

Published in final edited form as:

Eur J Clin Nutr. 2013 January ; 67(1): 101–107. doi:10.1038/ejcn.2012.124.

***INSIG2* variants, dietary patterns, and metabolic risk in Samoa**

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Abstract

Background/Objectives—Association of insulin-induced gene 2 (*INSIG2*) variants with obesity has been confirmed in several but not all follow-up studies. Differences in environmental factors across populations may mask some genetic associations and therefore gene-environment interactions should be explored. We hypothesized that the association between dietary patterns and components of the metabolic syndrome could be modified by *INSIG2* variants.

Subjects/Methods—We conducted a longitudinal study of adiposity and cardiovascular disease risk among 427 and 290 adults from Samoa and American Samoa (1990–95). Principal component analysis on food items from a validated FFQ was used to identify neo-traditional and modern dietary patterns. We explored gene-dietary pattern interactions with the *INSIG2* variants rs9308762 and rs7566605.

Results—Results for American Samoans were mostly non-significant. In Samoa, the neo-traditional dietary pattern was associated with lower triglycerides, BMI, waist circumference, systolic and diastolic blood pressure, and fasting glucose (all p-for-trend<0.05). The modern pattern was significantly associated with higher triglycerides, BMI, waist circumference, and lower HDL cholesterol (all p-for-trend<0.05). A significant interaction for triglycerides was found between the modern pattern and the rs9308762 polymorphism (p=0.04). Those from Samoa consuming the modern pattern have higher triglycerides if they are homozygous for the rs9308762 C allele.

Conclusions—The common *INSIG2* rs9308762 variant was associated with poorer metabolic control and a greater sensitivity of triglycerides to a modern dietary pattern. Environmental factors need to be taken into account when assessing genetic associations across and within populations.

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Conflict of interest: None

Keywords

INSIG2; dietary patterns; gene-diet interactions; metabolic risk; Samoa

Introduction

Insig2 is an endoplasmic reticulum protein that blocks the processing of sterol regulatory element-binding proteins (SREBPs) (1, 2). Dysregulation of SREBP-1c affects lipid metabolism and may lead to insulin resistance and obesity (3). Results from genome-wide association studies (GWAS) have shown that a variant in the *INSIG2* gene (rs7566605) on chromosome 2q14.2 is associated with obesity (4). However, this association has only been confirmed in several (5–11) but not all replication studies conducted in other populations (12–31), including our prior report in Samoans. The lack of replication may be due to variation in linkage disequilibrium among different populations if the variant is not causal (32). On the other hand, most obesity genes are unlikely to cause obesity in the absence of an obesogenic environment (33). Therefore, it is possible that differences in environmental factors across and within populations may mask some genetic associations. In particular, some genetic associations may not be evident in the absence of obesogenic dietary patterns. Therefore, it is important to study these associations in populations that still retain traditional dietary patterns.

The population of the Samoan Islands is characterized by high prevalence of obesity, metabolic syndrome, and diabetes (34–38). Rates of obesity have been increasing dramatically in the last decades as the population experienced rapid modernization (39). As a consequence of this rapid modernization, Samoans have undergone a nutrition transition, largely abandoning their neo-traditional diets and acquiring a modern dietary pattern and sedentary lifestyle (39, 40). In a study conducted in 2002–03 among 723 American Samoans and 785 Samoans, we have shown previously that a modern dietary pattern was significantly associated with metabolic syndrome in Samoa and with increased triglyceride levels in both polities (41). In addition, a neo-traditional dietary pattern was associated with higher HDL-cholesterol in American Samoa and lower waist circumference in both polities (41). In another study conducted in the Samoan islands from 1990 to 1995 with the study sample used here, we have shown also that a variant in the *INSIG2* gene (rs9308762) was associated with BMI and waist circumference in the combined Samoa and American Samoa sample and with BMI in the sample from Samoa. However, rs7566605 was not associated with BMI or waist circumference in the combined sample nor in either polity (32).

The goal of this study was to use principal component analysis to derive dietary patterns in the Samoan Islands population and evaluate their association with metabolic health in order to reproduce the associations previously seen in the 2002–03 population, and assess for the first time if the *INSIG2* variants, rs9308762 and rs7566605, modify these associations. Out of the 4 *INSIG2* variants that were previously genotyped in this study population, we chose to focus upon rs9308762 due to our earlier study (32) which showed association with BMI and waist circumference, and on rs7566605 due to the many prior studies of this single nucleotide polymorphism (SNP) in other populations (5–31).

Materials and Methods

Study Population

The study population consists of adults 25 to 55 years old with all four grandparents of Samoan ancestry. A detailed description of this longitudinal study has been reported previously (42, 43). Briefly, participants were recruited from 46 villages and worksites in

American Samoa in 1990 and nine villages in (then Western) Samoa in 1991, and followed up four years later in 1994 and 1995, respectively. All participants were free of self-reported history of heart disease, hypertension, or diabetes at baseline. At baseline, 563 and 694 individuals were recruited from American Samoa in 1990, and 1991 in Samoa, respectively. In 1994 and 1995 when outcome data were collected, 361 and 560 individuals were available for the four-year follow-up in American Samoa and Samoa, respectively.

Informed consent was obtained from all participants. All study protocols were approved by the human subjects committees of the Miriam Hospital of Providence, RI, The Department of Health, American Samoa and the Ministry of Health, Samoa.

Data collection

Dietary intake was measured at baseline with a validated 42-item food frequency questionnaire (FFQ) specifically designed for this population (41). Information on socio-demographic and lifestyle variables was obtained through a questionnaire, including number of hours farming (used as a proxy of physical activity (34)), education, and occupation. The outcome variables at the four-year follow-up included BMI, waist, leptin, triglycerides, HDL-cholesterol, LDL-cholesterol, fasting glucose, insulin, systolic blood pressure, and diastolic blood pressure. Anthropometric measures were taken with participants wearing light tropical clothing without shoes. Height was measured using a portable GPM anthropometer (Pfister Imports, New York, NY) and they were weighed with a calibrated spring-balance scale. Waist circumference was measured twice at the umbilicus level with a metal tape and the average of the two measurements was used in the analysis. Blood pressure was measured three times using a mercury sphygmomanometer with appropriately sized upper arm cuffs after the participants were seated and resting for 5 minutes. The average of the three measurements was used in the analysis. Blood was collected in the morning after a minimum of 10 hours fasting. Serum glucose was analyzed with a Berkman CX4 automatic analyzer. Total cholesterol and triglycerides were measured by enzymatic assays on a Gilford Impact 400 computer directed analyzer. HDL cholesterol was measured after precipitation of VLDL and LDL with heparin-Mn²⁺ reagent. Leptin was measured by radioimmunoassay (RIA) using a kit from ALPCO (Windham, NH, USA). Insulin was measured using standard RIA kits from Diagnostic Products Inc.

Genotyping

Genotyping data from four tagging SNPs spanning a region of 28kb in *INSIG2* including rs7566605 were already available for this study population (32). Details about the genotyping can be found in Deka et al. (32).

Of the four previously genotyped and analyzed SNPs (rs756605, rs1352083, rs2161829, rs9308762), only rs9308762, located in intron 3 of *INSIG2*, was associated with BMI and waist circumference in the Samoan study population (32). Therefore, we focused our main analysis of gene-dietary pattern interactions on this SNP. However, we also explored potential gene-dietary pattern interactions with rs7566605, i.e., the SNP found to be associated to obesity in GWAS of other populations, under the assumption that differences in environmental exposures may mask genetic associations. r^2 and D' for rs9308762 and rs7566605 were 0.37 and 0.95 respectively, as reported previously (32). Genotype frequencies were in Hardy-Weinberg equilibrium.

Data Analysis

There are no genetic differences between Samoans and American Samoans and genetically they can be considered a unique homogenous single population without evidence of population substructure as shown by our previous studies (32, 44, 45). However, there are

substantial economic disparities between the two countries. Because these economic disparities are reflected in different stages of the nutrition transition, we decided to explore our main aims stratifying by country.

In order to maximize power, only participants with missing information in age, sex, or the FFQ, or pregnant women were deleted from the analysis. The final sample size at baseline was 427 for Samoa and 290 for American Samoa (Table 1). However, due to missing values in some of the four-year outcome variables, potential confounders, and genotype information, the sample size varied from 290 to 390 in Samoa, and from 200 to 278 in American Samoa, in the multivariable regression models. Participants with missing values in the outcome variables or in the FFQ did not differ in socio-demographic baseline variables except for education in Samoa. They reported more education (0.6 y more on average).

We used principal component analysis (PCA) to derive dietary patterns. The factors obtained were rotated by an orthogonal transformation to achieve a simpler structure that assists interpretability. We considered eigenvalues >1 , the Scree test, and the general interpretability of the factors to determine the number of factors to retain (46). The standardized frequencies of intake for each food group were multiplied by the factor score coefficients and the sum of these products was the score for each derived factor. Adjusted least square means by quintiles of dietary pattern were estimated from linear regression models for each outcome variable. We used robust estimates of the variance (47) to overcome potential deviations from normality in the error distributions for linear regression models. To test for trends across quintiles of dietary patterns, the median intake of each quintile was assigned to each subject in the same quintile and treated as a continuous variable in regression analyses. These models were adjusted for age, sex, years of education, and number of hours farming at baseline. Adjustment for other potential confounders, like type of job, did not change the results. Age- and sex-adjusted least square means by genotype were estimated from linear regression models for each outcome variable and p-values from genetic additive models were reported. Analyzing the data under a co-dominant model did not change the significance of the models and conclusions. Genotypes were coded as follows: CC=0, CT=1, TT=2 for rs9308762, and GG=0, CG=1, CC=2 for rs756660. Standard regression diagnostics, such as Cook's d, leverage, and change in beta estimates were used to evaluate potential influential points. Points that were considered influential based on conventional cutoffs for the mentioned diagnostics were removed. Effect modification by genotype was assessed using an F test for the interaction term. P-values of 0.05 were considered significant for the metabolic-diet associations and we used the false discovery rate method to adjust the p-values of the interaction analysis. All analyses were conducted using Statistical Analyses System software version 9.2 (SAS Institute, Inc., Cary, NC).

Results

Descriptive characteristics of the population at baseline stratified by sex and country are shown in Table 1. Subjects from Samoa had lower waist circumference, BMI, systolic and diastolic blood pressure, fasting blood glucose, insulin, and leptin compared to American Samoans. They also reported higher number of hours of farm work and less years of education. There were not major differences in the frequency of the rs9308762 and rs7566605 genotypes by sex and polity.

Using principal component analysis, three factors were retained in each polity (Supplementary Table 1). Based on the loadings on neo-traditional or modern foods, factors retained in Samoa were named as “modern”, “neo-traditional 1”, and “neo-traditional 2”. Factors retained in American Samoa were named as “modern”, “transitional”, and “neo-

traditional”, respectively. The three factors retained in each polity explained 32% and 29% of the variability in diet in Samoa and American Samoa respectively. The neo-traditional dietary patterns were characterized by high intake of local foods, including coconut products, taro, tropical fruits and low intake of processed foods. The modern patterns were characterized by high intake of processed foods, non-traditional foods like milk, cheese, and eggs, and low intake of local foods. The transitional pattern in American Samoa is characterized by both local and non-local foods (Supplementary Table 1).

Adherence to the modern pattern in Samoa was consistently associated with a worse metabolic profile, including higher BMI, larger waist, higher leptin, higher triglycerides, lower HDL-cholesterol, higher fasting glucose, and higher insulin (Table 2). Absolute differences between extreme quintiles of adherence to the modern pattern were 3 Kg/m² for BMI, 6 cm for waist, 4.1 ng/mL for leptin, 43 mg/dL for triglycerides, 3 mg/dL for HDL cholesterol, 6 mg/dL for fasting glucose, and 5 mU/mL for insulin. On the other hand, adherence to the neo-traditional 2 pattern was consistently associated with a healthier metabolic profile, including lower BMI, lower waist, lower leptin, lower triglycerides, higher HDL-cholesterol, lower insulin, and lower systolic and diastolic blood pressure (Table 2). Absolute differences between extreme quintiles of adherence to the traditional 2 pattern were 2.1 Kg/m² for BMI, 5 cm for waist, 2.8 ng/mL for leptin, 22 mg/dL for triglycerides, 4 mg/dL for HDL cholesterol, 3.9 mU/mL for insulin, 7 mmHg for systolic blood pressure, and 5 mmHg for diastolic blood pressure. The neo-traditional 1 pattern was only associated with lower HDL-cholesterol with 6 mg/dL difference between extreme quintiles (Table 2). None of the dietary patterns were associated with LDL-cholesterol.

In American Samoa, none of the dietary patterns were significantly associated with any of the outcome variables analyzed (data not shown), except for the transitional pattern that was associated with glucose, insulin, and systolic blood pressure. Higher adherence to the transitional pattern was associated with higher glucose, higher insulin and higher systolic blood pressure (all p value <0.05).

The rs9308762 C allele was associated with higher BMI, and higher waist circumference, as reported previously, and higher leptin, and higher triglycerides, among people from Samoa. Absolute differences comparing homozygous for the variant with homozygous for the wild type were 2.9 Kg/m² for BMI, 6 cm for waist, 4 ng/mL for leptin, and 23 mg/dL for triglycerides. Interestingly, there were no significant associations between the rs9308762 genotype and metabolic variables among American Samoans (Table 3). Genotypes at rs7566605 were not associated with metabolic traits in this population as reported previously (data not shown).

We next explored interactions between dietary patterns and the rs9308762 genotype among participants from Samoa for BMI, waist circumference, and triglycerides (i.e., for those outcomes that had significant main effects). A significant interaction was detected for triglycerides and the modern pattern (Figure 1). The association between triglycerides and the modern pattern was most pronounced among people homozygous for the C allele and almost nonexistent among those homozygous for the T allele (p value for interaction = 0.04 after adjusting for multiple comparisons).

Finally, we explored if similar interactions with triglycerides could be found among those from Samoa for rs7566605, i.e., the SNP found to be associated with obesity in previous GWAS, even though rs7566605 was not associated as a main effect with metabolic traits in this population. We saw a potential interaction between rs7566605 and the neo-traditional 2 pattern on triglyceride concentrations that did not reach statistical significance after adjusting for multiple comparisons (data not shown).

Discussion

Out of the three dietary patterns identified in Samoa in 1991, the modern pattern was associated with a poorer metabolic profile four-years later while the neo-traditional 2 pattern was associated with a better metabolic profile. The rs9308762 C allele was associated with higher BMI, higher waist circumference, higher leptin, and higher triglycerides, among people from Samoa. Samoans consuming the modern pattern have higher triglycerides if they are homozygous for the rs9308762 C allele. Results from American Samoans were mostly non-significant.

Our results on dietary patterns and metabolic syndrome components are consistent with our previous published work of an independent study conducted in the Samoan Islands in 2002–2003 (41). In that study we used a different method to extract dietary patterns (partial least square regression) but findings were similar. The modern dietary pattern was significantly associated with metabolic syndrome in Samoa and with increased triglyceride concentrations in both polities. In addition, a neo-traditional dietary pattern was associated with higher HDL-cholesterol in American Samoa and lower waist circumference in both polities (41). Our results are reassuring that the previously seen associations are not due to chance but to a potential real biological mechanism.

As mentioned before, *INSIG2* is involved in the regulation of SREBPs that play an important role in lipid metabolism. Through SREBPs inhibition, upregulation of *insig2* decreases fatty acid and triglyceride synthesis (48). Consequently, the *INSIG2* polymorphism rs9308762 has been associated with adiposity measures in the Samoan population and in a population of 1,495 Hispanics (11). Although most studies have focused on the association of *INSIG2* variants with adiposity measures, others have also explored associations with related metabolic traits such as plasma lipids and glucose homeostasis (11, 17, 19, 21, 26, 28–30). Results from these studies are not consistent either. For example, similar to our findings, Wang et al. found the *INSIG2* variant rs7566605 to be associated with triglycerides among obese Chinese children (23). However, this association was not confirmed in other studies (26, 28, 29). Lack of replication may be due to gene-gene or gene-environment interactions. For example, the association between the *INSIG1* variant rs2721 and triglycerides was strengthened by the *INSIG2* rs7566605 variant in a U.S. family study of 1,560 Northern European-ancestry individuals (49). Likewise, our findings showing an interaction between dietary patterns and the *INSIG2* variant rs9308762 on triglyceride concentrations serve as a potential explanation of why results may not be homogenous across populations. It is noteworthy that we found a potential interaction, although not significant after correcting for multiple testing, between the neo-traditional 2 pattern and the rs7566605 variant on triglyceride concentrations among subjects from Samoa, regardless of not finding a significant genetic main effect for rs7566605. Therefore, our results offer an alternative explanation for the lack of consistent association across populations. Interactions between the *INSIG2* variant rs7566605 and other environmental factors have been reported in previous studies. In a population-based study of 6,514 Danish subjects, BMI was higher for rs7566605 C-allele carriers compared with G-allele carriers among physically inactive people (20). However, this interaction was not replicated in another study carried out in 2,003 European children (22).

Our mostly non-significant results in the American Samoa population offer some interesting insights. The lack of significant results could be attributed to lack of power, since the sample size in American Samoa was much lower. However, there were not even noticeable non-significant trends in our results. It is also possible that dietary patterns are not as clearly defined as in the Samoa study sample. On the other hand, the genetic results were also non-significant among American Samoans. Compared to most populations in the world,

overweight and obesity prevalence is much higher among people from the Samoan Islands (34, 50, 51), but there are still important differences between the two polities (Table 1). The American Samoa population has even a higher prevalence of obesity than the Samoa population. Therefore, it is possible that when BMI reaches very high values at the population level, and if there are not enough thin people as a reference, it becomes hard to find associations with environmental or even genetic factors. Since American Samoans and Samoans come from the same genetic pool, our study became a natural experiment that allowed us to evaluate the impact of dietary pattern variation on genetic associations. Our results emphasize that comparison of genetic associations across populations need to take into account environmental heterogeneity. The lack of main effects in American Samoa between dietary patterns and metabolic and cardiovascular phenotypes, and between allelic variants and those phenotypes precluded our exploration of possible gene by dietary pattern interactions.

Strengths of our study include its longitudinal design, the variability of dietary exposures in the population, and the use of dietary patterns that better capture the diet as a whole. Our study has some limitations. First, we had a relatively small sample size. Low power may have been responsible of lack of significant associations among American Samoans, and reaching significance for some of the potential interactions. Second, we excluded people with missing values, which may result in bias. Third, although we used number of hours farming to adjust for physical activity, we cannot totally rule out residual confounding. However, we have found an inverse association between participation in farming and adult BMI and percent body fat in both Samoa and American Samoa in another independent study (34). Furthermore, it could be argued that rather than confounding for physical activity, our dietary patterns may also reflect lifestyle patterns that include physical activity as one more component. As expected, number of hours farming was positively correlated with the neo-traditional and transitional patterns and negatively correlated with the modern pattern in both Samoa and American Samoa. Finally, we acknowledge that our results need replication before reaching any definitive conclusion.

For some of the studied metabolic outcomes, we found important differences between quintiles of adherence to the modern and neo-traditional 2 patterns and between genotypes. For example, differences in BMI between extreme quintiles of the modern and neotraditional 2 pattern translate into an increase in 9% and a decrease in 12% of the obesity prevalence, respectively. Similarly, differences in triglycerides between extreme quintiles of the modern and neotraditional 2 pattern translate into an increase in 17% and a decrease in 8% of the prevalence of high triglycerides, respectively. These differences in metabolic outcomes clearly have important public health implications and highlights that departure from traditional lifestyles is affecting the cardiometabolic risk of Samoans. Also, based on our interaction analysis, genetic background can increase or attenuate the effect of environmental factors. Although our interaction results need to be replicated in other populations and we are still far from performing genetic testing at the population level, it will be important to keep this heterogeneity in mind when considering general public health interventions.

In conclusion, we found that the modern dietary pattern in Samoa was associated with a poorer metabolic profile compared to the neo-traditional pattern. Homozygotes for the rs9308762 C allele were more susceptible to the hypertriglyceridemic effect of the modern pattern. We did not see any significant association between dietary patterns and SNPs with metabolic factors among American Samoans. Our findings present an interesting example of the complex interplay between genes and the environment on the risk of metabolic traits and suggest that results from GWAS may be attenuated or masked if gene-environment interactions are not taken into account.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported by NIH grants AG09375, HL52611, DK55406, and DK59642 and from the Dean's fund of the University of Cincinnati College of Medicine

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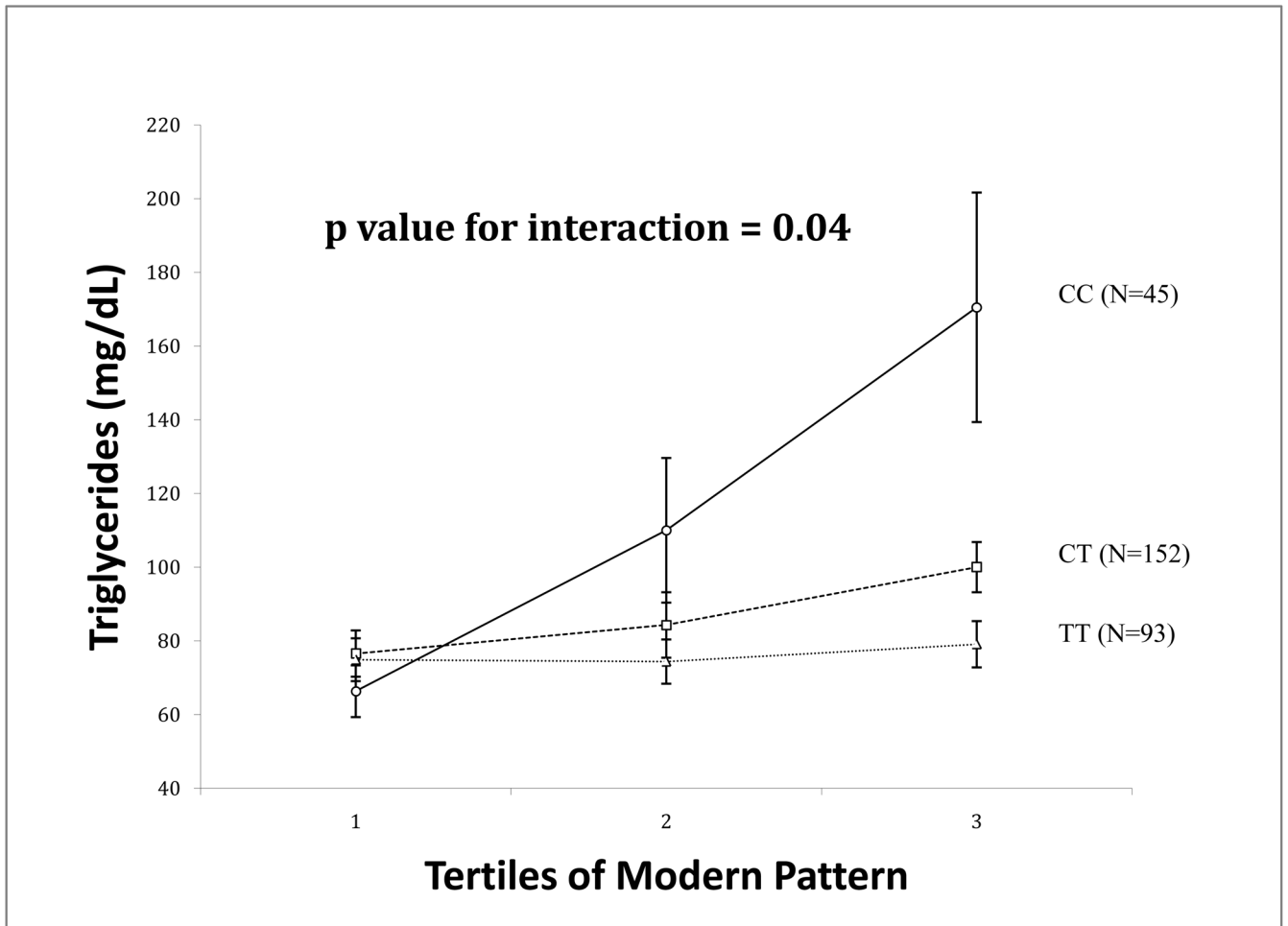


Figure 1. Association between triglycerides and tertiles of the modern dietary pattern by rs9308762 genotype among participants from Samoa (N=293). Triangles, squares, and circles are least square means adjusted by age, sex, and hours of farming, and bars are standard errors. CC are represented by circles, CT by squares, and TT by triangles.

Table 1

Characteristics of the study population at baseline (1990–1991) by polity and sex¹

	Samoa ²		American Samoa ³	
	Women (N=216)	Men (N=211)	Women (N=175)	Men (N=115)
Age (y)	38.5 (8.3)	39.3 (8.8)	39.3 (9.8)	40.3 (10.3)
Farm work (h/w)	4.9 (6.3)	13.5 (11.3)	2.5 (4.2)	5.9 (8.7)
Education (y)	10.1 (2.2)	9.1 (2.5)	12.6 (2.3)	12.8 (3)
Waist circumference (cm)	97.8 (12.7)	93.0 (13.0)	109.3 (15.9)	106.7 (14.5)
BMI (Kg/m ²)	31.1 (5.1)	29.2 (5.0)	35.9 (7.1)	33.9 (6.0)
Systolic blood pressure (mmHg)	120.1 (13.2)	124.6 (13.1)	124.1 (17.2)	125.2 (13.3)
Diastolic blood pressure (mmHg)	76.1 (9.6)	80.3 (11.2)	80.9 (10.9)	84.1 (10.9)
Fasting blood glucose (mg/dL)	86.3 (14.6)	87.9 (20.0)	96.6 (17.4)	101.7 (29.9)
Insulin (μU/mL)	12.5 (10.7)	10.7 (9.0)	22.3 (20.2)	23.7 (32.5)
Leptin (ng/mL)	17 (9.3)	4.3 (3.7)	25 (11.9)	10.3 (23.1)
rs9308762 genotype frequencies (%)				
CC	16	16	20	16
CT	52	51	47	50
TT	32	33	33	35
rs9308762 C allele frequency (%)	42	41	44	41
rs7566605 genotype frequencies (%)				
CC	15	10	19	13
CG	41	47	44	43
GG	44	43	37	44
rs7566605 C allele frequency (%)	35	34	41	35

¹Values are means (SD) for continuous variables and percentages for categorical values

²Missing values for farm work (20), education (15), waist circumference (11), BMI (7), systolic blood pressure (6), diastolic blood pressure (6), fasting blood glucose (4), insulin (6), leptin (47), rs9308762 genotype (87), and rs7566605 genotype (35)

³Missing values for farm work (11), education (24), waist circumference (10), BMI (9), systolic blood pressure (8), diastolic blood pressure (8), fasting blood glucose (3), insulin (4), leptin (76), rs9308762 genotype (9), and rs7566605 genotype (75)

Table 2

Adjusted least square means for metabolic parameters in Samoa at follow-up (1995) by quintiles of dietary patterns^{1,2}

	N	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p for trend
BMI (Kg/m²)							
Modern	372	28.5 (27.6, 29.5)	30.4 (29.2, 31.6)	29.7 (28.5, 30.9)	31.3 (30.2, 32.5)	31.5 (30.1, 33)	0.001
Neotraditional 1	372	28.9 (27.8, 30)	30 (28.6, 31.4)	30.6 (29.3, 31.8)	31.5 (30.4, 32.7)	30.2 (29.1, 31.3)	0.25
Neotraditional 2	372	31.2 (30, 32.3)	30.9 (30, 31.8)	30.5 (29.1, 31.9)	29.8 (28.5, 31)	29.1 (27.8, 30.3)	0.006
Waist (cm)							
Modern	377	91 (89, 94)	96 (93, 99)	94 (91, 97)	99 (96, 102)	97 (94, 100)	0.006
Neotraditional 1	377	91 (88, 94)	96 (93, 100)	96 (93, 99)	99 (96, 102)	95 (93, 98)	0.20
Neotraditional 2	377	98 (95, 101)	97 (95, 100)	95 (92, 99)	94 (91, 97)	93 (90, 96)	0.005
Lepin (ng/mL)							
Modern	354	8.4 (7.2, 9.7)	10 (8.3, 11.8)	10.3 (8.7, 12)	12.9 (11, 14.8)	12.5 (10.5, 14.5)	0.0005
Neotraditional 1	354	8.8 (7.2, 10.5)	10.1 (8.3, 11.9)	10.8 (9.4, 12.3)	13.1 (11.3, 14.9)	10.6 (8.9, 12.4)	0.18
Neotraditional 2	354	11.7 (10.1, 13.3)	11 (9.6, 12.4)	11.1 (9, 13.1)	11.2 (9.4, 13)	8.9 (7.2, 10.6)	0.03
Triglycerides (mg/dL)³							
Modern	363	72 (63, 81)	76 (67, 85)	78 (67, 90)	93 (80, 106)	115 (96, 134)	<0.0001
Neotraditional 1	363	89 (75, 104)	74 (65, 83)	79 (69, 89)	99 (82, 117)	87 (75, 100)	0.36
Neotraditional 2	363	94 (83, 105)	98 (79, 118)	93 (80, 105)	74 (66, 83)	72 (63, 81)	0.0002
HDL (mg/dL)							
Modern	366	44 (42, 47)	44 (42, 46)	45 (42, 47)	42 (39, 44)	41 (38, 44)	0.045
Neotraditional 1	366	46 (43, 49)	43 (41, 46)	44 (41, 46)	43 (40, 46)	40 (38, 42)	0.001
Neotraditional 2	366	41 (39, 43)	43 (40, 46)	44 (41, 46)	44 (41, 46)	45 (42, 47)	0.045
LDL (mg/dL)							
Modern	366	130 (125, 135)	138 (132, 145)	136 (130, 142)	144 (136, 151)	137 (128, 146)	0.23
Neotraditional 1	366	138 (131, 145)	139 (131, 146)	134 (127, 140)	137 (130, 144)	137 (130, 144)	0.96
Neotraditional 2	366	137 (131, 143)	136 (129, 143)	144 (136, 151)	132 (126, 139)	135 (129, 142)	0.45
Fasting glucose (mg/dL)³							
Modern	376	86 (83, 90)	85 (82, 88)	85 (82, 88)	89 (82, 95)	92 (86, 98)	0.04
Neotraditional 1	376	89 (84, 93)	86 (82, 90)	87 (83, 91)	86 (81, 92)	88 (84, 92)	0.97
Neotraditional 2	376	90 (85, 95)	84 (81, 87)	87 (83, 91)	86 (82, 90)	89 (84, 94)	0.95

	N	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	p for trend
Insulin (mU/mL)³							
Modern	373	8 (5.8, 10.1)	7.9 (6.5, 9.3)	10.2 (7.7, 12.6)	10.6 (8.1, 13)	13 (9.4, 16.5)	0.007
Neotraditional 1	373	9.2 (7.2, 11.3)	8.4 (5.8, 11)	9.4 (7, 11.8)	12.1 (9.3, 15)	9.5 (7.2, 11.8)	0.53
Neotraditional 2	373	10.9 (8.4, 13.4)	12.1 (8.9, 15.2)	10.1 (7.5, 12.8)	9.1 (7, 11.2)	7 (5.6, 8.4)	0.002
Systolic blood pressure (mmHg)							
Modern	390	120 (117, 124)	123 (120, 126)	123 (119, 126)	124 (121, 127)	123 (120, 126)	0.24
Neotraditional 1	390	122 (118, 125)	123 (119, 126)	123 (120, 126)	125 (122, 128)	122 (118, 125)	0.94
Neotraditional 2	390	128 (124, 131)	125 (122, 128)	121 (118, 123)	119 (116, 122)	121 (118, 124)	0.0004
Diastolic blood pressure (mmHg)							
Modern	390	77 (74, 79)	78 (76, 80)	78 (76, 81)	77 (75, 79)	79 (76, 81)	0.45
Neotraditional 1	390	77 (75, 80)	78 (75, 80)	77 (74, 79)	80 (78, 82)	77 (75, 80)	0.8
Neotraditional 2	390	80 (77, 83)	80 (78, 82)	77 (75, 79)	77 (75, 80)	75 (73, 77)	0.006

¹ Values are adjusted least square means and 95% confidence intervals in brackets from linear regression models with robust variances.

² Models are adjusted for age, sex, years of education, and number of hours farming.

³ Influential points removed in models for triglycerides (3), fasting glucose (2), and insulin (1).

Table 3

Adjusted least square means for metabolic parameters in Samoa and American Samoa at follow-up (1994–5) by rs9308762 genotype ^{1,2}

	rs9308762 genotype				p value from additive model
	N	CC	CT	TT	
BMI (Kg/m²)					
Samoa	317	32.3 (30.5, 34.1)	30.1 (29.4, 30.9)	29.4 (28.3, 30.4)	0.009
American Samoa	261	35 (32.6, 37.3)	35.8 (34.7, 36.9)	35.8 (34.6, 36.9)	0.61
Waist (cm)					
Samoa	321	100 (96, 104)	95 (93, 97)	94 (91, 96)	0.02
American Samoa	271	111 (106, 116)	111 (109, 114)	111 (109, 114)	0.92
Leptin (ng/mL)					
Samoa	301	13.6 (11.5, 15.7)	10.3 (9.2, 11.4)	9.6 (8.4, 10.9)	0.004
American Samoa	212	17.7 (14.6, 20.7)	19.4 (17.3, 21.5)	17.7 (16.1, 19.3)	0.75
Triglycerides (mg/dL)³					
Samoa	315	100 (83, 118)	89 (80, 98)	77 (71, 84)	0.007
American Samoa	232	138 (118, 159)	129 (112, 147)	125 (112, 137)	0.27
HDL (mg/dL)					
Samoa	315	40 (38, 43)	44 (42, 45)	44 (41, 46)	0.14
American Samoa	224	34 (31, 36)	35 (34, 37)	34 (32, 35)	0.77
LDL (mg/dL)					
Samoa	315	135 (126, 144)	137 (133, 142)	131 (126, 137)	0.29
American Samoa	224	137 (126, 148)	135 (128, 142)	143 (136, 149)	0.24
Fasting glucose (mg/dL)³					
Samoa	324	85 (81, 89)	88 (85, 91)	85 (82, 87)	0.58
American Samoa	235	104 (92, 116)	103 (96, 110)	100 (93, 107)	0.52
Insulin (mIU/mL)³					
Samoa	323	12.1 (8.5, 15.7)	9.3 (7.6, 11)	9.3 (7.7, 11)	0.22
American Samoa	234	20.7 (14.6, 26.8)	19.3 (15.7, 22.9)	17.7 (15, 20.4)	0.33
Systolic blood pressure (mmHg)					
Samoa	338	124 (121, 127)	123 (120, 125)	122 (119, 124)	0.28
American Samoa	278	131 (127, 136)	130 (127, 133)	131 (129, 134)	0.87

		rs9308762 genotype			p value from additive model	
		N	CC	CT		TT
Diastolic blood pressure (mmHg)						
Samoa		338	81 (78, 83)	77 (75, 78)	77 (75, 79)	0.07
American Samoa		278	86 (83, 90)	85 (83, 87)	85 (83, 88)	0.65

¹Values are adjusted least square means and 95% confidence intervals in brackets from linear regression models with robust variances. P values correspond to additive models

²Models are adjusted for age and sex.

³Influential points removed in models for leptin (1 in American Samoa), triglycerides (3 in Samoa), fasting glucose (1 in Samoa), and insulin (1 in Samoa and 1 in American Samoa)