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Original Contribution

Dairy, Magnesium, and Calcium Intake in Relation to Insulin Sensitivity: Approaches to Modeling a Dose-dependent Association

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Dairy intake has been inversely associated with insulin resistance, which may be partly due to the specific effects of calcium and magnesium. Data from the Insulin Resistance Atherosclerosis Study (1992–1999) for 1,036 US adults without diabetes at baseline were examined to evaluate the cross-sectional association of habitual dairy, magnesium, and calcium intake with insulin sensitivity at baseline and after 5 years of follow-up. Insulin sensitivity was directly measured with a validated, 12-sample, insulin-enhanced, intravenous glucose tolerance test with minimal model analysis. Dietary intake was assessed by a validated food frequency interview, and dietary supplement dose was confirmed by reviewing the supplement label. Several statistical approaches were used to ensure appropriate modeling of the dose-dependent association. No association was found between dairy intake and insulin sensitivity ($p = 0.41$); however, associations were positive for magnesium and calcium intake ($p = 0.016$) after adjusting for demographic, nondietary lifestyle and dietary factors, and food groups. Furthermore, magnesium intake was associated with insulin sensitivity in a threshold fashion, with a Bayesian method–estimated threshold (325 mg) ($\beta = 0.0607$/100 mg, $p = 0.0008$ for $<$325 mg of magnesium/day; and $\beta = -0.001$/100 mg, $p = 0.82$ for $\geq$325 mg of magnesium/day). This study suggests that magnesium and calcium intake specifically, but not dairy intake, is associated with insulin sensitivity.

calcium; dairy products; insulin resistance; magnesium

Abbreviations: AIC, Akaike's Information Criterion; BIC, Schwarz's Bayesian Information Criterion; IRAS, Insulin Resistance Atherosclerosis Study; S_I, insulin sensitivity index.

Dairy intake has been inversely associated with insulin resistance (1, 2) and type 2 diabetes (3). This finding may be partly due to the associations of some single nutrients in dairy products, such as magnesium and calcium, with insulin sensitivity. Magnesium has been postulated to play a role in glucose homeostasis and insulin action (4, 5). Several cross-sectional studies have observed an inverse association of plasma or erythrocyte magnesium with fasting insulin or the risk of type 2 diabetes (6–8). These findings indicate a potential role of magnesium status in the pathogenesis of type 2 diabetes. Magnesium intake is believed to be important in maintaining magnesium homeostasis (4). An inverse association between magnesium intake and the risk of type 2 diabetes has been found in most (9–13), but not all (14), prospective studies. Calcium has been shown to be associated with hypertension and obesity, components of the insulin resistance syndrome (15–18). One clinical trial found that calcium supplementation reduced fasting plasma insulin and increased insulin sensitivity in nondiabetic, essential hypertensive patients (19). However, there

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has been limited population-based research on the relation of dairy, magnesium, and calcium intake with insulin sensitivity, especially with regard to a dose-dependent relation.

It is important to examine the dose-dependent association for magnesium and calcium to find the optimal intake with respect to health-related outcomes, because excessive intake of magnesium and calcium could cause adverse effects such as decreased absorption of other minerals (20, 21). In epidemiologic research, one of the common approaches to evaluating the association is to classify the continuous exposure variable into several categories by percentiles and then to compare the outcome values among these categories. This approach could be used to assess the association without any strong assumptions, but it fails to take advantage of the continuous nature of data. A linear relation is frequently assumed in the dose-dependent association for continuous variables. Instead of being strictly linear, the dose-dependent association could be exhibited in a threshold fashion, which means that the strength of the linear relation would change when the exposure value attains the threshold point.

A threshold estimated from experience or percentiles is commonly used in the threshold relation. This approach might not be appropriate because an inaccurate threshold estimate could potentially underestimate or overestimate the underlying relation (22). The maximum likelihood method (23–25) and Bayesian method (26–28) have been developed to improve estimation of the threshold by fully utilizing all the information from the data, but wider use of these methods has been impeded by the difficulties in analytical and numeric analysis. Recently, the Markov Chain Monte Carlo method, an easy-to-apply sampling method to simulate distributions for unknown parameters by using mathematical methods, has been applied in the Bayesian approach to estimate the threshold (29–31). The conceptual simplicity of the Markov Chain Monte Carlo methods proves an attractive alternative to the analytical and numeric sophistication required by other methods in the Bayesian approach.

Methods for evaluating dose-dependent associations, especially for estimating the data-driven change point in the threshold effect, are rarely reported in epidemiologic research. Therefore, this study examined the cross-sectional association of habitual dairy, magnesium, and calcium intake with insulin sensitivity by using multiple statistical approaches to ensure appropriate modeling of the dose-dependent association. We used a Bayesian approach with the Markov Chain Monte Carlo method to objectively estimate the threshold. In addition, we examined possible mediation by magnesium and calcium of an association between dairy intake and insulin sensitivity, should such an association be detected.

MATERIALS AND METHODS

Study design

The multicenter Insulin Resistance Atherosclerosis Study (IRAS) explored the cross-sectional associations among insulin resistance and its risk factors in a large, multiethnic population. A baseline examination was conducted from October 1992 to April 1994. A total of 1,624 adults aged 40–69 years were recruited from four clinical centers in San Antonio, Texas; San Luis Valley, Colorado; Los Angeles, California; and Oakland, California. By design, sufficient numbers of individuals in different age, sex, ethnic, and glucose tolerance groups were included to enable efficient study of relations between and among these groups. The 5-year follow-up examination of the IRAS cohort began in February 1998 and was completed in July 1999. The response rate for the follow-up examination was 81 percent. More details of the IRAS study design have been reported previously (32).

Participants

Participants with type 2 diabetes at baseline were excluded from our analysis to avoid bias, because the diagnosis of type 2 diabetes generally leads to changes in dietary habits (33). This exclusion led to a total study sample of 1,087 nondiabetics. For participants lost to follow-up, who developed diabetes, or whose glucose tolerance status was unknown ($n = 329$) at the follow-up examination, we used their baseline data only. Thus, there were 992 nondiabetic participants at baseline and 663 nondiabetic participants at follow-up who had both diet and insulin sensitivity data. The combined data analysis incorporating baseline and follow-up was limited to those participants for whom diet and insulin sensitivity data were available for at least one of the two examinations ($n = 1{,}036$).

Variable measurements

Clinical measures. At baseline and follow-up, a clinical examination was conducted, which consisted of two 4-hour visits 1 week apart. Prior to each visit, participants were asked to fast for 12 hours and to refrain from drinking alcohol and participating in vigorous activity for 24 hours and from smoking on the day of the visit. A 2-hour 75-g oral glucose tolerance test (Orange-dex; Custom Laboratories, Baltimore, Maryland) was administered during the first visit. World Health Organization criteria (34) were used to determine glucose tolerance status. Individuals currently taking oral hypoglycemic medications were assumed to have type 2 diabetes regardless of the results of the oral glucose tolerance test.

Insulin sensitivity was measured during the second visit by using the frequently sampled intravenous glucose tolerance test (35) with minimal-model analysis (36). Two modifications were used: injection of insulin rather than tolbutamide (37) and a reduced number of plasma samples (12 rather than 30) (38). Glucose, in the form of a 50 percent solution (0.3 g/kg of body weight), and regular human insulin (0.03 U/kg) were injected over a 3-hour period. Defined as the dependence of fractional glucose disappearance on plasma insulin (39), the insulin sensitivity index (S_I) was calculated by mathematical modeling methods: the time course of plasma glucose was fitted by using nonlinear least-squares methods with the plasma insulin value as a known input into the system (MINMOD (36)). The unit

of insulin sensitivity is (minute^{-1} · pmol^{-1} · liter) × 10^{-5}; for a one-unit increase in 10^{-5} pmol/liter of plasma insulin, there will be an increase of 1 percent/minute in fractional glucose disappearance. A higher S_I value indicates better insulin sensitivity, and a zero value of S_I indicates being very insulin resistant (40).

Dietary intake. Usual dietary intake of foods and nutrients over the past year before baseline and follow-up was assessed by a 114-item food frequency questionnaire modified from the National Cancer Institute–Health Habits and History Questionnaire (41, 42) to accommodate regional and ethnic food choices. The nutrient database (HHHQ-DIETSYS Analysis Software, version 3.0; National Cancer Institute, Bethesda, Maryland, 1993) was expanded to account for additional foods on the IRAS food frequency questionnaire based on values obtained from the Minnesota Nutrition Data System (program version 2.3; Nutrition Coordinating Center, University of Minnesota, Minneapolis, Minnesota, 1990). Among participants who took nutritional supplements, usual frequency (number of pills per day, week, or month) and dosages were queried. Calcium and magnesium dosages were confirmed from product labels and summed across all products that contained calcium and magnesium. The validity and reproducibility of this questionnaire to measure intake of nutrients has been demonstrated in a subset of IRAS participants (43). Magnesium and calcium intake were calculated as the sum of intake from foods and supplements. Total dairy product intake was calculated as the sum across all dairy-containing food items in the respective food groups, including milk, milk drinks, butter, cheese, cottage cheese, yogurt, and ice cream. Usual alcohol intake was evaluated by using the food frequency questionnaire with additional questions about recent use and average lifetime use. Participants were classified as drinkers if their alcohol intake was greater than zero and nondrinkers if their alcohol intake was zero.

Other measurements. Standardized interviewing procedures were used to assess smoking status, which was classified as current smoking, past smoking, and never smoking. Ethnicity was determined by self-report. Body mass index was calculated as weight in kilograms divided by height in meters squared. Physical activity was assessed as usual frequency of vigorous activity by using five predefined responses that ranged from ''rarely to never'' to ''5 or more times per week'' (44, 45). Family history of diabetes was defined as at least one parent or sibling being diagnosed with diabetes.

Statistical methods

Descriptive statistics. Approximately 5 percent of participants had an S_I value of zero, and the distribution of S_I skewed to the right. Therefore, a constant was added to all values of S_I, and then the log transformation of the sum (Log ($S_I + 1$)) was used as the outcome variable. With this transformation, the distribution of resulting residual values approached normality. This approach has been used in other IRAS analyses (46, 47). Sample means, standard deviations, medians, and frequencies were calculated for all characteristics of interest. Calorie-adjusted Pearson's correlation coefficients between exposure variables (dairy, magnesium, and calcium intake), insulin sensitivity, and other relevant mediators or confounders were calculated at baseline and at follow-up separately.

Modeling approach for the dose-dependent association. Adjusting for demographic factors and other potential confounders in a stepwise manner, we used the following exploratory methods to elucidate the possible shape of dose-dependent associations without any assumption: we compared insulin sensitivity among categories of each exposure variable classified by quintiles and plotted insulin sensitivity across each exposure variable in the smoothing scatter plots, such as the locally weighted, running-line smoother. The following four modeling approaches for the dose-dependent association were used to search for the appropriate association with insulin sensitivity for each exposure variable:

1. Quintile model: the exposure variable was classified into five categories by quintile according to the respective distribution.
2. Linear model: assuming a linear association, the exposure variable was included as a continuous variable.
3. Quadratic model: assuming a nonlinear association, the continuous exposure variable and its quadratic terms were included.
4. Threshold model: assuming a threshold relation, the model included two continuous variables representing exposure lower than and greater than the threshold. A Bayesian approach with the Monte Carlo method was used to estimate the threshold.

The general linear model was used to examine dose-dependent associations at baseline and follow-up separately. After confirming internal consistency of the association between baseline and follow-up, a likelihood-based linear mixed model was used to assess the association of each exposure variable with insulin sensitivity, incorporating baseline and follow-up.

The linear mixed model is one of the powerful statistical methods used to analyze repeated-measures data (48–51). The basic assumption is that the repeated measures for each subject are from an unobserved, multivariate normal distribution (48). In this study, insulin sensitivity values from baseline and follow-up for each participant were assumed to follow a bivariate normal distribution, with the means determined by the fixed effects of exposure variable and covariate, where the fixed effect means that the regression coefficients for the exposure variable and covariates were assumed to be fixed parameters and were the same between baseline and follow-up. Subject-specific random intercepts were included in the linear mixed model to allow for heterogeneity between subjects and to indirectly generate a within-subject covariance structure, where the subject-specific random intercepts were assumed to follow a bivariate normal distribution. These random intercepts could account for unobserved covariates that induce dissimilarities in insulin sensitivity between subjects not properly accounted for by the observed exposures and covariates. By directly modeling the covariance structure, the linear mixed model greatly improves analysis of repeated-measures data by providing

a valid standard error (within-subject) and efficient statistics tests (51).

In the linear mixed model, the covariance structure was determined first. A restricted maximum likelihood estimation method was used to calculate fit statistics, Akaike's Information Criterion (AIC) and Schwarz's Bayesian Information Criterion (BIC), to find the best covariance structure (52). AIC and BIC are computed as follows: $AIC = -2L + 2P$ and $BIC = -2L + P\text{Log}(N)$, where L is the maximized log likelihood, P the number of parameters, and N the sample size (52). A smaller value indicates a better fit. For all exposure variables, an unstructured covariance structure fit the data better than other covariance structures did. Therefore, an unstructured covariance structure was used in all linear mixed models.

Different approaches to modeling dose-dependent associations were compared based on the goodness-of-fit statistics—model R squared—in the general linear model and on AIC and BIC in the linear mixed model. AIC penalizes for number of parameters only; BIC penalizes for number of parameters and sample size (48). After we determined the covariance structure, the maximum likelihood estimation method was used in calculating the fit statistics. Consistent evidence from the exploratory statistical methods and goodness-of-fit statistics for different modeling approaches were used as the criteria to determine a dose-dependent association. The model with the fewest assumptions about the does-dependent association, the quintile model, was chosen if there was no consistent evidence.

The association of each exposure variable with insulin sensitivity was first evaluated after adjustment for demographic factors (age, sex, ethnicity and clinical center, total calorie intake, and family history of diabetes). Additional potential confounders determined from previous work, such as nondietary lifestyle factors (smoking, drinking, and physical activity), dietary factors (dietary protein, fat, fiber, calcium, or magnesium intake), food groups (refined and whole grains, fruit, vegetables, fish, and meat), and body mass index, were adjusted for in a stepwise manner to assess the associations. Separate analyses were conducted for the total study sample and for the nonsupplement users of magnesium or calcium.

Change point estimation. With the same distribution assumption as for the linear mixed model, a Bayesian approach with the Markov Chain Monte Carlo method (31) was used to estimate the threshold. Insulin sensitivity at baseline and follow-up were assumed to have a bivariate normal distribution, with an unstructured covariance matrix and an expected value explained by exposure variables and covariates. Exposure variables and covariates were assumed to be fixed effects, the change point was assumed to be uniformly distributed within the range of the middle 95 percent of the exposure variable distribution, and regression coefficients of the exposure variable were assumed to be different between values less than and greater than the change point. All of the regression coefficients were given a prior normal distribution with mean zero and a large variance (10,000), and the covariance matrix was given a prior Wishart distribution. Convergence was checked by examining the Gelman and Rubin diagnostic test (53) and the time-series plot of two separate chains. After attaining convergence, the posterior mean of the change point was used as the estimator of the change point.

WinBUGS software (version 1.4; Imperial College and Medical Research Council, Cambridge, United Kingdom) was used for the Bayesian analyses. All other statistical analyses were performed by using the SAS statistical software program (version 8.2; SAS Institute, Inc., Cary, North Carolina). All p values were two sided, and $p < 0.05$ was defined as significant.

TABLE 1. Descriptive characteristics of participants without diabetes at baseline ($n = 1,087$), Insulin Resistance Atherosclerosis Study, United States, 1992–1994

Characteristic	Mean (SD*) or %
Age (years)	54.83 (8.44)
Gender	
Male	43.6
Female	56.4
Ethnicity	
African American	26.5
Hispanic	33.5
Non-Hispanic White	40.0
Family history of diabetes	41.4
Smoking	
Never	45.3
Past	38.2
Current	16.5
Physical activity	
Rarely/never	28.7
1–3 times/month	18.9
1 time/week	13.2
2–4 times/week	28.6
>4 times/week	10.6
Alcohol drinker	57.7
Glucose tolerance status	
Normal	66.0
Impaired	34.0
Body mass index (kg/m^2)	28.47 (5.72)
Insulin sensitivity (minute^{-1} · pmol^{-1} · liter) × 10^{-5}	2.17 (2.03)
Magnesium supplement user	5.4
Calcium supplement user	17.2

* SD, standard deviation.

RESULTS

Descriptive characteristics of the study participants at baseline are presented in tables 1 and 2. The average S_I was 2.17 (standard deviation, 2.03) (minute^{-1} · pmol^{-1} · liter) × 10^{-5}. The mean daily dairy intake was about 1.02 (standard

TABLE 2. Dietary intake of participants without diabetes at baseline ($n = 1,087$), Insulin Resistance Atherosclerosis Study, United States, 1992–1994

Dietary factor	Mean (SD*)	Median
Total energy (kcal/day)	1,885 (821)	1,743
Dairy (servings/day)	1.02 (0.73)	0.89
Total magnesium (mg/day)	403 (288)	326
Dietary magnesium (mg/day)	398 (287)	319
Total calcium (mg/day)	970 (647)	807
Dietary calcium (mg/day)	836 (485)	722
Dietary protein (g/day)	77.69 (34.65)	72.01
Dietary fat (g/day)	74.71 (39.50)	67.05
Dietary fiber (g/day)	13.47 (5.78)	12.85
Whole and refined grains (servings/day)	3.00 (1.57)	2.73
Vegetables (servings/day)	3.17 (1.89)	2.84
Fruit (servings/day)	2.29 (1.71)	1.96
Fish (servings/day)	0.24 (0.25)	0.17
Meat (servings/day)	0.98 (0.82)	0.81

* SD, standard deviation.

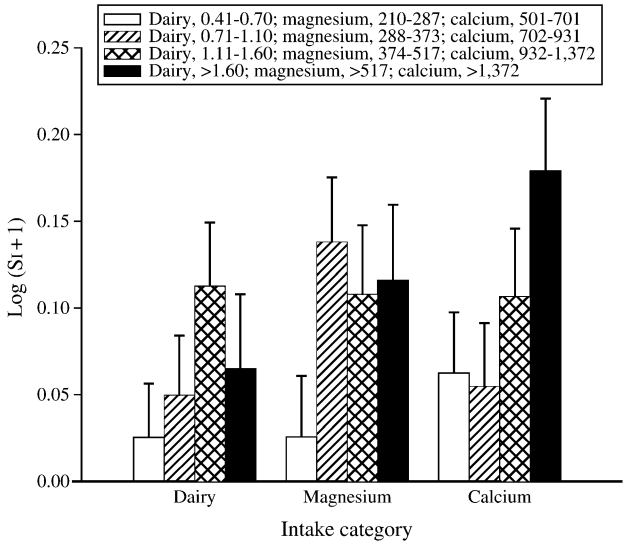

FIGURE 1. Differences in insulin sensitivity (Log ($S_I + 1$)) and upper 95% confidence intervals (vertical lines) according to quintiles of intake of dairy products (number of servings), magnesium (milligrams), and calcium (milligrams), with the lowest quintile as the reference group, incorporating baseline and follow-up data for 1,036 participants in the Insulin Resistance Atherosclerosis Study, United States, 1992–1999. After adjustment for demographic factors; p value for overall effect: dairy, 0.41; magnesium, 0.0001; calcium, 0.0005. S_I, insulin sensitivity index.

deviation, 0.73) servings. The average daily magnesium intake from diet and supplements was 403 (standard deviation, 288) mg. For the 5 percent of participants who took magnesium supplements, the median dose was 100 mg. The average daily calcium intake from diet and supplements was 970 (standard deviation, 647) mg. About 17 percent of the participants took calcium supplements, and the median dose was 500 mg. Positive correlations were found for dairy, magnesium, and calcium with insulin sensitivity and among them (table 3).

Results of the linear mixed models

Similar results for dairy, magnesium, and calcium intake were obtained from separate baseline and follow-up analyses (results not shown), which met the assumption for the linear mixed model. Therefore, this paper presents results from the linear mixed model.

Association of dairy intake with insulin sensitivity. Dairy intake was not statistically significantly associated with insulin sensitivity after we adjusted for demographic factors (figure 1). Further adjustment for nondietary lifestyle factors, dietary factors, and food groups including magnesium and calcium intake did not change the association (results not shown).

Association of magnesium intake with insulin sensitivity. The quintile model (figure 1) and smoothing scatter plots (results not shown) suggested a clear threshold association. Bayesian analyses produced a threshold of 325 (standard deviation, 99) mg, which was used in the threshold model. Fit statistics for the quintile, linear, quadratic, and threshold modeling approaches are shown in table 4. Compared with the other modeling approaches, the threshold model had the lowest AIC and BIC values, which suggested that the threshold association fit the data best. Given the consistent evidence from the different, above-described statistical methods, the threshold model was chosen for reporting the association of magnesium intake with insulin sensitivity. As shown in table 5, magnesium intake was significantly associated with insulin sensitivity when magnesium intake was lower than the threshold ($\beta = 0.0659$/100 mg, $p = 0.008$) and was not associated with insulin sensitivity when magnesium intake was higher than the threshold ($\beta = -0.0008$/100 mg, $p = 0.87$) after adjustment for demographics, nondietary lifestyle factors, dietary factors

TABLE 3. Pearson's correlations for insulin sensitivity (Log (S_I† + 1)) and for dairy, magnesium, and calcium intake after adjustment for total calorie intake at baseline ($n = 992$), Insulin Resistance Atherosclerosis Study, United States, 1992–1994

Measure	Log ($S_I + 1$)	Dairy (servings/day)	Magnesium (mg/day)	Calcium (mg/day)
Log ($S_I + 1$)	1.00	0.07*	0.09**	0.11***
Dairy		1.00	0.33***	0.43***
Magnesium			1.00	0.42***
Calcium				1.00

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.
† S_I, insulin sensitivity index.

TABLE 4. Goodness-of-fit comparisons of different modeling approaches for magnesium intake, incorporating baseline and follow-up data for 1,036 participants in the Insulin Resistance Atherosclerosis Study, United States, 1992–1999

Adjustment covariates and fit statistics	Modeling approach			
	Quintile model	Linear model	Quadratic model	Threshold model
Model 1: Demographics*				
AIC†	2,088.0	2,095.3	2,089.8	2,083.8
BIC†	2,186.9	2,179.4	2,178.8	2,172.7
Model 2: Demographics and nondietary lifestyle‡ and dietary§ factors				
AIC	2,045.4	2,050.3	2,046.1	2,041.9
BIC	2,193.7	2,183.8	2,184.5	2,180.3
Model 3: Demographics, nondietary lifestyle and dietary factors, and calcium intake (quintiles)				
AIC	2,036.9	2,041.9	2,040.9	2,035.7
BIC	2,204.9	2,195.1	2,199.0	2,193.8
Model 4: Demographics, nondietary lifestyle and dietary factors, calcium intake (quintiles), and food groups¶				
AIC	2,042.2	2,046.0	2,045.0	2,041.2
BIC	2,234.9	2,223.9	2,227.9	2,223.8
Model 5: Demographics, nondietary lifestyle and dietary factors, calcium intake (quintiles), food groups, and body mass index				
AIC	1,727.5	1,730.3	1,730.4	1,727.4
BIC	1,924.5	1,913.4	1,918.2	1,913.3

* Includes age, sex, ethnicity and clinical center, family history of diabetes, and total calorie intake per day.

† AIC, Akaike's Information Criterion; BIC, Schwarz's Bayesian Information Criterion.

‡ Includes smoking, physical activity, and alcohol drinking.

§ Includes dietary protein, dietary fat, and dietary fiber.

¶ Includes whole and refined grains, vegetables, fruit, fish, and meat.

including calcium, and food groups. Furthermore, adding body mass index slightly attenuated the association, but magnesium intake was still significantly associated with insulin sensitivity when magnesium intake was lower than the threshold ($\beta = 0.0607$/100 mg, $p = 0.008$). A diagrammatic graph of the linear and threshold association between magnesium intake and insulin sensitivity is shown in figure 2.

Association of calcium intake with insulin sensitivity. The quintile models (figure 1, table 6) and smoothing scatter plots (results not shown) suggested an overall linear relation, but with a possible threshold association. Bayesian analysis produced a threshold of 1,255 (standard deviation, 367) mg. The quintile, linear, quadratic, and threshold models were compared in terms of fit statistics (table 6). In general, none of the four dose-dependent modeling approaches fit the data consistently better than other models did. Therefore, we chose the quintile model to present the association of calcium intake with insulin sensitivity because it involved the fewest assumptions. As shown in table 7, higher calcium intake groups were associated with higher levels of insulin sensitivity after we adjusted for demographics, nondietary lifestyle factors, dietary factors including threshold magnesium intake, and food groups ($p = 0.016$ for overall effect). Further adjustment for body mass index attenuated the overall effect to be nonsignificant ($p = 0.13$).

Similar associations for magnesium and calcium intake were found for participants who did not use supplements of magnesium or calcium (data not shown).

DISCUSSION

This study, incorporating data from baseline and 5 years of follow-up, found no association between dairy intake and insulin sensitivity. Positive and independent associations of magnesium and calcium intake with insulin sensitivity were identified, and these associations were independent of other risk factors for insulin resistance. Furthermore, magnesium intake was associated with insulin sensitivity in a threshold fashion, which suggested that intake of more than the threshold of 325 mg might not provide further benefit in terms of insulin sensitivity. The association of calcium intake with insulin sensitivity did not appear to involve a threshold.

TABLE 5. Association of magnesium intake with insulin sensitivity (Log (S_I* +1)), incorporating baseline and follow-up data in the linear and threshold modeling approach (threshold, 325 mg) for 1,036 participants in the Insulin Resistance Atherosclerosis Study, United States, 1992–1999

Adjustment covariates	Linear effect model		Threshold effect model			
			Magnesium <325 mg		Magnesium ≥325 mg	
	Change in insulin sensitivity†	*p* value	Change in insulin sensitivity	*p* value	Change in insulin sensitivity	*p* value
Model 1: Demographics‡	0.0097	0.030	0.0888	<0.0001	0.0038	0.42
Model 2: Demographics and nondietary lifestyle§ and dietary¶ factors	0.0091	0.07	0.0803	0.0004	0.0050	0.33
Model 3: Demographics, nondietary lifestyle and dietary factors, and calcium intake (quintiles)	0.0038	0.53	0.0721	0.003	−0.0005	0.92
Model 4: Demographics, nondietary lifestyle and dietary factors, calcium intake (quintiles), and food groups#	0.0027	0.61	0.0659	0.008	−0.0008	0.87
Model 5: Demographics, nondietary lifestyle and dietary factors, calcium intake (quintiles), food groups, and body mass index	0.0023	0.65	0.0607	0.008	−0.0010	0.82

* S_I, insulin sensitivity index.
† Change in insulin sensitivity for every 100-mg increase in magnesium intake.
‡ Includes age, sex, ethnicity and clinical center, family history of diabetes, and total calorie intake per day.
§ Includes smoking status, physical activity, and alcohol drinking.
¶ Includes dietary protein, dietary fat, and dietary fiber.
# Includes whole and refined grains, vegetables, fruit, fish, and meat.

One strength of this study is that different dose-dependent modeling approaches were used to evaluate the association, especially the Bayesian approach with the Monte Carlo method to estimate the threshold with the goal of improving modeling of the threshold association. Linear association was commonly assumed in assessing the relation; however, this assumption would underestimate or fail to find the underlying relation in the case of threshold association in the studies with a limited sample size. In our study, the linear model failed to find a statistically significant association of magnesium intake with insulin sensitivity (table 4), whereas the threshold model found a statistically significant association when magnesium intake was less than 325 mg. Quintiles of magnesium intake have been commonly used in assessing the association of magnesium intake with fasting insulin and the incidence of type 2 diabetes (12, 13), which fail to take advantage of the continuous nature of magnesium intake. However, comparing the outcome values by quintiles of exposure could help to explore the dose-dependent association. In our study, the magnesium quintile model suggested a possible threshold association.

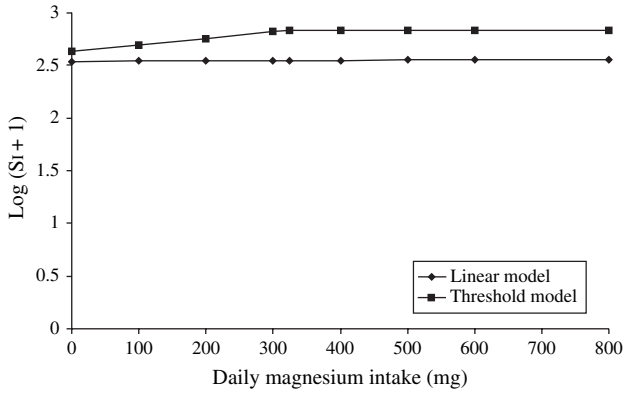

FIGURE 2. Comparison of the estimated dose-dependent association of magnesium intake with insulin sensitivity (Log (S_I + 1)), incorporating baseline and follow-up data for 1,036 participants in the Insulin Resistance Atherosclerosis Study, United States, 1992–1999. Here, magnesium intake was limited to <800 mg. In the linear model, the slope is 0.0023/100 mg; in the threshold model, the slope is 0.0607/100 mg when magnesium intake is <325 mg and –0.001/100 mg when it is ≥325 mg. Refer to model 5 in table 5 for the adjusting covariates. S_I, insulin sensitivity index.

When studying the threshold effect, the method of estimating the threshold is critical. In this study, we used a Bayesian approach with the Markov Chain Monte Carlo method to estimate the threshold for magnesium intake. The estimated threshold (325 mg) is close to the Recommended Dietary Allowance of magnesium for this study population (420 mg/day for men and 320 mg/day for women) (20). The resulting threshold model detected a significant association when magnesium intake was less than 325 mg and fit the data consistently better than other dose-dependent modeling approaches did.

There are other strengths of this study. Each study subject took part in two examinations 5 years apart, which enabled determination of internal consistency and use of the linear mixed model to summarize the associations and improve the power of data analysis. As a repeated-measures regression analysis, the linear mixed model helps to uncover the

TABLE 6. Goodness-of-fit comparisons of different modeling approaches for calcium intake, incorporating baseline and follow-up data for 1,036 participants in the Insulin Resistance Atherosclerosis Study, United States, 1992–1999

Adjustment covariates and fit statistics	Modeling approach			
	Quintile model	Linear model	Quadratic model	Threshold model
Model 1: Demographics*				
AIC†	2,086.1	2,090.3	2,091.8	2,085.4
BIC†	2,185.0	2,174.4	2,180.8	2,174.4
Model 2: Demographics and nondietary lifestyle‡ and dietary§ factors				
AIC	2,040.3	2,044.4	2,045.7	2,039.0
BIC	2,188.6	2,177.8	2,184.1	2,177.4
Model 3: Demographics, nondietary lifestyle and dietary factors, and threshold effect of magnesium				
AIC	2,035.7	2,037.2	2,039.1	2,036.1
BIC	2,193.8	2,180.5	2,187.4	2,184.4
Model 4: Demographics, nondietary lifestyle and dietary factors, threshold effect of magnesium, and food groups¶				
AIC	2,041.2	2,041.9	2,043.9	2,041.4
BIC	2,224.0	2,209.9	2,216.8	2,214.4
Model 5: Demographics, nondietary lifestyle and dietary factors, threshold effect of magnesium, food groups, and body mass index				
AIC	1,725.5	1,724.4	1,726.3	1,724.8
BIC	1,913.3	1,897.3	1,904.2	1,902.7

* Includes age, sex, ethnicity and clinical center, family history of diabetes, and total calorie intake per day.
† AIC, Akaike's Information Criterion; BIC, Schwarz's Bayesian Information Criterion.
‡ Includes smoking, physical activity, and alcohol drinking.
§ Includes dietary protein, dietary fat, and dietary fiber.
¶ Includes whole and refined grains, vegetables, fruit, fish, and meat.

TABLE 7. Differences in insulin sensitivity (Log (S_I* + 1)) (mean (SE*)) according to calcium category (with the lowest (<501 mg/day) as the reference group), incorporating baseline and follow-up data for 1,036 participants in the Insulin Resistance Atherosclerosis Study, United States, 1992–1999

Adjustment covariates	Calcium intake quintile (mg/day)				*p* for overall effect
	501–701	702–931	932–1,372	>1,372	
Model 1: Demographics†	0.0626 (0.0349)	0.0548 (0.0365)	0.1066 (0.0393)	0.1789 (0.0419)	0.0005
Model 2: Demographics and nondietary lifestyle‡ and dietary§ factors	0.0667 (0.0346)	0.0574 (0.0364)	0.1140 (0.0315)	0.1806 (0.0429)	0.0007
Model 3: Demographics, nondietary lifestyle and dietary factors, and threshold effect of magnesium	0.0326 (0.0364)	0.0152 (0.0391)	0.0688 (0.0423)	0.1435 (0.0456)	0.007
Model 4: Demographics, nondietary lifestyle and dietary factors, threshold effect of magnesium, and food groups¶	0.0290 (0.0365)	0.0108 (0.0393)	0.0626 (0.0428)	0.1339 (0.0467)	0.0157
Model 5: Demographics, nondietary lifestyle and dietary factors, threshold effect of magnesium, food groups, and body mass index	0.0053 (0.0341)	0.0097 (0.0366)	0.0477 (0.0398)	0.0922 (0.0435)	0.1288

* S_I, insulin sensitivity index; SE, standard error.
† Includes age, sex, ethnicity and clinical center, family history of diabetes, and total calorie intake per day.
‡ Includes smoking, physical activity, and alcohol drinking.
§ Includes dietary protein, dietary fat, and dietary fiber.
¶ Includes whole and refined grains, vegetables, fruit, fish, and meat.

underlying associations that the general linear model fails to find by providing a valid standard error and efficient statistics tests. Omission of participants for whom data on some variables are missing in the general linear model could bias the results, and the linear mixed model could include participants for whom data on some, but not all, variables are missing. In addition, our study directly measured insulin sensitivity to assess its association with dairy, magnesium, and calcium intake, while most studies use fasting insulin levels to represent insulin sensitivity.

Limitations of this study relate mainly to its observational nature, with the possibility of differential misclassification and residual confounding. Overweight and obesity are the main determinants of insulin sensitivity (54), and overweight and obese persons tend to underreport their dietary intake (55). Thus, for insulin-resistant persons, the percentage of underreporting of dietary intake might be higher, which might bias the association further away from the null. Although we adjusted for potential confounders, the possibility of residual confounding cannot be completely ruled out.

This study failed to find an association between dairy intake and insulin sensitivity. Given that insulin sensitivity is an underlying factor in the insulin-resistance syndrome and the main predictor of diabetes, this finding is not consistent with those from previous studies. Prospective studies have found an inverse association of dairy intake with risk of the insulin-resistance syndrome (1, 2) and type 2 diabetes (3). Different study outcomes might partly explain the inconsistent results. In addition, the limited variability of daily dairy intake in our study (interquartile range, 0.49–1.40 servings) might have precluded detection of a true effect. Furthermore, dairy intake is a surrogate measurement for nutrients and/or health-related behaviors, which is not specific in terms of a biologic effect. On the contrary, insulin sensitivity is a specific biologic measurement, which might partly explain why the specific nutrients, magnesium and calcium, instead of dairy, were associated with insulin sensitivity. Further studies are needed to elucidate the association of dairy intake with insulin sensitivity.

The positive association of magnesium intake with insulin sensitivity in our study is consistent with previous studies. Several prospective studies have found a significant inverse association between magnesium intake and diabetes risk (9–13), and two magnesium supplementation studies have found a beneficial effect of magnesium on insulin sensitivity (56, 57). Furthermore, one cross-sectional study found an inverse association between magnesium intake and plasma levels of fasting insulin for overweight women only (12). Limited studies have examined the association of calcium intake with insulin sensitivity. One clinical trial found that calcium supplementation (1,500 mg/day) for 8 weeks could increase insulin sensitivity in 20 nondiabetic, essential hypertensive patients (19). In our study, we found a positive association of calcium intake with insulin sensitivity after taking into account demographics, nondietary lifestyle and dietary factors including magnesium in a threshold fashion, and food groups. Further adjustment for body mass index attenuated the association for magnesium and calcium intake, which suggested that body mass index might be a potential effect confounder and/or mediator.

In conclusion, we found that magnesium and calcium intake were independently associated with insulin sensitivity. Furthermore, intake of magnesium over a threshold level might not provide further benefit in terms of insulin sensitivity, which supports the importance of examining the dose-dependent association for dietary intake.

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