Cord blood acrylamide levels and birth size, and interactions with genetic variants in acrylamide-metabolizing genes

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Abstract

Background Up to now, 3 epidemiological studies have shown clear inverse associations between prenatal acrylamide exposure and birth size. In addition to studying the association between acrylamide and birth size, we investigated the interaction between acrylamide and polymorphisms in acrylamide-metabolising genes, with the aim of probing the causality of the inverse relationship between acrylamide and fetal growth.

Methods We investigated the association between prenatal acrylamide exposure (acrylamide and glycidamide to hemoglobin adduct levels (AA-Hb and GA-Hb) in cord blood) and birth weight, length and head circumference in 443 newborns of the ENVIRONAGE (ENVIRONmental influence ON AGEing in early life) birth cohort. In addition, we studied interaction with single nucleotide polymorphism (SNPs) in CYP2E1, EPHX1 and GSTP1, using multiple linear regression analysis.

Results Compared to neonates in the lowest quartile of AA-Hb in cord blood, the body weight, length and head circumference of neonates in the highest quartile were -101 grams (95% CI: -208, 7; p for trend = 0.12), -0.13 centimetres (95% CI: -0.62, 0.36; p for trend = 0.69) and -0.41 centimetres (-0.80, -0.01; p for trend = 0.06) lower, respectively. For GA-Hb, the corresponding effect estimates were -222 grams (95% CI: -337, -108; p for trend = 0.001), -0.85 (95% CI: -1.38, -0.33; p for trend = 0.02) and -0.55 (95% CI: -0.98, -0.11; p for trend = 0.01), respectively. The associations for GA-Hb were similar or stronger in newborns of non-smoking mothers. There was no statistically significant interaction between acrylamide exposure and the studied genetic variations but there was a trend of stronger inverse associations with birth weight and head circumference among newborns with homozygous wildtypes alleles for the CYP2E1 SNPS and with variant alleles for a GSTP1 SNP (rs1138272).

Conclusions Prenatal dietary acrylamide exposure, specifically in the form of its metabolite glycidamide, was inversely associated with birth weight, length and head circumference. The interaction pattern with SNPs in CYP2E1, although not statistically significant, is an indication for the causality of this association. Other studies are needed to corroborate this finding.

Background

Acrylamide is a probable human carcinogen (IARC class 2A; based on rodent studies) present in various heat-treated carbohydrate-rich foods, such as cookies, potato chips, French fries and coffee, due to preparation of the foods at high temperatures.

In rodents, acrylamide has clearly been shown to cause cancer in various tissues.¹ The epidemiological evidence on the association between dietary acrylamide intake and human cancer risks is less clear; for some cancers, increased risks have been observed in some studies but not in all.² A 2015 meta-analysis on the association between acrylamide intake and cancer risk shows increased risk of renal cell, endometrial and ovarian cancer, the latter two in never-smokers.² In addition to the carcinogenic effects,

acrylamide has been shown to cause neurotoxicity in animals and occupationally exposed humans, and reproductive and developmental toxicity in animals.¹

With regard to developmental toxicity, a decrease in offspring body weight due to gestational acrylamide exposure has been observed in both mice and rats but only at doses that caused overt toxicity to the mothers.¹ Those doses are not reached through human dietary intake.¹

Acrylamide and its metabolite glycidamide readily pass the placental barrier, and a strong correlation between cord and maternal blood was observed, 0.69 ($p = 0.001$) and 0.78 ($p < 0.001$) for acrylamide and glycidamide, respectively.³

To date, all of the 3 epidemiological studies that have studied the link between prenatal acrylamide exposure and fetal growth have consistently shown that higher exposure is linked to reduced fetal growth.⁴⁻⁶ In light of these findings, the European Food Safety Authority (EFSA) in its 2015 acrylamide risk assessment called for more epidemiological research on the association between acrylamide intake and birth outcome.

Through the present study, we aim to contribute more evidence on the causality of the association between prenatal acrylamide exposure and birth outcomes by assessing the link between acrylamide exposure biomarkers and birth weight, birth length and birth head circumference, and studying the potential modifying role of single nucleotide polymorphisms (SNPs) in acrylamide-metabolising genes (*CYP2E1*, *EPHX1* and *GSTP*) in this association.

Materials And Methods

Study population and data collection

We recruited mother-newborn pairs in the East-Limburg Hospital in Belgium, between Friday 12 p.m. and Monday 7 a.m. from February 2nd 2010 until May 18th 2013. The catchment area of the hospital included the province Limburg in Belgium and combines both urban and suburban to rural areas with population densities of the municipalities ranging from 82 to 743 inhabitants/km². Participants were recruited if the mother was able to fill out a questionnaire in Dutch. The overall participation rate of eligible mothers was 61%. The ENVIRONAGE birth cohort was representative of all births in Flanders with regard to maternal age and education, parity, new-born's sex, ethnicity, and birth weight.

Upon arrival in the hospital for delivery, participating mothers gave written informed consent and completed questionnaires on among other age, pre-gestational body mass index (BMI), maternal education, occupation, smoking status, alcohol consumption, use of medication, parity and new-born's ethnicity in the postnatal ward after delivery.

Perinatal parameters, such as new-born's sex, birth date, birth weight and length, gestational age and Apgar score, were collected after birth. We included only full-term (≥ 36 weeks) and singleton

pregnancies. All included new-borns were healthy and free of anomalies confirmed by both prenatal ultrasound examination and postnatal assessment immediately by paediatricians. Further details of the study are reported elsewhere.⁷ The analysis described in this paper involved 443 mother-child pairs for which we had acrylamide to hemoglobin adduct measurements in umbilical cord blood and complete covariable data.

Acrylamide and glycidamide to hemoglobin adducts in cord blood

EDTA cord blood samples (n = 500, among which were 25 duplicate samples) were sent to the Centers for Disease Control and Prevention (CDC) Protein Biomarker Laboratory (Atlanta, USA) to measure acrylamide (AA-Hb) and glycidamide (GA-Hb) to hemoglobin adducts. Details of the methodology can be found elsewhere.^{8,9} Briefly, 300 µL of whole cord blood, of which plasma and buffy coat layers formed after centrifugation were removed, was analysed using an optimized Edman reaction and HPLC/tandem mass spectrometry (HPLC/MS/MS). Hemoglobin adducts of acrylamide and glycidamide with the N-terminal valine of the hemoglobin protein chains were measured as N-[2-carbamoyl-ethyl]valine-pentafluorophenylhydantion (PFPTH) derivative and N-[2-hydroxycarbamoyl-ethyl]valine-pentafluorophenylhydantion (PFPTH) derivative, respectively. Concentrations of AA-Hb and GA-Hb were reported relative to the amount of hemoglobin (pmol per g of Hb). and total hemoglobin was measured as cyanmethemoglobin, which is formed from methemoglobin by reaction with cyanide. The light absorption of the resulting red colored complex was measured spectrophotometrically at 540 nm. The lower limits of detection for this method are 3 pmol/g of Hb for acrylamide and 4 pmol/g of Hb for glycidamide.

Single nucleotide polymorphisms (SNPs) in acrylamide-metabolizing genes

In the context of the ENVIRONMENT birth cohort, a set of candidate SNPs (n = 210) in genes related to several domains of health (cognition, obesity, cardiovascular disease, ageing) were selected for genotyping. Within this set, there were 3 SNPs in *cytochrome P450 2E1 (CYP2E1)*, namely rs2480258, rs915906 and rs11101888 (the latter as a proxy for rs6413432), 1 in *epoxide hydrolase 1 (EPHX1)* (rs1051740) and 2 in *glutathione-s-transferase P1 (GSTP1)* (rs1695 and rs1138272); all are genes involved in acrylamide metabolism. CYP2E1 catalyses the epoxidation of acrylamide, resulting in glycidamide. EPHX1 hydrolyses glycidamide, resulting in glyceramide. GSTP1 is a member of the glutathione S-transferase family, which are phase-II metabolizing enzymes that enable detoxification by facilitating the conjugation of toxic compounds to glutathione, in this case both acrylamide and glycidamide.

Genomic DNA was isolated from placental tissue from the fetal side of the placenta using the QIAamp® DNA Mini Kit (Qiagen Inc., Venlo, The Netherlands), according to the manufacturer's instructions. In short, cells were lysed by using AL buffer and RNA and proteins were inactivated by RNase A and proteinase K,

respectively. To purify DNA, ethanol was added to the samples before applying them to the QIAamp Mini spin columns. Wash buffers and an elution buffer were used to ultimately collect the DNA from the samples. The concentration and purity of the isolated DNA were evaluated using the NanoDrop® ND–1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Genotyping was conducted using the Biotrove OpenArray SNP Genotyping Platform at the Harvard Medical School-Partners Healthcare Center for Genetics and Genomics. For the current analysis, we only included participants with a sample genotyping call rate $\geq 90\%$ across all of the 210 SNPs in the analyses.

Statistical analysis

We performed multiple linear regression analysis to assess the association between cord blood acrylamide and glycidamide to hemoglobin adducts and birth weight, birth length and birth head circumference. Covariables in the models were maternal age, education, pre-gestational BMI, ethnicity, the number of cigarettes smoked during pregnancy, parity, gestational age, date of delivery and newborn's sex. In sensitivity analyses, vegetable, fruit and fish intakes and consumption of soda drinks were included in the models as covariables.

We performed subgroup analyses for newborns of mothers who did not smoke during pregnancy. Furthermore, we analysed whether maternal smoking or new-born's sex modified the association between acrylamide exposure and birth outcomes.

Multiplicative interaction between acrylamide and SNPs was tested using product terms of the continuous acrylamide variable and the categorical genotype variable. For this analysis, we considered acrylamide hemoglobin adducts as a biomarker of exposure. We did not analyse the interaction between genotypes and glycidamide adducts because this marker is a representation of internal acrylamide exposure after metabolism, which is influenced by the studied genotypes. To show genotype strata-specific associations between acrylamide and birth outcomes, we used a dominant genetic model for all SNPs (homozygous wildtype versus heterozygous or homozygous variant). We calculated sum scores for *CYP2E1* and *GSTP1* by summing up variant allele numbers for the 3 SNPs in *CYP2E1* and the 2 SNPs in *GSTP1*, respectively. For the *CYP2E1* and *GSTP1* sum scores, we dichomotised the scores into 0 versus 1 or more variant alleles.

Lastly, we analysed the association between the SNPs in acrylamide-metabolising genes and the ratio of glycidamide to acrylamide hemoglobin adducts; the latter represents a marker of the degree of conversion of acrylamide to glycidamide in the body, which is possibly influenced by the studied SNPs.

Q-Q plots of the residuals were inspected in order to check the assumptions of linear models.

We used SAS software (version 9.4; SAS Institute Inc., Cary, NC, USA) for all statistical analyses.

Results

Based on the 25 samples that were analysed in duplicate, intraclass correlation coefficients (ICC) were 0.97 for acrylamide and 0.98 for glycidamide. For glycidamide, 1 sample was below the LOQ, and 57 samples were non-reportable due to an unacceptable signal-to-noise ratio in the instrument signal, and thus 417 out of the 475 samples had a result for glycidamide. The median acrylamide to hemoglobin adduct level in the 443 newborns that we had complete data for (including covariables data) was 13.2 (IQR: 10.4–17.6) pmol/ g of hemoglobin. The median glycidamide to hemoglobin adduct level was 13.3 (IQR: 10.2–18.0) pmol/ g of hemoglobin. The corresponding levels in newborns of non-smoking mothers were 12.5 (IQR: 10.2–15.6) and 12.2 (IQR: 9.8–15.6) pmol/ g of hemoglobin. Other characteristics of the study population are summarized in Table 1. The study population consisted of 229 (51.7%) boys and 214 (49.3%) girls. The median birth weight, birth length and head circumference of the newborns were 3430 (IQR: 3140–3720) grams, 50 (IQR: 49–52) centimeters and 34 (IQR: 33–35) centimeters, respectively. Several variables were associated with birth outcomes (results not shown). Boys had on average a higher birth weight, birth length and birth head circumference. The duration of the pregnancy was positively associated with all 3 birth outcomes, as was the weight gain of the mother during pregnancy. Cigarette smoking was inversely associated with birth weight and birth length, while parity was positively associated with birth weight and head circumference. The BMI of the mother before pregnancy was only associated with birth weight.

The frequencies of the genotypes and the corresponding p-value for Hardy-Weinberg equilibrium are shown in Table 2. None of the 6 SNPs deviated statistically significantly from Hardy-Weinberg equilibrium.

None of the SNPs was by itself statistically significantly associated with birth outcomes (Table 3).

In unadjusted analyses, cord blood acrylamide hemoglobin adduct levels were statistically inversely associated with birth weight, length and head circumference when acrylamide adduct levels were modelled as a continuous variable but there was only a significant linear trend over the quartiles of acrylamide adducts for head circumference (Table 4). In newborns of women who did not smoke during pregnancy, acrylamide adduct levels were inversely associated with birth weight and head circumference when acrylamide was modelled as a continuous variable but there was no clear linear trend over the quartiles (Table 4).

In multivariable-adjusted models, acrylamide adduct levels were still inversely associated with all 3 birth outcomes with regression coefficients of -40 (95% CI: $-71, -9$; $p: 0.01$) grams, -0.17 (95% CI: $-0.31, -0.03$; $p: 0.02$) centimeters, and -0.13 ($-0.24, -0.01$; $p: 0.03$) centimeters per increment of 10 pmol per gram of hemoglobin for birth weight, length and head circumference, respectively, but the effect estimates were slightly reduced compared to the unadjusted estimates (Table 4).

The associations with glycidamide adducts are stronger and show clearer dose-response relationships than those with acrylamide. In addition, glycidamide adducts show a clear inverse association with birth length, in contrast to what was observed for acrylamide adducts. In unadjusted analyses, glycidamide was inversely associated with all 3 birth outcomes and with a clear dose-response across the quartiles of

glycidamide adducts. In multivariable-adjusted analyses, the regression coefficients of a 10 pmol per gram hemoglobin increase in glycidamide adducts were -53 (95% CI: $-90, -16$; $p: 0.005$) grams, -0.24 (95% CI: $-0.41, -0.08$; $p: 0.004$) centimeters and -0.11 (95% CI: $-0.25, 0.03$; $p: 0.11$) centimeters for birth weight, length and head circumference, respectively. In newborns of women who did not smoke during pregnancy, the regression coefficients of the continuous glycidamide adduct level variable were roughly twice as large as those for the whole group.

For acrylamide, but particularly for glycidamide, the effect estimates were larger in newborns of mothers who did not smoke during pregnancy (Table 4). The interaction with smoking was not significant for birth weight (p interaction = 0.50), birth length (p interaction = 0.88) or head circumference (p interaction = 0.25) for acrylamide and birth length for glycidamide (p interaction = 0.15), but (borderline) significant for birth weight (p interaction = 0.04) and head circumference (p interaction = 0.07) for glycidamide. Newborn's sex did not statistically significantly modify the association between acrylamide hemoglobin adduct levels and birth weight (p interaction = 0.36) birth length (p interaction = 0.35), or head circumference (p interaction = 0.55) for acrylamide and the corresponding p values were 0.19, 0.34 and 0.39 for glycidamide.

The effect estimates of acrylamide and glycidamide did not change profoundly when we additionally adjusted for consumption of vegetables, fruits, fish and soda drinks (results not shown).

There was no statistically significant interaction between any of the genetic variables and acrylamide (Table 5). Some differences between genotypes are, however, worth mentioning. Among neonates of non-smoking mothers, there was only an inverse association between acrylamide and birth weight in those who were homozygous wild types for rs915906, rs2480258 and rs11101888 in *CYP2E1*. When the 3 *CYP2E1* SNPs were summed, neonates with no variant alleles had a stronger inverse association than those with one or more variant alleles. Furthermore, only neonates from non-smoking mothers with at least 1 variant allele of rs1138272 in *GSTP1* had an inverse association between acrylamide and birth weight. With regard to birth head circumference, similar differences were observed for the same genetic variants. The sum of the 2 *GSTP1* SNPs did not modify the association between acrylamide and birth outcomes. The above-mentioned differences between genotypes are shown in Figure 1, for birth weight and head circumference.

There were no associations between any of the SNPs and the ratio of glycidamide to acrylamide hemoglobin adducts (Table 6).

Discussion

We observed an inverse association between acrylamide to hemoglobin adducts and birth weight and birth head circumference. The association was similar in newborns of mothers who did not smoke during pregnancy.

There was a stronger association between acrylamide's metabolite glycidamide and birth weight and head circumference and glycidamide was additionally inversely associated with birth length. Glycidamide was stronger inversely associated with birth outcomes among newborns of non-smoking mothers, particularly for birth weight and head circumference.

Although with no statistically significant interaction between acrylamide and genotype, the inverse association between acrylamide exposure and birthweight and birth head circumference was stronger in children with a homozygous wildtype genotype for the studied SNPs in *CYP2E1* and children with at least 1 variant allele in rs1138272 in *GSTP1*.

The order of magnitude of the effect size of the association between gestational acrylamide exposure and fetal growth in our study is similar to that of maternal smoking. For instance, the effect estimate in the highest quartile of glycidamide adducts (–225 grams for birth weight, –0.81 for length and –0.63 for head circumference in neonates from non-smoking mothers) is similar to the effect size of maternal smoking in our study (–219 grams for birth weight, –0.97 for length and –0.45 for head circumference).

Up to now, 3 other studies have investigated the association between acrylamide hemoglobin adduct levels and birth outcomes, among which was 1 study that also used acrylamide and glycidamide to hemoglobin adducts as a biomarker of exposure. All 3 studies observed an inverse association between gestational acrylamide exposure and indicators of fetal growth.^{4–6} The consistency between all 4 studies that have investigated the association thus far and between the studies using food frequency questionnaire data and biomarkers is rather remarkable. All 4 studies adjusted largely for the same covariables that we adjusted for in this study.

Out of the 3 studies, including the current study, that investigated the link with birth head circumference,^{5,6} ours is the second study to observe an inverse association between prenatal acrylamide exposure and head circumference.

We did not observe statistically significant interaction between acrylamide and genetic variants in genes involved in acrylamide metabolism. However, there were clear differences in the strength of the association between acrylamide and birth weight and head circumference between genotypes of *CYP2E1* SNPs and a *GSTP1* SNP (rs1138272) in newborns of non-smoking mothers. For the *CYP2E1* SNPs, these differences suggest that glycidamide may be more important with regard to fetal growth than acrylamide because the wildtype allele of the studied SNPs is thought to have a higher enzymatic activity than the variant allele.^{10–12} A stronger effect of glycidamide than of acrylamide is reflected by the observation that the associations between glycidamide and birth outcomes in the current study were stronger than the associations with acrylamide. Strikingly, analyses on acrylamide and endometrial and ovarian cancer risks in a prospective cohort study showed that non-smoking women who were homozygous for the wild type *CYP2E1* alleles (of rs2480258, rs915906 and rs11101888) had higher risks of both endometrial and ovarian cancer than women with variant alleles for those 3 SNPs.¹³ This pattern is similar to what we saw in the current study, where newborns of non-smoking mothers who were homozygous wildtypes for

the same 3 SNPs showed stronger associations with birth weight and head circumference than newborns with variant alleles for these SNPs.

GSTP1 is an important phase II enzyme that protects against oxidative stress and it is involved of the metabolism of acrylamide, generating mercapturic acid derivatives of acrylamide and glycidamide that are excreted with the urine. The variant allele of the rs1138272 SNP probably leads to a reduced enzymatic activity of GSTP1.¹⁴ Therefore, it was expected that newborns with variant alleles of rs1138272 would show a stronger association between acrylamide and fetal growth.

We did not observe clear differences between the studied genotypes with regard to the glycidamide to acrylamide hemoglobin adduct ratio in cord blood. *CYP2E1* gene transcription is induced or inhibited by numerous factors, such as liver function, fasting, obesity, alcohol intake, medication and various dietary factors.¹⁵ In addition, post-transcriptional processes have an important influence on CYP2E1 enzyme activity. A more subtle effect of the genotype may be masked by those other factors and processes that influence enzyme activity.¹⁵ Nevertheless, Pelle *et al.* found that carriers of the variant allele (T allele) of rs2480258 in *CYP2E1* had a decreased enzyme activity, with a reduced *CYP2E1* expression phenotype at the mRNA, protein, and CYP2E1 enzyme activity level.¹⁰ In a separate study, they observed a decreased glycidamide to acrylamide ratio in homozygous carriers of the T allele.¹⁶ Duale *et al.* did not observe differences in the ratio of glycidamide to acrylamide for 2 SNPs in *CYP2E1* (rs6413419 and rs2515641); rs6413419 is a missense mutation. In addition, they did not observe a difference in the ratio of glycidamide to acrylamide for rs1051740 in *EPHX1*, similar to our study.¹⁷ Also similar to our study, Doroshenko *et al.* did not observe a difference in the ratio of glycidamide to acrylamide among genotypes of rs1695 and rs1138272 in *GSTP1* in human volunteers but the sample size of their study was very small (n = 16).¹⁸ In a study on 51 persons occupationally exposed to acrylamide, Huang *et al.* observed no influence on the glycidamide to acrylamide ratio of variant alleles of the *CYP2E1**5 SNP (rs2031920/rs3813867) nor of variant alleles of the rs1051740 SNP in *EPHX1*.¹⁹

Although the interactions between acrylamide and glycidamide and genotypes in the current study were not statistically significant because of the limited sample sizes and therefore have to be interpreted cautiously, they do point towards a role of *CYP2E1* and *GSTP1* in the association between gestational acrylamide exposure and fetal growth and thus give some suggestion that the association could be causal.

Currently, it is only possible to speculate about the possible mechanism or mechanisms behind acrylamide's possible adverse effect on fetal growth. Both acrylamide and glycidamide can bind to thiol groups in proteins and when those thiol groups are in important positions, the function of the protein may be impaired.²⁰ However, it is unknown what proteins could be involved in acrylamide's putative adverse influence on fetal growth. Acrylamide has been shown to be associated with reduced serum insulin levels in both rats²¹ and human adults.²² Insulin is a growth factor that plays an important role in fetal growth. Acrylamide was also shown to decrease levels of plasma thyroid hormones in rats,^{23,24} and among

Taiwanese adolescents, there was an inverse association between levels of a urinary acrylamide metabolite and serum free thyroxin (T4).²⁵ Thyroid hormones are also involved in fetal growth and development. Acrylamide may impair the function of enzymes involved in insulin and thyroid hormone regulation.

Our study has some limitations. We cannot exclude the possibility of residual confounding by factors that are associated with both acrylamide and glycidamide hemoglobin adduct levels and birth outcomes, such as another dietary exposure or a generally less healthy diet. However, when we additionally adjusted the analyses for variables that can be thought of as proxies for a healthy or unhealthy diet (consumption of vegetables, fruits, fish and soda drinks), the associations were virtually unchanged. In addition, associations were found in different study populations in which the dietary sources of acrylamide differ and were still present after adjustment for confounders and after exclusion of children from smoking mothers. Therefore, it is not likely that other factors could fully account for the observed associations. In addition, the possible effect modification by SNPs in *CYP2E1* eliminates a long list of potential confounding exposures that are not influenced by metabolism by CYP2E1.

In addition, we may have had too limited statistical power to observe statistically significant differences in the association between acrylamide hemoglobin adduct levels and birth outcomes in subgroups of genotypes.

Our study also has specific strengths. Our findings are generalizable as our study population is representative for the gestational segment of the population at large.⁷ Another advantage of our study was the use of acrylamide biomarkers. Biomarkers should not be assumed to always be superior to other methods of acrylamide exposure assessment, such as food frequency questionnaires, because a biomarker may not represent the exposure during the relevant period for disease etiology well, e.g., due to intra-individual variations of exposure in time. However, the hemoglobin adduct biomarker may well be superior in the case of a study on fetal growth because the hemoglobin adducts of acrylamide and glycidamide represent the exposure during the last 4 months of pregnancy. This part of the pregnancy is the period in which most growth takes place. The effects sizes in the study of Pedersen *et al.*⁶ and in the current study, which are the 2 studies that have used acrylamide biomarkers, are quite comparable but they are larger than the effect sizes in the studies that used food frequency questionnaires to estimate acrylamide intake. This may be due to the greater precision of the acrylamide exposure estimate that is reached by using the biomarker. Interestingly, the 2 biomarker studies were also the studies that observed an inverse association with birth head circumference, whereas the study that used a food frequency questionnaire did not.⁵

Our study strengthens the body of evidence that acrylamide intake at current dietary levels may have developmental effects. According to the Developmental Origins of Health and Disease hypothesis, suboptimal prenatal development likely predisposes to inferior health throughout life.²⁶ Reduced fetal growth has been associated with among other increased incidences of diabetes mellitus (type 2), obesity and cardiovascular disease.²⁷ Birth head circumference is an indication of fetal brain growth, and several

studies have shown associations between decreased birth head circumference and decreased cognitive skills in childhood, even among the children within the normal range of birth size.²⁸ It has also been shown to be associated with an increased risk of attention deficit hyperactivity disorder (ADHD).²⁹ The association between acrylamide exposure and reduced birth head circumference suggests that prenatal acrylamide exposure may have cognitive effects in later life. This study suggests that reducing acrylamide exposure during pregnancy can be beneficial for child development.

Further studies on the association between acrylamide hemoglobin adduct levels and birth outcomes in newborns, and the interaction with *CYP2E1* and *GSTP1* SNPs are needed to better understand the potential effects of acrylamide on fetal growth. Furthermore, more epidemiological research is needed on possible mechanisms of action underlying the association between prenatal acrylamide exposure and fetal development. We strongly encourage specifically epidemiological studies on this topic because the animal data suggested that no developmental effects on humans would occur at dietary doses.¹

Conclusion

In conclusion, *in utero* exposure to acrylamide and especially its metabolite glycidamide are associated with decreases in birth weight, birth length and birth head circumference. Preventative measures leading to reduced exposure of pregnant women to acrylamide should be considered.

Declarations

Ethics approval and consent to participate

The study protocol of the ENVIRONAGE birth cohort was approved by the Ethics Committee of Hasselt University and East-Limburg Hospital in Genk, Belgium. Participating mothers signed an informed consent form when they were at the hospital for delivery.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Author contributions: JGFH had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. TSN coordinates the ENVIRONAGE birth cohort and managed funding. HV supervised the measurements of the acrylamide and glycidamide to hemoglobin adducts. NM organized the field work with help of WG. IDV supervised the genotyping. JGFH obtained funding and did the statistical analysis and the quality control of the database. JGFH wrote the first draft of the manuscript. All authors have helped with data interpretation.

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Disclaimer:

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Tables

Table 1. Characteristics of mother-newborn pairs (n=443)

Characteristics	Median (IQR) or n (%)
Maternal	
Age, y	30 (27-32)
Pre-gestational BMI, kg/m ²	23.5 (21.3-26.6)
Gestational weight gain, kg	14 (11.0-17.5)
Maternal education	
Low	48 (10.9%)
Middle	142 (32.3 %)
High	250 (56.8 %)
Smoking during pregnancy	
Yes	63 (14.2 %)
No	380 (85.8%)
Number of cigarettes smoked per day*	9 (5-10)
Parity	
1	236 (53.3%)
2	152 (34.3%)
≥ 3	55 (12.4%)
Newborn	
Sex	
Male	229 (51.7%)
Female	214 (49.3%)
Ethnicity	
European	393 (88.7%)
Non-European	50 (11.3%)
Acrylamide to hemoglobin adducts (pmol/ g hemoglobin)	13.2 (10.4-17.6)
Glycidamide to hemoglobin adducts (pmol/ g hemoglobin)	13.3 (10.2-18.0)
Gestational age, w	40 (39-40)
Birth weight, g	3430 (3140-3720)
Birth height, cm	50 (49-52)
Birth head circumference, cm	34 (33-35)

IQR = interquartile range

* among the women who reported to have smoked during pregnancy

Table 2. Genotype frequencies

SNP ID	Homozygous wildtype n (%)	Heterozygous n (%)	Homozygous variant allele n (%)	Hardy-Weinberg p value
rs915906, <i>CYP2E1</i>	196 (70)	78 (28)	6 (2)	0.59
rs2480258, <i>CYP2E1</i>	174 (62)	99 (35)	9 (3)	0.26
rs11101888, <i>CYP2E1</i>	234 (83)	47 (17)	1 (0)	0.40
rs1051740, <i>EPHX1</i>	157 (54)	107 (37)	25 (9)	0.27
rs1695, <i>GSTP1</i>	125 (43)	140 (48)	26 (9)	0.13
rs1138272, <i>GSTP1</i>	234 (82)	50 (17)	2 (1)	0.70

Table 3. Associations between genotypes of acrylamide-metabolizing enzymes and birth outcomes

SNP*	Estimated change in birth outcome (95% CI)					
	<i>Birth weight (gram)</i>	<i>p</i>	<i>Birth length (cm)</i>	<i>p</i>	<i>Birth head circumference (CM)</i>	<i>p</i>
rs915906	24.5 (-36.9, 86.0)	0.43	-0.17 (-0.44, 0.10)	0.22	-0.04 (-0.26, 0.19)	0.74
rs2480258	19.1 (-40.3, 78.5)	0.53	-0.19 (-0.45, 0.07)	0.16	-0.02 (-0.24, 0.20)	0.85
rs11101888	18.8 (-56.0, 93.7)	0.62	0.05 (-0.28, 0.38)	0.77	0.19 (-0.07, 0.44)	0.15
rs1051740	-17.6 (-76.3, 41.1)	0.56	0.05 (-0.21, 0.31)	0.70	-0.02 (-0.23, 0.20)	0.89
rs1695	2.0 (-56.2, 60.2)	0.95	-0.02 (-0.28, 0.23)	0.86	0.04 (-0.17, 0.25)	0.71
rs1138272	-7.3 (-86.6, 72.1)	0.86	-0.09 (-0.44, 0.26)	0.60	-0.03 (-0.32, 0.27)	0.86

*modeled is the regression coefficient of 1 or 2 variant alleles of the SNP compared to the homozygous wildtype

Adjusted for: maternal age (yrs), maternal pre-pregnancy BMI (kg/m²), maternal smoking during pregnancy (number of cigarettes), maternal education level (low, middle, high), parity (n children), gestational age (weeks), newborn's sex and date of delivery

Table 4. Associations between acrylamide and glycidamide to hemoglobin adducts in cord blood and birth outcomes

		Unadjusted regression coefficient (95% CI)							Multivariable-adjusted *regression coefficient (95% CI)						
		Continuous exposure variable (per 10 pmol/g hemoglobin)		Quartiles of exposure					Continuous exposure variable (per 10 pmol/g hemoglobin)		Quartiles of exposure				
	n	Regression coefficient (95% CI)	p	Q1 REF	Q2	Q3	Q4	p for trend	Regression coefficient (95% CI)	p	Q1 REF	Q2	Q3	Q4	p for trend
Acrylamide to hemoglobin adducts among all participants															
Weight, g	443	-59 (-88, -31)	<0.001	1	-74 (-188, 41)	-98 (-212, 17)	-149 (-263, 34)	0.08	-40 (-71, -9)	0.01	1	-30 (-127, 67)	-102 (-200, -5)	-101 (-208, 7)	0.12
Length, cm	442	-0.24 (-0.37, -0.11)	<0.001	1	-0.22 (-0.73, 0.30)	0.17 (-0.35, 0.68)	-0.35 (-0.86, 0.16)	0.19	-0.17 (-0.31, -0.03)	0.02	1	-0.03 (-0.48, 0.41)	0.15 (-0.29, 0.60)	-0.13 (-0.62, 0.36)	0.69
Head circumference, cm	432	-0.15 (-0.24, -0.05)	0.003	1	-0.33 (-0.72, 0.06)	-0.51 (-0.90, -0.12)	-0.54 (-0.92, -0.15)	0.03	-0.13 (-0.24, -0.01)	0.03	1	-0.16 (-0.52, 0.19)	-0.45 (-0.81, -0.09)	-0.41 (-0.80, -0.01)	0.06
Acrylamide to hemoglobin adducts among neonates from non-smoking mothers															
Weight, g	380	-58 (-113, -4)	0.04	1	-67 (-181, 47)	-88 (-202, 25)	-30 (-170, 110)	0.45	-52 (-98, -5)	0.03	1	-26 (-123, 72)	-101 (-200, -2)	-77 (-197, 43)	0.19
Length, cm	379	-0.17 (-0.42, 0.08)	0.18	1	-0.21 (-0.73, 0.31)	0.19 (-0.33, 0.70)	0.27 (-0.37, 0.90)	0.35	-0.18 (-0.40, 0.04)	0.10	1	-0.04 (-0.49, 0.41)	0.15 (-0.31, 0.60)	-0.00 (-0.56, 0.55)	0.86
Head circumference, cm	369	-0.23 (-0.42, -0.04)	0.02	1	-0.32 (-0.72, 0.07)	-0.48 (-0.87, -0.08)	-0.36 (-0.85, 0.13)	0.12	-0.22 (-0.40, -0.05)	0.01	1	-0.16 (-0.52, 0.21)	-0.42 (-0.80, -0.05)	-0.48 (-0.94, -0.03)	0.07
Glycidamide to hemoglobin adducts among all participants															
Weight, g	393	-80 (-117, -44)	<0.001	1	-161 (-282, -40)	-175 (-297, -54)	-267 (-387, -146)	<0.001	-53 (-90, -16)	0.005	1	-144 (-246, -43)	-154 (-258, -49)	-222 (-337, -108)	0.001
Length, cm	392	-0.35 (-0.52, -0.19)	<0.001	1	-0.55 (-1.09, -0.01)	-0.59 (-1.13, -0.05)	-1.10 (-1.64, -0.56)	0.001	-0.24 (-0.41, -0.08)		1	-0.46 (-0.93, 0.01)	-0.48 (-0.96, -0.00)	-0.85 (-1.38, -0.33)	0.02
Head circumference, cm	382	-0.17 (-0.30, -0.05)	0.007	1	-0.69 (-1.10, -0.27)	-0.74 (-1.16, -0.33)	-0.74 (-1.15, -0.32)	<0.001	-0.11 (-0.25, 0.03)		1	-0.61 (-0.99, -0.23)	-0.61 (-1.00, -0.21)	-0.55 (-0.98, -0.11)	0.01
Glycidamide to hemoglobin adducts among neonates from non-smoking mothers															
Weight, g	331	-121 (-193, -50)	<0.001	1	-164 (-285, -43)	-176 (-296, -50)	-176 (-327, -25)	0.01	-104 (-165, -43)	0.001	1	-152 (-255, -50)	-161 (-268, -54)	-225 (-355, -95)	0.002
Length, cm	330	-0.48 (-0.80, -0.15)	0.004	1	-0.56 (-1.11, -0.01)	-0.61 (-1.17, -0.05)	-0.64 (-1.33, 0.05)	0.10	-0.42 (-0.70, -0.13)	0.004	1	-0.50 (-0.98, -0.02)	-0.56 (-1.06, -0.06)	-0.81 (-1.42, -0.21)	0.04
Head circumference, cm	320	-0.36 (-0.62, -0.11)	0.005	1	-0.69 (-1.11, -0.27)	-0.72 (-1.15, -0.29)	-0.55 (-1.09, -0.02)	0.003	-0.31 (-0.55, -0.07)	0.01	1	-0.61 (-1.01, -0.22)	-0.61 (-1.02, -0.19)	-0.63 (-1.14, -0.13)	0.007

*Regression coefficient (95% confidence interval) per 10 pmol/g hemoglobin increase in AA-Hb and GA-Hb adducts

Adjusted for: maternal pre-pregnancy BMI (kg/m²), maternal weight gain during pregnancy, maternal smoking during pregnancy (n cigarettes/day), parity (n children), gestational age (weeks), newborn's sex and date of delivery

Table 5. Interactions between genetic variants and cord blood acrylamide adduct levels on birth outcomes

	All				Non-smoking mothers			
	n	Acrylamide regression coefficient	95% CI	p for interaction	n	Acrylamide regression coefficient	95% CI	p for interaction
Birth weight								
<i>CYP2E1</i> rs915906 = 0*	196	-62.1	-130.3, 6.1	0.80	169	-37.0	-145.0, 71.0	0.87
<i>CYP2E1</i> rs915906 = 1*	83	-54.9	-143.4, 33.6		70	12.3	-167.4, 192.0	
<i>CYP2E1</i> rs2480258 = 0	174	-74.5	-146.3, -2.6	0.64	148	-66.9	-187.8, 54.4	0.59
<i>CYP2E1</i> rs2480258 = 1	107	-45.1	-121.9, 31.8		92	7.8	-135.3, 150.9	
<i>CYP2E1</i> rs11101888 = 0	234	-77.8	-136.4, -19.3	0.17	199	-57.9	-164.2, 48.4	0.34
<i>CYP2E1</i> rs11101888 = 1	47	10.2	-154.4, 174.7		41	35.3	-141.1, 211.7	
<i>EPHX1</i> rs1051740 = 0	157	-55.6	-141.8, 30.5	0.15	137	-83.1	-249.6, 83.3	0.49
<i>EPHX1</i> rs1051740 = 1	131	-43.9	-112.6, 24.8		109	7.8	-103.3, 118.9	
<i>GSTP1</i> rs1695 = 0	129	-51.4	120.4, 17.6	0.85	105	-34.4	-146.8, 78.0	0.82
<i>GSTP1</i> rs1695 = 1	166	-68.3	-162.2, 5.6		144	-42.4	-196.2, 111.4	
<i>GSTP1</i> rs1138272 = 0	237	-51.7	-109.2, 5.8	0.46	202	-0.1	-95.9, 95.7	0.28
<i>GSTP1</i> rs1138272 = 1	52	-121.5	-347.0, 104.1		43	-148.4	-415.6, 118.8	
Sum of 3 <i>CYP2E1</i> SNPs = 0	163	-78.0	-149.9, -6.0	0.38	138	-77.8	-200.5, 45.0	0.35
Sum of 3 <i>CYP2E1</i> SNPs ≥ 1	103	-36.3	-118.8, 46.2		88	44.4	-103.2, 191.9	
Sum of 2 <i>GSTP1</i> SNPs = 0	117	-42.5	-114.5, 29.5	0.59	99	-24.2	-137.9, 89.5	0.84
Sum of 2 <i>GSTP1</i> SNPs ≥ 1	166	-85.4	-168.9, -1.9		145	-63.7	-212.1, 84.7	
Birth length								
<i>CYP2E1</i> rs915906 = 0	196	-0.21	-0.50, 0.09	0.69	169	-0.09	-0.56, 0.39	0.42
<i>CYP2E1</i> rs915906 = 1	83	-0.19	-0.61, 0.24		70	0.21	-0.65, 1.06	
<i>CYP2E1</i> rs2480258 = 0	174	-0.22	-0.52, 0.09	0.76	148	-0.14	-0.66, 0.37	0.52
<i>CYP2E1</i> rs2480258 = 1	107	-0.19	-0.56, 0.17		92	0.07	-0.63, 0.78	
<i>CYP2E1</i> rs11101888 = 0	234	-0.22	-0.48, 0.04	0.32	199	-0.02	-0.49, 0.46	0.97
<i>CYP2E1</i> rs11101888 = 1	47	-0.23	-0.91, 0.45		41	-0.12	-0.92, 0.68	

= 1			0.46			0.67		
EPHX1 rs1051740 = 0	157	-0.23	-0.61, 0.16	0.50	138	0.28	-0.45, 1.01	0.85
EPHX1 rs1051740 = 1	131	-0.11	-0.43, 0.20		109	0.02	-0.50, 0.54	
GSTP1 rs1695 = 0	129	-0.27	-0.58, 0.04	0.33	105	-0.28	-0.80, 0.24	0.19
GSTP1 rs1695 = 1	166	-0.04	-0.40, 0.32		144	0.55	-0.11, 1.21	
GSTP1 rs1138272 = 0	237	-0.11	-0.35, 0.14	0.46	202	0.11	-0.31, 0.53	0.93
GSTP1 rs1138272 = 1	52	-0.81	-1.89, 0.27		43	-0.28	-1.75, 1.18	
Sum of 3 CYP2E1 SNPs = 0	167	-0.24	-0.54, 0.07	0.64	138	-0.16	-0.68, 0.36	0.55
Sum of 3 CYP2E1 SNPs ≥ 1	105	-0.21	-0.60, 0.17		88	-0.00	-0.71, 0.70	
Sum of 2 GSTP1 SNPs = 0	120	-0.22	-0.55, 0.12	0.59	99	-0.24	-0.77, 0.29	0.33
Sum of 2 GSTP1 SNPs ≥ 1	167	-0.10	-0.47, 0.27		145	0.44	-0.21, 1.09	
<i>Birth head circumference</i>								
CYP2E1 rs915906 = 0	192	-0.10	-0.38, 0.17	0.42	165	-0.38	-0.84, 0.07	0.56
CYP2E1 rs915906 = 1	81	-0.19	-0.47, 0.08		68	-0.14	-0.71, 0.43	
CYP2E1 rs2480258 = 0	171	-0.15	-0.44, 0.14	0.19	145	-0.53	-1.03, -0.03	0.22
CYP2E1 rs2480258 = 1	104	-0.16	-0.40, 0.08		89	-0.07	-0.54, 0.40	
CYP2E1 rs111101888 = 0	229	-0.22	-0.44, 0.01	0.36	194	-0.46	-0.88, -0.04	0.31
CYP2E1 rs111101888 = 1	47	-0.21	-0.71, 0.29		40	-0.13	-0.72, 0.46	
EPHX1 rs1051740 = 0	157	-0.23	-0.51, 0.06	0.54	137	-0.39	-0.92, 0.15	0.91
EPHX1 rs1051740 = 1	125	-0.09	-0.40, 0.22		103	-0.33	-0.86, 0.20	
GSTP1 rs1695 = 0	121	-0.13	-0.43, 0.17	0.31	101	-0.29	-0.83, 0.25	0.84
GSTP1 rs1695 = 1	163	-0.23	-0.50, 0.05		142	-0.46	-0.96, 0.04	
GSTP1 rs1138272 = 0	228	-0.16	-0.38, 0.06	0.39	197	-0.26	-0.64, 0.13	0.34
GSTP1 rs1138272 = 1	51	-0.44	-1.20, 0.33		42	-0.87	-1.81, 0.07	
Sum of 3 CYP2E1 SNPs = 0	160	-0.16	-0.46, 0.14	0.24	135	-0.58	-1.01, -0.06	0.15
Sum of 3 CYP2E1	100	-0.16	-0.41,		85	0.02	-0.46,	

SNPs ≥ 1			0.10				0.51	
Sum of 2 GSTP1	113	-0.07	-0.40,	0.70	95	-0.27	-0.82,	0.59
SNPs = 0			0.26				0.29	
Sum of 2 GSTP1	164	-0.27	-0.54,		143	-0.49	-0.96,	
SNPs ≥ 1			0.00				-0.01	

*0= homozygous wildtype, 1 = 1 or 2 variant alleles for SNPs

Adjusted for: maternal age (yrs), maternal pre-pregnancy BMI (kg/m²), maternal smoking during pregnancy (number of cigarettes), maternal education level (low, middle, high), parity (n children), gestational age (weeks), newborn's sex and date of delivery

Table 6. Associations between SNPs in acrylamide-metabolising genes and the ratio of glycidamide to acrylamide hemoglobin adducts

SNP ID	Homozygous wildtype			Heterozygous			Homozygous variant allele	
	n	Regression coefficient (95% CI)	n	Regression coefficient (95% CI)	p	n	Regression coefficient (95% CI)	p
All								
rs915906, <i>CYP2E1</i>	196	Ref	78	0.004 (-0.07, 0.08)	0.91	6	-0.12 (-0.37, 0.14)	0.37
rs2480258, <i>CYP2E1</i>	174	Ref	99	0.04 (-0.03, 0.10)	0.30	9	-0.06 (-0.26, 0.14)	0.56
rs11101888, <i>CYP2E1</i>	234	Ref	47	-0.04 (-0.13, 0.05)	0.35	1	0.83 (0.34, 1.32)	0.001
rs1051740, <i>EPHX1</i>	157	Ref	107	-0.05 (-0.12, 0.01)	0.10	25	-0.05 (-0.16, 0.06)	0.36
rs1695, <i>GSTP1</i>	125	Ref	140	-0.04 (-0.11, 0.02)	0.19	26	0.01 (-0.12, 0.13)	0.93
rs1138272, <i>GSTP1</i>	234	Ref	50	-0.01 (-0.10, 0.07)	0.77	2	0.20 (-0.15, 0.55)	0.27
Non-smokers								
rs915906, <i>CYP2E1</i>	169	Ref	64	0.03 (-0.06, 0.11)	0.56	6	-0.11 (-0.38, 0.16)	0.43
rs2480258, <i>CYP2E1</i>	148	Ref	84	0.05 (-0.03, 0.12)	0.21	8	-0.11 (-0.34, 0.13)	0.38
rs11101888, <i>CYP2E1</i>	199	Ref	40	-0.04 (-0.14, 0.07)	0.48	1	0.84 (0.32, 1.36)	0.002
rs1051740, <i>EPHX1</i>	137	Ref	87	-0.07 (-0.14, 0.01)	0.08	22	-0.05 (-0.17, 0.07)	0.39
rs1695, <i>GSTP1</i>	105	Ref	121	-0.05 (-0.12, 0.03)	0.20	23	0.02 (-0.12, 0.16)	0.76
rs1138272, <i>GSTP1</i>	202	Ref	41	-0.02 (-0.12, 0.08)	0.69	2	0.14 (-0.16, 0.44)	0.36

Adjusted for: maternal age (yrs), maternal pre-pregnancy BMI (kg/m²), maternal smoking during pregnancy (number of cigarettes), maternal education level (low, middle, high), parity (n children), gestational age (weeks), newborn's sex and date of delivery

Figures

Figure 1

the association between acrylamide to hemoglobin adducts and birth weight and head circumference in newborns from non-smoking mothers according to selected genotypes