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In Vitro Model of Vitamin D Synthesis by UV Radiation in an Australian Urban Environment

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ABSTRACT

Vitamin D, an important constituent of human health, is produced through exposure of human skin to short wave (280–315 nm) ultraviolet radiation (UV). We aimed to establish whether an urbanized environment with tall buildings in close proximity (an 'urban canyon') significantly reduced the capacity of sunlight to synthesize vitamin D, when compared to a typical suburban area (~2.5 km away); and to investigate the association of UV and vitamin D production with pollution, temperature, and humidity. Measurements of ambient UV (295–400 nm) (using a portable photometer/radiometer and detector) and synthesized vitamin D (from an *in vitro* model) were taken regularly at urban and control sites over three months in Brisbane, Australia. During a typical 20-minute measurement, urban and control sites received 0.26 and 1.03 W/m^2 mean total UV respectively ($p<0.001$), and produced 0.12 and 0.53 μg/mL mean vitamin D ($p<0.001$). Pollution, temperature and humidity were not associated with UV or vitamin D production. This demonstrates a large difference in vitamin D synthesis between an urban canyon and a nearby control site. Although the results cannot be directly applied to humans, they emphasize the need for further study of human vitamin D production in urban environments.

INTRODUCTION

A large number of studies have investigated both the positive and negative effects of human exposure to ultraviolet radiation (UV) (1-3). Exposure to the skin of UV, especially the UV-B part of the spectrum (280–315 nm wavelengths), can result in the photoproduction of vitamin D (4), which has benefits for human health. Negative effects of UV exposure can include sunburn, eye damage, and photoageing (5). UV exposure also induces DNA damage in skin epidermal cells, and thus is established as one of the main causes of skin cancer (5). A variety of geophysical factors influence whether or not the ambient UV is sufficient for humans to produce vitamin D and /or DNA damage such as latitude, season, time of day, cloud cover, aerosol content (fine, solid or liquid particles suspended in the air), and shade from natural or man-made structures (6-9); the final two being particularly relevant in urban environments.

An increasing proportion of the population worldwide live in urban environments (10), which are susceptible to high levels of pollution (9), and are associated with increased building density and height. The term 'urban canyon' has been used to refer to a three dimensional space of street bounded by tall buildings on either side (11). The environmental factors imply that within an urban canyon UV availability at street level would be reduced. One past study has measured this empirically; in a small urban canyon in Poland (12), levels of UV measured at street level were lower than those measured at a nearby weather monitoring station located outside the urban canyon. However, this study was limited by data collection on only four days.

If the availability of UV is lower within urban environments, this may lead to a subsequent decrease in the amount of vitamin D produced by those living and/or working in urban

canyons. Urban environments were first linked to vitamin D-deficiency related health conditions in the 1800s, when children living in industrialized cities were found to have an increased incidence of rickets (13). More recently, several small studies have shown that living in an urban environment can be related to vitamin D deficiency. In both a Thai and Belgian study, women living in urban areas were found to have higher odds of being vitamin D deficient, compared to those living in nearby suburban or rural areas (14-15). In India, a study found that urban adults had lower levels of vitamin D compared to rural adults (16). Furthermore, with regards to the effect of pollution, another Indian study found that young children living in an urban area of Delhi with high levels of air pollution had lower vitamin D compared to children living in a similar area of the city with lower levels of pollution (17).

No previous study has specifically measured the amount of available ambient UV (on the portion of the spectrum for which vitamin D is produced) within an urban canyon, or simultaneously measured subsequent vitamin D production. Therefore, the aim of this study was to establish whether ambient UV consistently differed between an urban canyon site and a control site over a period of time, and to determine whether vitamin D production was reduced at the urban site compared to the control, based on an *in vitro* model which responds to the same wavelengths as does human skin (18-19). We also aimed to investigate the effect of air pollution, humidity, and temperature on the availability of UV and vitamin D production.

MATERIALS AND METHODS

Setting: Brisbane, Australia, is a subtropical metropolitan city with approximately 1.5 million residents located at 27°S, 153°E, 29 m above sea level (ASL). The study was conducted during winter, in which weather conditions are commonly very stable, with very little cloud cover and very low precipitation. We chose four urban canyon sites within the central

business district (CBD), and one suburban control site. For a site to be classified as an 'urban canyon' it had to have at least 50% of each side bordered by buildings of no less than five stories and no more than 30% of each side of the street bordered by building of two stories or fewer. The control site was located approximately 2.5 kilometers from the CBD in an open, unshaded area.

***In vitro* vitamin D Model:** We assessed vitamin D production using an *in vitro* model slightly modified from a model described previously by others (8) to mirror the reactions that occur in human skin. In humans, the precursor molecule, 7-dehydrocholesterol (7-DHC), is converted into previtamin D when exposed to UV-B radiation. Over a period of 8 to 24 hours, previtamin D isomerizes into vitamin D by a thermal reaction that occurs at skin temperature. Vitamin D (cholecalciferol) is then transported into the bloodstream for hydroxylations in the liver and kidneys.

In the *in vitro* model used in this work, 7-DHC in powdered form (Sigma–Aldrich Castle Hill, Australia D4429-5 G 93.7% purity) was dissolved in ethanol (Sigma 270741-2 L Chromasolv for High Performance Liquid Chromatography (HPLC)). To create a sample, 2 mL of solution was placed into a UV-transmissive quartz cuvette (Starna Ptd. Ltd. Product code: 21/Q/10; 1 cm path-length with an 85% average transmission over the UV spectrum). After exposure to UV, the solution was incubated at 37ºC for a standardized time period of 24 hours to allow previtamin D to isomerize into vitamin D (cholecalciferol) (20).

A concentration of 300 µg/mL of 7-DHC in ethanol was used for all experiments, based on previous work with *in vitro* models of vitamin D synthesis (21-25). Samples were shielded from UV during transportation to the measurement sites, where they were exposed for 20 minutes and then replaced into the sleeves. Upon return to the laboratory, the samples were

promptly incubated as per the model, and then analyzed by HPLC. Care was taken to ensure no sources emitting UV sources were used during the laboratory analysis.

HPLC Analysis: This was performed using a method adopted from Porter et al. (26). The mobile phase was methanol:ethanol:water (86:10:4) (Ajax FineChem, UNICHROM Methanol, A2314-4 L, Taren Point, Australia; Sigma–Aldrich, CHROMASOLV, 270741-2 L, Castle Hill, Australia; >18 MΩ-cm MilliQ H_2O). We used a 150 mm (length) x 4.6 mm (diameter) reverse-phase C-18 column with 5 µm-silica particles (Phenomenex Gemini 110A, Lane Cove, Australia). Column temperature was stabilized to 40.0 ± 0.1 ºC during analysis. The flow rate was 1.5 mL/min, which caused cholecalciferol and 7-DHC to elute at 5.2 min and 6.1 min, respectively. The analysis time was 7 min per sample. Detection was with a dual-wavelength UV–VIS absorbance detector, which was optimized to the peak absorption wavelengths of 7-DHC and cholecalciferol, respectively.

Field Collection Protocols: During exposure of the cuvettes, ambient erythemal UV (295–400 nm) was measured at each site using a portable photometer/radiometer (Solar Light Company model PMA2100) and detector (Solar Light Company PMA2101). The UV irradiance was measured on a horizontal surface (all were undertaken using the same protocol at all locations). Instruments were calibrated against the UV radiometer located on the roof of the AusSun laboratory. In the field, a CCD spectrometer (StellarNet model epp2000) was used simultaneously to scan the total UV spectrum (biologically un-weighted). The ambient UV radiation was measured in one nanometre (nm) steps across the entire UV spectrum and this data was then summed into wave bands of total UV (295–400 nm), UV-A (315–400 nm), and UV-B (280–315 nm). Data were integrated over the entire exposure period (20 minutes) and a single value of erythemal UV was recorded for each exposure.

Field data were collected between June 22, 2007 and August 30, 2007. Data were collected at the four urban canyon sites following a rotating schedule of two sites per day, three times a day (9 am, 12 pm, and 3 pm), three days per week (Monday, Wednesday, Friday) (data collected at a total of 84 time-points). Data were collected at the control site at the same times (9 am, 12 pm, and 3 pm) twice a week (Tuesday, Thursday) (data collected at a total of 29 time-points).

Pollution monitoring: Measurements of visibility reducing particles (visibility loss due to haze), and PM-10 sized particles (particles less than 10 microns in diameter measured in $\mu g/m^3$) at time points corresponding to *in vitro* model exposures were extracted from freely available government data from a pollution monitoring station in the Brisbane CBD (27).

Environmental conditions: Temperature and humidity data for time points corresponding to *in vitro* model exposures were obtained from an AusSun Lab weather station (Davis Instruments, Vantage Pro2) located on the roof of a building near the control site.

Statistical analysis: Standard descriptive statistics were compiled to summarize the data and assess its distribution. Following from this, associations between variables were investigated using Pearson's correlation coefficients (r). Finally, multivariable linear regression modeling was conducted stratified for urban and control location in order to determine the independent contribution of the UV, pollution, temperature and humidity to predict the production of cholecalciferol.

RESULTS

Median temperatures for the measurements were: morning 16°C (range 12–22°C); noon 22°C (15–26°C), and afternoon 20°C (range 16–24°C). The median humidity overall was 40%

(range 16–100%), which was highest in the mornings (58%; 31–100%) and lower at noon (36%; 18–54%) and in the afternoon (38%; 16–86%). Overall, mean air pollution (PM-10 = 17.9 $\mu g/m^3$) in Brisbane during the study period was well below the National Environmental Protection Council air quality standard of 50 $\mu g/m^3$ for PM-10 particles (28).

Combined across all data collection days and times, during a typical 20-minute measurement, the urban canyon sites received a mean of 0.26 W/m^2 total UV, of which 0.254 W/m^2 was within the UV-A and 0.004 W/m^2 was within the UV-B spectrum. Overall the urban canyon sites received approximately one quarter of the mean total UV received by the control site (Table 1).

<Table 1>

The average time of morning data collection was 09:40 AEST, and afternoon data collection 15:30 AEST. The mean solar zenith angle (SZA) at these data collection time-points was 56° in the morning and 70° in the afternoon, respectively. Reflecting this, total UV was much lower at afternoon data collection time-points, and the proportion of UV captured at urban sites compared to the control site was greater (Table 1). A similar pattern occurred with erythemal UV measurements (Table 1).

The average amount of cholecalciferol produced during a single exposure at urban sites was 0.12 μg/mL compared to an average of 0.53 μg/mL at the control site ($p < 0.001$). Morning and noon data collection time-points contributed larger proportions to this average difference than afternoon data collection (Table 1).

In bivariate analysis, across all sites, cholecalciferol measurements were shown to correlate strongly with UV-A ($r = 0.90$), UV-B ($r = 0.91$), and erythemal UV ($r = 0.97$) ($p< 0.01$ for all correlations) measurements (Table 2). Measurements of cholecalciferol did not

correlate strongly with air pollution or environmental data (Table 2). All types of UV were more strongly correlated with cholecalciferol measurements at the control site compared to urban sites.

<Table 2>

Linear regression models showed that at both the urban (β=0.92, $p < 0.001$) and control sites (β=0.99, $p < 0.001$), erythemal UV was highly associated with cholecalciferol production. In contrast, PM-10 particles, visibility reducing particles, and humidity did not have a substantial impact on the prediction of cholecalciferol production at either the urban or control sites. Overall the model explained 89% of the variance in cholecalciferol production at the urban sites and 97% of the cholecalciferol at the control site (Table 3).

<Table 3>

DISCUSSION

This study was the first to directly compare ambient UV and subsequent vitamin D production within an urban canyon area and a nearby control site over an extended time period (three months). Compared to the control site, the urban canyon received significantly less total and erythemal UV, and produced a much lower amount of vitamin D (as measured by an *in vitro* model). UV measurements were strongly correlated with the production of vitamin D at both sites. However, neither temperature, humidity, nor pollution were found to be associated with these outcomes.

Previous research has linked lower UV levels in urban environments to air pollution (29, 9, 30). One study concluded that differences in vitamin D status in a sample of Indian children could be attributed to differing levels of pollution in their environment (17). Our study, in

contrast, found no correlation between pollution levels and the availability of UV or daily vitamin D production. However, our findings are not conclusive, as pollution during the study period was very low, and we did not take measurements adjacent to each study site.

Past work has also shown that levels of vitamin D are lower among those living in urban environments (14-15) (16); and our study provides further evidence for why this may be the case. A minimal erythemal dose (MED) is the time taken in minutes for UV radiation to produce barely perceptible sunburn. Samanek *et al* (31) suggest that around one sixth to one third of a MED would be sufficient to produce equivalent to the Australian recommended daily dose of vitamin D (200–600 IU). They estimated that in Brisbane during the winter, it would take a fair-skinned person 5-14 minutes to receive this dose (31). However, during an average 20 minute exposure in our study in winter, while the control site received over one third of a MED (0.40 MED) the urban canyon sites received just one tenth (0.10 MED). Furthermore, the *in vitro* model from urban sites produced less than a quarter of the vitamin D produced at the control site.

Strengths and Limitations

The use of the *in vitro* model enabled us to compare the vitamin D-effective doses of UV that humans could receive in urban environments under a 'best case scenario'. This plays a fundamental role in determining potential production of vitamin D within such environments. However, this should be interpreted carefully, as the vitamin D dosimeter is an imperfect model for production of vitamin D in humans. Although comparisons of previtamin D in skin samples with *in vitro* models show that production is comparable for a given UV dose

(4), previtamin D production is generally higher in organic solvents than in skin (32). Furthermore, it is known that the UV doses individuals receive depend on other behavioral and genetic factors (33) and this leads to differences in their vitamin D status (34).

There were also some limitations with data measurement. Ideally, data would have been collected from control and urban sites on the same days, rather than alternate days; however, this is unlikely to have had a great effect on the data as it was averaged over a period of time. Secondly, measurements of pollution were taken from a single monitoring station rather than adjacent to each site, which limited ability to discern its contribution to UV and vitamin D production at the two sites.

Conclusions

This study confirmed previous research findings (12) that the amount of available UV within an urban canyon was significantly lower than at a nearby site outside of the canyon; and extended previous results by demonstrating that subsequent vitamin D production was also much lower within the canyon. Our findings were not designed to inform practice, and cannot be directly applied to humans. However, they indicate that an exposure that leads to the production of a significant amount of vitamin D in a control area may result in little or no vitamin D production in an urban canyon. While this requires further research, it merits consideration when designing future public health messages about optimum UV exposure times.

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References

1. Berwick, M., B. Armstrong, L. Ben-Porat, J. Fine, A. Kricker, C. Eberle and R. Barnhill (2005) Sun exposure and mortality from melanoma. *J Natl Cancer Inst,* **97,** 195-9.

2. Lucas, R. M., A. J. McMichael, B. K. Armstrong and W. T. Smith (2008) Estimating the global disease burden due to ultraviolet radiation exposure. *Int J Epidemiol,* **37,** 654-67.

3. Lucas, R. M. and A. L. Ponsonby (2006) Considering the potential benefits as well as adverse effects of sun exposure: can all the potential benefits be provided by oral vitamin D supplementation? *Prog Biophys Mol Biol,* **92,** 140-9.

4. MacLaughlin, J. A., R. R. Anderson and M. F. Holick (1982) Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. *Science,* **216,** 1001-3.

5. International Agency for Research on Cancer (IARC) (2008) Vitamin D and Cancer. Vol. 5. IARC Working Group Reports, Lyon.

6. Moise, A. F. and R. Aynsley (1999) Ambient ultraviolet radiation levels in public shade settings. *Int J Biometeorol,* **43,** 128-38.

7. Parisi, A. V., J. Sabburg and M. G. Kimlin (2004) *Scattered and Filtered Solar UV Measurements.* Kluwer Academic Publishers.

8. Turnbull, D. J., A. V. Parisi and J. Sabburg (2003) Scattered UV beneath public shade structures during winters. *Photochem Photobiol,* **78,** 180-3.

9. Weatherhead, E. C. (2005) Report on Geographic and Seasonal Variability of UV Affecting Human and Ecological Health. U.S. Environmental Protection Agency.

10. United Nationals Publication (2004) 2003 Revision of World Urbanization Prospects. United Nations.

11. Nunez, M. and T. R. Oke (1977) The Energy Balance of an Urban Canyon. *Journal of Applied Meteorology,* **16,** 11-19.

12. Podstawczynska, A. and W. Pawlak (2002) Daily course of ultraviolet and total solar radiation in an urban canyon - Lodz case study. In *Fifth International Conference on Urban Climate*. International Association for Urban Climate (IAUC), Lodz, Poland.

13. Holick, M. F. and D. Feldman (2004) Photobiology of Vitamin D. In *Vitamin D*. (Edited by F. H. Glorieux and J. W. Pike), pp. 37 - 43. Academic Press, San Diego.

14. Soontrapa, S. and L. O. Chailurkit (2005) Difference in serum calcidiol and parathyroid hormone levels between elderly urban vs suburban women. *J Med Assoc Thai,* 88 Suppl 5**,** S17-20.

15. Manicourt, D. H. and J. P. Devogelaer (2008) Urban tropospheric ozone increases the prevalence of vitamin D deficiency among Belgian postmenopausal women with outdoor activities during summer. *J Clin Endocrinol Metab,* **93,** 3893-9.

16. Goswami, R., N. Kochupillai, N. Gupta, D. Goswami, N. Singh and A. Dudha (2008) Presence of 25(OH) D deficiency in a rural North Indian village despite abundant sunshine. *J Assoc Physicians India,* **56,** 755-7.

17. Agarwal, K. S., M. Z. Mughal, P. Upadhyay, J. L. Berry, E. B. Mawer and J. M. Puliyel (2002) The impact of atmospheric pollution on vitamin D status of infants and toddlers in Delhi, India. *Arch Dis Child,* **87,** 111-3.

18. Commission Internationale de L'Eclairage (CIE) (2006) Action spectrum for the production of previtamin D3 in human skin.

19. Terenetskaya, I. P. (2003) Solar UV-B dosimetry in situ with D-dosimeter: effect of ozone depletion on the vitamin D synthetic capacity of sunlight. *Agricultural and Forest Meteorology,* **120,** 45-50.

20. Olds, W. J., A. R. McKinley, M. R. Moore and M. G. Kimlin (2008) In vitro model of vitamin D3 (cholecalciferol) synthesis by UV radiation: dose-response relationships. *J Photochem Photobiol B,* **93,** 88-93.

21. Bolsee, D., A. R. Webb, D. Gillotay, B. Dorschel, P. Knuschke, A. Krins and I. Terenetskaya (2000) Laboratory facilities and recommendations for the characterization of biological ultraviolet dosimeters. *Appl Opt,* **39,** 2813-22.

22. Galkin, O. N. and I. P. Terenetskaya (1999) 'Vitamin D' biodosimeter: basic characteristics and potential applications. *J Photochem Photobiol B,* **53,** 12-9.

23. Salih, F. M. (2004) Effect of clothing varieties on solar photosynthesis of previtamin D3: an in vitro study. *Photodermatol Photoimmunol Photomed,* **20,** 53-8.

24. Terenetskaya, I. (2004) Two methods for direct assessment of the Vitamin D synthetic capacity of sunlight and artificial UV sources. *J. Steroid. Biochem. Mol. Biol.,* **89-90,** 623-6.

25. Terenetskaya, I. P. (2000) Spectral monitoring of biologically active solar UVB radiation using an in vitro model of vitamin D synthesis. *Talanta,* **53,** 195-203.

26. Porter, J. A., K. E. Young and P. A. Beachy (1996) Cholesterol modification of hedgehog signaling proteins in animal development. *Science,* **274,** 255-9.

27. Queensland Government and Environmental Protection Agency (EPA) (2009) Hourly air quality data. Available at: http://www.epa.qld.gov.au/projects/air/. Accessed on 10 April 2010.

28. Department of the Environment and Heritage (2005) National standards for criteria air pollutants in Australia: Air quality fact sheet. Available at: http://www.environment.gov.au/atmosphere/airquality/publications/standards.html#fn2. Accessed on 30 August 2010.

29. Liu, C. M. (2000) Surface Erythemally-weighted UVB Flux in Taiwan. *Terrestral, Atmospheric and Ocean Sciences (TAO),* **11,** 879-894.

30. Zerefos, C., C. Meleti, A. Bais and A. Lambros (1995) The recent UVB variability over southeastern Europe. *J Photochem Photobiol B,* **31,** 15-19.

31. Samanek, A. J., E. J. Croager, P. Giesfor Skin Cancer Prevention, E. Milne, R. Prince, A. J. McMichael, R. M. Lucas and T. Slevin (2006) Estimates of beneficial and harmful sun exposure times during the year for major Australian population centres. *Med J Aust,* **184,** 338-41.

32. Webb, A. R., L. Kline and M. F. Holick (1988) Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab,* **67,** 373-8.

33. Gies, P., C. Roy, A. McLennan and D. Tomlinson (1999) Trends in ultraviolet radiation. In *Trends in sun protection seminar.* Anti-Cancer Council of Victoria, Melbourne.

34. Webb, A. (2006) Who, what, where and when-influences on cutaneous vitamin D synthesis. *Prog Biophys Mol Biol,* **92,** 17-25.

Table 1. Mean (SD) UV, erythemal UV, and cholecalciferol (Vitamin D) production in urban sites compared to the control site

	Morning		Noon		Afternoon		Overall	
Total UV (W/m^2)								
Urban Sites	0.29	(0.18)	0.32	(0.27)	0.17	(0.14)	0.26	(0.21)
Control	1.18	(0.33)	1.47	(0.31)	0.45	(0.15)	1.03	(0.52)
Difference (95% CI)	-0.89‡	(-1.17, -0.61)	-1.15‡	(-1.37, -0.93)	-0.29‡	(-0.40, -0.18)	-0.77‡	(-0.98, -0.56)
Urban as % of suburban	23.9%		21.7%		37.8%		24.3%	
Erythemal UV (MED)								
Urban Sites	0.09	(0.03)	0.15	(0.08)	0.04	(0.03)	0.10	(0.07)
Control	0.44	(0.16)	0.66	(0.15)	0.11	(0.04)	0.40	(0.26)
Difference (95% CI)	-0.35‡	(-0.41, -0.30)	-0.51‡	(-0.56, -0.46)	-0.07‡	(-0.08, -0.06)	-0.31‡	(-0.36, -0.26)
Urban as % of control	20.5%		22.7%		36.4%		25.0%	
Cholecalciferol (μg/mL)								
Urban Sites	0.11	(0.06)	0.21	(0.15)	0.05	(0.05)	0.12	(0.12)

	Morning		Noon		Afternoon		Overall	
Control	0.58	(0.30)	0.91	(0.28)	0.11	(0.06)	0.53	(0.08)
Difference (95% CI)	-0.47[†]	(-0.70, -0.23)	-0.70[‡]	(-0.91, -0.50)	-0.06[†]	(-0.10, -0.02)	-0.41[‡]	(-0.57, -0.25)
Urban as % of control	19.0%		23.1%		45.5%		22.6%	

[†] Significant at $p<0.05$ level

[‡] Significant at $p<0.001$ level

Table 2. Pearson's correlations between cholecalciferol and other variables at all sites

	Cholecalciferol		
	Urban	Control	Overall
UV-A	.703‡	.908‡	.899‡
UV-B	.759‡	.955‡	.907‡
Erythemal UV	.929‡	.974‡	.974‡
PM-10	.186†	-.145	.176‡
Visibility reducing particles	.035	-.156	.010
Temperature	.289‡	.309†	.311‡
Humidity	-.092‡	-.130†	-.082

† Correlation is significant at $p<0.05$ level

‡ Correlation is significant at $p<0.01$ level

Table 3. Multivariable linear regression model of factors associated with cholecalciferol production

	Urban		Control	
	β[†]	p-value	β[†]	p-value
Erythemal UV	0.92	<0.001	0.99	<0.001
Pollution PM-10	0.04	0.64	0.03	0.54
Visibility reducing particles	-0.03	0.59	-0.13	0.04
Temperature	0.09	0.12	-0.03	0.57
Humidity	0.06	0.17	0.06	0.25
Total r^2	**0.89**		**0.97**	

[†] β: standardised beta coefficient (0–1 scale); values indicate the relative amount by which cholecalciferol increases (positive values) or decreases (negative values) for each increasing unit of the explanatory variable.