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Gestational acrylamide exposure and biomarkers of fetal growth: Probing the mechanism underlying the association between acrylamide and reduced fetal growth

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ABSTRACT

Introduction: Four epidemiological studies have shown a negative association between prenatal acrylamide exposure and birth size. In order to shed light on the possible underlying mechanism(s), we analysed associations between acrylamide biomarkers and biomarkers related to fetal growth.

Methods: In newborns of the ENVIRONAGE birth cohort (n ranges from 215 to 434), we investigated the association between prenatal acrylamide exposure (acrylamide and glycidamide hemoglobin adduct levels in cord blood) and thyroid hormones (TSH, T3, T4 and the ratio of T4 to T3 in cord plasma), insulin-related factors (cord plasma insulin and IGF1, and placental IGF2), neurotrophins (cord plasma BDNF, and placental NGF, NT3 and NT4), and cord plasma homocysteine and progesterone, using multiple linear regression analysis. In addition, we investigated whether the biomarkers mediated the associations between prenatal acrylamide exposure and birth outcomes.

Results: We observed lower cord plasma TSH (−10.2% [95% CI: −15.0, −4.3]) and higher placental NGF levels (10.0% [95% CI 3.7, 17.4]) for a twofold increase of acrylamide adducts, a decrease in the ratio of cord plasma free T4 and free T3 with higher acrylamide and glycidamide adducts of −2.9% (95% CI: −5.7, −0.1) and −3.9% (95% CI: −6.2, −1.6) for a twofold increase in acrylamide and glycidamide adduct levels, respectively, and higher cord plasma free T3 with increases in both acrylamide and glycidamide adducts of 2.8% (95% CI: 0.2, 5.6) and 3.6% (95% CI: 0.8, 6.6) for a twofold increase in acrylamide and glycidamide adduct levels, respectively. Additionally, a twofold increase in glycidamide adducts was associated with lower cord plasma insulin levels, particularly among newborns of non-smoking mothers (−11.2% [95% CI: −19.5, −0.1]).
Cord plasma insulin seemed to mediate the association between glycidamide adducts and birth weight.

Conclusions: A decrease in cord plasma insulin levels may be (a marker of) a mechanism by which gestational acrylamide exposure is associated with decreased fetal growth. The possible health consequences of the associations between gestational acrylamide exposure and thyroid hormones and neurotrophins warrant future study.

1. Introduction

Acrylamide is abundantly present in our diets. Many potato and grain-containing foods that are prepared at high temperatures (>120 °C), such as cookies, potato chips, French fries and coffee, contain high levels of acrylamide (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2015).

Acrylamide and its metabolite glycidamide are small, reactive compounds and for that reason they can cause various toxic effects. In rodents, acrylamide has been shown to cause cancer in various tissues, neurotoxicity, and reproductive and developmental toxicity (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2015).

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 hemoglobin adducts, respectively (von Stedingk et al., 2011). In a subsequent larger study (171 mother-newborn pairs), the

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correlations were even stronger: 0.95 ($p < 0.001$) for acrylamide and 0.94, $p < 0.001$ for glycidamide (Pedersen et al., 2012). Four epidemiological studies, among which our own study (Pedersen et al., 2012; Kadawathagedara et al., 2016; Duarte-Salles et al., 2013; Hogervorst et al., 2021); have shown a negative association between gestational acrylamide exposure and fetal growth. The mechanism underlying this association is unknown. This is one of the reasons that the European Food Safety Authority (EFSA) did not use the epidemiological studies on fetal growth for their 2015 risk assessment of the health effects of dietary acrylamide. EFSA did, however, call for more epidemiological research on the association between acrylamide intake and birth outcomes (EFSA CONTAM Panel (EFSA Panel on Contaminants in the Food Chain), 2015).

Through the present study, we aimed to shed light on the possible mechanism(s) underlying the association between prenatal acrylamide exposure and birth outcomes, thereby addressing the knowledge gap put forward by EFSA. We analyzed the associations between acrylamide exposure biomarkers and several possible biomarkers of fetal growth selected based on the literature: thyroid hormones (Forhead and Fowden, 2014; Janssen et al., 2017) (thyroid-stimulating hormone (TSH), triiodothyronine (T3), thyroxine (T4) and the ratio of T4 to T3), insulin-related factors (Kadakia and Josefson, 2016; Ahmad et al., 2016) (insulin, insulin-like growth factor 1 (IGF1) and 2 (IGF2)), neurotrophins (Sahay et al., 2015) (brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin 3 (NT3) and neurotrophin 4 (NT4)), progesterone (Diemert et al., 2017) and homocysteine (Bergen et al., 2016). In addition, we were interested in possible effect modification by the sex of the newborn because acrylamide is hypothesized to affect sex hormone levels (Hogervorst et al., 2013). Through this effect, acrylamide could exert different effects on growth biomarkers in boys and girls. Furthermore, we investigated the mediation of the growth-related biomarkers in the association between acrylamide exposure and fetal growth.

2. Materials and methods

2.1. Study population and data collection

The study protocol of the ENVIRONAGE birth cohort was approved by the Ethics Committee of Hasselt University and East-Limburg Hospital in Genk, Belgium. We recruited mother-infant pairs in the East-Limburg Hospital in Belgium, between Friday 1200 h and Monday 0700 h 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/km^2. Participants were recruited if the mothers were able to fill out a questionnaire in Dutch. Enrolment was spread equally over all seasons of the year. 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 (Janssen et al., 2017).

Upon arrival in the hospital for delivery, participating mothers provided written informed consent and completed study questionnaires on age, pre-gestational body mass index (BMI), maternal education, occupation, smoking status, alcohol consumption, place of residence, 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, Apgar score, and ultrasonography data, were collected after birth. We included only full-term (≥36 weeks) and singleton pregnancies. All new-borns were healthy and free of anomalies confirmed by both prenatal ultrasound examination and postnatal assessment immediately after birth by paediatricians. Further details of the study are reported elsewhere (Janssen et al., 2017). We selected the mother-child pairs for acrylamide exposure assessment from the total number of 1387 mother-newborn pairs included into the birth cohort at the time. At the moment, inclusion for this cohort is still ongoing and there are now more than 2000 mother-newborn pairs included. The selection of mother-newborn pairs for acrylamide exposure assessment was based on whether the children had participated in the follow-up study at 4 years of age (we preferentially included those), on missing data for possible covariables (we chose mother-newborn pairs with the least missing data) and on the volume of the cord blood samples that was available. The analysis described in this paper involved 215–434 mother-child pairs, depending on the biomarker, for which we had acrylamide hemoglobin adduct measurements.

2.1.1. Cord blood samples

Directly after delivery, umbilical cord blood was collected in BD Vacutainer™ Plastic Blood Collection Tubes coated with K2EDTA (BD, Franklin Lakes, NJ, USA). To obtain plasma, samples were centrifuged at 3,200 rpm for 15 min and stored in Eppendorf® tubes at −80 °C until further analysis.

2.1.2. Placental tissue samples

Within 10 min after delivery, placentas were collected and biopsies were taken according to a standardized protocol previously described by Adibi et al.. Briefly, placental tissue biopsies of approximately 1 cm^3 were taken at 4 equidistantly spread sites across the fetal side of the placenta, 4 cm from the umbilical cord and 1–1.5 cm below the chorioamniotic membrane. To minimize site variability, the largest umbilical cord artery served as a reference point to position the placenta facing upwards and the first biopsy was taken at the right side of the reference point. After washing with PBS, placental tissue samples were stored in Eppendorf® tubes containing RNALater (Qiagen, KJ Venlo, the 11 Netherlands), since biopsies were taken for multiple types of measurements. Samples were incubated at 4 °C for 24 h before storage at −20 °C.

2.1.3. Acrylamide and glycidamide hemoglobin adducts in cord blood

EDTA cord blood samples (n = 500, of which 25 were duplicate samples) were sent to the Center for Disease Control and Prevention (CDC) Protein Biomarker Laboratory (Atlanta, USA) to measure acrylamide and glycidamide haemoglobin adducts. Details of the methodology can be found elsewhere (Ospina et al., 2005; Vesper et al., 2008). Briefly, 300 μL of whole cord blood was analyzed using HPLC/tandem mass spectrometry (HPLC/MS/MS). The levels of acrylamide and glycidamide haemoglobin adducts were reported relative to the amount of hemoglobin (pmol per g of Hb). The lower limits of detection for this method are 3 pmol/g of Hb for acrylamide, and 4 pmol/g of Hb for glycidamide. One sample had a glycidamide hemoglobin adduct level below the detection limit and we excluded this sample from the analyses. For 57 samples, the glycidamide hemoglobin adduct level could not be reliably determined due to an unfavorable signal to noise ratio and these samples were also omitted from the analysis.

2.1.4. Biomarkers of fetal growth

The cord plasma levels of free T4, free T3, TSH, IGF1, insulin, homocysteine and progesterone were measured with electrochemiluminescence immunoassays using the Modular E170 automatic analyzer (Roche, Basel, Switzerland) at the clinical laboratory of East-Limburg Hospital.

Cord plasma protein levels of BDNF were measured by using a sandwich enzyme-linked immunosorbent assay (ELISA) (Boster Human PicoKineTM ELISA kit; Boster Biological Technology, Pleasanton, CA, USA), according to the manufacturer's instructions. The Human IGF2 ELISA kit by Cusabio (Wuhan, Hubei, China) was used for the measurement of IGF2 proteins in the plasma samples.

Protein levels of NGF, NT-3 and NT-4 were not detectable in our umbilical cord plasma samples using commercially available ELISA kits. Therefore, these neurotrophins were measured in the placental tissue samples. After thawing, placental tissue biopsies were homogenized in chilled PBS (7.5 μL/mg tissue) containing cOmplete™ ULTRA Mini

Protease Inhibitor Cocktail (Roche Diagnostics GmbH, Mannheim, Germany) by using two stainless steel beads and the Retsch Mixer Mill MM 400 (Retsch, Haan, Germany). Tissue samples were disrupted 2 times for 2 min at 30 Hz and kept on ice for 5 min between and after disruption. After centrifugation at 16,000*g* for 20 min at 4 °C, supernatant was aliquoted and stored at −20 °C or used immediately for NGF, NT3 and NT4 protein measurement by commercially available ELISA kits (Boster Human PicoKineTM ELISA kit; Boster Biological Technology, Pleasanton, CA, USA).

Total protein concentrations in placental tissue supernatants were estimated with the Bio-Rad protein assay (Bio-Rad Laboratories, Temse, Belgium) according to the manufacturer's instructions. Placental levels of NGF, NT3 and NT4 were standardized according to the protein content of the sample by dividing their levels by the concentration of total protein in the sample.

2.2. Statistical analysis

In multivariable-adjusted linear regression models, we analysed the associations between biomarkers of fetal growth and birth outcomes. The studied biomarkers of fetal growth were: thyroid hormones (TSH, free T3, free T4, free T4/free T3), insulin-related factors (insulin, IGF1, IGF2), neurotrophins (BDNF, NGF, NT-3, Nt-4) and progesterone. The results of our analyses on the associations between the acrylamide and glycidamide haemoglobin adduct levels and birth outcomes have been presented previously (Hogervorst et al., 2021). Further, we performed multiple linear regression analysis to assess the association between cord blood acrylamide hemoglobin adducts and biomarkers of fetal growth. We only included variables in the linear regression models models as covariables if they were associated ($p < 0.05$) with any of the biomarkers of fetal growth. We checked the following variables for their association with the biomarkers of fetal growth: maternal age, pre-gestational BMI, maternal weight gain during pregnancy, parity, gestational age, date of delivery, the number of cigarettes smoked during pregnancy, maternal education level and newborn's sex. The variables that were associated with one or more of the biomarkers of fetal growth were pre-gestational BMI, maternal weight gain during pregnancy, parity, gestational age, date of delivery, the number of cigarettes smoked during pregnancy, and newborn's sex. We adjusted for this same set of covariables for all the biomarkers of fetal growth. In sensitivity analyses, vegetable, fruit and fish intakes and consumption of soda drinks were additionally included in the models as covariables to check whether they, as proxies for unhealthy dietary habits, were confounders of the association between acrylamide exposure and biomarkers of fetal growth. Q-Q plots of the residuals were inspected in order to check the assumptions of linear models. Both the acrylamide and glycidamide biomarkers, and the biomarkers of fetal growth were log10-transformed in order to improve the distribution of the residuals. Because of the log10 transformation of both the independent and dependent variables, the results reflect the percent difference in the dependent variable for a 2-fold increase of the independent variable.

We performed subgroup analyses for newborns of mothers who did not smoke during pregnancy because smoking is an important source of acrylamide and adversely affects fetal growth. Furthermore, we analysed whether the newborn's sex modified the association between acrylamide exposure and birth outcomes by calculating the p-value of the interaction term between acrylamide or glycidamide hemoglobin adducts and sex.

Finally, we studied mediation by the biomarkers of fetal growth on the association between acrylamide exposure and fetal growth by decomposing the total effect into a direct effect (effect of exposure on outcome at a fixed level of the mediator) and an indirect effect (effect of exposure on outcome that operates through the mediator), as described by Valeri *et al.* (Valeri and Vanderweele, 2013).

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

3. Results

Characteristics of the study population are summarized in Table 1.

The study population consisted of 229 (51.7%) boys. The median levels of cord blood acrylamide and glycidamide hemoglobin adduct levels were 13.2 (IQR: 10.4–17.6) and 13.3 (IQR: 10.2–18.0) pmol/g of hemoglobin, respectively.

Supplemental Table 1 shows the characteristics of the total cohort, the cohort for which we had acrylamide haemoglobin adduct level data

Table 1
Characteristics of mother-newborn pairs.

Characteristics	Sample size	Median (IQR) or n (%)
Maternal		
Age, y	443	30 (27–32)
Pre-gestational BMI, kg/m^2	443	23.5 (21.3–26.6)
Gestational weight gain, kg	443	14 (11.0–17.5)
Maternal education*	440	
Low		48 (10.9%)
Middle		142 (32.3%)
High		250 (56.8%)
Smoking during pregnancy		
Yes	443	63 (14.2%)
No		380 (85.8%)
Number of cigarettes smoked per day**		9 (5–10)
Parity	443	
1		236 (53.3%)
2		152 (34.3%)
≥ 3		55 (12.4%)
Newborn		
Sex	443	
Male		229 (51.7%)
Female		214 (49.3%)
Ethnicity	443	
European		393 (88.7%)
Non-European		50 (11.3%)
Gestational age, w	443	40 (39–40)
Birth weight, g	443	3430 (3140–3720)
Birth length, cm	443	50 (49–52)
Birth head circumference, cm	443	34 (33–35)
Cord blood biomarkers		
Acrylamide hemoglobin adducts (pmol/g globin)	443	13.2 (10.4–17.6)
Glycidamide hemoglobin adducts (pmol/g globin)	393	13.3 (10.2–18.0)
TSH (mU/L)	433	8.6 (5.9–13.7)
Free T3 (pmol/L)	414	2.46 (2.15–2.78)
Free T4 (pmol/L)	434	16.2 (14.8–17.9)
Free T4/free T3 (no unit)	413	6.6 (5.7–7.6)
Homocysteine (μmol/L)	432	7.2 (5.7–9.2)
Insulin (pmol/L)	432	33 (19–54)
IGF1 (ng/mL)	235	909 (689–1152)
IGF2 (ng/mL)	292	379 (273–485)
Progesterone (ng/mL)	215	82 (61–105)
BDNF (pg/mL)	354	925 (657–1404)
Placental biomarkers		
NGF (pg/mg protein)	216	536 (425–626)
NT3 (pg/mg protein)	315	549 (442–690)
NT4 (pg/mg protein)	312	183 (150–223)

The numbers of observations per characteristic are the numbers for all mother-child pairs that had full data on acrylamide hemoglobin adduct levels and covariables available. The number of observations varies per biomarker.
IQR = interquartile range, BMI = body mass index, TSH = thyroid stimulating hormone, T3 = triiodothyronine, T4 = thyroxine, IGF1 = insulin-like growth factor 1, IGF2 = insulin-like growth factor 2, BDNF = brain-derived neurotrophic factor, NGF = nerve growth factor, NT3 = neurotrophin 3, NT4 = neurotrophin 4.

* Maternal education: low = no diploma or primary school, middle = high school, high = college or university degree.

** Among the women who reported to have smoked during pregnancy.

and covariable data, and the cohort for which we had acrylamide haemoglobin adduct level data, covariable data and NGF data. The differences between the cohorts for these demographic variables are minor.

3.1. Associations between biomarkers of fetal growth and birth outcomes

Table 2 shows the multivariable-adjusted effect estimates of the associations between the biomarkers of growth and birth outcomes.

Of the studied biomarkers, cord plasma free T4, insulin and progesterone were positively associated with birth weight, length and head circumference ($p < 0.06$ for all associations). In addition, cord plasma TSH was positively associated with birth length ($p = 0.02$) and cord plasma IGF2 was negatively associated with birth length ($p = 0.046$).

3.2. Associations between acrylamide hemoglobin adduct levels and biomarkers of fetal growth

Table 3 shows the multivariable-adjusted effect estimates of the associations between acrylamide and glycidamide hemoglobin adduct levels and the biomarkers of growth.

A twofold increase in the acrylamide adduct level was associated with a 10.2% (95% CI: −15.0, −4.3; $p = 0.001$) lower cord plasma TSH level among all newborns and a 9.3% (95% CI: −15.6, −1.7; $p = 0.02$) lower cord plasma TSH level among newborns of non-smoking mothers.

Acrylamide hemoglobin adducts were also negatively associated with the ratio of free T4 to free T3, with a decrease of 2.9% (95% CI: −5.7, −0.1; $p = 0.01$) for each twofold increase in the acrylamide adducts, and the corresponding estimate among newborns of non-smoking mothers was −3.1% (95% CI: −5.9, −0.03; $p = 0.06$). Among all newborns, a doubling of the acrylamide adduct levels was associated with a 2.8% (95% CI: 0.2, 5.6; $p = 0.04$) higher free T3 level but this association was not statistically significant among newborns of non-smoking mothers (2.0% [95% CI; −1.2, 5.6; $p = 0.22$]). Furthermore, acrylamide hemoglobin adduct levels were positively associated with placental NGF levels among all neonates with a 10.0% (95% CI: 3.7, 17.4; $p < 0.001$) increase for a doubling of the acrylamide adducts and 11.7% (95% CI: 4.0, 20.7; $p = 0.002$) among neonates of non-smoking mothers. Placental NT4 was positively associated with acrylamide adducts, with an increase of 3.5% (95% CI: −1.0, 8.4; $p = 0.13$) and 5.2% (95% CI: −0.3, 11.5; $p = 0.06$) for a doubling of the acrylamide adducts among all neonates and neonates of non-smoking mothers, respectively.

Acrylamide was not statistically significantly associated with free T4 levels or with any biomarkers of the insulin system, homocysteine or progesterone.

The associations mentioned above did not change substantially when we excluded the three newborns with the highest acrylamide levels.

Newborn's sex did not statistically significantly modify the association between acrylamide hemoglobin adduct levels and any of the markers of fetal growth (results not shown).

3.3. Associations between glycidamide hemoglobin adduct levels and biomarkers of fetal growth

Similar to acrylamide, glycidamide was positively associated with cord plasma free T3 levels (3.6% [95% CI: 0.8, 6.6; $p = 0.01$]) but this association was weaker among newborns of non-smoking mothers (3.1 [95% CI −0.3, 6.9; $p = 0.08$]). Further, glycidamide was negatively associated with cord plasma free T4 levels but only among neonates of non-smoking mothers: −1.9% (95% CI: −3.7 – 0.2; $p = 0.08$). There was a negative association between glycidamide adducts and the ratio of free T4 to free T3 among all newborns (-3.9% [95% CI; −6.2, −1.6; $p = 0.002$]) and among newborns of non-smoking mothers (-4.5% [95% CI: −7.3, −1.5; $p = 0.004$]) for a doubling of glycidamide adducts. The association with placental NGF that was observed for acrylamide was less strong for glycidamide and only statistically significant among all neonates with an increase of 6.8% (95% CI: 0.6, 13.9; $p = 0.03$), not among neonates of non-smoking mothers. Cord plasma insulin levels were negatively associated with a doubling of the glycidamide adduct levels (-9.0% [95% CI: −16.3, 0.3; $p = 0.06$]), particularly among newborns of non-smoking mothers (-11.2% [95% CI: −19.5, −0.1; $p = 0.048$]). The associations mentioned above did not change substantially when we excluded the three newborns with the highest glycidamide adduct levels.

Newborn's sex did not statistically significantly modify the association between glycidamide hemoglobin adduct levels and any of the markers of fetal growth (results not shown).

3.4. Mediation analysis

Based on their associations with birth outcomes in our study population, we studied cord plasma insulin and free T4 as a mediator for the association between glycidamide and birth weight, length and head circumference; the ratio between cord plasma free T4 and free T3 as a mediator for the association between both acrylamide and glycidamide

Table 2
Associations between biomarkers of growth and fetal growth outcomes.

	Birth weight			Birth length			Birth head circumference		
	Effect estimate	95% CI	P value	Effect estimate	95% CI	P value	Effect estimate	95% CI	P value
Cord plasma biomarkers									
TSH (mU/L)	0.40	−0.40, 1.19	0.33	0.31	0.06, 0.56	0.02	0.01	−0.26, 0.28	0.93
Free T3 (pmol/L)	1.13	−1.11, 3.41	0.33	0.07	−0.63, 0.78	0.84	0.17	−0.60, 0.95	0.67
Free T4 (pmol/L)	12.5	8.7, 16.5	<0.0001	1.96	0.83, 3.11	<0.001	3.07	1.85, 4.30	<0.0001
Free T4/free T3 (no unit)	3.49	1.21, 5.83	0.003	0.69	−0.01, 1.41	0.06	0.88	0.13, 1.63	0.02
Homocysteine (μmol/L)	0.74	−0.62, 2.12	0.29	0.20	−0.23, 0.63	0.37	0.16	−0.31, 0.62	0.51
Insulin (pmol/L)	2.26	1.73, 2.80	<0.0001	0.34	0.17, 0.51	<0.0001	0.30	0.12, 0.48	0.001
IGF1 (ng/mL)	−0.82	−2.42, 0.82	0.32	0.11	−0.39, 0.62	0.66	−0.29	−0.88, 0.30	0.34
IGF2 (ng/mL)	−0.78	−1.85, 0.31	0.16	−0.34	−0.67, −0.01	0.046	0.20	−0.18, 0.59	0.30
Progesterone (ng/mL)	10.7	8.8, 12.7	<0.0001	1.38	0.79, 1.97	<0.0001	1.79	1.11, 2.47	<0.0001
BDNF (pg/mL)	−0.63	−1.92, 0.67	0.34	0.09	−0.31, 0.50	0.65	−0.05	−0.51, 0.42	0.84
Placental biomarkers									
NGF (pg/mg protein)	−1.14	−3.70, 1.48	0.39	−0.03	−0.84, 0.79	0.95	−0.22	−1.22, 0.78	0.66
NT3 (pg/mg protein)	0.07	−1.85, 2.03	0.94	−0.26	−0.86, 0.35	0.40	−0.30	−0.99, 0.40	0.41
NT4 (pg/mg protein)	0.72	−1.36, 2.85	0.50	0.04	−0,62, 0.70	0.90	0.08	−0.69, 0.85	0.84

Adjusted for: maternal pre-pregnancy BMI (kg/m^2), 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. 95% CI = 95% confidence interval, BMI = body mass index, TSH = thyroid stimulating hormone, T3 = triiodothyronine, T4 = thyroxine, IGF1 = insulin-like growth factor 1, IGF2 = insulin-like growth factor 2, BDNF = brain-derived neurotrophic factor, NGF = nerve growth factor, NT3 = neurotrophin 3, NT4 = neurotrophin 4. *Effect size (95% confidence interval) represents difference (%) in birth outcome expressed for a twofold increase in cord blood and placental biomarkers.

Table 3
Associations between acrylamide and glycidamide hemoglobin adducts in cord blood and biomarkers of fetal growth.

All participants								
	Acrylamide				*Glycidamide*			
	n	Effect size	CI	p	n	Effect size	CI	p
TSH	433	−10.2	−15.0, −4.3	0.001	383	−5.7	−11.7, 1.3	0.11
Free T3	414	2.8	0.2, 5.6	0.04	365	3.6	0.8, 6.6	0.01
Free T4	434	−0.3	−1.9, 1.2	0.65	384	−0.8	−2.4, 0.8	0.30
Free T4/free T3	413	−2.9	−5.7, −0.1	0.01	364	−3.9	−6.2, −1.6	0.002
Insulin	432	−6.4	−13.8, 2.7	0.15	383	−9.0	−16.3, 0.3	0.06
IGF1	235	−2.7	−8.2, 3.9	0.41	211	1.5	−5.2, 9.4	0.68
IGF2	292	−1.2	−9.2, 8.9	0.80	261	4.1	−6.2, 17.5	0.47
Progesterone	215	−3.7	−8.6, 2.0	0.19	191	−3.2	−8.7, 3.3	0.32
BDNF	354	−2.0	−7.5, 4.5	0.52	315	−1.0	−7.3, 6.4	0.80
NGF	216	10.0	3.7, 17.4	0.001	192	6.8	0.6, 13.9	0.03
NT3	315	1.0	−3.0, 5.6	0.62	280	1.2	−3.1, 5.9	0.61
NT4	312	3.5	−1.0, 8.4	0.13	277	1.0	−3.5, 6.0	0.67
Homocysteine	431	3.0	−1.2, 7.8	0.17	381	4.1	−0.6, 9.3	0.09
Non-smokers								
	Acrylamide				*Glycidamide*			
	n	Effect size	CI	p	n	Effect size	CI	p
TSH	375	−9.3	−15.6, −1.7	0.02	325	−4.1	−11.8, 5.5	0.37
Free T3	359	2.0	−1.2, 5.6	0.22	310	3.1	−0.3, 6.9	0.08
Free T4	375	−0.8	−2.7, 1.2	0.43	325	−1.9	−3.7, 0.2	0.08
Free T4/free T3	358	−3.1	−5.9, −0.03	0.06	309	−4.5	−7.3, −1.5	0.004
Insulin	373	−5.8	−14.9, 6.4	0.31	324	−11.2	−19.5, −0.1	0.048
IGF1	201	−3.7	−10.9, 5.1	0.38	177	1.5	−7.0, 12.0	0.76
IGF2	248	−4.5	−14.3, 8.5	0.45	217	2.7	−10.2, 21.2	0.72
Progesterone	185	1.2	−6.0, 9.8	0.76	162	1.7	−5.7, 10.7	0.67
BDNF	303	−3.7	−10.1, 4.0	0.34	264	−1.5	−9.1, 7.8	0.80
NGF	187	11.7	4.0, 20.7	0.002	163	5.5	−1.4, 13.7	0.13
NT3	273	1.3	−3.6, 7.0	0.61	238	1.7	−3.6, 7.8	0.54
NT4	272	5.2	−0.3, 11.5	0.06	237	1.0	−4.4, 7.3	0.72
Homocysteine	375	1.6	−3.5, 7.4	0.55	325	2.9	−2.7, 9.4	0.32

Adjusted for: maternal pre-pregnancy BMI (kg/m^2), 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. 95% CI = 95% confidence interval, BMI = body mass index, TSH = thyroid stimulating hormone, T3 = triiodothyronine, T4 = thyroxine, IGF1 = insulin-like growth factor 1, IGF2 = insulin-like growth factor 2, BDNF = brain-derived neurotrophic factor, NGF = nerve growth factor, NT3 = neurotrophin 3, NT4 = neurotrophin 4. *Effect size (95% confidence interval) represents difference (%) in biomarker expressed for a twofold increase in the acrylamide or glycidamide hemoglobin adducts.

Table 4
Estimates of mediation by biomarkers of fetal growth of the association between acrylamide and glycidamide hemoglobin adducts in cord blood and fetal growth.

	Birth weight				Birth length				Birth head circumference			
	Acrylamide		Glycidamide		Acrylamide		Glycidamide		Acrylamide		Glycidamide	
	Mediation*, % (95% CI)	p-value	Mediation, % (95% CI)	p-value	Mediation, % (95% CI)	p-value	Mediation, % (95% CI)	p-value	Mediation, % (95% CI)	p-value	Mediation, % (95% CI)	p-value
All												
Insulin	NA	NA	11.9 (−1.4, 25.2)	0.08	NA	NA	7.0 (−2.2, 16.1)	0.13	NA	NA	5.8 (−4.5, 16.0)	0.27
TSH	NA	NA	NA	NA	9.5 (−9.4, 28.4)	0.33	NA	NA	NA	NA	NA	NA
fT4	NA	NA	5.8 (−5.6, 17.2)	0.32	NA	NA	2.6 (−3.2, 8.4)	0.38	NA	NA	8.1 (−8.0, 24.1)	0.32
fT4/fT3	2.0 (11.7, −7.7)	0.68	−1.3 (−11.7, 9.1)	0.81	−2.0 (−16.1, 12.1)	0.78	−3.7 (−15.0, 7.7)	0.53	5.0 (−6.5, 16.5)	0.39	8.5 (−8.6, 25.6)	0.33
Non-smokers												
Insulin	NA	NA	12.8 (−1.6, 27.2)	0.08	NA	NA	8.0 (−3.4, 19.3)	0.17	NA	NA	2.1 (−6.6, 10.8)	0.63
TSH	NA	NA	0.3 (−2.9, 3.5)	0.86	NA	NA	NA	NA	NA	NA	NA	NA
fT4	NA	NA	4.2 (−3.4, 11.8)	0.28	NA	NA	2.6 (−3.2, 8.4)	0.38	NA	NA	13.2 (−3.4, 29.7)	0.12
fT4/fT3	1.6 (13.2, −10.1)	0.79	−2.6 (−13.6, 8.5)	0.65	NA	NA	−7.2 (−21.3, 6.9)	0.32	2.8 (−6.1, 11.8)	0.53	5.0 (−9.2, 19.1)	0.49

Adjusted for: maternal pre-pregnancy BMI (kg/m^2), 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 95% CI = 95% confidence interval, NA = not applicable, BMI = body mass index, TSH = thyroid stimulating hormone, T3 = triiodothyronine, T4 = thyroxine, IGF1 = insulin-like growth factor 1, IGF2 = insulin-like growth factor 2, BDNF = brain-derived neurotrophic factor, NGF = nerve growth factor, NT3 = neurotrophin 3, NT4 = neurotrophin 4.

* Effect size (95% confidence interval) represents % mediation.

and birth weight, length and head circumference; and cord plasma TSH as a mediator for the association between acrylamide and birth length (Table 4).

Our data only suggest a mediating effect of cord plasma insulin, specifically for the association with birth weight. The proportion of the association between glycidamide and birth weight, length and head circumference mediated by insulin was 11.9% ($p = 0.08$), 7.0% ($p = 0.13$) and 5.8% ($p = 0.27$), respectively. Among neonates of non-smoking mothers, the corresponding proportions were 12.8% ($p = 0.08$), 7.5% (0.18) and 2.1% ($p = 0.63$).

4. Discussion

The key finding of our study is that prenatal acrylamide exposure was associated with endocrine and metabolic targets important in fetal growth and neurodevelopment. We observed negative associations between cord blood acrylamide hemoglobin adducts and cord plasma TSH and the ratio of free T4 to free T3, particularly among newborns of non-smoking mothers. Acrylamide was positively associated with cord plasma free T3 but this association was less strong among newborns of non-smoking mothers, and with placental NGF and NT4, also among neonates of non-smoking mothers. Glycidamide hemoglobin adducts were negatively associated with the ratio of cord plasma free T4 to free T3, insulin and free T4 (the latter two particularly in newborns of non-smoking mothers), and positively associated with free T3.

In particular, the observed associations between acrylamide and glycidamide and cord plasma TSH, free T4, the ratio of free T4 to free T3 and insulin are interesting leads of a possible mechanism underlying the association between gestational acrylamide exposure and fetal growth because those markers were positively associated to fetal growth in the literature and also in our own study population. TSH was positively associated with birth length, and the ratio of free T4 to free T3, free T4 and insulin were positively associated with birth weight, length and head circumference in our study population. In our previous analysis on acrylamide and fetal growth, birth weight, length and head circumference were more strongly associated with glycidamide than with acrylamide itself (Hogervorst et al., 2021). In the current analysis, the ratio of free T4 to free T3, free T4 and insulin showed stronger associations with glycidamide than with acrylamide.

Previously, a cross-sectional study in Taiwanese adolescents showed a statistically significant negative association between urinary levels of the acrylamide metabolite N-acetyl-S-(propionamide)-cysteine and serum free T4 levels (Lin et al., 2015). The study also found a negative and borderline statistically significant association between the acrylamide metabolite in urine and TSH (Lin et al., 2015). Animal studies have also shown effects of acrylamide on the thyroid or thyroid hormone levels but the effects of acrylamide on thyroid hormone levels in animal studies seemed to vary with dosages and exposure durations (Khan et al., 1999; Hamdy et al., 2012; Mannaa et al., 2006; Bowyer et al., 2008; Dourson et al., 2008). The relevance of these animal studies for the human scenario can be questioned as they were carried out with acrylamide doses several fold higher than human dietary doses and there are important differences in thyroid physiology between rodents and humans, which make extrapolation to human dietary doses problematic.

It has to be noted that TSH, free T4 and the ratio of free T4 to free T3 did not seem important mediators of the association between acrylamide or glycidamide and birth outcomes in the current study. Although thyroid hormones thus may not be an underlying mechanism for the association between acrylamide exposure and birth size, their potential role in a possible effect of acrylamide on cognitive function in later life is a relevant topic for further study because of the important role of prenatal thyroid status for neurocognitive development. Increased cord blood TSH levels were associated with worse cognition in boys so the negative association between acrylamide and TSH would not specifically suggest a reason for concern. The effect that acrylamide exposure may have on cognition in later life through its possible negative association on the ratio of free T4 to free T3 is unclear. However, the results of this study suggest that prenatal acrylamide exposure disrupts the prenatal thyroid status and thus studies on prenatal acrylamide exposure and cognitive development are warranted.

Insulin has previously been shown to be negatively associated with acrylamide in 1,356 adults in the cross-sectional NHANES study, with a statistically significant trend across the quartiles of acrylamide exposure, which was represented by the level of acrylamide hemoglobin adducts in blood (Lin et al., 2009). This association is supported by a study in rats, in which the rats were given a daily oral acrylamide dose that was comparable to daily human acrylamide intake. In this study, the acrylamide-exposed rats had significantly lower insulin levels (Totani et al., 2007).

In our analysis, insulin mediated the association between glycidamide adducts and birth weight by 11.9% ($p = 0.08$) among all neonates and by 12.9% ($p = 0.08$) among neonates of non-smoking mothers. Thus, the association between acrylamide exposure and birth size may to some extent be explained by an effect of acrylamide on insulin levels but there are likely still other mechanisms that play a role.

The biochemical mechanisms by which acrylamide exposure may affect cord plasma thyroid and insulin levels are unclear. Acrylamide and glycidamide are soft electrophiles that bind to cysteine residues in proteins (Friedman, 2003). Through this, they could disrupt key proteins in thyroid and insulin metabolism.

NGF and NT4 belong to the neurotrophin family and play a role in pre and postnatal brain development and neuroprotection, and they are suggested to be involved in placental and fetal growth (Sanchez-Infantes et al., 2018). In one study, children born small for gestational age (SGA) had higher cord blood NGF levels than children that were born appropriate for gestational age (Sanchez-Infantes et al., 2018); and in another study, cord blood NGF levels were negatively associated with birth weight (Kilari et al., 2011). However, in a third study, NGF in cord blood was positively associated with birth weight (Malamitsi-Puchner et al., 2007). In our study population, placental NGF levels were not associated with fetal growth outcomes. Thus, it is unclear how to interpret the association between acrylamide and NGF in terms of a possible mechanism underlying the link between acrylamide and fetal growth. As far as we know, there are no other studies on a link between acrylamide exposure and NGF. There is little information on the relationship between NT4 and fetal growth. In a case-control study, blood NT4 levels from postnatal day 1 were lower, but not statistically significantly, in very preterm growth-restricted neonates than in non-growth-restricted very preterm neonates and this association was stronger and statistically significant at postnatal day 21 and 28 (Leviton et al., 2017). The relevance of this study population for a healthy term population of neonates is unclear. In another case-control study, there was no difference in cord blood NT4 levels between intrauterine growth-restricted fetuses and appropriate for gestational age fetuses (Malamitsi-Puchner et al., 2007). In the current study, there was no association between placental NT4 levels and birth outcomes. Similar to NGF, it is unclear how to interpret the association between acrylamide and NT4 in terms of a possible mechanism that may underlie the link between acrylamide and fetal growth. Apart from a possible role in fetal growth, NGF and NT4 might be relevant to study with regard to a possible association between gestational acrylamide exposure and cognitive function in children. We encourage future studies on that topic.

Our study has some limitations, one of which is its cross-sectional nature; the independent (acrylamide biomarkers) and dependent (biomarkers of fetal growth) variables were measured at the same time point; right after birth in cord blood. This means that, if we assume that the associations we observed are causal, we cannot tell what was the cause and what was the effect. Insulin, for example, has been shown to negatively affect the activity of CYP2E1, the major enzyme responsible for acrylamide metabolism to glycidamide. Thus, the negative association we observed between glycidamide hemoglobin adducts and cord blood insulin levels, if causal, could theoretically be explained by insulin

causing a decrease in CYP2E1 activity (Woodcroft et al., 2002); rendering less glycidamide. That would translate to the more insulin, the less glycidamide, or vice versa, the more glycidamide, the less insulin, which is what we observed. However, this explanation is not likely because acrylamide hemoglobin adducts were also negatively associated (albeit less strong and not statistically significant) to insulin levels, whereas a positive association would have been expected if it just reflected the positive effect of insulin on CYP2E1 activity. To overcome the limitation related to the cross-sectional nature of the study, the association between gestational acrylamide exposure and biomarkers of fetal growth could be studied using a dietary assessment method, such as a food frequency questionnaire, to estimate acrylamide intake.

In addition, we performed many statistical tests and therefore some of the statistically significant findings may be chance findings. However, this is the first study into the links between gestational acrylamide exposure and markers of fetal growth. Thus, the findings presented here are exploratory and should serve as input for further studies. We did not adjust the p-values of our analyses for multiple testing. The downside of adjusting for multiple testing is that it strictly controls for type-I errors and thereby lowers the statistical power to (often) unacceptable levels. Especially in explorative studies investigating something for the first time, like this study, that would be very unfortunate. If we had strictly applied correction for multiple testing, that would have led to the conclusion that there was no association between acrylamide or glycidamide and biomarkers of fetal growth (for all or some of the biomarkers), which would suggest there is no clear need for follow-up of this topic in future studies. Instead of adjusting for multiple comparisons, interpretation of an observed association should be based on critical evaluation of the quality of the study (in terms of precision and validity), on the size of the observed effect, and on the plausibility of the association in terms of what is known from other lines of evidence (e.g. laboratory studies), while reporting all the associations that were tested within the research that is presented in the same manuscript (Perneger, 1998; Rothman, 1990; Savitz and Olshan, 1995); which we did.

We cannot exclude the possibility of residual confounding by factors that are associated with both acrylamide and glycidamide hemoglobin adduct levels and the biomarkers of fetal growth, such as another dietary exposure or a generally less heathy diet. However, when we additionally adjusted the analyses for variables that can be considered proxies for a healthy or unhealthy diet (consumption of vegetables, fruits, fish and soda drinks), the associations were virtually unchanged (results not shown).

Our study also has specific strengths. The ENVIR*ON*AGE study population is representative for the gestational segment of the population at large and therefore our findings have external validity (Janssen et al., 2017). Another advantage of our study was the use of acrylamide biomarkers. Biomarkers may not always outperform other methods of acrylamide exposure assessment, such as food frequency questionnaires, because a biomarker may not accurately capture the exposure during the relevant period for disease etiology, for instance because exposure may show considerable intra-individual variation in time. However, the hemoglobin adduct biomarker may well be superior in the case of a study on biomarkers related to 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.

In order to further strengthen the body of evidence on gestational acrylamide exposure and fetal growth and development, we encourage other epidemiological studies on the association between cord blood acrylamide hemoglobin adduct levels and insulin, neurotrophins and thyroid hormones.

5. Conclusion

In conclusion, we found associations between prenatal acrylamide exposure and the endocrine system; a higher *in utero* exposure to acrylamide was associated with lower cord blood TSH, a lower ratio of free T4 to free T3, and higher placental NGF and NT4 levels, while higher levels of its metabolite glycidamide were associated with higher cord blood free T3 and placental NGF, a lower ratio of free T4 to free T3, and lower insulin levels in cord blood. Insulin seems a particularly promising candidate for further study on the mechanisms of action underlying the association between gestational acrylamide exposure and reduced fetal growth. Possible health consequences of the association between gestational acrylamide exposure and thyroid hormones and neurotrophins warrant future study.

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. JGF obtained funding and did the statistical analysis and the quality control of the database. JGF wrote the first draft of the manuscript. TSN coordinates the ENVIR*ON*AGE birth cohort and managed funding. Fetal growth biomarker measurements were performed by NDS. All authors have helped with data interpretation and critically reviewed the manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106668.

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