Apolipoprotein Polymorphisms and Phenotypic Variability in American Samoans: Preliminary Data

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ABSTRACT Human apolipoprotein genetic variation is associated with phenotypic variability in body habitus and in lipid and glucose metabolism, interrelated aspects of human physiology. In this study, structural variation at the apolipoprotein E and H loci, documented for 67 residents of American Samoa, was investigated for associations with body habitus, plasma glucose, glycated hemoglobin, pulse rate, and blood pressure. Compared to men with the common APO E*3 allele, those with APO E*2 had higher weight, percent trunk fat, ratio of subscapular to triceps skinfold, and larger subscapular, suprailiac, and medial calf skinfolds; men with the APO E*4 allele had lower weight, body mass index (BMI), upper arm circumference, estimated arm muscle circumference, and a smaller suprailiac skinfold. Such variability by apolipoprotein E types was not observed in Samoan women nor when men and women were combined for analysis with or without statistical control for sex. At the APO H locus, little difference in aspects of body habitus was observed between men carrying the most common APO H*2 allele and the less common APO H*1 allele. Percent trunk fat and the subscapular to triceps skinfold ratio tended to be larger in women with the APO H*1 allele. Plasma glucose and glycated hemoglobin were lower in Samoans with the APO E*2 and E*4 alleles, but higher in those with the APO H*1 allele. Blood pressure and pulse rate were lower and higher, respectively, in those with the APO E*4 allele. The observed associations differ from those observed in Caucasian, Japanese, and Hispanic samples, and support suggestions that associations between apolipoprotein polymorphisms and morphological measures vary across ethnic groups. © 1993 Wiley-Liss, Inc.

Many proteins have been identified as components of various lipoprotein particles and hence have been termed “apolipoproteins.” These gene products participate in lipoprotein synthesis, secretion, processing, and catabolism. Several apolipoproteins have been characterized in detail with respect to their structure, biochemical and physiological properties, and chromosomal localizations (Breslow, 1988; Hegele and Breslow, 1987). In general, apolipoproteins have three main functions. As structural components of various lipoprotein fractions, apolipoproteins provide stability and maintain lipid solubility in aqueous body fluids. They also act as ligands for receptor-mediated transport in the distribution of lipid particles across cell membranes. Apolipoproteins are also active as allosteric effectors for various enzymes involved in lipid metabolism. Variation in the structure of apolipoproteins potentially influences interindividual differences in susceptibility to cardiovascular diseases and non-insulin-dependent diabetes mellitus (NIDDM) (Davignon et al., 1988; Humphries et al., 1987; Kamboh et al., 1991; Kottke et al., 1986).

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Marked ethnic differences in frequencies have been reported in populations that have been screened for apolipoprotein genetic variation (Davignon et al., 1988; Kamboh and Ferrell, 1990; Utermann, 1987). This variation has been shown to be associated with differences in plasma lipid levels and aspects of body habitus (Davignon et al., 1988; Eto et al., 1986, 1988a,b; Fumeron et al., 1988; Humphries et al., 1987; Pouliot et al., 1990; Sing and Davignon, 1985). It has also been suggested that polymorphism at the apolipoprotein E (APO E) locus modulates the effect of obesity on lipids and lipoprotein metabolism (Fumeron et al., 1988). In particular, the relationship between abdominal obesity and plasma lipoprotein levels appear to differ across APO E genotypes (Pouliot et al., 1990). Lipid metabolism also is commonly abnormal in diabetic patients (Laker, 1987) and apolipoprotein variants have recently been reported to be associated with plasma glucose levels in Hispanic women with NIDDM and with insulin levels in Anglo men with NIDDM (Kamboh et al., 1991). Apolipoprotein H (APO H), also referred to as B2-glycoprotein I, is associated with all of the major lipoprotein particles (Polz and Kostner, 1979a,b). Although the exact role of APO H in lipid metabolism is unknown, the available data suggest that it is involved in triglyceride clearance (Wurm et al., 1982) and that it may be an activator of lipoprotein lipase (Nakaya et al., 1980).

Samoans, a Polynesian population of the South Pacific, are noted for high levels of obesity and high prevalence of and mortality rates from diabetes (Bindon and Baker, 1985; Crews, 1988; Crews et al., 1991a; Crews and MacKeen, 1982; Pawson and Janes, 1981; Zimmet et al., 1981). One theory advanced to explain the high prevalence of obesity and diabetes in populations such as the Samoans is the "thrifty genotype" (Neel, 1962, 1982). Although recourse to this model of diabetes is frequently encountered, little data have been presented illustrating any differences in genetically determined proteins associated with energy or glucose metabolism in populations with high diabetes rates. Recently, plasma samples from 67 residents of American Samoa were screened to detect genetic variation at six apolipoprotein loci: A-I, A-II, A-IV, C-II, E, and H with use of isoelectric focusing (IEF)-immunoblotting methods (Crews et al., 1991b). Three were monomorphic (A-I, A-II, C-II) and only limited variation was observed at the A-IV locus. To examine the hypothesis that genes involved with lipid metabolism contribute to phenotypic variation in cardiovascular risk factors, the association of alleles identified at the APO E and H loci with variability in body dimensions and indices of body habitus, plasma glucose, percent glycated hemoglobin, systolic and diastolic blood pressure, and pulse rate were determined in this sample of Samoans.

MATERIALS AND METHODS

Sample

Background. Since 1976, the population of American Samoa has been the object of an ongoing study to examine the effects of sociocultural change on health and well-being. This project is generally known as the Samoan Studies Project (SSP) (Baker et al., 1986). Data collected as part of the SSP have been used to show that Samoans are very overweight compared to the general U.S. population (Bindon and Baker, 1985). Studies have also documented that Samoan women are significantly larger in average body mass index (BMI = 33 kg/m^2) and triceps skinfold thickness (39 mm) than Samoan men (BMI = 30 kg/m^2, triceps skinfold = 17 mm) (Crews, 1988). However, the sexes differ less in total or high density lipoprotein (HDL)-cholesterol than U.S. samples (Pelletier and Hornick, 1986). Given this risk profile, the population of American Samoa experiences a lower age-adjusted mortality rate from cardiovascular diseases than do residents of the U.S., but experiences more than twice the mortality rate from diabetes (Crews and MacKeen, 1982).

Data. Data for this study were obtained during a follow-up of 32 families who participated in the 1976 SSP survey (Crews, 1988; McGarvey and Baker, 1979). Methods of participant recruitment, sampling design, data protocols, sample preparation, and analyses have been detailed elsewhere (Crews et al., 1991a,b), but are briefly described here. The sample was stratified to include 32 probands, their spouses, and 2 children for apolipoprotein typing, glucose measurement, and blood pressure follow-up. Given household membership and inability to obtain samples from some participants, only 67 samples were obtained for apolipoprotein typing. In only 10 households was the ideal sampling unit obtained. Plasma
samples were available from 26 men, 19 of their spouses, 20 of their children/step-children, and 2 more distant relatives or unrelated individuals 20–71 years of age.

Allele frequencies have been reported for the entire sample (N = 67) and with children/step-children of the 26 probands excluded (N = 47). No significant differences in allele frequencies at the polymorphic loci were observed when children/step-children were excluded and results are reported here for the larger sample (Crews et al., 1991b). Although no corrections were made for the inclusion of related individuals in the analyses, the effect of their inclusion should be to decrease the variability in the dependent variables and reduce the likelihood of observing significant differences across apolipoprotein variants. All analyses were repeated on the smaller sample and observed trends (not reported) were similar to those reported here for the total sample.

Methods

Laboratory. Blood samples were drawn by venipuncture into tubes with ethyldiaminetetraacetic acid (EDTA) as an anticoagulant. These were stored in a styrofoam ice chest with frozen cold packs for 3–4 hours until transport to the Hematology Laboratory at the Lyndon B. Johnson Tropical Medical Center. The blood was centrifuged at 2,000 rpm for approximately 15 minutes, after which the plasma was decanted into several separate 1 ml aliquots and stored in a –20°C freezer along with the packed cells until transport to the United States in styrofoam ice chests with frozen cold packs. All samples were then stored at –70°C until extraction of DNA or transport to the Department of Human Genetics, Graduate School of Public Health, University of Pittsburgh for apolipoprotein typing. When the packed cells were thawed for extraction of DNA, a 1 ml sample was decanted and saved for later determinations of glycated hemoglobin. All plasma samples were in good condition for typing. Frequency data for alleles and phenotypes at the APO E and H loci have been reported elsewhere (Crews et al., 1991b). The APO E and APO H loci were each represented by three commonly reported alleles, representing six possible phenotypes at each locus. Capillary blood glucose levels were determined in the field with use of the finger-stick method and a GLUCOSCAN® Blood Glucose Meter and GLUCOSCAN® Test Strips manufactured and donated by Lifescan Inc. (Mountain View, CA 94043). Capillary glucose values obtained were later converted to their approximate plasma equivalents through multiplication by 1.15.

Body habitus. Six dimensions (weight; the triceps, subscapular, suprailiac and medial calf skinfolds; upper arm circumference) and five indices (BMI, percent trunk fat, ratio of subscapular to triceps skinfolds, ratio of subscapular to medial calf skinfolds, and estimated arm muscle circumference) are examined in this analysis. All measurements were made according to standard techniques described by Weiner and Lourie (1969), that have been used throughout the SSP and have been described in detail (Bindon, 1984a,b; Bindon and Baker, 1985; Crews, 1985, 1988; McGarvey and Baker, 1979). For participants with skinfolds larger than the maximum width of the skinfold calipers (60 mm), a value of 61 mm was assigned.

A number of calculated indices of fat patterning and muscularity have been developed and examined as correlates of chronic diseases and their associated risk factors. The most familiar of these may be the BMI (weight [kg]/height [m]²). Sometimes BMI is interpreted as an index of obesity (Garrow, 1981; Garrow and Webster, 1985), and at others as an index of overweight (Simopolulos and VanItallie, 1984). Here, BMI is interpreted as an index of overweight (Bindon, in press; Najjar and Rowland, 1987). Percent trunk fat (subscapular/(subscapular + triceps) skinfolds * 100) is an index of the distribution of subcutaneous fat on the trunk as opposed to the upper limb. The ratios, subscapular to medial calf and subscapular to triceps skinfolds, also index the degree to which subcutaneous fat shows an upper body distribution. Although these indices only provide an assessment of the distribution of subcutaneous fat, similar indices have been positively linked to risks for cardiovascular disease and diabetes (Bjornstorp, 1988, Joos et al., 1984; Mueller and Reid, 1979). Estimated arm muscle circumference (upper arm circumference – 3.14 * triceps skinfold) is a useful measure for assessment of nutritional status (Frisancho, 1974). Although estimated arm muscle circumference is not an indicator of lean body mass, it may usefully index muscularity in contrast to fat. Samoans as a population tend toward large body size; however it re-
TABLE 1. Associations of apolipoprotein alleles at the E locus with measurements and indices of body habitus in Samoan men

<table>
<thead>
<tr>
<th>APO E*3</th>
<th>APO E*2 (N = 4)</th>
<th>APO E*4 (N = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean Diff 95% CI</td>
<td>Mean Diff 95% CI</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.4</td>
<td>114.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.3</td>
<td>36.4</td>
</tr>
<tr>
<td>Triceps (mm)</td>
<td>17.4</td>
<td>18.3</td>
</tr>
<tr>
<td>Subscap (mm)</td>
<td>35.3</td>
<td>50.0</td>
</tr>
<tr>
<td>Trunk (%)</td>
<td>66.8</td>
<td>73.9</td>
</tr>
<tr>
<td>SFratio (mm/mm)</td>
<td>2.12</td>
<td>2.98</td>
</tr>
<tr>
<td>Arm cir (cm)</td>
<td>34.6</td>
<td>35.3</td>
</tr>
<tr>
<td>Arm mus (cm)</td>
<td>29.1</td>
<td>29.6</td>
</tr>
<tr>
<td>Suprail (mm)</td>
<td>39.6</td>
<td>54.5</td>
</tr>
<tr>
<td>Med calf (mm)</td>
<td>13.8</td>
<td>23.7</td>
</tr>
<tr>
<td>Sub/med (mm/mm)</td>
<td>2.98</td>
<td>2.30</td>
</tr>
</tbody>
</table>

1Diff, differences between means; 95% CI, 95% confidence intervals; P, P values; BMI, body mass index; triceps, triceps skinfold; subscap, subscapular skinfold; trunk, percent trunk fat; SFratio, ratio subscapular to triceps skinfolds; arm cir, upper arm circumference; arm mus, estimated arm muscle circumference; suprail, suprailiac skinfold; med calf, medial calf skinfold; sub/med, ratio of subscapular to medial calf skinfold.

mains unclear the degree to which this size is due to fat, muscle, or skeletal components. Therefore, estimated arm muscle circumference is included in these analyses to provide some contrast to variables, such as the BMI and skinfolds, used to assess overweight and fat placement.

Blood pressure and pulse rate. Systolic and diastolic blood pressure along with pulse rate were measured prior to the anthropometry after the participant had been seated for at least 10 minutes. Blood pressure and pulse rate were measured twice on the right arm using a mercury sphygmomanometer following protocols from the systolic hypertension in the elderly program (SHEP). Most anthropometric and blood pressure measurements were completed by a single investigator (D.E.C.).

Statistics. For comparisons between apolipoprotein alleles, the most frequent allele was used as the base group and mean differences from this base, along with 95% confidence intervals (CIs) and P values for the differences, were determined with use of linear regression for each variant allele. The variable of interest (e.g., BMI, skinfold ratio) was entered as the dependent variable and each variant allele, represented as a dichotomous 0,1 (0 = common allele; 1 = variant allele) variable, was entered as the independent variable in separate regression equations combining both sexes, by each sex, and combining sexes but including a control variable for sex, and also with age included as an independent variable. Only the age-adjusted analyses by sex are reported for body habitus, while for all other comparisons age-adjusted analyses for both sexes combined are reported. Because of the exploratory nature of these analyses and the low level of statistical power available for most comparisons, trends rather than significance levels are emphasized, although P values are presented for all comparisons. In addition, because non-normal distributions and non-linear associations may characterize some of the relationships of the apolipoprotein variants with measures and indices of body habitus, glucose metabolism, and blood pressures in Samoans, Mann-Whitney non-parametric rank sum tests, based on dichotomies of the alleles at the APO E and H loci, were completed in addition to the general linear regression models. Results from these analyses are reported as adjuncts to the parametric tests.

RESULTS

Body habitus

Mean differences in measures and indices of body habitus were determined by sex for variant alleles compared to the most common allele at the APO E locus: men APO E*3 (N = 57), APO E*2 (N = 4), APO E*4 (N = 11); women APO E*3 (N = 50), APO E*2 (N = 2), APO E*4 (N = 10). Among men almost every measurement and index of body habitus was larger in those carrying the APO E*2 alleles than in those with the most common E*3 allele (Table 1). Differences in weight and the subscapular and suprailiac skinfolds were of borderline statistical significance, while differences in the
TABLE 2. Associations of apolipoprotein alleles at the H locus with measurements and indices of body habitus in Samoan men

<table>
<thead>
<tr>
<th>APO H* locus</th>
<th>APO H*1 (N = 9)</th>
<th>Mean Diff</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 62</td>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>95.3</td>
<td>93.7</td>
<td>-1.6</td>
<td>-15.5, 12.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>33.0</td>
<td>32.1</td>
<td>-0.9</td>
<td>-5.2, 3.4</td>
</tr>
<tr>
<td>Triceps (mm)</td>
<td>17.2</td>
<td>16.5</td>
<td>-1.6</td>
<td>-7.2, 4.0</td>
</tr>
<tr>
<td>Subscap (mm)</td>
<td>35.5</td>
<td>32.7</td>
<td>-2.8</td>
<td>-13.1, 7.6</td>
</tr>
<tr>
<td>Trunk (%)</td>
<td>67.0</td>
<td>66.9</td>
<td>-0.1</td>
<td>-4.1, 3.9</td>
</tr>
<tr>
<td>SFratio</td>
<td>2.13</td>
<td>2.14</td>
<td>0.01</td>
<td>-0.45, 0.45</td>
</tr>
<tr>
<td>Arm cir (cm)</td>
<td>34.2</td>
<td>33.5</td>
<td>-0.7</td>
<td>-3.7, 2.3</td>
</tr>
<tr>
<td>Arm mus (cm)</td>
<td>28.8</td>
<td>28.6</td>
<td>-0.2</td>
<td>-2.3, 1.8</td>
</tr>
<tr>
<td>Suprail (mm)</td>
<td>39.1</td>
<td>37.6</td>
<td>-1.5</td>
<td>-12.9, 9.9</td>
</tr>
<tr>
<td>Med calf (mm)</td>
<td>13.8</td>
<td>16.3</td>
<td>2.5</td>
<td>-3.6, 8.7</td>
</tr>
<tr>
<td>Sub/med</td>
<td>2.93</td>
<td>2.41</td>
<td>-0.52</td>
<td>-1.49, 0.44</td>
</tr>
</tbody>
</table>

1Diff, differences between means; 95% CI, 95% confidence intervals; P, P values; BMI, body mass index; triceps, triceps skinfold; subscap, subscapular skinfold; trunk, percent trunk fat; SFratio, ratio subscapular to triceps skinfolds; arm cir, upper arm circumference; arm mus, estimated arm muscle circumference; suprail, suprailiac skinfold; med calf, medial calf skinfold; sub/med, ratio of subscapular to medial calf skinfold.

APOLIPOPROTEIN POLYMORPHISMS AND PHENOTYPIC VARIABILITY

Medial calf skinfold, percent trunk fat, and the ratio of subscapular to triceps skinfolds attained standard levels of statistical significance, even with the small number of E*2 alleles available (Table 1). With use of the Mann-Whitney rank-sum test, differences in both weight (P = .054) and the medial calf skinfold (P = .068) attained similar levels of statistical significance.

On the other hand, most measures and indices of body habitus were smaller among men with the APO E*4 allele than in those with the APO E*3 allele (Table 1). In this case, differences in weight and upper arm circumference attained standard levels of statistical significance, while those for the BMI, suprailiac skinfold, and estimated arm muscle circumference were of borderline significance. Based on the Mann-Whitney rank-sum test, only the differences in weight (P = .084) and estimated arm muscle circumference (P = .082) attained borderline levels of statistical significance.

Only one other polymorphic locus, APO H, was represented by a number of variant alleles among men sufficient to conduct preliminary analyses. APO H*1 occurred in nine men. For almost every measure and index of body habitus, the APO H*1 allele was associated with somewhat lower means than were observed for the APO H*2 allele in men (N = 62) (Table 2), although none of the differences attained standard levels of statistical significance.

When the same measurements and indices of body habitus among women were examined for associations with alleles at these two polymorphic loci, means were little different for the APO E*4 variant (N = 10) than observed for the common APO E*3 allele (N = 50) (results not shown). Similarly, the APO H*1 allele (N = 9) was associated with means that were only slightly different from those for the APO H*2 allele (N = 53). None of the differences approached standard levels of statistical significance. Women with the APO H*1 allele tended to be younger than those carrying the APO H*2 allele (P = .039), and age is inversely correlated with BMI and weight in the general female population of American Samoa. Combining men and women and examining these associations while controlling for differences in either age or sex did not greatly alter the results. Although age had little influence on the outcomes, sex was highly correlated with most measures and indices of body habitus, and led to slightly greater differences between the variant and common allele. Differences observed across apolipoprotein alleles were not observed when data were examined by genotypes.

**Glucose and glycated hemoglobin**

Mean plasma glucose and percent glycated hemoglobin have been determined for this sample (Crews et al., 1991a). Associations of APO E and APO H alleles with these measures are described here. Both sexes are combined since neither of the measures varied by sex. Individuals carrying either variant APO E allele exhibited lower average plasma glucose and percent glycated hemoglobin than those with the common APO E*3 allele (Table 3). However, only the difference in percent glycated hemoglobin between the APO E*2 and the APO E*3 alleles attained borderline statistical significance. At the APO H locus, although there were no significant differences, mean plasma glucose and percent glycated hemoglobin were higher among individuals carrying the less common APO H*1 allele than among those carrying the common APO H*2 allele (Table 4). Results from Mann-Whitney analyses were not substantially different.

In an alternative analysis, elevated casual glucose, based on a random plasma glucose of 140 mg/dl or more, was observed in associ-
TABLE 3. Associations of apolipoprotein alleles at the E locus with age-adjusted mean plasma glucose, glycated hemoglobin, pulse rate, and blood pressure in American Samoans

<table>
<thead>
<tr>
<th></th>
<th>APO E*3 Mean</th>
<th>APO E*2 (N = 6) Mean</th>
<th>APO E*4 (N = 21) Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 107</td>
<td>Diff 95% CI P</td>
<td>Diff 95% CI P</td>
</tr>
<tr>
<td>Glucose</td>
<td>154.0</td>
<td>-11.6 -83.2, 60.1 .75</td>
<td>147.3 -6.7 -43.9, 30.4 .72</td>
</tr>
<tr>
<td>% Glycated hemoglobin</td>
<td>10.3</td>
<td>-1.0 -4.2, 2.3 .56</td>
<td>8.6 -1.7 -3.5, 0.1 .07</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>79.3</td>
<td>5.2 -3.1, 13.4 .22</td>
<td>83.6 4.3 -0.2, 8.9 .06</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>136.4</td>
<td>-2.3 -18.2, 13.7 .78</td>
<td>127.6 -8.8 -17.9, 0.4 .06</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>86.2</td>
<td>4.7 -6.6, 15.9 .42</td>
<td>82.4 -3.9 -10.6, 2.8 .25</td>
</tr>
</tbody>
</table>

1Diff, differences between means; 95% CI, 95% confidence intervals; P, P values; BP, blood pressure.

TABLE 4. Associations of apolipoprotein alleles at the E locus with age-adjusted mean plasma glucose, glycated hemoglobin, pulse rate, and blood pressure in American Samoans

<table>
<thead>
<tr>
<th></th>
<th>APO H*2 Mean</th>
<th>APO H*1 (N = 18) Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 115</td>
<td>Diff 95% CI P</td>
</tr>
<tr>
<td>Glucose</td>
<td>154.4</td>
<td>22.1 -17.9, 62.1 .28</td>
</tr>
<tr>
<td>% Glycated hemoglobin</td>
<td>10.0</td>
<td>0.4 -1.6, 2.3 .74</td>
</tr>
<tr>
<td>Pulse rate</td>
<td>79.6</td>
<td>3.4 -1.5, 8.3 .17</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>134.8</td>
<td>1.2 -8.8, 9.2 .81</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>85.4</td>
<td>2.0 -5.2, 9.2 .58</td>
</tr>
</tbody>
</table>

1Diff, differences between means; 95% CI, 95% confidence intervals; P, P values; BP, blood pressure.

Blood pressure and pulse rate

As with glucose and glycated hemoglobin, no large sex differences were observed in blood pressure or pulse rate; hence, both sexes were combined for the analyses. At the APO E locus, the E*4 allele was associated with higher mean pulse rate, but somewhat lower systolic and diastolic blood pressures than the common E*3 allele (Table 3). The first two differences were of borderline statistical significance. At the APO H locus, mean pulse rate and systolic and diastolic blood pressures were higher in association with the H*1 allele than in those with the H*2 allele, but none of the differences approached standard levels of statistical significance (Table 4).

DISCUSSION

Unfortunately, only 67 blood samples from residents of American Samoa were available for this exploratory analysis of the association of apolipoprotein polymorphisms with cardiovascular risk factors—body habitus, glucose metabolism, pulse rate, and blood pressure. Thus, the data are of a preliminary nature and most useful for developing hypotheses for further testing. The sample was, however, representative of the total population with respect to specific background factors, such as education and area of residence. Some members of the sample were related, and this likely acted to reduce phenotypic variability in a number of the measures examined. However, although these considerations reduce the power of this study to detect small differences between groups that may be significant with a larger sample, it is likely that any statistically strong or physiologically large differences observed in this sample will be replicated in a larger study. That is, in a small sample, there is limited power (1-beta) to detect small differences between groups, and no firm conclusions may be made regarding the lack of a difference. However, when the observed difference is large and
statistically significant, the likelihood that it will remain statistically significant in a larger study is equal to \(1 - \alpha\).

A solution to this problem is to forego reporting of significance (\(P\) values) and instead to report only \(95\%\) CIs for all comparisons, drop all recourse to statistical significance tests, and concentrate on the possible physiological and epidemiological relevance of observed differences with regard to future health outcomes (see Rothman, 1990). The question then becomes whether variant alleles, for example, at the APO E locus, that are associated with differences from the wild type allele of, for example, \(+3.1\) kg/m\(^2\) (E*2) or of \(-3.7\) kg/m\(^2\) (E*4) of BMI are likely to alter the risk of morbidity and mortality from cardiovascular disease or diabetes. A previous study of mortality in American Samoans reported that differences in BMI between men who survived 6 years and those who died from cardiovascular diseases and diabetes equaled 1.8 kg/m\(^2\) (Crews, 1989) and 2.3 kg/m\(^2\), respectively (Crews, 1985). Differences between variant and wild type APO E alleles reported here are 1.5 to 2 times as large as those reported here are 1.5 to 2 times as large as differences between 6-year survivors and decedents in these earlier analyses. In a further analysis of diabetes mortality, estimated arm muscle circumference was 3.4 mm, systolic 23.1 mmHg, and diastolic blood pressure 5.9 mmHg higher in Samoans who died with diabetes as their underlying cause of death, while the triceps skinfold was 7.8 mm lower than in survivors. Differences associated with the APO E*4 allele for systolic and diastolic blood pressure, estimated arm muscle circumference, and the triceps skinfold are 38\% and 66\%, 53\%, and 45\% of these levels.

Based on these data, a hypothesis that apolipoprotein genetic variation is associated with variability in body habitus, glucose metabolism, and blood circulation in Samoan Americans is suggested for future testing. Furthermore, such future studies might usefully address the hypothesis that associations of apolipoproteins with cardiovascular risk factors differ by sex in Samoans. An additional suggested hypothesis is that the APO E*2 allele is associated with a more obese and APO E*4 with a thinner body habitus in Samoan men.

APO E is a constituent of triglyceride-rich lipoproteins and HDL (Fumeron et al., 1988) and is intimately involved in lipid catabolism (Mahley and Innerarity, 1983). Furthermore, the APO E 2-2 phenotype has been observed at a higher frequency in samples of patients with type III hyperlipidemia than has been observed in the general population (Utermann, 1987; Brewer et al., 1983; Eto et al., 1988a,b). In the general population, the less common E*4 allele is associated with lower very low density lipoprotein (VLDL)-associated lipids and higher low density lipoprotein (LDL)-cholesterol, contrary to the common E*3 allele which is associated with higher VLDL and lower LDL-cholesterol (Davignon et al., 1988; Utermann, 1987). Based upon its association with lipid metabolism, it has been suggested that APO E genetic variation may be linked to obesity (Eto et al., 1988a, 1991; Fumeron et al., 1988). However, data from neither Japanese nor French samples of obese individuals showed a significantly higher frequency of any allele at the APO E locus compared to normal weight persons (Eto et al., 1988a, 1991; Fumeron et al., 1988). In the French sample, the APO E*4 allele was associated with the risk of obesity-induced hypertriglyceridemia (Fumeron et al., 1988). Furthermore, several studies have shown convincingly that variation at apolipoprotein loci is a major determinant of differences in the plasma lipoprotein-lipid levels in the general population (Berg, 1989). There are also indications that genetic impact on lipoprotein-lipid levels may be influenced by socioeconomic and ethnic backgrounds (Utermann, 1987; Sepherina et al., 1989). It appears to be essential, therefore, to screen additional populations, with diverse ethnic backgrounds, for apolipoprotein genetic variations and their impact on lipoprotein-lipid levels and body habitus.

This preliminary study was undertaken to determine the extent to which genetic variation at two apolipoprotein loci, APO E and APO H, may influence phenotypic variability in cardiovascular risk factors in the Polynesian population of American Samoa. Samoans residing on Tutuila, the main island of American Samoa, show high levels of obesity (Crews, 1988) and fasting plasma triglycerides, men 156 mg/dl; women 136 mg/dl (Pelletier and Hornick, 1986), somewhat above those observed, for example, in U.S. samples. APO E*2 is associated with lower cholesterol and the APO E*4 allele with high cholesterol in several populations studied to.
date (Davignon et al., 1988). In Samoan men, the APO E*2 may be associated with a larger and the APO E*4 with a smaller body habitus, and the finding at variance with results from both Japanese and French samples and a hypothesis in need of additional research.

The tendency for percent glucose and glycated hemoglobin to be lower in Samoans with the APO E*4 and E*2 alleles suggests possible influences of apolipoprotein genetic variation on some aspects of glucose metabolism in this ethnic group, an association that is possibly secondary to an association of APO E variation with body habitus. The APO E*4 allele also may be associated with lower blood pressure and slightly lower pulse rate than the more common APO E*3 allele, two factors related to variability in the development of coronary heart disease (CHD). Hypothetically, such an association could also be mediated through a primary association with body habitus and lipid metabolism. The APO H locus in Samoans is characterized by a frequency of APO H*1 among the highest observed in any world population (Crews et al., 1991b). The highly frequent APO H*1 allele does not appear to be strongly related to variability in body habitus or blood pressure. However, the possible association of APO H*1 with higher plasma glucose needs further investigation, particularly given the high prevalence of NIDDM in several Polynesian populations and the elevated levels of fasting triglyceride frequently reported among persons with diabetes.

The apolipoprotein genetic data examined here are the first of their kind for any Polynesian population. In general, most such data are restricted to European populations with homogeneous gene frequencies (Kambho et al., 1990). The available cross-cultural data suggest not only that marked interethnic variation exists in gene frequencies, but also that the association of apolipoprotein alleles with body habitus and other risk factors for cardiovascular disease may vary across ethnic groups. The data from a small sample of Samoan Americans suggest that further study of apolipoprotein variation in this population may add to existing data indicating that apolipoprotein variation is not identically associated with morphological or metabolic variability in different ethnic groups. For example, the detection of several significant and borderline associations of apolipoprotein variants with body habitus, glucose metabolism, pulse rate, and blood pressure, all established risk factors for cardiovascular diseases, in this Samoan sample suggests that such variation may be associated with risk of CHD and NIDDM in Polynesians and other ethnic groups. Detailed future studies of apolipoprotein genetic variation in Pacific populations should be of potential significance in determining the manner in which these alleles are related to health and disease in these and other populations.

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LITERATURE CITED


