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ORIGINAL ARTICLE

The association between birth weight and plasma fibrinogen is abolished after the elimination of genetic influences

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Summary. Low birth weight is associated with an increased risk of atherothrombosis, which may be related in part to the association between low birth weight and high plasma fibrinogen. The association between birth weight and fibrinogen may be explained by intrauterine, socio-economic or genetic factors. We examined birth weight and fibrinogen in 52 dizygotic and 56 adolescent monozygotic (genetically identical) twin pairs. The dizygotic but not the monozygotic twins with the lowest birth weight from each pair had a fibrinogen level that was higher compared with their co-twins with the highest birth weight [dizygotic twins: $2.62 \pm 0.46\,g\,L^{-1}$ vs. $2.50 \pm 0.41\,g\,L^{-1}$ ($P = 0.04$); monozygotic twins: $2.42 \pm 0.45\,g\,L^{-1}$ vs. $2.49 \pm 0.39\,g\,L^{-1}$ ($P = 0.2$)]. These findings suggest that the association between birth weight and plasma fibrinogen is abolished after the elimination of genetic influences and therefore that this association has genetic causes. Improvement of intrauterine nutrition may not lower fibrinogen levels in later life.

Keywords: birth weight, fibrinogen, genetics, twins.

Introduction

Indices of fetal growth, such as birth weight, are inversely associated with the prevalence and mortality of coronary heart disease and stroke [1,2]. It has been suggested that increased plasma concentrations of fibrinogen in later life may play a role in these associations [3–5]. The inverse association between birth weight and fibrinogen has been attributed to a programmed response to intrauterine malnutrition that induces permanent changes in the structure and function of organs, which cause increased levels of fibrinogen in later life [1]. This theory may be supported by a study demonstrating that people who were exposed to the Dutch famine in early gestation had slightly elevated levels of fibrinogen in later life [5]. If the association between birth weight and fibrinogen is due to intrauterine nutrition, improvements in intrauterine nutrition may lower fibrinogen levels in later life. However, the alternative view is that some factors other than intrauterine nutrition may influence both growth *in utero* and levels of fibrinogen. Environmental causes, particularly those associated with socio-economic status, as well as genetic factors have been proposed as alternative explanations [6]. If the association between birth weight and fibrinogen is due to socio-economic or genetic causes, improvement of intrauterine nutrition may not lower fibrinogen levels in later life. Studies in dizygotic and monozygotic twin pairs still living with their parents offer a unique opportunity to distinguish between intrauterine, socio-economic and genetic influences [7–9]. Studying differences in twin pairs avoids socio-economic factors that could confound the association between size at birth and cardiovascular disease in later life. Furthermore, investigating differences in monozygotic (genetically identical) twin pairs allows elimination of the influence of genotype on this association.

We investigate herein the association between birth weight and fibrinogen in a group of adolescent dizygotic and monozygotic twin pairs still living with their parents. The underlying hypotheses for the within-pair analyses were that if intrauterine nutrition is responsible for the association between birth weight and fibrinogen, the association would be present within both dizygotic and monozygotic twin pairs. If socio-economic factors are responsible, the association would be absent within both dizygotic and monozygotic twin pairs. If a genetic predisposition is responsible, birth weight would be associated with fibrinogen within dizygotic twin pairs, but not within monozygotic twin pairs.

Methods

Subjects

This study is part of a larger project in which cardiovascular risk factors were studied in 160 adolescent twin pairs and their parents [8–10]. Addresses of twins living in Amsterdam and neighboring cities were obtained from City Council population registries. Twins still living with their biological parents were contacted by letter. A questionnaire was used to gather information on various factors including the use of medication and smoking behavior. The maternal questionnaire included questions regarding birth weight and gestational age of the children.

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This questionnaire was sent to the mothers a few weeks ahead of their visit to our department, allowing them to obtain birth data from birth certificates [8,9]. Opposite-sex dizygotic twin pairs ($n = 28$) were excluded from the analyses because of the effects of sex differences within a pair on both birth weight and fibrinogen. Subjects using oral contraceptives were also excluded (six dizygotic twin pairs and seven monozygotic twin pairs). None of the subjects used any other medication that may affect plasma concentrations of fibrinogen. Thus, 52 dizygotic and 56 monozygotic twin pairs were available for analysis.

Measurements

Height and weight were measured in a standardized way. After acclimatization blood was obtained between 08.30 and 10.30 am by venipuncture after overnight fasting. Total fibrinogen antigen in EDTA plasma was determined with an enzyme immunoassay that uses a pool of rabbit anti-human fibrinogen immunoglobulins (IgGs) as catching antibodies [11] and peroxidase-conjugated monoclonal antibodies against fragment DD (DD13) [12] as tagging antibodies. Pooled plasma of healthy volunteers is used as a standard (100%) from which the fibrinogen content was determined additionally with a gravimetric method to express fibrinogen in grams per liter [13].

Statistical analysis

The paired Student's *t*-test was used to compare twins with the lowest birth weight from each pair with their co-twins with the highest birth weight. For this analysis, two dizygotic twin pairs and one monozygotic twin pair had to be excluded because the birth weight of the twins within a pair was equal. Differences in dizygotic twin pairs and in monozygotic twin pairs were compared using the independent samples *t*-test. In addition, linear regression analysis was used to analyze whether intrapair differences in birth weight influenced intrapair differences in fibrinogen before and after adjustment for differences in current weight or body mass index (BMI; including the three twin pairs in which the birth weight of the twins within a pair was equal). Interaction analysis was performed to investigate whether zygosity or differences in current weight influenced the associations between intrapair differences in birth weight and fibrinogen. Linear regression analysis was used to analyze the association between birth weight and fibrinogen in the overall sample. Interaction analysis was performed to investigate whether zygosity influenced this association. A two-tailed *P*-value <0.05 was considered significant. All analyses were performed on a personal computer using the statistical software package SPSS version 9.0 (SPSS Inc).

Results

As a first intrapair analysis, we compared co-twins with the lowest birth weight from each pair with their co-twins with the highest birth weight. The dizygotic but not the monozygotic twins with the lowest birth weight from each pair had fibrinogen levels that were higher compared with their co-twins with the highest birth weight (Table 1). The differences in fibrinogen between the co-twins with the lowest and the co-twins with the highest birth weight were significantly different in dizygotic compared with monozygotic twin pairs ($P = 0.02$). In both the dizygotic and the monozygotic twins, current BMI and smoking habits were similar in co-twins with the lowest and the co-twins with the highest birth weight. In an additional analysis, intrapair differences in birth weight were associated with differences in fibrinogen in dizygotic twins {regression coefficient $-0.25\,\mathrm{g\,L^{-1}\,kg^{-1}}$ [95% confidence interval (CI) -0.49 to -0.01], $P < 0.05$} suggesting that the larger the difference in birth weight, the higher the fibrinogen in the twin with the lowest birth weight compared with the co-twin with the highest birth weight. In monozygotic twins, however, intrapair differences in birth weight were not associated with differences in fibrinogen [$+0.16\,\mathrm{g\,L^{-1}\,kg^{-1}}$ (95% CI -0.12–0.45), $P = 0.3$]. Interaction analyses confirmed that these associations were significantly different between dizygotic and monozygotic twins ($P = 0.03$). In the overall sample of twins, birth weight was not associated with fibrinogen [regression coefficient $0.01\,\mathrm{g\,L^{-1}\,kg^{-1}}$ (95% CI -0.10–0.13), $P = 0.8$, adjusted for age and sex]. The results were similar after adjustment for (differences in) current weight, BMI or smoking. In addition, the results were similar after the exclusion of smokers. Interaction analyses demonstrated that the (intrapair) associations between birth weight and fibrinogen were not different between men and women (*P* for interaction was always >0.4).

Table 1 Clinical characteristics of the co-twins with the lowest and the highest birth weight in dizygotic and monozygotic twin pairs

	Dizygotic twin pairs			Monozygotic twin pairs		
	Co-twins with the lowest birth weight	Co-twins with the highest birth weight	*P*	Co-twins with the lowest birth weight	Co-twins with the highest birth weight	*P*
Birth weight (g)	2226 ± 477	2604 ± 540	<0.001	2339 ± 524	2637 ± 475	<0.001
Gestational age (weeks)	37 ± 2.8	37 ± 2.8	–	36 ± 8.4	36 ± 8.4	–
n (male/female)	50 (29/21)	50 (29/21)	–	55 (29/26)	55 (29/26)	–
Age (years)	17.0 ± 1.7	17.0 ± 1.7	–	16.0 ± 1.8	16.0 ± 1.8	–
Current BMI ($\mathrm{kg\,m^{-2}}$)	20.0 ± 1.9	20.3 ± 2.2	0.5	19.5 ± 2.3	19.8 ± 2.3	0.2
Smoking (*n*)	7	9	–	4	4	–
Fibrinogen ($\mathrm{g\,L^{-1}}$)	2.62 ± 0.46	2.50 ± 0.41	0.04	2.42 ± 0.45	2.49 ± 0.39	0.2

Mean ± SD. BMI, body mass index.

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Discussion

We found that low birth weight was associated with high fibrinogen within dizygotic twin pairs, but not within monozygotic twin pairs. These data provide the first evidence that the association between birth weight and fibrinogen is abolished after the elimination of genetic influences. Importantly, these findings contradict the hypothesis that improvement of intrauterine nutrition may lower fibrinogen levels in later life.

It could be argued that besides genetic factors, intrauterine factors may also differ between dizygotic and monozygotic twins and may be the cause of the difference in the intrapair association between birth weight and fibrinogen. However, previous studies have demonstrated that the associations between low birth weight and increased cardiovascular risk factors in overall samples of twins were similar in dizygotic and monozygotic twins [8,9,14]. In addition, intrapair differences in birth weight were related to differences in diabetes [15], high density lipoprotein (HDL) cholesterol [9], and height [16,17] in both dizygotic and monozygotic twins. These studies suggest that intrauterine differences between dizygotic and monozygotic twins do not explain the differences in the intrapair association between birth weight and fibrinogen in dizygotic and monozygotic twins.

It could be suggested that twin pairs cannot be used as a model to study the association between birth weight and cardiovascular risk factors in singletons. However, birth weight in twins has been associated with many variables that have been related to birth weight in singletons [8,9,14–17]. In addition, fibrinogen levels in our adolescent twins were not different from levels found in studies in singletons [18,19].

The absence of an association between birth weight and fibrinogen in the overall sample is consistent with several studies in singletons [20–22], but not all [3,4]. Our results in dizygotic twin pairs demonstrate that the association between birth weight and fibrinogen may be strengthened after the elimination of socio-economic factors. In contrast, this association is abolished after the elimination of genetic factors. Genetic and socio-economic influences may be different across populations and may explain the contradictory findings of studies in singletons. Interestingly, it has been demonstrated that although size at birth was not associated with plasma levels of fibrinogen in people born around the Dutch famine, people who were exposed to famine in early gestation had slightly elevated levels of fibrinogen in later life [5].

The results from the Dutch famine birth cohort could be interpreted as a specific effect of the intrauterine environment on fibrinogen levels [5]. However, an alternative explanation is that this finding reflects a selective survival advantage of fetuses genetically susceptible to an increased cardiovascular risk [23]. During the famine, the number of conceptions was about 50% lower than the prefamine level and perinatal mortality as well as mortality in the first year after birth were highest in those who were born during the famine [24].

Our findings suggest that genetic factors account for the association between birth weight and fibrinogen. However, the genetic factors that may be responsible are not known. Interestingly, it has recently been demonstrated that several inherited risk factors for thrombophilia were related to low birth weight in Caucasian children [25]. Therefore, further research into the genetic factors responsible for the association between birth weight and fibrinogen is warranted.

In summary, we found that low birth weight was associated with high levels of fibrinogen within dizygotic twin pairs, but not within monozygotic twin pairs. These data suggest that genetic factors account for the association between birth weight and fibrinogen. Therefore, improvements in intrauterine nutrition may not lower fibrinogen levels in later life.

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