

Associations of Serum Concentrations of 25-Hydroxyvitamin D and Parathyroid Hormone with Surrogate Markers of Insulin Resistance among U.S. Adults without Physician-Diagnosed Diabetes: National Health and Nutrition Examination Survey, 2003-2006

Running title: Vitamin D, Parathyroid Hormone and Insulin Resistance

GUIXIANG ZHAO, MD, PHD
EARL S. FORD, MD, MPH
CHAOYANG LI, MD, PHD

Division of Adult and Community Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia

Corresponding author:
Guixiang Zhao, M.D., Ph.D.
Email: GZhao@cdc.gov

Submitted 20 May 2009 and accepted 13 October 2009.

This is an uncopyedited electronic version of an article accepted for publication in *Diabetes Care*. The American Diabetes Association, publisher of *Diabetes Care*, is not responsible for any errors or omissions in this version of the manuscript or any version derived from it by third parties. The definitive publisher-authenticated version will be available in a future issue of *Diabetes Care* in print and online at <http://care.diabetesjournals.org>.

Objective– To examine whether concentrations of serum 25-hydroxyvitamin D (25[OH]D) and parathyroid hormone (PTH) are associated with surrogate markers of insulin resistance (IR) in U.S. adults without physician-diagnosed diabetes.

Research design and methods– Cross-sectional data (n=3,206) from the 2003-2006 National Health and Nutrition Examination Survey were analyzed.

Results– The age-adjusted prevalence of hyperinsulinemia, high homeostatic model assessment-IR, high glycohemoglobin, and fasting and 2-hour hyperglycemia decreased linearly across quintiles of 25(OH)D but increased linearly across quintiles of PTH (except for a quadratic trend for fasting hyperglycemia). After extensive adjustment for potential confounders, the relationships between 25(OH)D and the markers of IR and 2-hour hyperglycemia persisted. Only hyperinsulinemia was positively associated with PTH ($P<0.05$).

Conclusions– Among U.S. adults without physician-diagnosed diabetes, low concentrations of serum 25(OH)D were associated with markers of IR. The role of PTH in IR deserves further investigation.

The role of vitamin D and parathyroid hormone (PTH) in metabolic syndrome and diabetes is receiving increased attention. Insulin resistance (IR) may represent a potential mechanism linking vitamin D and PTH to these conditions. The inverse associations between vitamin D and fasting insulin concentrations or the homeostatic model assessment of IR [HOMA-IR] index have been reported in some (1-5) but not all studies (6). Moreover, evidence linking PTH to markers of IR is limited and inconsistent (7-9). This study examined whether serum 25-hydroxyvitamin D (25[OH]D) and PTH are associated with surrogate markers of IR in U.S. adults without physician-diagnosed diabetes.

RESEARCH DESIGN AND METHODWe used data from the National Health and Nutrition Examination Survey (NHANES) 2003-2006. Participants who were aged ≥ 20 years, attended the morning examination after fasting ≥ 8 hours, and had not been diagnosed with diabetes were included as were participants with undiagnosed diabetes (fasting glucose ≥ 126 mg/dL or glycohemoglobin $\geq 6.5\%$) (10). Serum 25(OH)D concentrations were measured using a radioimmunoassay procedure. Serum PTH concentrations were measured on the Elecsys 1010 analyzer using an electrochemiluminescent procedure. The quintiles of 25(OH)D and PTH were created after taking into account the sampling weights.

Plasma concentrations of fasting and 2-hour glucose, fasting insulin, and glycohemoglobin were adjusted for differences in laboratory methodology between NHANES 2003-2004 and 2005-2006. Oral glucose tolerance test data were available only for NHANES 2005-2006. We defined fasting hyperglycemia as a fasting glucose ≥ 100 mg/dL, 2-hour hyperglycemia as a 2-hour glucose ≥ 140

mg/dL, and high glycohemoglobin as a value of $\geq 6.0\%$. HOMA-IR index was calculated as (fasting plasma insulin [mU/L] x fasting plasma glucose [mmol/L])/22.5. Hyperinsulinemia and high HOMA-IR were defined using the weighted 75th percentiles.

Covariates in our analyses included age, sex, race/ethnicity, education, smoking, physical activity, alcohol drinking, body mass index (BMI), abdominal obesity, and serum calcium concentrations. From 3,551 participants without physician-diagnosed diabetes, 3,206 (1,582 men, 1,624 women) remained in our analyses after excluding those who had missing values for study variables. The prevalence of surrogate markers of IR was age-standardized to the 2000 U.S. population. Linear trends across quintiles of 25(OH)D and PTH were tested using orthogonal contrasts in SUDAAN software. Multiple logistic regression analyses were conducted to test associations of 25(OH)D or PTH with markers of IR.

RESULTS

Among 3,206 participants, 118 had undiagnosed diabetes. The age-adjusted prevalence was 5.7% (95% confidence interval [CI]: 4.8-6.7%) for high glycohemoglobin, 30.6% (95% CI: 27.8-33.6%) for fasting hyperglycemia, and 20.4% (95% CI: 17.7-23.4%) for 2-hour hyperglycemia. The prevalence of all outcome measures and the multivariate-adjusted odds ratios for hyperinsulinemia, high HOMA-IR, high glycohemoglobin, and 2-hour hyperglycemia decreased linearly across quintiles of 25(OH)D ($P < 0.05$ for all, Table 1). After excluding participants without physician-diagnosed diabetes but who had diabetes based on fasting glucose or glycohemoglobin values, similar results were observed except the significant association between high glycohemoglobin and 25(OH)D disappeared.

Interactions between race/ethnicity and 25(OH)D for outcome measures were not significant in the full models.

The prevalence of hyperinsulinemia, high HOMA-IR, high glycohemoglobin, and 2-hour hyperglycemia increased linearly, and the prevalence of fasting hyperglycemia increased nonlinearly across quintiles of PTH. After adjusting for demographic and lifestyle factors, the odds ratios for hyperinsulinemia, high HOMA-IR, and high glycohemoglobin were significantly higher in the highest than in the lowest quintile of PTH, and significantly increasing trends existed for all measures except for 2-hour hyperglycemia. After further adjusting for all potential confounders, most of the associations lost statistical significance; only an increasing trend for hyperinsulinemia across quintiles of PTH persisted ($P < 0.05$). These results did not change much after excluding participants without physician-diagnosed diabetes but who had diabetes based on fasting glucose or glycohemoglobin values.

CONCLUSIONS

Our findings of an inverse association between 25(OH)D and IR among adults without physician-diagnosed diabetes are consistent with previous findings from cross-sectional (1-4) and prospective (5) studies. These results offer further support that lower concentrations of 25(OH)D may be a predictor of increased likelihood of diabetes in the population (11-14).

Compared with previous studies that examined the associations of 25(OH)D with IR (1-4), an advantage of our study was that we were able to simultaneously examine the associations between serum 25(OH)D and PTH—both of which play an essential role in regulating calcium homeostasis—and IR. In addition, we were able to adjust for overall obesity (i.e., BMI) and abdominal obesity, which were strong confounders for the analyses. The expression of vitamin D

receptors in both pancreatic β -cells and skeletal muscle cells, which, upon activation by vitamin D supplementation, result in increased insulin release and responsiveness to insulin for glucose transport (14), may serve as an underlying mechanism.

Our results regarding possible racial/ethnic disparities in the associations of 25(OH)D with IR conflict with those from Scragg et al.(3). Given the high proportion of African-Americans with vitamin D deficiency or insufficiency, the issue of possible racial/ethnic disparities deserves further investigation (15).

Primary hyperparathyroidism was associated with impaired glucose tolerance, insulin insensitivity and diabetes (7). However, a significant correlation between PTH and HOMA-IR was observed in adults aged 70 years with an average BMI of 27 kg/m² (8), but not in middle-aged, morbidly obese adults (an average BMI: 44.7 kg/m²) (9). These studies were conducted in selected populations and the data analyses did not adequately control for potential confounders. Our study based on a nationally representative sample showed a significant association between PTH and hyperinsulinemia after adjusting for potential confounders. However, analyses limited to participants with concentrations of 25(OH)D <30 ng/mL (n=2,518), a point at which PTH concentrations begin to increase, revealed no associations between PTH and hyperinsulinemia or HOMA-IR.

Our study was limited by the inability to establish the causality between 25(OH)D and IR based on our cross-sectional study, and by the inability to account for sunlight exposure due to lack of data.

In conclusion, low concentrations of 25(OH)D were associated with markers of IR among U.S. adults without physician-diagnosed diabetes. Future prospective studies and intervention trials are needed to confirm

the associations of 25(OH)D with IR, and to further investigate the role of PTH in IR.

ACKNOWLEDGEMENTS

Disclosure: No potential conflicts of interests relevant to this article were reported.

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Sources of financial support: None.

REFERENCES

1. Liu E, Meigs JB, Pittas AG, McKeown NM, Economos CD, Booth SL, Jacques PF: Plasma 25-hydroxyvitamin d is associated with markers of the insulin resistant phenotype in nondiabetic adults. *J Nutr* 139:329-334, 2009
2. Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, Li X, Yang X, Chen Y, Lin X. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. *Diabetes Care* 32:1278-1283, 2009
3. Scragg R, Sowers M, Bell C: Serum 25-hydroxyvitamin D, diabetes, and ethnicity in the Third National Health and Nutrition Examination Survey. *Diabetes Care* 27:2813-2818, 2004
4. Chiu KC, Chu A, Go VL, Saad MF: Hypovitaminosis D is associated with insulin resistance and beta cell dysfunction. *Am J Clin Nutr* 79:820-825, 2004
5. Forouhi NG, Luan J, Cooper A, Boucher BJ, Wareham NJ: Baseline serum 25-hydroxy vitamin d is predictive of future glycemic status and insulin resistance: the Medical Research Council Ely Prospective Study 1990-2000. *Diabetes* 57:2619-2625, 2008
6. Gannagé-Yared MH, Chedid R, Khalife S, Azzi E, Zoghbi F, Halaby G. Vitamin D in relation to metabolic risk factors, insulin sensitivity and adiponectin in a young Middle-Eastern population. *Eur J Endocrinol* 160:965-971, 2009
7. Khaleeli AA, Johnson JN, Taylor WH: Prevalence of glucose intolerance in primary hyperparathyroidism and the benefit of parathyroidectomy. *Diabetes Metab Res Rev* 23:43-48, 2007
8. Ahlstrom T, Hagstrom E, Larsson A, Rudberg C, Lind L, Hellman P: Correlation between plasma calcium, parathyroid hormone and the metabolic syndrome in a community-based cohort of men and women. *Clin Endocrinol (Oxf)* 2009 (DOI: 10.1111/j.1365-2265.2009.03558.x)
9. Hjelmessaeth J, Hofso D, Aasheim ET, Jenssen T, Moan J, Hager H, Roislien J, Bollerslev J: Parathyroid hormone, but not vitamin D, is associated with the metabolic syndrome in morbidly obese women and men: a cross-sectional study. *Cardiovasc Diabetol* 8:7-13, 2009
10. International Expert Committee. International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care* 32:1327-1334, 2009
11. Knekt P, Laaksonen M, Mattila C, Harkanen T, Marniemi J, Heliovaara M, Rissanen H, Montonen J, Reunanen A: Serum vitamin D and subsequent occurrence of type 2 diabetes. *Epidemiology* 19:666-671, 2008
12. Pittas AG, Dawson-Hughes B, Li T, Van Dam RM, Willett WC, Manson JE, Hu FB: Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care* 29:650-656, 2006
13. Ford ES: Vitamin supplement use and diabetes mellitus incidence among adults in the United States. *Am J Epidemiol* 153:892-897, 2001
14. Borissova AM, Tankova T, Kirilov G, Dakovska L, Kovacheva R: The effect of vitamin D3 on insulin secretion and peripheral insulin sensitivity in type 2 diabetic patients. *Int J Clin Pract* 57:258-261, 2003
15. Ginde AA, Liu MC, Camargo CA Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. *Arch Intern Med* 169:626-632, 2009

Table 1. Age-adjusted prevalence and adjusted odds ratios (with 95% confidence intervals) of the surrogate markers of insulin resistance by quintiles of serum 25-hydroxyvitamin D and parathyroid hormone concentrations among U.S. adults aged ≥ 20 years without physician-diagnosed diabetes, NHANES 2003-2006 (n=3,206)*

| | N | Hyperinsulinemia | High HOMA-IR | High glycohemoglobin | Fasting hyperglycemia | 2-hour hyperglycemia† |
|-----------------------------|-----|------------------|------------------|----------------------|-----------------------|-----------------------|
| Quintiles of 25(OH)D | | | | | | |
| Prevalence (%) | | | | | | |
| Q1 (<15 ng/mL) | 690 | 37.0 (32.3-42.0) | 37.6 (32.8-42.7) | 10.8 (8.6-13.4) | 32.2 (27.4-37.2) | 26.0 (22.9-29.3) |
| Q2 (15-<21 ng/mL) | 731 | 33.5 (29.2-38.1) | 34.2 (29.8-38.9) | 6.8 (5.1-8.9) | 32.6 (29.1-36.3) | 23.0 (18.8-27.8) |
| Q3 (21-<25 ng/mL) | 558 | 23.6 (19.9-27.9) | 22.7 (18.5-27.4) | 5.3 (3.6-7.5) | 31.8 (28.0-35.9) | 21.0 (16.8-25.9) |
| Q4 (25-<31 ng/mL) | 629 | 17.6 (13.2-23.1) | 17.7 (13.3-23.0) | 4.6 (3.2-6.6) | 31.4 (26.4-36.9) | 16.8 (11.7-23.5) |
| Q5 (≥ 31 ng/mL) | 598 | 13.3 (9.7-18.0) | 13.9 (10.4-18.5) | 2.8 (1.8-4.3) | 25.7 (21.4-30.6) | 13.6 (8.2-21.6) |
| <i>P-trend</i> | | <0.001 | <0.001 | <0.001 | 0.035 | <0.001 |
| Model 1 | | | | | | |
| Q1 (<15 ng/mL) | 690 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q2 (15-<21 ng/mL) | 731 | 0.81 (0.61-1.08) | 0.81 (0.62-1.07) | 0.56 (0.39-0.81) | 0.96 (0.74-1.24) | 0.82 (0.58-1.16) |
| Q3 (21-<25 ng/mL) | 558 | 0.49 (0.38-0.62) | 0.44 (0.33-0.60) | 0.42 (0.26-0.68) | 0.89 (0.67-1.18) | 0.73 (0.48-1.12) |
| Q4 (25-<31 ng/mL) | 629 | 0.34 (0.23-0.51) | 0.33 (0.23-0.47) | 0.36 (0.22-0.59) | 0.86 (0.61-1.20) | 0.52 (0.30-0.90) |
| Q5 (≥ 31 ng/mL) | 598 | 0.24 (0.17-0.36) | 0.25 (0.17-0.36) | 0.20 (0.12-0.33) | 0.64 (0.48-0.86) | 0.39 (0.20-0.76) |
| <i>Wald- Chisq P</i> | | <0.001 | <0.001 | <0.001 | 0.006 | 0.002 |
| <i>P-trend</i> | | <0.001 | <0.001 | <0.001 | 0.005 | 0.001 |
| Model 2 | | | | | | |
| Q1 (<15 ng/mL) | 690 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q2 (15-<21 ng/mL) | 731 | 0.80 (0.61-1.06) | 0.80 (0.61-1.05) | 0.69 (0.45-1.07) | 0.94 (0.70-1.25) | 0.77 (0.52-1.15) |
| Q3 (21-<25 ng/mL) | 558 | 0.45 (0.36-0.58) | 0.42 (0.31-0.56) | 0.58 (0.35-0.96) | 0.88 (0.66-1.18) | 0.68 (0.43-1.05) |
| Q4 (25-<31 ng/mL) | 629 | 0.32 (0.21-0.47) | 0.31 (0.21-0.44) | 0.52 (0.29-0.93) | 0.84 (0.58-1.23) | 0.48 (0.28-0.85) |
| Q5 (≥ 31 ng/mL) | 598 | 0.22 (0.15-0.34) | 0.23 (0.15-0.34) | 0.30 (0.17-0.51) | 0.65 (0.46-0.92) | 0.36 (0.16-0.77) |
| <i>Wald- Chisq P</i> | | <0.001 | <0.001 | <0.001 | 0.034 | 0.003 |
| <i>P-trend</i> | | <0.001 | <0.001 | <0.001 | 0.016 | 0.002 |
| Model 3 | | | | | | |
| Q1 (<15 ng/mL) | 690 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q2 (15-<21 ng/mL) | 731 | 1.04 (0.74-1.46) | 1.02 (0.73-1.41) | 0.75 (0.46-1.23) | 1.01 (0.77-1.32) | 0.79 (0.54-1.15) |
| Q3 (21-<25 ng/mL) | 558 | 0.63 (0.43-0.92) | 0.54 (0.36-0.80) | 0.74 (0.43-1.29) | 1.08 (0.82-1.42) | 0.75 (0.50-1.14) |
| Q4 (25-<31 ng/mL) | 629 | 0.44 (0.27-0.73) | 0.41 (0.26-0.63) | 0.70 (0.39-1.26) | 1.07 (0.78-1.47) | 0.58 (0.34-0.98) |
| Q5 (≥ 31 ng/mL) | 598 | 0.42 (0.24-0.71) | 0.41 (0.25-0.66) | 0.46 (0.25-0.82) | 0.87 (0.64-1.17) | 0.50 (0.23-1.01) |

| | | | | | |
|----------------------|--------|--------|-------|-------|-------|
| <i>Wald- Chisq P</i> | <0.001 | <0.001 | 0.098 | 0.489 | 0.082 |
| <i>P-trend</i> | <0.001 | <0.001 | 0.019 | 0.362 | 0.024 |

Quintiles of PTH

| | | | | | | |
|----------------------|-----|------------------|------------------|------------------|--------------------|------------------|
| Prevalence (%) | | | | | | |
| Q1 (<27 pg/mL) | 564 | 16.0 (12.6-20.1) | 15.6 (11.7-20.5) | 4.2 (2.3-7.6) | 30.3 (24.7-36.6) | 13.2 (8.2-20.5) |
| Q2 (27-<34 pg/mL) | 571 | 18.1 (14.4-22.4) | 20.0 (15.5-23.5) | 3.1 (1.9-5.1) | 29.2 (24.9-33.9) | 20.0 (16.1-24.7) |
| Q3 (34-<42 pg/mL) | 635 | 24.1 (21.0-27.5) | 23.4 (20.2-26.9) | 5.0 (3.7-6.8) | 29.4 (24.4-35.0) | 20.7 (15.8-26.7) |
| Q4 (42-<54 pg/mL) | 685 | 31.3 (27.3-35.7) | 31.9 (28.2-35.9) | 6.8 (5.1-9.1) | 28.9 (25.2-32.9) | 24.4 (19.5-30.1) |
| Q5 (≥ 54 pg/mL) | 751 | 34.4 (29.9-39.2) | 33.4 (28.7-38.6) | 8.5 (6.9-10.3) | 37.3 (33.1-41.7) | 20.7 (16.5-25.6) |
| <i>P-trend</i> | | <0.001 | <0.001 | <0.001 | 0.036 [‡] | 0.007 |
| Model 1 | | | | | | |
| Q1 (<27 pg/mL) | 564 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q2 (27-<34 pg/mL) | 571 | 1.28 (0.94-1.75) | 1.46 (1.00-2.14) | 0.87 (0.37-2.06) | 1.07 (0.74-1.54) | 1.79 (0.93-3.45) |
| Q3 (34-<42 pg/mL) | 635 | 1.83 (1.33-2.52) | 1.76 (1.19-2.62) | 1.38 (0.66-2.86) | 1.06 (0.71-1.58) | 2.05 (1.21-3.48) |
| Q4 (42-<54 pg/mL) | 685 | 2.70 (1.92-3.80) | 2.81 (1.98-3.99) | 1.96 (1.03-3.70) | 1.05 (0.71-1.56) | 2.59 (1.30-5.17) |
| Q5 (≥ 54 pg/mL) | 751 | 3.16 (2.34-4.27) | 3.08 (2.13-4.45) | 2.50 (1.30-4.79) | 1.59 (1.08-2.34) | 1.98 (1.08-3.64) |
| <i>Wald- Chisq P</i> | | <0.001 | <0.001 | <0.001 | 0.005 | 0.040 |
| <i>P-trend</i> | | <0.001 | <0.001 | <0.001 | 0.008 | 0.052 |
| Model 2 | | | | | | |
| Q1 (<27 pg/mL) | 564 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q2 (27-<34 pg/mL) | 571 | 1.26 (0.93-1.73) | 1.44 (0.98-2.11) | 0.82 (0.35-1.92) | 1.02 (0.72-1.46) | 1.71 (0.86-3.41) |
| Q3 (34-<42 pg/mL) | 635 | 1.72 (1.25-2.37) | 1.66 (1.11-2.47) | 1.30 (0.65-2.58) | 1.00 (0.67-1.49) | 1.89 (1.04-3.46) |
| Q4 (42-<54 pg/mL) | 685 | 2.52 (1.79-3.56) | 2.61 (1.82-3.74) | 1.72 (0.92-3.19) | 0.99 (0.66-1.47) | 2.38 (1.09-5.20) |
| Q5 (≥ 54 pg/mL) | 751 | 2.82 (2.04-3.90) | 2.72 (1.84-4.02) | 1.98 (1.04-3.74) | 1.48 (0.97-2.26) | 1.73 (0.86-3.47) |
| <i>Wald- Chisq P</i> | | <0.001 | <0.001 | <0.001 | 0.024 | 0.016 |
| <i>P-trend</i> | | <0.001 | <0.001 | 0.001 | 0.031 | 0.209 |
| Model 3 | | | | | | |
| Q1 (<27 pg/mL) | 564 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Q2 (27-<34 pg/mL) | 571 | 1.12 (0.87-1.46) | 1.33 (0.92-1.94) | 0.75 (0.31-1.83) | 0.97 (0.66-1.43) | 1.55 (0.75-3.23) |
| Q3 (34-<42 pg/mL) | 635 | 0.98 (0.70-1.38) | 0.92 (0.60-1.41) | 1.01 (0.48-2.11) | 0.82 (0.56-1.20) | 1.52 (0.86-2.70) |
| Q4 (42-<54 pg/mL) | 685 | 1.37 (0.94-1.99) | 1.42 (0.98-2.05) | 1.19 (0.62-2.26) | 0.74 (0.49-1.10) | 1.67 (0.78-3.55) |
| Q5 (≥ 54 pg/mL) | 751 | 1.39 (0.99-1.96) | 1.29 (0.85-1.96) | 1.20 (0.61-2.37) | 1.13 (0.75-1.72) | 1.06 (0.57-1.94) |
| <i>Wald- Chisq P</i> | | 0.119 | 0.006 | 0.327 | 0.019 | 0.096 |
| <i>P-trend</i> | | 0.049 | 0.231 | 0.163 | 0.430 | 0.267 |

* The weighted 75th percentile cutoff points were 12.4 mU/L for fasting plasma insulin and 3.1 for HOMA-IR. Model 1: adjusted for age and sex, Model 2: adjusted for age, sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, and other), education (< high-school diploma, high school graduate, and > high-school diploma), smoking (current, former and never), heavy alcohol drinking (>2 drinks/day in men and >1 drink/day in women), and physical activity (engaging in moderate or vigorous physical activity daily for at least 10 minutes), Model 3: adjusted for variables in model 2 plus abdominal obesity (waist circumference >102 cm for men and WC>88 cm for women), body mass index (continuous, calculated from measured weight and height), and serum concentrations of calcium (continuous) and PTH (or vitamin D). †Data from NHANES 2005-2006 only (n=1,412 in total), ‡for a quadratic trend.