Periconceptional maternal biomarkers of one-carbon metabolism and embryonic growth trajectories: the Rotterdam Periconceptional Cohort (Predict Study)

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Objective: To study the associations between the periconceptional maternal biomarkers of one-carbon metabolism and embryonic growth.
Design: Prospective, periconceptional hospital-based birth cohort.
Setting: Tertiary medical care center.
Patient(s): Between 2010 and 2014, 236 women with early singleton ongoing pregnancies that resulted in 139 strictly dated spontaneous pregnancies and 97 pregnancies conceived after assisted reproductive technology.
Intervention(s): None.
Main Outcome Measure(s): Maternal serum vitamin B_{12} and plasma total homocysteine (tHcy) assessed at enrollment, and longitudinal first-trimester crown-rump length (CRL), embryonic volume (EV), and absolute growth rates obtained via three-dimensional ultrasound (3D-US) and virtual reality.
Result(s): In early pregnancy, we performed a median of five 3D-US scans (range: 1–7). Vitamin B_{12} concentrations were positively associated with CRL and EV measurements in the total population (CRL: β 5^10^{-4} (1^10^{-4} to 9^10^{-4}) $\sqrt{}$mm; EV: β 2^10^{-4} (0^10^{-4} to 4^10^{-4}) $\sqrt[3]{}$cm^3) and in the strictly dated spontaneous pregnancy subgroup. The tHcy concentration was negatively associated with embryonic growth in all study groups. High tHcy concentrations (+2 standard deviation [SD], 10.3 μmol/L) were associated with a 1.7 mm smaller CRL (−13.4%) at 7 weeks and a 3.6-mm smaller CRL (−7.1%) at 11 weeks compared with −2 SD tHcy (−3.0 μmol/L). A high tHcy concentration was also associated with a 0.10 cm^3 smaller EV (−33.3%) at 7 weeks and a 1.65 cm^3 smaller EV (−16.1%) at 11 weeks. The embryonic growth rate was positively associated with vitamin B_{12} and negatively associated with tHcy.
Conclusion(s): Minor variations in periconceptional maternal concentrations of one-carbon metabolism biomarkers are associated with human embryonic growth. (Fertil Steril® 2017;107:691–8. ©2016 by American Society for Reproductive Medicine.)
Key Words: Crown-rump length, embryonic volume, homocysteine, maternal one-carbon metabolism, virtual reality

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Over the last few decades, impaired reproductive health has been associated with poor parental nutrition and lifestyle; the impact on the development of gametes, embryos, and fetuses has long-term implications for the health and noncommunicable diseases of the offspring (1–5). Derangements in one-carbon (I-C) metabolism represent one of the causal links between parental poor nutrition, lifestyle habits, and reproductive failures (6). Clinical biomarkers of this metabolic pathway comprise folate, vitamin B_{12}, and total homocysteine (tHcy), with

Received June 24, 2016; revised and accepted November 30, 2016; published online January 6, 2017.
F.P. has nothing to disclose. M.R. has nothing to disclose. A.H.J.K. has nothing to disclose. S.P.W. has nothing to disclose. I.C. has nothing to disclose. E.A.P.S. has nothing to disclose. R.P.M.S.-T. is CSO of the startup company Slimmere Zorg and CEO of eHealth Care Solutions.
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Fertility and Sterility® Vol. 107, No. 3, March 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.11.030

elevated tHcy concentrations representing the most sensitive marker of I-C metabolism derangement (6, 7).

For many years, researchers have studied the association between folate and reproductive outcome (6). Of interest is that research also is focusing now on the effects of vitamin B_{12} on perinatal health, showing significant associations with birth defects and weight (8). Elevated plasma tHcy has been associated with an increased risk of congenital malformations, small-for-gestational-age fetuses, low placental weights, preterm births, preeclampsia, and a poor cardiovascular risk profile in childhood and adulthood (6,9–13). Moreover, in the last decade the periconceptional period (14 weeks before conception to 10 weeks after conception) has been recognized as one of the most important time windows in life, during which gametogenesis, embryogenesis, and placentation take place (6). These processes are influenced by genetic and environmental factors that affect mechanisms such as epigenetic programing, further explaining the associations among periconceptional health, pregnancy outcome, and health of the offspring in adult life.

The introduction of high-frequency probes and three-dimensional ultrasound (3D-US) scans has markedly improved first-trimester embryonic evaluations and the precision of crown-rump length (CRL) measurements. Additionally, the use of the Barco I-Space, an immersive virtual reality (VR) system, provides real depth perception and interaction with 3D-US data sets in an intuitive manner. The V-Scope VR application allows offline CRL and embryonic volume (EV) measurements in the I-Space, important and highly reliable noninvasive biomarkers for embryonic growth (14, 15). Our study evaluated the association between periconceptional maternal vitamin B_{12} and tHcy concentrations and embryonic growth as assessed by longitudinal CRL and EV measurements performed in the Barco I-Space VR system.

MATERIALS AND METHODS

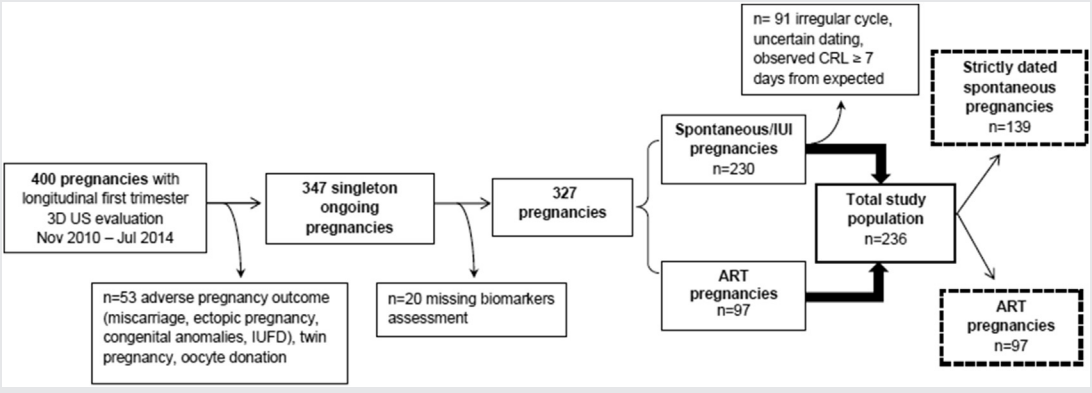
Our study was performed in the Rotterdam Periconception Cohort (Predict study), a prospective periconceptional tertiary hospital-based birth cohort study conducted at the Department of Obstetrics and Gynecology of the Erasmus MC, University Medical Centre in Rotterdam, the Netherlands. This ongoing cohort study started in 2009 with the aim of investigating the periconceptional determinants of first-trimester and pregnancy outcomes and the biological mechanisms associated with offspring health during the life course (16). The protocol has been approved by the local medical ethics committee, and all women sign a written, informed consent form before participation.

Study Population

Between November 2010 and July 2014, all women at least 18 years of age with an early first-trimester (<8 weeks of gestation) ongoing singleton pregnancy were eligible for enrollment. Figure 1 shows a flowchart summarizing the excluded and included participants for the current study. Women who conceived spontaneously, after intrauterine insemination (IUI), or assisted reproductive technology (ART), including in vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI), or cryopreserved embryo transfer, were eligible for participation. Among spontaneously conceived pregnancies, the exclusion criteria were unknown first day of the last menstrual period, self-reported irregular cycle, or observed CRL $\geq$7 days different from the expected CRL according to the Robinson curves (17).

The total study population included 236 pregnancies defined by reliable pregnancy dating, comprising of 97 ART pregnancies and 139 strictly dated spontaneous pregnancies. Because the association between maternal blood biomarkers and embryonic growth could eventually be confounded by the conception mode, we adjusted the analysis in the total population for this potential confounder, and we further stratified the analysis in the two subgroups. In this way we could investigate the effect of maternal biomarkers among pregnancies with reliable pregnancy dating only, considering the influence of different conception modes on the resulting associations. Gestational age was defined from the first day

FIGURE 1



Flowchart of the study population. ART = assisted reproductive technology; CRL = crown-rump length; 3D-US = three-dimensional ultrasound; IUFD = intrauterine fetal death; IUI = intrauterine insemination.

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of the last menstrual period for spontaneous pregnancies (adjusted for duration of the menstrual cycle if <25 or >31 days); from the first day of the last menstrual period or insemination date plus 14 days for IUI pregnancies, from the day of oocyte retrieval plus 14 days for IVF-ICSI pregnancies; and from embryo transfer day plus 17 or 18 days in pregnancies derived from transfer of cryopreserved embryos.

General Data and Laboratory Assays

At enrollment all women completed a self-administered general questionnaire covering details on age, height, weight, ethnicity, education, obstetric and medical history, and periconceptional lifestyle (smoking, alcohol use, folic acid and multivitamin supplements). One fasting venous blood sample per pregnancy was collected at enrollment before 8 weeks of gestation for routine determination of serum vitamin B_{12} and plasma tHcy among others and drawn in a Vacutainer ethylenediamine tetraacetate (EDTA) tube and in a dry Vacutainer tube (BD Diagnostics). The dry Vacutainer tubes were centrifuged at 2,000 × *g*, and serum was collected and analyzed for vitamin B_{12} concentrations using an immunoelectrochemoluminescence assay (E170; Roche Diagnostics GmbH). Plasma was separated by centrifugation within 1 hour for determination of tHcy by using a sensitive liquid chromatography tandem mass spectrum method (HPLC-Tandem MS, Waters Micromass Quattro Premier XE Mass Spectrometer with Acquity UPLC system; Waters Corporation).

Ultrasound Data

All women received longitudinal transvaginal 3D-US scans from enrollment up to 13^{+0} weeks of pregnancy with a 6–12 MHz transvaginal probe using GE Voluson E8 equipment and 4D View software (General Electric Medical Systems). In the pilot study, we performed weekly 3D-US scans during the first trimester, resulting in a maximum of seven scans per pregnancy (18). However, these data showed that an accurate modeling of growth trajectories could be obtained also with three 3D-US scans per pregnancy, leading to this reduction after 2013 (16). The 3D-US data sets were transformed to Cartesian (rectangular) volumes using 4D View and transferred to the Barco I-Space (Barco N.V.) at the Department of Bioinformatics, Erasmus MC, University Medical Centre, Rotterdam, to perform offline CRL and EV measurements using the V-Scope software.

The CRL measurements were performed three times using a length-measuring tool available in V-Scope, and the average was used in the analysis. The calipers were placed from crown to caudal rump in a straight line. The correct position in the midsagittal plane was verified by rotating the hologram (19). A semiautomated volume-measuring application, based on gray-scale differences, was used to perform EV measurements as previously validated by Rousian et al. (15). The EV measurements were performed once by the investigator on the same data set selected for CRL measurement. The reliability, technique, and methods used for CRL and EV measurements have been extensively studied and described before (15, 19).

Statistical Analysis

Maternal baseline characteristics and biomarkers were compared between included and excluded pregnancies and between the ART and strictly dated spontaneous pregnancy subgroups using chi-square or exact tests for ordinal variables and Student's *t*-test or the Mann-Whitney *U* test for continuous variables. Univariable linear regression was performed to evaluate associations between maternal baseline characteristics and biomarker concentrations. Linear mixed models were estimated in the total study population and in the ART and strictly dated spontaneous pregnancy subgroups to model longitudinal CRL and EV measurements, taking into account the existing correlation between serial measurements within the same pregnancy, and to analyze associations with maternal biomarkers with adjustment for potential confounders.

Square root transformation of CRL data and third root transformation of EV data were performed to obtain a normal distribution of observations, as required by linear mixed models. This transformation also resulted in approximate linearity with gestational age and an almost constant variance independent from gestational age. First, we performed the analysis with adjustment for gestational age only (model 1); second, we adjusted for additional potential confounders (parity, smoking, alcohol use, folic acid supplement use, age, body mass index, comorbidity, and fetal gender) (model 2). Additionally, we investigated the associations between maternal biomarker concentrations and embryonic size parameters separately at enrollment (first available 3D-US scan, <8 weeks of gestation) and in the late first trimester (last available 3D-US scan, >10 weeks of gestation).

Finally, linear mixed models were used to study the associations between maternal biomarkers and the embryonic absolute growth rate defined as (CRL1 − CRL2)/(GA1 − GA2) and (EV1 − EV2)/(GA1 − GA2) at two consecutive 3D-US scans. A random intercept was used to model the within-subject correlation. $P<.05$ was considered statistically significant. All analyses were performed using SPSS Statistics for Windows, version 21.0 (IBM Inc.) and R version 3.2.1 (R Foundation for Statistical Computing).

RESULTS

Baseline Characteristics of the Study Population

Table 1 shows maternal baseline characteristics, with comparisons between the included and excluded pregnancies and between the ART and strictly dated spontaneous pregnancy subgroups. The prevalence of hyperhomocysteinemia in the total study population was 2.1% (>13 μmol/L). In the univariable linear regression, maternal vitamin B_{12} showed a positive association with age (β 0.01; 95% confidence interval [CI], 0.002–0.02; $P=.03$) and a negative association with tHcy (β −0.07; 95% CI, −0.10 to −0.04; $P<.001$).

Embryonic Growth Trajectories

We included 236 pregnancies with a total of 1,207 3D-US data sets in the analysis. The CRL measurements could be performed in 1,029 data sets (85.3%) and the EV measurements in 941 data sets (78.0%) with good quality. The median gestational

TABLE 1

Maternal baseline characteristics of included and excluded pregnancies.

Characteristic	Total study population (n = 236)	M	Excluded pregnancies (n = 116)	M	*P* value	Strictly dated spontaneous pregnancies (n = 139)	ART pregnancies (n = 97)	*P* value
Age (y), median (range)	32 (22–42)	0	30 (21–44)	0	.00	32 (22–42)	32 (24–42)	.16
Geographic origin		2		4	.16			.43
Dutch, n (%)	196 (83.1)		100 (86.2)			119 (85.6)	77 (79.4)	
Other Western	11 (4.7)		3 (2.6)			4 (2.9)	7 (7.2)	
Non-Western,	27 (11.4)		9 (7.8)			15 (10.8)	12 (12.4)	
Educational leve, n (%)		2		4	.36			.70
High	136 (57.6)		64 (55.2)			82 (59)	54 (55.7)	
Intermediate	93 (39.4)		45 (38.8)			52 (37.4)	41 (42.3)	
Low	5 (2.1)		3 (2.6)			4 (2.9)	1 (1)	
BMI (kg/m^2), median (range)	24.2 (17.0–42.6)	1	25.8 (17.8–45.0)	2	.03	24.2 (18.6–42.6)	24.4 (17.0–38.4)	.90
Nulliparous, n (%)	74 (31.4)	2	39 (33.6)	2	.68	30 (21.6)	44 (45.4)	.00
Alcohol use, n (%)	83 (35.2)	3	38 (32.8)	6	.09	61 (43.9)	22 (22.7)	.00
Periconceptional smoking, n (%)	33 (14)	2	20 (17.2)	7	.01	21 (15.1)	12 (12.4)	.81
Folic acid supplementation (<6 wk), n (%)	224 (94.9)	5	108 (93.1)	2	.57	128 (92.1)	96 (99.0)	.05
Comorbidity, n (%)	27 (11.4)	0	20 (17.2)	0	.13	22 (15.8)	5 (5.2)	.01
Vitamin B_{12} (pmol/L), median (range)	300 (95–953)	0	287.5 (109–915)	25	.47	290 (95–953)	315 (124–713)	.12
tHcy (μmol/L), median (range)	6.4 (3.7–17.6)	4	6.4 (3.4–13.6)	22	.79	6.6 (4.0–16.3)	6.1 (3.7–17.6)	.02

Note: Excluded pregnancies comprise women with irregular cycle or uncertain pregnancy dating and women without blood samples for biomarkers assessment. Comorbidity includes cardiovascular, autoimmune, endocrine, and metabolic diseases. The comparison among groups was performed using chi-square or exact tests for ordinal variables and Student's *t*-test or Mann-Whitney *U* test for continuous variables. ART = assisted reproductive technology; M = missing values; tHcy = plasma total homocysteine.

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age at recruitment was 7^{+1} weeks (range: 6^{+0} to 11^{+5}), and the median number of 3D-US examinations per pregnancy was five (range: 1–7). The mean absolute CRL and EV growth rates were 1.43 mm/day and 0.46 cm^3/day, respectively.

First we compared differences in CRL and EV measurements between the ART and strictly dated spontaneous pregnancy subgroups, showing no statistically significant differences: model 2, CRL analysis: β 0.01 $\sqrt{}$mm (95% CI, −0.03 to 0.05), P=.65; EV analysis: β 0.01 $\sqrt[3]{}$cm^3 (95% CI, −0.01 to 0.03), P=.60. Table 2 shows the resulting associations between I-C metabolism biomarkers and longitudinal embryonic CRL and volume measurements using linear mixed modeling. Vitamin B_{12} was positively associated with longitudinal CRL measurements in the total study population and in the strictly dated spontaneous pregnancy subgroup for both models 1 and 2.

The tHcy concentration was negatively associated with longitudinal CRL measurements in the total study

TABLE 2

Effect estimates from the linear mixed models for associations between maternal biomarkers of one-carbon metabolism and longitudinal embryonic crown-rump length and volume measurements.

	Total population	Strictly dated spontaneous pregnancies	ART pregnancies
Biomarker		**Effect estimates CRL β (95% CI), $\sqrt{}$mm**	
Vitamin B_{12}			
Model 1	4^{-4} (1^{-4} to 7^{-4})[a]	7^{-4} (3^{-4} to 1^{-3})[b]	-1^{-4} (-4^{-4} to 3^{-4})
Model 2	5^{-4} (1^{-4} to 9^{-4})[a]	8^{-4} (4^{-4} to 1^{-3})[b]	-1^{-4} (-5^{-4} to 3^{-3})
tHcy			
Model 1	−0.04 (−0.06 to −0.02)[b]	−0.04 (−0.08 to −0.00)[c]	−0.04 (−0.05 to −0.02)[b]
Model 2	−0.03 (−0.05 to −0.01)[b]	−0.03 (−0.07 to −0.00)[c]	−0.03 (−0.05 to −0.01)[a]
		Effect estimates EV β (95% CI), $\sqrt[3]{}$cm^3	
Vitamin B_{12}			
Model 1	2^{-4} (0^{-4} to 4^{-4})[a]	3^{-4} (1^{-4} to 5^{-4})[a]	-2^{-5} (-2^{-4} to 2^{-4})
Model 2	2^{-4} (0^{-4} to 4^{-4})[a]	3^{-4} (1^{-4} to 5^{-4})[a]	-2^{-5} (-2^{-4} to 2^{-4})
tHcy			
Model 1	−0.02 (−0.03 to −0.01)[b]	−0.02 (−0.04 to −0.00)[c]	−0.02 (−0.03 to −0.01)[b]
Model 2	−0.02 (−0.03 to −0.01)[b]	−0.01 (−0.03 to 0.01)	−0.02 (−0.03 to −0.01)[b]

Note: Effect estimates represent the amount of change in square root CRL ($\sqrt{}$mm) and third root EV ($\sqrt[3]{}$cm^3) per unit increase of the biomarker's concentrations. Model 1 is adjusted for gestational age. Model 2 includes adjustment for parity, alcohol use, smoking habit, folic acid supplement use, maternal age, body mass index, comorbidity, and fetal gender. Vitamin B_{12} concentrations are measured in serum and tHcy in plasma. CI = confidence interval; CRL = crown-rump length; EV = embryonic volume; tHcy = total homocysteine.

[a] P< .01.
[b] $P\leq$.001.
[c] $P\leq$.05.

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population and in both subgroups for models 1 and 2. In the total study population and after transformation to the original scale, a high tHcy concentration (+2 standard deviation [SD], corresponding to 10.3 μmol/L) was associated with a 1.7 mm smaller CRL (−13.4%) at 7 weeks and a 3.6 mm smaller CRL (−7.1%) at 11 weeks of gestation compared with a low tHcy concentration (−2 SD, corresponding to 3.0 μmol/L).

Regarding the EV analysis, vitamin B_{12} was statistically significantly associated with increased longitudinal EV measurements in the total study population and in the strictly dated spontaneous pregnancy subgroup for models 1 and 2. The tHcy concentration was negatively associated with longitudinal EV measurements in the total study population and ART subgroup for both models, whereas model 2 lost statistical significance in the strictly dated spontaneous pregnancy subgroup. The transformation to the original scale in the total study population showed that high tHcy concentrations (+2 SD) statistically significantly lowered EV measurements with a mean of 0.10 cm^3 (−33.3%) at 7 weeks and 1.65 cm^3 (−16.1%) at 11 weeks of gestation compared with low tHcy concentrations (−2 SD). Figure 2 shows the average regression lines for tHcy in the total study population (model 2). Supplemental Table 1 (available online) shows the associations between maternal biomarker concentrations and early or late first trimester embryonic size, confirming the detected associations particularly during the late first trimester.

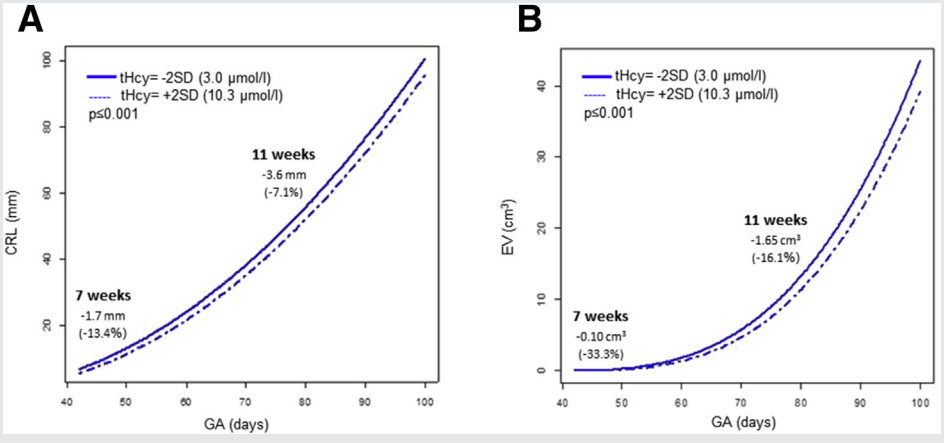
Finally, in the total study population the analysis on embryonic growth rate per day showed statistically significant positive associations for vitamin B_{12} (model 2, CRL growth rate: β 0.0002 mm/day [95% CI, 0.0001–0.0003], $P=.02$; EV growth rate: β 2.7∧10−5 cm^3/day [95% CI, 5.0∧10−6 to 4.9∧10−5], $P=.02$) and negative associations for tHcy (model 2, CRL growth rate: β −0.0001 mm/day [95% CI, −0.009 to 0.009], $P=.87$; EV growth rate: β −0.001 cm^3/day [95% CI, −0.002 to −0.000], $P=.05$).

DISCUSSION

This study demonstrates that periconceptional maternal tHcy concentrations are negatively associated and vitamin B_{12} concentrations are positively associated with embryonic growth depicted by longitudinal CRL and EV measurements and absolute growth rates. In agreement with previous results showing EV as a more effective measurement of first trimester growth restriction compared with CRL, the strongest effect in our study was observed for EV, with a reduction of more than one-third of this volume in case of high tHcy concentrations at 7 gestational weeks (20). The obtained mean EV measurements in our population were comparable with previous results (21, 22). In particular, a 10-week-old embryo showed a mean volume of 5.6 cm^3, which is in agreement with measurements previously reported at the same gestational age (4.2–6.2 cm^3) (21, 22). Small variations could be explained by differences in methods (VR versus 3D-US), maternal baseline characteristics, and pregnancy dating procedures (21, 22). The obtained CRL measurements were also in line with the reference curves provided by the Robinson chart (17).

The effect size of tHcy concentration on CRL is comparable with the estimates previously reported for maternal age, whereas the effect size is 10 times stronger than for alcohol consumption (CRL β −0.0025 √mm [95% CI, −0.0047 to −0.0003]) (18). We previously showed in a smaller study population that maternal periconceptional smoking (>10 cigarettes per day) was associated with a 7 times stronger effect sizes on CRL compared with the tHcy concentration (β −0.202 √mm [95% CI, −0.404 to −0.001]) (18). In the Generation R study, maternal age, diastolic blood pressure, hematocrit value, smoking, and folic acid supplement use were

FIGURE 2



Fully adjusted models for (**A**) crown-rump length (CRL) and (**B**) embryonic volume (EV) in relation to total homocysteine (tHcy) concentrations in the total study population. The tHcy concentration is expressed as +2 standard deviations (SD) (*dashed line*, corresponding to 10.3 μmol/L in the total study population) and −2 SD (*continuous line*, corresponding to 3.0 μmol/L). Gestational age (GA) is expressed in days. Full adjustment for parity, alcohol use, smoking, folic acid use, maternal age, body mass index, comorbidity, and fetal gender was performed.

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statistically significantly associated with late first-trimester CRL measurements in a multivariable adjusted model, where no use of folic acid supplement revealed the strongest effect (β −1.33 mm [95% CI, −2.41 to −0.24]) (12). In our model, the effect estimate of high tHcy (+2 SD) on CRL is almost three times higher. In regard to vitamin B_{12}, the associations, albeit statistically significant, showed small effect sizes on CRL and EV (Table 2). The analysis on CRL and EV growth rates confirmed a statistically significant association with maternal I-C metabolism, pointing out associations not only with embryonic size at a specific step point, but also with growth velocity.

The associations between maternal biomarkers and embryonic growth differed between the two subgroups. We suggest that the ovarian stimulation treatment, the extrauterine development of the preimplantation embryo, and the use of varying culture media can influence embryonic responses to periconceptional biomarker exposure. Moreover, despite the adjustments, it is difficult to disentangle the impact of the causes of subfertility as an independent factor affecting embryonic growth (23, 24).

Our results underline that maternal periconceptional I-C metabolism is significantly associated with embryonic growth, which substantiates previous findings on associations with embryonic development and congenital anomalies (6, 7, 25, 26). Moreover, our data mean to integrate the known association between an optimal maternal long-term folate status and increased longitudinal CRL measurements, finally confirming the importance of maternal I-C metabolism for first-trimester embryonic outcomes (27).

Several molecular and biological processes may represent the causal link between maternal I-C metabolism derangements and impaired embryonic growth and development, including direct cytotoxic effects, excessive oxidative stress, impaired DNA synthesis and repair, and increased apoptosis (6, 9, 28). Recent data indicate that I-C metabolism is also crucial in the programming and development of mammalian oocytes and preimplantation embryos (29, 30). The addition of Hcy to the culture medium suppresses blastocyst development in animal models (31). In the ART population, we previously demonstrated statistically significant associations between high tHcy concentrations in follicular fluid and poor embryo quality, reduced chances of ongoing pregnancy, smaller size of ovarian follicles, and a lower number of oocytes retrieved (32, 33). Moreover, the development of mammalian embryos represents a critical stage for epigenetic perturbations, a programing mechanism that can explain the associations shown in animal models between a periconceptional environment deficient in vitamin B_{12}, folate, and methionine and offspring exhibiting hypertension, obesity, insulin resistance, and global changes in liver methylation status (34). These data substantiate again the involvement of maternal I-C metabolism as early as the periconceptional period for growth and development of the human embryo with implications for future health.

The main strengths of this study are the longitudinal prospective design starting very early in pregnancy and the highly precise and repeated measurements of CRL and EV in the same data set, thereby taking into account embryonic growth and development in three dimensions. By performing a median of five scans per pregnancy, we considered first-trimester embryonic growth as a continuous and evolving process, providing highly accurate growth curves. Conversely, only two measurements per pregnancy could have not depicted the detailed trajectory of embryonic development, indicating only the starting and ending point of the growth process.

Semiautomated volume measurements can be performed using full immersive VR systems (15). Compared with other methods, which reckon on manually drawing the contours in different rotational or longitudinal planes and exclude embryonic limb involvement, the V-Scope software enables volume measurements that are less sensitive to individual variations, further including the whole embryonic body into the measurement (15). The selection of ART pregnancies and spontaneous pregnancies with strict pregnancy dating makes the assessment of gestational age highly reliable and minimizes the risk of confounding of embryonic growth by gestational age.

The concentrations of tHcy were statistically significantly lower in the ART subgroup compared to the strictly dated spontaneous pregnancy subgroup, which fits with a higher frequency of preconceptional initiation of folic acid supplement use in the ART subgroup compared with the spontaneous subgroup. The high frequency of folic acid supplement use in women undergoing ART treatment and their high educational level explain the low prevalence of hyperhomocysteinemia in our population. This makes the results even stronger because clinically normal tHcy concentrations close to the upper limit already seem to impact embryonic growth.

Some limitations inherent to the observational design of our study also need to be addressed. Despite adjustment for several potential confounders, this cohort study was conducted in a tertiary hospital population with a high proportion of comorbidity and high-risk pregnancies, which reduces the external validity of our findings.

First-trimester embryonic growth has been strongly associated with second- and third-trimester fetal growth and birth weight (35). Moreover, a smaller embryo depicted by CRL measurements has been associated with an increased risk of preterm birth, low birth weight, small for gestational age, and a poor cardiovascular risk profile in early childhood (12,35–37). These data together with our findings illustrate the importance of the periconceptional period and emphasize the need for early first-trimester antenatal visits to improve the maternal modifiable conditions involved in I-C metabolism (38, 39). In this regard, inadequacies in dietary B vitamins and lifestyle (i.e., smoking and alcohol and coffee consumption) have led to an average increase in plasma tHcy concentrations of 1–4 μmol/L over the last several decades (6). In clinical settings, a random plasma tHcy measurement represents an overall stable marker, with no seasonal variation, within the same individual (40). This is probably due to the general stability of individual exposures highly associated with tHcy concentrations (i.e., nutritional and lifestyle habits). Moreover, serial tHcy measurements have been recently shown to be constant in uncomplicated pregnancies (41). This suggests that a random tHcy measurement is reflective of an individual's average concentration and represents a potential useful predictor of

disease, supporting its widespread use as a predictor and prognostic marker of cardiovascular diseases in both high-risk and low-risk populations (42). Although our study did not mean to provide a prediction model of embryonic growth, our results suggest that a random maternal tHcy evaluation as early as the preconceptional period could impact and possibly optimize embryonic growth. On the other hand, it seems too early to provide recommendations on periconceptional vitamin B_{12} assessment for embryonic health, mainly due to the small effect size detected in our results.

CONCLUSIONS

We have shown that small variations in biomarkers of periconceptional maternal I-C metabolism are associated with human embryonic growth. Because a smaller embryo is associated with a higher risk of adverse pregnancy outcomes and increased risks of noncommunicable diseases in later life, periconceptional maternal I-C metabolism may be used as future predictor of embryonic health and perhaps health during the life course. Nevertheless, further research is recommended to elucidate whether the observed associations also apply to the general pregnant population.

Acknowledgments: The authors thank all participating women and the whole Predict study group for the great effort they put into the Rotterdam Periconception cohort from 2009 and onward.

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SUPPLEMENTAL TABLE 1

Effect estimates from the linear mixed models for associations between maternal biomarkers of one-carbon metabolism and early or late first trimester crown-rump length (CRL) and embryonic volume (EV) measurements.

	Early first trimester (< 8 wk)		Late first trimester (> 10 wk)	
Biomarker	**CRL β (95% CI), $\sqrt{}$mm**	**EV β (95% CI), $\sqrt[3]{}$cm^3**	**CRL β (95% CI), $\sqrt{}$mm**	**EV β (95% CI), $\sqrt[3]{}$cm^3**
Total population				
B_{12}	0.0004 (0.0001; 0.0007)[a]	0.0001 (−0.00002; 0.0002)	0.001 (0.0002; 0.001)[b]	0.0002 (0.0000; 0.0004)[a]
tHcy	−0.02 (−0.04; 0.00)	−0.01 (−0.02; −0.00)[a]	−0.04 (−0.06; −0.02)[c]	−0.02 (−0.03; −0.01)[b]
Strictly dated spontaneous pregnancies				
B_{12}	0.0006 (0.0001; 0.001)[a]	0.0002 (0.00002; 0.0004)[a]	0.001 (0.0005; 0.001)[c]	0.0004 (0.0001; 0.001)[b]
tHcy	−0.03 (−0.06; 0.00)	−0.01 (−0.03; 0.00)	−0.04 (−0.08; −0.00)[a]	−0.02 (−0.04; −0.00)[a]
ART pregnancies				
B_{12}	0.000 (−0.001; 0.001)	−0.0001 (−0.0003, 0.0001)	−0.0002 (−0.001; 0.000)	−0.000 (−0.000; 0.000)
tHcy	−0.02 (−0.04, 0.00)	−0.01 (−0.02; 0.00)	−0.04 (−0.07; −0.01)[b]	−0.03 (−0.04; −0.00)[b]

Note: Effect estimates represent the amount of change in square root CRL ($\sqrt{}$mm) and third root EV ($\sqrt[3]{}$cm^3) per unit increase of the biomarker's concentrations. A fully adjusted model is shown including adjustment for parity, alcohol use, smoking habit, folic acid supplement use, maternal age, body mass index, comorbidity, and fetal gender. ART = assisted reproductive technology; CI = confidence interval; CRL = crown-rump length; EV = embryonic volume; tHcy = total homocysteine.

[a] $P \leq .05$.

[b] $P < .01$.

[c] $P \leq .001$.

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