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RESEARCH ARTICLE

Pre- and Post-Pubertal Leptin Levels in Subjects Born Very Preterm and Full-Term Small for Gestational Age in Relation to Measurements of Adipose Tissue

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ABSTRACT

Background: Children born with low birth weight may be at risk of developing the metabolic syndrome.

Aim: Determine body composition and metabolic profile with focus on adipose tissue and leptin from childhood to young adulthood in a cohort of subjects born very preterm with LBW or term-born small for gestational age (SGA) compared to controls.

Methods: This follow-up cohort study included 82 adults (57% women), mean age 21 (range 19-22) years. Seventy-four (90%) of these had taken part in a pre-pubertal study at age 9 years and were re-examined. The adult cohort comprised of subjects born very preterm (<30 gestational weeks) (n= 31) (preterm), born SGA (n=24) and full-term normal-weight controls (n= 27). Demographics including target height were collected. Adipose tissue was estimated by impedance. Fasting levels of glucose, c-peptide, leptin, insulin, insulin growth factor (IGF) binding protein (BP)-1, IGF-I and glucagon were determined.

Results: Preterm women weighed less ($p<0.01$) and had lower height SDS ($p<0.05$) compared with control women at follow-up. Preterm women had lower total adipose tissue percentage (24 (20–27) *vs* 29 (25–32) %, $p=0.037$), and trunk fat mass (19 (15–23) *vs* 26 (22–30)%, $p=0.029$) compared with control women, not seen in preterm men. Glucose or insulin did not differ between groups, but mean C-peptide was higher in SGA women compared with preterm women ($p<0.05$). Leptin levels did not differ between groups ($p=0.86$). Leptin was closely correlated with total adipose content in men ($r= 0.79$, $p< 0.001$) and in women ($r= 0.84$, $p< 0.001$). Leptin adjusted for adipose tissue was higher in all preterm compared with controls ($p=0.017$). In multiple regression analyses, 78% of the leptin variability was explained by adipose tissue percentage, insulin and gender ($p<0.0001$, n=76). In preterm men IGF-I was strongly correlated with adipose tissue percentage ($r=0.77$, $p<0.001$).

Conclusion: In young adulthood, preterm women weigh less, were shorter and had lower adipose tissue percentage, and all preterm had higher leptin levels adjusted for adipose tissue. We speculate that the higher leptin levels in relation to adipose tissue in preterm might serve as protection to enhance insulin sensitivity. The higher C-peptide could indicate peripheral insulin resistance in SGA women. IGF-I could be of special importance to adipose tissue in preterm men.

Keywords: Preterm children, SGA, body mass index (BMI), insulin resistance, leptin, IGFBP-1

Abbreviations

AGA: Appropriate for gestational age
BMI: Body mass index
HOMA-IR: homeostasis model assessment insulin resistance index
IGF: Insulin growth factor
IGFBP-1: Insulin growth factor binding protein-1
IUGR: Intrauterine growth retardation
LBW: Low birth weight
OGTT: Oral glucose tolerance test
SGA: Small for gestational age
tb-SGA: term born small for gestational age
VPT-LBW: very preterm low birth weight

Introduction

Approximately 5000 individuals are born preterm, (before week 37) in Sweden. Of these 900 subjects are born before week 32 and surviving the first year. In Sweden up to 100000 subjects have been born before week 37.

Being born with low birth weight (LBW) (less than 2500 grams) could either be due to preterm birth or being born full-term with intrauterine growth restriction (IUGR), small for gestational age (SGA) or a combination of these two. The short-term effects, during early postnatal weeks and childhood in terms of growth, metabolic and cardiovascular out-come of a preterm or SGA birth have been studied quite extensively [1, 2] but in adults, clinical studies are still scarce [3-6] and we are lacking information on long-term effects and beneficial systems.

Low birth weight is associated with an increased risk for type 2 diabetes, as shown by one meta - analysis [7], probably due to a reduced insulin sensitivity [8] in adulthood. In studies of individuals born with LBW, insulin resistance have been detected in children [9] and at young adulthood [10-12] but the mechanism behind these relationships are poorly understood. At 25 years, very LBW, especially women, were shown to display a less favorable metabolic profile compared with controls [13].

In a study of 9-year-old children born SGA at term higher insulin levels compared to controls when adjusted for body mass index (BMI) were found and these results were interpreted as early signs of peripheral insulin resistance [14].

Leptin is a hormone derived from the adipocytes that reflects adipose tissue mass and obesity [15]. Leptin levels correlate to adipose tissue percentage [16]. Lipodystrophy, a rare form of heterogenous disorder characterized by adipose tissue deficiency, gives rise to a severe hyperglycemia and intractable diabetes. Leptin replacement therapy (metreleptin) has been found to improve metabolic parameters in many patients with lipodystrophy [15].

Several large studies found lower fat mass in preterm born children at 5-7 years [2, 17, 18]. Female sex was a strong predictor for higher fat mass in these studies [2]. However, at age 20 higher total fat mass, adjusted for height standard deviation score (SDS), was present in a large cohort of preterms compared with full-term controls [4]. Prepubertal hyperglycemic SGA females exhibited relatively higher leptin levels in relation to BMI SDS, rising speculation that this might be a protective feature to prevent hyperglycemia in this group [19]. Leptin receptors are found in human liver cells [20] and it has been speculated that leptin may act by hepatic receptors to enhance the hepatic insulin sensitivity. In a study of 9-year-old preterm subjects indications of a reduced hepatic insulin sensitivity were found [14].

The aim of this study was to determine leptin levels in relation to adipose tissue measurements as well as to insulin and predicted target height in a group of adults born with LBW due to preterm birth or SGA. In a large part of the cohort these hormones were also studied at age 9. Furthermore, we aimed to study differences in pre-and post-pubertal levels and to evaluate leptin in relation to gender and growth to further comprehend the influence of this hormone.

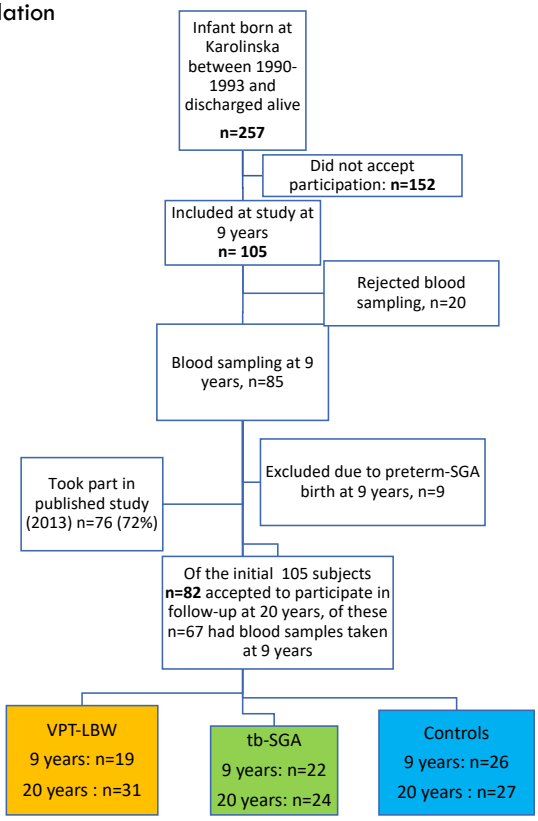
Material and methods

SUBJECTS

See flowchart fig 1. The characterization and modes of selection of the children at 9 years of age has previously been described [14, 19]. In brief, 257 children born at the Karolinska University Hospital between 1990 and 1993 were invited to take part in a follow-up study at 9 years of age, 105 of them accepted to participate. At the time of investigation at 9 years of age all children were at Tanner stage < 2. The study participants (n=105) were then reinvited at age 20 and of these 82 subjects accepted to participate and were reexamined after puberty. Of those who agreed to participate as adults, 67 (84%) subjects also had blood samples taken at age 9.

In the adult group 57% (46/82) were women and mean age was 21 (range 19-22) years at reexamination. The adult cohort comprised of subjects born preterm (<30 gestational weeks) (n= 31) (preterm), born SGA at term (n=24) (tb-SGA), and full-term normal-weight controls (n= 27).

Fig 1. Flowchart of the study population



Methods

DATA COLLECTION

Gestational age (GA) for the children was determined by ultrasound in early pregnancy. SGA was defined as a birth weight ≤2 SD (i.e. below the sex specific 2.5th centile for gestational age) according to Swedish reference data for normal fetal growth [21]. SDS are based on the Swedish reference curve at birth [21] and at the time of the study [22]. In children, target height was calculated according to parental height [(father´s height + mother´s height)/2 + 6.5 (boys) – 6.5 cm (girls)] cm, and target height SDS according to the Swedish reference material in adult age [22]. Parental/target height was missing in one preterm individual.

Neonatal characteristics were collected from the Swedish Birth Records. Paternal and maternal anthropometric characteristics (for target height calculations) were obtained by the written/oral questions asked prior to or at the time of the hospital visit at 20 years.

The same research nurse preformed all anthropometric measurements and blood samplings in the children and the same physician for the adult subjects. The same physician performed 90% of the physical exams in children and another physician performed all the physical exams in the adult subjects. At the time of the study all children and adults were in good health. Written consent was obtained from the parents of the children and from the children at the first follow-up and once more from the study subjects at the second follow-up in adulthood. The study was approved by the Regional Ethical Review Board, Stockholm (number 2012/525-31/1).

Fasting blood values and homeostasis model assessment insulin resistance index (HOMA-IR)

Blood samples were drawn in the morning after an overnight fast. Serum leptin was analyzed with a human leptin RIA KIT, where ^{125}I activity is measured by gamma counter (Linco Research, Inc., Missouri, USA). The interval for measuring leptin is 0.5-100 µg/L, reference interval BMI 16-25 kg/m2, and women <15 µg/L. Reference intervals for leptin levels in children are not available.

In-house RIA was used for IGFBP-1 with individual serum samples in the same assay. The RIA for IGFBP-1 was performed according to Povoa et al 1984 with intra- and inter-assays CV 3% and 10%, respectively [23, 24]. Blood glucose readings were performed and documented by the nursing staff using HemaCue 201`s method [25]. In children commercial kits were used for S-insulin by electrochemiluminescence immunoassays (Roche

Diagnostics GmbH, Mannheim, Germany). HOMA-IR in children and adult subjects was calculated from the formula (fasting glucose* fasting insulin /22.5). In-house RIA was also used for IGF-I with individual serum samples in the same assay. IGF-I was measured after ethanol extraction and cryoprecipitation and using des(1-3) IGF-I as a ligand [26]. The intra- and inter-assay CV were 4% and 11%, respectively Human adiponectin (only in adult subjects) was analyzed by adiponectin radio-immuno-acid (RIA) kit, Linco Research, Inc., Missouri, USA. Laboratory analyses for HbA1c, c-peptid, Cholesterol, HDL/LDL, triglycerides. Prolactin with reference values (range): women (102-496) mIE/L, and men (86-324) (mIE/L), Glucagon, s-Cortisol, TSH and T4 were measured according to standard laboratory procedures.

Statistics

Anthropometric data in boys, girls, men and women are presented as median (range). Serum leptin, insulin, HOMA-IR, IGFBP-1, IGF-I, adiponectin, s-Prolactin, s-Glucagon, s-Cortisol, values were log-transformed, due to lack of normal distribution, and are presented as geometrical mean ± 95% confidence intervals (CI).

The comparison between three independent groups was assessed by analyses of variances (parametric ANOVA) test, followed by post-hoc Fischer´s test for comparisons between the separate groups. Forward stepwise multiple regression analyses were also performed. A p-value of <0.05 was considered significant. The statistical analyses were performed using Statistical Stat Soft, version 20 (Tulsa, OK, USA).

Results

ANTHROPOMETRIC DATA

Weight at follow-up and catch-up from birth to follow-up

Growth variables for study subjects at birth are shown in Table 1, and in adulthood in Table 2. Median age at last follow-up was 20.6 years, taken all groups together, tb-SGA were slightly younger. VPT-LBW women weighed less (mean 56 (95% CI:51–61) *vs* 66 (61–71) kg, p< 0.01), and had lower height (SDS –0.61 (–1.1–0.13) *vs* 0.11 (–0.38–0.60), p= 0.041) compared with control women at follow-up but did not differ in BMI (Table 2).

Table 1. Characteristics at the time of birth for subjects born very preterm (<30 weeks) (VPT-LBW), term and small for gestational age (tb-SGA) and term with normal birth weight (controls).

At birth	VPT-LBW (n=31)	tb-SGA (n=24)	Controls (n=27)
Girls, n (%)	17 (55%)	14 (58%)	15 (56%)
GA (weeks)	26.7 (26.1–7.4)	39.4 (38.8–40.0)	39.4 (38.9–40.0)
Birth weight (gram)	970 (830–1110)	2480 (2340–2610)	3450 (3320–3580)
Birth weight–SDS	–0.8 (–1.2– (–0.3)	–3.1 (–3.5– (–2.6))	–0.4 (–0.8–0.0)
Birth length (cm)	35 (34–36)	47 (46–48)	50 (49–51)
Birth length–SDS	–1.3 (–1.7– (–0.4))	–2.6 (–3.3– (–2.0))	–0.7 (–1.3– (–0.1))
Head circumference (cm)	24 (23–24)	33 (32–33)	35 (34–35)
Head circumference–SDS	–0.7 (–1.2– (–0.3))	–1.3 (–1.7– (–0.9))	0.3 (0.1–0.7)

CI: confidence interval, GA: gestational age, SDS: standard deviation score. Data are presented as mean (95% CI).

Table 2. Characteristics of the study cohort born very preterm (<30 weeks) (VPT-LBW), term and small for gestational age (tb-SGA) and term with normal birth weight (controls) at 20.6 years.

At follow-up 20 y	VPT-LBW (n=31)	tb-SGA (n=24)	Controls (n=27)	P-value
Age (years)	20.9 (20.6–21.3)	20.2 (19.8–20.6)	20.8 (20.4–21.1)	<0.05#
Weight (kg)	64 (59–68)	64 (59–68)	70 (66–75)	0.06
Men	73 (67–79)	68 (61–77)	77 (71–83)	0.18
Women	56 (51–61)	61 (55–66)	66 (61–71)	<0.01*
Weight–SDS	–0.35 (–0.8–0.1)	–0.24 (–0.7–0.2)	0.46 (0.0–0.9)	<0.05* <0.05#
Height (cm)	170 (167–174)	169 (165–172)	174 (170–177)	0.13
Height-SDS	–0.48 (–0.9–0.1)	–0.70 (–1.1–0.25)	0.16 (–0.26–0.57)	<0.05* <0.01#
Men	–0.31 (–1.0–0.33)	–0.89 (–1.7–0.12)	0.22 (–0.51–0.96)	0.12
Women	–0.61 (–1.1–0.13)	–0.55 (–1.1– 0.03)	0.11 (–0.38–0.60)	0.041*
BMI (kg/m^2)	22 (21–23)	22 (21–24)	23 (22–25)	0.25
Men	23 (21–25)	22 (20–24)	23 (21–25)	0.79
Women	21 (19–23)	23 (21–25)	23 (21–25)	0.16

At follow-up 20 y	VPT-LBW (n=31)	tb-SGA (n=24)	Controls (n=27)	P-value
Waist circumference (cm)	78 (74–81)	78 (74–81)	82 (78–85)	0.24
Hip circumference (cm)	97 (94–101)	97 (94–100)	98 (93–102)	0.99
Fat mass (%)	19 (16–22)	22 (18–25)	23 (19–26)	0.26
Men	14 (11–17)	14 (10–17)	14 (11–18)	0.92
Women	24 (20–27)	28 (24–31)	29 (25–32)	0.037*
Fat mass (kg)	12 (9–15)	14 (11–17)	16 (14–19)	0.12
Men	10 (7–13)	10 (6–13)	11 (8–15)	0.72
Women	14 (10–17)	17 (13–21)	20 (16–23)	0.016*
Trunk fat mass (%)	17 (14–20)	19 (16–23)	21 (18–25)	0.18
Men	14 (11–18)	13 (9–17)	15 (11–19)	0.79
Women	19 (15–23)	24 (19–28)	26 (22–30)	0.029*
Target height (cm)	174 (171–177) (n=1 missing)	171 (168–174)	172 (169–175)	0.44
Catch up WSDS from 9 y to 20 y	0.81 (0.1–1.6)	–1.1 (–1.8–0.33)	0.02 (–0.7–0.7)	<0.01* <0.05#
Catch up LSDS from birth to 20 y	0.7 (–0.1–1.4)	1.9 (1.2–2.7)	0.8 (0.2–1.5)	<0.05#
Catch up LSDS from 9 y to 20 y	0.1 (–0.3–0.5)	–0.3 (–0.7–0)	–0.2 (–0.5–0.1)	0.25
Diff from pred target height (cm)	–3 (–5– (–1))	–2 (–5– (–0.2))	2 (0.1–4)	<0.01* <0.01#
Diff from pred target height SDS	–0.5 (–0.8–(–0.2))	–0.4 (–0.7–0.0)	0.3 (0–0.6)	<0.01* <0.01#
Diff from pred target height SDS Men	–0.5 (–1–0)	–0.5 (–1–0.1)	0.5 (0–1.1)	<0.01* 0.02#
Diff from pred target height SDS Women	–0.5 (–0.9–(–0.1))	–0.3 (–0.8–0.1)	0.2 (–0.2–0.6)	0.016*

*vs VPT-LBW *vs* control. # vs tb-SGA *vs* control.
SGA: small for gestational age, GA: gestational age, SDS: Standard deviation score, LSDS: Height Standard deviation score BMI: body mass index, pred: predicted, diff: difference, WSDS: weight standard deviation score, Target height SDS, for description see Methods.
Data are presented as number (%) or mean (95% CI). $P < 0.05$ was considered significant according to analysis of variance (ANOVA) or Pearson's chi–square comparing all groups, and according to Fisher's post hoc test for comparison between two groups.

Height at follow-up and catch-up from birth to follow up

The majority (82 %, n= 68/82) of the cohort had reached their target height SDS (± 1 SD) at age 20. Of the 14 subjects that did not reach their target height (± 1 SD) 10 were VPT-LBW, thus, 33% (10/30, one missing) of the VPT-LBW had a final height less than –1 SD from predicted target height. Corresponding figures in the other groups were 13% (3/24) in tb-SGA and 4% (1/27) in controls.

ADIPOSE TISSUE CONTENT

Adipose tissue content in total and percentage as well as trunk fat mass and muscle mass are shown in Table 2. The VPT-LBW women had lower total adipose tissue percentage (24 (20–27) *vs* 29 (25–32) %, p= 0.037), and trunk fat mass trunk fat mass (19 (15–23) *vs* 26 (22–30)%, p=0.029) compared with control women, not seen in VPT-LBW men (Table 2 and Fig 2).

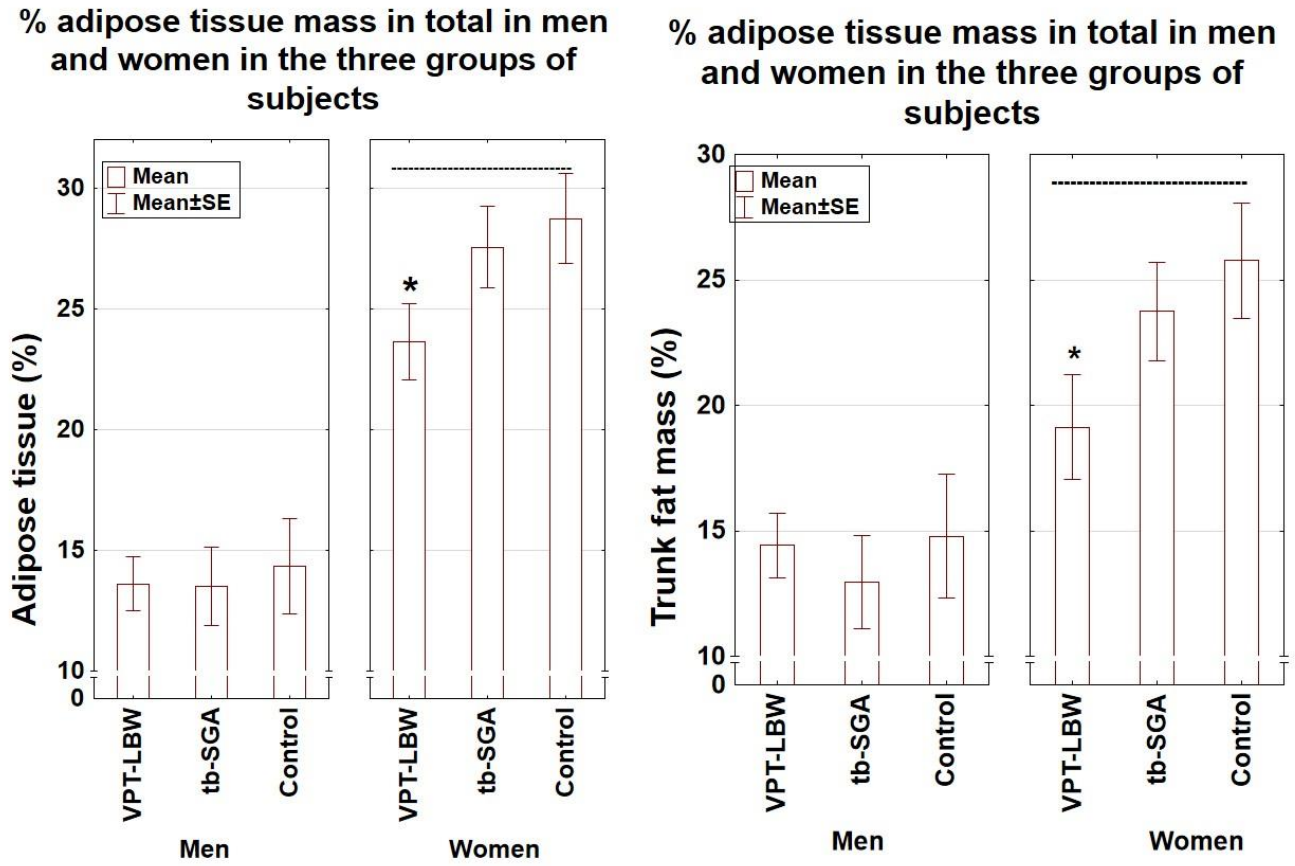


Fig 2. % adipose tissue mass and trunk fat mass in the three adult groups of men and women (VPT-LBW, tb-SGA and controls). VPT-LBW women had lower adipose tissue fat mass and lower trunk fat mass (in percentage) compared with control women.
(* p <0.05)

LEPTIN

Leptin levels in relation to childhood levels and in men and women

Fasting serum levels of leptin, IGFBP-1, insulin, glucose, IGF-I and adiponectin as well as the calculated HOMA-IR are shown in Table 3. No difference in mean leptin values were observed between the three groups grouped according to gender. Leptin levels did not differ between groups (p=0.86), despite less adipose tissue in VPT-LBW women. Leptin at 9 and 20 years are shown in Fig 3. In young adulthood all women had four times higher leptin levels compared to men (mean 15.2 *vs* 3.94 µg/L, p< 0.001). Leptin levels in the adult tb-SGA women were four to five times higher compared with prepubertal levels (mean (95% CI) 4.3 (2.5-7.4) to 19 (13-26) µg/L). In VPT-LBW and control women leptin were two times higher compared with prepubertal levels. Leptin at 9 years correlated positively with leptin at 20 years in men (r = 0.48, p= 0.011) but not in women (r = – 0.04, p = 0.81).

Table 3. Blood samples in the study cohort born very preterm (<30 weeks) (VPT-LBW), term and small for gestational age (tb-SGA) and term with normal birth weight (controls) at 20.6 years.

At follow-up 20 y	VPT-LBW (n=31)	tb-SGA (n=24)	Controls (n=27)	P-value
Insulin (pmol/L)	7.3 (5.8–9.2)	8.4 (6.8–10.4)	7.6 (6.1–9.6)	0.64
Men	7.0 (4.5–10.5)	7.3 (4.8–11.1)	7.9 (5.3–11.7)	0.90
Women	7.6 (5.7–10.1)	9.4 (7.7–11.3)	7.5 (6.2–9.0)	0.25
HbA1c (mmol/mol)	32 (31–34)	32 (32–33)	32 (31–33)	0.84
Glucose (mmol/L)	4.8 (4.6–5.0)	4.8 (4.6–4.9)	4.6 (4.4–4.8)	0.23
Men	5.0 (4.7–5.3)	4.8 (4.5–5.0)	4.7 (4.3–5.0)	0.23
Women	4.6 (4.4–4.8)	4.8 (4.6–5.0)	4.6 (4.4–4.8)	0.25
C–peptide (nmol/L)	0.69 (0.60–0.77)	0.74 (0.64–0.85)	0.66 (0.58–0.74)	0.36
Men	0.60 (0.46-0.75)	0.65 (0.49-0.81)	0.64 (0.49-0.80)	0.89
Women	0.68 (0.59–0.76)	0.81 (0.72–0.90)	0.69 (0.60–0.78)	*0.042*¤
HOMA-IR	1.50 (1.26-1.79)	1.79 (1.46-2.20)	1.60 (1.25-2.04)	0.48
Men	1.49 (1.09-2.03)	1.54 (0.98-2.44)	1.63 (0.93-2.83)	0.95
Women	1.52 (1.20-1.91)	1.99 (1.64-2.41)	1.57 (1.26-1.97)	0.14
IGF–I inhouse (µg/L)	355 (311–406)	333 (289–382)	348 (317–382)	0.73
IGF–I SD–Score	0.97 (0.48–1.45)	0.67 (0.18–1.17)	0.86 (0.53–1.19)	0.62
IGF–I (µg/L)	246 (210–287)	237 (206–27)	248 (225–275)	0.85
IGFBP–1 (µg/L)	26 (18–38)	25 (18–35)	23 (17–33)	0.86
IGFBP–3 (µg/L)	4983 (4310–5760)	4745 (4270–5270)	4890 (4490–5320)	0.82
Leptin (µg/L)	8.4 (5.6–12.6)	9.2 (5.6–15.2)	7.8 (5.2–11.7)	0.86
Men	4.5 (2.9–6.9)	3.7 (2.3–6.0)	3.6 (2.2–5.7)	0.74
Women	13 (10–18)	19 (13–26)	15 (11–20)	0.30
Leptin (µg/L) adjusted for adipose (kg)	9.7 (8.1–11.6)	8.9 (7.3–10.9)	7.1 (5.8–9.6)	0.017*
Adiponectin (mg/L)	10.4 (8.7–12.4)	7.6 (6.1–9.5)	8.1 (6.2–10.5)	0.14
Total cholesterol (mmol/L)	4.4 (4.0–4.8)	4.3 (4.0–4.6)	4.4 (4.1–4.7)	0.96
LDL/HDL	1.8 (1.4–2.2)	1.8 (1.4–2.2)	2.1 (1.8–2.4)	0.44
LDL–cholesterol (mmol/L)	2.5 (2.2–2.9)	2.4 (2.1–2.8)	2.7 (2.4–2.9)	0.39
Triglycerides (mmol/L)	0.73 (0.5–0.9)	0.82 (0.64–1.0)	0.80 (0.6–1.0)	0.78
HDL–cholesterol (mmol/L)	1.5 (1.3–1.7)	1.5 (1.3–1.6)	1.4 (1.2–1.5)	0.39
Prolactin (mIE/L)	204 (158–264)	240 (209–277)	215 (181–255)	0.44
Men	154 (118–200)	221 (170–287)	240 (187–307)	0.01*, 0.04¤
Glucagon (pmol/L)	67 (64–71)	65 (60–71)	70 (65–75)	0.35
Cortisol (nmol/L) morning	520 (421–643)	556 (457–676)	531 (443–637)	0.88
T4, free (pmol/L)	11.3 (10.4–12.2)	10.9 (20.2–11.6)	10.5 (9.8–11.2)	0.32
TSH (mE/L)	2.4 (1.3–3.5)	1.7 (0.8–2.7)	2.0 (1.6–2.4)	0.73

¤VPT-LBW *vs* tb-SGA. *VPT-LBW *vs* control.

SGA: small for gestational age, GA: gestational age, HBA1c: Hemoglobin A1 c, IGF–I: insulin growth factor –1, IGFBP–1: insulin growth factor binding protein–1, IGFBP–3: insulin growth factor binding protein–3, LDL: low density lipoprotein, HDL: high density lipoprotein, for description see Methods.

Values are presented as mean or geometrical mean (insulin, HOMA-IR, IGF–I inhouse, IGF–I, IGFBP–1 and 3, leptin, adiponectin, prolactin, glucagon) (95% CI). $P < 0.05$ was considered significant according to analysis of variance (ANOVA) or Pearson's chi–square comparing all groups, and according to Fisher's post hoc test for comparison between two groups.

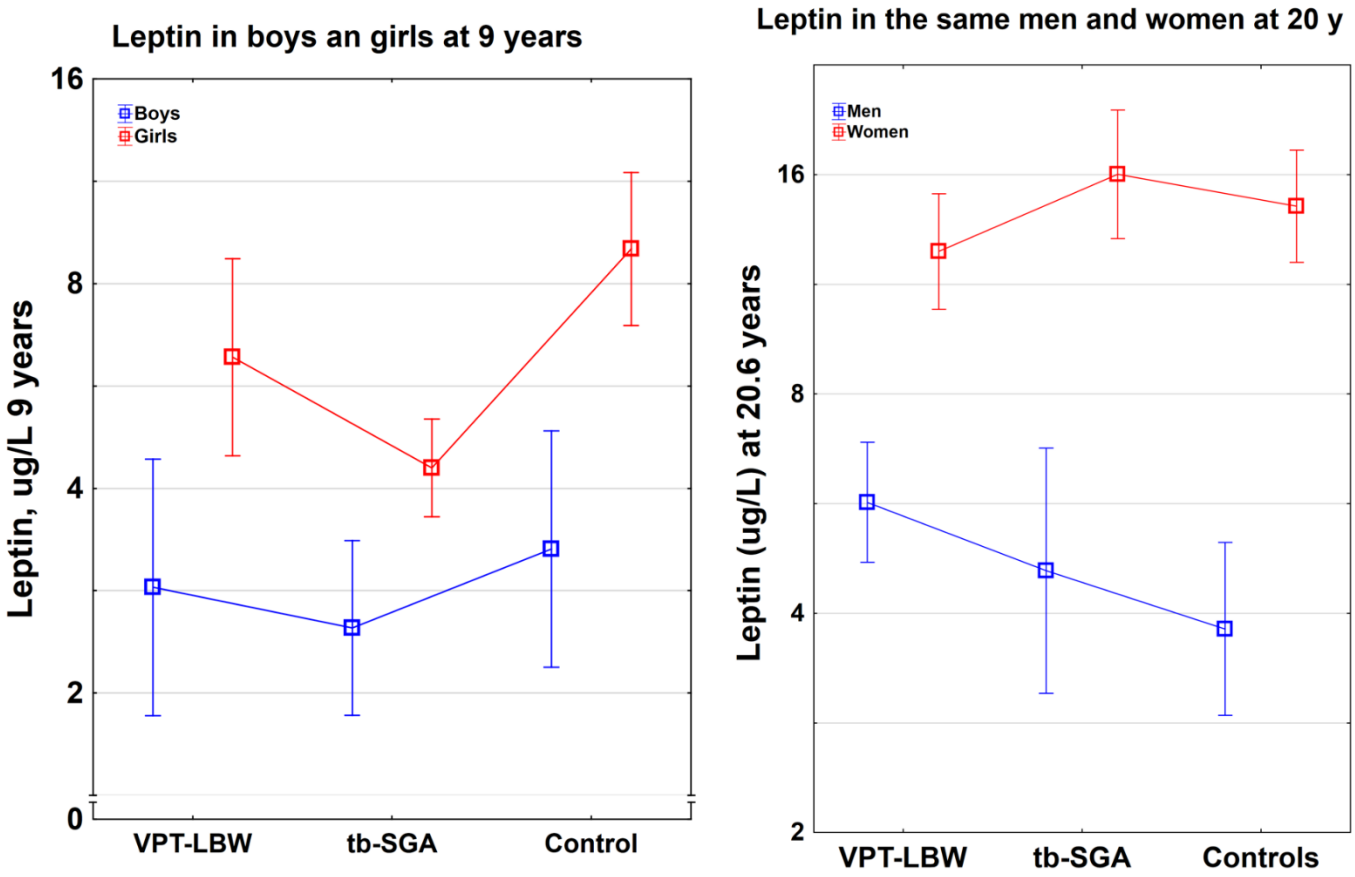


Fig 3. Comparison between leptin levels in girls (A) to women (B) (red) and in boys (A) to men (B) (blue). In young adulthood all women had four times higher leptin levels compared to men (mean 15.2 *vs* 3.94 µg/L, p< 0.001). Leptin levels in the adult tb-SGA women were four to five times higher compared with pre-pubertal levels (mean (95% CI) 4.3 (2.5-7.4) to 19 (13-26) µg/L).

Glucose or insulin did not differ between groups, but mean C-peptide was higher in tb-SGA women compared with VPT-LBW women (0.68 (0.59–0.76) *vs* 0.81 (0.72–0.90) nmol/L, p= 0.042) (Table 3). Prolactin was lower in VPT-LBW men compared with tb-SGA and control men (p<0.05, (Table 3).

Adipose tissue content and leptin

Leptin was closely associated to adipose tissue percentage in all men (r= 0.79, p< 0.001) and all women (r= 0.84, p< 0.001), (Fig 4). Leptin adjusted for adipose tissue was higher in all VPT-LBW compared to controls (9.7 (8.1–11.6) *vs* 7.1 (5.8–9.6) µg/L, p= 0.017). In men and women leptin correlated with insulin (r= 0.64, p< 0.001 (men) and (r= 0.48, p< 0.001) (women)) (fig 5). Leptin was also positively correlated with HOMA-IR in both men (r=0.62, p<0.001) and women (r=0.53, p<0.001).

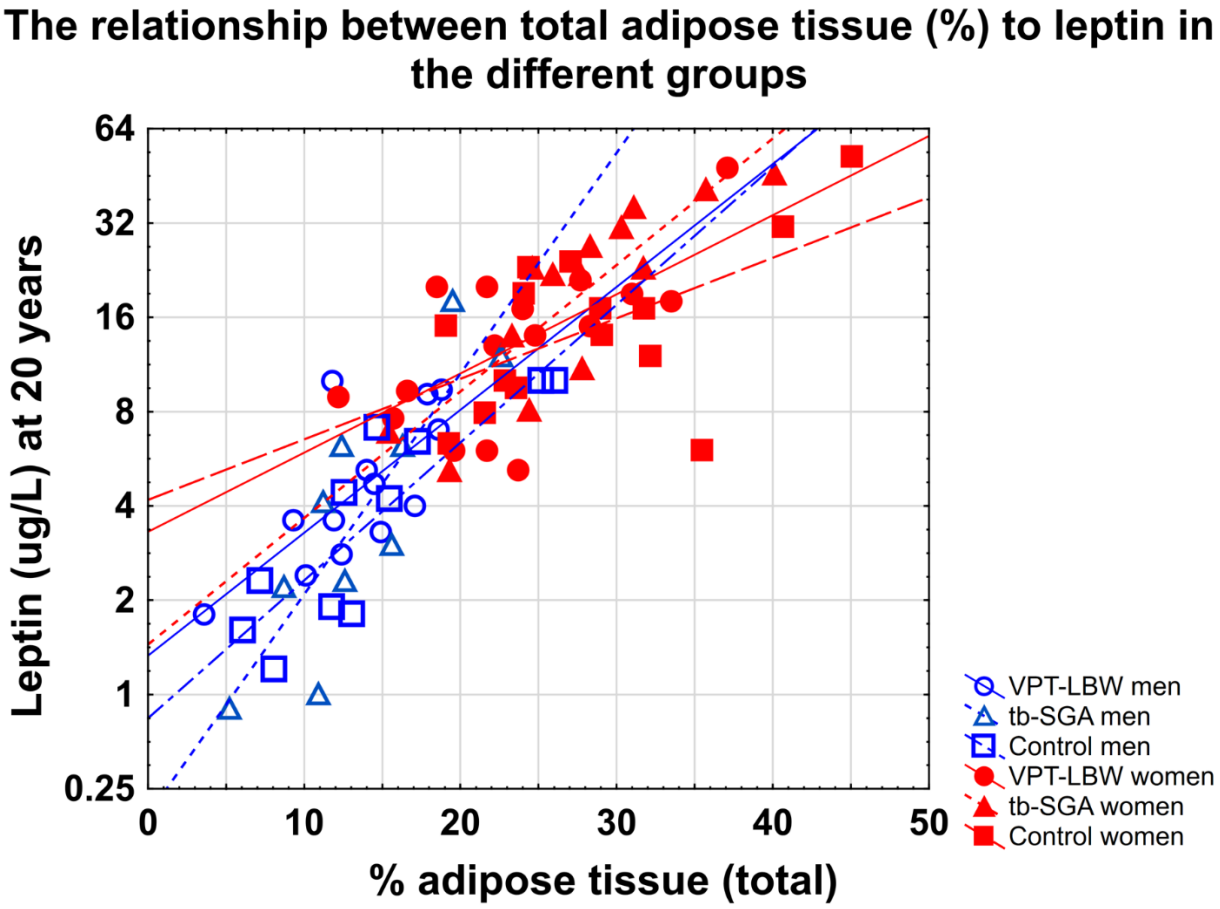


Fig 4. Leptin to adipose tissue % total in the different groups of men and women. The different symbols of men are blue VPT-LBW =, square tb-SGA = triangle and control= rectangle and women are red VPT-LBW = square, tb-SGA = triangle and control = rectangle. All groups had strong correlations between leptin levels and total adipose tissue (%). In men: the association between the variables was VPT-LBW r =0.71, p<0.01, tb-SGA r=0.85, p<0.01 and controls r=0.87, p<0.01 and women: VPT-LBW r =0.66, p<0.01, tb-SGA r=0.86, p<0.001 and controls r=0.57, p=0.03. In all men the regression line was: 2Log y=-0.262+0.163*x in all women: 2Logy=1.635+ 0.087*x.

In multiple regression analyses, 78% of the leptin variability was explained by adipose tissue percentage, insulin and gender (p<0.0001, n=76).

IGFBP-I TO INSULIN AT 9 AND 20 YEARS

At age 20 the regression line of insulin to IGFBP-1 was suppressed in VPT-LBW men compared with women not seen in the other groups (Fig 6).

23% of the IGFBP-I variability was explained by insulin, leptin (p=0.06) and gender. The relationship between leptin and IGFBP-1 was positively correlated in VPT-LBW men (r = –0.61, p= 0.022) not seen in the combined group of full-term men (r = –0.31, p= 0.19).

SUBGROUP OF MALES THAT DEVIATED -1 SD FROM PREDICTED TARGET HEIGHT

In VPT-LBW men IGF-I/ IGF-I SD was strongly correlated with adipose tissue percentage (r= 0.77, p< 0.001) (Fig 7a), not seen in preterm women or the other groups. One third of the VPT-LBW did not reach their target height (> –1 SD).

VPT-LBW men that deviated > -1 SD from predicted target height (n=6) had lower IGF-I (214 (180–255) *vs* 278 (230–336) µg/L, p= 0.031) (Table 4) and less trunk fat mass percentage 11 (6–17) *vs* 17 (14–19) %, p= 0.037) compared with VPT-LBW males (n=8) that had reached their target height (± 1SD). A more positive deviation from target height correlated with higher IGF-1 in VPT-LBW men. (Fig 7b).

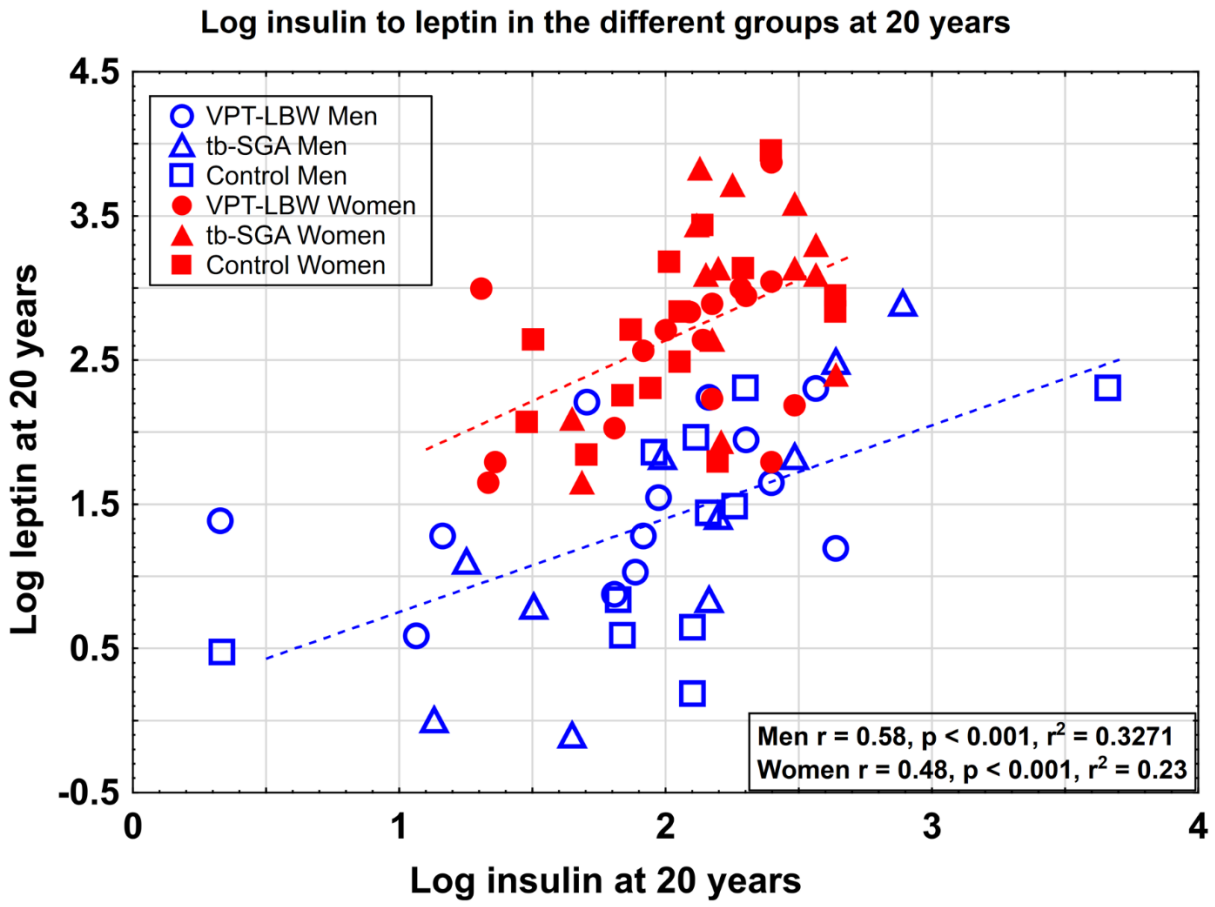


Fig 5. Log leptin levels in relation to log insulin at age 20 in men and women. The different symbols of men are blue VPT-LBW =, square tb-SGA = triangle and control= rectangle and women are red VPT-LBW = square, tb-SGA = triangle and control = rectangle. The relationship was significant in all men r=0.57, p<0.001 (Log y= 0.106+0.647 * Log x), and in all women r=0.47, p<0.001 (Log y = 0.954+0.841 * Log x).

Table 4. Adipose tissue mass and blood samples in preterm men that differed >—1 SD in height compared with target height compared with men that did not, at 20.6 years.

	VPT-LBW men differed > –1 SD in predicted target height (n=6)	VPT-LBW men differed ≤ –1 SD in predicted target height (n=8)	P-value
Fat mass (kg)	7 (4–11)	12 (10–15)	0.017
Trunk fat mass (%)	11 (6–17)	17 (14–19)	0.037
BMI (kg/m²)	22 (20–24)	24 (21–26)	0.24
Insulin (pmol/L)	5.0 (3.3–7.5)	7.7 (3.7–16)	0.25
Glucose (mmol/L)	4.9 (4.5–5.3)	6.2 (3.3–9.1)	0.32
C–peptide (nmol/L)	0.55 (0.31–0.79)	0.65 (0.39–0.90)	0.51
HbA1c (mmol/mol)	30 (27–33)	37 (24–50)	0.28
IGF–I (µg/L)	214 (180–255)	278 (230–336)	0.031
IGF–I SD score	0.16	1.3	0.011

HbA1c: Hemoglobin A1 c, IGF–I: Insulin growth factor 1. Analyses by variances (ANOVA), t-test between groups. Values are presented as mean or geometrical mean (insulin, IGF–I) (95% CI).

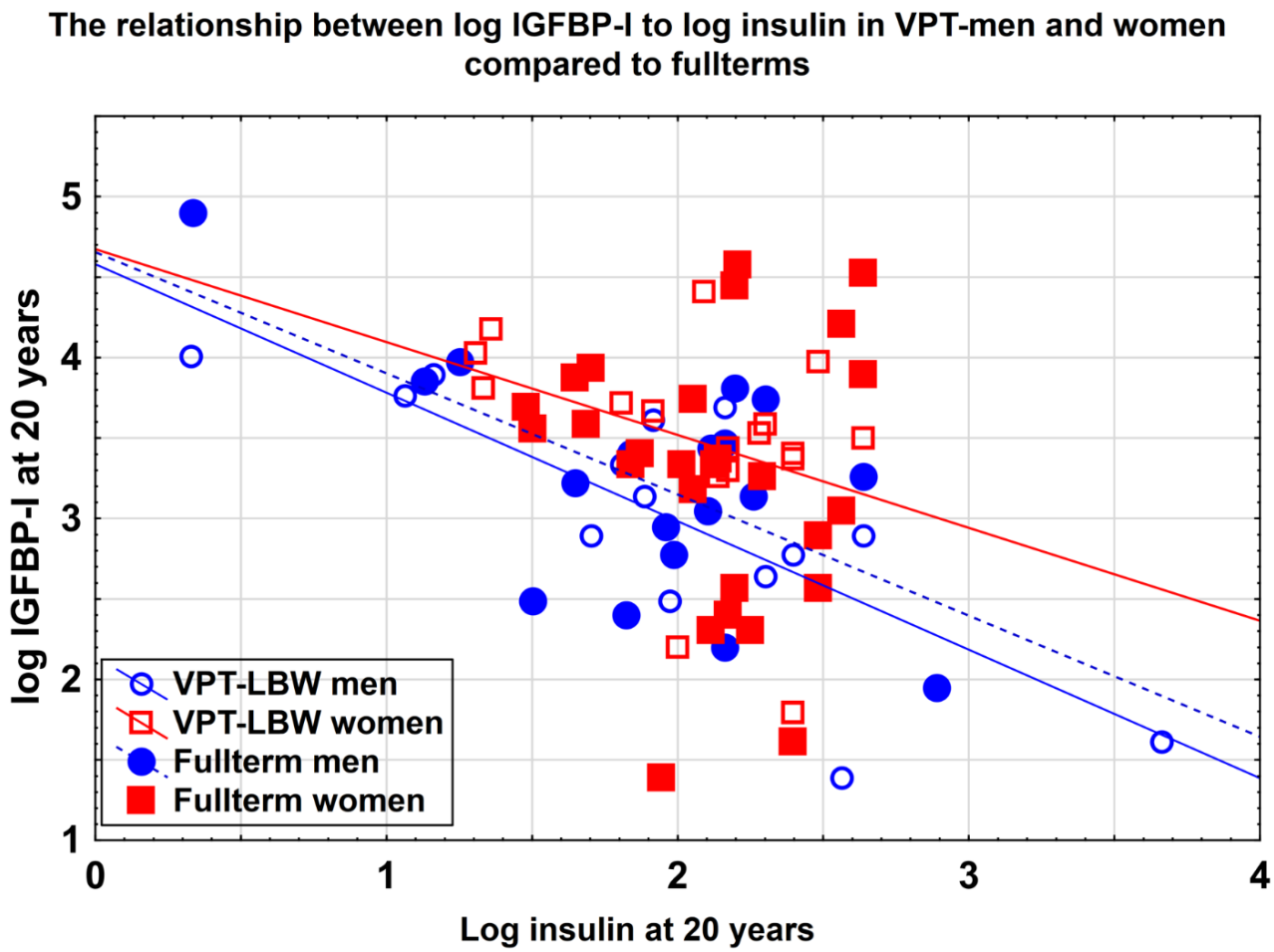


Fig 6. The relationship between log insulin to log IGFBP-I in VPT-LBW men and women compared with the combined full-term group of men and women. A significant relationship was seen in VPT-LBW men ($r=-0.79$, $p<0.001$) and full-term men ($r=-0.62$, $p<0.01$) but not in women's groups. VPT-LBW men had a reduced regression line compared to the regression line of VPT-LBW women ($p<0.01$).

Discussion

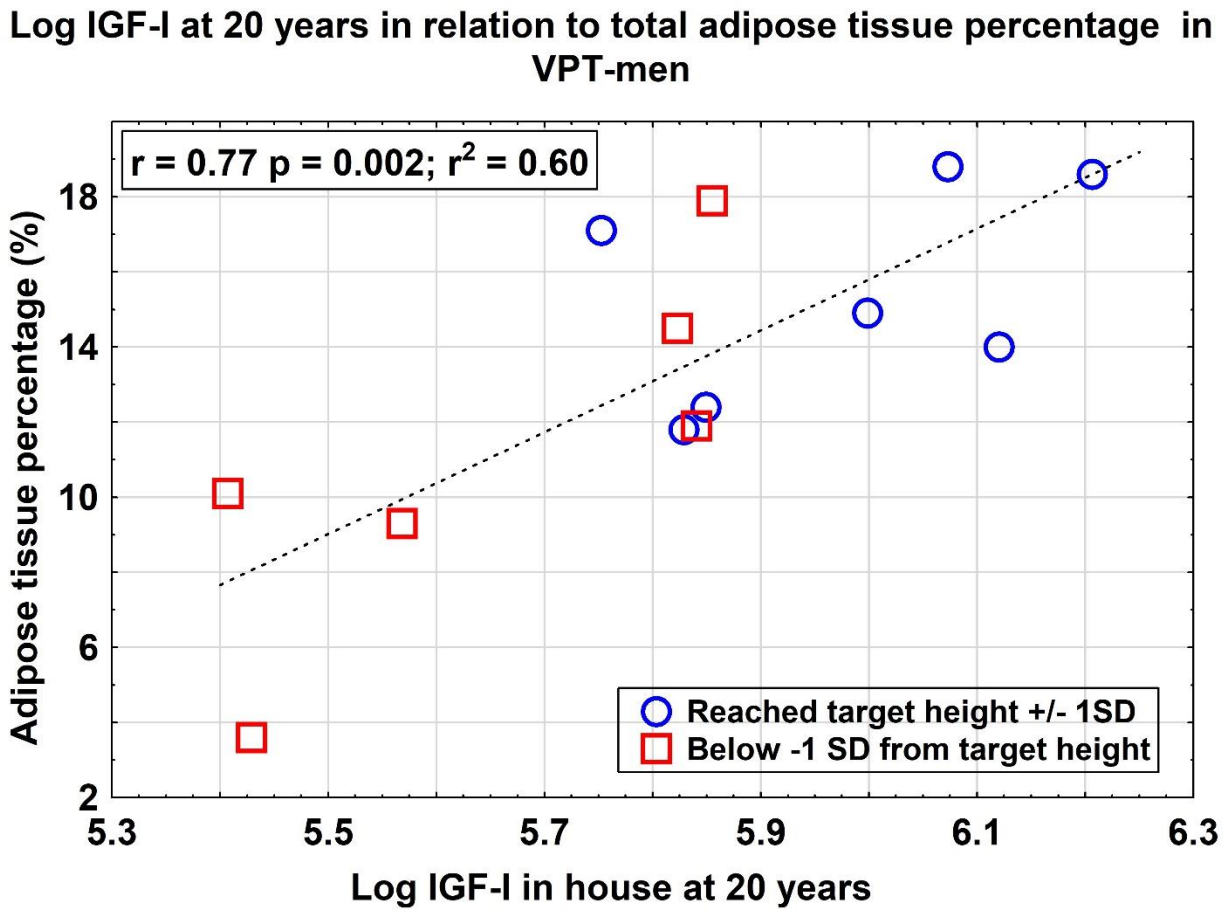
In this follow-up study of young adults born VPT-LBW or tb-SGA we found strong correlations between leptin and adipose tissue content in all groups and both genders. Our study is the first to present leptin levels in the same VPT-LBW cohort both during pre-and post-puberty. Almost 80% of the leptin variability was explained by adipose tissue percentage, insulin, and gender. The VPT-LBW women had lower total adipose tissue percentage and trunk fat mass compared with control women, not seen in VPT-LBW men. Although VPT-LBW tended to have lower adipose tissue content compared with the other groups their leptin levels did not differ. Leptin in former VPT-LBW at 20 years was higher compared with tb-SGAs and controls when adjusting for adipose tissue mass. Leptin levels increased more in women compared with men after puberty. Pre-and postpubertal leptin levels was correlated in men, not seen in women. One third of the VPT-LBWs did not reach their target height and in VPT-LBW men IGF-I was strongly correlated with adipose tissue.

We found no differences in glucose, HOMA or insulin levels. The Tb-SGA women had higher c-peptide levels compared with VPT-LBW women, not seen in men. The VPT-LBW men had a suppressed regression line of insulin to IGFBP-1 and lower prolactin compared with controls, although their prolactin levels were within reference values.

Serum leptin levels reflect body fat and adipose tissue content [27] in humans. Insulin has been suggested to increase leptin mRNA and its release by adipocytes [28]. We also found distinct correlations between insulin and leptin levels in all groups and gender.

In contrast to our results, Breukhoven *et al* found higher percentage of fat mass and trunk fat mass, adjusted for height SDS, determined by dual-energy x-ray (dexa) in 167 preterms aged between 18-24 years in whom half were born SGA

compared with full-terms controls. [4]. In the study PT-LBW born before week 36 were included but it is unclear how many subjects were born < 30 week. Also, the influence of gestational age was not studied separately between groups. In another large study, fat mass was determined by air-displacement plethysmography during different ages in PT-LBW born between week 34-36. In this study, a lower fat mass was present in childhood but a higher in adulthood [29]. Jussinniemi *et al* found no differences in fat mass between VPT-LBW and controls at age 36, however those born VPT-LBW were shorter and had lower lean body mass [6]. In a study in which 8-12 years olds were investigated by DEXA, 200 preterms (consisting of both very preterms and late preterms) were enrolled [2]. After adjustment for height preterm born subjects had lower fat mass but no differences in fat free mass compared to full-terms. Female sex was a strong predictor for more fat mass. In 2-year-old preterm subjects lower BMI, lower fat mass but higher glucose compared with full-terms was found [30]. Our study found reduced fat mass in VPT-LBW women but not in men and the influence of puberty on adipose tissue content is more profound in women compared with men.



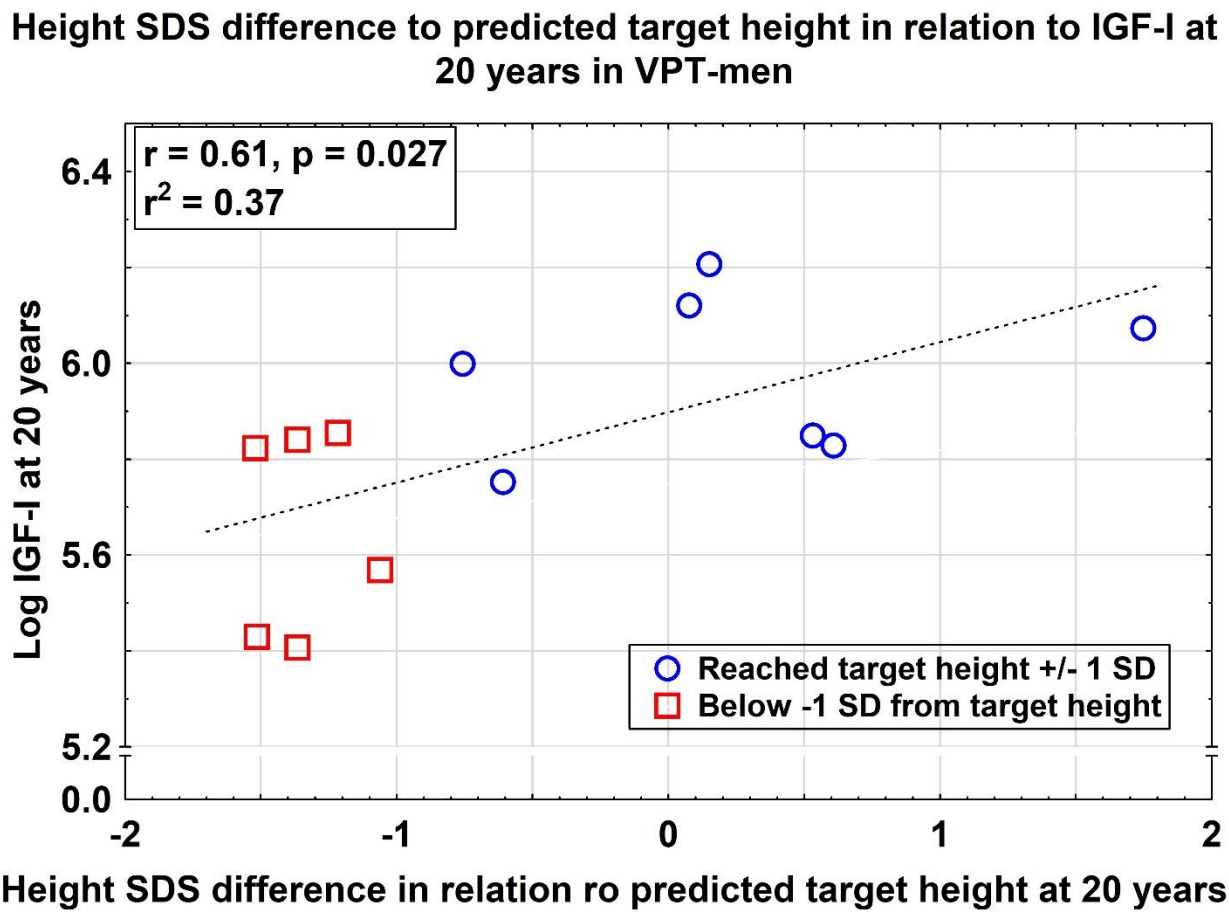


Fig 7a. Log IGF-I to adipose tissue in VPT-LBW men (n=13). The different symbols of men are blue = no deviation from predicted target height) red => -1 SD deviation from predicted target height). b. **Log IGF-I to Height SDS difference in relation to predicted target height at adulthood in VPT-LBW men.** Same symbols as in 7a.

We found relatively increased leptin levels in relation to adipose tissue in the VPT-LBW group. Leptin has been proposed to exert an insulin sensitizing effect, partly by increasing fatty acid and decrease storage of triglyceride in the muscle [31] and decrease glucose [32]. Leptin injections can partly replace the absence of adipose tissue in lipodystrophic patients, who develop insulin resistant diabetes and elevated triglycerides [15]. In mice, leptin was shown to improve hepatic insulin sensitivity in mice of both genders, whereas only male mice also displayed an improved peripheral insulin sensitivity [13]. Increased leptin levels in relation to adipose tissue could be a compensatory event in subjects born preterm to enhance insulin hepatic sensitivity. The ratio leptin- to adipose tissue content may be especially important in VPT-LBW subjects in risk of hyperglycemia in adulthood. VPT-LBWs most at risk for hyperglycemia and type 2 diabetes could be those where leptin levels in relation to adipose tissue are reduced [33].

Prepubertal hyperglycemic tb-SGA females exhibited relatively higher leptin levels in relation to BMI SDS, rising speculation that this might a protective feature to prevent hyperglycemia in this group [19]. However, in adulthood this relation is no longer apparent. In female tb-SGA c-peptide levels were higher compared with VPT-LBW women. C-peptide has been suggested to mimic beta cell function and was shown to be inversely correlated with insulin sensitivity in early type 2 diabetes [34]. The higher C-peptide as well as a slightly higher HOMA-IR might indicate a peripheral insulin resistance in female tb-SGA.

In 1992 Hales and Barker stated the epitet "the thrifty-phenotype" [35] in the etiology of type 2 diabetes. They proposed that the low-birth-weight infant has an impaired growth of the beta-cells. While the individual persists in an undernourished state there is no need to produce much insulin. However, exposed to rapid catch-up growth and overnutrition the reduced state of beta-cell function is insufficient which may lead to diabetes. The authors did not discuss the possible importance of fat mass in their hypothesis since the adipocyte derived hormone leptin was first discovered in

1994 [36]. Embleton *et al* investigated the relationship between weight gain and metabolic outcome in children born VPT-LBW at age 12 and found strong associations between more rapid childhood weight gain to higher fat mass, higher insulin levels and reduced insulin sensitivity [1]. In our study the VPT-LBW male subjects that deviated more than –1 SD from predicted target height, (n=6) had, not surprisingly, lower IGF-I and less adipose tissue content compared with preterm males that had reached their target height.

IGF-I has been proposed to be a stimulator in adipogenesis [37]. A positive association between IGF-I and subcutaneous adipose tissue has been shown in normal weight men [38]. The robust correlation between IGF-I and adipose tissue in VPT-LBW men indicate that IGF-I might be of special importance in this group. During infancy and childhood, up to 8 years, leptin but also in some extent IGF-I positively correlated with fat mass in PT-LBW [39].

Increased insulin levels reduce IGFBP-1 and the inverse relationship between the hormones is well-known. IGFBP-1 has been used as a surrogate marker of hepatic glucose output [40]. In the present study we found that the IGFBP-1 levels in VPT-LBW men were reduced in relation to insulin compared with VPT-LBW women, not seen at 9 years. In particular, VPT-LBW men might be at risk of developing type 2 diabetes. Low IGFBP-1 in relation to insulin predicted abnormal glucose regulation and type 2 diabetes 10 years before the event in men [41]. In middle-aged women IGFBP-1 levels rose after the onset of diabetes, indicating a loss of insulin sensitivity in the liver [24]. In our whole prepubertal cohort, leptin and insulin were found to influence 50% of the IGFBP-I variability. In adulthood, after puberty, the influence of leptin on IGFBP-I is weaker. Although an inverse association was present between IGFBP-1 and leptin in VPT-LBW men.

We also found lower prolactin in VPT-LBW men, compared with the other groups although within normal reference values. The reason for the relatively lower prolactin levels in VPT-LBW men could be reduced or altered testosterone to estrogen levels which in turn would affect prolactin secretion, although sex hormones were not studied in the present study. Dopamine is another determinant of prolactin.

The obvious disadvantage in the present study is the restricted number of subjects in each group and using impedance to measure adipose tissue.

Conclusion

In summary, we found elevated leptin levels in relation to adipose tissue in men and women born very preterm. We speculate that the higher leptin levels could be a protective event to enhance hepatic insulin sensitivity in this group. IGFBP-1 levels in relation to insulin were suppressed in preterm men compared with preterm women. There might be an increased risk for very preterm men compared with women to risk enduring hyperglycemia and type 2 diabetes as they grow older. Very preterm men with less fat mass and lower IGF-I might be a special vulnerable group in terms of reduced insulin sensitivity. SGA women born full-term showed signs of peripheral insulin resistance. In conclusion, the impact of very preterm birth appears to be a risk factor for metabolic changes later in life, especially in men.

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Declaration of interest

The authors have nothing to declare.

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