Article

Stress resilience in young men mediates the effect of childhood trauma on their offspring's birth weight – An analysis of 250,000 families

Kristiina Rajaleid[a,b,*], Denny Vågerö[a]

[a] Centre for Health Equity Studies, Department of Public Health Sciences, Stockholm University, Stockholm, Sweden
[b] Stress Research Institute, Stockholm University, Stockholm, Sweden

A B S T R A C T

Experiencing the death of a parent during childhood is a severe trauma that seems to affect the next generation's birth weight. We studied the consequences of parental loss during childhood for men's psychological and physiological characteristics at age 18, and whether these were important for their first-born offspring's birth outcomes. We used a structured life-course approach and four-way decomposition analysis to analyse data for 250,427 three-generation families retrieved from nationwide Swedish registers and found that psychological resilience was impaired and body mass index was higher in men who had experienced parental death. Both characteristics were linked to offspring birth weight. This was lower by 18.0 g (95% confidence interval: 5.7, 30.3) for men who lost a parent at ages 8–17 compared to other ages. Resilience mediated 40% of this influence. Mediation by body mass index, systolic and diastolic blood pressure was negligible, as was the effect of parental loss on length of gestation. There was no mediation by the education of the men's future spouse.

Previous literature has indicated that the period before puberty, the "slow growth period", is sensitive. Our evidence suggests that this may be too narrow a restriction: boys aged 8–17 appear to be particularly likely to respond to parental loss in a way which affects their future offspring's birth weight. We conclude that the observed transgenerational influence on birth weight is mediated by the father's psychological resilience but not by his body mass index or blood pressure.

1. Introduction

The question of whether environmental exposure in one generation can trigger a response in later generations is particularly intriguing on the paternal side. While it is widely recognized that a woman's environment is formative for future generation(s) (Barker, 1992, 1994), this is much less obvious for the man, who is going to be a father. Nevertheless, if he has been exposed to noxious chemicals, radiation or tobacco smoke, his offspring may be affected (Olsson et al., 2018; Shea, Little, & the ALSPAC Study Team, 1997; Pembrey et al., 2006). This has usually been seen as mediated by damage to the DNA sequence. However, new evidence suggests that environmental stimuli may alter the epigenetic profile in the male germ line, with consequences for the next generation(s). Emma Whitelaw (2006), who disliked the one-sided focus on maternal factors in developmental origins of health and disease (DOHaD) research, ironically coined the phrase "the sins of the fathers, and their fathers". Pembrey et al. (2006) hypothesized the existence of "a sex-specific male line transgenerational effect in humans". This hypothesis was given support by a recent three-generational study (Vågerö, Pinger, Aronsson, & van den Berg, 2018). Paternal origins of health and development (POHaD) is now seen as a new research field (Soubry, 2018).

Paternal characteristics strongly influence offspring development via social and cultural forces as well as via shared genes (Vågerö,

Koupilová, Leon, & Lithell, 1999; Lunde, Melve, Gjessing, Skjaerven, & Irgens, 2007; De Stavola, Leon, & Koupil, 2011). However, to draw conclusions about the importance of paternal factors it is necessary to consider their potential association with maternal factors. Selection processes, such as assortative mating, may confound or mediate any observed association between a father's pre-conception environment and his offspring's development. Assortative mating based on education is a particularly well-known and powerful phenomenon (Domingue, Fletcher, Conley, & Boardman, 2014). Even without assortative mating, the environments of spouses tend to be similar, as they constitute a household together, share their resources and influence each other's habits and attitudes.

In terms of epigenetic pathways, further pertinent questions for the study of paternal origins are whether and when the male germ-line might be open for epigenetic reprogramming and how events in somatic cells (including the brain) can signal to the germ cells, thereby breaking the classical Weismann barrier (Weismann, 1892). According to Weismann, information could pass from germ cells to somatic cells, but no information could go the other way. Soubry et al. (2014) suggested four "windows of susceptibility" for epigenetic reprogramming, one of them being the period just before puberty. A window of susceptibility defines a critical or sensitive period (Ben-Shlomo & Kuh, 2002; Kuh et al., 2003) in intergenerational influences. Chen, Yan, and Duan (2016) described a mechanism whereby the paternal environment could

influence the sperm epigenome, thus bypassing the Weissman barrier. Rodgers et al. (2013) highlighted the role of the hypothalamic-pituitary-adrenal (HPA) axis in epigenetic inheritance with the help of animal experiments.

Experience of parental death differs from periodic experience of hunger or food abundance, which has been found to influence epigenetic or phenotypic characteristics in later life (Heijmans et al., 2008) and in later generations (Pembrey et al., 2006; Vågerö et al., 2018; Veenendaal et al., 2013). The exposure to hunger or food surplus disappears entirely when the food situation normalizes again. Loss of a parent is an acute trauma, but it makes sense to see it also as a long-term stressor, causing an accumulation of stress load. Life-course mechanisms related to both sensitive periods and accumulation of exposure (Ben-Shlomo & Kuh, 2002) may thus operate simultaneously.

The term allostatic load refers to a permanent engagement of the HPA axis, which will eventually exhaust itself and cause physiological changes, possibly affecting blood pressure and body mass index (BMI) (Johnson, Cavallaro, & Leon, 2017; McEwen, 2000). We could think about allostatic load as the result of an accumulation of stress over time (Ben-Shlomo & Kuh, 2002). Psychological resilience (Bonanno, 2004) may buffer these effects, but may also itself be affected by prolonged distress, thus potentially modifying or mediating the effect of childhood trauma on outcomes in the next generation.

A study of three consecutive generations showed that trauma experience in childhood can give rise to a transgenerational response among both men and women (Vågerö & Rajaleid, 2017a). Boys exposed to parental death at age 8–12, and to a lesser extent, those exposed at age 13–17, fathered children with lower birth weight. We see this as support for the hypothesis (Pembrey et al., 2006) that a transgenerational effect through the male germ line could be triggered by exposure in pre-puberty ("the slow growth period"). The age of girls at parental loss seemed less important, thus suggesting gender-specific pathways for any transgenerational response. No effort was made, in that study to look more closely at which factors mediate influences along the male pathway.

In this study we focus on male characteristics pre-conception in order to further understand the previously observed age-dependent associations between boys' severe childhood trauma and their offspring's birth outcomes. We aimed to disentangle the effects of sensitive periods and accumulation, and shed light on the mechanisms behind these associations. Data collected at military conscription provided physiological and psychological characteristics at around age 18 for the future fathers. In addition, we used data on the education of their future spouses. Data on socioeconomic and demographic characteristics of generation 1 and generation 2 (G1 and G2), and birth outcomes of G3 were available.

2. Theory

David Barker's hypothesis that adult health and disease are already programmed in utero (Barker, 1992, 1994) has been replaced by a broader theoretical framework, referred to as the developmental origins of adult health and disease (DOHaD) (Heindel & Vandenberg, 2015). There is now considerable consensus that the early environment, interacting with the genome, influences the development of individuals, a process often referred to as "biological programming".

Barker's original hypothesis focused heavily on the maternal environment, including the environment of the woman before conception. "The womb is more important than the home", he wrote in 1990 (Barker, 1990), highlighting in particular the importance of nutrition to the growing foetus. The view that the womb is more important than the home often overlooked the fact that the womb always existed in a particular home and family, which provided a specific environment, both material and psychological, for the pregnant woman. It also tended to downplay the importance of the father.

Social class differences in birth weight, persistent over generations

(Vågerö et al., 1999), demonstrate the importance of broader social factors such as education, income and general standing in the community for foetal outcomes. These influences often crucially depend on the father. Life course approaches to adult health and disease incorporate influences from both parents and accept that all periods of life might contribute; however, they usually assume that early life factors are especially important for the biological programming of individual characteristics.

Disentangling influences that operate during the life course, for instance accumulation of health risks over time, the existence of critical periods and the effect of social mobility from childhood to adulthood, proved to be a formidable task (Hallqvist, Lynch, Bartley, Lang, & Blane, 2004). Only in recent years has strong methodology to address this problem been available (Mishra et al., 2009; VanderWeele, 2014).

The pathway from mother's environment to her foetus's growth and its health as an adult illustrates the existence of intergenerational influences on health. This causal pathway is better understood, and more intuitive, than any corresponding pathway from the father to his offspring's adult health. The paternal pathway could be seen as genetic, epigenetic, cultural or social. Sex differences in the developmental biology of the germ cell (sperm and egg) result in differences in the timing of germ cell programming in boys and girls (Hanson & Skinner, 2016). According to Soubry et al. (2014) sensitive periods for germ cell reprogramming in boys, that is modification of the male germ cell epigenome, may include the period before puberty, when primordial germ cells differentiate into spermatogonia.

It is the latter hypothesis that we set out to explore here. We refer to the Pembrey-Bygren hypothesis, most distinctly formulated in their 2006 paper, where they claim "proof-of-principle that a sex-specific male-line transgenerational effect exists in humans". This effect, they believed, was primarily epigenetic and triggered by events during the so-called slow growth period, the period just before puberty. The existence of this male-line effect was supported by empirical data on nutrition and smoking (Pembrey et al., 2006). In animal experiments there is evidence that psychological trauma can be transmitted from traumatized males to their offspring, with methylation of genes which are expressed both in germ cells and in the brain of offspring (Franklin et al., 2010).

2.1. Hypotheses and aims

Our theoretical proposal and a priori hypothesis is therefore that a specific period of boys' childhood, the slow growth period, is a sensitive period, meaning that trauma during this period could potentially influence male germ cells and lead to consequences in the next generation, such as reduced foetal growth. We hypothesize that part of this effect is mediated by an altered function of the HPA-axis and that low stress resilience is therefore a potential mediator.

Our aims were therefore to study:

1) how age at parental (G1) death predicts stress resilience, BMI, diastolic and systolic blood pressure in G2 boys at age 18 and how it predicts birth weight and length of gestation in their offspring (G3);
2) the association between G2 characteristics at age 18 and G3 birth weight and length of gestation;
3) whether G2 characteristics at age 18, or those of their spouses, mediate the association between the traumatic experience of parental loss during G2 childhood and G3 birth characteristics.

3. Methods

3.1. Study population

The population was retrieved from the Social Mobility Database, a large, temporary and anonymized database covering all individuals resident in Sweden at any time after 1960 and born 1932–2002 (Vågerö

& Rajaleid, 2017a). The Swedish Multigeneration Register provided links with their biological parents, even if the parents were born before 1932. Information from several linked registers was used: death dates (for G1) from the Cause of Death Register (from 1961 and onwards); social class in 1960 (for G1) from the Population and Housing Census; characteristics at age 18 (for G2) from the Swedish Military Conscription Register (1981 and onwards); education of spouse (G2) from the 1990 Census and birth outcomes (for G3) from the Swedish Medical Birth Register (established 1973).

We extracted all singleton births from the Medical Birth Register as G3 and established ancestral lineages by tracing their parents (G2) and grandparents (G1). Only lineages including the first born G3 of the G2 father were considered, irrespective of sex, because our previous study suggests that transgenerational response is independent of G3 parity (through G2 males) and sex (through males and females) (Vågerö & Rajaleid, 2017a). Thus, each man (G2) was represented once, together with his first-born child and information on both parents. We excluded a family if G3 gestational age was < 28 weeks, or if the G2 man underwent the military conscription examination before age 18 (in order to make sure parental death was measured before conscription) or after age 20 (as the characteristics could in this case be affected by age rather than by trauma experience). Three-generation families with complete links between G1, G2 and G3, with complete information for all included variables, were eligible for the analyses, giving us 250,427 families.

3.2. Variables

3.2.1. Exposure

Age at parental (G1) death during childhood (of G2) was divided into four categories with ± 2 years intervals around the ages 0, 5, 10 and 15, i.e. 0–2, 3–7, 8–12, and 13–17 years, similar to our previous study (Vågerö & Rajaleid, 2017a). During each interval a boy could have both parents alive (not exposed, 0), or could have experienced the death of a parent (exposed, 1). This gave an exposure profile with four values. For example, a boy who lost a parent at age 10 had the profile (0, 0, 1, 1). Boys of 18 with both parents alive had the profile (0, 0, 0, 0) and constituted the reference category. If both parents died, age at the first death was used.

Four measurement intervals and two possible categories could result in $2^4 = 16$ exposure patterns. However, as losing a parent is an irreversible event, "backwards" mobility between categories is not possible and thus the number of possible patterns is reduced (see Table 1). The lack of mobility also allows us to generalize the structured approach to four time points (Mishra et al., 2009).

3.2.2. Mediators

Several characteristics were measured at military conscription. *Stress resilience* of G2 men was evaluated as the ability to control nervousness and cope with stress in military service. It was rated by a psychologist during a 20–25 min semi-structured interview and included dimensions of social maturity, leisure interests, psychological energy and emotional stability. This produced a score, varying between

1 and 9, and followed normal distribution on population level. Higher values indicate greater stress resilience. *BMI* was calculated as (weight in kg)/(height in m²). *Diastolic* and *systolic blood pressure* were analysed as continuous variables. Blood pressure measurements were made on the first day of the conscription examination, after 5–10 min of rest (sitting). Only one measurement was made, unless systolic blood pressure exceeded 145 mm Hg or diastolic blood pressure was outside the interval of 50–85 mm Hg. In that case, a second measurement was made on the day after and the resulting value registered (Leon, Johansson, & Rasmussen, 2000).

Education of spouse was considered a potential mediator and defined as short (compulsory, 8–9 years; 26% of the 250,427 mothers), medium (secondary, usually 12 years; 51%), long (postsecondary/university; 8%) or missing (15%). Missing constituted a separate category in order not to systematically exclude the youngest part of G2. Education was analysed as a continuous variable with category value 0 for those with missing information and 1, 2, 3 representing short, medium and long education, respectively.

3.2.3. Outcomes

Birth weight (in grams) and *length of gestation* (in days) were treated as continuous variables.

3.2.4. Confounders

All the analyses adjusted for *social class of G1 father in 1960,* a*ge of G1 mother* at G2 birth, and *birth year of G2*, categorized as shown in Table 2.

3.3. Analytic approach

We applied the structured life-course approach (Mishra et al., 2009) and explored the effect of G2 age at parental death on his own stress resilience, BMI, diastolic and systolic blood pressure, and on (the future) offspring's birth weight and length of gestation. The method defines a number of regression models corresponding to different life-course hypotheses, and uses a model selection procedure to identify the model that explains the most amount of variance in outcome (Howe et al., 2016). The competing hypotheses were: accumulation; sensitive period during any age interval; no effect. Mishra et al. (2009) originally used the term critical period (as contrasted to sensitive period). We discuss this contrast in the Discussion section.

Our a priori hypothesis for offspring effects was that ages 8–12 years was a sensitive period. An additional post-hoc hypothesis with age interval 8–17 as the sensitive period was tested. Models representing the hypotheses were compared to the "saturated" or "all-inclusive" model that allows exposure during each age interval to contribute independently. All the outcomes were measured on a continuous scale and linear regression models were used. The competing models were compared with partial *F*-test (Mishra et al., 2009). The results guided us when defining exposure categories in the four-way decomposition analyses.

Associations between G2 characteristics at conscription and offspring birth outcomes were visualized by dividing all the characteristics into nine categories with cut-points at approximately the same percentiles as for the stress resilience score (resulting in category sizes of approximately 1%, 6%, 10%, 14%, 27%, 18%, 16%, 6% and 2%, respectively), and calculating average birth weight and length of gestation for each category.

The four-way decomposition analysis (VanderWeele, 2014) was used to decompose the effect of age of G2 at parental death on G3 birth outcomes, assuming mediation by G2 men's stress resilience, BMI, diastolic or systolic blood pressure. To address the issue of assortative mating we also tested whether the men's choice of a spouse with a similar education was a mediating factor. The method allows one to study the overall effect of an exposure on an outcome when a mediator is present, and decomposes this effect into four components: due to

Table 1
Exposure profiles and the life course hypotheses relevant for each profile.

Age at parental death	Critical periods covered				Accumulation score
	0–2 years	3–7 years	8–12 years	13–17 years	
0–2 years	yes	yes	yes	yes	4
3–7 years	no	yes	yes	yes	3
8–12 years	no	no	yes	yes	2
13–17 years	no	no	no	yes	1
18 + years	no	no	no	no	0

Table 2
Description of the three generations included in the analyses: $n = 250{,}427$ men (generation 2), their parents (generation 1) and firstborn offspring (generation 3), stratified by generation 2 age at parental death.

	Age of G2 at death of G1				
	0–2 years $n = 863$	3–7 years $n = 2162$	8–12 years $n = 3253$	13–17 years $n = 4553$	≥ 18 years $n = 239{,}596$
Characteristics of G1					
Social class of G1 father in 1960, n (%)					
Non-manual occupation	150 (17%)	402 (19%)	584 (18%)	850 (19%)	43,816 (18%)
Self-employed	112 (13%)	247 (11%)	401 (12%)	540 (12%)	27,847 (12%)
Manual occupation	417 (48%)	1060 (49%)	1564 (48%)	2236 (49%)	111,455 (47%)
Other	121 (14%)	278 (13%)	419 (13%)	582 (13%)	39,057 (16%)
Missing	63 (7%)	175 (8%)	285 (9%)	345 (8%)	17,421 (7%)
Age of G1 mother at G2 birth, n (%)					
≤ 20 years	126 (15%)	250 (12%)	364 (11%)	445 (10%)	34,183 (14%)
21–25 years	280 (32%)	660 (31%)	863 (27%)	1227 (27%)	91,061 (38%)
26–34 years	325 (38%)	912 (42%)	1382 (42%)	2004 (44%)	96,687 (40%)
≥ 35 years	132 (15%)	340 (16%)	644 (20%)	877 (19%)	17,665 (7%)
Characteristics of G2					
Birth year, n (%)					
1963–1966	254 (29%)	654 (30%)	1030 (32%)	1423 (31%)	67,411 (28%)
1967–1970	356 (41%)	846 (39%)	1280 (39%)	1833 (40%)	97,780 (41%)
1971–1975	204 (24%)	545 (25%)	747 (23%)	1036 (23%)	61,493 (26%)
1976–1986	49 (6%)	117 (5%)	196 (6%)	261 (6%)	12,912 (5%)
Stress resilience[1] at age 18, mean (SD)	4.93 (1.67)	4.84 (1.69)	4.79 (1.69)	4.91 (1.71)	5.21 (1.61)
Body mass index (kg/m^2) at age 18, mean (SD)	22.0 (2.71)	22.0 (2.79)	22.0 (2.84)	22.1 (3.03)	21.9 (2.70)
Diastolic blood pressure (mm Hg) at age 18, mean (SD)	64.5 (9.81)	64.5 (10.04)	65.2 (10.01)	64.9 (9.81)	64.7 (9.83)
Systolic blood pressure (mm Hg) at age 18, mean (SD)	128.1 (10.8)	128.0 (10.5)	128.4 (10.6)	128.1 (10.8)	128.2 (10.6)
Characteristics of G3					
Birth weight (grams), mean (SD)	3520 (527)	3516 (552)	3488 (558)	3498 (549)	3508 (542)
Length of gestation (number of days), mean (SD)	279 (13)	279 (13)	279 (13)	279 (13)	279 (13)

Abbreviations: G, generation; SD, standard deviation.

[1] possible values 1 … 9, higher values indicate greater stress resilience.

mediation only, to interaction between the exposure and mediator only, to both mediation and interaction, and to neither mediation nor interaction. In these analyses we treated the exposure variable (age at parental death) as suggested by the structured life-course analyses described above. The effect decomposition was performed at the most prevalent values of the confounders and at the 15th or 85th percentile of the mediators. We chose the level 15% as it identifies men with stress resilience score 1–3 which is considered low (Bergh et al., 2015). Cut point at 85% for BMI and blood pressure identifies a risk group of the same size.

We used StataSE 14 (Stata Corp LP, College Station, Texas, USA).

4. Results

4.1. Description of the data

Table 2 presents the three generations by G2 age at parental death.

4.2. Structured life-course approach

Table 3 shows estimated differences in outcomes in G2 and G3. Stress resilience was lower for all the < 18 years age categories than for unexposed G2; BMI was higher in those who lost a parent at age 8–12 or 13–17; offspring birth weight and length of gestation were lower in those who lost a parent at age 8–12. The differences were statistically significant but small (e.g. length of gestation was half a day less and BMI was 0.1 units higher, which corresponds to < 300 g for persons whose height is 170 cm).

Table 4 shows P values from the partial F-test comparing models representing the different life-course models with the saturated model. P values > 0.05 indicate that the simpler model is not significantly worse than the saturated model and is thus suitable for describing the data. None of the models for stress resilience had $P > 0.05$. The no effect model ($P = 0.3614$) indicates that parental death is not a predictor of systolic blood pressure. However, overall the models for the

critical period around ages 8–12 and 13–17 tended to have the highest P values, thus suggesting that these periods are sensitive. Consequently, we defined a post-hoc hypothesis with ages 8–17 as the sensitive period. The models for age 8–17 as the sensitive period described the data well, especially for G3 outcomes. We therefore used this age categorization in the four-way decomposition below.

4.3. Paternal characteristics at 18 and offspring birth outcomes

The average birth weight of G3 increased monotonically across the categories of G2 stress resilience and BMI, with almost 100 g difference between the lowest and highest category; the increase across categories of diastolic and systolic blood pressure was less clear (Fig. 1). Length of gestation of G3 did not show clear trends across categories of G2 characteristics (not shown).

4.4. Four-way decomposition analysis

The total effect of G2 parental death at age 8–17 (vs all other ages) on G3 birth weight was estimated at around -18 g (Table 5), and decomposed into four components for each of the hypothesised mediators. For stress resilience, both components indicating mediation of the effect (pure indirect effect and mediated interaction) had the same direction and were statistically significant at 0.05-level. Thus, we conclude that stress resilience mediated the association between parental death and offspring birth weight. The sum of these two components (i.e. the total indirect effect), was around -7.3 g, or about 40% (95% confidence interval: 8%–72%) of the total effect. The estimated values of controlled direct effect and reference interaction changed when the level of stress resilience was varied (not shown). Their sum (i.e. the pure direct effect (VanderWeele, 2014)), indicating the effect operating through other pathways independent of stress resilience, was always around -10.7 g. Decomposing the total effect for the other three hypothesized mediators indicated no mediation, as point estimates of pure indirect effect and mediated interaction were close to zero. Testing spouse education as a

Table 3

Difference in outcomes according to age at parental death, estimated by linear regression models: $n = 250,427$ men (generation 2), and their firstborn offspring (generation 3).

	Outcomes in exposed generation, G2, at age 18										Outcomes in offspring generation, G3			
	Stress resilience (score 0 … 9)		Body mass index (kg/m^2)		Diastolic blood pressure (mm Hg)		Systolic blood pressure (mm Hg)			Birth weight (grams)		Length of gestation (days)		
	β	95% CI	β	95% CI	β	95% CI	β	95% CI		β	95% CI	β	95% CI	
G2 age at parental death														
0–2 years	−0.26	−0.37, −0.16	0.09	−0.09, 0.27	−0.2	−0.9, 0.4	−0.1	−0.8, 0.6		10.9	−24.6, 46.6	0.0	−0.8, 0.9	
3–7 years	−0.36	−0.43, −0.29	0.05	0.06, 0.17	−0.2	−0.6, 0.3	−0.3	−0.7, 0.2		6.5	−16.9, 29.9	−0.2	−0.7, 0.4	
8–12 years	−0.40	−0.46, −0.35	0.10	0.00, 0.19	0.5	0.1, 0.8	0.1	−0.2, 0.5		−22.1	−41.4, −2.9	−0.5	−1.0, −0.1	
13–17 years	−0.30	−0.35, −0.25	0.12	0.04, 0.20	0.2	−0.1, 0.5	−0.2	−0.5, 0.1		−12.0	−28.1, 4.1	−0.3	−0.6, 0.1	
18 + years	0	Referent	0	Referent	0	Referent	0	Referent		0	Referent	0	Referent	

Abbreviations: CI, confidence interval; G, generation

mediator gave a point estimate of 0% mediation (95% confidence interval: −0.0%-0.1%).

The total effect of G2 parental death on G3 length of gestation was around −0.4 days and further decomposition of this effect did not give meaningful results (Table 4).

5. Discussion

Early severe stress produces a cascade of neurobiological events with the potential to cause enduring changes in brain development. These changes include neurohumoral (especially the HPA axis) mechanisms (Teicher et al., 2003). Parental death during childhood/adolescence may thus have lasting effects on the HPA axis, even in the absence of psychopathology (Nicolson, 2004). In our study, psychological resilience was influenced by parental loss, predicted offspring birth weight, and mediated the association between parental loss and offspring's birth weight. It is conceivable that a man with a low tolerance of stress influences his partner's pregnancy in a negative way, suggesting a cultural pathway for such mediation. However, since this mediation was not observed for boys who experienced parental death before age 8, we see this cultural pathway as a less likely explanation.

We considered the role of memory. If we assume that the earlier the loss occurred, the milder the consequences will be for the boy (he does not remember his dead parent and a step-parent may have filled his/her place), we would expect to see a "reversed" accumulation effect. The effect on stress resilience would in this case be strongest for the oldest age group and gradually weaker for those boys who lost the parent at younger ages. We tested the possible accumulation effect, thinking mainly about the opposite alternative, i.e. the longer the time without a parent, the greater the effect. However, these two alternative analyses are actually statistically equivalent. Table 4 shows that the accumulation hypothesis was not supported at all for stress resilience

($P = 0.000$). Thus, we consider it less likely that a more recent and vivid memory of parental death explains the observed effect in offspring.

The role of BMI in the association between parental loss and offspring birth weight was difficult to disentangle because several simultaneously operating mechanisms may have cancelled each other out. Long term stress load and experience of stressful life events are both associated with less healthy behaviors, including less physical exercise (increases BMI) and more smoking (reduces BMI) (Stults-Kolehmainen & Sinha, 2014). In our data, parental loss in mid- and late childhood was linked to increased BMI at age 18. Higher BMI, in turn, predicted increased offspring birth weight. Paternal obesity is linked to loss of methylation in the *IGF2* gene in newborn offspring (Soubry et al., 2013), suggesting a mechanism by which paternal exposures may affect offspring growth in utero. However, the total effect of parental loss on next generation's birth weight in our study was negative and we detected practically no mediation by BMI.

Continuity in social disadvantage across generations could result in an association between parental death and low birth weight of offspring. Controlling for family social class and mother's age at childbirth gave no support for such an explanation.

Men who lost their parents were slightly less likely to get a long education (not shown). It is possible that they were more often smokers as well (Kennedy et al., 2019). We considered the possibility that they also tended to partner women with a lower education which, in turn, could correlate with health behaviours (smoking in pregnancy being the factor with the strongest potential to affect next generation's birth weight). Such assortative mating allows us to see maternal characteristics as downstream mediators in a causal chain departing from the boy's childhood trauma, leading to his low stress resilience and operating further through pathways involving his own health behaviours (including smoking), as well as through the characteristics of the
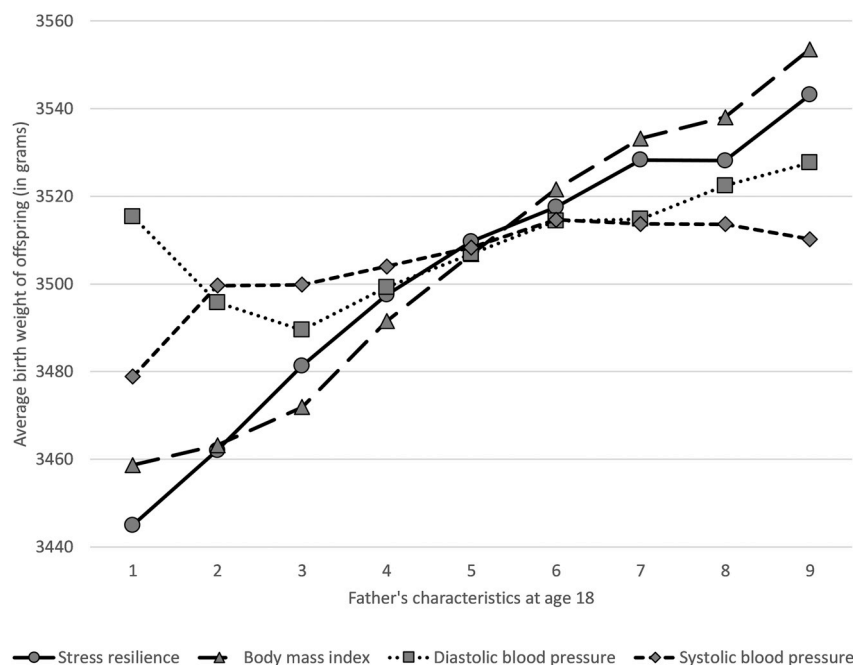
Table 4

P values from partial F-test comparing models representing the different life course models against saturated model, studying outcomes in 250,427 men (generation 2) and their firstborn offspring (generation 3).

	Outcomes in exposed generation, G2, at age 18				Outcomes in offspring generation, G3	
	Stress resilience (score 0 … 9)	Body mass index (kg/m^2)	Diastolic blood pressure (mm Hg)	Systolic blood pressure (mm Hg)	Birth weight (grams)	Length of gestation (days)
Critical period models:						
0–2 years	0.0000	0.0042	0.0172	0.2371	0.0510	0.0493
3–7 years	0.0000	0.0056	0.0233	0.4238	0.0591	0.0525
8–12 years	0.0000	0.0296	0.0293	0.2388	0.0721	0.2553
13–17 years	0.0155	0.8430	0.0719	0.4205	0.1732	0.5907
8–17 years	0.0000	0.5563	0.5036	0.2717	0.7249	0.7917
Accumulation of risk	0.0000	0.1316	0.0219	0.3538	0.0622	0.2324
No effect	0.0000	0.0067	0.0317	0.3614	0.0862	0.0971

Abbreviations: G, generation

Fig. 1. Average birth weight of offspring (generation 3) by categories of stress resilience, body mass index, diastolic and systolic blood pressure of father (generation 2) at age 18.

partner he chooses. We analysed the mediating effect of maternal education, but could not detect any such mediation at all. We therefore conclude that spouse education and related factors do not explain our observed results. Assortative mating based on education is considerably stronger than that based on shared genes (Domingue et al., 2014). Vågerö and Rajaleid (2017b) showed previously that correlation of birth weights between spouses in this population was only 0.02.

We considered whether parental loss might influence the next generation primarily within a specific age window of susceptibility or through the consequences of trauma that accumulate over a long period. Our evidence supported the first alternative, but the two alternatives are not mutually exclusive.

Critical periods are seen as limited time windows during development in which exposures can have long-lasting effects on function and health outcomes (Ben-Shlomo & Kuh, 2002; Kuh et al., 2003). The notion of sensitive periods, on the other hand, is less absolute and allows for modification or reversal of changes as well as weaker effects outside the specific time frame. We consider it a more appropriate term in relation to our analyses and findings.

A sensitivity period between ages 8 and 17 gave the best data fit for outcomes in the next generation. In the literature there is some ambiguity about when sensitivity periods occur, although there seems to be consensus that they are gender-specific, due to the differential development of germ cells in boys and girls (Hanson & Skinner, 2016). Several studies suggest that pre-puberty may be open for epigenetic reprogramming of male germ cells (Northstone, Golding, Davey Smith, Miller, & Pembrey, 2014; Pembrey et al., 2006; Soubry et al., 2014). A study of offspring asthma in relation to early paternal smoking defined the sensitive period as before age 15, "the mean of completed puberty" (Svanes et al., 2017; Accordini et al., 2018). Two studies (Soubry et al., 2014; Wu et al., 2015) added "the reproductive cycle", a 74-day long process when spermatogonia are transformed into mature sperm in a constantly repeated cycle, from puberty into adulthood. In experiments on mice it has been difficult to separate peri-pubertal influences from influences during puberty and indeed adult life. Thus, Rodgers et al. (2013) concluded that "male exposure to stress, either throughout puberty, or in adulthood, reprograms paternal germ cells and results in transmission of an offspring HPA stress axis dysregulation phenotype".

Support for a cumulative effect comes from a study of pre-fatherhood betel chewing and offspring metabolic syndrome (Yen et al., 2016).

5.1. Methodological considerations

The key strength of our nationwide study sample is that it spans three linked generations. Its size allows analyses of rare exposures and the identification of small effects. These may have little clinical significance, but nevertheless be of great theoretical value.

Men with chronic illness or disability (approximately 4%) did not attend the military assessment. The assessment was discontinued if a condition that made the man ineligible for conscription was identified. Thus, the sample is somewhat selected for better health (Bergh et al., 2015).

The interviewing psychologist may have known about any parental death, which may have influenced scoring of resilience. It is less probable that this bias was dependent on the age when the death occurred. Measurements of BMI and blood pressure cannot be biased by parental loss. They showed partly consistent results with stress resilience (were predicted by parental loss; predicted next generation's birth weight) thus strengthening the results observed for stress resilience. Death of a parent is an objective measure of a severely stressful event. Birth outcomes are recorded with high quality and errors in the Medical Birth Register cannot be related to grandparental death.

Our analyses rely on certain assumptions: no unmeasured confounding between the exposure and the mediator, the exposure and the outcome, or mediator and the outcome; and no measurement error, particularly in the mediator (VanderWeele, 2014). We adjusted the analyses for G1 father's social class; G1 mother's age at the birth of G2; and birth year of G2. As social class, age at family formation, and birth years are correlated within married/cohabiting couples and across generations of the same family, these adjustments crudely control for factors related to historical time, age and social background, while avoiding over-adjustment (e.g. collinearity problems).

5.2. Conclusion

We found evidence that parental loss in boys at age 8–17 could

Table 5
Results from four-way decomposition analysis: n = 250,427 men (generation 2), and their firstborn offspring (generation 3).

Exposure	Mediator	Outcome	Total effect	Controlled direct effect (due neither to mediation nor interaction)	Reference interaction (due to interaction only)	Mediated interaction (due to mediation and interaction)	Pure indirect effect (due to mediation only)
Parental death at age 8-17	Stress resilience	Birth weight	***-18.0 (−30.3, −5.7)	*63.5 (−10.1, 137.1)	**,-74.2 (−143.7, −4.8)	**-3.0 (−5.8, −0.2)	***-4.3 (−4.9, −3.6)
	Body mass index		***-18.4 (−30.7, −6.2)	7.9 (−253.7, 269.4)	−27.4 (−289.4, 234.6)	0.1 (−0.5, 0.6)	***1.1 (0.6, 1.5)
	Diastolic blood pressure		***-18.4 (−30.6, −6.2)	*-26.4 (−54.0, 1.1)	7.9 (−17.3, 33.0)	−0.1 (−0.6, 0.3)	***0.3 (0.1, 0.5)
	Systolic blood pressure		***-18.5 (−30.8, −6.3)	22.0 (−28.9, 72.8)	−40.5 (−90.1, 9.0)	0.1 (−0.1, 0.4)	−0.1 (−0.2, 0.1)
Parental death at age 8-17	Stress resilience	Length of gestation	***-0.4 (−0.7, −0.1)	0.8 (−0.9, 2.5)	−1.1 (−2.7, 0.5)	−0.0 (−0.1, 0.0)	***-0.0 (0.0, 0.0)
	Body mass index		***-0.4 (−0.7, −0.1)	3.4 (−2.7, 9.4)	−3.8 (−9.9, 2.3)	0.0 (−0.0, 0.0)	0.0 (−0.0, 0.0)
	Diastolic blood pressure		***-0.4 (−0.7, −0.1)	***-0.9 (−1.6, −0.3)	*0.6 (−0.0, 1.1)	−0.0 (−0.0, 0.0)	0.0 (−0.0, 0.0)
	Systolic blood pressure		***-0.4 (−0.7, −0.1)	−0.0 (−1.2, 1.1)	−0.4 (−1.5, 0.8)	0.0 (−0.0, 0.0)	−0.0 (−0.0, 0.0)

All the analyses were adjusted for social class of G1 father in 1960, age of G1 mother at G2 birth and birth year of G2.

*** statistically significant, $P < 0.01$.

** statistically significant, $P < 0.05$.

* statistically significant, $P < 0.1$.

influence offspring birth outcomes. Our a priori hypothesis was that the 8–12 year period was the most sensitive one. However, a longer age interval gave the best fit to data. We found no evidence that assortative mating is likely to explain our results.

Psychological resilience, measured by in-depth interview at age 18, was itself influenced by parental loss and in addition mediated a large part of its influence on offspring birth weight. Resilience to stress has been defined as "the overall pattern of HPA responses to challenge, encompassing the rate of initial response to challenge, the magnitude of the response, and the rate of recovery of the HPA axis to the basal state" (Seeman & Robbins, 1994).

Could the HPA-axis response to early-life trauma in some way be captured by the gametes, and thus influence the male germ line epigenome? According to previous biological thinking, the "Weismann barrier" forbids soma to germ line communication. However, Sharma (2017) speculated about "a hypothetical nervous system-spermatogenesis axis", which bypasses this barrier. Exactly how, if at all, psychological resilience or BMI influence male germ cells we leave open (Baxter & Drake, 2019). We are only at the beginning of understanding this phenomenon.

Conflicts of interest

The authors declare that they have no conflict of interest.

Funding

Author contributions

KR and DV designed the research; KR analyzed data; KR and DV wrote the paper.

Ethical approval statement

Stockholm Ethical Review Board gave ethical permission [2009/1115–32]. All analyses used anonymized routine registry data.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ssmph.2019.100429.

References

Accordini, S., Calciano, L., Johannessen, A., et al. (2018). A three-generation study on the association of tobacco smoking with asthma. *International Journal of Epidemiology, 47*(4), 1106–1117.

Barker, D. (1990). The foetal and infant origins of adult disease. The womb might be more important than the home. *BMJ, 301*, 1111.

Barker, D. (Ed.). (1992). *Fetal and infant origins of adult disease.* London: BMJ Publications.

Barker, D. (Ed.). (1994). *Mothers, babies and disease in later life.* London: BMJ Publishing Group.

Baxter, F. A., & Drake, A. J. (2019). Non-genetic inheritance via the male germline in mammals. *Philosophical Transactions of the Royal Society, 374*, 20180118. https://doi:org/10.1098/rstb.2018.0118.

Ben-Shlomo, Y., & Kuh, D. (2002). A life course approach to chronic disease

epidemiology: Conceptual models, empirical challenges and interdisciplinary perspectives. *International Journal of Epidemiology, 31*, 285–293. https://doi.org/10.1093/ije/31.2.285.

Bergh, C., Udumyan, R., Fall, K., et al. (2015). Stress resilience and physical fitness in adolescence and risk of coronary heart disease in middle age. *Heart, 101*(8), 623–629.

Bonanno, G. A. (2004). Loss, trauma and human resilience: Have we underestimated the human capacity to thrive after extremely aversive events? *American Psychologist, 59*, 20–28.

Chen, Q., Yan, W., & Duan, E. (2016). Epigenetic inheritance of acquired traits through sperm RNAs and sperm RNA modifications. *Nature Reviews Genetics, 17*, 733–743.

De Stavola, B. L., Leon, D. A., & Koupil, I. (2011). Intergenerational correlations in size at birth and the contribution of environmental factors: The Uppsala Birth Cohort Multigenerational Study, Sweden, 1915-2002. *American Journal of Epidemiology, 174*(1), 52–62. https://doi.org/10.1093/aje/kwr032.

Domingue, B., Fletcher, J., Conley, D., & Boardman, J. D. (2014). Genetic and educational assortative mating among US adults. *Proceedings of the National Academy of Sciences, 111*, 7996–8000.

Franklin, T. B., Russig, H., Weiss, I. C., Gräff, J., Linder, N., Michalon, A., et al. (2010). Epigenetic transmission of the impact of early stress across generations. *Biological Psychiatry, 68*(5), 408–415.

Hallqvist, J., Lynch, J., Bartley, M., Lang, T., & Blane, D. (2004). Can we disentangle life course processes of accumulation, critical period and social mobility? An analysis of disadvantaged socio-economic positions and myocardial infarction in the stockholm heart epidemiology program. *Social Science & Medicine, 58*(8), 1555–1562.

Hanson, M., & Skinner, M. (2016). Developmental origins of epigenetic transgenerational inheritance. *Environmental Epigenetics*. https://doi.org/10.1093/eep/dvw002.

Heijmans, B. T., Tobi, E. W., Stein, A. D., et al. (2008). Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proceedings of the National Academy of Sciences, 105*(44), 17046–17049.

Heindel, J. J., & Vandenberg, L. N. (2015). Developmental origins of health and disease: A paradigm for understanding disease etiology and prevention. *Current Opinion in Pediatrics, 27*(2), 248–253. https://doi.org/10.1097/MOP.0000000000000191.

Howe, L. D., Smith, A. D., Macdonald-Wallis, C., et al. (2016). Relationship between mediation analysis and the structured life course approach. *International Journal of Epidemiology, 45*(4), 1280–1294.

Johnson, S. C., Cavallaro, F. L., & Leon, D. A. (2017). A systematic review of allostatic load in relation to socioeconomic position: Poor fidelity and major inconsistencies in biomarkers employed. *Social Science & Medicine, 192*, 66–73. https://doi.org/10.1016/j.socscimed.2017.09.025.

Kennedy, B., Chen, R., Fang, F., et al. (2019). Low stress resilience in late adolescence and risk of smoking, high alcohol consumption and drug use later in life. *Journal of Epidemiology & Community Health, 73*(6), 496–501.

Kuh, D., Ben-Shlomo, Y., Lynch, J., et al. (2003). Life course epidemiology. *Journal of Epidemiology & Community Health, 57*(10), 778–783.

Leon, D. A., Johansson, M., & Rasmussen, F. (2000). Gestational age and growth rate of fetal mass are inversely associated with systolic blood pressure in young adults: An epidemiologic study of 165,136 Swedish men aged 18 years. *American Journal of Epidemiology, 152*, 597–604.

Lunde, A., Melve, K. K., Gjessing, H. K., Skjaerven, R., & Irgens, L. M. (2007). Genetic and environmental influences on birth weight, birth length, head circumference, and gestational age by use of population-based parent-offspring data. *American Journal of Epidemiology, 165*(7), 734–741.

McEwen, B. (2000). Allostasis and allostatic load: Implications for neuropsychopharmacology. *Neuropsychopharmacology, 22*, 108–124.

Mishra, G., Nitsch, D., Black, S., et al. (2009). A structured approach to modelling the effects of binary exposure variables over the life course. *International Journal of Epidemiology, 38*(2), 528–537.

Nicolson, N. (2004). Childhood parental loss and cortisol levels in adult men. *Psychoneuroendocrinology, 29*(8), 1012–1018.

Northstone, K., Golding, J., Davey Smith, G., Miller, L., & Pembrey, M. (2014). Prepubertal start of father's smoking and increased body fat in his sons: Further characterization of paternal transgenerational response. *European Journal of Human Genetics, 22*, 1382–1386.

Olsson, A., Togawa, K., Schüz, J., et al. (2018). Parental occupational exposure to solvents and heavy metals and risk of testicular germ cell tumours in sons (NORD-TEST Denmark). *Scandinavian Journal of Work, Environment & Health, 44*(6), 658–669.

Pembrey, M. E., Bygren, L. O., Kaati, G., et al. (2006). Sex-specific, male-line transgenerational responses in humans. *European Journal of Human Genetics, 14*(2), 159–166.

Rodgers, A., Morgan, C., Bronson, S., et al. (2013). Paternal stress exposure alters sperm microRNA content and reprograms offspring HPA stress axis regulation. *Journal of Neuroscience, 33*(21), 9003–9012.

Seeman, T., & Robbins, R. (1994). Aging and hypothalamic-pituitary-adrenal response to challenge in humans. *Endocrine Reviews, 15*(2), 233–260.

Sharma, A. (2017). Transgenerational epigenetics: Integrating soma to germ line communication with gametic inheritance. *Mechanisms of Ageing and Development, 163*, 15–22.

Shea, K. M., & Little, R. E. the ALSPAC Study Team. (1997). Is there an association between preconception paternal x-ray exposure and birth outcome? *American Journal of Epidemiology, 145*(6), 546–551.

Soubry, A. (2018). POHaD: Why we should study future fathers. *Environ Epigen, 4*(2), dvy007. https://doi.org/10.1093/eep/dvy007.

Soubry, A., Hoyo, C., Jirtle, R., et al. (2014). A paternal environmental legacy: Evidence for epigenetic inheritance through the male germ line. *BioEssays, 36*, 359–371.

Soubry, A., Schildkraut, J., Murtha, A., et al. (2013). Paternal obesity is associated with *IGF2* hypomethylation in newborns: Results from a newborn epigenetics study (NEST) cohort. *BMC Medicine, 11*, 29.

Stults-Kolehmainen, M. A., & Sinha, R. (2014). The effects of stress on physical activity and exercise. *Sports Medicine, 44*(1), 81–121.

Svanes, C., Koplin, J., Skulstad, S. M., Johannesen, A., Bertelsen, R. J., Benediktsdottir, B., et al. (2017). Father's environment before conception and asthma risk in his children: A multigeneration analysis of the respiratory health in norhtern europe study. *International Journal of Epidemiology, 46*, 235–245. https://doi.org/10.1093/ije/dyw151.

Teicher, M. H., Andersen, S. L., Polcari, A., et al. (2003). The neurobiological consequences of early stress and childhood maltreatment. *Neuroscience & Biobehavioral Reviews, 27*(1–2), 33–44.

Vågerö, D., Koupilová, I., Leon, D. A., & Lithell, U. B. (1999). Social determinants of birthweight, ponderal index and gestational age in Sweden in the 1920s and the 1980s. *Acta Paediatrica, 88*(4), 445–453.

Vågerö, D., Pinger, P. R., Aronsson, V., & van den Berg, G. (2018). Paternal grandfather's access to food predicts all-cause and cancer mortality in grandsons. *Nature Communications, 9*(1), 5124. https://doi.org/10.1038/s41467-018-07617-9.

Vågerö, D., & Rajaleid, K. (2017a). Does childhood trauma influence offspring's birth characteristics? *International Journal of Epidemiology, 46*(1), 219–229.

Vågerö, D., & Rajaleid, K. (2017b). Transgenerational response and life history theory: A response to Peeter Hõrak. *International Journal of Epidemiology, 46*, 233–234.

VanderWeele, T. J. (2014). A unification of mediation and interaction: A four-way decomposition. *Epidemiology, 25*(5), 749–761.

Veenendaal, M., Painter, R., de Rooij, S., et al. (2013). Transgenerational effects of prenatal exposure to the 1944–45 Dutch famine. *BJOG: An International Journal of Obstetrics and Gynaecology, 120*, 548–554.

Weismann, A. (1892). *Das Keimplasma: eine Theorie der Vererbung.* Jena: Fischer.

Whitelaw, E. (2006). Epigenetics: Sins of the fathers, and their fathers. *European Journal of Human Genetics, 14*(2), 131–132.

Wu, H., Rauser, R., Krawetz, S., et al. (2015). Environmental susceptibility of the sperm epigenome during windows of male germ cell development. *Current Environmental Health Reports, 2*(4), 356–366.

Yen, A. M., Boucher, B. J., Chiu, S. Y., et al. (2016). Longer duration and earlier age of onset of paternal betel chewing and smoking increase metabolic syndrome risk in human offspring, independently, in a community-based screening program in Taiwan. *Circulation, 134*(5), 392–404.