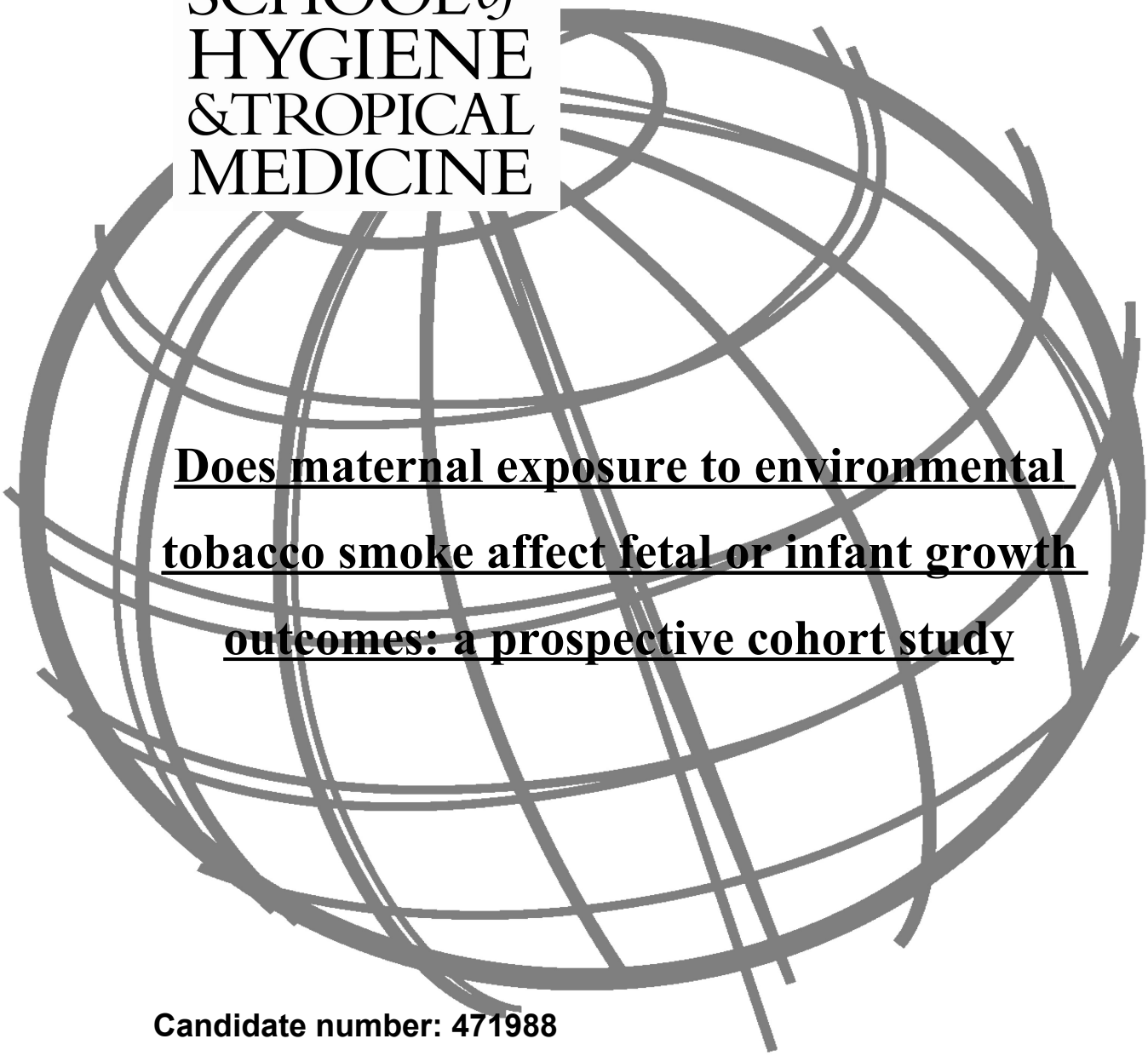

LONDON SCHOOL of HYGIENE &TROPICAL MEDICINE

Does maternal exposure to environmental tobacco smoke affect fetal or infant growth outcomes: a prospective cohort study

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Abbreviations

The following abbreviations are used in this document:

95% CI	95% confidence interval
BMI	Body mass index
cm	centimetre
ETS	environmental tobacco smoke
g	grams
kg	kilogram
m	metre
OFC	occipital-frontal (head) circumference

Abstract

Background: Adult health problems related to early developmental events are an increasing public health concern. Numerous studies have been conducted in developed countries, but evidence from countries passing through the epidemiological transition is limited. Birth size is a proxy for fetal growth and can indicate intrauterine growth restriction; infants subject to this often display "catch-up growth" in infancy. Both restricted and "catch-up" growth patterns are associated with adult problems.

Tobacco has been shown to have negative effects on placental function, fetal development and subsequent growth. Maternal exposure to environmental tobacco smoke (ETS) may have similar effects; however, the effect of ETS on postnatal growth has not previously been investigated. Data from the Geração XXI cohort in Porto, Portugal, was used to investigate the relationship between maternal ETS exposure and birth size and growth outcomes during the first two years of life.

Methodology: Inclusion criteria were healthy, normal singletons born between 24-42 weeks gestation. Data was translated from Portuguese to English and maternal ETS exposure categorised according to time period of exposure (pre-pregnancy, trimesters one, two and three). Simple regression analysis adjusted birth outcomes for gestational age; growth outcomes were treated as clustered data and adjusted for chronological age. Extended analysis provided adjustment for multiple potential confounding variables (Maternal: age, body size, smoking habits, alcohol use, country of birth, parity. Infant: sex, breastfeeding, nutrition, gestational age. Socioeconomic: housing, parental education, luxury possessions).

Results: 258 mother-infant pairs were available for analysis; 19 did not meet inclusion criteria. Of the remaining 239, half were male and most had good postnatal follow-up (10+ appointments). All birth outcomes were associated with maternal ETS exposure in all time periods in simple analysis; extended analysis adjusted for gestational age (weeks) demonstrated a borderline association for birth weight only (trimester 1: -162g with highest level of ETS exposure; 95% confidence interval -335 – 10, p= 0.07). Among infant growth outcomes, only OFC showed an association in the simple analysis (linear trend p=0.02 for

all time periods except trimester 1, p=0.03), and a borderline association in the extended analysis (linear trend p=0.05 except for trimester 3, p=0.06).

Conclusions: Maternal ETS exposure during pregnancy is associated with size at birth – particularly weight – and, in this study, with head growth in infancy. These results may be due to differential or non-differential misclassification, or chance, with the possibility of residual confounding remaining. However, maternal ETS exposure is likely to have small but important effects on many different measures of growth during early life and these warrant further investigation. Additionally, data from the Geração XXI cohort are of sufficient quality to provide useful information.

Introduction

The problem: adult illness

The global increase in adult health problems related to events in early development is rapidly becoming a major public health concern. Many problems such as obesity, type II diabetes mellitus, hypertension, stroke, coronary artery disease, osteoarthritis and other bone diseases, chronic respiratory disease, certain types of cancer, and impaired neurological development have been demonstrated to be related to size at birth, as well as numerous other genetic and environmental factors such as growth velocity in infancy or genetic susceptibilities to environmental triggers[1-3]. The public health implications of this increase are enormous, with consequences affecting not only healthcare provision at community population levels, but impacting also on measures of economic productivity due to effects on the workforce. While much research has been done in developed countries with studies such as the follow-up investigations of the 1958 National Child Development Study[4] in the UK and of the populations exposed to the Dutch Famine in 1944-45[5], the Generation R study in Rotterdam (Netherlands)[6] or the Avon Longitudinal Study of Parents and Children[7], less is known about societies undergoing an epidemiological transition. Fortunately, studies such as the Pelotas (Brazil) Birth Cohort studies[8] are providing valuable data.

Smoking

Tobacco smoke has been repeatedly shown to be harmful to the individual and also, in pregnant mothers, to the developing fetus. The mechanism by which maternal smoking is thought to affect the fetus is through changes in placental blood flow and composition directly mediated by chemicals in the inhaled cigarette smoke. This is supported by studies looking at the effects of inhaled tobacco smoke on vascular function[9]. As a consequence, mothers who smoke have smaller babies[6]. Postnatally,it has been shown that these infants display “catch-up” growth, and differences between these infants and those of never-smokers are eliminated by 6 months of age[10]. There may, however, be other mechanisms that are not directly linked to maternal smoking which also impact on fetal growth.

Environmental tobacco smoke

One factor which may have an impact is passive smoking: maternal exposure to environmental tobacco smoke (ETS). While an association between maternal smoking and decreased birth weight has been apparent for many years, the link with ETS has been more difficult to elicit. Several studies have now shown that ETS contributes to fetal growth restriction as measured through weight at birth.[11,12] This contribution is difficult to quantify precisely, but is likely to be small, given the differences between studies in the exposure measurements and study designs employed – for example, prospective collection of biological samples linked with contemporaneous maternal surveys, or retrospective questionnaires. Indeed, a recent meta-analysis found a decrease of just 33 grams (95% confidence intervals (95% CI) 16 – 51g) in mean birth weight at term in babies born to ETS exposed mothers in 17 prospective studies[11]. A relationship between maternal ETS exposure during pregnancy and postnatal growth is even harder to demonstrate: no studies were found specifically addressing this question.

Developmental origins hypothesis

The developmental origins of health and adult disease hypothesis was developed during the 1990s following the observation that birth weight was associated with the development of hypertension in later life[13]. Birth weight, though, is just a proxy measure for *in-utero* growth. Much investigation has taken place since looking at such intermediate proxy markers linking early life influences to later outcomes. For example, it has been established that maternal nutrient deprivation during pregnancy is associated with low birth weight[5]; thus, evidence suggests that risk of hypertension in adulthood is associated with restricted maternal diet during pregnancy. Similarly, childhood growth trajectories have been used as an indicator of subsequent obesity[14].

Growth itself is determined by genetic and environmental mechanisms. Prenatally, environmental influences are those factors which impact on the normal functioning of the uterus. These may be non-modifiable, such as maternal age or height, or modifiable factors such as smoking status and nutritional intake. Together, the modifiable and non-modifiable factors restrict the rate of fetal growth beyond the full genetic potential and are collectively termed "maternal constraint."[2,15] The most obvious manifestation of this occurs in multiple pregnancies: lack of space in the uterus inhibits fetal growth causing the products of the pregnancy to be smaller than a singleton of comparative gestational age. Consequently, these

pregnancies are often characterised by premature delivery – when the womb is no longer able to cope with increasing fetal sizes.

Postnatally, all infants are exposed to a less-confined environment and are normally able to compensate for *in-utero* growth restriction, often expressing 'catch-up' growth during the first six months of life. This period of early postnatal growth has also been associated with subsequent health consequences[14]. Life-history theory, part of the developmental origins of adult disease hypothesis, postulates the mechanism as being resource diversion from cellular repair mechanisms to compensatory growth[2]. This results in increased susceptibility to damage in later years due to those mechanisms no longer being available for further repair. Importantly, the idea is dependent upon the concept of *developmental plasticity*, referring to a period of an organ's development during which it is particularly susceptible to environmental influences[13]. An example is the need for optical stimulation during the first weeks of life in order to prevent blindness.

Birth size, early growth and consequences

Weight, length and occipital-frontal circumference (OFC) at birth are all closely related. Depending upon the conditions present during pregnancy, growth of these three variables is differentially affected: fetal growth restriction will tend to first inhibit weight growth, then head growth and finally growth in length. These changes are reflected in medical terminology: asymmetric growth restriction is when the abdominal size of the fetus is decreased – reflecting lower weight growth – but head size is maintained; symmetric growth restriction occurs when there are severe restrictions and weight as well as head growth is restricted. They also reflect the impact on adiposity (weight) and linear growth (length, OFC). Therefore, any effect on fetal growth is likely to be detected more easily looking at birth weight than at length or OFC.

Following birth, there is widespread adaptation to the new environment. Infants who were subject to intrauterine growth restriction may be exposed to an environment where they can “catch-up” with what would otherwise have been their normal growth trajectory. Particularly, this has been noted in infants affected by maternal smoking[10], and is associated with obesity and other disease processes in later life[14,16].

Portugal

Prevalence rates of obesity in Portuguese adults approach levels found elsewhere in Europe and North America[17]. Additionally, Portugal is one of the last European countries to have passed through the demographic and epidemiological transition and, as such, a high proportion of the population continue to live on a low income and in poorer socio-economic conditions than other parts of western Europe. In 2006, 18% of the population lived below the poverty line, and unemployment was around 7.5% in 2007[18]. Currently, average life expectancy at birth is 78 years (male and female combined) and the population structure is stable with the birth rate approximately equal to the death rate. Total population is 10.67 million[18].

Geração XXI

The Geração XXI (Generation 21) study is the first cohort study to be set up in Portugal looking at the growth and development of approximately 10,000 individuals from birth to adulthood. It was established in 2005 in the Oporto region of Northern Portugal (see map) surrounding Porto, the capital of the region and second largest city in Portugal.

Study design

Geração XXI is a cohort study looking at factors affecting human growth and development. The design involved gathering information about 10,000 newborn babies by enrolling pregnant mothers and prospectively collecting information about the entire family. Information included questionnaire and biological data (e.g. height, weight, blood samples for later analysis). The children have then been followed from birth, and it is planned to continue until they reach adulthood. Data from the cohort was made available for this pilot study through Professor Isabel dos Santos Silva (London School of Hygiene and Tropical Medicine), one of the principal investigators.

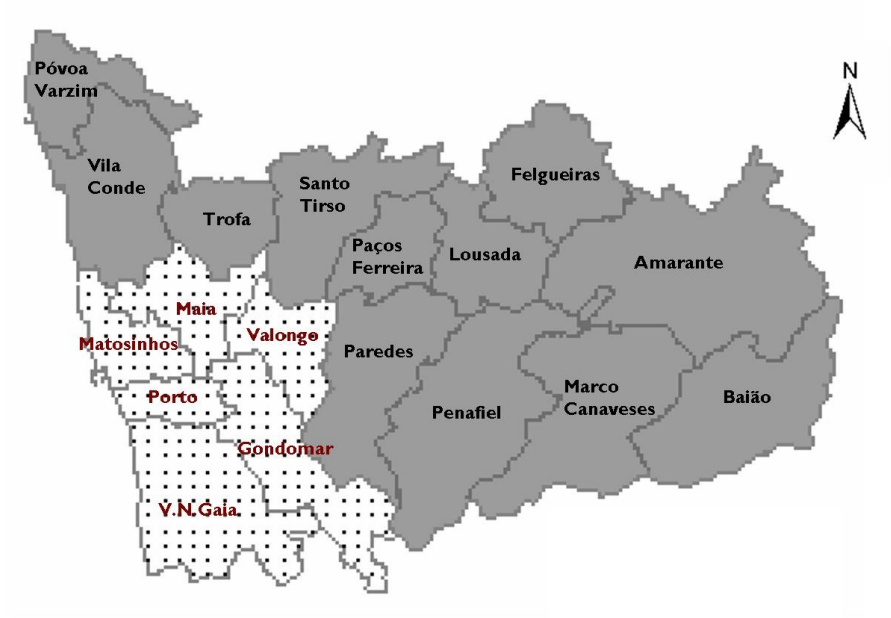


Figure 1: Portugal's Northern Region with district names; the Oporto region, representing the study catchment area, is highlighted.

Background to data collection

Maternal data from pregnancy and the two-year follow-up was collected prospectively using nationally standardised techniques to gather and record information. Mothers were enrolled in the study during an appointment 2 weeks before their due date; if the baby was born earlier, the mothers were enrolled while in hospital or as soon as possible thereafter. Families were invited to participate between May 1, 2005 and August 31, 2006; in total, 8654 infants have been born to mothers in the Geração XXI study, recruited from public hospitals (in which ~92% of births in the region occur)[19], and representing approximately 95% of those eligible[20]. All mothers completed a detailed questionnaire. Trimesters of pregnancy were defined as fewer than 18 weeks, 18-28 weeks and greater than 28 weeks gestation.

Child growth data was collected prospectively in the parent held health records whenever the child was seen by a health professional; values were subsequently collated at the 2 year follow-up for entry into the database. Mothers were offered an incentive of a free dental check to attend this appointment[20]. Maternal and infant biometric measurements were made using methods and equipment available at the time of assessment by the health professional in accordance with national guidelines and standardised by institution, and were *not* verified in any way[20].

Background to data entry

Pregnancy and birth data was single entered into the database contemporaneously. Follow-up data was single entered into the master database after collation at the 2 year follow-up[20].

Ethics

Ethical approval for the Geração XXI study was obtained from the Faculty of Medicine in the University of Porto, Portugal. See Appendix A.

Funding

The Geração XXI cohort was set up with an initial budget of 700,000 Euros, 75% of which came from the European Union and 25% from the Portuguese national government.

Project Aims

Initial question

This pilot study was initially conceptualised towards the end of 2007 and finalised by the middle of February 2008. At that time, it was thought that data from a majority of the complete cohort would be available from the questionnaires administered to both parents during pregnancy. It was also thought that growth data for children up to 6 months of age would be available for around 1000 children. Thus, the initial aims were to investigate:

- whether paternal smoking was an indicator of socio-economic factors that have a causal impact or confound the impact of maternal smoking on *in utero* fetal growth that has been observed in other studies;
- whether there was a differential effect on post-natal growth in infants whose parents smoked during pregnancy, resulting in "catch-up growth" patterns during the first few months of life.

Available data

Two year follow-up for the first infants to pass through the Geração XXI cohort occurred in January 2008. Data from these infants was coded and entered into the database, hence follow-up data was available from a greater time period than had been initially envisaged. Unfortunately, data from the paternal questionnaires have not been completely entered into the database, and so it wasn't possible to directly answer the original questions. Data about maternal exposure to environmental tobacco smoke was available, however, and it was possible to adapt the study questions.

Adapted question

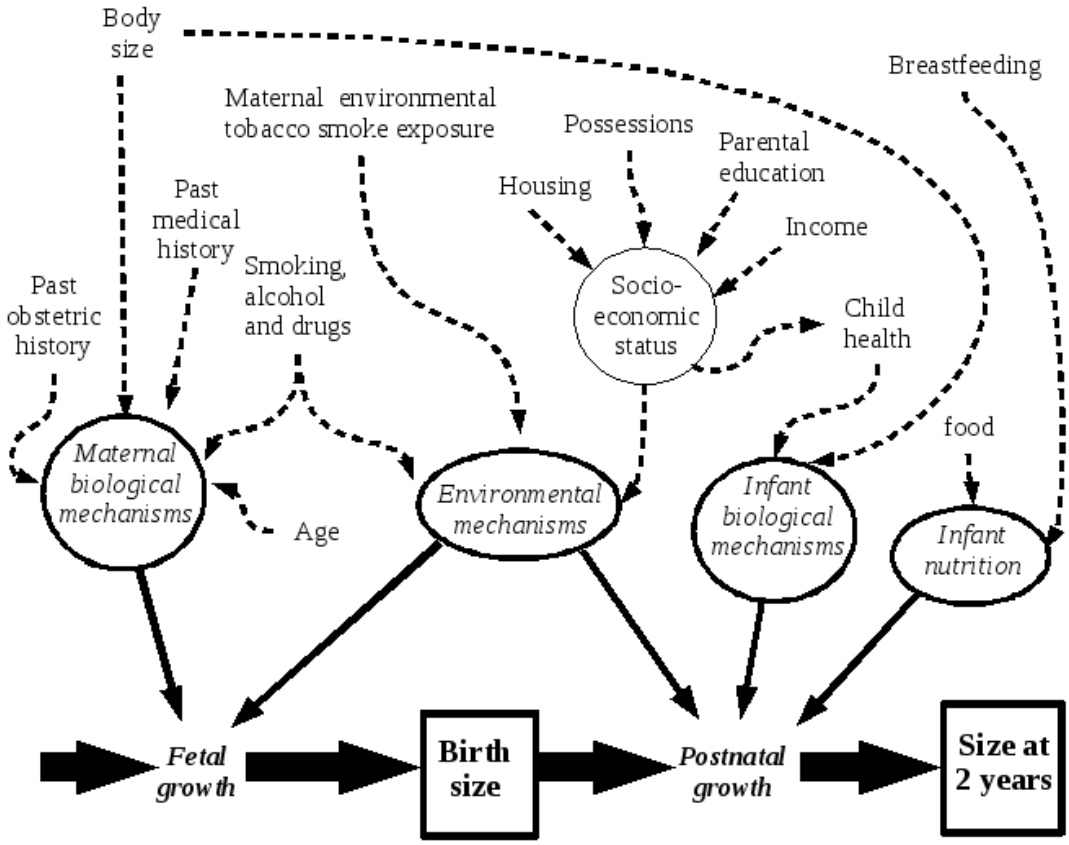
The original hypothesis was that paternal smoking may be a proxy marker for any socio-economic or environmental factors confounding the relationship of maternal smoking with infant birth size and postnatal growth. Maternal ETS exposure may be a proxy marker for these other factors but may also, on its own, contribute to a difference in birth size. As data

was available regarding ETS exposure at different time points before and during pregnancy, it was decided to focus this pilot study on whether there was any difference in outcome with different exposure levels in this population, and whether the period of exposure was important.

Causal pathways

Potentially influential factors on pre- and postnatal growth were categorised into four major groups: maternal biological, infant biological, infant nutritional, and environmental. Environmental factors affected growth either directly, on the child, or indirectly via the mother. From these categorisations, a causal diagram showing important factors and how they could potentially influence infant size and growth was constructed (Figure 2). To guide analysis, only variables measured in the Geração XXI cohort were included. The diagram was then able to be used to identify *a priori* confounders for the separate outcomes.

Figure 2: Causal pathways diagram showing variables measured in the Geração XXI study and how they may potentially contribute to fetal and infant growth. Dashed lines link variables into broad categories which affect growth.



Methods

Ethical approval

This pilot study was granted ethical approval by the London School of Hygiene and Tropical Medicine. See Appendix B.

Translation

Data was translated into English prior to analysis using computer-generated translations (Babelfish, Yahoo! Inc., CA. USA - http://babelfish.yahoo.com/), double-checked by a non-native Portuguese speaker. Complex translations that were unclear or did not make sense were verified with two native Portuguese speakers. Additionally, information about the Portuguese educational systems and other data requiring specialised local knowledge were verified with the project supervisor.

Merging databases

Data was available in the original Portuguese in four data sets which had been prospectively collected. Collected survey data was available from enrolment (maternal survey data), during pregnancy and at birth (maternal drug exposure data; infant birth data) and at the final two year follow-up appointment. Biological and nutritional data for the babies, detailing prospectively measured growth parameters, was collated at the two year follow-up appointment.

The databases containing maternal information were merged with each other, as were the two databases containing information about the babies. Maternal information was then merged into the infant database, providing one complete row per baby. The data was reshaped to convert the multiple outcome measurements (weight, length and OFC at different follow-up times) into single variables (weight, length, OFC), thus creating multiple entries for each infant.

Data cleaning

Inclusion criteria

All healthy, normal, singleton infants born without major complications between 24 and 42 weeks and enrolled around the time of delivery were eligible for inclusion in this pilot study.

Exclusion criteria

Infants were excluded if:

- they were the product of a multiple pregnancy;
- there was a major congenital abnormality noted antenatally, at birth or in the first year of life;
- the recorded gestational age was not feasible (<24 weeks or > 42 weeks gestation)
- there were any serious problems during the neonatal period that were likely to impact on subsequent growth (e.g. severe neurological or gastrointestinal damage)

Hypotheses

The hypothesis being tested was that maternal ETS exposure pre- and during pregnancy affected *in utero* growth, thus resulting in smaller size at birth. Secondary to this, the study hypothesised that those infants whose mothers were exposed to higher levels of ETS had an increased growth rate during the first two years of life ("catch-up growth"). The alternative hypotheses were that there was no difference in birth size for those mothers with a higher rate of ETS exposure, and that the growth trajectories in the first two years of life did not differ from those of infants of mothers who had lower ETS exposure.

Primary outcomes

The primary outcome measures in this study were infant weight (to the nearest 5g), length (nearest half centimetre) and OFC (measured to the nearest 0.1 cm). Measurements were taken at the time of birth and during routine child health checks thereafter using available (non-standardised) equipment.

Primary exposures

The main exposure under investigation was maternal exposure to environmental tobacco smoke (ETS). This was divided into 4 time periods: the 3 months prior to becoming pregnant, and the first, second and third trimesters. Additionally, mothers' exposure to ETS had been asked about in 3 locations: at home, at work, and elsewhere; and exposure had been classified as "none," "sporadic," "<1 hour per day," "1-3 hours/day," ">3 hours/day." From these parameters, new variables were constructed by summating total exposure during each of the time periods and weighting them according to the number of locations in which maternal exposure occurred (see Appendix C for detail). This resulted in a ranking score which was collapsed into 4 broader categories due to insufficient numbers in some groups. ETS exposure during each time period was then investigated as a separate potential factor impacting on birth size and postnatal growth.

Secondary exposures

Potential maternal confounding exposures considered in this analysis were age at time of infant's birth, body size, past medical and past obstetric history, place of birth, smoking and alcohol consumption, details of current pregnancy (including whether it was planned or not) and socioeconomic status (based upon questions relating to parental educational level, housing situation, family income, numbers of 'luxury' possessions, employment and marital status). Infant factors were gestational age, child health, child nutrition and length of breast feeding.

Maternal data

Maternal body size was assessed in several different ways. Average reported body weight from the 2 years prior to pregnancy was used (either as reported directly or calculated from the mean of the highest and lowest reported weights in that time); additionally, "current" – at the 2 year follow-up – maternal weight was used (measured value or, where not available, reported value). Data for maternal height was also available from the beginning of pregnancy (self-reported) and at the 2 year follow-up (measured). Weight and height values were used to calculate maternal body mass index (BMI; weight(kg)/height^2 (m)) pre-pregnancy and at the final follow-up appointment. Due to missing data and imprecision of self-reported measurements, only follow-up BMI was used in the analysis.

Mothers were directly asked about specific medical and obstetric conditions that they experienced either prior to or during the pregnancy. Answers were categorised in binary format (yes/no) with the option to provide detail if required (for example, if the woman suffered from allergies, she was able to state specific allergens). The total number of complications experienced during pregnancy was recorded, as was detailed information relating to each individual complaint and to any hospital admissions. These data were cross-checked against each other and, where appropriate, with neonatal data.

Data on racial origin is not permitted to be collected in Portugal; as a proxy, the place of birth of the mother and her parents was documented. This was analysed as a binary variable indicating whether the mother was born in Portugal or not.

Socioeconomic variables

Data about maternal and paternal education levels, housing situation, marital status, overall family income and access to 'luxury' items (see box: total number of luxuries was summated to create a 9-point scale) was collected at the first maternal interview. These were all assessed separately in the analysis.

Parental occupational status was inquired about antenatally; however, the data available for analysis was considered insufficiently detailed to use as a discriminatory variable. At the two-year follow-up, the mother (or principle carer) was asked for further information about who the principle carers were for the child. This information was also not included in the analysis due to limited relevance.

Luxury items

- Car ownership
- Television ownership
- Bicycle ownership
- Receiving home help (1+ times/week)
- Going on holiday (1+ weeks/year)
- Possessing a telephone
- Dishwasher ownership
- Central heating

Infant data

Gestational age was estimated by first trimester ultrasound if available; if not, maternal dates were used. A continuous scale demonstrated grouping at weekly intervals, hence gestational age was analysed in weeks rather than days. It was further recategorised into a binary variable indicating prematurity (<37 weeks gestation) or not. Chronological age was calculated in days from the date of birth.

At birth, information was recorded about sex of the child, type of delivery and any complications, including admission to the neonatal intensive care unit.

Subsequent assessment of child health considered hospital admission as the main discriminator; detail of admissions were individually assessed for the presence of any condition that might affect growth. The number of symptoms of illness a child experienced in the 12 months prior to the 2-year follow-up was also recorded, although not the total number of episodes of illness. Hence, if a child was ill a few times, but had many symptoms, s/he could get a higher score than if s/he was frequently ill but with few symptoms. This was considered a poor discriminator of child health and, therefore, was not used in the analysis.

Data was recorded at the 2-year follow-up, when all families were also asked about breast-feeding status, including reporting length of time of exclusive (breast milk only) and total (breast milk as well as other milk or solids) breast-feeding. If length of exclusive breast-feeding was longer than the reported total time, the value was adjusted to be the same as total time.

Mothers were asked about the availability to the children of the following 'junk' food groups: fizzy drinks, refrigerated soft drinks, coffee/barley/tea, pre-prepared meals, crisps, pizza, hamburgers, dried sausage, sauces (e.g. ketchup, mayonnaise, mustard), pastries, cream pastries, ice cream, chocolate, sugar, gum/sweets . These were counted if they formed part of the regular diet of the infant (defined as being available at least once a week), and the total number of junk foods per child was summated.

Extreme results

Follow-up variables were converted to missing if they were reported as 'unknown' or 'not reported'. Growth values of weight, length or OFC which were less than 80% of the birth value, or which were widely discrepant from the expected trajectory of that subject (greater than 2 kg, 5cm or 5 cm discrepancy from preceding and following values for weight, length and OFC respectively) were also converted to missing.

Nonsensical exposure variables were either converted to the nearest sensible answer (for example, if exclusive breast-feeding was recorded as lasting longer than the total breast-feeding time, it was replaced with the total time) or analysed as "missing data."

Statistical analysis

Data was converted from Microsoft access format to Stata-10 format using Stat/Transfer 9 (Circle systems, Inc. Seattle, WA. USA). All statistical analysis was then performed using STATA 10 (StataCorp, TX. USA).

Descriptive analysis

An initial descriptive analysis was carried out to examine baseline characteristics of the study cohort. This included summary statistics of continuous variables, tabulations of binary variables and manual inspection of explanatory descriptions. Continuous outcome variables were assessed for normality, kurtosis and skewedness through visual display with superimposed normal distribution curves. Growth parameters were plotted against chronological age to look for evidence of heteroscedasticity and non-linearity, as well as identifying outliers for closer examination.

Simplified analysis

Outcome variables were individually regressed with each other to look for associations between the variables. They were then regressed with the primary and secondary exposure variables, and the primary exposures were also regressed with each other. Due to the time-specificity of the outcome variables, infant age was included in all these analyses. Birth measures were analysed first accounting for prematurity, and then using gestational age in weeks as either a continuous variable or as independent categorical measures. Postnatal growth data was investigated by treating the multiple growth measurements for each child as clustered data and using a quadratic transformation of the chronological age of the child to account for the variation in age (see formula). Strength of association was assessed using partial F-Tests for the exposure categories. Primary and secondary variables were examined for collinearity using chi-squared test for binary and categorical variables, or else using simple linear regression.

Formula for postnatal growth using a quadratic transformation of age with multiple regression ('*' represents multiplication):

$$growth = a + b_1*(chronological\ age) + b_2*(chronological\ age)^2 + b_3*ETS\ exposure + b_4*confounder + \ldots$$

Extended analysis

The multiple regression analysis was extended for all the outcome data. Birth outcomes were assessed by using the available mother-infant data to construct models. Growth outcomes were similarly assessed, but made use of gestational age as well as the quadratic transformation of chronological age and clustering, as described above. For both sets of outcomes, partial F-tests were used to construct models.

It was hypothesised that gestational age and maternal smoking and alcohol status would confound all outcomes. Maternal drug use was also considered as a confounder but thought to be an unreliable indicator due to reporting bias and the small numbers likely to be affected in this study. Other socio-economic marker variables were considered *a priori* as

confounders, although it was unclear which – if any – would be sufficiently discriminatory to have an impact in this study. It was thought, however, that previous obstetric experience (parity) and pregnancy planning may be associated with both ETS exposure and growth *in-utero*, and thus confound the birth outcomes.

Markers of maternal health before and during pregnancy were considered as potential confounders for all outcomes, although it was felt unlikely that they would provide sufficient power in a small population and hence were excluded *a priori*. Maternal body size was thought to be an important explanatory factor for all the outcomes, particularly birth weight and postnatal weight growth; but, due to the different ways in which it had been recorded, it was unclear whether there would be an association with ETS and thus was also not included *a priori*. Breast feeding status, however, was considered as a potentially influential factor on postnatal growth, as well as being potentially related to tobacco exposure, thus was included as a possible confounder.

All variables considered *a priori* as confounders were included in the full adjusted analysis. Further variables were also included if they were weakly associated ($P<0.1$) with both the outcome and one of the exposure variables (ETS pre- or during pregnancy) in the simplified analysis. Additionally, infant gender was considered as an explanatory variable for all outcomes.

Sensitivity analysis

Sensitivity of the results was checked by two methods: firstly, by restricting the inclusion criteria by gestational age (from 35, 36 and 37 weeks gestation); secondly, by excluding any apparent outliers. Following each of these, analyses were re-run.

Results

Exclusions

Records from 258 infants were available for analysis. Of these, 17 infants were the product of a multiple pregnancy and one child was recorded as having a gestational age of 43 weeks plus 1 day. Of the remaining 240 mother-infant pairs, 1 baby was recorded as suffering from "cerebral depression and coma" as well as being premature (34 weeks gestation); additionally, the mother was recorded as having diabetes since 6 years of age – but not requiring insulin – and also suffered from hypertension during the pregnancy. This pair was thus excluded, leaving 239 complete datasets available for analysis.

Baseline characteristics

Infants

118 of 239 were male (49.4%), with no important differences in primary or secondary exposures by sex. The youngest recorded gestational age was 31 weeks + 3 days, and the majority were 37 weeks or over; no baby was born after 41 weeks. Mean birth weight was 3175 grams, length 48.9 cm and OFC 34.3 cm (Table 1 contains detailed characteristics). Four children had confirmed or suspected congenital malformations, and one was suspected of developmental delay during infancy; however, these children did not differ from the others in any other way and hence were included in all analyses. Seven children were admitted to hospital during the study period, none more than once. The majority of children had frequent follow-up observations made (Table 2, figure 3).

Table 1: Baseline infant characteristics (n 239) from the pilot study of environmental tobacco smoke exposure, nested within the Geração XXI cohort in Porto, Northern Portugal.

	Number	**Mean (range)**
[1]Birth weight (g)	238	3175 (1460-4355)
[1]Birth length (cm)	232	48.9 (39-54)
[1]Birth OFC (cm)	232	34.3 (27-38)
Gestational age (weeks)		38.9 (31.4 – 41.0)
< 37	18 (7.5%)	
37+	221 (92.5%)	
Male sex	119 (49.4%)	
[2]*Type of delivery*		
Vaginal	118 (54.9%)	
Ventouse	33 (15.4%)	
Forceps	4 (1.9%)	
Caesarean	60 (27.9%)	
Breastfed	225 (94.1%)	
[3]*Junk food consumption (foods/week)*		
0	41 (17.2%)	
1-2	78 (32.8%)	
3-5	90 (37.8%)	
6-12	29 (12.2%)	

[1] Sex was strongly associated with birth length ($F(1,230)$ 6.61; $p = 0.0107$) and birth OFC ($F(1,230)$ 9.55; $p=0.0022$), but not with birth weight ($F(1,236)$ 1.60; $p=0.2078$); gestational age was very strongly associated with all 3 birth outcomes ($p<0.0001$)

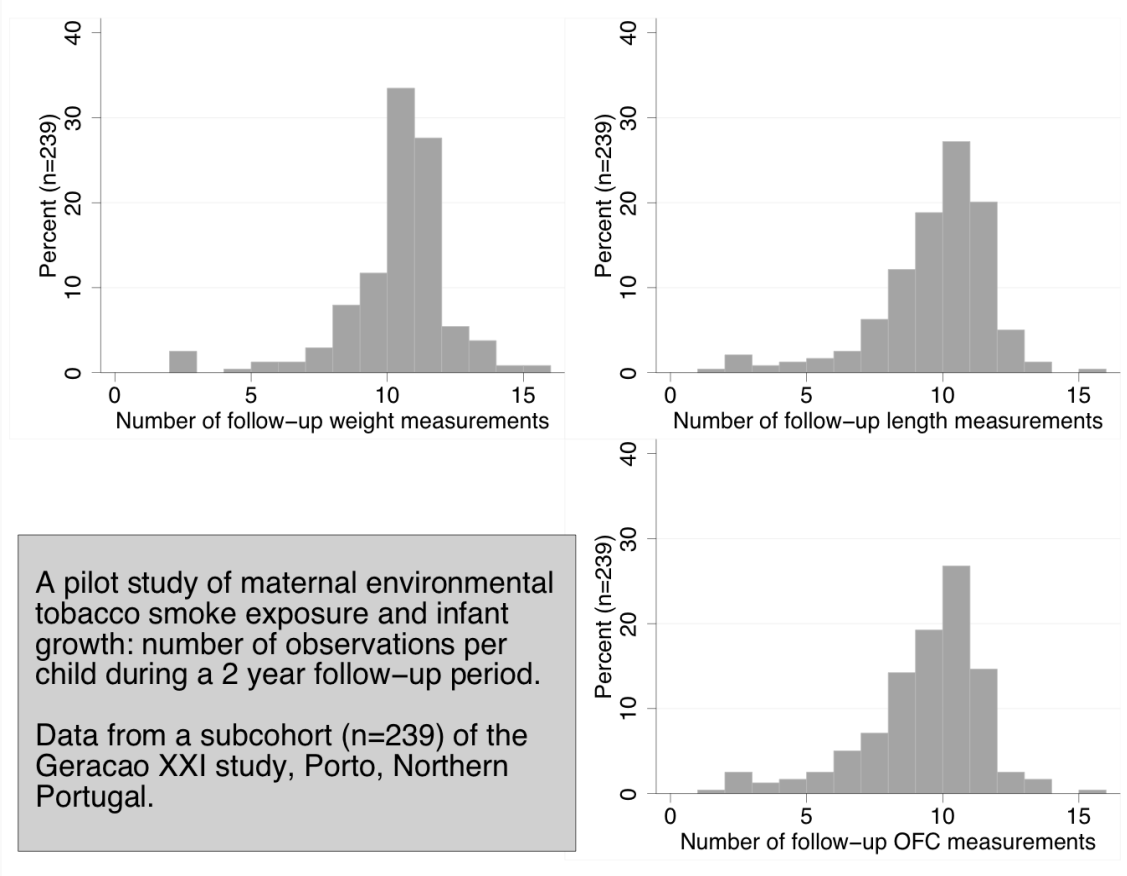
[2] N = 215.

[3] The number of different types of junk food consumed at least once per week was summated. N = 238.

Table 2: Number of visits at which measurements were made of infants in a pilot study from the Geração XXI cohort, Porto, Northern Portugal, by outcome (n 239).

Number of visits	Weight	Length	OFC
<8	20 (8.4%)	36 (15.1%)	49 (20.5%)
8-9	47 (19.7%)	74 (31.0%)	80 (33.5%)
10	80 (33.5%)	65 (27.2%)	64 (26.8%)
11	66 (27.6%)	48 (20.1%)	35 (14.6%)
12+	26 (10.9%)	16 (6.7%)	11 (4.6%)

Figure 3: Frequency distribution of the number of follow-up growth measurements per child participating in the study.



Maternal

Mothers ranged in age from 17.6 to 43.7 years, with a mean of 30.4 years. 113 of 238 (49.4%) were primiparous, and only 12 (5.1%) had more than 2 previous pregnancies. The majority of women were in stable relationships (231/239, 95.7%) with planned pregnancies (166/238, 69.8%). 19/239 (7.9%) were born overseas (Brazil, Africa, France, Eastern Europe and unrecorded).

Of the included women, only 4 were recorded as having cardiac problems prior to pregnancy, 7 as having hypertension and none with diabetes. During pregnancy, 14 mothers developed high blood pressure, 13 developed diabetes and 14 had threatened preterm labour; overall, 30 women were hospitalised.

ETS exposure was similar both prior to and throughout pregnancy, as shown in Table 3. Only 6 women reported no ETS exposure at all, hence were included with the next lowest category (sporadic exposure in just one setting); similarly, only 9-10 women reported the highest level of exposure (equivalent, in this study, to >1 hour daily exposure in 2 locations, plus sporadic or daily exposure in the third location) and hence were included with the next highest group. There were, therefore, 4 risk categories of environmental tobacco smoke exposure: low, low-moderate (low-mod), high-moderate (high-mod) and high.

Table 3: Distribution of mothers by environmental tobacco smoke exposure (ETS) categories for 4 different time periods: 3 months prior to pregnancy, first, second and third trimesters of pregnancy.

Time period	**ETS risk category [***number (percentage)***]**			
	Low	**Low-mod**	**High-mod**	**High**
Pre-pregnancy	81 (34.0)	61 (25.6)	63 (26.5)	33 (13.9)
Trimester 1	85 (35.7)	60 (25.2)	61 (25.6)	32 (13.4)
Trimester 2	89 (37.4)	56 (23.5)	61 (25.6)	32 (13.4)
Trimester 3	92 (38.7)	56 (23.5)	58 (24.4)	32 (13.4)

Just over half of women had never smoked, and about 60% had never drunk alcohol, as indicated in Table 4. There was a strong correlation between ETS exposure and tobacco consumption ($p<0.001$ for all time periods). Not included in subsequent analyses were 9 (3.8%) women who admitted to having consumed drugs: this figure was considered unreliable and didn't contribute to an important difference in the regression modelling.

Sufficient pre-pregnancy maternal weight and height measurements were only available to calculate body mass index for 167 women; at the 2 year follow-up, both measurements were available for 201 women in total. At this time, the mean BMI was 26.1 (range 17.6 – 44.6); height ranged from 147.8 to 175 (mean 159.1) cm and weight from 40.1 to 110.8 (mean 66.1) kg.

Table 4: Baseline maternal characteristics from the pilot study of environmental tobacco smoke exposure, nested within the Geração XXI cohort in Porto, Northern Portugal.

	Number	**Mean (range)**
Age at delivery (years)	239	30.4 (17.6 – 43.7)
Body size		
Pre-pregnancy weight (self-reported, kg)	217	61.4 (41.0 – 98.0)
[1]Current weight (measured, kg)	201	66.1 (40.1 – 110.8)
Height (measured, cm)	203	159.1 (147.8 – 175.0)
[1]Current BMI	201	26.1 (17.6 – 44.6)
[2]*Pregnancy order*		
First pregnancy	113 (47.5%)	
Second or third pregnancy	113 (47.5%)	
More than 3 previous pregnancies	12 (5.0%)	
[2]*Smoking status*		
Never-smoker	134 (56.3%)	
Ex-smoker	34 (14.3%)	
Smoker, stopped in pregnancy	32 (13.5%)	
Current smoker	38 (16.0%)	
[2]Had ever consumed alcohol	95 (39.9%)	

[1] "Current" = at the 2 year follow-up.

[2] Lifetime alcohol consumption (n = 238).

Socio-economic

The majority of the families in the cohort were poorly educated: of the 217 fathers with education recorded, 148 (68.2%) had completed 4 or fewer years and only 32 (14.7%) had completed at least 12 years of schooling. These figures were echoed by the mothers: 172 (76.8%) with 4 or few years and only 23 (20.3%) with 12 or more years education. Like the measures of education, family income was right skewed with most families (144./220, 65.5%) earning between 500-1500 Euros, and only 31 (14.1%) earning more than 2000 Euros. The number of luxury possessions per family provided better discrimination (Table 5) but, in contrast, housing assessment was a poor discriminator, with most families living in their own accommodation (data not shown).

Table 5: Baseline socio-economic characteristics of families (n 239) from the pilot study of environmental tobacco smoke exposure, nested within the Geração XXI cohort in Porto, Northern Portugal.

	Number (%)
Married/living 'de facto'	231 (96.7)
Maternal educational level (n 224)	
< 5 years education	172 (76.8)
5-11 years education	29 (12.9)
> 11 years education	23 (10.3)
Family income (n 220)	
<1000 E /month	90 (40.9)
1000-1499 E /month	63 (28.6)
1500+ E/month	67 (30.3)
Total number of luxuries (n 238)	
0 – 4	75 (35.2)
5 – 6	75 (35.2)
7 – 8	68 ((29.6)

Multivariate analysis

Unsurprisingly, each of the three birth and growth outcomes were very strongly linked to each other ($p<0.001$ for all parameters). Maternal ETS exposure was also strongly related to both prematurity and gestational age ($p<0.001$ in all time periods); however, there was no association between any of the birth or growth outcomes and maternal smoking status.

Birth size

In the birth outcome model adjusted for prematurity, an association with decreased birth size in those infants whose mothers had a higher level of ETS exposure was seen for all parameters (Table 6). However, when adjusted for gestational age, only birth weight was strongly linked with maternal ETS exposure (tests for linear trend: $p=0.01$ for pre-pregnancy and trimester 1 exposure; $p<0.01$ for exposure in trimesters 2 and 3); birth length and OFC did not demonstrate an association for any of the exposure periods. Regression plots are shown in appendix D.

Table 6: Simple regression analysis, by exposure period, for birth outcomes in relation to environmental tobacco smoke (ETS) exposure before and during pregnancy, adjusted for prematurity[1]; data from Geração XXI cohort in Porto, Northern Portugal.

	Weight (kg)		**Length (cm)**		**OFC (cm)**	
	mean (95% CI)	*p-value*[2]	mean (95% CI)	*p-value*[2]	mean (95% CI)	*p-value*[2]
Observations	*237*		*231*		*231*	
Pre-pregnancy ETS exposure						
Low [3]	3.299 (3.207 - 3.391)	--	49.2 (48.8 – 49.7)	--	34.5 (34.2 - 34.8)	--
Low-mod	3.274 (3.166 – 3.386)	*0.72*	49.4 (48.9 – 49.9)	*0.56*	34.7 (34.4 – 35.1)	*0.26*
High-mod	3.182 (3.076 – 3.288)	*0.10*	49.0 (48.5 – 49.5)	*0.45*	34.2 (33.9 – 34.5)	*0.22*
High	3.022 (2.876 – 3.167)	*0.002*	48.3 (47.7 – 49.0)	*0.03*	34.0 (33.5 – 34.4)	*0.05*
P-value (trend)[4]		*0.001*		*0.04*		*0.03*
Trimester 1 ETS exposure						
Low[3]	3.304 (3.214 - 3.395)	--	49.2 (48.8 – 49.7)	--	34.5 (34.2 - 34.8)	--
Low-mod	3270 (3.161 – 3.378)	*0.63*	49.4 (48.9 – 49.9)	*0.63*	34.7 (34.3 – 35.0)	*0.52*
High-mod	3.167 (3.060 – 3.275)	*0.05*	48.9 (48.4 – 49.4)	*0.31*	34.2 (33.8 – 34.5)	*0.12*
High	3.025 (2.878 – 3.173)	*0.002*	48.4 (47.7 – 49.1)	*0.03*	34.0 (33.6 – 34.5)	*0.08*
P-value (trend)[4]		*0.001*		*0.03*		*0.03*

(Continued on next page)

Table 6 (continued)

	Weight (kg)		Length (cm)		OFC (cm)	
	mean (95% CI)	*p-value*[2]	mean (95% CI)	*p-value*[2]	mean (95% CI)	*p-value*[2]
Trimester 2 ETS exposure						
Low[3]	3.317 (3.229 - 3.406)	--	49.3 (48.9 - 49.7)	--	34.5 (34.3 - 34.8)	--
Low-mod	3.256 (3.144 – 3.367)	*0.39*	49.4 (48.9 – 49.9)	*0.84*	34.6 (34.3 – 35.0)	*0.68*
High-mod	3.159 (3.052 – 3.267)	*0.02*	48.9 (48.4 – 49.4)	*0.21*	34.2 (33.9 – 34.5)	*0.14*
High	3.025 (2.878 – 3.172)	*<0.001*	48.4 (47.7 – 49.1)	*0.02*	34.0 (33.6 – 34.5)	*0.07*
P-value (trend)[4]		*<0.001*		*0.02*		*0.03*
Trimester 3 ETS exposure						
Low[3]	3.318 (3.231 - 3.405)	--	49.3 (48.9 - 49.7)	--	34.5 (34.3 - 34.8)	--
Low-mod	3.250 (3.139 – 3.361)	*0.34*	49.3 (48.8 – 49.9)	*0.91*	34.7 (34.3 – 35.0)	*0.61*
High-mod	3.148 (3.038 – 3.259)	*0.02*	48.9 (48.4 – 49.4)	*0.23*	34.2 (33.8 – 34.5)	*0.12*
High	3.035 (2.888 – 3.182)	*0.001*	48.4 (47.7 – 49.1)	*0.02*	34.0 (33.6 – 34.5)	*0.07*
P-value (trend)[4]		*<0.001*		*0.02*		*0.03*

[1] less than 37 weeks gestational age.

[2] T-test for difference from the baseline group.

[3] The baseline group – which all other comparisons are with – comprised term babies (37+ weeks gestation) whose mothers had the lowest level of ETS exposure.

[4] T-test for linear trend between the exposed groups.

In the extended analysis, adjusted for prematurity, maternal ETS exposure was only associated with birth weight, but not length or OFC. Results for trimester 1 are shown in tables 7-9; results were extremely similar for the other time periods (data not presented). Prematurity accounted for the most important difference in all outcomes. Sex was an important discriminator for length and OFC, but not weight. Maternal smoking status did not show any associations, but ever having consumed alcohol was associated with changes in weight and length.

When adjustment was made for gestational age instead of prematurity, the association between ETS exposure and birth weight disappeared, other than for the highest category of exposure for which it remained borderline (Trimester 1: regression coefficient -162 (grams), 95% CI -335 – 10, p=0.07; test for linear trend p=0.09).

Table 7: Extended analysis for birth weight (g) compared with maternal ETS exposure during trimester 1 (<18 weeks gestational age) accounting for prematurity; data from Geração XXI cohort in Porto, Northern Portugal.

	Mean (95% CI)	***p-value*[1]**
Baseline[2]	3551 (3097 - 4004)	*<0.001*
ETS exposure category		
Low	3551 (3097 - 4004)	--
Low-mod	3553 (3097 – 4010)	*0.97*
High-mod	3444 (3008 – 3881)	*0.18*
High	3296 (2851 – 3742)	*0.007*
P-value (trend)[3]		*0.005*[3]
Female sex	3530 (3093 – 3968)	*0.73*
Prematurity[4]	2914 (2398 – 3430)	*<0.001*
Planned pregnancy	3396 (2948 – 3844)	*0.02*
Maternal age	3550 (3106 – 3993)	*0.91*
Maternal smoking status		
Never smoker	3551 (3097 - 4004)	--
Ex-smoker	3615 (3141 – 4089)	*0.45*
Stopped in pregnancy	3406 (2922 – 3890)	*0.11*
Current smoker	3460 (2985 – 3936)	*0.28*
P-value (trend)[3]		*0.13*[3]
Mother ever used alcohol	3423 (2947 – 3900)	*0.04*
Luxury items[5]	3531 (3090 – 3973)	*0.20*
Previous pregnancies[6]	3589 (2398 – 3430)	*0.24*

[1] T-test for difference from the baseline group.

[2] Baseline is a male infant born to a non-smoking primiparous mother who never drank alcohol, had not planned her pregnancy and did not have own any luxury items. Comparisons for all variables were made with this baseline.

[3] T-test for linear trend between the exposed groups.

[4] Less than 37 weeks gestation.

[5] Change per luxury item owned.

[6] Number.

Table 8: Extended analysis for birth length (cm) compared with maternal ETS exposure during trimester 1 (<18 weeks gestational age) by gestational maturity (p<0.001); data from Geração XXI cohort in Porto, Northern Portugal.

	Mean (95% CI)	***p-value*[1]**
Baseline[2]	50.2 (48.1 – 52.3)	
ETS exposure category		
Low	50.2 (48.1 – 52.3)	
Low-mod	50.4 (48.3 – 52.5)	*0.55*
High-mod	49.9 (47.9 – 51.9)	*0.32*
High	49.6 (47.6 – 51.6)	*0.15*
P-value (trend)[3]		*0.11*
Female sex	49.6 (47.6 – 51.7)	*0.03*
Prematurity[4]	47.8 (45.5 – 50.2)	*<0.001*
Planned pregnancy	49.7 (47.7 – 51.7)	*0.08*
Maternal age	50.2 (48.2 – 52.2)	*0.96*
Maternal smoking status		
Never smoker	50.2 (48.1 – 52.3)	
Ex-smoker	50.4 (48.2 – 52.6)	*0.57*
Stopped in pregnancy	50.0 (47.7 – 52.2)	*0.53*
Current smoker	49.6 (47.4 – 51.8)	*0.12*
P-value (trend)[3]		*0.11*
Mother ever used alcohol	49.6 (47.4 – 51.8)	*0.03*
Luxury items[5]	50.2 (48.2 – 52.2)	*0.88*
Previous pregnancies[6]	50.6 (48.5 – 52.7)	*0.02*

[1] T-test for difference from the baseline group.

[2] Baseline is a male infant born to a non-smoking primiparous mother who never drank alcohol, had not planned her pregnancy and did not have own any luxury items. Comparisons for all variables were made with this baseline.

[3] T-test for linear trend between the exposed groups.

[4] Less than 37 weeks gestation.

[5] Change per luxury item owned.

[6] Number.

Table 9: Extended analysis for birth OFC (cm) compared with maternal ETS exposure during trimester 1 (<18 weeks gestational age) by gestational maturity (p<0.001); data from Geração XXI cohort in Porto, Northern Portugal.

	Mean (95% CI)	***p-value*[1]**
Baseline[2]	35.5 (34.1 – 36.9)	
ETS exposure category		
Low	35.5 (34.1 – 36.9)	--
Low-mod	35.7 (34.3 – 37.1)	*0.36*
High-mod	35.1 (33.8 – 36.5)	*0.17*
High	35.1 (33.7 – 36.5)	*0.19*
P-value (trend)[3]		*0.08*
Female sex	35.1 (33.7 – 36.5)	*0.04*
Prematurity[4]	*33.8 (32.2 – 35.4)*	*<0.001*
Planned pregnancy	35.1 (33.7 – 36.5)	*0.06*
Maternal age	35.5 (34.1 – 36.9)	*0.98*
Maternal smoking status		
Never smoker	35.5 (34.1 – 36.9)	--
Ex-smoker	36.0 (34.5 – 37.5)	*0.07*
Stopped in pregnancy	35.2 (33.7 – 36.8)	*0.39*
Current smoker	35.3 (33.8 – 36.8)	*0.56*
P-value (trend)[3]		*0.39*
Mother ever used alcohol	35.2 (33.7 – 36.6)	*0.10*
Luxury items[5]	35.5 (34.1 – 36.8)	*0.62*
Previous pregnancies[6]	35.5 (34.1 – 36.9)	*0.76*

[1] T-test for difference from the baseline group.

[2] Baseline is a male infant born to a non-smoking primiparous mother who never drank alcohol, had not planned her pregnancy and did not have own any luxury items. Comparisons for all variables were made with this baseline.

[3] T-test for linear trend between the exposed groups.

[4] Less than 37 weeks gestation.

[5] Change per luxury item owned.

[6] Number.

Growth outcomes

Maternal ETS exposure during pregnancy only showed an association during the first two years of life on head growth (test for linear trend: p=0.02 in all periods except trimester one when p=0.03), and did not impact weight or length. In the extended analysis, with adjustment for prematurity and other potential confounding factors, the association remained for head growth; however, with full adjustment for gestational age (weeks) as a continuous variable, the effect was only borderline, as shown for the second trimester in table 10 (the other time periods were similar with the exception of trimester 3 for which the p-value for the test for trend was 0.06).

Sensitivity analysis

All the effects seen in the simple and extended analyses remained similar when the inclusion criteria were restricted to infants of 37 weeks gestation or above.

Table 10: Simple and extended regression analyses of OFC growth in the first 2 years of life in relation to maternal ETS exposure during the second trimester, adjusted for postnatal age.

	Simple analysis		Extended analysis	
	RC[1] (95% CI)	***p-value*[2]**	**RC[1] (95% CI)**	***p-value*[2]**
Observations (clusters)	*1991 (238)*		*1991 (238)*	
ETS exposure category[3]				
Low ETS exposure	--	--	--	--
Low-mod ETS exposure	-0.2 (-0.6 – 0.2)	*0.28*	-0.1 (-0.5 – 0.2)	*0.39*
High-mod ETS exposure	-0.2 (-0.6 – 0.1)	*0.21*	-0.2 (-0.5 – 0.1)	*0.19*
High ETS exposure	-0.6 (-1.1 – -0.1)	*0.02*	-0.4 (-0.8 – 0.1)	*0.11*
P-value (trend)[4]		*0.02*		*0.05*
Gestational age (weeks)[5]			-0.2 (-0.3 – -0.1)	*<0.001*
Female sex			-1.0 (-1.2 – -0.7)	*<0.001*
Ever breastfed			-0.2 (-0.9 – 0.4)	*0.48*
Maternal age			0.0 (--)[6]	*0.83*
Maternal smoking status[3]				
Never smoker				
Ex-smoker			0.4 (0.0 – 0.7)	*0.03*
Stopped in pregnancy			-0.2 (-0.6 – 0.3)	*0.46*
Current smoker			0.3 (-0.1 – 0.7)	*0.12*
P-value (trend)[4]				*0.25*

[1] RC: regression coefficient (centimetres difference from baseline).

[2] T-test for difference from the baseline group.

[3] The baseline was a male infant of 40 weeks gestation, never breastfed, born to a non-smoking mother with low ETS exposure. All calculations are based upon a quadratic function of postnatal age.

[4] T-test for linear trend between the exposed groups.

[5] Change per week for gestational age < 40 weeks. (change is positive if infant was born post-dates)

[6] Confidence intervals were less than +/- 0.1

Discussion

The data presented here from a pilot study of 239 eligible mother-infant pairs from the Geração XXI cohort indicate that maternal environmental tobacco smoke exposure before and during pregnancy is associated with birth size. This association is strongest with birth weight, an association that remained after adjusting for potential confounding factors, followed by OFC and then birth length, and is consistent with reports from other studies[11].

Amongst the growth outcomes, only head growth during the first two years of life shows an association with maternal ETS exposure – which also persists following adjustment for possible confounders. This appears to be a novel outcome which has not been reported before; indeed, no studies were found looking at the association between maternal ETS exposure and postnatal growth. The question of why the head is affected more than weight or linear growth remains to be answered.

It should also be noted that results were broadly the same across the 4 time periods examined in this study. With the ETS exposure data having been collected retrospectively, this raises a question about the accuracy of the reporting and whether recall bias plays an important role. Additionally, there is a strong possibility of residual confounding by factors – particularly socioeconomic factors – unmeasured or otherwise not included in the study: this is emphasised given the lack of association between maternal smoking status and any of the outcomes.

Limitations

There are a number of limitations to the conclusions that can be drawn from this pilot study – importantly, the small number of eligible subject pairs (239) who had data available for analysis, which is discussed in more detail below. The population may not be representative of the population of Portugal, or even the surrounding region; indeed, there were extremely low levels of education among both the mothers and the fathers in the study and almost half the families had an income below 1000 E/month. While this would be more important in a study designed for public health purposes – this project was looking at a possible causal

relationship – it does suggest that the sample is not as diverse as it might otherwise have been.

The failure to find any association between birth size or subsequent growth and maternal smoking status in this pilot study is concerning, even without including maternal ETS exposure, as smoking should plausibly have a much greater effect than ETS exposure. This may be due to the strong correlation between ETS exposure and smoking status, and that the categorisation of ETS in this study was a better discriminant than smoking status. Additionally, it is known that smoking has a dose-response effect on the fetus[21], but the quantity smoked was not investigated here. Nor are the numbers of heavy smokers likely to have been high, given that only about a quarter were smokers at the beginning of pregnancy.

These statistics provide a suggestion of selection bias when viewed in comparison with the same characteristics of the overall study population (see Appendix E), which show different levels of socio-economic indicators than those seen in this pilot study. Additionally, knowing that follow-up attendance was high (the majority of infants had 10 or more sets of anthropological measurements made during the two year follow-up, equating to at least one infant surveillance check every 3 months) and mothers were offered an incentive to attend the 2-year interview, this strongly suggests a sub-population with lower economic means than the overall population.

The methodologies used may have contributed to a non-differential classification bias, thus attenuating the magnitude of any association. Some of the problems are inherent within the overall Geração XXI study design, due to limitations imposed by Portuguese law (for example, restricting the recording of ethnicitity data) or finances – which, for example, precluded the possibility of double data-entry. Other limitations are part of the pilot study itself. Translation was undertaken by a non-native Portuguese speaker who sought clarification if there were concerns; however, errors may have occurred which were not double-checked. Data was recorded as reported by the mothers, and this may also have contributed to comprehension difficulties: in several cases, data was clearly entered as descriptive text that was similar or identical to one of the tick-box options on the maternal questionnaires (e.g. a number of women described all the symptoms of pre-eclampsia but clearly did not realise they had it).

The classification schema used in this pilot study were also novel. While methods for determining tobacco consumption are relatively established (e.g. classification into number of cigarettes smoked per day), numerous methods have been described for measuring ETS exposure. Classification, moreover, is dependent upon how the data is gathered and, for ETS, exposure is difficult to quantify. What has been shown is that self-reporting is able to discriminate adequately between low and high exposures[22]: for this study, this means there is likely to be some validity in the categorisation used and differences seen in outcome in the highest exposure groups are likely to be true.

Other scoring systems used were also novel: the scale of 'luxury' possessions, and the measure of 'junk' food consumption by infants. Neither of these are precise – perhaps explaining why they did not produce important results in the analysis – but were the best indicators that could be constructed from the available data, and are easily replicable. Similarly, the lack of data on ethnicity makes it difficult to assess the impact of genetics; use of maternal grand-parental data showed borderline associations with some outcomes but did not contribute in this study of environmental tobacco smoke exposure. These borderline associations may simply be due to chance, or may reflect a useful proxy measure for an individual's genetic constitution.

Another concern is the lack of data used in the analysis that related to postnatal growth. There was inadequate data available to assess infant health – a result of the way data was able to be collected for the cohort – and a paucity of data on postnatal infant exposure to ETS. It is likely, for example, that mothers with a high level of ETS exposure also had infants with a high level of exposure, possibly affecting their growth. Additionally, parental body size was either available in insufficient detail (mothers) or not available at all (fathers – who it is suggested have a greater influence on infant body size[14]), hence making adjustment for these factors impossible.

Chance

Ideally, an *a priori* estimation for a study to investigate an effect representing a 1% change in birth weight, from 3300g to 3270g and with a standard deviation of 250g, would have

suggested about 1700 mother-infant pairs were required in the lowest ETS exposure group and about 800 in the highest group for 80% power at a significance level of 0.05. Assuming a 1cm decrease in birth length or OFC with a standard deviation of 5cm would have required about 600 and 300 mother-infant pairs for the lowest and highest exposure groups, respectively. As this pilot study was based upon a dataset that had already been provided, an *a priori* estimation was not appropriate; *post-hoc* sample size calculation has also been argued to be inappropriate given complete statistical reporting[23]. Instead, we should bear in mind that there is a possibility of a type II error occurring – that is, that although no effect has been seen, the sample size may have been too small to adequately demonstrate an effect.

Furthermore, actual statistical methodologies employed were crude: advanced techniques have been developed for analysing hierarchical data such as that available[24], but were beyond the scope of this study. Few adjustments were made for non-linearity of data, and there could have been more detailed assessment of whether the assumptions of the regression modelling were met (i.e. whether or not there was a constant variance about the predicted regression line and whether the true residuals observed a normal, Gaussian distribution or not).

Strengths

Importantly, this pilot study has a number of strengths, particularly in relation to further work. The sample contained a balance of male and female infants with a plausible spread of the outcome measures as well as important characteristics like gestational age and type of birth. The growth measurements followed the correct trajectories for the first 2 years of life and are within the ranges expected from international standardised prediction scales (e.g. weight 10kg at 1 year and 12kg at 2 years)[25]. There are no available standardised Portuguese guidelines for growth such as produced in the UK[26] or by the World Health Organisation[27], however, hence a full comparison is not possible – but this was not the aim of the present study. Further confirmation of the usefulness of this cohort are the differences in birth outcome seen between the lowest and highest groups of maternal ETS exposure: these are in line with those expected from other studies[11,12]. Additionally, there appears to be enough growth data available per child of sufficient quality to produce useful results. This is particularly reassuring in relation to the limited resources that were able to be invested in the

Geração XXI study – including the collection and recording of data which mean that there is a higher chance of error being introduced than might be seen with a stricter data regime.

Finally, it should be emphasised that the Geração XXI study was designed – and the data collected – prospectively, hence minimising differential misclassification. Overall, Geração XXI has fairly comprehensive population coverage due to recruitment having occurred via the public hospital system, so it would be possible for expanded analyses to incorporate a broader and more representative population. This provides the opportunity for results from the study cohort to influence local and national health and preventive medicine (incorporating other governmental departments such as transport, education or environment) planning. Results may also be used in comparison with other studies to provide international supporting evidence for associations that have been demonstrated in other populations.

Further work

The results from this pilot study indicate that maternal ETS exposure is likely to have small but important effects on many different measures of growth during early life. Birth weight has been extensively investigated but subtle differences, particularly in postnatal growth, have not previously been described and warrant further investigation. Replication of the findings – either in the main cohort or elsewhere or, ideally, both – would be reassuring that the differences seen were real. Using more advanced statistical techniques would also increase confidence in the results and be useful in quantifying any change in outcome more precisely.

Exploring the biological and socio-economic determinants of early development will continue to be an important area of research, particularly as more countries undergo the demographic, epidemiological and nutritional transitions that have characterised recent history of the developed world. Data from this study has indicated that information from the Geração XXI cohort is of suitable quality to allow for deeper investigation into these early life determinants. Consequently, potential exists to not only extend the questions asked in this pilot study to involve the entire cohort, but also to explore other factors which may be of importance in determining infant growth and future health status.

Conclusions

In summary, this study has demonstrated that maternal ETS exposure during pregnancy may have a detrimental effect on fetal and infant growth. This effect may be seen particularly on birth outcomes, but there may be differences that remain important postnatally. These findings warrant further investigation. Additionally, data collection from the Geração XXI cohort appears to be of sufficient quality to allow sophisticated analysis and, while the statistical methodologies used in this pilot study could be improved, it is important to recognise that simpler ones may actually be suitable to provide adequate explanation of the data.

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Statement of authorship

This project originally stemmed from an idea by Professor Isabel dos Santos Silva using data that she had available from Portugal. The author helped develop the idea further and conducted the background literature search and prepared the data for analysis, including performing a majority of the translations. The analytical models used were developed by the author, under the guidance of Costanza Pizzi; the discussion and conclusions are the work of the author.

Acknowledgements

Thank you to Isabel dos Santos Silva for her invaluable guidance, Renato Oliveira-e-Souza for help with translations, Costanza Pizzi for assistance with the statistics and Sara Thomas for moral support.

Appendix A

Geração XXI ethical approval

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BIOÉTICA

PARECER

SOBRE O PROJECTO DE INVESTIGAÇÃO "GERAÇÃO XXI - NASCER E CRESCER NO INÍCIO DO MILÉNIO" PROPOSTO PELO SR. PROF. DOUTOR HENRIQUE DE BARROS DA FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO.

Considerando que este estudo epidemiológico tem como objectivos principais:

1- Constituir e acompanhar uma coorte de 10.000 recém-nascidos e criar as condições para o seguimento regular desta coorte;
2- Produzir informação e monitorizar o estado de saúde materno-infantil desta coorte contribuindo assim para medir e estimular os ganhos de saúde da população portuguesa;
3- Identificar factores genéticos e ambientais que condicionam o desenvolvimento intra-uterino e da criança nos primeiros anos de vida;
4- Descrever práticas e identificar determinantes do aleitamento materno e dos modelos de cuidados infantis;
5- Desenvolver programas informáticos que permitam criar um sistema de armazenamento de informação recolhida durante os períodos peri e pós-natal e que permita no futuro manter a vigilância epidemiológica e do estado de saúde dos sujeitos avaliados;

Que a investigação de natureza epidemiológica deve pautar-se por regras éticas diferenciadas[1], designadamente no atinente à obtenção de consentimento informado por parte do sujeito tendo em atenção o relevante interesse público envolvido;

Que a prevalência dos interesses da pessoa sobre os interesses exclusivos da ciência, afirmando o primado da dignidade do ser humano e materializando o valor intrínseco não-instrumental da pessoa humana, não impedem a realização de estudos desta natureza quando se perspectivam importantes benefícios para a sociedade e sobretudo para as gerações futuras;

Que os direitos dos sujeitos se encontram genericamente salvaguardados;

[1] Ver The European Epidemiology Group: Good Epidemiological Practice: Proper Conduct in Epidemiologic research; assim como International Guidelines for Ethical Review of Epidemiological Studies. Prepared by the Council for International Organisations of Medical Sciences (CIOMS) in collaboration with the World Health Organisation (WHO), Geneva, 1991; e CIOMS: Ethics and Epidemiology: International Guidelines. Edited by Z. Bankowski, J. Briant, & J. Last. Council for International Organizations of Medical Sciences, Geneva, 1993.

1

Que está em causa com este estudo a criação de uma base de dados pessoais sendo fundamental garantir o direito à privacidade e a confidencialidade dos dados obtidos, no estrito cumprimento das disposições éticas e jurídicas existentes;

Pelo que é um imperativo ético a imposição de limites ao acesso não autorizado a informação de natureza individual, implicando o rigoroso cumprimento do segredo profissional por parte de todos os agentes envolvidos no tratamento dos dados pessoais, biológicos ou genéticos, bem como o arquivamento escrupuloso destes dados, independentemente do suporte em que se encontrem;

Que a razão risco benefício para o sujeito (individualmente considerado) parece ser claramente a favor do estudo em causa, dado que nenhuma das intervenções propostas tem um risco superior ao mínimo;

Que está prevista a constituição de um banco de produtos biológicos e de uma base de dados genéticos[2] sendo que sobre estas matérias existem importantes instrumentos normativos que devem ser respeitados, desde logo:

1- A Lei n.º 67/98 de 26 de Outubro – Lei de Protecção de Dados Pessoais que transpõe para a ordem jurídica portuguesa a Directiva n.º 95/46/CE, do Parlamento Europeu e do Conselho, de 24 de Outubro de 1995, relativa à protecção das pessoas singulares no que diz respeito ao tratamento dos dados pessoais e à livre circulação desses dados[3].

2- A Lei n.º 12/2005 de 26 de Janeiro sobre Informação Genética Pessoal e Informação de Saúde tendo sido objecto de Relatório/Parecer do Conselho Nacional de Ética para as Ciências da Vida[4];

---

[2] Por base de dados genéticos entende-se qualquer registo, informatizado ou não, que contenha informação genética sobre um conjunto de indivíduos ou famílias (incluindo os dados decorrentes da informação proteómica). Está em causa o armazenamento e utilização de resultados de testes genéticos e não de amostras biológicas (sangue, DNA) sendo importante distinguir entre perfis electroforéticos de bandas (*DNA-Fingerprints*), SNPs (*Single Nucleotide Polymorphisms*) de zonas não codificantes e resultados de testes para a detecção de genes de susceptibilidade ou determinantes da ocorrência de doença.

[3] Esta Lei define por dados pessoais "qualquer informação, de qualquer natureza e independentemente do respectivo suporte, incluindo som e imagem, relativa a uma pessoa singular identificada ou identificável (titular dos dados)".

[4] Relatório/Parecer 43/CNECV/2004 do Conselho Nacional de Ética para as Ciências da Vida sobre o Projecto de Lei N.º 28/IX Informação Genética Pessoal e Informação de Saúde (Relator: Rui Nunes) aprovado por unanimidade em sessão plenária do CNECV dia 11 de Maio de 2004.

3- A Recomendação da Entidade Reguladora da Saúde n.º R/01/ERS/05 Sobre a Colheita e Conservação de Células Estaminais do Sangue do Cordão Umbilical de Recém-Nascidos[5];

4- A Directiva 2004/23/CE do Parlamento Europeu e do Conselho de 31 de Março de 2004 relativa ao estabelecimento de normas de qualidade e segurança em relação à dádiva, colheita, análise, processamento, preservação, armazenamento e distribuição de tecidos e células de origem humana que será num futuro próximo transposta para a ordem jurídica portuguesa.

Somos de parecer que este estudo pode prosseguir, não apresentando restrições de natureza ética. Porém, fazem-se as seguintes sugestões:

1- O estudo em apreço deve ser efectuado no mais profundo respeito pelas normas éticas internacionais, entre outras[6] e desde logo, a última versão da Declaração de Helsínquia. Pelo que os questionários devem ser avaliados na perspectiva ética antes de serem aplicados;

2- O documento de informação e de consentimento deverá ser oportunamente submetido para apreciação do ponto de vista ético. Em todo o caso deve constar do documento de informação a finalidade da investigação (incluindo a colheita de produtos biológicos), os presumíveis benefícios, o tempo de conservação e o destino a dar ao material biológico;

3- Deve igualmente ser submetido para apreciação o laboratório que ficará fisicamente encarregado de preservar o material biológico, sendo que este deve estar dotado dos requisitos de qualidade e de licenciamento previstos na Lei;

---

[5] Entidade Reguladora da Saúde, Porto, Abril de 2005 (Relatores: Rui Nunes, Cordeiro Tavares e Paulo Freitas)

[6] Podem destacar-se ainda sobre esta temática a Carta dos Direitos Fundamentais da União Europeia (n.º 2 do artigo 3.º), a Declaração Internacional sobre Dados Genéticos Humanos Aprovada por consenso em Paris na 32ª Sessão da Conferência Geral da UNESCO, a 29 de Setembro – 7 de Outubro de 2003, e Convenção para a Protecção dos Direitos do Homem e da Dignidade do Ser Humano face às Aplicações da Biologia e da Medicina, aberta à assinatura dos Estados-Membros do Conselho da Europa em Oviedo, em 4 de Abril de 1997 (Resolução da Assembleia da República n.º 1/2001, Diário da República Número 2, I-Série, 3 de Janeiro de 2001).

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4- Deve a Comissão Nacional de Protecção de Dados Pessoais pronunciar-se sobre as condições em que serão efectuados os registos informáticos que contenham informação pessoal.

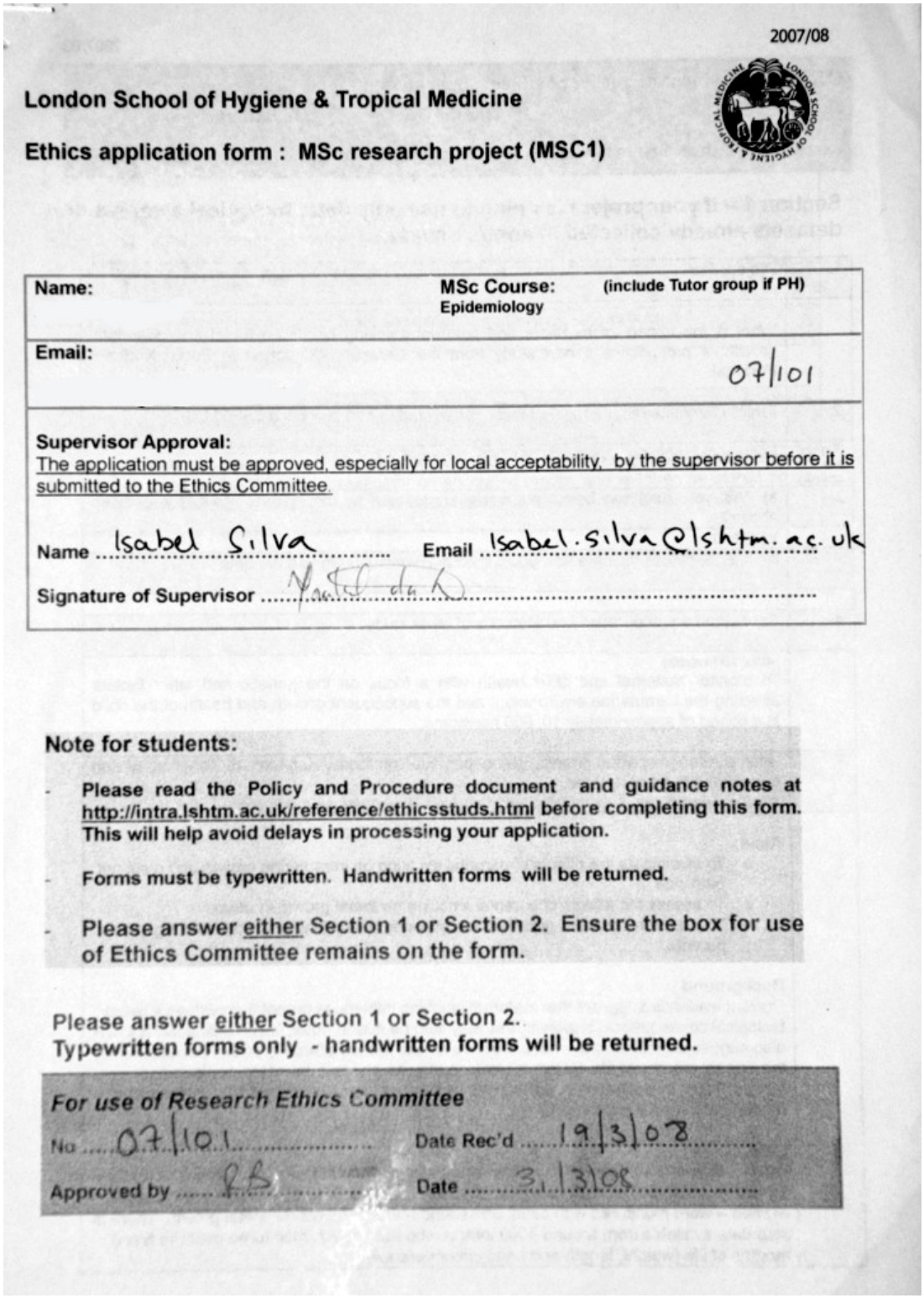
Porto, 27 de Abril de 2005

O Relator

PROF. DOUTOR RUI NUNES
DIRECTOR DO SERVIÇO DE BIOÉTICA E ÉTICA MÉDICA
FACULDADE DE MEDICINA DA UNIVERSIDADE DO PORTO

Appendix B

LSHTM ethics form



2007/08

London School of Hygiene & Tropical Medicine

Ethics application form : MSc research project (MSC1)

Name:	**MSc Course:** Epidemiology	**(include Tutor group if PH)**
Email:		07/101

Supervisor Approval:
The application must be approved, especially for local acceptability, by the supervisor before it is submitted to the Ethics Committee.

Name Isabel Silva Email Isabel.silva@lshtm.ac.uk

Signature of Supervisor .....

Note for students:

- **Please read the Policy and Procedure document and guidance notes at http://intra.lshtm.ac.uk/reference/ethicsstuds.html before completing this form. This will help avoid delays in processing your application.**
- **Forms must be typewritten. Handwritten forms will be returned.**
- Please answer either Section 1 or Section 2. Ensure the box for use of Ethics Committee remains on the form.

Please answer either Section 1 or Section 2.
Typewritten forms only - handwritten forms will be returned.

For use of Research Ethics Committee

No 07/101 Date Rec'd 19/3/08

Approved by RB Date 31/3/08

Overall assessment of quality of ethical application

☐ **Excellent** ☑ **Good** ☐ **Satisfactory**

Comment by Ethics Committee .....

Section 1 – If your project is going to use only data, biological samples or datasets already collected in another study.

1.	Project title:
	What is the impact of maternal and paternal smoking habits on foetal and post-natal growth: a prospective cohort study from the Geração XXI cohort in Porto, Northern Portugal.
2.	Ethics Committee that approved that original study - and number if LSHTM.
(Expand box to answer)	Faculty of Medicine, University of Porto, Portugal, April 2005
3.	a) Will your analyses be for purposes not covered by the original application detailed above?
	YES/**NO**
	b) If YES, please indicate how you will obtain permission to use the data.
4.	Brief summary of purpose and methods of other study
	(max 100 words) To monitor maternal and child health with a focus on the genetic and other factors affecting the intrauterine environment and the subsequent growth and health of the child in a cohort of approximately 10, 000 newborns.
5.	Brief summary of your project, giving purpose, methods, numbers of participants and procedures to be performed. *See Guidance notes at http://intra.lshtm.ac.uk/reference/ethicsstuds.html*
	Aims: • To investigate the effects of parental smoking on intrauterine growth and resultant birth size • To assess the effects of parental smoking on foetal growth *in utero*. • To compare postnatal growth in the offspring of smoking and non-smoking parents. **Background** Current evidence suggests that maternal smoking influences prenatal growth as a direct biological consequence. However, this may also be due to other, confounding factors. It is also suggested that infants whose mothers smoke exhibit altered growth velocities during the first six months of life. Paternal smoking may be an indicator of the confounding factors. Thus, investigation of differences between maternal and paternal smoking and related outcomes is warranted. **Dataset** Data from approximately 8,500 women and their offspring in Porto, Northern Portugal is available. About 50% includes data for the male partners. A further subset – around 250 women – were monitored with serial ultrasound measurements for foetal growth. There is also data available from around 1000 infants who had growth monitored over the first 6 months of life (weight, length and head circumference).

	Methods All the data has already been entered into databases and is available for analysis, requiring only minimal cleaning. First, a descriptive analysis will be performed, before using advanced techniques to control for confounding variables and allow for hierarchical analysis.
6.	Does project involve: a) analysis of documentary data already collected
	YES/NO Please see description above.
	b) analysis of the results of tests on biological material already collected
	YES/**NO** If yes, specify tests
7.	Specify how confidentiality will be maintained. When small numbers are involved, indicate how possible identification of individuals will be avoided. *Guidance notes at* http://intra.lshtm.ac.uk/reference/ethicsstuds.html
	All data has been anonymised prior to data entry. It will be transported from Portugal to England by the Principle Investigator on encrypted media, and will then be stored on an encrypted hard drive which has restricted access from where it will be analysed.

Appendix C

Construction of summary environmental tobacco smoke exposure categories for different time periods from the Geração XXI dataset

Environmental tobacco smoke (ETS) exposure was measured in 4 time periods: the 3 months prior to becoming pregnant, and the first, second and third trimesters. Mothers were asked about exposure in 3 locations: at home, at work, and elsewhere. For each location and each time period, they were provided with a categorical measure of exposure: none, sporadically exposed, <1 hour per day, 1-3 hours/day, >3 hours/day. These therefore needed to be condensed to produce a summary measure for each time period.

A matrix was constructed for each time period (table 1: pre-pregnancy exposure is used as an example). The exposures were re-ranked according to the scale on the right of the matrix. Each woman than had an overall score assigned by adding up the sum of the exposures from the different locations and dividing the total by the number of localities that had contributed. For example, if she reported exposure at home and work and during her leisure time, the sum was divided by 3; if she only reported information for home and during her leisure time, the sum was divided by 2. Table 2 shows the numbers of women who reported ETS exposure in 2 or 3 localities.

The resulting scores were naturally organised into 8 groups, representing differing degrees of exposure. The lowest and the highest groups for each time period contained small numbers of subjects, hence were collapsed together to make 4 risk categories. This is shown in table 3. Consistently throughout all the time periods surveyed, there were less than 8 women who claimed no environmental tobacco smoke exposure.

Table 1: Number of women from a sub-cohort of the Geração XXI study reporting different levels of environmental tobacco smoke exposure in three different environments during the 3 months prior to becoming pregnant: initial scoring system and adjusted ranking

Initial rank	Detail	Home	Work	Leisure	Adjusted ranking
1	**none**	168	92	9	0
2	**sporadic**	18	40	185	1
3	**<1 hr/day**	14	25	36	
4	**1-3 hrs/day**	25	10	5	2
5	**>3 hrs/day**	13	44	3	
	Missing	1	28	1	
	Total	**239**	**239**	**239**	

Table 2: numbers of women reporting environmental tobacco smoke exposure in different numbers of locations, by time period (before and during pregnancy)

	Number of locations*		
Time period	**3**	**2**	**0**
Pre-pregnancy	211	27	1
Trimester 1	207	31	1
Trimester 2	202	36	1
Trimester 3	192	46	1

* No women reported environmental tobacco smoke exposure in only 1 location.

Table 3: Regrouping of environmental tobacco smoke exposure summary score distribution and division into 4 risk categories. Summary scores were calculated by summating the adjusted ranking(s) and dividing by the number of location(s) in which the woman was exposed to ETS.

Final exposure category	**Summary score**								**Total**
	0	**1/3**	**1/2**	**2/3**	**1**	**4/3**	**3/2**	**5/3**	
Low	6	60	15	0	0	0	0	0	81
Low-mod	0	0	0	61	0	0	0	0	61
High-mod	0	0	0	0	63	0	0	0	63
High	0	0	0	0	0	17	6	10	33
Total	6	60	15	61	63	17	6	10	238

Appendix D

Regression curves for birth and growth parameters from a pilot study looking at maternal exposure to environmental tobacco smoke (ETS) using data from the Geração XXI cohort in Porto, Northern Portugal

This appendix contains the figures listed below. Each figure contains regression lines representing the 4 different levels of maternal ETS exposure and the effect on growth – either as measured at birth, or over the first two years of life.

Birth outcomes

These are adjusted for gestational age:

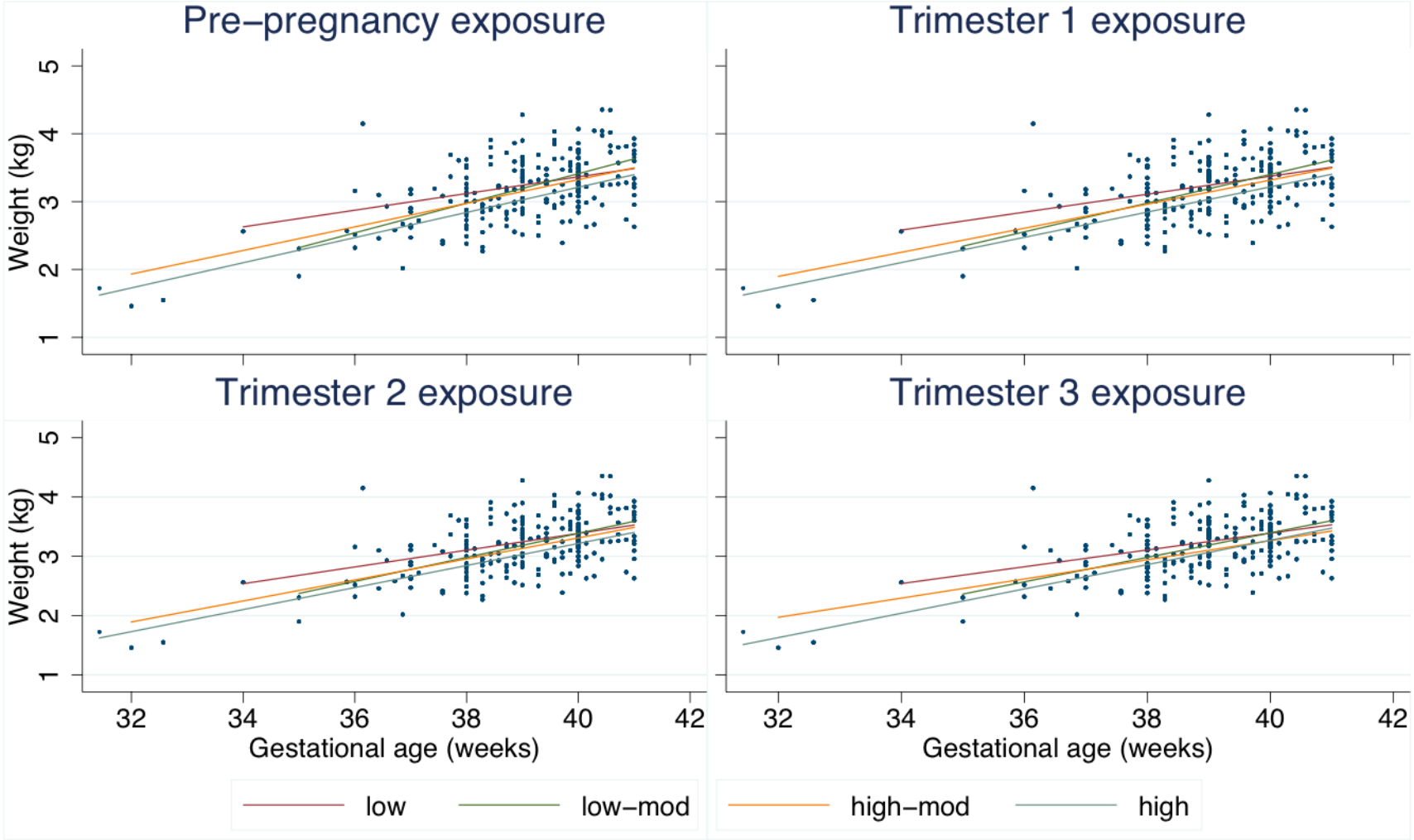
- Effect on birth weight of maternal environmental tobacco smoke exposure status
- Effect on birth length of maternal environmental tobacco smoke exposure status
- Effect on birth OFC of maternal environmental tobacco smoke exposure status

Infant growth outcomes

These are adjusted for chronological age:

- Effect of maternal environmental tobacco smoke exposure status on weight growth in the first 2 years of life
- Effect of maternal environmental tobacco smoke exposure status on length growth in the first 2 years of life
- Effect of maternal environmental tobacco smoke exposure status on OFC growth in the first 2 years of life

Effect on birth weight of maternal environmental tobacco exposure status

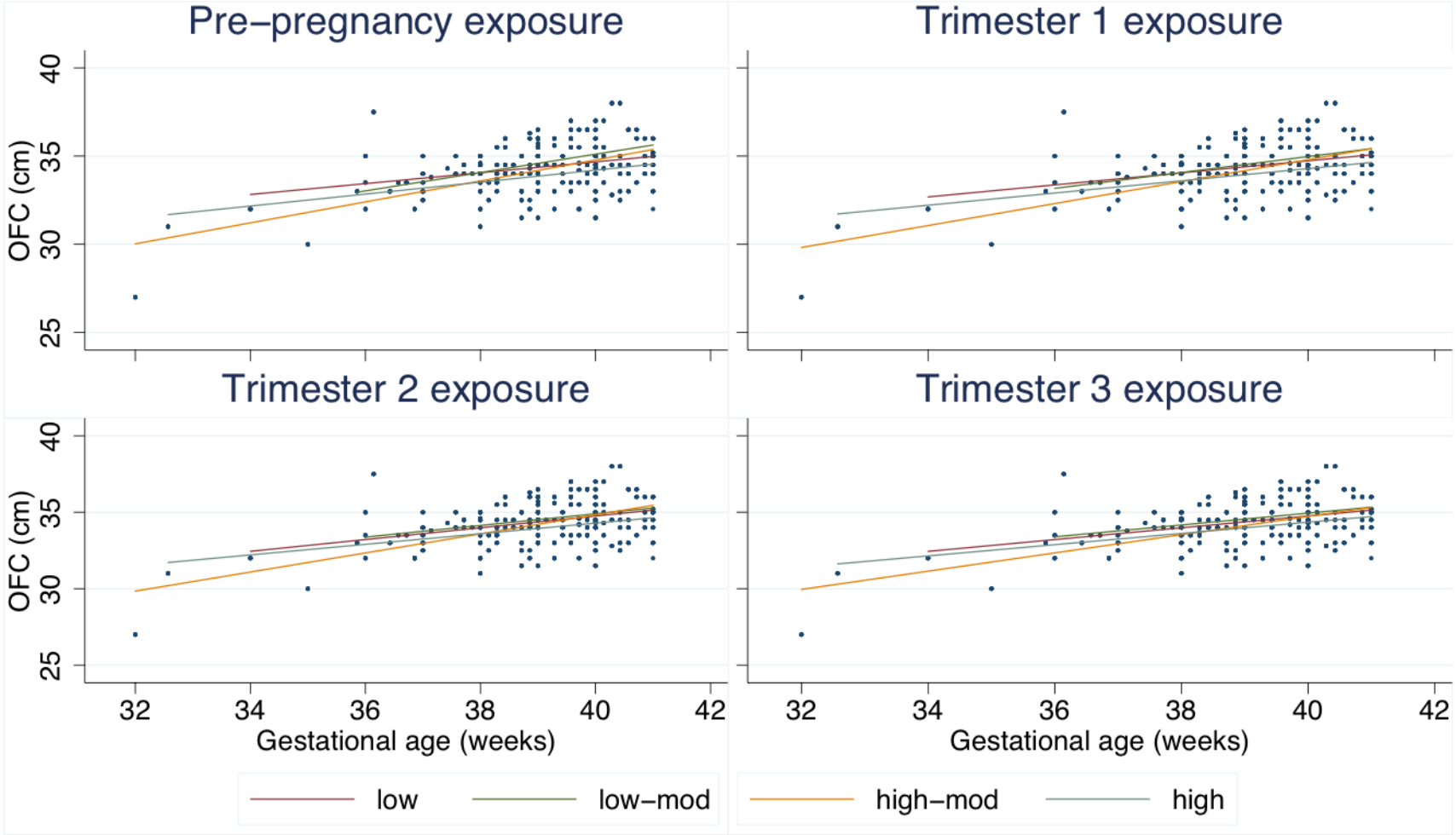


Data from a subcohort (n=239) of the Geracao XXI study, Porto, Northern Portugal.

Effect on birth length of maternal environmental tobacco exposure status

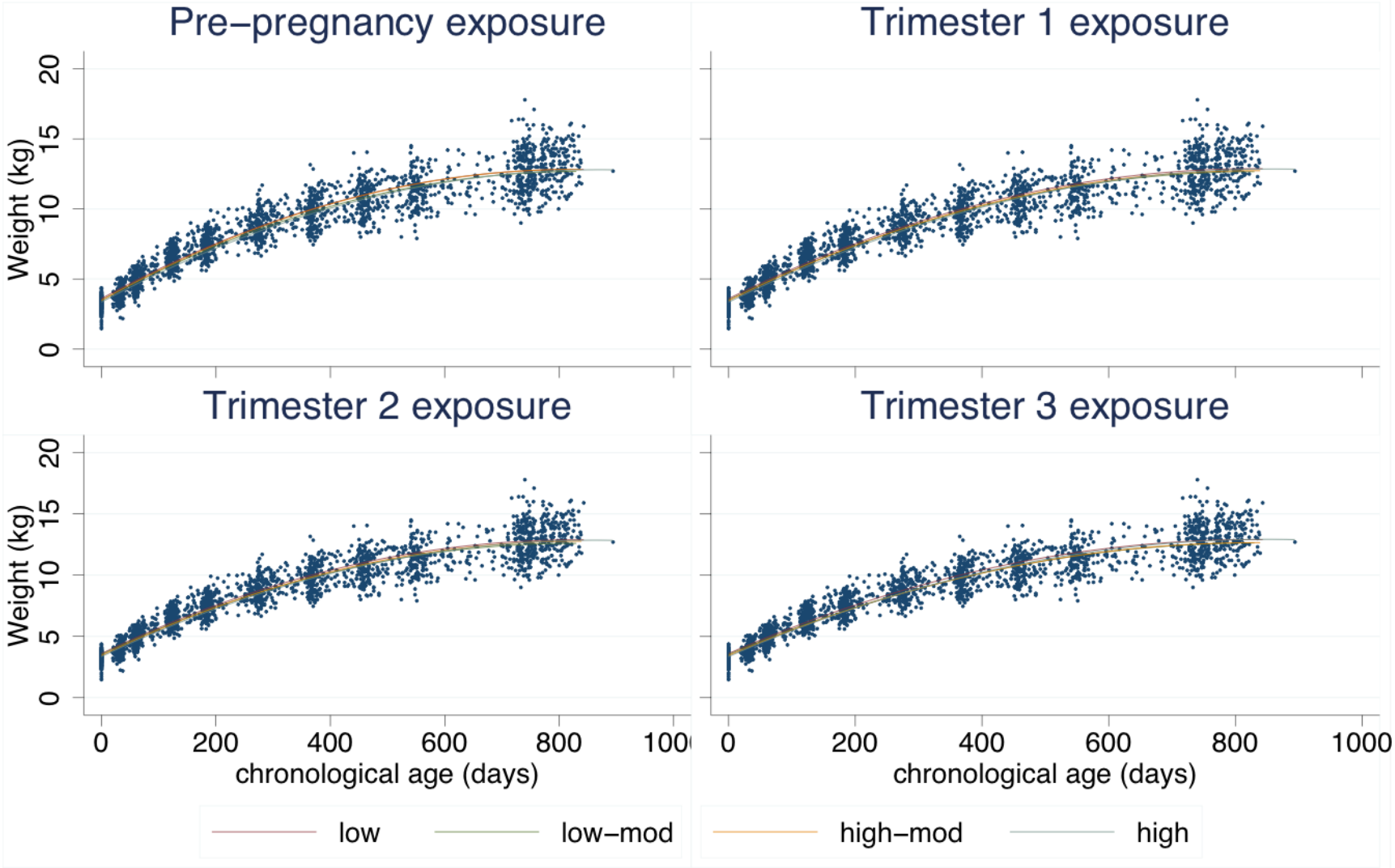
Data from a subcohort (n=239) of the Geracao XXI study, Porto, Northern Portugal.

Effect on birth OFC of maternal environmental tobacco exposure status



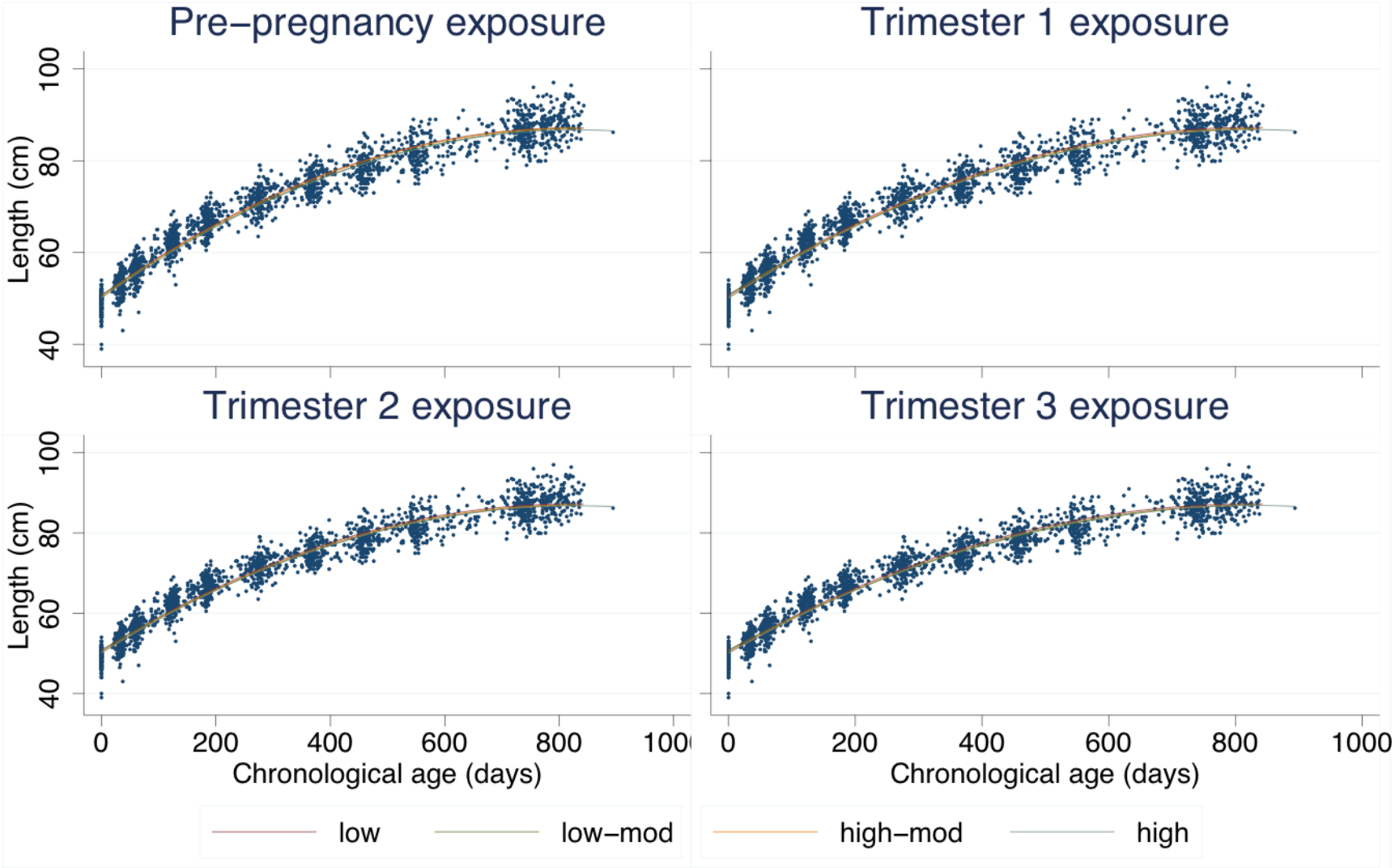
Data from a subcohort (n=239) of the Geracao XXI study, Porto, Northern Portugal.
OFC = occipitalfrontal (head) circumference

Effect of maternal environmental tobacco smoke exposure status on weight growth in the first 2 years of life



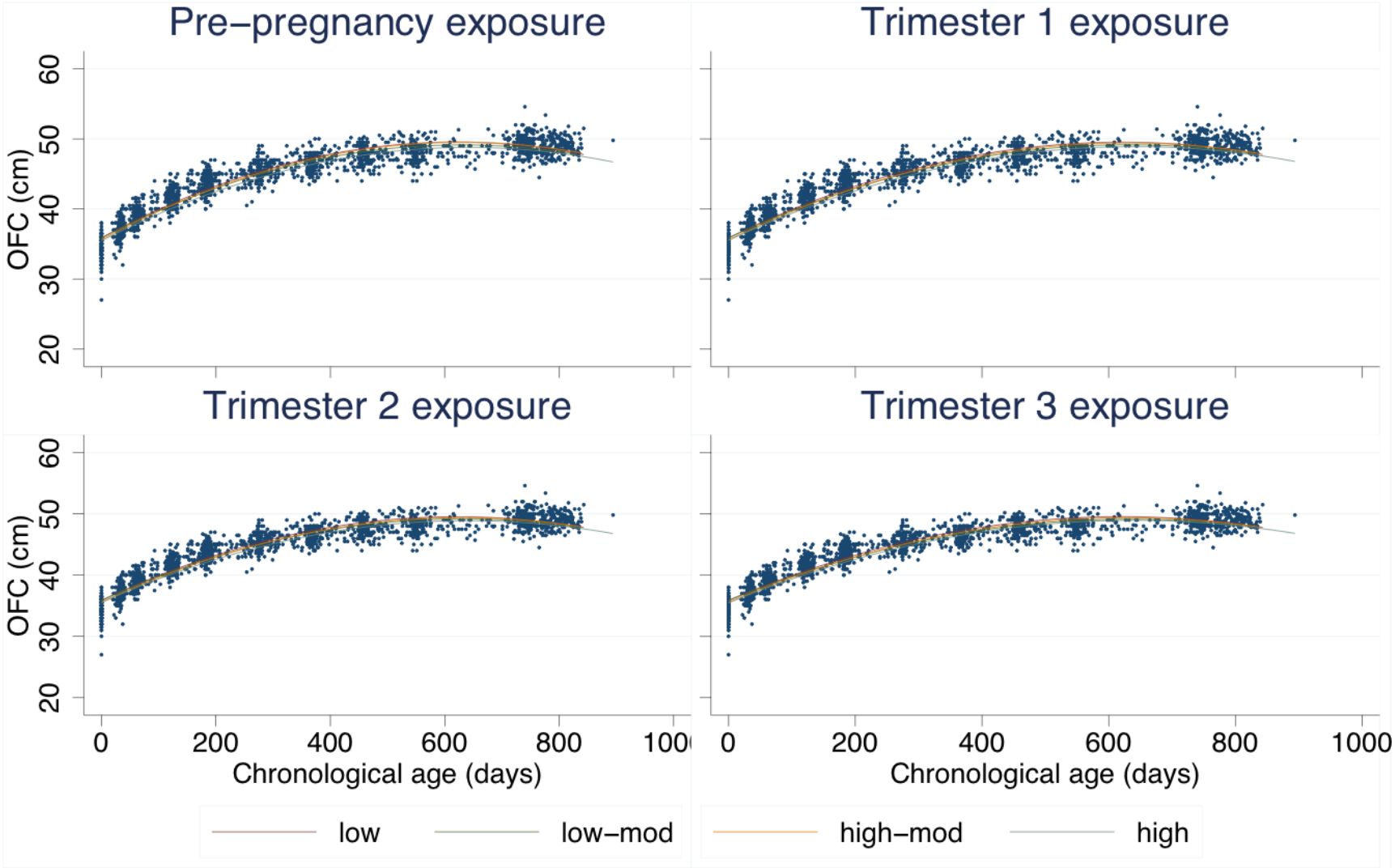
Data from a subcohort (n=239) of the Geracao XXI study, Porto, Northern Portugal.

Effect of maternal environmental tobacco smoke exposure status on length growth in the first 2 years of life



Data from a subcohort (n=239) of the Geracao XXI study, Porto, Northern Portugal.

Effect of maternal environmental tobacco smoke exposure status on OFC growth in the first 2 years of life



Data from a subcohort (n=239) of the Geracao XXI study, Porto, Northern Portugal.

Appendix E

Characteristics of the main cohort from the Geração XXI study in Porto, Northern Portugal

The tables on the following pages were adapted from an unpublished report from November 2007 detailing the background to the Geração XXI cohort. They are included with the kind permission of Professor Isabel dos Santos Silva, London School of Hygiene and Tropical Medicine.

Table 1: Maternal socio-demographic characteristics from the Geração XXI study in Porto, Northern Portugal.

	n*	%
Maternal age (years)		
<20	396	5,5
20-24	1118	16,4
25-29	2154	29,7
30-34	2329	32,1
35-39	997	13,7
≥40	185	2,6
Marital situation		
Married/living *de facto*	6789	94,0
Single	381	5,3
Widowed/Separated/Divorced	54	0,7
Education (years)		
≤ 4	576	8,0
5-6	1107	15,3
7-9	1840	25,4
10-12	1930	26,7
≥13	1778	24,6
Employment status		
Employed	5147	71,4
Unemployed	1395	19,3
Housewife	450	6,2
Student	189	2,6
Other	32	0,4
Family income (€)		
<500	469	6,6
501-1000	2100	29,7
1001-1500	1754	24,8
1501-2000	974	13,8
>2000	1017	14,4
Don't know	645	9,1
Didn't answer	102	1,4

*n different totals for different variables due to missing data.

Table 2: Characteristics of mothers at the time of the pregnancy with which they were enrolled in the Geracao XXI study in Porto, Northern Portugal.

	n*	%
Parity		
1	3481	48,0
≥2	3767	52,0
Planned pregnancy		
Yes	4861	67,2
No		
Type of antenatal care		
Private	2816	39,2
Gestational age at 1st appointment (wks)**		
≤ 12	6241	86,7
> 12	800	11,1
Unknown	159	2,2
Number of antenatal consultations		
Less than 3	57	0,8
3 to 6	470	6,6
7 to 9	1360	19,0
10 or more	3766	52,6
Unknown	1501	21,0
Number of altenatal ultrasound scans		
Less than 3	374	5,3
3	1974	28,0
4 to 6	3173	45,0
More than 6	1467	20, 7
Unknown	70	1,0
Complications during pregnancy	2925	40,8
Yes		
No		
Hospitalised during pregnancy	802	11,2
Yes		
No		
Smoking (7103)		
Ever smoked	2625	37,0
Smoked in the:		
3 months pre-pregnancy	1794	25,3
1st Trimester	1623	22,9
2nd Trimester	1145	16,1
3rd Trimester	1038	14,6

Table 2 (continued)

	n*	%
Alcohol consumption		
Ever drunk	2485	35,0
Drunk in the:		
1st Trimester	796	11,2
2nd Trimester	690	9,7
3rd Trimester	683	9,6

* Not all equal due to missing data.
** Of the mothers with at least one consult during pregnancy.