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This is an electronic version of an article published in:

Hammar, M., Larsson, E., Bladh, M., Finnström, O., Gäddlin, P O, Leijon, I., Theodorsson, E., Sydsjö, G., (2018), A long-term follow-up study of men born with very low birth weight and their reproductive hormone profile, *Systems biology in reproductive medicine*, 64(3), 207-215.

<https://doi.org/10.1080/19396368.2018.1448901>

Original publication available at:

<https://doi.org/10.1080/19396368.2018.1448901>

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# **A Long-term Follow-up Study of Men Born with Very Low Birth Weight and Their Reproductive Hormone Profile**

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## List of abbreviations:

ADHD	Attention deficit hyperactive disorder
AGA	Average for gestational age
BMI	Body Mass Index
CP	Cerebral Palsy
DHT	Dihydrotestosterone
FSH	Follicle stimulating hormone
LBW	Low birth weight
LH	Luteinizing Hormone
SAD	Sagittal Abdominal Diameter
SGA	Small for gestational age
SHBG	Sex hormone binding globulin
TSH	thyroid stimulating hormone
T3	triiodothyronine
T4	Thyroxin
VLBW	Very Low Birth Weight

## **Abstract (243 words)**

Environmental factors during the fetal period may adversely affect reproductive functions in men being born with very low birth weight (VLBW, <1500 g). The objective of this prospective, controlled cohort study was to investigate if VLBW men have an altered reproductive hormone profile compared with men born at term. The study group initially consisted of all VLBW boys live-born between 1<sup>st</sup> February 1987 and 30 April 1988 in the south-east region of Sweden (n=47). A control child was chosen born at term, at the same hospital, with the same parity, without malformations, and next in order after each VLBW child who survived the first four weeks (n=45). The present follow-up was performed when the men were 26-28 years of age and included measurements of serum hormone levels, hair testosterone concentration, and anthropometric data. Also life-style questionnaires were collected from 26 VLBW men and 19 controls.

The VLBW group (n=26) had higher median levels of serum estradiol, 84.5 pmol/L than controls (n=19), 57.5 pmol/L (P=0.008). There was no significant correlation between serum estradiol and BMI (r=0.06, P=0.74). There were no differences in other hormone levels or the reproductive pattern between the groups. In conclusion, even though there was a statistically significant difference in estradiol levels between the groups, both groups had low normal mean levels with questionable clinical significance. The reproductive pattern was similar in the two groups and in this study being born VLBW does not seem to affect measured aspects of reproduction.

**Key words:** Very low birth weight, Preterm, Reproduction, Follow-up study

**Capsule:** Men born with very low birth weight had higher levels of serum estradiol but did not differ in other hormone levels or in reproductive pattern compared with controls,

**Running title:** Hormone profile in men born with very low weight. (49 characters)

## **Introduction**

There are approximately 110,000 childbirths every year in Sweden, and about 5.9% of them are prematurely born (gestational week<37) and 4.2% with low birth weight (LBW<2500g). About 0.5% of all singleton children have very low birth weight (VLBW<1500g) and 2.3% are small for gestational age (SGA, i.e. birthweight < -2 standard deviations for gestational age) (Welfare, 2003).

The neonatal mortality has decreased substantially since 1973 when official measurements started in Sweden and also in the western world. This is probably due to the development of modern neonatology that started in the 60's (Philip, 2005; Welfare, 2003). Even though many of the children born preterm or with LBW survive, they have an increased risk for numerous disabilities, both in the adolescent period and in later adulthood (Gaddlin et al. 2007; Gaddlin et al., 2008; Hack et al. 2002; Monfils Gustafsson et al. 2009; Nosarti et al. 2012). There are inconclusive results from different parts of the world regarding the prognosis for children born VLBW e.g. concerning educational level, social life, occupation and self-perceived health in adulthood (Gaddlin et al. 2009; Hack et al. 2002). According to a recent review, some studies found preterm born adults to be more likely to live with their parents, not to cohabite, not have intercourse and had fewer sexual partners (Saigal 2014). A large population-based study from Norway reported that preterm born children are less likely to have children of their own, and only 13.9% of very preterm born men had become biological fathers compared with 50% of term born men (Saigal 2014). A Swedish population-based study found similar results with reduced reproduction among those born very preterm or with VLBW (deKeyser et al. 2012). Men seeking treatment for infertility had significantly lower birth weight than controls (Francois et al. 1997).

Significantly shorter stature has also been seen among young adults born VLBW and one study reported that men born VLBW had significantly lower BMI, and lean body mass corrected for height in adulthood (Hovi et al. 2007; Mollee et al. 2011).

The “Barker hypothesis”, which attempts to explain the association between cardiovascular disease and LBW, states that the in utero environment can permanently

influence the fetal metabolism, physiology and structure. Organs and tissues have time windows (in utero) when they are plastic and sensitive to environmental influences and might therefore be permanently damaged (Barker, 2007). The combination of intrauterine growth restriction and subsequent postnatal excess weight gain may therefore increase the risk for metabolic and cardiovascular disease in adult life (Hodgson, 2006). Prematurity and low birth weight, both seem to negatively affect reproduction due to fetal environmental factors that are not fully understood. Cryptorchidism and hypospadias are associated with impaired testicular function but also with LBW (Main et al., 2006). Ibanez et al. (Ibanez et al., 2002) found higher FSH levels in men born small for gestational age (SGA), compared with men born average for gestational age (AGA). Their interpretation was that the gonads among men born SGA needed higher FSH levels to stimulate Sertoli cells to support normal spermatogenesis and to produce inhibin B levels to suppress FSH from the pituitary. Testicular volume and testosterone levels have been found to be lower and LH levels higher in SGA boys in some (Ibanez et al., 2002) but not all studies (Boonstra et al. 2008).

A study including normal stature adult men born SGA, found them to have raised serum levels of estradiol, dihydrotestosterone (DHT) and inhibin B compared with AGA or large for gestational age born men (Allvin et al. 2008). The increased ratio estradiol/testosterone and DHT/testosterone suggested an increased aromatase and 5- $\alpha$ -reductase activity or decreased function of the elimination of estradiol and DHT from the liver and kidney (Allvin et al., 2008). A review and metaanalysis regarding BMI, semen parameters and reproductive hormones found evidence for a negative relationship between BMI and serum testosterone levels. This negative relationship was also true for BMI and SHBG and to a lesser extent for BMI and free testosterone. However, even in those subjects with a markedly reduced level of free testosterone there was no clinical signs of hypogonadism, and it was concluded that the reduced level of free testosterone therefore had little biological effect. Despite these alterations in hormone profiles there was no evidence for an association between BMI and semen parameters (MacDonald et al. 2010).

Thus studies report conflicting results regarding levels of gonadotropins and sex steroid hormones as well as reproductive function in SGA and AGA men. Since men born

VLBW may be even more affected by their intrauterine environment we hypothesized that there is a difference regarding levels of sex-hormones between adults born VLBW and adult control men indicating lower reproductive function in the VLBW men. We also introduced a new method measuring testosterone in hair – a potential new measure which may mirror the exposition to bioavailable testosterone over a given period of time (Slezak et al. 2017). The aim of this study was to investigate whether men born VLBW have an altered hormonal profile and reproductive pattern indicating lower reproductive function, compared with matched controls born at term.

## Results

A non-response analysis did not show a statistical differences regarding birth weight between VLBW subjects who gave blood samples and those who did not ( $P=0.764$ ). This was also true for controls ( $P=0.765$ ). From previous data from this study cohort four VLBW boys have had undescended testis of which two participated in the current study but leaving their data out or using them did not change any results. No boy had been diagnosed with an endocrine disease or aberration at any of the previous or the present follow-ups.

Table 1 shows median hormone levels in the two groups. VLBW males had significantly higher estradiol levels compared with controls ( $P=0.008$ ). Levels of gonadotropins and thyroid hormones were similar in the groups. No differences regarding total testosterone, free testosterone, bioavailable testosterone, testosterone in hair, SHBG or albumin could be seen between VLBW males and controls.

There was no significant correlation between testosterone and estradiol levels ( $r=-0.086$ ,  $P=0.616$ ) or between estradiol and BMI ( $r=0.058$   $P=0.743$ ). However, estradiol correlated negatively to birth length ( $r=-0.396$   $P=0.025$ ).

Testosterone is often clinically divided into subgroups were serum levels below 8 nmol/L are considered pathologic, levels between 8-15 constitute a grey zone and levels above 15 are considered normal (Lunenfeld et al., 2012). After categorizing the percentage of men in each subgroup according to testosterone levels there was no difference in distribution between the VLBW and controls (data not shown).

Table 2 summarizes the anthropometric data. Clearly birth weight and birth length differed between the groups. At this follow-up the participants were 26-28 years old and now no difference in weight and length was found. Sagittal abdominal diameter as well as BMI were similar between VLBW males and their controls.

**Sociodemographic and reproductive data.** There were no significant differences between the two groups regarding marital status, desire to have children, if they had fathered a child or made a women pregnant. Furthermore, there were no significant differences in tobacco and alcohol use between the groups (Table 3).

## Discussion

The striking observation of this long term, controlled follow-up study of boys born VLBW was that they did not differ from matched controls born at term regarding reproductive sex hormones, BMI, marital and reproductive status, desire to have children, and tobacco and alcohol use. We interpret this as a sign of normal developmental potency in these VLBW children who survived the neonatal period.

Although many variables were similar between the two groups, estradiol levels were significantly higher in VLBW men compared with controls. However, both groups had low normal mean levels of estradiol with questionable clinical significance of the observed difference. The main production of estradiol in adult men is by means of aromatization of testosterone, e.g. in adipose tissue. Overweight men express higher serum estradiol levels (Akingbemi, 2005). This, however, does not clearly explain the significantly higher estradiol levels in VLBW men since they did not have higher BMI or weight than the controls. It should be emphasized, however, that BMI is a rough measure of the amount of adipose tissue and in relation to muscle tissues. It is therefore possible that the VLBW group could have a higher proportion of adipose tissue and less muscle tissue than the control group still leading to similar BMI in the two groups. Other measures than BMI have to be used in order to prove such a hypothesis, but similar sagittal abdominal diameters in the two groups suggest that the difference is not

explained by different amounts of intraabdominal adipose tissue. Yet another explanation could be overexpression of aromatase CYP 18 which has been found in men with Klinefelter's syndrome (Wosnitzer and Paduch, 2013) and in men with diabetes and the metabolic syndrome (Kalyani and Dobs, 2007).

Interestingly, our results are in accordance with a study by Allvin et al. (Allvin et al., 2008) that also found higher estradiol levels among SGA men than men born large or average for gestational age. In that study, those born SGA also had higher levels of dihydrotestosterone (DHT) and inhibin B. In line with our results, there was no difference in testosterone, SHBG, LH or FSH between the groups. However, they found the ratio between estradiol and testosterone to be significantly higher for SGA males than AGA males. This was also in line with our data showing the ratio between estradiol and testosterone to be slightly higher for VLBW males although it did not reach statistical significance ( $P=0.077$ ). In the study by Allvin et al (2008), as in ours, there was no a correlation between estradiol levels and current BMI or weight. The authors speculated that the higher estradiol and DHT levels among SGA men could be due to possible impaired fetal organ development which in turn could lead to a decreased metabolism or excretion of estradiol and DHT by the kidneys and liver, since both hormones are excreted into urine and bile. They also suggested that the men born SGA might have higher aromatase activity compared with controls, which could account for the higher estradiol levels (Allvin et al., 2008).

There are other studies indicating that being born SGA does not negatively influence testicular function. There were no differences regarding LH, FSH, Inhibin B, or testosterone between males born SGA and controls born AGA (Boonstra et al., 2008; Jensen et al., 2007). One of these studies also included measurement of testicular volume but found no differences between SGA men and healthy controls (Jensen et al., 2007).

There are shortcomings in our study that might contribute to making the statistically significant difference in estradiol uncertain. Firstly, the groups were small and estradiol levels were missing for one VLBW individual and one control. Moreover, serum samples of estradiol were below the detection limit for three VLBW and four controls, and were

thus not included in the statistical comparison of estradiol levels. Despite these shortcomings, the power for the comparison of estradiol means was 91.4%. It could be argued that a higher number of controls for each VLBW boy would have increased the power for all comparisons made. This is definitely true, but since the original study was designed several decades ago the cohort may not later be changed and increased.

Blood samples were drawn at a time convenient for each participant, meaning the blood was drawn at different hours of the day, the majority in the afternoons and evenings. This makes our results less reliable since, especially, testosterone is recommended to be sampled in the morning when the serum levels are highest and more sensitive to show differences between groups (Boyce et al., 2004). This is probably the reason why the majority of both groups had testosterone levels categorized within the “grey zone (8-15 nmol/L)” rather than within the normal zone (>15nmol/L). It should be pointed out that four VLBW men, two participating in this study, had congenital undescended testis but none in the control group. A review concluded that LBW increases the risk for congenital undescended testis and that undescended testis is linked to impaired testicular function (Main et al., 2006). However, excluding the two men with cryptorchidism did not change the results significantly.

Although reproductive hormone levels might give a clue about fertility, the number of pregnancies is evidently more relevant. In our small study, only four men in the VLBW group reported to have made a woman pregnant and reported to be fathers. However, a vast majority in both groups wanted children or more children in the future. This indicates that a majority had not yet tried to have children. Moreover, in both our groups less than half of the participants did not cohabit. We can therefore not confirm the results from earlier studies that have found associations between VLBW and a reduced chance of reproducing (deKeyser et al., 2012; Swamy et al. 2008). Of course, this could also be related to social factors besides reduced fertility. However, at the 20-year follow up of this cohort, there were no significant differences regarding self-perceived health between VLBW individuals and their controls. Frequency of graduation from upper secondary school as well as way of living were also similar between the groups (Gaddlin et al., 2008). While some studies have found VLBW subjects to have lower BMI and shorter

stature as adults (Hovi et al., 2007; Mollee et al., 2011), we could not see these associations but rather no differences in height, weight and BMI between VLBW men and their controls.

Abdominal height or SAD, has been shown to better correlate with cardiovascular risk factors such as blood pressure, insulin, glucose, cholesterol and triglycerides than other obesity measures (Gustat et al. 2000). SAD measurements were similar between the two groups, and if one would want to better measure the potentially increased risk for cardiovascular diseases that VLBW subjects may have, other surrogate markers should have been analyzed as well.

We expected a participation rate of 75% since the participation rates at the 20-year follow up were 91.5% for VLBW men and 79.1 % for controls but at this follow up we had a lower participating rate. A potential explanation to the low participation rate is that this study was more time consuming compared with the 20 year follow up, which only consisted of questionnaires that could be answered at home.

One of the strengths of this study is the matched controls and the extensive information that has been collected through several follow-up studies since it started already from birth.

To get a better understanding about the fertility in the groups it would have been ideal to measure testicular volume and include semen analyses that would have given valuable information about sperm count, morphology and motility. These measurements were planned but were not given permission in the Ethical Committee. Also, there is a substantial risk that adding these elements to the study would have affected the already low participation rate negatively. A compromise could have been to measure serum inhibin B since it correlates to testicular volume and, to a lesser extent, total sperm count, and is, thus, often used to measure potential for spermatogenesis and stimulated Sertoli cells. Also, the weakness of not including the diurnal variations in hormone levels could

have been reduced if all blood samples had been drawn at the same time of the day, preferably in the morning.

The higher estradiol level among men born VLBW seen in this study is unlikely to have any clinical relevance *per se*, especially considering that the median estradiol levels for both groups were below the reference range. On the other hand it could be speculated that the higher estradiol levels in the VLBW group is an early marker for decreasing insulin sensitivity (Kalyani and Dobs, 2007) known to be prevalent in men born SGA or with LBW (Barker, 2007).

As the absolute number of children born VLBW is rising and as we, nowadays, are able to rescue even lighter children it is of great importance to make valid follow up studies on the long term effects of being born VLBW. Current follow up studies may only follow up men who are still relatively young and have not yet reached the age when reproduction peaks or middle age with the increased risk for cardiovascular and metabolic diseases. Hence, there is a continuous and strong need for further follow-up studies trying to investigate their long-term health and the potentially negative aspects of being born very preterm, later on in adult life in order to identify preventive methods to support their long term health.

## Materials and Methods

### **Participants**

The study group initially consisted of all VLBW children, live-born between 1<sup>st</sup> February 1987 and 30 April 1988 in the south-east region of Sweden. There were 107 VLBW children (girls and boys) and they accounted for 0.72% of all deliveries in this region during that period,

63 of them (59%) at the University Hospital in Linköping. Out of the 60 boys born VLBW 47 survived their neonatal period, i.e. the first four weeks.

A control child was chosen born at term and next in order after each VLBW child who survived the neonatal period, i.e. the first four weeks. We used no exclusion criteria such as intersex, inability to naturally reproduce (e.g. Klinefelter's syndrome) etc. The

control child was born at the same hospital, with the same gender, parity and without malformations and the control group consisted of 45 boys (Figure 1).

The 47 VLBW boys surviving the first four weeks were born between week 25 – 37 and had a mean birth weight of 1197 g (SD 208g, range 685-1495gr) and six of them had extremely low birth weight (ELBW, <1000 g). The control boys were born in week 37 – 42 with a mean birth weight of 3645g (SD 520g, range 2230-4570g). In the group with VLBW 51% were also small for gestational age and had a mean gestational age of 32.4 weeks versus a gestational age of 29.4 weeks for the 49% of the VLBW boys who were average for gestational age. Seven VLBW boys were later diagnosed with Cerebral Palsy (CP) and five with Attention Deficit Hyperactivity Disorder (ADHD). Later on two control boys emigrated and another died.

### **Data collection**

Information about the present follow-up study was sent by mail in 2014 to the 47 VLBW men and the 42 control men living in Sweden, together with a consent paper form. Those who accepted to participate were sent questionnaires to be answered at home. A day suitable for each participant's examination and blood sampling was scheduled via telephone. Two research nurses and two medical students carried out examinations at two hospitals in the region. For practical reasons most examinations and blood samples were drawn in the afternoon. A reminder was sent twice to non-responders.

Those who found it difficult to visit the hospitals were asked to answer questionnaires and give blood samples at their local health care center. Figure 1 shows a flowchart of the number of VLBW and control males participating in different aspects of this study.

A total of 62% VLBW and 50% control males accepted to participate in the study and all of these men answered the questionnaires. In the VLBW and control group respectively, 55% and 40% took part in examinations and 55% and 45% gave blood samples. In total 55% of the VLBW and 38% of the controls participated in the study according to the protocol, i.e. participated in all three parts of the study (Figure 1). Out of the seven VLBW men diagnosed with CP three participated in the study according to protocol. One

of five VLBW diagnosed with ADHD and two out of four VLBWs who have had undescended testis participated in the study according to the protocol.

### **Anthropometric methods**

Weight was measured in kilograms using a digital scale with one decimal (SECA scale class III model 704, Hamburg, Germany), and with the participants wearing underwear. Height was measured in centimeters using a digital wall-mounted stadiometer (Ulmer stadiometer, Prof. Henze, Ulm, Germany). Data as weight and length at birth, at 40 weeks from conception and from the follow up studies at 6, 12 and 18 months, 4,9,12 and 15 years of age have been retrieved from the original data from the previous follow-ups. Abdominal circumference was measured in cm using a plastic measuring tape. Abdominal height was measured in centimeters with one decimal using a Sagittal Abdominal Diameter (SAD) equipment during expiration in lying position with legs lightly bent.

### **Questionnaires**

The study-specific questionnaires included questions on marital status, education, income, tobacco use, alcohol use, activities, diseases, desire to have children and reproductive history.

### **Hormone Assays**

Blood samples from three control men have been drawn at their local health care centers and were transported to the laboratory at either of the participating hospitals. Venous blood samples were drawn using an intravenous catheter to measure serum concentrations of thyroid stimulating hormone (TSH), free thyroxin (T4), free triiodothyronine (T3), sex hormone binding globulin (SHBG), luteinizing hormone (LH), follicle-stimulating hormone (FSH), total testosterone, estradiol and plasma levels of albumin. All participants were asked if they were using or have ever used anabolic steroids or testosterone, no one answered affirmatively.

Plasma albumin was measured using ADVIA 1800 (Siemens Healthcare Diagnostics).

The total coefficient of variation (CV%) was 4.04%.

Serum SHBG, testosterone and estradiol were measured using the 2<sup>nd</sup> generation chemiluminescence techniques of Cobas e602 (Roche Diagnostics). The inter-assay CV% were 2.3%, 5.5%, 10.7%, respectively.

Free testosterone and bioavailable testosterone were calculated from total testosterone, plasma albumin and serum SHBG using the calculator at the website of International Society for Study of the Aging Male ((ISSAM)).

Serum LH and FSH were measured using Immulite 2000 XPi (Siemens Healthcare Diagnostics). The interassay CV% was 4.7%.

Testosterone concentration in hair is a new potential indicator of the exposure to bioavailable testosterone that has occurred over time and was measured as follows (Slezak et al 2017). A lock of hair was cut as close as possible to the scalp, the proximal end marked before wrapped in foil. The hair samples were stored in a freezer until analyses.

Testosterone in hair was measured using a competitive radioimmunoassay in speedvaced methanol extracts of homogenized hair. The radioligand was <sup>125</sup>I labelled testosterone-3-CMO-histamine and rabbit antiserum (T4276, Sigma Aldrich) was used which crossreacts 23.0, 1.5, 0.2, and 1.7 % for 5 $\alpha$ -dihydrotestosterone, 17 $\alpha$ -epitestosterone, dehydroepiandrosterone and androstenedione, respectively. The calibrator was testosterone (Sigma Aldrich T5411) verified with a European pharmacopoeia reference standard (EDQM, Strasbourg, France). All hair samples were analysed in the same assay, the intra-assay CV % for testosterone in hair was 9.6% at 10 pg/ml, 3.0 % at 45 pg/ml and 2.9 % at 90 pg/ml.

Serum TSH, T3 and T4 were measured using ADVIA Centaur XP (Siemens Healthcare Diagnostics). The interassay CV% were 4.23%, 4.6% and 3.3%, respectively. Three VLBW males and four control males had serum samples of estradiol below the detection limit for the analysis (depending on the laboratory, less than 44, 50 or 37 pmol/L) and were therefore left out when analyzing estradiol. Moreover, estradiol values were missing for one person in each group, and albumin and TSH values were missing for another control. Totally the number of samples, collected and statistically analyzed for each hormone, varied between 22 - 26 in the VLBW group and between 14-19 in the control group.

### **Statistical analyses**

Statistical analyses were performed using the Statistical Package for Social Science version 22 (IBM SPSS Inc., Armonk, NY, USA). Because not all variables were normally distributed nonparametric tests were chosen, for comparisons between groups, Mann-Whitney U-test. Hormone levels are presented as median and interquartile range. Correlation analyses were made with Spearman non-parametric rank correlation. Qualitative variables were analyzed using the Chi-square or Fisher's exact test. Due to the limited number of observations in this follow-up, no multivariate comparisons were performed. The significance level was set to 5% for all analyses.

### **Ethics**

Written informed consent was obtained from all the participants. Ethical approval was received from the Regional Ethical Review Board, Linköping, Sweden, N:o 2013/394-31.

**Authors contributions statement:** All authors conceived and designed the experiments; Elvar Theodorsson was responsible for the hair analyses, Marie Bladh for the statistical analyses, all authors analyzed the data: Elvar Theodorsson contributed reagents, Ingemar Leijon, Per-Olof Gäddlin and Orvar Finnström contributed with the original study cohort, Gunilla Sydsjö, Erika Larsson, Per-Olof Gäddlin and Mats Hammar arranged the practical handling of the visits; Erika Larsson, Gunilla Sydsjö and Mats Hammar had the main responsibility for writing the manuscript with inputs from the others and all authors approved revisions and the final paper.

**Acknowledgements:** We are deeply thankful to research nurses Christina Fuxin and Karin Fridolfsson who with greatest skills arranged and administered the visits and performed measurements of physical variables and blood sampling.

**Declaration of interest:** The authors report no conflicts of interest.

Financial support: The study had financial support from the County Council of Östergötland (ALF grants) and Futurum – the Academy for Health and Care, Region Jönköping County, Sweden.

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**Table 1 Hormone analyses in men born VLBW and controls born at term and with normal weight.**

*Serum hormones and SHBG levels as well as testosterone in hair (pg testosterone/mg hair) in men born with VLBW (n=22-26) and controls (n=14-19). Albumin was measured in plasma. Hormone levels are presented as median and interquartile ranges shown in parenthesis. 10 nmol/L of testosterone is 0.347 ng/dL and 10 pmol/L of estradiol is 2.724 pg/mL.*

<b>Endocrine status</b>	<b>VLBW</b>	<b>Controls</b>	<b>P-value</b>	<b>Reference range</b>
S-estradiol (pmol/L)	84.50 (23.00)	57.50 (36.50)	0.008	90-190
Total S-testosterone (nmol/L)	13.85 (7.50)	13.00 (7.50)	0.936	7.6-31
S-estradiol/S-testosterone	5.91 (4)	4.81 (3)	0.077	
Free testosterone (nmol/L)	0.30 (0.15)	0.27 (0.08)	0.535	
Free testosterone (%)	2.05 (0.48)	1.99 (0.60)	0.302	
Bioavailable testosterone (nmol/L)	7.55 (3.6)	6.96 (2.075)	0.487	
Bioavailable testosterone (%)	52.85 (14.1)	49.4 (12.7)	0.176	
S-SHBG (nmol/L)	30.00 (14.75)	32.0 (18.0)	0.535	15-64
S-testosterone/S-SHBG	0.46 (0.29)	0.41 (0.11)	0.50	
Testosterone/hair (pg/mg; mean/SD)	1.34 (0.48)	1.37 (0.71)	0.87	
	1.30 (0.96-1.70)	1.18 (0.86-1.63)	0.493	
S-LH (IE/L)	3.45 (2.90)	3.20 (2.20)	0.352	1.7-8.6
S-FSH (IE/L)	3.55 (3.25)	3.20 (2.20)	0.542	1-13
P-albumin (g/L)	46.00 (3.25)	46.00 (4.25)	0.440	36-48
S-TSH (mIE/L)	1.45 (0.82)	1.65 (1.25)	0.214	0.4-4
S-T3 (pmol/L)	5.55 (0.92)	5.30 (1.00)	0.082	3.5-6
S-T4 (pmol/L)	17.10 (4.45)	15.75 (3.00)	0.061	11.0-22.0

**Table 2 Present weight and length and at birth in men born VLBW and controls born at term and with normal weight.**

*(Presented as median and interquartile range in parenthesis )*

	<b>VLBW</b>	<b>Controls</b>	<b>p-value</b>
Birth weight (g)	1155 (320)	3330 (767)	<0.001
Birth length (cm)	38.0 (3.5)	51.5 (2.5)	<0.001
Weight (kg)	76.40 (16.9)	78.4 (26.8)	0.207
Height (cm)	175.3 (8.3)	179.0 (10.2)	0.062
BMI (kg/m <sup>2</sup> )	24.66 (3.5)	24.69.11 (8.0)	0.532
Sagittal abdominal diameter (cm)	20.3 (2.4)	20.0 (6.2)	0.660

**Table 3. Socio-demographic and reproductive data in men born VLBW and controls born at term and with normal weight. The percentages within brackets denote percentage out of the number of men who had answered that specific question.**

	<b>VLBW</b>	<b>Controls</b>	<b>p-value*</b>
	n (%)	n (%)	
Desire to have children	18 (100.0)	15 (93.8)	0.471
Fathered a child	4 (14.3)	0 (0.0)	0.130
Self perceived health	26 (92.9)	19 (90.5)	1.000
Regular use of medication	6 (20.7)	4 (19.0)	1.000
Married/cohabiting	14 (48.3)	7 (33.3)	0.387
University degree	9 (31.0)	12 (57.1)	0.086
Use of tobacco	12 (42.9.0)	8 (38.1)	0.777
Use of alcohol	26 (96.2)	15 (88.2)	0.552

\* Fisher's exact test

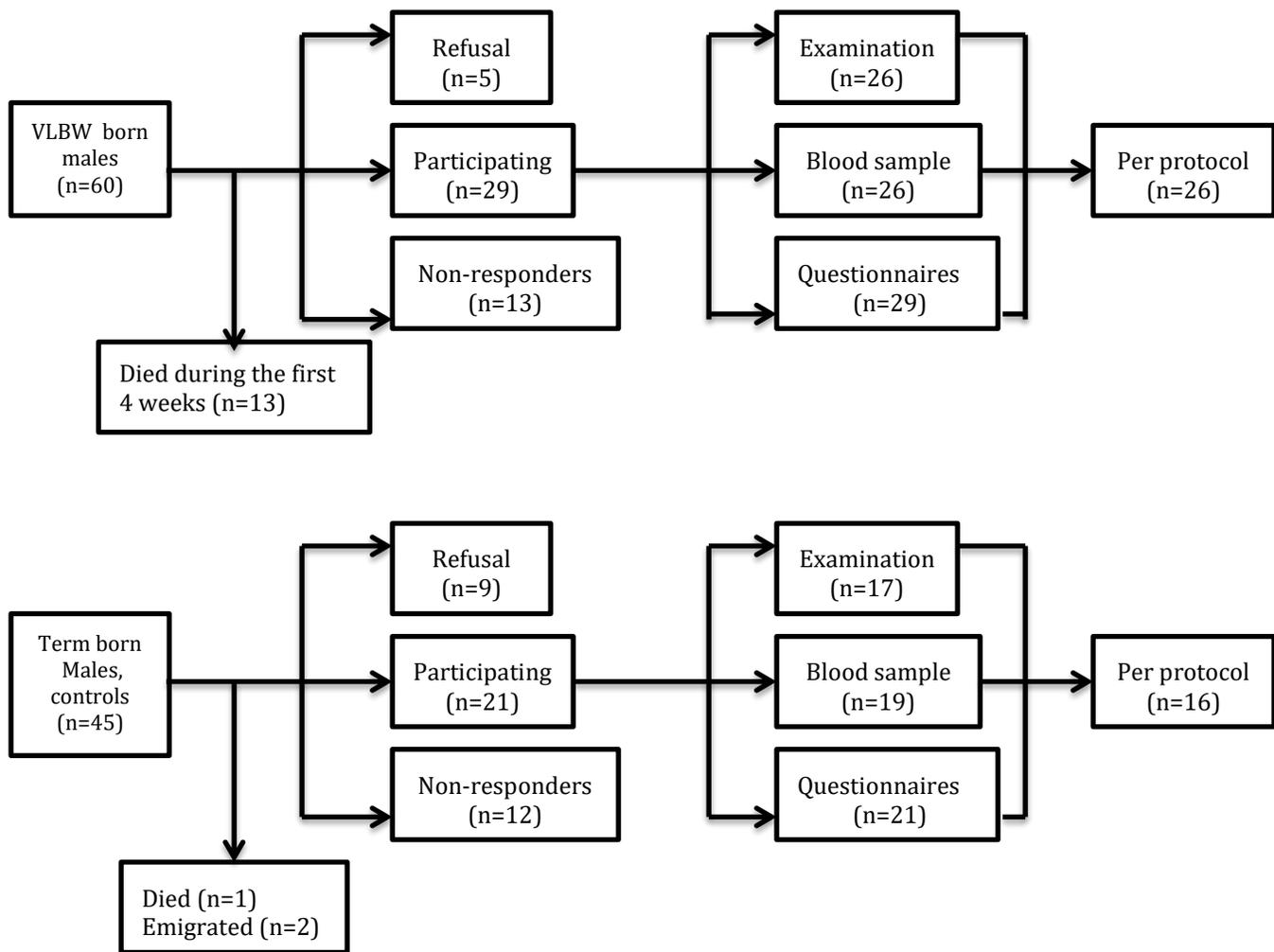


Figure title:

**Figure 1. A flowchart of the number of participants in a follow-up study of 60 men born with very low birth weight (VLBW) and 45 controls born healthy at term.**

**Figure legend:** A healthy control boy was selected for each of the 47 VLBW boys who survived the neonatal period, i.e. the first four weeks. This follow-up performed when the men were 26-28 years old included questionnaires (which could be sent to the men who did not accept to come to a personal visit), physical examinations and blood sampling for a number of hormonal analyses. The figure shows the number of men in each group who participated in different steps of the study and also the drop-outs from the original groups.



