Maternal and Neonatal Vitamin D Status are not associated with Risk of Childhood Type 1 Diabetes: a Scandinavian Case-Cohort Study

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Funding: Copenhagen University; Danish Heart Association; Danish National Research Foundation; Danish Pharmaceutical Association; Danish Ministry of Health; Danish National Board of Health; Herlev University Hospital; Innovation Fund Denmark; March of Dimes Birth Defects Foundation; Novo Nordisk Foundation; National Institutes of Health; Oak Foundation; The Capital Region of Denmark; and The Research Council of Norway.

Conflict of interest: The authors report no conflicts of interests.

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Abstract

Studies on vitamin D status during pregnancy and risk of type 1 diabetes (T1D) lack consistency, and are limited by small sample sizes or single measures of 25-hydroxyvitamin D (25(OH)D). We investigated whether average maternal 25(OH)D plasma concentrations during pregnancy are associated with risk of childhood T1D. In a case-cohort design, we identified 459 children with T1D and a random sample (n=1,561) from the Danish National Birth Cohort (n=97,127) and Norwegian Mother and Child Cohort Study (n=113,053). Participants were born between 1996 and 2009. The primary exposure was the estimated average 25(OH)D concentration based on serial samples from the first trimester until delivery, and umbilical cord plasma. We estimated hazard ratios using weighted Cox regression adjusting for multiple confounders. The adjusted hazard ratio for T1D per 10 nmol/L increase in the estimated average 25(OH)D concentration was 1.00 (95% confidence interval: 0.90–1.10). Results were consistent in both cohorts, in multiple sensitivity analyses, and when we analyzed mid-pregnancy or cord blood separately. In conclusion, our large study demonstrated that normal variation in maternal or neonatal 25(OH)D is unlikely to have a clinically important effect on risk of childhood T1D.

Keywords: Adolescent; Etiology; Child; Diabetes Mellitus, Type 1; Epidemiology; Vitamin D/immunology

Abbreviations: 25(OH)D: 25-hydroxyvitamin D; BMI: body mass index; DNBC: Danish National Birth Cohort; HR: Hazard Ratio; MoBa (a Norwegian acronym): The Norwegian Mother and Child Cohort Study; T1D: Type 1 diabetes

Type 1 diabetes (T1D) is a chronic autoimmune disease with severe long-term complications (1). There has been a marked increase in the incidence of childhood T1D worldwide during the last four decades (2). Genetic predisposition combined with unknown environmental factors early in life are thought to trigger a loss of self-tolerance for the insulin-producing pancreatic β-cells (3,4).

Maternal vitamin D status during pregnancy is critical for determining fetal 25-hydroxyvitamin D (25(OH)D) concentration (5). The fetus may regulate the concentrations of both 25(OH)D and the bioactive metabolite 1,25 dihydroxyvitamin D from an early stage, suggesting an important evolutionary role for vitamin D metabolites during pregnancy. In addition, the role of vitamin D in the fetus may not be restricted to the development of healthy bones (6,7). Experimental studies primarily using animals and *in vitro* human immune cell lines have demonstrated that vitamin D is involved in maintaining immunological self-tolerance (8,9).

An inverse association was reported between a high dose of vitamin D supplements in the first year of life and the risk of childhood T1D (10). However, only two studies have investigated the relationship between maternal 25(OH)D concentrations during pregnancy and the risk of childhood T1D, with inconsistent results (11,12). Partially inconsistent results were obtained from two additional studies that investigated the association between 25(OH)D_3 measured in neonatal dried blood spots and the risk of childhood T1D (13,14). These variations could be due to methodological issues including single measurements of 25(OH)D, the lack of data on potential confounders, or limited sample sizes. We tested the hypothesis that there is an association between maternal vitamin D status and the risk of childhood T1D, using a series of 25(OH)D measurements in samples taken from early in pregnancy through until delivery, in two of the largest cohorts of pregnant women in

the world. A secondary aim was to examine whether maternal vitamin D supplements taken during pregnancy influenced childhood T1D risk.

METHODS

Overview of study design

This bi-national study consists of a case-cohort sample from the Danish National Birth Cohort (DNBC) and the Norwegian Mother and Child Cohort Study (MoBa), which are prospective population-based pregnancy cohort studies conducted by the Statens Serum Institut in Denmark and the Norwegian Institute of Public Health, respectively. In the DNBC, pregnant women were recruited across Denmark between 1996 and 2002. Approximately 50% of all general practitioners participated in the recruitment process and 60% of women invited agreed to participate (15). In the MoBa, pregnant women were recruited across Norway between 1999 and 2008, and 41% of eligible women participated (16).

Study sample and identification of T1D

We linked the cohorts with the Danish Childhood Diabetes Registry and the Norwegian Diabetes Childhood Registry, respectively, to identify children who had developed T1D according to the World Health Organization's criteria (17,18). These diabetes registers have nearly complete nationwide coverage and record high-quality prospective data on children with T1D. We included all 459 children diagnosed with T1D (270 from the DNBC and 189 from the MoBa) who had available blood samples, and a random cohort sample of 1,561 children (985 from the DNBC and 576 from the MoBa) from 97,127 (DNBC) and 113,053 (MoBa) eligible children in our study population (Fig. 1).

All participants had a minimum of one plasma sample assayed for 25(OH)D and 91% of the mother/child pairs were represented by two or three blood samples.

Exposure assessment

Collection and storage of blood samples

For the DNBC, maternal venous blood was drawn at approximately weeks 7–9 and 24–25 of gestation, and from the umbilical cords of newborn infants (15). For the MoBa, maternal venous blood was drawn at approximately week 17–18 of gestation, shortly after delivery, and from the umbilical cords of newborn infants (19,20). DNBC plasma samples were stored at –20°C or in liquid nitrogen (15). MoBa plasma samples were stored at –80°C (20).

Assessment of vitamin D status

We used liquid chromatography-tandem mass spectrometry (LC-MS/MS) to measure plasma 25(OH)D_2 and 25(OH)D_3 separately (see Web appendix 1 for details). Our exposure variable was defined as the sum of 25(OH)D_2 and –D_3, hereafter referred to as 25(OH)D. All samples were assayed by two technicians in a single laboratory at the Statens Serum Institute (Copenhagen, Denmark) from July through until October 2015. All samples were processed in random order (i.e., independently of their cohort or case-status) and the technicians were blinded to the case-status of the samples. Repeated measurements of standards gave inter-assay coefficients of variation (CV) for 25(OH)D_3 of 3.4% and 7.9% for concentrations of approximately 33 nmol/L and 80 nmol/L, respectively.

Other variables

Birth weight, maternal age at delivery, and mode of delivery details were obtained from the nationwide Medical Birth Registry of Norway and the National Hospital Discharge Registry in Denmark (15,16). Information regarding maternal pre-pregnancy body mass index (BMI) and

smoking during pregnancy was obtained from telephone interviews (DNBC) and questionnaires administered mid-pregnancy (MoBa). Information on intake of vitamin D, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from supplements was obtained from food frequency questionnaires administered during the second trimester in both cohorts (15,19). See Web Appendix 1 for details on questionnaires. For the DNBC, information regarding any type of maternal diabetes was obtained from the Danish National Diabetes Register. For the MoBa, data on maternal T1D were obtained from questionnaires and the Norwegian Patient Registry.

Statistical analysis

Statistical analyses were performed with R software for statistical computing (ver. 3.3.1; R Foundation for Statistical Computing, Vienna, Australia; http://www.r-project.org) using the *lava* package (ver. 1.4.5) and the *survey* package (ver. 3.31).

All details of the analysis plan were determined *a priori*. The primary analysis was a two-stage analysis, utilizing all available 25(OH)D measurements to estimate the hazard ratio (HR) of childhood T1D per 10 nmol/L increase in estimated average 25(OH)D concentration during pregnancy and at birth. The first stage generated a measurement error (structural equation) model for the estimated average 25(OH)D concentration i.e., "latent variable". Concentrations of 25(OH)D were adjusted for the time of year (season) of blood sampling using cosinor modelling (21). In the second stage, we used the estimated average 25(OH)D concentration stratified by cohort (DNBC/MoBa) as the continuous exposure variable in a weighted Cox regression model, with time since birth as the baseline. The Cox model was modified to account for the case-cohort design by applying inverse probability weights (22). Further details of the statistical analysis as well as our pre-study power calculations are described in the Web Appendix 1.

We assessed the linearity assumption by a categorical analysis using quartiles. Based on a graphical presentation of the log cumulative-hazard functions in strata defined by quartiles of 25(OH)D, the proportional-hazards assumption was valid.

The primary analysis was adjusted for the following covariates: maternal diabetes, age at time of delivery (continuous), pre-pregnancy BMI (categorical variable with boundaries at 18.5 kg/m^2, 25 kg/m^2 and 30 kg/m^2), child's sex, and birth weight (continuous).

Sensitivity analyses

In a series of sensitivity analyses, we examined: i. the primary analysis after additional adjustment for mode of delivery (caesarean delivery: yes or no), maternal smoking during pregnancy (yes or no), and maternal eicosapentaenoic acid and docosahexaenoic acid intake, or vitamin D supplements taken during pregnancy; ii. the sensitivity of our results to missing covariate data, using inverse probability weighting by propensity for missing data; iii. the primary analysis without adjusting for season of blood sampling (to test the hypothesis that absolute 25(OH)D concentrations during pregnancy predict childhood T1D); and iv. the separate associations of 25(OH)D concentrations in mid-pregnancy or in umbilical cord blood samples, with the risk of childhood T1D. We assessed the sensitivity of our results to deviation from the assumption of normality in the measurement error model by using a Gaussian Mixture model with two or three components. Finally, we investigated potential heterogeneity between the DNBC and MoBa cohorts by running cohort-specific measurement error models in stage one of the analysis.

Ethics

The DNBC study was approved by the Danish National Ethics Board and the Danish Data Protection Agency. The MoBa study was approved by the Norwegian Data Protection Authority and the Regional Ethics Committee for Medical Research of South East Norway. All women provided written informed consent.

RESULTS

The characteristics of the study participants are presented in Table 1. The median age at T1D diagnosis was 7.4 years (range 0.7–14.9), and the median follow-up time for the random cohort sample was 12.0 years (range 4.7–16.2). There was a positive correlation between seasonally-adjusted 25(OH)D concentrations in mid-pregnancy and umbilical cord blood samples ($r = 0.40$, $P < 0.001$). Cohort-specific correlations are presented in Web Fig. 1. The seasonal variation in 25(OH)D concentrations are shown in Web Fig 2.

Estimated average maternal 25(OH)D concentration and childhood T1D

Our primary analysis demonstrated that there was no association between estimated average seasonally-adjusted 25(OH)D concentration and childhood T1D [adjusted HR per 10 nmol/L increase: 1.00; 95% confidence interval (CI): 0.90–1.10]. There was also no indication of any threshold (nonlinear) association (Fig. 2).

Sensitivity analyses

The lack of association between maternal/cord blood 25(OH)D and the risk of childhood T1D was demonstrated consistently in a series of sensitivity analyses. These included an analysis adjusted for maternal intake of the long chain n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid, separate analyses for mid-pregnancy and umbilical cord blood samples (Fig. 3), and analyses using absolute rather than seasonally-adjusted 25(OH)D concentrations (Web Fig. 3). First trimester samples were only available from DNBC, and showed a suggestive but non-significant inverse association with childhood type 1 diabetes [adjusted hazard ratio: adjusted HR per 10 nmol/L increase: 0.95; 95% CI: 0.88–1.02]. The primary association was essentially equal in boys and girls [p(interaction)=0.99].

Vitamin D supplementation and association with risk of childhood T1D

In support of our main finding, we found no association between maternal self-reported vitamin D supplementation during pregnancy and the risk of childhood T1D when used as a binary variable (HR: 0.91; 95% CI: 0.57–1.43) or when used as a continuous variable (HR per increased µg of vitamin D/day: 1.01; 95% CI: 0.98–1.03).

DISCUSSION

In this study, which utilises two of the world's largest cohorts of pregnant women, we present novel data on an unresolved issue in T1D aetiology. Our results show that normally varying 25(OH)D concentrations in a series of maternal and umbilical cord plasma measurements were not associated with risk of childhood T1D. In addition, maternal intake of vitamin D supplements during pregnancy was not associated with risk of childhood T1D.

Comparison with other studies

Two previous studies investigated maternal 25(OH)D during pregnancy (11,12), and two studies investigated 25(OH)D_3 in neonatal dried blood spots,(13,14) all relative to childhood T1D. The results were inconsistent but there were important differences and limitations to take into account. In a Norwegian nested case-control study with 109 cases, Sørensen *et al*. reported a two-fold increase in T1D risk for children born to women with late pregnancy 25(OH)D concentrations in the first compared with the fourth quartile (12). In an updated analysis of the same individuals, Sørensen *et al*. found no association between first and second trimester 25(OH)D concentrations and childhood T1D risk (23). A Finnish study of 343 case-control pairs found no association between maternal concentrations of 25(OH)D during the first trimester and the risk of childhood T1D (11). In light of this latter study (11), our suggestive but non-significant inverse association observed for first trimester samples available in DNBC only, were likely due to chance.

One small Italian case-control study (67 cases with T1D) reported that increased neonatal dried blood spot 25(OH)D concentrations were associated with a lower risk of childhood T1D in an immigrant subgroup, but there was no significant association in the Italian subgroup or the two subgroups combined (14). A large Danish study, with 1,090 T1D cases, found no association

between neonatal concentrations of 25(OH)D_3 in dried blood spots and the risk of childhood T1D (13,14). Dried blood-spot 25(OH)D concentrations are substantially lower but correlate strongly with plasma measurements (24). In the Danish study, the median concentrations of 25(OH)D_3 ranged from 21.1 to 24.3 nmol/L (13), whereas in the Italian study, the mean 25(OH)D_3 concentrations were extremely low in both groups (< 5 nmol/L) (14).

The current study is to our knowledge the first to assess cord blood 25(OH)D in relation to childhood T1D, and our results are consistent with those of the larger Danish study on neonatal 25(OH)D concentrations (13). Importantly, we show with precision that the lack of association was not limited to a specific trimester or sample type.

As a biomarker, 25(OH)D provides an objective measurement of vitamin D that integrates both dietary intake and endogenous production in the skin in response to ultraviolet irradiation. Some previous studies have investigated dietary intake of vitamin D during pregnancy in relation to childhood T1D. In these studies, a retrospective (case-control) design would be inferior due to the high risk of recall and selection bias, and prospective designs are preferable. Vitamin D intake from food or supplements during pregnancy was not associated with islet autoimmunity (a preclinical stage of T1D) in genetically susceptible Finnish children (25). In addition, the results from a Swedish population-based study were consistent with those from our larger, prospective study in demonstrating that the use of vitamin D supplements during pregnancy was not associated with risk of childhood T1D (26).

Strengths and weaknesses

The strengths of this study included its large-scale, which provided precise risk estimates and its prospective approach. In addition, multiple measurements were made during pregnancy and also from umbilical cord blood, which allowed the average 25(OH)D concentration to be estimated from pregnancy through to delivery. Another strength of the study was the concurrent assessment of maternal vitamin D supplementation.

Some study limitations should also be considered. As in any observational study, we cannot exclude the possibility that unknown confounding factors may have influenced our results. We did not have information on human leukocyte antigen (HLA), the major genetic determinant of T1D, or single nucleotide polymorphisms of the vitamin D pathway. Therefore, we could not examine potential genetic-environmental interactions. However, we do not expect that our null finding could be attributable to a confounding factor, genetic variation in the vitamin D-pathway (27), or an HLA genotype (13,28). Furthermore, we did not measure plasma vitamin D-binding protein, which could have been helpful in estimating the free 25(OH)D fraction. On the other hand, Sørensen et al. did not find any association between the estimated free maternal 25(OH)D during pregnancy and childhood T1D (23), and the relevance of the free 25(OH)D is debated. Participants in the DNBC and MoBa studies may not be representative of the general population of pregnant women in Denmark and Norway (e.g., they may be better educated or have healthier lifestyles), but this does not necessarily confound exposure-outcome associations (29,30). While our results should be largely generalizable to other similar European and European origin populations, we cannot exclude the possibility that results may not be generalizable to populations with much lower vitamin D status.

Implications and future perspective

While sufficient vitamin D concentrations during pregnancy could have other benefits, the results of our study do not support recommending vitamin D supplements during pregnancy to reduce the risk of T1D in the offspring. Only a large scale, long-term randomized controlled trial can establish whether increasing 25(OH)D during pregnancy beyond the concentrations we observed can alter the risk of childhood T1D. However, our results do not favor the initiation of such a trial.

Conclusion

Our large-scale Scandinavian study shows that normal variation in maternal or neonatal 25(OH)D is unlikely to have a clinically important effect on risk of childhood T1D.

Acknowledgments

Funding

This research has been conducted using the Danish National Biobank resource, supported by the Novo Nordisk Foundation. DNBC is supported by the March of Dimes Birth Defects Foundation, the Danish Heart Association, the Danish National Research Foundation, the Danish Pharmaceutical Association, the Ministry of Health, the National Board of Health, and grant no. 09-067124 from the Innovation Fund Denmark. MoBa is supported by grants UO1 NS 047537-01 and UO1 NS 047537-06A1 from the National Institutes of Health and grant 151918/S10 from the Research Council of Norway/FUGE. Steffen U. Thorsen was supported by a scholarship from Copenhagen University and by a grant from Herlev University Hospital. Ketil Størdal was supported by an unrestricted grant from the Oak Foundation, Geneva, Switzerland. The 25-hydroxyvitamin laboratory measurements of the DNBC samples were funded by a grant from the Capital Region of Denmark. Costs of all data acquisition, including laboratory assays in MoBa (the sub-study PAGE; Prediction of Autoimmune Diabetes and Celiac Disease in Childhood by Genes and Perinatal Environment), was supported by grant 2210909/F20 from the Research Council of Norway (Dr. Lars C. Stene).

Presentation at meeting

The main results in this manuscript was presented as an abstract at the Immunology of Diabetes Society meeting in San Francisco, January 19-21, 2017.

Conflicts of interest

The authors report no conflicts of interests. The sponsors of the study had no role in the interpretation or presentation of the results. The authors alone are responsible for the content and the writing of the paper.

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Figure legends

Figure 1. The Study Population from The Danish National Birth Cohort (DNBC) and The Norwegian Mother and Child Cohort Study (MoBa) in a childhood type 1 diabetes (T1D) case-cohort design. Four children in the DNBC random sample were also T1D cases. There was insufficient plasma for 25(OH)D analysis in 12 vials from the DNBC sample population. Two of the MoBa random sample subjects were also T1D cases. 25(OH)D: 25-hydroxyvitamin D.

Figure 2. Survival curve illustrating the lack of association between quartiles of estimated average 25-hydroxyvitamin D during pregnancy and the risk of childhood type 1 diabetes ($P = 0.51$, df = 3). 25-hydroxyvitamin D cut-offs for each quantile were: 0% − 24.9%: 37.5 − 61.9 nmol/L; 25% − 49.9%: 62.0 − 69.1 nmol/L; 50% − 74.9%: 69.2 − 77.4 nmol/L; 75% − 100%: 77.5 – 130.3 nmol/L. Note that the y-axis does not begin at zero.

Figure 3. Sensitivity analyses for the association between maternal/cord blood vitamin D status and the risk of childhood type 1 diabetes (hazard ratio per 10 nmol/L increase in plasma 25-hydroxyvitamin D concentration). The primary model was adjusted for maternal diabetes, age at time of delivery, pre-pregnancy BMI, child's sex, birth weight, and the time of year/season that each blood sample was taken. The subsequent lines shows the main association after additional adjustment: Maternal EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) intake from diet and supplements during pregnancy (both continuous variables; 40% had missing data for these covariates). Missing covariates: this shows the result of the primary model with additional adjustment for missing covariates using inverse probability weighting by propensity for missing information on at least one of the primary covariates. The two lower lines shows the results of the primary model (same covariates), but using cord blood only or midpregnancy samples only, for 25-

hydroxyvitamin D concentration. Details regarding missing covariates are shown in the footnote of Table 1. CI: Confidence interval. BMI: Body mass index.

Table 1. Characteristics of cases with childhood type 1 diabetes and subjects randomly selected from the Danish National Birth Cohort (DNBC) and the Norwegian Mother and Child Cohort Study (MoBa). Participants were recruited from 1996 to 2008, and followed up to February 2014 with respect to type 1 diabetes.[a]

	DNBC				MoBa			
	Case (n = 270)		Cohort[b] (n = 985)		Case (n = 189)		Cohort[b] (n = 576)	
Plasma 25(OH)D (nmol/L)								
Maternal first trimester sample	50.4	36.5, 67.6	54.2	40.8, 69.4				
Maternal mid-gestation sample	61.1	41.0, 79.4	60.0	41.4, 80.9	56.2	41.6, 77.4	57.5	42.5, 74.8
Umbilical cord blood sample	37.7	26.6, 54.9	38.9	26.6, 52.1	31.7	21.5, 45.3	31.9	21.3, 46.1
Maternal postpartum sample					42.5	34.1, 63.2	45.7	30.6, 64.3
Age at diagnosis of T1D (years)	9.0	5.7, 11.1			5.7	3.6, 7.9		
Female children	138	51.1	497	50.4	93	49.2	285	49.5
Maternal age at delivery (years)	30	26.8, 32.6	30	27, 33	30	27, 33	30	27, 33
Birth weight (kg)	3.5	3.2, 3.9	3.5	3.3, 3.9	3.7	3.3, 4.0	3.6	3.3, 4.0
Maternal pre-pregnancy BMI[c]								
< 18.5	17	6.9	42	4.5	7	4.0	17	3.2
18.5–24.9	155	63.0	648	70.4	90	51.7	362	68.6
25–29.9	48	19.5	162	17.6	49	28.2	109	20.6
≥ 30	26	10.6	68	7.4	28	16.1	40	7.6
Maternal diabetes diagnosis [d]	15	5.6	29	2.9	7	3.7	0	0.0
Maternal vit. D supplements (μg/d)[e]	10.0	5.0, 10.0	9.3	5.0, 10.0	4.8	1.3, 10.0	4.6	2.2, 10.0
Maternal vit. D from foods (μg/d)[f]	2.6	1.9, 4.1	2.9	2.0, 4.1	2.7	1.8, 4.2	3.2	1.9, 4.4
Maternal EPA suppl. (mg/d)[e, g]	63.1	336.2	25.2	157.3	214.5	317.4	199.4	290.4
Maternal EPA foods (mg/d)[f,g]	89.9	77.0	94.7	80.5	155.5	150.9	170.2	171.8
Maternal DHA suppl. (mg/d)[e,g]	52.7	318.1	17.7	106.5	232.8	328.2	223.9	304.6
Maternal DHA foods (mg/d)[f,g]	223.6	177.3	238.5	195.4	250.4	205.7	270.2	226.2
Maternal smoking in pregnancy	56	21.3	266	27.3	14	33.3	50	38.2
Caesarean delivery	39	14.4	149	15.1	36	19.0	59	10.2

DNBC: Danish National Birth Cohort. DHA: Docosahexaenoic acid; EPA: eicosapentaenoic acid; MoBa: The Norwegian Mother and Child Cohort Study (a Norwegian acronym). T1D: Type 1 diabetes; 25(OH)D: 25-hydroxyvitamin D.
[a]Data are medians (25-percentile, 75-percentile) for continuous variables or n (% of those with non-missing data) for categorical variables, unless otherwise specified. Missing values out of 2,020 individuals: maternal age at delivery (n = 1); birth weight (n = 4); maternal pre-pregnancy body mass index (BMI) (n = 152); maternal vitamin D supplementation (n = 811); maternal vitamin D intake from foods (n = 396); maternal EPA intake from supplements (n = 811); maternal DHA intake from supplements (n = 811); maternal EPA and DHA intake from diet (n = 719); maternal smoking during pregnancy (n = 611). [b]A randomly selected sample from the cohort (sub cohort in case-cohort design). [c]BMI: Body Mass Index: Weight (kg)/height $(m)^2$). [d]Maternal type 1 diabetes in the MoBa, maternal diabetes of any type in the DNBC. [e]Intake of from supplements of vitamin D, EPA, or DHA, reported during weeks 22–25 of gestation. [f] Maternal intake of vitamin D, EPA, or DHA from foods, estimated from food frequency questionnaires administered in the second trimester. [g]Data are mean, standard deviation.