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Birth Weight, Head Circumference, and Prenatal Exposure to Acrylamide from Maternal Diet: The European Prospective Mother-Child Study (NewGeneris)

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Running title

Prenatal exposure to acrylamide and birth weight

Keywords

Biomarker, children, diet, intrauterine growth restriction, in utero exposure

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Conflict of interest

HvS, PR and MT are stakeholders in Adduct Analys AB, Stockholm, Sweden, the company which owns the patent for the analytical method used for the hemoglobin adduct measurements. The other authors and collaborators declare that they have no actual or potential competing financial interests.

List of abbreviations and definitions

BiB Born in Bradford birth cohort
BMI Body mass index
CI confidence interval
FIRE fluorescein isothiocyanate R Edman
FFQs food-frequency questionnaires
Hb hemoglobin
INMA INfancia y Medio Ambiente [Spanish for Environment and Childhood] birth cohort
MoBa Den norske Mor & Barn- undersøkelsen [Norwegian for Norwegian Mother and Child Cohort study]
RR relative risk
SD standard deviation

Abstract

Background: Acrylamide is a common dietary exposure that crosses the human placenta. It is classified as a probable human carcinogen, and developmental toxicity has been observed in rodents.

Objectives: We examined the associations between prenatal exposure to acrylamide and birth outcomes in a prospective European mother-child study.

Methods: Hemoglobin (Hb) adducts of acrylamide and its metabolite glycidamide were measured in cord blood (reflecting cumulated exposure in the last months of pregnancy) from 1101 singleton pregnant women recruited in Denmark, England, Greece, Norway and Spain, 2006-2010. Maternal diet was estimated through food-frequency questionnaires.

Results: Both acrylamide and glycidamide Hb adducts were associated with a statistically significant reduction in birth weight and head circumference. The estimated difference in birth weight for infants in the highest versus lowest quartile of acrylamide Hb adduct levels after adjusting for gestational age and country was -132 grams (95% confidence interval (CI): -207, -56); the corresponding difference for head circumference was -0.33 cm (95%CI: -0.61, -0.06). Findings were similar in infants of non-smokers, were consistent across countries, and remained after adjustment for factors associated with reduced birth weight. Maternal consumption of foods rich in acrylamide, such as fried potatoes, was associated with cord blood acrylamide adduct levels and with reduced birth weight.

Conclusions: Dietary exposure to acrylamide was associated with reduced birth weight and head circumference. Consumption of specific foods during pregnancy was associated with higher acrylamide exposure *in utero*. If confirmed, these findings suggest that dietary intake of acrylamide should be reduced among pregnant women.

Introduction

Acrylamide is neurotoxic in humans and animals, and is classified as a probable human carcinogen (IARC 1994). Occupational exposure and smoking were originally regarded as the main sources of exposure to acrylamide in humans (IARC 1994), but a decade ago it was unexpectedly discovered that acrylamide formed in a wide variety of carbohydrate-containing foods during frying or baking at high temperatures (Tareke et al. 2002). Worldwide concern about potential health effects of dietary exposure to acrylamide followed the finding of acrylamide in commonly consumed foods such as fried potatoes, potato chips, biscuits, breakfast cereals, and coffee (EFSA 2010; JECFA 2011; Manson et al. 2005). Prenatal exposure to acrylamide is of particular concern as reproductive and developmental toxicity of acrylamide has been reported in rodents, including dose-dependent body weight reduction and skeletal malformations in offspring exposed *in utero* (El Sayyard et al. 2011; Manson et al. 2005; Tyl and Friedman 2003).

Acrylamide and its metabolite glycidamide are reactive and may form adducts with nucleophilic sites in proteins and DNA. Glycidamide has a much higher reactivity towards DNA than acrylamide, and a considerably higher genotoxicity than the parent compound (Rice 2005). Hemoglobin (Hb) adducts can serve as biomarkers of internal dose in the blood for reactive compounds like acrylamide (Törnqvist et al. 2002). Detection of Hb adducts from acrylamide in human umbilical cord blood (Schettgen et al. 2004; von Stedingk et al. 2011), and studies of acrylamide in *ex vivo* placenta perfusion studies (Annola et al. 2008), show that acrylamide crosses the human placenta.

In view of the possible health effects associated with the widespread dietary exposure to acrylamide (EFSA 2010; IARC 1994; JECFA 2011; Manson et al. 2005) starting *in utero*

(Brantsaeter et al. 2008), we investigated the association between prenatal exposure to acrylamide, measured as Hb adducts in cord blood, and birth outcomes in a multicentre European study. We also assessed prenatal exposure to acrylamide through maternal food-frequency questionnaires (FFQs) and associations between maternal dietary exposure to acrylamide, cord blood adduct levels, and birth outcomes, hypothesising that higher maternal intake of acrylamide-rich food during pregnancy would be associated with reduced intrauterine growth.

Methods

Study population

The study was conducted by the NewGeneris consortium (www.newgeneris.org) as part of research exploring the impact of diet during pregnancy on child health (Merlo et al. 2009). Pregnant women enrolled during 2006-2010 in eleven maternity units located in Copenhagen, Denmark; Heraklion, Greece; Oslo and Akershus, Norway; Barcelona and Sabadell, Spain; and Bradford, England (Chatzi et al. 2011; Magnus et al. 2006; Pedersen et al. 2009; Raynor et al. 2009). Specific eligibility criteria were applied in the baseline cohorts for the participation of mothers (see Supplemental Material, Table S1 for details). Precise participation rates for the present analysis cannot be estimated because several filters were applied for inclusion of mothers in the NewGeneris study, such as giving birth during the periods of cord blood collection and processing, getting sufficient volume of cord blood, successful blood processing and biomarker analysis.

Questionnaire information on maternal characteristics and cord blood Hb adduct measurements was available for 1151 mother-child pairs. We excluded 16 twins and 34 pairs with missing information on maternal smoking, gestational age, birth weight, and/or sex of

the child leaving 1101 mother-child pairs for analysis. Information on birth weight, head circumference, gestational age, sex, and mode of delivery was obtained from maternity records.

Gestational age for participants from Denmark, Greece and Spain was estimated from the interval between last menstrual period and date of the delivery and corrected by ultrasound measurement if there was a discordance of seven days or more between both estimates. Ultrasound-based estimation was provided for the majority of participants from England and Norway. We defined small-for-gestational-age children as those who weighed less than the 10th percentile of the cohort-specific reference of fetal growth, stratified by completed week of gestation and sex.

Ethical approval was obtained from the research ethics committee in each country: the Regional Ethical Review Board in Stockholm, Sweden, the Capital Region of Denmark, the Ethical Committee of the University Hospital in Heraklion, Crete, Greece, the Norwegian Regional Committee for Medical and Health Research Ethics, the Clinical Research Ethics Committee of Barcelona, Spain, the Bradford Local Research Ethics Committee, Bradford, the United Kingdom. Further details are provided in Supplemental Material. Written informed consent for participation of the women and their children was obtained from all participating women.

Acrylamide and glycidamide Hb adducts in cord and maternal blood

Cord blood was collected in heparin tubes from the placenta by umbilical puncture immediately after delivery, following a common protocol at each site. Erythrocytes were separated by centrifugation and stored at -20°C before and after shipment on dry ice. Hb

adducts from acrylamide and glycidamide were simultaneously determined by the "adduct FIRE procedure" (fluorescein isothiocyanate R Edman) in 1101 cord blood samples and 172 maternal blood samples (von Stedingk et al. 2010, 2011). In brief, adducted N-terminal valines were detached using the Edman reagent fluoresceine-5-isothiocyanate. The detached analytes were purified by solid phase extraction and analyzed by liquid chromatography/mass spectrometry.

Ethylene oxide Hb adduct levels that are associated with exposure to tobacco smoke were also measured in 1074 cord blood samples (to assess exposure to tobacco smoke during pregnancy) using the same methods (von Stedingk et al. 2011).

Maternal diet

Detailed information on maternal diet during pregnancy was obtained from the mothers using FFQs collected before or at the time of delivery (Merlo et al. 2009). We grouped food and drink items into eight groups known to contain potentially high levels of acrylamide based on similarities in the composition and processing of the foods in each group, specifically: fried potatoes, potato chips, breakfast cereals, crisp bread, coffee, cookies, fine bakery products, bread and toast (Brantsaeter et al. 2008; EFSA 2010; JECFA 2011). We developed an acrylamide food score for non-smokers following the same approach used for the evaluation of the Mediterranean diet (Trichopoulou et al. 2003). The score was based on consumption of the eight food groups (as mentioned above) and each non-smoking woman received a score of zero for each food if her consumption was below the country-specific median, and a score of one if her consumption was above the median. Scores were then added for each woman and ranged from zero (lowest intake) to eight (highest). In all dietary analyses we excluded women with missing FFQs (n=146), those who smoked during the last four months of

pregnancy (n=129) and 25 women with a total energy estimate of less than 500 or above 6000 kcal/day (Butte and King 2005; Willett 1998).

Statistical analysis

We used linear regression models for birth outcomes evaluated on a continuous scale (birth weight, head circumference) and calculated beta-coefficients and 95% confidence intervals (CI). Logarithmic-binomial regression models were used to relative risks (RR) and 95% CI for small-for-gestational-age. Cord blood Hb adduct levels were modeled on a continuous scale (effect estimated per 10 pmol/grams Hb increments) or categorized according to quartiles of the distribution. All models were adjusted for country of the child's birth and gestational age (continuous in completed weeks). Additional adjustment for gestational age as a quadratic term (continuous completed weeks^2) gave nearly identical results (not shown). Further adjustment was based on *a priori* selection of potential risk factors for reduced birth weight including maternal smoking (no, yes), passive smoking (no, yes), sex, pre-pregnancy body mass index (BMI, kg/m^2), parity (0, 1+), maternal age (years), maternal ethnicity (White, Non-White), and maternal education (low, middle, high) as a marker of socio-economic position. In addition, models were adjusted for high or low maternal consumption during pregnancy (according to country-specific median intake) of fruit and vegetables (based on 20 to 61 questionnaire items), fish (fresh, canned, dried and prepared fish and shellfish, including items with high and low fat content), and soft drinks (e.g. coca cola, other soft drinks, energy soft drinks including regular and light products) as markers of more versus less healthy dietary patterns.

The average lifespan of erythrocytes is four months in adults (Törnqvist et al. 2002). Therefore women who never smoked or who quit smoking before the last four months of

pregnancy were categorized as “non-smokers” while those who never smoked were categorized as “never smokers”. All associations were examined for the full study population (n=1101) and separately for children born to non-smokers (n=972).

In addition to estimating associations adjusted for country, we estimated country-specific associations between acrylamide Hb adducts and birth weight, and performed a meta-analysis to derive pooled estimates of effect. Only results from fixed effects models are shown since heterogeneity of effects between centres were not statistically significant.

Associations of maternal diet with acrylamide Hb adduct levels in cord blood, and with birth weight, were estimated using linear regression models that were adjusted as described above. Exposure-response curves were estimated using a generalized additive model with the dietary score modeled as a smoothed spline with two degrees of freedom, adjusted for country and gestational age. We used alpha levels of 5% as reference value for statistical significance and Stata S.E. version 10.0 for the statistical analyses (StataCorp, Texas, USA).

Results

Levels of Hb adducts from acrylamide

Acrylamide and glycidamide Hb adducts in cord blood were detectable in all children (N=1101). The median Hb adduct level from acrylamide was 14.4 pmol/grams Hb (range 4.4–147.6) and for glycidamide was 10.8 pmol/grams Hb (2.0–117.6). There was a statistically significant correlation between glycidamide and acrylamide adduct levels in cord blood (Pearson correlation coefficient r=0.85, P<0.001). Median acrylamide adduct levels were higher in cord blood from children of mothers who smoked (n=129) than in children of non-smokers (n=972; 30.5 versus 13.8 pmol/grams Hb, P<0.001). Corresponding levels for

glycidamide adducts were 20.7 versus 10.1 pmol/grams Hb (P<0.001), and for ethylene oxide adducts (as a marker of exposure to cigarette smoke) were 24.5 versus 8.9 pmol/grams Hb, (P<0.001).

The median acrylamide Hb adduct levels in cord blood were approximately half of the levels in paired maternal blood (Figure 1). Hb adduct levels in cord blood were positively correlated with both maternal acrylamide (r=0.95, P<0.001, n=171) and glycidamide Hb adducts (r=0.94, P<0.001, n=171). Among non-smokers the highest median level of acrylamide adducts was detected in children from England (23.6 pmol/grams Hb) and the lowest in children from Denmark (12.0 pmol/grams Hb).

Factors associated with birth weight

The participating mothers were mainly white, non-smoking, in their late-20s to early-30s with pre-pregnancy BMI in a range of 18.5–24.9 kg/m^2 and already parous (Table 1). The prevalence of smokers was lowest in Norway and Denmark and Norway (2.4% and 3.8%, respectively) and highest in Greece (21%). Birth weight was significantly increased in association with maternal age, parity (parous versus nulliparous), pre-pregnancy BMI, non-smoking, male sex, and gestational age (Table 2). On average, children from the Northern European countries weighed more than children from Spain and Greece (P<0.001).

Hb adduct levels and birth weight

Higher levels of acrylamide and glycidamide adducts in cord blood were associated with a significant decrease in birth weight (Table 3). The mean birth weight was reduced by 35 grams (95% CI: -51, -19) with each 10-pmol/g Hb increase in acrylamide adduct levels after adjusting for country of birth and gestational age in the total population, and by 20 grams (95% CI: -46, 6) among 972 non-smokers. A 10-pmol/g Hb increase in glycidamide adducts

was associated with a 60 gram reduction in birth weight (95% CI: -87, -34) in the total population, and a 53 gram reduction (95% CI: -95, -10) in non-smokers. Results were similar among the subgroup of 889 women who were never-smokers with a mean birth weight reduction of 26 grams (95% CI: -54, 3; p=0.074) for a 10-pmol/g Hb increase in acrylamide adducts when adjusted for gestational age and country of birth; the corresponding reduction in birth weight for glycidamide adducts was 65 grams (95% CI: -111, -19).

Birth weight decreased monotonically with increasing quartiles of exposure (Table 3). The estimated difference in birth weight for infants in the highest versus lowest quartile of acrylamide Hb adduct levels after adjusting for gestational age and country was -132 grams (95% CI: -207, -56) in the total population and -107 grams (95% CI: -188, -27) when restricted to non-smokers. The estimated difference in birth weight for infants in the highest versus lowest quartile of glycidamide adducts was -136 grams (95% CI: -212, -60) in the total population and -103 grams (95% CI: -182, -23) among non-smokers (Table 3). Among term deliveries (n=1063), the difference in birth weight for children in the highest versus lowes quartile of acrylamide Hb adduct levels was -137 grams (95%CI: -214, -60); a similar difference was observed for 939 term babies of non-smokers (-120 grams; 95%CI: -201, -38).

Associations between Hb adducts and birth weight adjusted for country and gestational age were not substantially modified when further adjustments were made for other potential risk factors for reduced birth weight, including sex, pre-pregnancy BMI, parity, maternal age, ethnicity, education, passive smoking and smoking and dietary variables that could be related with a healthy (or unhealthy) eating pattern such as intakes of vegetables and fruits, fish or soft drinks (Table 3). The population in the comprehensively adjusted models (n=747) is

smaller than the population in models adjusted only for country and gestational age (n=1101). We also estimated associations adjusted for ethylene oxide Hb adduct levels in cord blood as a biomarker for active/passive smoking, in addition to the other covariates listed above. The difference in birth weight for infants of non-smokers (n=656) in the highest versus lowest quartile of acrylamide Hb adduct levels remained statistically significant after adjustment for ethylene oxide adducts (-142 grams; 95% CI: -246, -38) while the corresponding difference was borderline significant for glycidamide Hb adducts (-82 grams; 95% CI: -182, 17; P=0.11).

Negative associations between acrylamide and glycidamide Hb adduct levels in cord blood and birth weight were observed for all countries (Figure 2). A meta-analysis of country-specific estimates gave similar results to estimates based on combined data adjusted for country, with a 10-pmol/g Hb increase in acrylamide adducts associated with a 36 gram (95% CI: -53, -19) decrease in birth weight, and a 10-pmol/g Hb increase in glycidamide adduct levels associated with a 63 gram (95% CI: -90, -36) decrease in birth weight.

The pattern was also consistent among non-smokers (-26 grams, 95% CI: -52, 1 and -63 grams, 95%CI: -106, -20 for a 10-pmol/g Hb increase in acrylamide and glycidamide, respectively), with no significant heterogeneity between countries (P=0.51 and P=0.46) although in England the association with birth weight was minimal (-2 grams, 95% CI: -44, 41 and -18, 95% CI: -95, 59).

Hb adduct levels and small-for-gestational age

Small-for-gestational-age (birth weight < 10^{th} percentile for the cohort according to week of gestation and sex) was increased in association with a 10-pmol/grams Hb increase in

acrylamide adduct levels in the full population (RR=1.20, 95% CI: 1.08, 1.33) based on 891 observations and 72 small-for-gestational age births) and for infants of non-smokers (RR=1.35, 95% CI: 1.10, 1.65, based on 794 observations and 60 small-for-gestational age births). The corresponding estimates for glycidamide were RR=1.36 (95% CI: 1.13,1.64) for all and RR=1.42 (95% CI: 1.00, 2.02) for non-smokers.

Hb adduct levels and birth head circumference

The highest versus lowest quartile of acrylamide Hb adduct levels was associated with a significant reduction in head circumference of 0.33 cm (95% CI: -0.61, -0.06) in the full population and among non-smokers, with similar results were observed for glycidamide (Table 4). Similar to the associations with birth weight, there was a monotonic reduction in birth head circumference with increasing quartiles of exposure. This pattern was also found after further adjustment in the smaller study population with available information on potential risk factors (Table 4). These associations, however, were not statistically significant when adjusted for the other potential risk factors, as above.

Maternal diet, acrylamide Hb adduct levels in cord blood and birth weight

A one-unit increase in the acrylamide-rich food score was associated with higher Hb cord blood adduct levels (Figure 3) for acrylamide (0.68 pmol/grams Hb, 95% CI: 0.30, 1.06) and glycidamide (0.39 pmol/grams Hb, 95% CI: 0.15, 0.63) based on food score modeled as a simple continuous variable. Consistent with the associations observed between Hb adducts from acrylamide and birth weight, a 1-unit increase in the acrylamide food score was associated with a 16 gram decrease in birth weight (95% CI: -33, 1; P=0.066) after adjustment for country and gestational age. Additional adjustment did not change this association (Figure 3). Higher food scores were associated with a non-significant reduction in

birth head circumference of -0.01 cm (95% CI: -0.07, 0.05; P=0.72) among children of non-smoking women with acrylamide food score data (n=726).

Discussion

This study provides strong evidence that higher prenatal exposure to acrylamide through maternal diet during pregnancy is associated with reduced birth weight and head circumference. Prenatal exposure to acrylamide was evaluated using Hb adducts, which are well-established biomarkers for acrylamide exposure (Vikström et al. 2011). Birth weight decreased monotonically with increasing acrylamide and glycidamide adduct cord blood levels. These findings were consistent between countries, were shown in the full study population as well as in non-smokers. Maternal consumption of acrylamide-rich foods was associated with higher levels of acrylamide and glycidamide Hb adducts in cord blood, and with lower birth weight.

Prenatal exposure assessment using biomarkers is a key strength of the study. The measurement of Hb adduct levels in cord blood enabled a more accurate estimation of prenatal exposure to acrylamide compared to estimates based solely on dietary questionnaires or maternal Hb adduct levels. By measuring cord blood Hb adduct levels, variation related to transplacental exposure, uptake, and metabolism between children is taken into account. In line with our observation of higher Hb adduct levels in children from England compared with other study countries, higher levels have also been reported in non-smoking British adults compared with other European adults (Vesper et al. 2008). Furthermore our study population was large and detailed information on maternal characteristics, including smoking and dietary habits during pregnancy, was collected in a manner that enabled us to evaluate potential sources of exposure contributing to *in utero* formation of Hb adducts and reduce potential biases through adjustment in a large subset of the study population.

In addition to dietary intake of acrylamide (Bransaeter et al. 2008; Tran et al. 2010; Vikström et al. 2011), it is possible that acrylamide adducts were acting as a proxy marker for another dietary exposure or mix of exposures that were responsible for the associations observed, such as other Maillard reaction products that, like acrylamide, are formed during processing of food at high temperatures (Chaundhry et al. 2006; Tareke et al. 2002). Alternatively, acrylamide adducts may have been acting as a marker of a less healthy diet in general. Acrylamide may also be one of many contributors to the observed association. Adjusting for indicators of healthy and unhealthy eating habits, such as fruits and vegetables, fish, and soft drink intakes, maternal BMI, and indicators of socioeconomic status, did not substantially alter associations among the subset of the population with available data. Furthermore, foods that are generally considered to be part of a healthy diet, such as crisp bread and certain breakfast cereals, may contain high concentrations of acrylamide (EFSA 2010). Finally, Hb acrylamide and glycidamide adducts could also reflect exposure to tobacco smoke (von Stedingk et al. 2011). Monotonic dose-response associations of adduct levels with birth outcomes were observed in women who were non-smokers in pregnancy, as well as in never-smokers, even after adjusting for passive smoking based on self-reporting or using ethylene oxide adducts as biomarkers of exposure to tobacco smoke. We cannot rule out uncontrolled confounding or the possibility that adduct levels are serving as a proxy marker for some other causal factor; however, given the consistency of our findings in different population subgroups and after adjustment for multiple potential confounders, it seems unlikely that prenatal exposure to tobacco smoke or other dietary compounds could fully explain the associations observed between acrylamide and birth weight.

A limitation of exposure assessment based on dietary information is the difficulty of evaluating exposure to toxic agents, such as acrylamide, for which concentrations may vary substantially among similar foods depending on manufacturing or preparation methods (EFSA 2010; JECFA 2011; Manson et al. 2005; Tareke et al. 2002). Quantitative estimation of dietary intake is particularly complex in international studies. Nonetheless, intakes of key food types have been found to be predictive of higher acrylamide Hb adduct levels in other study populations (Tran et al. 2010; Vikström et al. 2011), as well as in the present study population.

Decreases in offspring body weight following maternal acrylamide exposure during gestation have been consistently observed in mice and rats (El Sayyad et al. 2011; Tyl and Friedman 2003). A US National Toxicology Program evaluation panel concluded that acrylamide causes decreased birth weight in rodents (Manson et al. 2005), although the mechanisms underlying the effects of acrylamide on birth weight are not understood. Acrylamide is classified as a probable carcinogen to humans on the basis of animal studies and genotoxicity (IARC 1994) and its genotoxicity is thought to be largely due to metabolic conversion to the genotoxic epoxide glycidamide (Rice 2005). Acrylamide Hb adduct levels in adults were associated with decreased serum insulin and reduced insulin resistance (Lin et al. 2009). In addition, oxidative stress causing increased production of reactive oxygen radicals and inflammation was reported in fourteen healthy volunteers after ingestion of 160 grams of fried potato crisps daily for 4 weeks, which correspond to a daily acrylamide exposure that is approximately three times higher than the currently calculated ingestion of ~50 μg/day in the Western diet (Naruszewicz et al. 2009). The ability of acrylamide to readily react with sulfhydryl and amino residues in proteins, including enzymes, receptors, and cytoskeletal proteins, can affect a multitude of cellular processes and has been suggested to form the basis

of some of acrylamide's toxic effects (IARC 1994) and may contribute to the associations with birth outcomes observed in our study population.

The potential public-health implications of our findings are substantial. Increases in head circumference are an important indication of continued brain growth, and reduced birth head circumference has been associated with delayed neurodevelopment (Gale et al. 2006). Reduced birth weight is a risk factor for numerous adverse health effects early in life, and has been associated with multiple adverse outcomes later in life such as reduced stature, increased incidence of cardiovascular disease, type 2 diabetes mellitus, and osteoporosis (Gluckman et al. 2008). The estimated difference in mean birth weight among children the highest acrylamide exposed quartile compared with children in the lowest quartile was around 100 grams, consistent with the reduction in birth weight observed for children exposed *in utero* to maternal smoking (Li et al. 1993). Many commonly consumed foods, e.g. fried potatoes and related products, crisp bread, and coffee, contain high concentrations of acrylamide (EFSA 2010; JECFA 2011; Manson et al. 2005; Tareke et al. 2002). The amount of acrylamide in specific foods varies widely depending on precursor levels and processing, and, crucially, is amenable to appropriate interventions in the preparation of food.

In summary, this large population-based study provides the first epidemiological evidence of a significant association between prenatal exposure to acrylamide and reduced birth weight and head circumference. If confirmed in other studies, these findings provide evidence supporting the need for changes in food production and for providing clear public health advice to pregnant women to reduce their dietary intake of foods that may contain high concentrations of acrylamide.

References

Annola K, Keski-Rahkonen P, Vahakangas K, Lehtonen M. 2008. Simultaneous determination of acrylamide, its metabolite glycidamide and antipyrine in human placental perfusion fluid and placental tissue by liquid chromatography-electrospray tandem mass spectrometry. J Chromatogr B Analyt Technol Biomed Life Sci 876:191–197.

Brantsaeter AL, Haugen M, Mul A, Bjellaas T, Becher G, Klaveren JV et al. 2008. Exploration of different methods to assess dietary acrylamide exposure in pregnant women participating in the Norwegian Mother and Child Cohort Study (MoBa). Food Chem Toxicol 46:2808–2814.

Butte NF, King JC. 2005. Energy requirements during pregnancy and lactation. Public Health Nutr. 8:1010–1027.

Chatzi L, Mendez M, Garcia R, Roumeliotaki T, Ibarluzea J, Tardón A et al. 2011. Mediterranean diet adherence during pregnancy and fetal growth: INMA (Spain) and RHEA (Greece) mother-child cohort studies. Br J Nutr 1–11.

Chaundhry MQ, Cotterill J, Watkins R, Price N. 2006. The potential of molecular modeling for the prediction of toxicity of compounds generated during heat treatment of foods. In Acrylamide and Other Health Hazardous Compounds in Heat-Treated Foods (Skog K, Alexander J ed). Woodhead Publishing, Cambridge, England, 132–160.

European Food Safety Authority. 2010. Results on acrylamide levels in food from monitoring year 2008. EFSA, Parma, Italy.

El Sayyad HI, Abou-Egla MH, El Sayyad FI, El-Ghawet HA, Gaur RL, Fernando A et al. 2011. Effects of fried potato chip supplementation on mouse pregnancy and fetal development. Nutrition 27:343–350.

Gale CR, O'Callaghan FJ, Bredow M, Martyn CN. 2006. The influence of head growth in fetal life, infancy, and childhood on intelligence at the ages of 4 and 8 years. Pediatrics 118:1486–1492.

Gluckman PD, Hanson MA, Cooper C, Thornburg KL. 2008. Effect of in utero and early-life conditions on adult health and disease. N Engl J Med. 359:61–73.

International Agency for Research on Cancer. 1994. Some industrial chemicals. IARC monographs on evaluation of carcinogen risks to humans, Vol. 60. IARC, International Agency for Research on Cancer, Lyon, France.

Joint FAO/WHO Expert Committee on Food Additives. 2011. Evaluation of certain contaminants in food. JECFA, Joint FAO/WHO Expert Committee on Food Additives, Rome, Italy.

Li CQ, Windsor RA, Perkins L, Goldenberg RL, Lowe JB. 1993. The impact on infant birth weight and gestational age of cotinine-validated smoking reduction during pregnancy. JAMA. 269:1519–1524.

Lin CY, Lin YC, Kuo HK, Hwang JJ, Lin JL, Chen PC et al. 2009. Association among acrylamide, blood insulin, and insulin resistance in adults. Diabetes Care 32:2206–2211.

Magnus P, Irgens LM, Haug K, and the MoBa Study Group. 2006. Cohort profile: The Norwegian Mother and Child Cohort Study (MoBa). Int J Epidemiol 35:1146–1150.

Manson J, Brabac MJ, Buelke-Sam J, Carlson GP, Chapin RE, Favor JB et al. 2005. NTP-CERHR expert panel report on the reproductive and developmental toxicity of acrylamide. Birth Defect Research 74:17–113.

Merlo DF, Wild CP, Kogevinas M, Kyrtopoulos S, Kleinjans J. 2009. NewGeneris: A European study on maternal diet during pregnancy and child health. Cancer Epidemiol Biomarkers Prev 18:5–10.

Naruszewicz M, Zapolska-Downar D, Kosmider A, Norwicka G, Kozlowska-Wojciechowska M, Vikström AS et al. 2009. Chronic intake of potato chips in humans increases the production of reactive oxygen radicals by leukocytes and increases plasma C-reactive protein: a pilot study. Am J Clin Nutr 89:773–777.

Pedersen M, Wichmann J, Autrup H, Dang DA, Decordier I, Hvidberg M et al. 2009. Increased micronuclei and bulky DNA adducts in cord blood after maternal exposures to traffic-related air pollution. Environ Res 109:1012–1020.

Raynor P. Born in Bradford Collaborative Group. 2008. Born in Bradford, a cohort study of babies born in Bradford, and their parents: protocol for the recruitment phase. BMC Public Health 8:327.

Rice JM. 2005. The carcinogenicity of acrylamide. Mutat Res 580:3–20.

Schettgen T, Kutting B, Hornig M, Beckmann MW, Weiss T, Drexler H et al. 2004. Trans-placental exposure of neonates to acrylamide-a pilot study. Int Arch Occup Environ Health 77:213–216.

Tareke E, Rydberg P, Karlsson P, Eriksson S, Törnqvist M. 2002. Analysis of acrylamide, a carcinogen formed in heated foodstuffs. J Agric Food Chem 50:4998–5006.

Törnqvist M, Fred C, Haglund J, Helleberg H, Paulsson B, Rydberg P. 2002. Protein adducts: quantitative and qualitative aspects of their formation, analysis and applications. J Chromatogr B Analyt Technol Biomed Life Sci 778:279–308.

Tran NL, Barraj LM, Murphy MM, Bi X. 2010. Dietary acrylamide exposure and hemoglobin adducts-National Health and Nutrition Examination Survey (2003-04). Food Chem Toxicol 48:3098–3108.

Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. 2003. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 348:2599–2608.

Tyl RW, Friedman MA. 2003. Effects of acrylamide on rodent reproductive performance. Reprod Toxicol 17:1–13.

Vesper HW, Slimani N, Hallmans G, Tjonneland A, Agudo A, Benetou V et al. 2008. Cross-sectional study on acrylamide hemoglobin adducts in subpopulations from the European Prospective Investigation into Cancer and Nutrition (EPIC) Study. J Agric Food Chem 56:6046–6053.

Vikström AC, Abramsson-Zetterberg L, Naruszewicz M, Athanassiadis I, Granath FN, Törnqvist MA. 2011. In vivo doses of acrylamide and glycidamide in humans after intake of acrylamide-rich food. Toxicol Sci 119:41–49.

von Stedingk H, Rydberg P, Törnqvist M. 2010. A modified Edman produce for analysis of N-terminal valine adducts in hemoglobin by LC-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 878:2483–2490.

von Stedingk H, Vikström AC, Rydberg P, Pedersen M, Nielsen JKS, Segerbäck D et al. 2011. Analysis of hemoglobin adducts from acrylamide, glycidamide and ethylene oxide in paired mother/cord blood samples from Denmark. Chem Res Toxicol 24:1957–1965.

Willett WC. 1998. Nutritional Epidemiology. New York Oxford Press.

Table 1. Study population characteristics (N=1101).

Characteristics		All (N=1101) n (%)	Non-smokers * (n=972) n (%)
Country	Greece	236 (21)	186 (19)
	Spain	220 (20)	185 (19)
	Norway	247 (22)	241 (25)
	England	186 (17)	156 (16)
	Denmark	212 (19)	204 (21)
Maternal ethnicity	White	912 (83)	792 (82)
	Non-white	187 (17)	179 (18)
	Missing	2	1
Maternal age (years, mean ± SD)		30.9 ± 5.2	31.1 ± 5.1
Maternal education	High	357 (36)	342 (39)
	Middle	371 (38)	329 (38)
	Low	251 (26)	207 (24)
	Missing	122	94
Parity	Nulliparous	388 (36)	356 (37)
	Parous	688 (64)	594 (63)
	Missing	25	22
Pre-pregnancy BMI (kg/m^2)		24.1 ± 4.9	24.0 ± 4.8
	Missing	110	89
Second hand smoke during pregnancy	No	652 (65)	622 (70)
	Yes	354 (35)	272 (30)
	Missing	95	78
Gender	Boys	550 (50)	489 (50)
	Girls	551 (50)	483 (50)
Gestational age (completed weeks)	<37	38 (3)	33 (3)
	≥37	1063 (97)	939 (97)
Birth weight (grams)	<2500	18 (2)	11 (1)
	≥2500	1083 (98)	961 (99)
Small-weight-for-gestational age	No	819 (92)	734 (92)
	Yes	72 (8)	60 (8)
	Missing	210	178
Birth head circumference (cm)		34.8 ± 1.5	34.8 ± 1.5
	Missing	96	82
Cord blood hemoglobin adduct (pmol/g Hb)			
	Acrylamide	19.7 ± 16.5	16.8 ± 11.1
	Glycidamide	13.6 ± 10.1	11.8 ± 6.6
	Ethylene oxide	13.2 ± 13.6	10.5 ± 6.7
	Missing	27	24

* Women who never smoked or who quit smoking before the last four months of pregnancy.

Table 2. Differences in birth weight according to socio-demographic, reproductive and life-style factors.

	Total population (n = 1101)			Non-smokers * (n = 972)		
Characteristics	n	ß (95%CI)	p	n	ß (95%CI)	p
Country						
Greece	236	Ref.: 3205 g		186	Ref.: 3219 g	
Spain	220	78 (-1, 157)	0.050	185	81 (-4, 185)	0.063
Norway	247	197 (117, 277)	<0.001	241	203 (119, 286)	<0.001
England	186	258 (178, 337)	<0.001	156	228 (140, 316)	<0.001
Denmark	212	256 (178, 337)	<0.001	204	258 (175, 341)	<0.001
Ethnicity (white vs. non-white)	1099	11 (-69, 91)	0.79	972	-39 (-123, 46)	0.37
Maternal age (years)	1101	6 (1, 11)	0.015	972	5 (0, 10)	0.086
Maternal education						
High	357	Ref.: 3512 g		342	Ref.: 3523 g	
Middle	371	-54 (-114, 7)	0.082	329	-58 (-122, 6)	0.074
Low	251	-51 (-120, 17)	0.143	207	-39 (-111, 34)	0.30
Parity (nulliparous vs. parous)	1076	115 (61, 170)	<0.001	950	110 (54, 166)	<0.001
Pre-pregnancy BMI (kg/m^2)	991	12 (7, 18)	<0.001	883	12 (6, 18)	<0.001
Maternal smoking (no vs. yes)	1101	-142 (-221, -63)	<0.001			
Passive smoke (no vs. yes)	1006	-39 (-101, 23)	0.22	894	7 (-60, 73)	0.85
Ethylene oxide (10 pmol/g Hb)	1074	-31 (-50, -12)	0.001	948	-27 (-67, 13)	0.185
Gender (boy vs. girl)	1101	-140 (-189, -91)	<0.001	972	-141(-193, -90)	<0.001
Gestational age (weeks)	1101	113 (93, 192)	<0.001	972	110 (91, 130)	<0.001

Beta-coefficients (β) correspond to the difference in birth weight in grams and are estimated from linear regression models adjusted for country and gestational age (completed weeks).
95% CI=95% Confidence intervals.
* Women who never smoked or who quit smoking before the last four months of pregnancy

Table 3. Prenatal exposure to acrylamide and glycidamide measured as hemoglobin (Hb) adducts in cord blood, and associations with birth weight.

Variable	Acrylamide Hb adducts			Glycidamide Hb adducts		
	n	ß (95%CI)	p	n	ß (95%CI)	p
Basic adjusted*						
All						
Change per 10 pmol/g Hb	1101	-35 (-51, -19)	<0.001	1100	-60 (-87, -34)	<0.001
Quartile 1 (lowest)	288	Ref.: 3460 g		283	Ref.:3492 g	
Quartile 2	263	-65 (-136, 5)	0.066	269	-53 (-124, 18)	0.145
Quartile 3	275	-110 (-180, -39)	0.002	276	-61 (-131, 11)	0.094
Quartile 4 (highest)	275	-132 (-207, -56)	0.001	272	-136 (-212, -60)	0.001
Non-smokers						
Change per 10 pmol/g Hb	972	-20 (-46, 6)	0.187	972	-53 (-95, -10)	0.016
Quartile 1 (lowest)	247	Ref.: 3445 g		249	Ref.: 3503 g	
Quartile 2	242	-26 (-99, 48)	0.50	239	-73 (-147, 1)	0.053
Quartile 3	241	-105 (-181, -31)	0.006	244	-76 (-150, -1)	0.046
Quartile 4 (highest)	242	-107 (-188, -27)	0.009	239	-103 (-182, -23)	0.012
Further adjusted**						
All						
Change per 10 pmol/g Hb	747	-23 (-51, 5)	0.10	746	-22 (-67, -23)	0.33
Quartile 1 (lowest)	208	Ref.: 3509 g		214	Ref.: 3527 g	
Quartile 2	194	-65 (-139, 19)	0.14	199	-80 (-159, -1)	0.046
Quartile 3	205	-110 (-207, -48)	0.002	189	-50 (-131, -31)	0.022
Quartile 4 (highest)	140	-157 (-256, -58)	0.002	144	-110 (-207, -12)	0.028
Non-smokers						
Change per 10 pmol/g Hb	675	-34 (-72, 4)	0.078	674	-52 (-112, 8)	0.088
Quartile 1 (lowest)	174	Ref.: 3445 g		186	Ref.: 3542 g	
Quartile 2	183	-19 (-102, 64)	0.65	180	-67 (-150, 16)	0.12
Quartile 3	190	-132 (-216, -49)	0.002	171	-89 (-173, -4)	0.035
Quartile 4 (highest)	128	-149 (-248, -50)	0.003	137	-97 (-193, -1)	0.05

Beta coefficients (β) are from linear regression analyses and correspond to change in birth weight (grams) per 10 pmol/g Hb adducts, or relative to the lowest quartile of acrylamide or glycidamide adduct levels. Acrylamide adduct quartiles for all: ≤10.9, >10.9 – ≤14.4, >14.4 – ≤21.7, >21.7 and for non-smokers: ≤10.5, >10.5 – ≤13.8, >13.8 – ≤19.2, >19.2 pmol/g Hb. Glycidamide adduct quartiles for all: ≤7.9, >7.9 – ≤10.8, >10.8 – ≤15.7, >15.7 and for non-smokers: ≤7.6, >7.6 – ≤10.1, >10.1 – ≤14.2, >14.2 pmol/g Hb.

* Adjusting for gestational age (completed weeks) and country.

** Additional adjusted for maternal smoking at the end of pregnancy (no, yes), passive smoking (no, yes), sex (boy, girl), pre-pregnancy BMI (kg/m^2), parity (0, 1+), maternal age (years), maternal ethnicity (white, non-white), maternal education (low, middle, high) and maternal consumption of fruit and vegetables, fish and soft drinks (low, high)

Table 4. Prenatal exposure to acrylamide and glycidamide measured as hemoglobin (Hb) adducts in cord blood, and associations with birth head circumference.

Variable	Acrylamide Hb adducts			Glycidamide Hb adducts		
	n	ß (95%CI)	p	n	ß (95%CI)	p
Basic adjusted*						
All						
Change per 10 pmol/g Hb	1005	-0.06 (-0.12, 0.00)	0.034	1004	-0.10 (-0.20, 0.00)	0.040
Quartile 1 (lowest)	272	Ref.: 34.89 cm		268	Ref.: 34.99 cm	
Quartile 2	237	-0.10 (-0.35, 0.15)	0.44	251	-0.03 (-0.29, 0.22)	0.79
Quartile 3	251	-0.18 (-0.43, 0.08)	0.17	251	-0.14 (-0.40, 0.11)	0.27
Quartile 4 (highest)	245	-0.33 (-0.61, -0.06)	0.018	234	-0.38 (-0.65,-0.10)	0.007
Non-smokers						
Change per 10 pmol/g Hb	890	-0.07 (-0.16, 0.03)	0.184	889	-0.16 (-0.33, 0.00)	0.049
Quartile 1 (lowest)	232	Ref.: 34.86 cm		235	Ref.: 35.05 cm	
Quartile 2	220	-0.02 (-0.29, 0.25)	0.88	222	-0.15 (-0.42, 0.12)	0.27
Quartile 3	222	-0.13 (-0.40, 0.14)	0.36	224	-0.20 (-0.47, 0.07)	0.14
Quartile 4 (highest)	216	-0.35 (-0.65, -0.05)	0.021	208	-0.34 (-0.63, -0.05)	0.023
Further adjusted**						
All						
Change per 10 pmol/g Hb	713	0.02 (-0.08, 0.12)	0.71	712	-0.01 (-0.17, 0.16)	0.93
Quartile 1 (lowest)	201	Ref.: 34.98 cm		208	Ref.: 35.08 cm	
Quartile 2	182	-0.08 (-0.37, 0.21)	0.57	192	-0.08 (-0.36, 0.21)	0.60
Quartile 3	198	-0.08 (-0.37, 0.21)	0.60	177	-0.07 (-0.36, 0.23)	0.66
Quartile 4 (highest)	132	-0.22 (-0.59, 0.14)	0.23	135	-0.26 (-0.62, -0.09)	0.15
Non-smokers						
Change per 10 pmol/g Hb	645	-0.05 (-0.09, 0.19)	0.51	644	-0.05 (-0.27, 0.17)	0.64
Quartile 1 (lowest)	168	Ref.: 34.95 cm		181	Ref.: 3542 g	
Quartile 2	173	0.01 (-0.30, 0.32)	0.96	173	-0.08 (-0.38, 0.22)	0.61
Quartile 3	180	-0.10 (-0.41, 0.21)	0.52	160	-89 (-0.52, 0.10)	0.19
Quartile 4 (highest)	124	-0.21 (-0.57, 0.16)	0.27	130	-0.23(-0.58, 0.12)	0.20

Beta coefficients (β) are from linear regression analyses and correspond to change in birth head circumference (cm) per 10 pmol/g Hb adducts, or relative to the lowest quartile of acrylamide or glycidamide adduct levels. Acrylamide adduct quartiles for all: ≤10.9, >10.9 – ≤14.4, >14.4 – ≤21.7, >21.7 and for non-smokers: ≤10.5, >10.5 – ≤13.8, >13.8 – ≤19.2, >19.2 pmol/g Hb. Glycidamide adduct quartiles for all: ≤7.9, >7.9 – ≤10.8, >10.8 – ≤15.7, >15.7 and for non-smokers: ≤7.6, >7.6 – ≤10.1, >10.1 – ≤14.2, >14.2 pmol/g Hb.

* Adjusting for gestational age (completed weeks) and country.

** Additional adjusted for maternal smoking at the end of pregnancy (no, yes), passive smoking (no, yes), sex (boy, girl), pre-pregnancy BMI (kg/m^2), parity (0, 1+), maternal age (years), maternal ethnicity (white, non-white), maternal education (low, middle, high) and maternal consumption of fruit and vegetables, fish and soft drinks (low, high)

Figure legends

Figure 1.

Acrylamide hemoglobin (Hb) adduct levels (pmol/grams Hb) in mother-child pairs (n=172).

Figure 2.

Forest plot of the association between acrylamide hemoglobin adducts (highest relative to the lowest quartile *) and birth weight by country and combined meta-analytic estimate, adjusted for gestational age (completed weeks) in the full population (top), and in non-smokers (bottom).

The gray squares superimposed over the country-specific point estimates are proportional to the country-specific weights used in the meta-analyses, and the associated 95% confidence intervals are shown as horizontal black lines. The summary ß, which correspond to the change in birth weight (grams) for the highest relative to the lowest quartile of acrylamide, is indicated with a red dashed vertical line and blue diamond, and the associated 95% CI are indicated by the lateral tips of the diamond. The solid vertical line refers to no change in birth weight. The names of the country are shown on the left and the country-specific ßs, 95%CI and weights of each study on the right.

Full population (N=1101): Test for heterogeneity: Q=2.5 on four degrees of freedom (p=0.640)

Non-smokers (N=972): Test for heterogeneity: Q=1.0 on four degrees of freedom (p=0.902)

*Acrylamide adduct quartiles for all: ≤10.9 vs. >21.7 and for non-smokers: ≤10.5 vs. >19.2 pmol/grams Hb.

Figure 3.

Association of maternal acrylamide exposure through diet among non-smokers (N=801) estimated through an acrylamide-rich food score with acrylamide hemoglobin adducts in cord blood (top) and birth weight (bottom). Generalized additive model with a smoothed spline for acrylamide-rich food score adjusting for country, and gestational age (completed weeks).

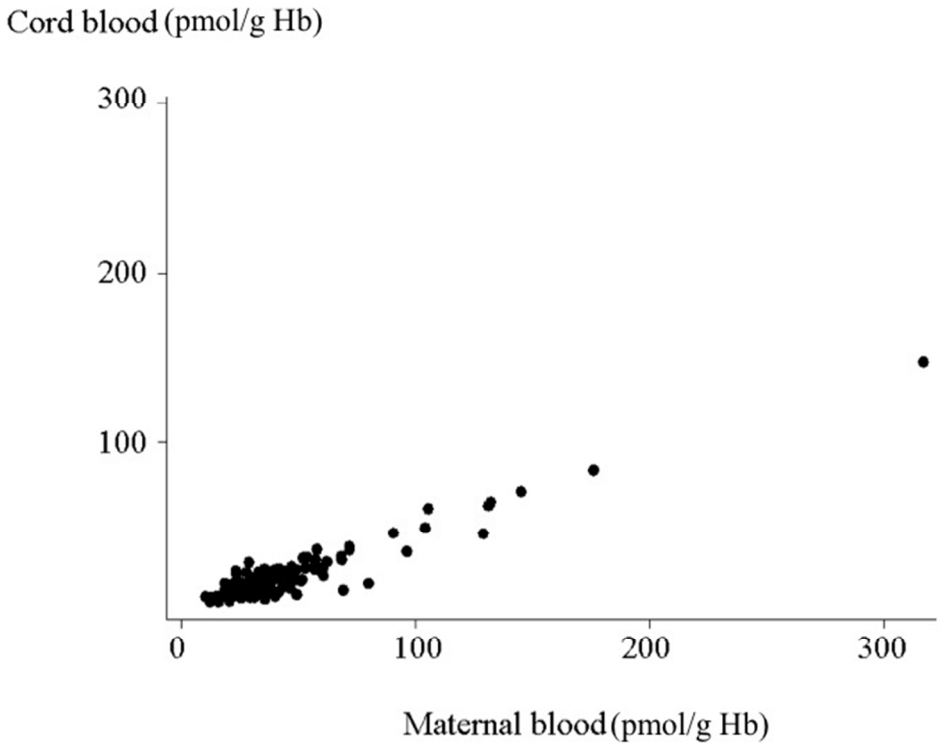

Figure 1.
254x190mm (72 x 72 DPI)

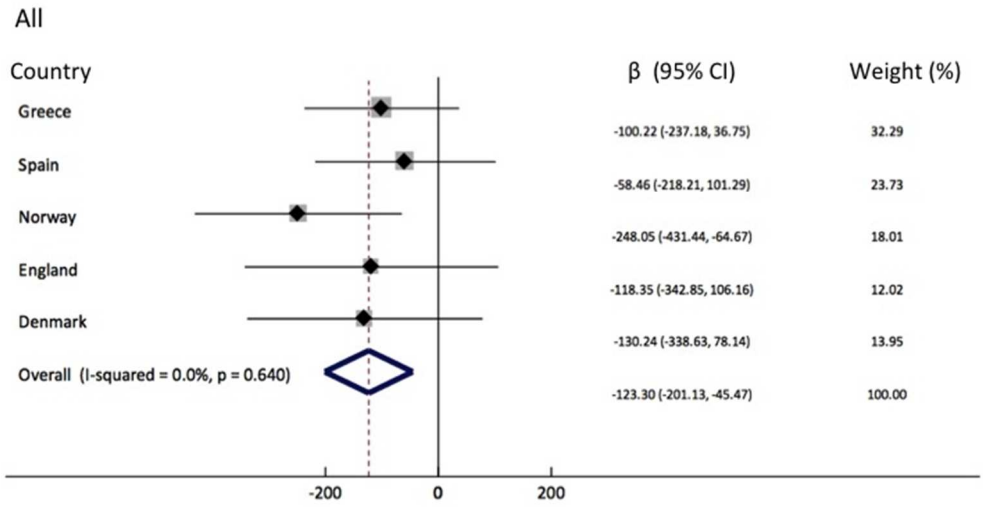

Figure 2, part 1.
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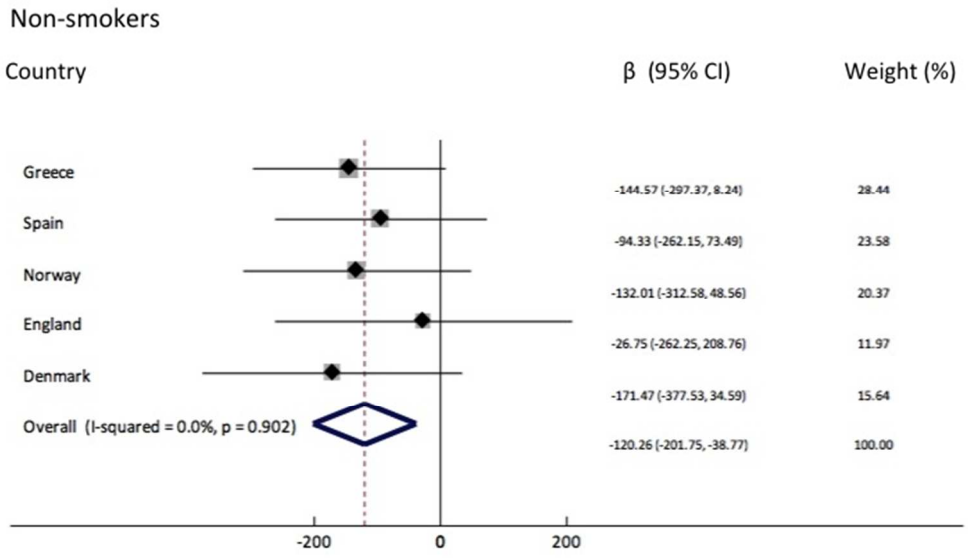

Figure 2, part 2.
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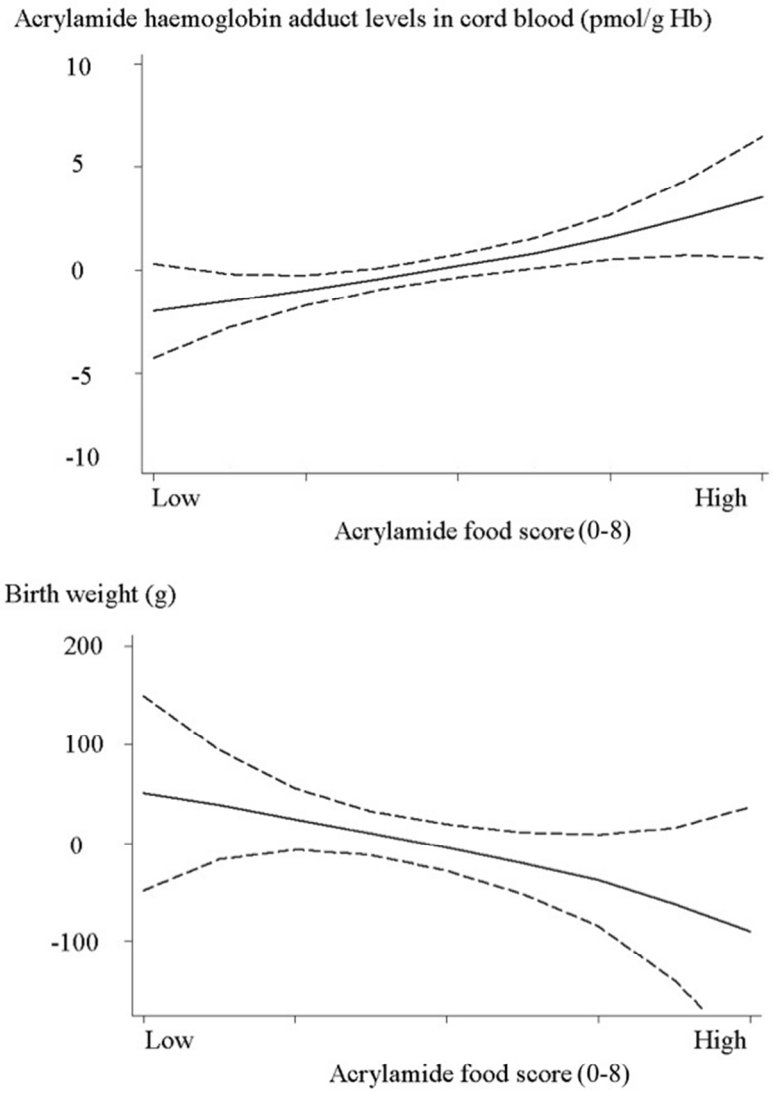

Figure 3.
254x338mm (72 x 72 DPI)