Low Serum 25-Hydroxyvitamin D Is Associated with Increased Risk of the Development of the Metabolic Syndrome at Five Years: Results from a National, Population-Based Prospective Study (The Australian Diabetes, Obesity and Lifestyle Study: AusDiab)

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Context: Serum 25-hydroxyvitamin D [25(OH)D] concentration has been inversely associated with the prevalence of metabolic syndrome (MetS), but the relationship between 25(OH)D and incident MetS remains unclear.

Objective: We evaluated the prospective association between 25(OH)D, MetS, and its components in a large population-based cohort of adults aged 25 yr or older.

Design: We used baseline (1999–2000) and 5-yr follow-up data of the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab).

Participants: Of the 11,247 adults evaluated at baseline, 6,537 returned for follow-up. We studied those without MetS at baseline and with complete data (n = 4164; mean age 50 yr; 58% women; 92% Europids).

Outcome Measures: We report the associations between baseline 25(OH)D and 5-yr MetS incidence and its components, adjusted for age, sex, ethnicity, season, latitude, smoking, family history of type 2 diabetes, physical activity, education, kidney function, waist circumference (WC), and baseline MetS components.

Results: A total of 528 incident cases (12.7%) of MetS developed over 5 yr. Compared with those in the highest quintile of 25(OH)D (≥34 ng/ml), MetS risk was significantly higher in people with 25(OH)D in the first (<18 ng/ml) and second (18–23 ng/ml) quintiles; odds ratio (95% confidence interval) = 1.41 (1.02–1.95) and 1.74 (1.28–2.37), respectively. Serum 25(OH)D was inversely associated with 5-yr WC ($P < 0.001$), triglycerides ($P < 0.01$), fasting glucose ($P < 0.01$), and homeostasis model assessment for insulin resistance ($P < 0.001$) but not with 2-h plasma glucose ($P = 0.29$), high-density lipoprotein cholesterol ($P = 0.70$), or blood pressure ($P = 0.46$).

Conclusions: In Australian adults, lower 25(OH)D concentrations were associated with increased MetS risk and higher WC, serum triglyceride, fasting glucose, and insulin resistance at 5 yr. Vitamin D supplementation studies are required to establish whether the link between vitamin D deficiency and MetS is causal. **(*J Clin Endocrinol Metab* 97: 1953–1961, 2012)**

It is unclear whether serum 25-hydroxyvitamin D [25(OH)D] concentrations predict the risk of developing the metabolic syndrome (MetS), although several cross-sectional studies have reported an inverse association between 25(OH)D and the prevalence of the metabolic syndrome (MetS) in adults (1–10) and in children or adolescents (11–13). To our knowledge, only one small prospective study involving 524 adults has evaluated the

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Abbreviations: BMI, Body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; HDL, high-density lipoprotein; HOMA2-IR, homeostasis model assessment for insulin resistance; 2-h PG, 2-h plasma glucose after oral glucose tolerance test; 25(OH)D, 25-hydroxyvitamin D; MetS, metabolic syndrome; OR, odds ratio; WC, waist circumference.

association between 25(OH)D and incident MetS risk, using a MetS risk z-score (14). It was reported that baseline 25(OH)D was associated inversely with 10-yr risk of MetS after adjusting for age, sex, smoking status, season, body mass index (BMI) and baseline MetS z-score. Here we investigated the prospective association between 25(OH)D and the MetS and its components in a large, population-based cohort of Australian adults followed for 5 yr.

Subjects and Methods

Study population

Details of the baseline (1999–2000) and 5-yr follow-up AusDiab studies have been described previously (15–17). Briefly, 20,347 noninstitutionalized Australian adults aged 25 yr or older from 42 randomly selected districts completed a household interview in 1999–2000. Of those, 11,247 (55.3%) attended a biomedical evaluation, provided a fasting blood sample, and underwent a 75-g 2-h oral glucose tolerance test. Five years later, 6537 returned for a repeat biomedical evaluation. After excluding those with the MetS at baseline (n = 1905) and missing data at baseline or follow-up (n = 468), 4164 remained for this analysis. Written informed consent was obtained from all participants, and ethical approval was provided by the International Diabetes Institute Ethics Committee and the Monash Committee on Ethics in Research Involving Humans.

MetS definition

The MetS was defined using the 2009 harmonized definition (18), which required at least three of the following criteria: waist circumference (WC) at least 102 cm for men and at least 88 cm for women of European origin or at least 90 cm for men and at least 80 cm for women of non-European origin, triglycerides at least 1.7 mmol/liter (150.4 mg/dl), high-density lipoprotein (HDL)-cholesterol below 1.0 mmol/liter (38.6 mg/dl) for men and below 1.3 mmol/liter (50.2 mg/dl) for women, systolic blood pressure at least 130 and/or diastolic blood pressure at least 85 mm Hg or on treatment, fasting plasma glucose (FPG) at least 5.6 mmol/liter (100.9 mg/dl) or known diabetes.

Measurements

Concentrations of 25(OH)D were measured in serum, having been stored at −80 C for 10 yr, using the Liaison 25OH vitamin D TOTAL (DiaSorin Inc., Stillwater, MN), which had an interassay coefficient variation of 7.0% at 45 nmol/liter and 6.3% at 93 nmol/liter. This method gives results that align with the National Institute of Standards and Technology (NIST) targets for serum 25(OH)D. Details of the 25(OH)D analysis have been described elsewhere (16). Season of blood sampling was divided into autumn-winter (April to September) and spring-summer (October to March). The latitude of each of the 42 districts was determined using the Google GPS tool (range 12° S to 43° S). FPG, 2-h plasma glucose after oral glucose tolerance test (2-h PG), and serum lipids were measured by enzymatic methods, whereas serum creatinine was assessed by the modified kinetic Jaffe reaction using an Olympus AU600 automated analyzer (Olympus Optical, Tokyo, Japan) (17). The estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease formula. Serum insulin at baseline was measured using a human insulin-specific RIA kit (Linco Research, Inc., St. Charles, MO), whereas at follow-up, an automated chemiluminescence immunoassay (Bayer ADVIA Centaur, Chicago, IL) was used. The insulin values, obtained with these different assays, were not directly comparable. Although both methods correlated well (r = 0.98), there was a negative intercept (constant low bias) of 24 pmol/liter by the RIA method used at baseline compared with the chemiluminescence immunoassay method used in the follow-up study. A validated formula was thus used to align the values from both methods so that the insulin results at baseline and follow-up were comparable: adjusted baseline serum insulin (picomoles per liter) = −24.4 + 0.99 × baseline insulin values (Linco-RIA measures) (19). The adjusted baseline serum insulin values were then used in all the statistical analyses. Due to cost constraints, insulin assays were conducted only in participants older than 35 yr and in those who were not on insulin therapy. Homeostasis model assessment for insulin resistance (HOMA2-IR) was calculated using the HOMA2 calculator version 2.2 (20, 21). As recommended by the HOMA2 calculator software, observations with extreme values of FPG (<3 or >25 mmol/liter) and/or serum insulin (<20 or >300 pmol/liter) were excluded. In analyses that included HOMA2-IR, the number of participants dropped due to the lack of measurement of serum insulin in participants younger than 35 yr (n = 535), missing serum insulin data at baseline (n = 30) or at follow-up (n = 3), and exclusion of extreme insulin values at baseline (n = 265) or at follow-up (n = 49).

Data on age, sex, ethnicity (Europid *vs.* non-Europid), smoking (current/ex-smoker *vs.* never smoker), tertiary education (university or technical and further education), family history of type 2 diabetes (first-degree relative with type 2 diabetes), leisure-time physical activity (minutes per week), and television-viewing time (minutes per week) were collected by trained interviewers using standardized questionnaires as previously reported (17). Total leisure-time physical activity for the previous week was calculated using the validated Active Australia questionnaire (22). Total time spent watching television or videos in the previous week was self-reported. Blood pressure, height, weight, and WC were assessed using standard procedures described previously (17). Total energy, alcohol, and dietary calcium and magnesium intake were assessed using a self-administered validated food frequency questionnaire (23). Dietary intake of calcium and magnesium was adjusted for total energy intake (24).

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Statistical analyses

Analyses were performed using SPSS version 19.0 (Chicago, IL) and SAS version 9.3 (Cary, NC). Normality of the data was verified before analysis. Logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (CI) for the association between 25(OH)D (as a continuous variable or in quintiles) and MetS risk. Tests of linear trend were conducted by assigning median values of 25(OH)D in quintiles as a continuous variable. Variables that were associated with MetS risk in a multivariable model ($P \leq 0.05$) comprising only variables with a $P < 0.1$ in univariable analyses were considered as potential confounders: model 1 included age, sex, ethnicity, season, and latitude; model 2 included the variables in model 1 plus smoking, physical activity, family history of type 2 diabetes, education, and eGFR; and model 3 included the variables in model 2 plus HOMA2-IR.

In a separate analysis, we investigated whether the inclusion of BMI in the multivariable model affected the association between serum 25(OH)D (as a continuous variable) and MetS risk. Because we found a significant interaction between BMI and 25(OH)D for MetS risk, we split the group in two based on median BMI (<25 kg/m^2 *vs.* ≥ 25 kg/m^2). No interaction was found between age, sex, ethnicity, and 25(OH)D for MetS risk. Finally, we calculated the relative contribution of serum 25(OH)D in comparison with the other risk factors in the overall MetS risk. To do so, we first entered the standardized values of the risk factors in a univariable logistic regression model to obtain Nagelkerke R-squared values. We then entered one by one, in a stepwise logistic regression, the risk factors with the highest to the lowest Nagelkerke R-squared values. The relative contribution of the risk factor was calculated by dividing its percent increment in Nagelkerke R-squared by the Nagelkerke R-squared value obtained when all the risk factors were included.

Associations between baseline 25(OH)D and each of the components of the MetS at 5 yr (WC, FPG, fasting triglycerides, HDL-cholesterol, and blood pressure) plus 2-h PG and HOMA2-IR were assessed using a logistic regression. In the analyses looking at the associations between serum 25(OH)D and the components of the MetS at 5 yr, we further adjusted for baseline MetS components and WC in model 1 and 2, because they were the strongest predictors of MetS components at 5 yr. Finally, to evaluate the shape of the relationship between serum 25(OH)D and MetS risk, we used a logistic regression with a generalized additive model smoothing term, as described by Hastie and Tibshirani (25). We fitted a model between the binary variable MetS and the independent variable serum 25(OH)D using a spline smoother for the outcome (MetS). We adjusted this association for the variables in model 2. We then plotted the adjusted probability of developing MetS at 5 yr against serum 25(OH)D concentrations at baseline.

Results

Serum 25(OH)D and risk factors of MetS at baseline

A total of 528 incident cases of MetS (12.7%) developed over 5 yr. Those who developed MetS had lower 25(OH)D concentrations at baseline than those who did not (mean 25 *vs.* 27 ng/ml; $P < 0.001$) [to convert serum 25(OH)D to nanomoles per liter, multiply by 2.5], but intakes of dietary calcium and magnesium did not differ (Table 1). Par-

TABLE 1. Baseline characteristics of the participants by MetS status at 5 yr

Characteristic	MetS	No MetS	*P* value[a]
n	528	3636	
Age (yr)	52.3 ± 12.0	49.5 ± 12.8	<0.001
Women (%)	49	59	<0.001
Europids (%)	90	93	0.01
Tertiary education (%)	35	45	<0.001
BMI (kg/m^2)	28.1 ± 3.9	24.9 ± 3.6	<0.001
Current/ex-smoker (%)	43	38	0.02
Alcohol intake (g/d)	6.8 (1.0–20.4)	7.2 (1.1–19.0)	0.94
Physical activity (min/wk)	145 (41–358)	180 (60–430)	<0.001
Television viewing time (min/wk)	660 (330–1020)	600 (300–960)	0.09
eGFR (ml/min)	75 ± 11	77 ± 12	<0.01
Waist circumference (cm)	100 ± 11	87 ± 11	<0.001
Hypertension (%)	47	31	<0.001
Serum FPG (mg/dl)	97.3 (91.9–102.7)	93.7 (88.3–99.1)	<0.001
Serum 2-h PG (mg/dl)	108.1 (91.9–124.3)	97.3 (82.9–113.5)	<0.001
Serum triglycerides (mg/dl)	123.9 (95.6–149.6)	88.5 (64.6–119.5)	<0.001
Serum HDL-cholesterol (mg/dl)	50.6 (43.6–57.9)	58.7 (49.8–68.7)	<0.001
HOMA2-IR (U)	1.51 (1.14–2.03)	1.13 (0.83–1.51)	<0.001
Serum 25(OH)D (ng/ml)	25 ± 9	27 ± 10	<0.001
Dietary calcium intake (mg/d)	882 (751–1,048)	898 (751–1,073)	0.25
Dietary magnesium intake (mg/d)	297 (262–334)	299 (264–340)	0.92

Data are presented as mean ± SD, median (interquartile range), or percent. Dietary calcium and magnesium intakes were adjusted for total energy intake. To convert 25(OH)D to nanomoles per liter, multiply by 2.5; to convert glucose to millimoles per liter, divide by 0.0555; to convert triglyceride levels to millimoles per liter, divide by 0.0113; to convert HDL-cholesterol to millimoles per liter, divide by 0.0259.

[a] Independent *t* test or Mann-Whitney *U* test for continuous variables or χ^2 test for categorical variables.

TABLE 2. Risk of developing MetS at 5 yr by quintiles of baseline serum 25(OH)D

Quintile of serum 25(OH)D (range in ng/ml)	n	Incident cases, n (%)	OR (95% CI)		
			Model 1	Model 2	Model 3
1 (<18)	811	108 (13.3)	1.47 (1.07–2.02)	1.41 (1.02–1.95)	1.15 (0.80–1.67)
2 (18–23)	829	134 (16.1)	1.83 (1.35–2.48)	1.74 (1.28–2.37)	1.47 (1.04–2.09)
3 (24–27)	828	98 (11.8)	1.24 (0.90–1.69)	1.19 (0.87–1.64)	1.10 (0.77–1.57)
4 (28–33)	843	103 (12.2)	1.26 (0.93–1.72)	1.23 (0.90–1.68)	1.13 (0.79–1.61)
5 (34–93)	853	85 (10.0)	1.00 (reference)	1.00 (reference)	1.00 (reference)
P for trend			<0.01	<0.01	0.21

Model 1 was adjusted for age, sex, ethnicity, season, and latitude. Model 2 is model 1 plus smoking, family history of type 2 diabetes, physical activity, education and eGFR. Model 3 is model 2 plus HOMA2-IR. For model 3, the number of participants decreased from 4164 to 3334 due to the exclusion of extreme values and to missing data for baseline insulin. To convert serum 25(OH)D to nanomoles per liter, multiply by 2.5.

ticipants with incident MetS were also older, had a higher BMI, and were more likely to be of non-Europid origin, current or ex-smokers, less educated, less active, and to have a lower eGFR.

Associations between serum 25(OH)D and risk of developing MetS at 5 yr

For each 10-ng/ml decrease in 25(OH)D, the risk of developing the MetS at 5 yr increased by 23% [OR (95% CI) = 1.23 (1.11–1.37)] and by 22% [OR (95% CI) = 1.22 (1.09–1.36)], after adjusting for the confounders in model 1 and 2, respectively. The association between 25(OH)D and MetS risk was attenuated and became nonsignificant after further adjustment for HOMA2-IR [model 3 OR (95% CI) = 1.13 (0.99–1.28); $P = 0.052$]. Compared with participants in the highest quintile of 25(OH)D (≥34 ng/ml), the risk of developing the MetS was increased significantly until baseline 25(OH)D reached 24 ng/ml (Table 2). Indeed, participants in the lowest quintile of 25(OH)D (<18 ng/ml) had a 41% increased risk, whereas those in the second quintile (18–23 ng/ml) had a 74% increased risk of developing the MetS at 5 yr after adjusting for the confounders in model 2 [OR (95% CI) for quintile 1 = 1.41 (1.02–1.95) and for quintile 2 = 1.74 (1.28–2.37)] (Table 2). Although participants in the second quintile of 25(OH)D appeared to have a higher risk of developing the MetS than those in the first quintile, the risk between the two groups was not statistically significant [OR (95% CI) for the second *vs.* the first quintile after adjusting for the confounders in model 2 = 1.24 (0.93–1.64); $P = 0.14$]. When we further adjusted for baseline insulin resistance (HOMA2-IR), the association between baseline serum 25(OH)D and risk of developing the MetS was no longer evident (P for trend = 0.21) (Table 2).

Effect of BMI status on the association between serum 25(OH)D and MetS risk and relative contribution of serum 25(OH)D to the overall incidence of MetS

In participants with a normal BMI (<25 kg/m^2), the association between serum 25(OH)D and MetS risk was not significant, with or without the inclusion of BMI as a confounder [OR (95% CI) per 10-ng/ml decrease in 25(OH)D = 0.93 (0.73–1.17) and 0.96 (0.76–1.21), respectively] (Table 3). Conversely, in participants who were overweight or obese (BMI ≥25 kg/m^2), each 10-ng/ml decrease in 25(OH)D was associated with a 15% increased risk of developing the MetS at 5 yr [OR (95% CI) = 1.15 (1.01–1.32)], independent of BMI. However, with further adjustment for HOMA2-IR, the association disappeared [OR (95% CI) = 1.08 (0.93–1.24)].

Altogether, the risk factors associated with the development of the MetS in model 2 explained 16.4% of the variance in MetS risk. BMI explained 75.6% of this variance, followed by age (5.5%), sex (4.9%), and physical

TABLE 3. Risk of developing MetS at 5 yr per 10-ng/ml decrease in baseline serum 25(OH)D stratified by BMI status

	BMI <25 kg/m^2 (n = 2087)			BMI ≥25 kg/m^2 (n = 2066)		
	Incident cases [n (%)]	OR (95% CI)	*P* value	Incident cases [n (%)]	OR (95% CI)	*P* value
Cases	98 (4.7)			428 (20.7)		
Model 1		1.01 (0.81–1.27)	0.92		1.21 (1.06–1.37)	<0.01
Model 2		0.96 (0.76–1.21)	0.72		1.20 (1.05–1.36)	<0.01
Model 3		0.93 (0.73–1.17)	0.53		1.15 (1.01–1.32)	0.03
Model 4		0.99 (0.76–1.31)	0.97		1.08 (0.93–1.24)	0.30

Model 1 was adjusted for age, sex, ethnicity, season, and latitude. Model 2 is model 1 plus smoking, family history of type 2 diabetes, physical activity, education, and eGFR. Model 3 is model 2 plus BMI. Model 4 is model 3 plus HOMA2-IR. For model 4, the number of participants decreased to 1579 in the group with a BMI below 25 kg/m^2 and to 1746 in the group with a BMI of 25 kg/m^2 or higher due to the exclusion of insulin extreme values and to missing data for insulin at baseline. To convert 25(OH)D to nanomoles per liter, multiply by 2.5.

TABLE 4. Risk of developing components of the MetS at 5 yr per 10-ng/ml decrease in baseline serum 25(OH)D

MetS component	Model 1		Model 2	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
WC ≥102 cm (Europid men), ≥90 cm (non-Europid men), ≥88 cm (Europid women), or ≥80 cm (non-Europid women)	1.20 (1.10–1.33)	<0.001	1.20 (1.10–1.33)	<0.001
FPG ≥100.9 mg/dl	1.14 (1.03–1.25)	<0.01	1.14 (1.03–1.25)	<0.01
Triglycerides ≥150.4 mg/dl	1.14 (1.03–1.25)	0.01	1.16 (1.05–1.28)	<0.01
HDL-cholesterol <38.6 mg/dl for men or <50.2 mg/dl for women	1.02 (0.92–1.14)	0.70	1.02 (0.92–1.14)	0.70
Blood pressure: systolic ≥130 mm Hg and/or diastolic ≥ 85 mm Hg or on treatment	1.04 (0.95–1.14)	0.32	1.03 (0.94–1.14)	0.46
2-h PG ≥140.4 mg/dl	1.09 (0.94–1.25)	0.23	1.08 (0.93–1.25)	0.29
HOMA2-IR ≥0.89 U (above median)	1.19 (1.09–1.30)	<0.001	1.20 (1.09–1.31)	<0.001

Model 1 was adjusted for age, sex, ethnicity, season, latitude, and MetS component at baseline (categorical variable) plus WC. Model 2 is model 1 plus smoking, family history of type 2 diabetes, physical activity, education, and eGFR. To convert serum 25(OH)D to nanomoles per liter, multiply by 2.5.

activity (4.9%). The contribution of serum 25(OH)D to MetS risk was low (1.2%), which was comparable to the contribution of kidney function (0.6%), smoking (1.2%), family history of type 2 diabetes (1.8%), education (1.8%), and ethnicity (2.4%).

Associations between serum 25(OH)D and risk of developing the components of the MetS at 5 yr

When we examined the associations between baseline 25(OH)D and the components of the MetS at 5 yr, we found that there was a significant inverse relationship with WC ($P < 0.001$), triglycerides ($P < 0.01$), FPG ($P < 0.01$), and HOMA2-IR ($P < 0.001$) but not with 2-h PG ($P = 0.29$), HDL-cholesterol ($P = 0.70$), or blood pressure ($P = 0.46$) (Table 4). Of note, these associations remained significant after taking into account the confounders in model 2 plus the baseline MetS component and WC. All these results remained essentially unchanged after substituting WC for BMI or after adding dietary calcium and magnesium intakes as potential confounders to all models (data not shown).

Figure 1 displays the graphical representation of the relationship between the adjusted probabilities of developing MetS at 5 yr based on baseline serum 25(OH)D concentrations. It suggests that MetS risk was highest in individuals with baseline serum 25(OH)D below approximately 20 ng/ml and that it decreases progressively above this cut-point.

Discussion

To our knowledge, our study is the largest prospective study to have evaluated the association between serum 25(OH)D and the future risk of developing MetS. In this national, population-based sample of Australian adults, we have shown that serum 25(OH)D concentrations of 23 ng/ml or lower were associated with up to a significant 74% increased risk of developing MetS compared with those with concentrations of 34 ng/ml or higher. Low concentrations of serum 25(OH)D were also associated with higher WC, FPG, serum triglyceride levels, and insulin resistance (HOMA2-IR) at 5 yr, which are likely the basis of this increased MetS risk. Of note, these associations were independent of measures of adiposity at baseline. Contribution of serum 25(OH)D to the overall incidence

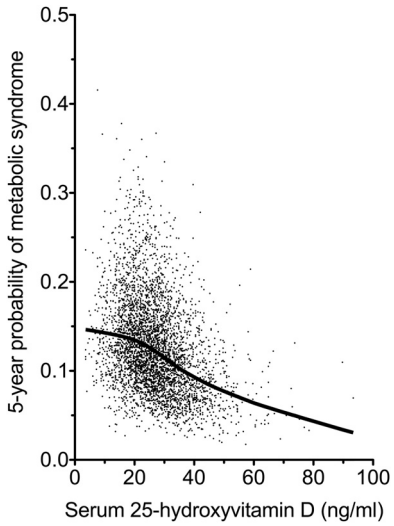

FIG. 1. Relationship between baseline serum 25(OH)D and adjusted probabilities of developing the metabolic syndrome at 5 yr. Spline regression model adjusted for age, sex, ethnicity, season, latitude, smoking, family history of type 2 diabetes, physical activity, education, and eGFR. To convert serum 25(OH)D to nanomoles per liter, multiply by 2.5.

of MetS was low, explaining about 1% of the variance in MetS risk.

We believe that the findings from our study confirm earlier suggestions (26) and provide strong evidence that serum 25(OH)D is an independent risk factor for the development of MetS. This is consistent with the findings reported by the only other small prospective study to have investigated this association. In this 10-yr follow-up of 524 randomly selected adults without diabetes, Forouhi *et al.* (14) reported that higher 25(OH)D was associated with significant decreases in MetS risk z-scores after adjusting for age, sex, smoking, season, BMI, and baseline MetS z-score ($P = 0.048$). However, this association was attenuated after taking into account other confounders [PTH, serum calcium, IGF-I, physical activity, and occupational social class ($P = 0.06$)], which could have been affected by low study power, given its limited sample size.

In our study, we found that the risk of developing MetS was highest (1.4- to 1.7-fold increase) in people with serum 25(OH)D concentrations below 24 ng/ml. When baseline serum 25(OH)D concentrations exceeded this threshold, MetS risk was not significantly increased compared with those with serum 25(OH)D of 34 ng/ml or higher. Moreover, the graphical representation of the spline regression model suggested that MetS risk was highest in individuals with baseline serum 25(OH)D concentrations below approximately 20 ng/ml. This serum 25(OH)D concentration is between the cutoffs suggested as adequate for bone health as recommended by the Institute of Medicine (20 ng/ml) and by The Endocrine Society (30 ng/ml) (27, 28). Although our findings suggest that MetS risk decreases sharply once serum 25(OH)D concentrations increase above 20 ng/ml and that MetS risk seems to somewhat plateau at a serum 25(OH)D concentration of approximately 50 ng/ml, caution should be applied when interpreting these data. Indeed, the number of observations is considerably lower at concentrations above 50 ng/ml than below, and the graphical representation does not allow the calculation of precise cutoffs. Although these findings should be viewed as exploratory to inform future studies, other studies have tried to estimate whether there is a threshold concentration for 25(OH)D above which MetS is decreased. In a cross-sectional study involving 1286 elderly Dutch men and women within the Longitudinal Aging Study Amsterdam, Oosterwerff *et al.* (8) found that MetS risk was highest in people with baseline serum 25(OH)D of approximately 16–20 ng/ml, based on their graphical representation of the adjusted spline regression model. Our findings are similar and indicate that for metabolic risk reduction, serum 25(OH)D concentrations need to be at least 20–23 ng/ml. However, to minimize MetS risk, serum 25(OH)D concentrations may need to be much higher but randomized controlled trials of vitamin D supplementation are required to answer this question.

To our knowledge, only one prospective study has specifically investigated the effects of increasing serum 25(OH)D concentration on MetS risk (29). Fifty-nine overweight and nondiabetic Saudis were asked to increase their sun exposure and dietary vitamin D intake for 1 yr, which led to a significant reduction in the prevalence of MetS from 25 to 13% ($P = 0.002$). However, there was no control group, and the serum 25(OH)D increased only from 8 to 11 ng/ml. Thus, although this study suggests that increasing serum 25(OH)D concentrations may reduce MetS risk, randomized controlled trials of vitamin D supplementation using vitamin D doses sufficient to increase serum 25(OH)D concentrations up to at least 20–30 ng/ml are necessary.

An important finding from our study was that the association between baseline serum 25(OH)D and the risk of developing MetS persisted after adjusting for BMI only in overweight or obese people but disappeared after taking into account baseline insulin resistance (HOMA2-IR). This suggests that insulin resistance mediates the association between 25(OH)D and MetS risk in people with a BMI of 25 kg/m^2 or higher. In line with this finding, we found that baseline 25(OH)D was inversely associated with a number of MetS components including WC, serum triglyceride, FPG levels, and HOMA2-IR at 5 yr and that these associations were independent of measures of obesity. Of note, the lack of association between serum 25(OH)D and MetS in our subgroup analysis of people with a BMI below 25 kg/m^2 should be interpreted with caution, because the number of incident MetS cases in this group was low.

Most previous cross-sectional studies in adults and children have also reported that 25(OH)D was inversely associated with WC (1–5, 7–9, 12, 13). It remains unknown whether low 25(OH)D concentrations are solely a consequence of obesity (30) or whether vitamin D deficiency plays a causal role in the accumulation of adipose tissue in the omental depot. Indeed, data from *in vitro* studies suggest that vitamin D is an important inhibitor of adipocyte differentiation (31). Our finding of an inverse association between 25(OH)D and serum triglyceride levels is also consistent with the majority of previous research (1–7, 9) and could be related to the activation of lipoprotein lipase by vitamin D in adipocytes (32). There are several studies supporting the idea that vitamin D might be involved in the maintenance of glucose homeostasis via the demonstration of an inverse association between 25(OH)D and insulin resistance (4, 7, 12, 14, 16), fasting insulin (4, 5, 7, 14), FPG (1, 3, 4), 2-h PG (14), glycated hemoglobin (2, 4),

and risk of developing type 2 diabetes (16, 33–35). The mechanisms through which vitamin D may affect glucose homeostasis have been described in detail previously (36). It may be related to an indirect effect of 25(OH)D on glucose homeostasis via maintenance of normocalcemia intracellularly. It may also be due to direct effects on insulin production and secretion and insulin sensitivity via an increase in insulin receptor transcription and translocation of glucose transporters to the cell membrane of insulin-sensitive cells. Finally, reports on 25(OH)D and blood pressure have yielded inconsistent findings with many studies finding no association (1, 5, 8, 9, 14), whereas others have reported an association with either systolic (3) or diastolic blood pressure (4) alone, or both (2, 7). These discrepancies may be explained by the different definitions of hypertension used and the confounders used as adjustments in these studies. In addition, although evidence from animal studies suggest that vitamin D plays a role in the inhibition of the renin-angiotensin system, supplementation trials with vitamin D for improving blood pressure in humans have been inconsistent (37).

Our study has several limitations. First, we did not have data on PTH levels, which may be important because it is strongly related to 25(OH)D and has been shown, in some studies, to be associated with MetS and its components (38, 39). Second, the drop in participant numbers in the analyses involving the insulin resistance index HOMA2-IR could have affected the results in two ways: 1) the decrease in statistical power could explain the disappearance of the association between serum 25(OH)D and MetS risk after we adjusted for HOMA2-IR; and 2) in addition, some characteristics of the participants who were excluded from the HOMA2-IR analyses (n = 882) were significantly different from those who were included (n = 3282): they were younger, more educated, and had a better kidney function and higher serum 25(OH)D concentration and a lower WC and serum triglyceride level. Third, compared with the original AusDiab cohort, participants who were included in this analysis had higher baseline 25(OH)D and had a lower MetS risk profile (younger, more physically active, less likely to be current/ex-smokers and hypertensive, and with lower baseline WC, FPG, 2-h PG, serum triglyceride, and HDL-cholesterol), which could have biased our results. Fourth, serum 25(OH)D was measured only at baseline, which may not reflect long-term vitamin D status. However, data from a 14-yr prospective study showed that serum 25(OH)D concentrations track within z-score quintiles, especially for those with the highest and lowest concentrations (40). Finally, our results cannot be extrapolated to non-Europids given that their number in our study was very small. Nevertheless, our study has a number of strengths. It is the largest prospective study to have evaluated the association between 25(OH)D and incidence of the MetS. More importantly, serum 25(OH)D was measured in the entire population at baseline and in the one laboratory, and thus we had adequate study power to account for several important potential confounders in our analyses.

In conclusion, we have confirmed in a large prospective, population-based cohort of Australian adults that lower serum 25(OH)D concentrations are associated with an increased risk of developing MetS at 5 yr and that this increased risk is likely to be related to an inverse association between 25(OH)D and WC, serum triglyceride, FPG levels, and insulin resistance, measured by HOMA2-IR. Furthermore, the association between serum 25(OH)D and MetS risk was evident among those with high BMI at baseline. However, the contribution of serum 25(OH)D to MetS risk was low, explaining about 1% of the variance in MetS risk. Randomized, placebo-controlled trials of vitamin D supplementation are required to establish causality between low serum 25(OH)D concentrations and future risk of developing MetS.

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