Prenatal influences on childhood psychological development

Summary of main findings

Birth weight and childhood psychological development

In **chapter 2**, we used multivariate linear regression analyses adjusted for maternal and childhood sociodemographic characteristics to investigate the association between the intrapair birth-weight difference and the difference in each problem behaviour scale in preschool-age monozygotic (MZ) and dizygotic (DZ) twins. The discordant-twin study design automatically controls for common genetic and environmental factors between co-twins, allowing the investigation of the effects of non-shared influences (which are exclusively environmental in nature in MZ twins, but genetic and environmental in DZ twins) responsible for the difference in birth weight (11,12). In the subgroup of MZ twins, we found that increasing birth-weight difference was associated with increasing differences in total problems (β= -5.95; 95% confidence interval, CI: -11.08, -0.82), driven by internalising behaviours (β= -4.17; 95% CI: -7.65, -0.69) and, specifically, emotional reactiveness (β= -2.70; 95% CI: -5.23, -0.17). Furthermore, increasing birth-weight difference was associated with larger predicted-score differences (*i.e.*, more negative predicted-score differences) in the three previously identified scales in MZ twins. This suggests that infants with a low birth weight might be more at risk of developing internalising problem (note that differences in problem-behaviour scores were calculated by subtracting the score of the smaller-at-birth twin from the score of the larger-at-birth twin; see **chapter 2** for details). Conversely, no associations were observed when examining DZ twins or MZ and DZ twins combined. Although none of the identified associations remained statistically significant after controlling for multiple testing, these results are in line with other studies done both in older twins (13–15) and singletons (*e.g.*, (16–19)), which reported an inverse relationship between birth weight and internalising behaviours. A similar trend is sometimes reported for externalising behaviours, although with smaller effect sizes (15,20–22). By contrast, a few other studies did not report any association between birth weight and childhood (23) or adolescence (24) problem behaviours in singletons.

Maternal weight before and during pregnancy and childhood psychological development

Maternal pre-pregnancy body mass index

The study described in **chapter 3** aimed to examine the association between maternal pre-pregnancy BMI and infant twins' temperament. The twin study design accounted for genetic and

environmental factors shared between co-twins (25), while allowing the assessment of the overall effect of maternal pre-pregnancy BMI on three temperamental scales (*i.e.,* activity level, distress to limitation and duration of orienting). We found a negative association with DZ twins' distress to limitation (β= -0.04; 95% CI: -0.065, -0.013), indicating that children born to women with a higher pre-pregnancy BMI would display fewer distress when confined in a place or position. However, the analyses in DZ twins are susceptible to residual confounding, as genetic influences cannot be completely controlled for. Furthermore, the estimate was very close to the null value and not significant after controlling for multiple testing. By contrast, no associations were found when examining the other temperamental scales or in MZ twins.

Previous studies in singletons reported poorer regulatory behaviours in infants born to women with pre-pregnancy overweight or obesity (26) or pre-pregnancy obesity in combination with excessive GWG (27). Maternal pre-pregnancy BMI was also associated with infants' negative affectivity (28) and negative behaviours (29), although in the last study the effect was reduced by high total n-3 PUFA concentrations in the last trimester of pregnancy. By contrast, maternal pre-pregnancy BMI was not found to be related with offspring temperament in children aged 1 year (30). However, while in two cases results should be considered preliminary due to the small sample size (*i.e.*, n=16 and n=68, (28,29)), overall no attempt to control for genetic or common environmental factors was made, which might have affected previous studies' results.

Gestational weight gain

The purpose of the study presented in **chapter 4** was to investigate the association between GWG and problem behaviours in school-age children. In addition, we assessed the possible effect modification of maternal pre-pregnancy BMI status, since previous studies reported a higher likelihood of excessive GWG in women with pre-pregnancy overweight or obesity (31). With this pooled analysis of individual data from the MEFAB and Rhea cohorts, we found evidence for higher problem behaviours associated with excessive GWG in pre-pregnancy overweight or obese women, while no evidence of an association was found in the pre-pregnancy normal-weight group. On average, children born to overweight/obese women who gained 0.5 kg/week scored 25 points higher (on a 0-100 scale) in both total problems and internalising behaviours than children whose mothers gained only 0.2 kg/week. Similarly, children born to overweight or obese women who gained 0.5 kg/week tended to have higher externalising problems, although the difference with children of women who gained less weight was smaller (*i.e.*, 18 points). Notably, being overweight

or obese and gaining 0.5 kg/week was predictive of problem behaviour scores in the clinical range of symptomatology as defined by the Child Behaviour Checklist (CBCL, *i.e.*, over 63/100).

These results are supported by previous findings showing higher infants' neurobehaviour and childhood attention deficit/hyperactivity disorder (ADHD) risk in children of pre-pregnancy overweight or obese women who gained excessive weight during pregnancy (27,32,33). Higher hyperactivity/impulsivity symptoms were also observed in children of women with insufficient compared to adequate GWG (33). By contrast, the only study that assessed specifically childhood problem behaviours, as well as ADHD symptoms, did not find any evidence of an association with GWG (34). However, the latter study examined data from a high-risk population, comprising a large proportion of young, never-married women with a low income who made use of illicit drugs, alcohol and marijuana during pregnancy. As we will discuss below (pages 239-240), the peculiarity of this study population precludes direct comparisons with our study, whose population can be considered at low risk, being largely composed of highly educated, double-parent families.

Maternal diet during pregnancy and childhood psychological development

Maternal PUFA concentrations

In the study presented in **chapter 5**, we focused on maternal PUFAs during pregnancy in relation to social competence and problem behaviours in school-age children from the MEFAB cohort. Since maternal PUFA concentrations change physiologically during pregnancy (35,36), we calculated the trend of change of maternal PUFAs during pregnancy. The results indicated that increasing maternal arachidonic acid (AA, a n-6 PUFA) concentration in late pregnancy (*i.e.,* defined as the period after the 30th week of gestation) might be associated with a small improvement in social competence. Furthermore, a larger decrease in total n-6 PUFAs in late gestation was associated with a small decrease in externalising behaviours. The clinical relevance of the two associations was modest, with predicted scores falling within the normal-development range as defined by the CBCL (*i.e.,* over 40/100 and less than 63/100 for social competence and externalising behaviours, respectively). In addition, associations fell short of statistical significance after controlling for multiple testing.

Previous observational studies analysed PUFA concentrations in the umbilical cord or in maternal blood at specific time-points, and reported largely inconsistent results (37–41). The only attempt to examine the whole prenatal exposure longitudinally was done by estimating maternal n-3 PUFA levels by assessing fish and seafood intake during pregnancy. Small improvements in hyperactivity and prosocial behaviour were reported in children of women with the highest fish or seafood intake

(42–44). However, assessing maternal fish and seafood intake alone limits the investigation to maternal n-3 PUFAs, while fatty acids of the n-6 family might also have a role. Furthermore, examining maternal PUFA concentration at a specific time-point or estimating n-3 PUFA intake cannot provide an adequate representation of prenatal PUFA exposure, which changes over time based on both maternal dietary intakes and the release of fatty acids from maternal adipose tissue (35,36,45). Finally, the role of prenatal PUFA exposure on neurocognitive and behavioural outcomes has been investigated by only a few, small randomised controlled trials (RCTs), which reported no effect of maternal supplementation on infants' outcomes (46).

Maternal diet quality

In **chapter 6**, we examined the association between maternal diet quality during pregnancy, measured with the MD or the DASH scores, and problem behaviours in 1,543 school-age children from the Rhea and the INMA cohorts. These two dietary scores were selected because they are based on healthy, practicable and understandable diet plans (47–49) that have been associated with reduced risk of psychiatric disorders in children and adults (*e.g.,* (50–52)). The results showed that a one-point increase in Mediterranean diet (MD) score, which could range from 0 to 8, was associated with lower odds of externalising behaviours (OR: 0.88; 95% C.I.: 0.79, 0.98). Overall, we estimated that the prevalence of childhood externalising behaviours in this population would be 16.4% lower (*i.e.*, from 28.9% to 12.5%) in children whose mothers had the highest compared to lowest MD score. Conversely, maternal diet quality assessed by adherence to the DASH diet was not associated with childhood problem behaviours. Compared to the MD, in the DASH score used for these analyses, intakes of fish and seafood are not assessed, while it is an integral part of the MD score and the MD dietary pattern in general. Furthermore, intake of fish and seafood was more modestly correlated with DASH score in this study population than with MD score, suggesting that prenatal exposure to fish and seafood might be beneficial to prevent later externalising behaviours.

Previous studies that examined the association between maternal dietary patters during pregnancy and childhood problem behaviours in other European populations found reduced odds of externalising behaviours in children of women following a "healthy" dietary pattern that resembled the MD, reporting overall similar effect estimates compared to those observed in our study (53–55). In addition, a re-analysis of the data to support findings obtained with principal component analysis found a small reduction in the odds of childhood externalising behaviours associated with higher adherence to the MD score (54). Moreover, higher adherence to a diet quality index characterised by high intakes of fruit, vegetables, fish and whole grains, was associated with a small reduction in

both internalising and externalising behaviours in preschool-age Norwegian children (56). Finally, maternal adherence to the MD at the time of conception was associated with fewer internalising and externalising behaviour symptoms in 2-year-old children from the USA. These associations corresponded to changes in the methylation patterns in the control regions of several imprinted genes, suggesting a possible mechanism underlying the association between maternal diet quality prenatally and childhood problem behaviours (57).