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ScienceDirect
Metabolism Clinical and Experimental 56 (2007) 1412 – 1417
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Growth hormone binding protein levels in children are associated with birth weight, postnatal weight gain, and insulin secretion

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Received 18 April 2006; accepted 6 June 2007

Abstract

Rapid infancy weight gain is associated with subsequent higher circulating insulin-like growth factor (IGF) I levels in normal children. We hypothesized that circulating levels of growth hormone binding protein (GHBP), a putative marker of GH sensitivity, may also be associated with postnatal weight gain and insulin secretion. In 751 normal children aged 7 to 8 years, we measured insulin, glucose, GHBP, IGF-I, IGF binding protein (IGFBP) 1, and IGFBP-3 levels in a fasting venous blood sample. Insulin secretion was assessed by measuring insulin and glucose levels 30 minutes after an oral glucose load. After adjustment for current weight, birth weight was inversely related to IGF-I and GHBP levels. Children with lower birth weight and rapid weight gain between birth and 3 years had higher IGF-I and GHBP levels and also lower IGFBP-1 levels than other children. Allowing for current body mass index, GHBP levels were positively related to insulin secretion. In conclusion, children who showed rapid early postnatal weight gain after low birth weight have higher levels of GHBP than other children. Increased GH sensitivity in such children could contribute to links between rapid infancy weight gain and subsequent faster rates of childhood growth and maturation.

1. Introduction

There seems to be a wide range of growth hormone (GH) sensitivity in normal individuals. In a recent study, the insulin-like growth factor (IGF) I increment to a single injection of GH was 80% higher in obese children and 36% higher in tall children compared with controls; in contrast, short children had a stimulated IGF-I absolute level below that of the controls [1]. We previously observed in normal children that differences in circulating levels of IGF-I were predicted by birth weight and early weight gain between 0 and 2 years [2]. Relatively low birth weights and rapid postnatal weight gain predicted higher IGF-I levels at age 5 years [2]. We hypothesized that variations in early postnatal weight gain may predict further markers of receptor sensitivity and bioactivity of the GH/IGF-I axis at age 8 years.

Insulin-like growth factor I generation is dependent on the combination of both GH and insulin [3-5]. Growth hormone binding protein (GHBP) is the cleaved extramembranous portion of the GH receptor (GHR) [6]. In Laron syndrome, genetic defects in the GHR typically lead to absent GHBP, low IGF-I levels, and poor growth despite high GH secretion [7,8]. Hepatic GHR numbers and circulating GHBP levels are regulated by insulin activity [9,10]. Children and adolescents with type 1 diabetes mellitus are relatively GH resistant, reflecting their portal insulin insufficiency [4]. In contrast, increased GH sensitivity in obese children [1] could reflect their hyperinsulinemia, which up-regulates GHBP levels and IGF-I generation and also increases IGF-I bioactivity by lowering levels of IGF binding protein (IGFBP) 1 [11].

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0026-0495/$ – see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.06.004

The relationship between circulating GHBP levels and GH sensitivity is not straightforward, as a number of processes may differentially regulate GHBP and the cellular GHR [12]. However, in addition to the above findings in experimental animal models and in rare human disease states, higher GHBP levels in obese adults have recently been reported to correlate with their increased GH sensitivity, as assessed by higher serum IGF-I responses to GH administration [13].

We have now measured circulating levels of IGF-I, GHBP, and the IGFBPs (IGFBP-3 and IGFBP-1) at age 7 to 8 years in a large representative birth cohort study to examine their relationships with birth weight, postnatal weight gain, and fasting and stimulated insulin levels.

2. Methods

2.1. Subjects

The Avon Longitudinal Study of Parents and Children (ALSPAC) is a prospective study of 14 541 pregnancies recruited from all pregnancies in 3 Bristol-based District Health Authorities with expected dates of delivery between April 1991 and December 1992 [14].

The data analyzed in this study relate to 751 unselected full-term, singleton children in whom GHBP levels were measured at age 8 years from a fasting plasma sample. The children who attended this fasting study [15] were invited from 2 randomly selected ALSPAC subcohorts: "Children in Focus" (n = 1335 term singletons) and a second subcohort (n = 1000) who had postnatal growth measurements at age 3 years. These children did not differ from other ALSPAC children with regard to size at birth. Details of antenatal data collection and measurements of body size from birth to 5 years have been previously described [16] (http://www.alspac.bris.ac.uk). Body weight was measured using electronic scales, standing height was measured by stadiometer (Leicester height measure, Child Growth Foundation, London, United Kingdom), and waist circumference was measured midway between the lowest rib and the iliac crest by tape measure (Harpenden anthropometric tapes, Holtane, Crosswell, Dyfed, United Kingdom). Ethical approval was obtained from the ALSPAC and the local ethics committees. Signed consent was obtained from a parent, and verbal assent was obtained from the child.

2.2. Blood samples

At age 8 years (mean ± SD age, 8.2 ± 0.1 years; range, 8.0-8.5 years), children attended the research clinic in the morning (mean ± SD time, 8:50 AM ± 0:22; range, 8:06-10:30 AM) after fasting from at least midnight the previous day [15]. A venous blood sample was collected after application of topical analgesic cream (EMLA cream, AstraZeneca, Luton, United Kingdom). Children were then given an oral glucose load (1.75 g/kg; maximum, 75 g) as a drink (Lucozade Energy Original, SmithKline Beecham, Middlesex, United Kingdom) over 5 minutes; and 30 minutes after completion of oral glucose, a second venous blood sample was taken for glucose and insulin. Fasting was validated by questionnaire, and data were excluded if children were taking oral steroids or had any current infection. The IGF-I and IGFBP-3 levels were measured in venous blood samples collected in either the fasting samples at age 8 years (n = 410) or the nonfasting samples at age 7 years (n = 270). All samples were placed immediately onto ice, centrifuged within 30 minutes, and stored at −70° until assay.

2.3. Assays

Serum levels of IGF-I were determined in venous blood by radioimmunoassay using a monoclonal antibody (Blood Products, Elstree, Hertfordshire, United Kingdom) and recombinant peptide (Pharmacia, Stockholm, Sweden) for standard and tracer after iodination using the chloramine-T method. Samples were analyzed after acid-acetone extraction to remove the IGFBPs, with an excess of IGF-II added to the extract to saturate any residual binding proteins. Serum levels of IGFBP-3 were determined by radioimmunoassay using an in-house polyclonal antibody raised against recombinant nonglycosylated IGFBP-3. The assay was calibrated against recombinant glycosylated IGFBP-3 (Dr C Maack, Celitrix, Santa Clara, CA). The average coefficients of variation (CVs) for IGF-I and IGFBP-3 were 6.7% and 3.6% for intraassay variability and 12% and 14% for interassay variation.

Plasma IGFBP-1 levels were measured by enzyme-linked immunosorbent assay using a commercial kit (DSL, London, United Kingdom) according to the manufacturer's instructions. The sensitivity of this assay was 0.04 ng/mL.

Glucose was measured by the glucose oxidase method on a YSI 2300 STAT Plus Analyzer (YSI, Farnborough, Hants, United Kingdom). The intraassay CV was 1.5% at 4.1 mmol/L; and interassay CVs were 2.8% and 1.7% at 4.1 and 14.1 mmol/L, respectively. Insulin was measured by enzyme-linked immunosorbent assay using a commercial kit (DSL). Sensitivity was 0.26 mU/L. Intraassay CVs were 4.4% and 5.1% at 10.3 and 35.8 mU/L, and equivalent interassay CVs were 8.7% and 2.9%; this assay has no cross-reactivity with proinsulin at levels up to 1000 pmol/L.

Growth hormone binding protein was assayed using an in-house double antibody radioimmunoassay as previously described [17] (first antibody, rabbit anti–recominant human GHBP; second antibody, goat anti-rabbit). Recombinant human GHBP from Pharmacia was used for tracer (I125) and standard. Sensitivity of the assay was 0.1 ng/mL; and intra- and interassay CVs were 2.8% and 8.3%, respectively.

2.4. Calculations

The associations between IGF-I levels with current body size or birth weight were similar whether IGF-I was measured at 7 years or at 8 years; and therefore, these data were combined. Although our data are not longitudinal, there

Table 1
Hormone and growth factor levels at age 7 to 8 years by sex

	Male	Female	Sex difference
8 y	n = 412	n = 339	
GHBP (ng/mL)	2.1 (1.8-2.4)	2.3 (1.9-2.7)	$P < .0001$
IGFBP-1 (ng/mL)	65 (53-76)	59 (46-74)	$P < .0001$
Fasting insulin (mU/L)	4.1 (2.9-7.1)	5.3 (3.5-8.5)	$P < .0001$
Insulin secretion [a] (mU/mmol)	12.2 (7.6-19.1)	13.0 (8.4-22.0)	$P < .0001$
	n = 234	n = 176	
IGF-I (ng/mL)	146 (110-193)	160 (123-200)	$P = .09$
IGFBP-3 (ng/L)	5110 (4382-6191)	5442 (4614-6651)	$P = .007$
7 y	n = 140	n = 130	
IGF-I (ng/mL)	112 (88-145)	140 (107-171)	$P = .0003$
IGFBP-3 (ng/L)	3500 (2752-4832)	3914 (3153-4562)	$P = .046$

Medians (interquartile ranges) are displayed.
[a] Insulinogenic index (insulin 30 − insulin 0)/(glucose 30 − glucose 0).

was a surprising increase in IGF-I and IGFBP-3 levels between age 7 and 8 years. It is very unlikely that many children would have progressed into puberty during this early age range, and there were no differences in the assay methodology. We therefore adjusted for these differences by calculating age- and sex-adjusted internal SD scores for IGF-I and IGFBP-3 levels.

Insulin secretion was estimated from fasting and 30-minute insulin and glucose data using the insulinogenic index: (insulin 30 − insulin 0)/(glucose 30 − glucose 0) [18].

2.5. Statistics

Sex differences in outcome variables were compared using *t* tests. Correlations were tested using multiple linear regression to adjust for sex and age differences and to identify independent effects by coentering multiple variables, for example, fasting insulin and current body weight. Standardized regression coefficients (β) are reported, which represent the number of SD change in the outcome variable for a 1-SD change in the exposure. Correction for multiple testing was not deemed necessary because of the significant intercorrelation between the various determinants and outcome variables.

3. Results

3.1. Hormone levels by age, sex, and current body size

Table 1 shows GHBP, IGF-I, IGFBP-3, and IGFBP-1 levels at 7 to 8 years summarized by sex. Girls tended to have higher levels of IGF-I, GHBP, and IGFBP-3 and lower levels of IGFBP-1 than boys. Age- and sex-adjusted SD scores for IGF-I and IGFBP-3 were used in all subsequent analyses. The GHBP levels were positively related to current body weight ($\beta = .45$, $P < .0005$) and also less closely related with current height ($\beta = .17$, $P < .0005$) and IGF-I levels ($\beta = .14$, $P < .005$).

3.2. Birth weight and early postnatal growth

The IGF-I levels at 7 to 8 years were inversely related to birth weight ($\beta = -.12$, $P < .005$). Further adjustment for current weight strengthened the IGF-I correlation with birth weight ($\beta = -.20$, $P < .0005$) and also revealed an inverse birth weight association with GHBP levels ($\beta = -.12$, $P < .0005$). Therefore, the highest IGF-I and GHBP levels were seen in children with the combination of lowest birth weight and highest current weight tertiles (Fig. 1). Such children also had the lowest IGFBP-1 levels (data not shown).

To demonstrate the timing of their postnatal weight changes, we compared age- and sex-adjusted weight SD scores from birth to age 8 years between quartile groups of IGF-I and GHBP at age 7 to 8 years (Fig. 2). Significant differences in early postnatal weight gain patterns were seen (repeated-measures analysis of variance, $P < .0001$), with children in the highest IGF-I or GHBP quartiles showing the greatest weight gain in particular during the first 2 to 3 years from birth (Fig. 2).

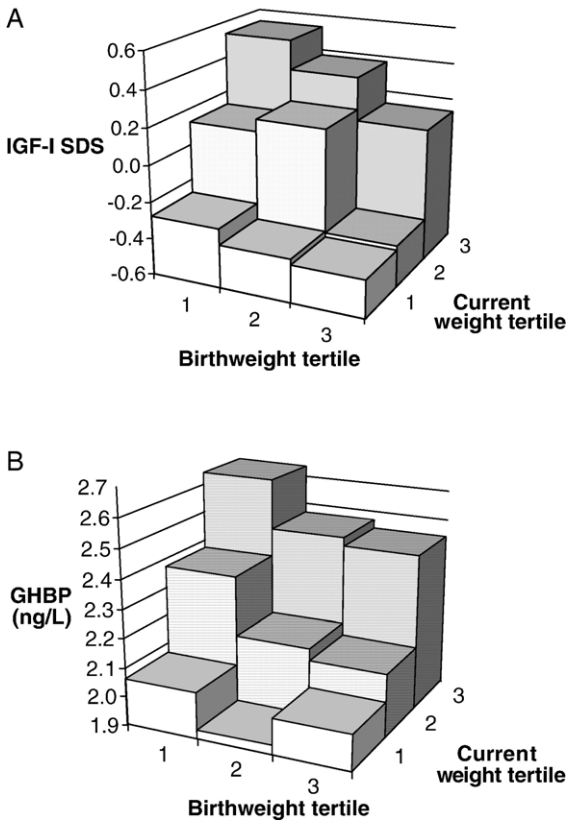

Fig. 1. Levels of (A) IGF-I at age 7 to 8 years (n = 680) and (B) GHBP at age 8 years (n = 781) by tertiles of birth weight and current weight. In multiple regression models both IGF-I and GHBP levels were each inversely related to birth weight ($P < .0005$ for both) and positively related to current weight SDs ($P < .0005$ for both).

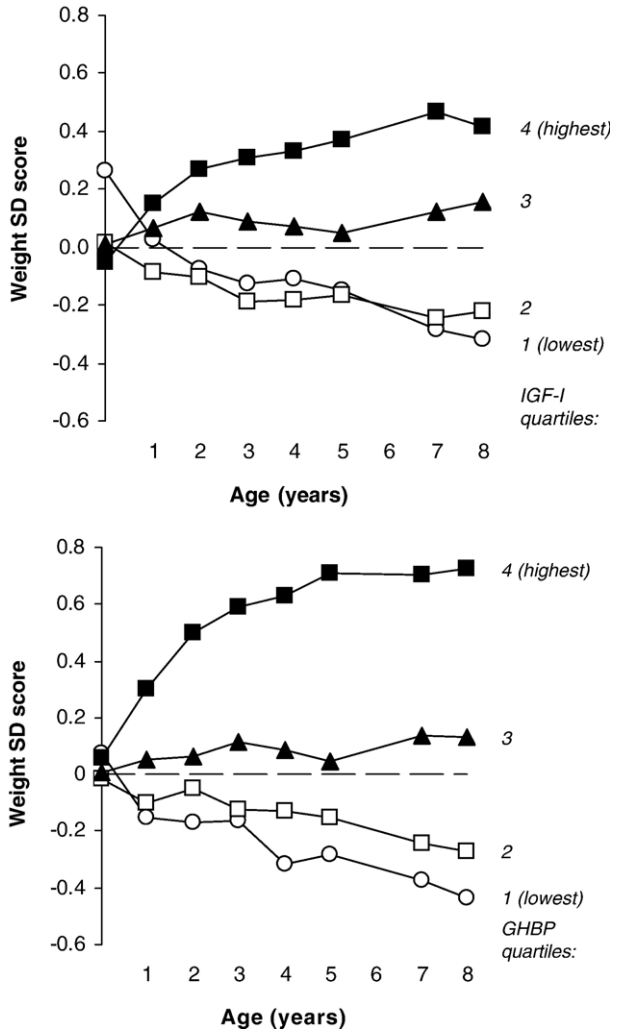

Fig. 2. Divergent early postnatal weight gain patterns between quartiles of circulating IGF-I or GHBP levels at age 8 years. Mean age- and sex-adjusted weight SD scores are displayed for each quartile. Repeated-measures analysis of variance: $P < .0005$ for differences in weight SD score between both IGF-I and GHBP quartiles.

Multivariate analyses were performed to distinguish whether these early postnatal weight changes reflect fatness (body mass index [BMI]) or skeletal (stature) growth. The GHBP levels at age 8 years were positively related to gain in BMI SD scores ($P < .0005$) but not to statural growth ($P = .8$) between birth and 3 years.

3.3. Fasting insulin and insulin secretion

The IGF-I and GHBP levels at 8 years were positively related to fasting insulin levels ($\beta = .16$, $P < .005$ and $\beta = .16$, $P < .0005$, respectively) and to insulin secretion post oral glucose load at 8 years ($\beta = .15$, $P < .005$ and $\beta = .24$, $P < .0005$, respectively). Further adjustment for current body weight abolished most of these associations, except for the positive association between GHBP levels and insulin secretion ($\beta = .14$, $P < .005$; adjusted for current weight).

4. Discussion

In this large population-based United Kingdom birth cohort, we confirmed our earlier findings at age 5 years [2] by showing that higher circulating IGF-I levels at age 7 to 8 years are related to rapid postnatal weight gains after lower birth weight. We also show that the transition from lower birth weight to larger childhood size is associated with higher circulating GHBP levels at age 7 to 8 years.

We recognize the limitations in drawing causal mechanisms from observational data. We did not measure GHBP levels earlier in these children; and it is possible that, rather than being a consequence of rapid growth, higher GHR numbers may have contributed to faster infancy weight gain. However, the GHBP association with infancy gains in BMI rather than statural growth suggests that such a reverse causality is unlikely. Furthermore, this proviso does not apply to the higher IGF-I levels we observed in low birth weight children who then grew rapidly, as we have previously reported that their IGF-I levels were initially low at birth [19].

Extrapolation from the relevance of GHBP levels in rare genetic conditions [7,8] and in adults [13] might suggest that our children with rapid infancy growth and higher GHBP levels may have greater GHR numbers and increased GH sensitivity with regard to IGF-I production. Insulin-like growth factor I has direct actions on the growth plate [20], and it has also been proposed to stimulate adrenal androgen production [21]. Thus, higher childhood GHBP and IGF-I levels could therefore mediate the rapid tempo of childhood growth and the earlier pubertal onset that typically follows in utero growth restraint and rapid "catch-up" infancy growth [22,23]. Circulating IGF-I levels in adults have also been associated with risks for a variety of adult diseases, including cancer, type 2 diabetes mellitus, and cardiovascular disease [24-27]. Dynamic testing of GH sensitivity as performed in other smaller studies [1] is not feasible in a large population-based study. However, follow-up of this cohort through puberty and beyond will allow us to test the associations between infancy growth, childhood GHBP and IGF-I levels, and timing of puberty, and also to explore the predictive ability of these variables on later markers of adult disease.

The IGFBP-3 levels were unrelated to birth weight and showed weaker correlations with childhood weight gain compared with IGF-I (data not shown). Rapid infancy weight gain may therefore have an even greater impact on free, or bioavailable, IGF-I than on total IGF-I levels. Furthermore, rapid postnatal weight gain and higher insulin levels were associated with lower IGFBP-1 levels; and this could further increase IGF-I biovailability [28].

The GHBP levels were positively related to insulin secretion even after adjustment for current body size. Our results are consistent with the hypothesis that long-term programming of higher IGF-I levels after rapid early weight gain could be mediated by increased insulin levels promoting

GHR numbers and GH sensitivity, as indicated by higher GHBP levels. The link between GHBP and insulin levels has been previously implicated in humans through study of obese and anorexic individuals [11] and in children with type 1 diabetes mellitus [4,29], and we now report the first support for a physiological relationship in normal children.

However, some difficulties remain in fully resolving these causal associations. For example, it is possible that increased hepatic insulin activity could increase IGF-I generation [9] or, conversely, higher IGF-I levels may stimulate or maintain the β-cell insulin secretory response [25]. In addition to experimental physiological studies in children, identification of functional genetic polymorphisms in the GH/GHR/IGF-I pathway and exploration of their associations with growth and glucose metabolism would help clarify these relationships.

In conclusion, these data in a large representative childhood cohort provide the first evidence to suggest that children with rapid infancy postnatal weight gain develop increased circulating GHBP levels at age 8 years suggestive of increased GH sensitivity, which could explain their higher IGF-I levels. As previously shown in obese and anorexic individuals, higher circulating GHBP levels in these normal children were also associated with higher insulin secretion. Such programming of the GH/GHR/IGF system could underlie the links between rapid infancy weight gain and subsequent faster rates of growth and maturation in later childhood.

Acknowledgments

The United Kingdom Medical Research Council, the Wellcome Trust, and the University of Bristol provide core support for ALSPAC.

The authors are extremely grateful to all the families who took part in this study and to the midwives for their help in recruiting them. We thank the whole ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses.

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