

## ORIGINAL ARTICLE

# Increasing paternal age at childbirth is associated with taller stature and less favourable lipid profiles in their children

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## Abstract

**Background** Paternal age at childbirth has been increasing worldwide, and we assessed whether this increase affects growth, body composition and metabolism in their children.

**Methods** We studied 277 children (aged 3–12 years) born to fathers aged 19.8–51.8 years. Clinical assessments were height and weight adjusted for parental measurements, DEXA-derived body composition, fasting lipids, glucose homeostasis and hormonal profiles.

**Results** Children born to fathers aged 31–35 ( $P = 0.009$ ) and >35 years ( $P = 0.021$ ) were 2 cm taller than those of fathers aged  $\leq 30$  years. Children of fathers aged >35 years at childbirth had a lower body mass index (BMI) ( $-0.32$  SDS) than offspring of fathers aged 31–35 ( $-0.01$  SDS;  $P = 0.043$ ) and  $\leq 30$  ( $0.22$  SDS;  $P = 0.019$ ). There were marked effects of paternal age at childbirth on childhood blood lipids. LDL-C concentrations in children born to fathers aged >35 years were 11% and 21% higher than in children of fathers aged 31–35 and  $\leq 30$  years, respectively ( $P < 0.01$ ). Total cholesterol to HDL-C ratio was also higher among the children of fathers aged 31–35 (12%;  $P = 0.014$ ) and >35 (16%;  $P = 0.004$ ) years at childbirth compared with the  $\leq 30$  group. In addition, HOMA-IR in girls (but not boys) born of fathers aged 31–35 ( $0.99$ ) and >35 years ( $1.11$ ) indicated better insulin sensitivity compared with offspring in the  $\leq 30$  group ( $1.63$ ;  $P < 0.05$ ).

**Conclusions** Increasing paternal age at childbirth is associated with a more favourable phenotype in their children (taller and slimmer, with better insulin sensitivity in girls) but with a less favourable lipid profile.

(Received 21 December 2012; returned for revision 17 June 2013; finally revised 18 June 2013; accepted 19 June 2013)

## Introduction

There has been a major shift in reproductive behaviour over the past several decades with an increasing number of couples having children in their thirties and forties.<sup>1</sup> While the increase in maternal age at childbirth has received much attention, an upwards shift in paternal age at childbirth is also taking place. In most developed countries, the age at first fatherhood has increased from an average age of 29 to 32 years between 1980 and 2000.<sup>2</sup> As a result, in 2003, more than 40% of children in the UK were born to fathers aged 35 years or older, and this trend towards older age at fatherhood shows no signs of abating.<sup>2</sup> Many reasons may account for the trend towards postponement of fatherhood, including economic pressures and personal choice (see review by Roberts et al<sup>3</sup>).

Some studies suggest that male fertility and sperm DNA quality start to decline after approximately 35 years of age.<sup>4</sup> Such a decline leads to an increased risk of spontaneous gene mutations, DNA damage and possibly epigenetic changes in sperm genes. However, unlike maternal age, there appears to be no clear threshold beyond which male gamete quality starts to decline, or when the risk of adverse childhood outcomes definitively increases with increasing paternal age at childbirth. The available evidence suggests that 20–30 years may represent the lowest paternal age-related risk of adverse health outcomes in the offspring,<sup>5</sup> with this risk becoming greater thereafter. There is some evidence that a paternal age at childbirth of approximately 35–37 years could mark the start of a nonlinear increase in the risk to the offspring.<sup>5,6</sup>

Increasing paternal age at childbirth is associated with a small, but appreciable risk of genetic and birth defects, as well as a slight increase in offspring risk of autism, adult psychiatric disorders and some childhood cancers.<sup>2,7</sup> A recent study found that increasing paternal age is associated with a higher rate of obesity among offspring in adulthood.<sup>8</sup> Increasing maternal age at childbirth is associated with alterations in offspring height and body composition<sup>9</sup> and type 1 diabetes risk.<sup>10</sup> However, the possible effects of paternal age on childhood growth and metabolism have not previously been examined. Therefore, we aimed to assess whether increasing paternal age at childbirth would be associated with changes in the height, body composition, metabolism and hormonal profiles in the offspring in childhood.

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## Methods

### Study cohort

We undertook a large project examining the effects of parental and prenatal factors in the offspring. From this larger project, we have examined the impact of conception with ovarian stimulation drugs on the growth and metabolism of children.<sup>11</sup> Children conceived after ovarian stimulation were asked to invite 4–5 family friends and school friends who were naturally conceived to participate in the study as controls,<sup>11</sup> so that these controls were recruited by study participants, and were of similar age group, ethnicity and socio-economic status. Thus, in this current study, we assessed the entire naturally conceived cohort that was recruited from this larger project (between October 2010 and October 2012).

Only healthy, developmentally normal, prepubertal children aged 3–12 years, born 37–41 weeks gestation were studied. All children were of New Zealand European ethnicity, naturally conceived, born of singleton pregnancies, and of birthweight appropriate-for-gestational-age (birthweight  $>-2$  and  $<2$  standard deviation scores (SDS)). Exclusion criteria also included signs of puberty (Tanner stage 2 breast development in girls and testicular volume  $>3$  ml in boys or evidence of adrenarche), receiving medication that could affect insulin sensitivity or growth, as well as having a first-degree relative with prediagnosed diabetes. Children were excluded if born to mothers with gestational diabetes, chronic illnesses or prolonged maternal drug use (including tobacco and alcohol). All participants were of higher socio-economic status according to their residential address and the 'decile score' of the school they attended. A decile score reflects the socio-economic status of the school communities, and it is a comprehensive assessment of community affluence. It takes into account a number of factors such as household income, parental occupation, parents' educational qualifications, number of occupants per dwelling size and government welfare benefits. All participants in the study were of decile 9 or 10 (highest) socio-economic status.

### Clinical assessments

All clinical assessments were carried out by a single researcher at the Maurice & Agnes Paykel Clinical Research Unit (Liggins Institute, University of Auckland). Standing height was measured using a Harpenden stadiometer. Children's weight and body composition were assessed using dual-energy X-ray absorptiometry (DEXA Lunar Prodigy 2000; General Electric, Madison, WI, USA). Apart from total body fat percentage, the DEXA-derived parameters of interest were percentage truncal fat, android fat to gynoid fat ratio (an indicator of abdominal adiposity) and bone mineral density (L1–L4). Each child also had a bone age X-ray to assess biological maturity, which was blindly assessed by a single paediatric endocrinologist using pre-established standards.<sup>12</sup>

Maternal and paternal height, weight and body mass index (BMI) were measured and recorded. Maternal obstetric history was also recorded to clarify parity and relevant medical history.

Children's birthweight, height and BMI were transformed into SDS.<sup>13,14</sup> Mid-parental height SDS (MPHSDS) was calculated for each child.<sup>15</sup> Children's heights SDS (HtSDS) were then individually corrected for their genetic potential (parents' heights), using the formula: height standard deviation score corrected for mid-parental height standard deviation score ('HtSDS–MPHSDS'). Parents' BMI were transformed into SDS, and the mean parental body mass index standard deviation score (MPBMISDS) was calculated for each child.

Following an overnight fast, blood samples were drawn from each child for assessment of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglyceride, insulin-like growth factor I (IGF-I), IGF-II and IGF-binding protein 3 (IGFBP-3) concentrations. Children also had glucose and insulin levels measured, and insulin sensitivity evaluated using the homeostasis model assessment of insulin resistance (HOMA-IR).<sup>16</sup>

Plasma insulin was measured using an Abbott AxSYM system (Abbott Laboratories, Abbott Park, IL, USA) by microparticle enzyme immunoassay (Abbott Diagnostics, Wiesbaden, Germany) with an interassay coefficient of variation (CV) of  $<5\%$ . Glucose, triglyceride, total cholesterol, HDL-C and LDL-C concentrations were measured on a Hitachi 902 autoanalyser (Hitachi High Technologies Corporation, Tokyo, Japan) by enzymatic colorimetric assay (Roche, Mannheim, Germany) with an interassay CV of 1.2% for glucose, and  $<5\%$  for total cholesterol, triglycerides, HDL-C and LDL-C. Commercially available ELISAs (R&D Systems, Minneapolis, MN, USA) were used to measure plasma IGF-I (DSL-100, intraassay CV 2.8%, interassay CV 9.2%), IGFBP-3 (DSL-10-6600, intraassay CV 3.1%, interassay CV 9.9%) and IGF-II (Meddiagnost, Reutlingen Germany; E-30, intraassay CV 1.9%, interassay CV 6.3%).

### Ethics approval

Ethics approval for this study was provided by the Northern Y Regional Ethics Committee (Ministry of Health, New Zealand). Written informed consent was obtained from parents or guardians, as well as verbal or written consent from each child as was appropriate to their age.

### Statistical analysis

Children in our study were divided into paternal age (at child-birth) subgroups approximating potentially relevant paternal age thresholds, while also reflecting age ranges known to be important for maternal age effects. As a result, to examine the possible nonlinear effects of paternal age at childbirth on measured outcomes, subjects were stratified into three groups: children born to fathers aged less than or equal to 30 years of age ( $\leq 30$ ), 31 to 35 years (30–35 years), and  $>35$  years ( $>35$ ).

Mean ages among groups were compared using one-way ANOVA in Minitab v.16 (Pennsylvania State University, State College, PA, USA). Other comparisons between paternal age groups were carried out using linear mixed models in SAS v.9.3 (SAS Institute, Cary, NC, USA), which included paternal identification

number as a random factor to account for the clustering of siblings. All models accounted for important confounding factors, namely gender, birthweight SDS, gestational age, birth order and maternal age. Other factors were controlled for as required, depending on the outcome response of interest: for lipids, hormones and outcomes associated with glucose homeostasis – children's age and body mass index standard deviation score (BMISDS) were included; for anthropometric data – the appropriate parental factor (i.e. MPBMISDS or MPHSDS). Following a global test, pairwise comparisons were carried out to identify specific differences between groups. Subgroup analyses were also run including only data on the recruited siblings. Note that there was no association between paternal age and the parameter used to assess genetic height potential (MPHSDS):  $r = -0.05$  and  $P = 0.44$ . Note also that, to ensure the validity of our multivariate models, any parameter used to obtain a specific dependent variable (e.g. MPHSDS) was not used in the same model, being neither included as an independent variable nor used in the construction of an independent variable.

The interaction effects between paternal age groups and gender were tested in all models, and outcomes only assessed separately for boys and girls if there was indication of a differential response between genders. Data on parameters associated with glucose homeostasis were log-transformed to approximate a normal distribution. Age data are provided as means  $\pm$  standard deviation; other data are means and 95% confidence intervals adjusted for confounders in the multivariate models, back-transformed were appropriate.

## Results

A total of 343 children volunteered to participate, but 31 were excluded: 22 children were born small-for-gestational age and/or premature, five were pubertal, three were born of a mother with gestational diabetes/glucose intolerance and one child was on medication known to influence growth (Figure S1). Of the remaining 312 controls, a further 35 had to be excluded due to lack of paternal age data (Figure S1). Thus, our study cohort consisted of 277 children (126 girls and 151 boys) aged  $7.4 \pm 2.2$  years (range 3–12 years). The offspring of 196 fathers were included in this study, as there were 71 sibling groups of 2 or 3 children ( $n = 153$ ). Paternal age at childbirth was  $35.4 \pm 5.4$  years (range 19.8–51.8) (Fig. 1).

Age, sex ratio, birthweight and gestational age were similar among paternal age subgroups (Table 1). There were also no differences between subgroups in parental anthropometric characteristics, including height SDS and BMISDS (data not shown). Importantly, children across paternal age subgroups had similar duration and rates of breastfeeding (data not shown), and there were no differences in biological maturity between subgroups based on bone age X-rays (Table 1).

### Anthropometry

When heights were corrected for genetic potential (HtSDS–MPHSDS), children of fathers aged 31–35 (0.35 SDS;  $P = 0.009$ )

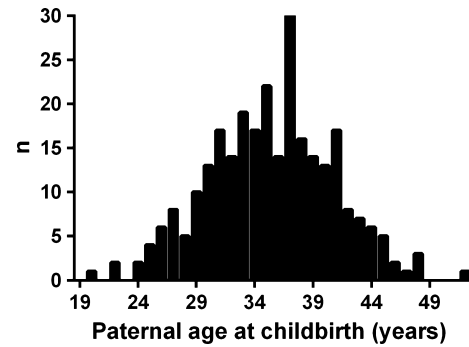


Fig. 1 Distribution of paternal age at childbirth in the study cohort.

Table 1. Birth outcomes and biological maturity in our study cohort according to paternal age at childbirth. Data are means  $\pm$  SD. Biological maturity is represented by the bone age minus the chronological age

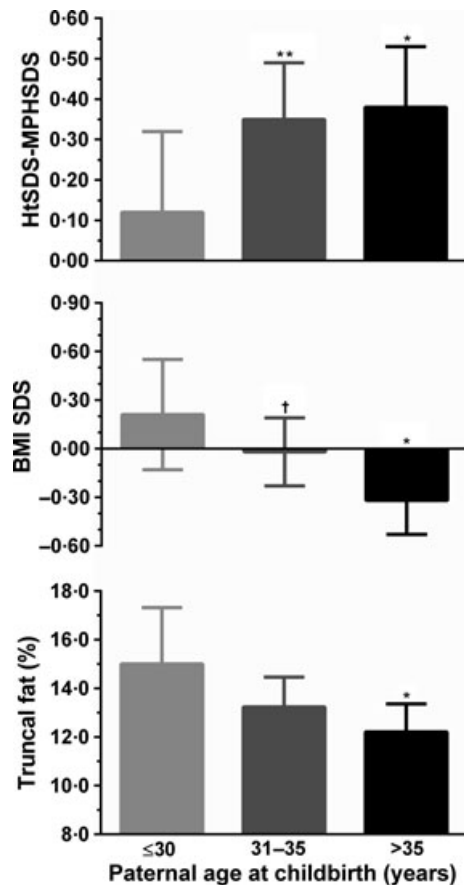
	Paternal age at childbirth		
	$\leq 30$ years	31–35 years	$> 35$ years
<i>n</i>	59	90	128
Sex ratio (boys/girls)	35/24	52/38	64/64
Age (years)	$7.55 \pm 2.22$	$7.59 \pm 2.22$	$7.12 \pm 2.15$
Gestational age (weeks)	$39.8 \pm 1.3$	$39.8 \pm 1.1$	$39.5 \pm 1.2$
Birthweight SDS	$0.24 \pm 1.02$	$0.26 \pm 0.93$	$0.14 \pm 0.95$
Biological maturity	$0.01 \pm 0.80$	$-0.21 \pm 0.87$	$-0.20 \pm 0.74$

and  $> 35$  (0.39 SDS;  $P = 0.019$ ) years at childbirth were taller than those of fathers aged  $\leq 30$  years (0.12 SDS) (Fig. 2). There was also some evidence of reduced adiposity in the offspring with increasing paternal age at childbirth (Fig. 2). BMISDS among children born of fathers aged  $> 35$  years ( $-0.32$  SDS;  $P = 0.043$ ) and  $\leq 30$  (0.22 SDS;  $P = 0.019$ ) years (Fig. 2). Truncal fat (central adiposity) was lower in children of fathers aged  $> 35$  years at childbirth than in the offspring of fathers aged  $\leq 30$  years (12.2 vs 15.0%;  $P = 0.029$ ) (Fig. 2). There were no differences between groups in total body fat, android to gynoid fat ratio or bone mineral density (data not shown).

### Lipid profiles

Increasing paternal age at childbirth was strongly associated with less favourable plasma lipid profiles in the offspring. Plasma total cholesterol concentrations among children of fathers aged  $> 35$  years at childbirth (4.52 mM) were higher than in both the 31–35 (4.18 mM;  $P = 0.002$ ) and  $\leq 30$  (4.20 mM;  $P = 0.046$ ) groups. Similarly, LDL-C concentrations were higher among the children born to fathers aged  $> 35$  years at childbirth (2.65 mM) compared with children of fathers aged 31–35 (2.38 mM;  $P = 0.005$ ) and  $\leq 30$  (2.22 mM;  $P = 0.003$ ) years (Fig. 3).

HDL-C concentrations in the children of fathers aged 31–35 years at childbirth were lower than in the  $\leq 30$  group (1.31 vs 1.46 mM;  $P = 0.012$ ) but similar to the  $> 35$  group



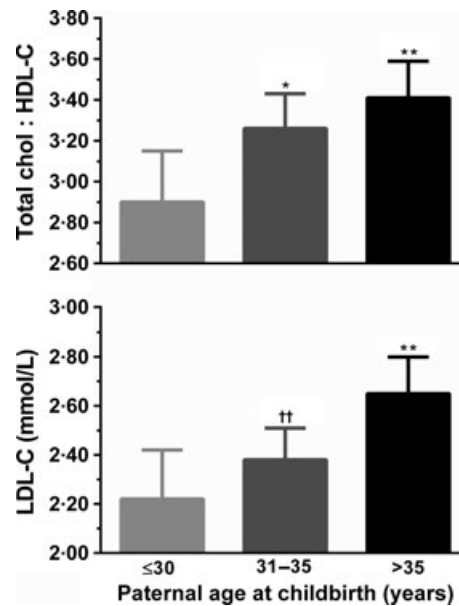
**Fig. 2** Height standard deviation score (SDS) corrected for mid-parental height SDS (HtSDS-MPHSDS) and measures of adiposity among the children of fathers of different ages at childbirth. Data are means and 95% confidence intervals, adjusted for other confounding factors in multivariate models, including maternal age. \* $P < 0.05$  and \*\* $P < 0.01$  vs children of fathers aged  $\leq 30$  years at childbirth; † $P < 0.05$  vs children of fathers aged  $> 35$  years. BMI SDS, body mass index standard deviation score.

(1.37 mm;  $P = 0.19$ ). However, the total cholesterol to HDL-C ratio was increased among the children of fathers aged 31–35 (3.37;  $P = 0.014$ ) and  $> 35$  (3.47;  $P = 0.004$ ) years at childbirth compared with the  $\leq 30$  group (3.00), due to higher LDL-C concentrations (Fig. 3). An identical pattern was also observed for the LDL-C to HDL-C ratio (data not shown).

### Growth factors and glucose homeostasis

There were no significant differences in IGF-I and IGFBP-3 concentrations between groups, but paternal age had a sex-dependant effect on IGF-II (Table 2). Among girls, IGF-II concentrations were lower in the daughters of fathers aged 31–35 ( $P = 0.002$ ) and  $> 35$  ( $P = 0.009$ ) years at childbirth compared with the  $\leq 30$  group (Table 2). In contrast, the exact opposite pattern was observed among boys ( $P < 0.05$ ; Table 2).

There was also a sex-dependant effect on glucose homeostasis, with paternal age at childbirth affecting girls but not boys (Table 2). Fasting insulin concentrations were lower in girls



**Fig. 3** Lipid profile among the children of fathers of different ages at childbirth. Data are means and 95% confidence intervals, adjusted for other confounding factors in multivariate models, including maternal age. \* $P < 0.05$  and \*\* $P < 0.01$  vs children of fathers aged  $\leq 30$  years at childbirth; †† $P < 0.01$  vs children of fathers aged  $> 35$  years. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; Total chol, total cholesterol.

**Table 2.** Growth factors and parameters of glucose homeostasis among boys and girls according to paternal age at childbirth. Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models (including maternal age)

	Paternal age at childbirth		
	$\leq 30$ years	31–35 years	$> 35$ years
IGF-I ( $\mu\text{g/l}$ )	119 (103–136)	107 (97–118)	108 (97–118)
IGF-II ( $\mu\text{g/l}$ )			
Boys	693 (652–734)	741 (715–767)*	753 (723–783)*
Girls	823 (775–868)	736 (708–765)**	741 (715–767)**
IGFBP-3 (ng/ml)	2481 (2179–2782)	2615 (2429–2802)	2816 (2616–3016)
HOMA-IR			
Boys	0.95 (0.077–1.18)	0.99 (0.87–1.13)	0.98 (0.85–1.14)
Girls	1.63 (1.21–2.20)	0.99 (0.83–1.19)**	1.11 (0.95–1.30)*
Fasting insulin (mU/l)			
Boys	4.51 (3.70–5.50)	4.49 (3.96–5.08)	4.63 (4.06–5.29)
Girls	7.38 (5.63–9.69)	4.84 (4.11–5.69)**	5.22 (4.51–6.04)*

\* $P < 0.05$  and \*\* $P < 0.01$  vs children of fathers aged  $\leq 30$  years at childbirth. Data for outcomes with a differential response between boys and girls are provided separately for each sex;  $P$ -values for interaction between paternal age group and sex were 0.02, 0.02 and 0.05 for IGF-II, HOMA-IR and fasting insulin, respectively.

born to fathers aged 31–35 ( $P = 0.005$ ) and  $> 35$  ( $P = 0.042$ ) years at childbirth compared with the girls born to younger fathers (Table 2). Thus, girls in the 31–35 and  $> 35$  groups were

more insulin sensitive (i.e. had lower HOMA-IR) than girls born to fathers aged  $\leq 30$  years at childbirth ( $P = 0.003$  and  $P = 0.040$ , respectively; Table 2).

### Siblings

Analyses of specific outcomes among sibling groups corroborated most findings on the larger cohort, although weaker  $P$ -values were obtained in view of the reduction in  $n$  (from 277 to 153). There was a gradual increase in HtSDS-MPHSDS with increasing paternal age at childbirth, and nonsignificant data suggesting a steady reduction in adiposity (Fig. 4). Regarding lipid profiles, children born to older fathers had higher LDL-C concentrations as well as higher total cholesterol to HDL-C ratio (Fig. 4).

### Maternal age

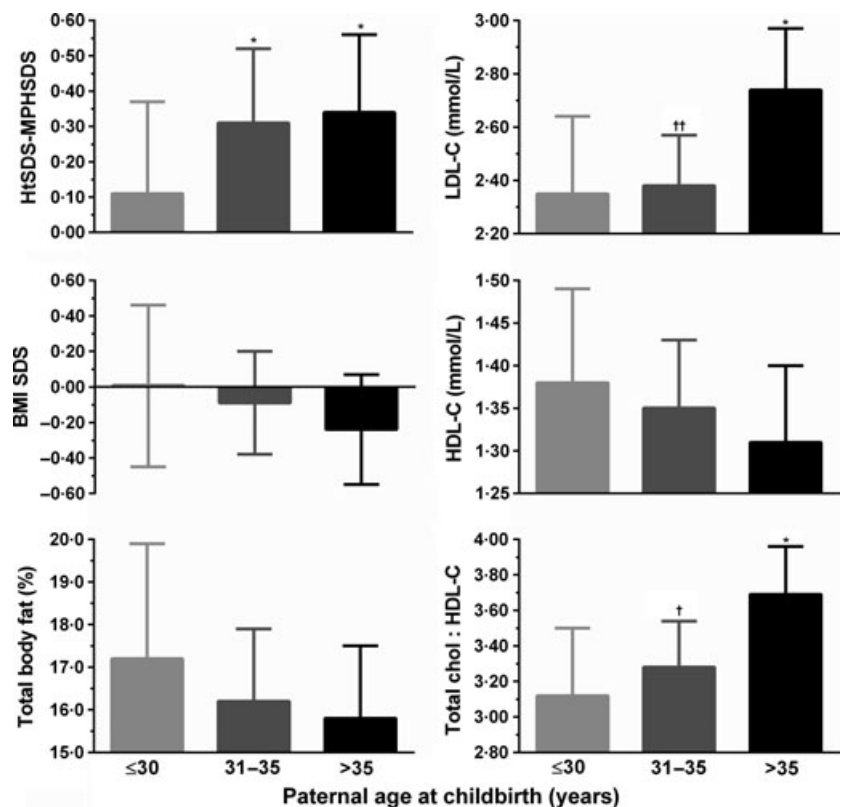
There was a high correlation between paternal and maternal ages ( $r = 0.75$ ;  $P < 0.001$ ). As a result, similar stratified analyses were carried out, examining maternal age groups (stratified as per the paternal age thresholds) with paternal age included as a continuous variable. These data are provided in the supplementary Table S1. The observed patterns of progressive improvement in adiposity and worsening lipid profiles were not observed for maternal age (Table S1). However, there was a significant increase in HtSDS-MPHSDS from the youngest group to those born to mothers aged 31–35 years ( $P = 0.012$ ; Table S1), with paternal age (as a continuous variable) remaining associated with HtSDS-MPHSDS ( $P = 0.033$ ).

## Discussion

Our study shows that increasing paternal age at childbirth is associated with taller stature and reduced adiposity but a less favourable lipid profile in their children. These changes were associated with a relatively young paternal age at childbirth (35.4 years), when one considers that in the USA, for example, birth rates among fathers aged 35–39 and 40–44 increased by approximately 50% over the last three decades.<sup>17</sup> The influence of paternal age at childbirth on height or metabolism in their children has not been described previously.

Children born to fathers over 30 years of age were approximately 2 cm taller than those of fathers aged  $\leq 30$  years. Notably, this height difference was present after correction for genetic height, the most important determinant of childhood height.<sup>15</sup> In addition, children in the paternal age subgroups had similar biological maturity according to bone age X-rays. Thus, the taller stature among children of fathers aged 30 years or more was unlikely to be associated with earlier pubertal development, and the observed height differences are likely to persist into adulthood.<sup>15</sup>

As paternal age at childbirth increased, their children displayed a reduction in BMI and truncal fat. As BMI in childhood is predictive of adult BMI, our findings suggest that the slimmer children of older fathers may have a lower risk of obesity in adulthood.<sup>18</sup> Increased truncal fat is a component of the metabolic syndrome, so that the children born to fathers aged over 30 years may be at a lower risk of metabolic disease and obesity. Importantly, the observed improvement in insulin sensitivity



**Fig. 4** Anthropometry and lipid profile among siblings born of fathers of different ages ( $n = 153$ ). Data are means and 95% confidence intervals, adjusted for other confounding factors in multivariate models, including maternal age. \* $P < 0.05$  vs children of fathers aged  $\leq 30$  years at childbirth; † $P < 0.05$  and †† $P < 0.01$  vs children of fathers aged  $>35$  years. BMI SDS, body mass index standard deviation score; HDL-C, high-density lipoprotein cholesterol; HtSDS-MPHSDS, height standard deviation score corrected for mid-parental height standard deviation score; LDL-C, low-density lipoprotein cholesterol; Total chol, total cholesterol.

seen among girls born of older fathers would support this hypothesis, as a reduction in insulin sensitivity is predictive of the metabolic syndrome in adulthood.<sup>19</sup> Nonetheless, there are conflicting reports regarding the effects of paternal age at childbirth on offspring obesity. In contrast to our study, a recent large investigation found an increased risk of obesity in young adult offspring in association with increasing paternal age. However, this was only observed when groups at the extreme of the paternal age spectrum were compared (<20 vs >50 years).<sup>8</sup> Furthermore, unlike our study, they only examined males and parental BMI was not accounted for in their analyses to correct for genetically determined obesity.<sup>8</sup>

However, increasing paternal age at childbirth was also associated with less favourable lipid profiles in their children. Specifically, children of fathers over 30 years of age had higher total cholesterol to HDL-C ratios compared with the children of younger fathers. Childhood lipid profiles worsened as paternal age at childbirth increased further, so that the children of fathers aged over 35 years had higher total cholesterol (due to higher LDL-C concentrations) than children of fathers aged  $\leq 35$  years. Childhood lipid profiles track or accentuate into adulthood.<sup>20</sup> It is therefore possible that the less favourable lipid profiles in these children may deteriorate further later in life, placing them at a greater risk of cardiovascular disease in adulthood.

There is no clear explanation for our observed findings in children in association with increasing paternal age at childbirth. This is particularly so for the observation of less favourable lipid profiles but lower adiposity in offspring with increasing paternal age, which is contradictory to findings on lipid profiles and adiposity in children and adults.<sup>21,22</sup> Thus, the triggers and mechanisms responsible for the influence of paternal age on offspring phenotype and metabolism require further investigation. Consequently, the factors leading to a taller and slimmer phenotype are potentially different to those associated with a less favourable lipid profile. Factors responsible for these differences may be prenatal, such as epigenetic changes in paternal gametes or paternal environmental factors (diet and toxins), or due to differences in the post-natal child-rearing environment. Similarly, while higher IGF-II concentrations among the girls born to youngest fathers are likely a result of their greater adiposity,<sup>23</sup> the opposite pattern for IGF-II concentrations observed among boys cannot be easily explained by differences in adiposity.

Sperm DNA may undergo subtle gene alterations with increasing paternal age, including epigenetic changes<sup>24</sup> that may be associated with phenotypic changes in the offspring. Increasing age is associated with a greater frequency of epigenetic modifications in both somatic<sup>25</sup> and germ cells.<sup>24</sup> Such gene changes may link advanced paternal age at childbirth with increased rates of autism and schizophrenia in the offspring.<sup>26,27</sup> It is therefore possible that increasing age leads to epigenetic changes in paternal sperm genes regulating growth and metabolism. We speculate that such gene changes may be subsequently transmitted to the offspring, leading to programmed changes in phenotype and metabolism in childhood.

Increasing paternal age is also associated with longer duration of exposure to common environmental toxicants (e.g. air

pollution and pesticides used on commonly consumed foods), which can affect sperm quality or induce epigenetic changes. Paternal alcohol consumption can also induce epigenetic changes in sperm,<sup>28</sup> with possible consequences to the offspring. Although not yet demonstrated, it is possible that such environmental factors may lead to phenotypic changes and other alterations in offspring outcomes. In addition, even paternal dietary habits may affect the offspring.<sup>29</sup> For example, a recent animal study found that a preconceptional paternal diet rich in carbohydrates was associated with adverse lipid profiles in the offspring.<sup>30</sup>

The post-natal child-rearing environment across the socio-economic spectrum is also known to affect phenotype and other health outcomes in childhood.<sup>31,32</sup> The fact that increasing maternal age was also associated with greater stature suggests that environmental factors could have a role affecting offspring height. A recent study on nearly 80,000 British children showed that increasing maternal age was associated with improved health and better developmental outcomes in the offspring in early childhood.<sup>33</sup> Children reared in families of higher socio-economic status tend to be taller and slimmer than those from lower socio-economic backgrounds.<sup>34,35</sup> However, our homogenous cohort of children was entirely from higher socio-economic families, meaning that our findings are unlikely to be explained by differences in child-rearing environment. Further, a more favourable child-rearing environment in association with increasing parental age would not explain the contrasting finding of a less favourable lipid profile in the offspring of older fathers.

A possible limitation of our study is that we studied a homogenous group of children (same ethnicity and higher socio-economic status), which may limit application of our study findings to the general population, particularly to those of lower socio-economic status. We also acknowledge that our measure of socio-economic status ('decile' scores) integrates a number of affluence variables, but does not provide detailed information about individual parent's income and education. Nonetheless, we believe that our relative homogenous cohort has eliminated much of the phenotypic and metabolic variability associated with both ethnicity and socio-economic status, allowing us to better address the potential effects of paternal age on measured outcomes. It is also important to note that the observed associations with paternal age do not necessarily prove causality. Prospective studies are needed to specifically assess possible mechanisms through which paternal age may affect growth and metabolism in the offspring in childhood.

## Conclusion

Our study showed that increasing paternal age at childbirth was associated with taller stature and reduced adiposity, but a less favourable lipid profile in their children. Further investigation of the possible triggers and mechanisms responsible for these findings is warranted. As paternal age at childbirth continues to increase worldwide, it is important to further evaluate the possible long-term effects on offspring health.

## Acknowledgements

We acknowledge the Paykel Trust for long-term funding of the Maurice & Agnes Paykel Clinical Research Unit at the Liggins Institute, University of Auckland. We thank Gravida: National Centre for Growth and Development and the Australasian Paediatric Endocrine Group for financial support. We also thank Eric Thorstensen (Liggins Institute) and his group for assistance with laboratory work, Dr Yannan Jiang (Department of Statistics, University of Auckland) for valuable statistical input, and all the participants and their families for their time and help with this study. All authors have no financial or nonfinancial interests to declare that may be relevant to this submitted work. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. T Savage, W Cutfield and JGB Derraik are the guarantors and take overall responsibility for the data and accuracy of the data analysis.

## Funding

This study was supported by grants from Gravida: National Centre for Growth and Development.

## Author contributions

All authors conceived and designed the study. TS performed the clinical study. TS, HLM, FM and JGBD compiled the data, which were analysed by JGBD. TS wrote the initial drafts of the manuscript, which were revised by JGBD and WSC, with input from HLM, FM and PLH. TS and WSC are the guarantors.

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### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Summary of the study's recruitment process.

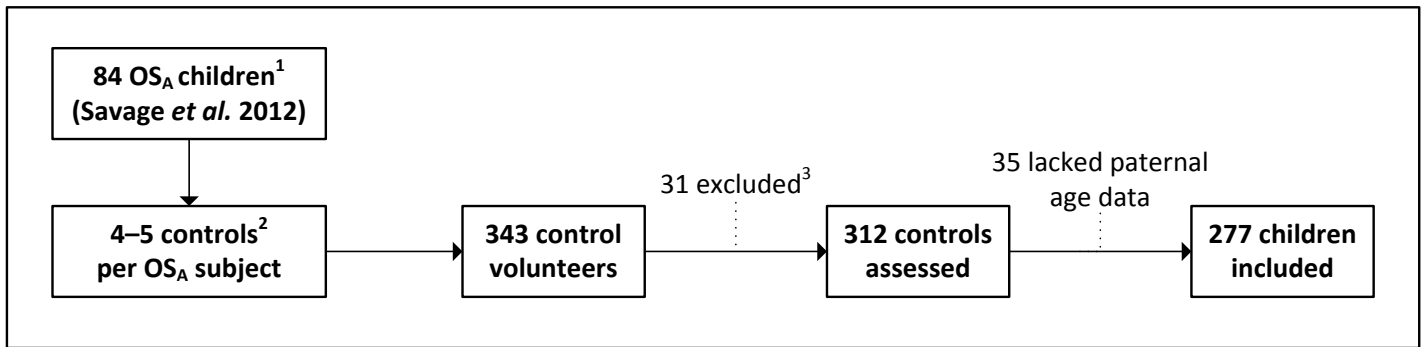
**Table S1.** Study outcomes among the children of mothers of different ages at childbirth.



**Table S1.** Study outcomes among the children of mothers of different ages at childbirth. Data are means and 95% confidence intervals, adjusted for other confounding factors in multivariate models, including paternal age. Data are means and 95% confidence intervals adjusted for other confounding factors in the multivariate models. \*p<0.05 vs children of mothers aged ≤30 years at childbirth; †p<0.05 vs children of mothers aged >35 years.

		≤30 years	31–35 years	>35 years
<b>n</b>		77	126	74
<b>Anthropometry</b>				
HtSDS–MPHSDS		0.18 (0.00–0.35)	0.37 (0.24–0.51)*	0.39 (0.21–0.56)
BMI SDS		-0.18 (-0.47–0.12)	-0.12 (-0.31–0.06)	-0.02 (-0.32–0.27)
Total body fat (%)		-0.14 (-0.42–0.14)	-0.03 (-0.21–0.15)	0.14 (-0.14–0.43)
Truncal fat (%)		-0.16 (-0.43–0.11)	-0.02 (-0.19–0.15)	0.17 (-0.11–0.44)
<b>Glucose homeostasis</b>				
HOMA-IR	Boys	0.91 (0.75–1.10)	1.02 (0.91–1.14)	0.97 (0.78–1.21)
	Girls	1.24 (0.97–1.59)	1.06 (0.91–1.23)	1.20 (0.97–1.59)
Fasting insulin (mU/l)	Boys	4.41 (3.68–5.28)	4.62 (4.16–5.12)	4.54 (3.72–3.54)
	Girls	5.76 (4.59–7.23)	5.07 (4.43–5.80)	5.70 (4.68–6.94)
<b>Hormonal concentrations</b>				
IGF-I (ng/ml)		101 (87–115)	114 (105–122)	113 (98–127)
IGF-II (ng/ml)	Boys	761 (725–797)	732 (709–755)	705 (662–748)
	Girls	781 (742–821)	729 (705–754)*	770 (736–805)
IGFBP-3 (ng/ml)		2691 (2428–2955)	2647 (2480–2815)	2705 (2434–2976)
<b>Lipid profile</b>				
Total cholesterol (mmol/l)		4.40 (4.19–4.63)	4.28 (4.15–4.42)	4.35 (4.14–4.58)
LDL-C (mmol/l)		2.52 (2.33–2.72)	2.46 (2.35–2.58)	2.40 (2.22–2.59)
HDL-C (mmol/l)		1.43 (1.34–1.52)	1.30 (1.25–1.36)*†	1.43 (1.33–1.52)
Total cholesterol : HDL-C		3.14 (2.93–3.37)	3.37 (3.23–3.52)	3.13 (2.91–3.36)

**Figure S1.** Summary of the study's recruitment process.



<sup>1</sup> OS<sub>A</sub> children had been conceived via ovarian stimulation and were examined in Savage *et al.* (Ovarian stimulation leads to shorter stature in childhood, *Human Reproduction* 2012, 27, 3092-3099).

<sup>2</sup> Controls were friends of OS<sub>A</sub> children to ensure similar age group, ethnicity, and socio-economic status

<sup>3</sup> 22 children were born small-for-gestational age and/or premature; 5 were pubertal; 3 were born of a mother with gestational diabetes/glucose intolerance; and 1 child was on medication known to influence growth.