of n-3 fatty acids may result in an impaired assembly of apo(a) with LDL or in enhanced catabolism.<sup>25</sup> However, to confirm these hypotheses, further studies are required to elucidate the

molecular mechanisms by which n-3 PUFAs decrease the levels of Lp(a) in plasma.

In conclusion, this is the first population-based, genetically controlled study to provide evidence that the dietary content of n-3 PUFAs is able to interfere with the expression of the atherogenic Lp(a) lipoprotein.

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Santica M. Marcovina, Hal Kennedy, Gabriele Bittolo Bon, Giuseppe Cazzolato, Claudio Galli, Edoardo Casiglia, Massimo Puato and Paolo Pauletto

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