Low IGF-I and Elevated Testosterone During Puberty in Subjects With Type 1 Diabetes Developing Microalbuminuria in Comparison to Normoalbuminuric Control Subjects

The Oxford Regional Prospective Study

RAKESH AMIN, MRCPCH[1]
CARL SCHULTZ, MD, PHD[1]
KEN ONG, MRCPCH[1]
JAN FRYSTYK, MD, PHD[2]
R. NEIL DALTON, PHD[4]
LES PERRY, PHD[3]
HANS ØRSKOV, MD, DMSC[2]
DAVID B. DUNGER, FRCPCH[1]

OBJECTIVE — To describe longitudinal variations in pubertal hormonal variables in subjects with and without microalbuminuria (MA).

RESEARCH DESIGN AND METHODS — Blood samples collected annually from subjects recruited at diagnosis of type 1 diabetes and followed prospectively through puberty (median follow-up 9.3 years, range 4.7–12.8) were analyzed for total and free IGF-I, IGF binding protein-1, testosterone, sex hormone–binding globulin, and HbA_{1c}. A total of 55 subjects who developed MA (MA^+ group) were compared with 55 age-, sex-, and duration-matched control subjects who did not develop MA (MA^- group).

RESULTS — For female subjects, total IGF-I (MA^+ 1.2 mU/l vs. MA^- 1.4 mU/l, $P = 0.03$) and free IGF-I levels (MA^+ 1,767 ng/l vs. MA^- 2010 ng/l, $P = 0.002$) were lower, whereas the free androgen index (MA^+ 2.4 vs. MA^- 2.0, $P = 0.03$) was higher in those with MA. These changes were less pronounced in male subjects. For both sexes, in a Cox model after adjusting for puberty, the presence of MA was associated with lower free IGF-I levels, higher testosterone standard deviation score (SDS), and poor glycemic control. We found that 22 of 55 case subjects (40%) developed persistent MA, whereas 33 (60%) had transient MA. In the persistent MA group compared with the transient and control groups, total IGF-I levels were lower (1.1 vs. 1.3 vs. 1.4 mU/l, $P = 0.002$) as were free IGF-I levels (1,370.9 vs. 1,907.3 vs. 1,886.7 ng/l, $P < 0.001$), whereas HbA_{1c} levels were higher (11.8 vs. 10.3 vs. 9.9%, $P < 0.001$).

CONCLUSIONS — Poor glycemic control and differences in IGF-I levels and androgens, particularly in female subjects, accompany development of MA at puberty. These differences may in part account for the sexual dimorphism in MA risk during puberty and could relate to disease progression.

Diabetes Care 26:1456–1461, 2003

From the [1]University Department of Pediatrics, Addenbrookes Hospital, Cambridge, U.K.; the [2]Medical Research Laboratories, Aarhus University Hospital, Aarhus, Denmark; the [3]Department of Clinical Biochemistry, St Bartholomew's Hospital, London, U.K.; and the [4]Children Nationwide Kidney Research Laboratory, Guy's Hospital, London, U.K.

Address correspondence and reprint requests to Prof. David B. Dunger, University Department of Paediatrics, Box 116, Level 8, Addenbrookes Hospital, Hills Road, Cambridge, CB2 2QQ, U.K. E-mail: dbd25@cam.ac.uk.

Received for publication 10 December 2002 and accepted in revised form 5 February 2003.

Abbreviations: ACR, albumin-to-creatinine ratio; CV, coefficient of variation; FAI, free androgen index; GH, growth hormone; IGFBP-1, IGF binding protein-1; MA, microalbuminuria; mAb, monoclonal antibody; ORPS, Oxford Regional Prospective Study of Childhood Diabetes; PCOS, polycystic ovarian syndrome; QC, quality control; RIA, radioimmunoassay; SDS, standard deviation score; SHBG, sex hormone–binding globulin.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Microalbuminuria (MA) is a marker of incipient nephropathy in adult subjects with type 1 diabetes (1). There is an unequivocal relationship between poor glycemic control and the development of MA (2). However, independent of poor glycemic control, early diabetic complications risk is increased with the onset of puberty (3). In addition, MA risk is twofold greater in pubertal female patients compared with male patients (3), in contrast to lifetime risk of diabetic nephropathy, which is greater in male patients (4). Sexual dimorphism is also present during puberty for risk of attenuated growth, weight gain, and retinopathy, and again these observations are independent of glycemic control (5–7). These data suggest that the development of diabetic microvascular complications may be associated with abnormalities in hormonal variables related to pubertal development.

Cross-sectional data describe abnormalities of the growth hormone (GH)/IGF-I axis in relation to the development of diabetic complications (8). In type 1 diabetes, bioavailability of circulating IGF-I is low and GH secretion exaggerated (9,10), and these abnormalities may be more marked in female patients (10). Despite evidence of hepatic GH resistance, integrity of GH pathways in other tissues remain intact, and both elevated GH and local paracrine IGF-I generation have been implicated in the development of diabetic nephropathy in humans and rats (11,12). Hyperandrogenism and low sex hormone–binding globulin (SHBG) levels in female patients have also been linked to MA risk (13), and this may relate to the reported increased prevalence of polycystic ovarian syndrome (PCOS) in young women with type 1 diabetes (5).

Cross-sectional studies during puberty are easily confounded by variables such as diabetes duration, pubertal stage, and inadequate selection of control subjects. A further confounding factor is the variability in urine albumin excretion, since it is thought that MA may resolve in up to 50% of cases during adolescence (3). To address these issues, we report longitudinal changes in IGF-I and sex steroids in a cohort of well-characterized male and female subjects who were recruited at diagnosis of type 1 diabetes and followed longitudinally through puberty, comparing those who went on to develop MA against carefully matched control subjects without MA.

RESEARCH DESIGN AND METHODS

Study design

Subjects. The Oxford Regional Prospective Study of Childhood Diabetes (ORPS) was established in 1986, and the characteristics of the cohort have been previously described (3). Children under 16 years of age were recruited within 3 months of diagnosis of type 1 diabetes and were assessed at the end of the first year from diagnosis and annually thereafter. Assessments consisted of measurements of height, weight, and three consecutive early-morning (first void) urine specimens for the albumin-to-creatinine ratio (ACR). Blood samples were collected for the central measurement of HbA_{1c}, and annual nonfasting blood samples were stored on each patient. Ethical approval was obtained from the regional ethics committees, with written consent from the parents and assent from the children.

MA was defined as an ACR >3.5 mg/mmol in male subjects and >4.0 mg/mmol in female subjects and <35 mg/mmol in two of three consecutive early-morning urine collections (3). This corresponded to an albumin excretion rate of between 20 and 200 mcg/min, as determined in 304 timed overnight urine samples using linear regression equations.

Of the 494 subjects recruited, 63 had an ACR that fell within our definition of MA on at least one annual assessment. A total of 55 subjects had sufficient blood samples for analysis, and these were designated as case subjects (MA^+ group). Case subjects were matched for age, sex, and duration of diabetes to control subjects selected from the remaining subjects who, to date, have no evidence of MA (MA^- group). For each subject, all available longitudinally collected blood samples were analyzed for the relevant hormones.

Methods

Auxology. BMI standard deviation scores (SDSs) were calculated using data based on the British 1990 Growth Reference and Cole's LMS method (14).

Albumin assay. Details of sample collection and storage were reported previously (4). Albumin was measured centrally by an enzyme-linked immunosorbent assay method (3). The within- and between-assay coefficients of variation (CVs) was 6 and 12%, respectively.

Creatinine. Creatinine was measured using a Jaffe method (Unimate 7; Roche Diagnostic Systems, Basel, Switzerland) on a Cobas Mira (Roche Diagnostic Systems) spectrophotometer. The CV was 2% at 2.2 mmol/l.

HbA_{1c}. HbA_{1c} was measured centrally, initially by an electrophoretic method (Ciba Corning Diagnostics, Halstead, U.K.), which was replaced by high-performance liquid chromatography (DIAMAT; Bio-Rad, U.K.) in 1992 (15). The relationship between the two methods has been described previously (3). The within-batch CV was 2.2 and 1.3% at a level of 9.8 and 10.1%, respectively. The between-batch CV was 3.5 and 2.2% at 5.6 and 10.1%, respectively. The normal range for the assay is 4.4–6.2%.

Free IGF-I. Serum free IGF-I levels were determined using ultrafiltration by centrifugation at conditions approaching those in vivo (16), using Amicon YMT 30 membranes and MPS-1 supporting devices (Amicon Division, Beverly, MA). Serum samples were diluted (1 in 11) in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 50 g/l human serum albumin (Behring, Marburg, Germany). Then, 600 μl were applied to the membranes and incubated (30 min at 37°C) and centrifuged (1,500 r/min at 37°C). The assay detection limit was 40 ng/l. The overall within- and between-assay CVs averaged 20%.

Total IGF-I. Total IGF-I levels were determined by radioimmunoassay (RIA) after acid-acetone extraction using rabbit anti-serum developed by L. Underwood (North Carolina University, Chapel Hill, NC). The assay was standardized against a pool of normal human serum, defined as containing 1.0 units IGF-I/ml, equivalent to 159 ng/ml of a purified preparation of IGF-I (17). The intra- and interassay CVs were 6.2 and 3.5%, respectively.

IGF binding protein-1. IGF binding protein-1 (IGFBP-I) was determined by a novel in-house RIA based on a monoclonal antibody (mAb), which recognizes all phosphorylated isoforms of IGFBP-I (mAb 6303; Medix Biochemica, Kainiainen, Finland) (18). Microtiter plates (Nunc, Roskilde, Denmark) were coated overnight at 5° with 4 mg/l anti-mouse IgG (Sigma Aldrich, Copenhagen, Denmark) in 15 mmol/l sodium carbonate, 35 mmol/l sodium hydrogen carbonate, pH 9.6. After washing (50 mmol/l Tris-HCl, pH 8.0, 0.9% [wt/vol] NaCl, 0.5% [vol/vol] Tween 20, and 0.05% [wt/vol] NaN_3), all wells were blocked with 1% (wt/vol) BSA in 40 mmol/l phosphate buffer with 0.05% (wt/vol) NaN_3, pH 8.0, for 2 h at room temperature. After washing, 100 μl of standard (purified amniotic human IGFBP-I; HyTest, Turku, Finland) or diluted serum (1 in 4), 50 μl of ^{125}I-labeled IGFBP-I (~10.000 cpm), and 50 μl of specific antibody (mAb 6303, 12.5 μg/l) were added to all wells. All reagents were dissolved in 40 mmol/l phosphate buffer containing 0.2% (wt/vol) BSA, 0.9% (wt/vol) NaCl, 0.2% (wt/vol) Tween 20, and 0.05% (wt/vol) NaN_3. The plates were then incubated for 2 days at 5°C, washed three times, and counted in a γ-counter. The working range of the assay was 1–200 μg/l, with ED_{50} ~25 μg/l. The lower detection limit was <2.5 μg/l, and the within- and between-assay CVs were <5 and <16%, respectively. The cross-reactivity of IGF-I, IGF-II, and IGFBP-2, -3, -4, and -5 was <1% (up to 10.000 μg/l).

Testosterone. Samples were analyzed on a Bayer Technicon Immuno-1 fully automated immunoassay analyzer (Bayer, Neubury, U.K.), using a competitive magnetic separation format and an enzymatic end-point detection system. The assay has three "in-house" quality control (QC) pools, two female and one male. The two female pools' interassay imprecision is: QC low mean 1.7 nmol/l, CV 8.0%, n = 114; and QC medium mean 2.9 nmol/l, CV 4.6%, n = 143.

SHBG. Samples were analyzed on an Immulite semiautomated immunoassay analyzer (Diagnostic Products, Llanberis, Gwynedd, Wales), using a solid-phase two-site chemiluminescent immunoas-

say. The assay has three "in-house" internal QC pools. Imprecision is as follows: QC low mean 19.5 nmol/l, CV 6.0%, n = 84; and QC medium mean 38.0 nmol/l, CV 5.7%, n = 84; and QC high mean 86.0 nmol/l, CV 5.7%, n = 84.

Statistical methods

All data were normally distributed, except IGFBP-1, testosterone, SHBG, and free androgen index (FAI; FAI = testosterone × 100/SHBG), which were log transformed to allow parametric analyses. Levels of each hormone were compared between groups using an ANOVA model. To adequately consider the effects of puberty, a Cox proportional hazards model was fitted with duration of diabetes as the time variable, MA as the outcome, and pubertal onset as a time-dependent covariate (using age 11 years as a surrogate marker for puberty onset). For this model, data from each subject was summarized by calculating the mean of all measurements. The covariates examined included HbA_{1c}, free IGF-I, daily insulin dose, and testosterone SDS. Testosterone SDSs were used to allow both sexes to be considered together and were derived from comparison of data against comparable age- and sex-matched nondiabetic subjects using the Growth Analyser Program (Dutch Growth Foundation). To display the longitudinal changes in hormone levels over time, we used a multilevel modeling software (MLwiN version 1.0 beta; Institute of Education, London) (19). This is an extension of multiple regression, using repeated-measures data and analyses within and between individual effects, allowing consideration of individual curves and their summation by predefined groups. SPSS version 10.0 was used for analysis. Data are presented as the mean ± SD or median (interquartile range). A P value <0.05 was considered significant.

RESULTS

Cohort characteristics

There were no significant differences between the groups in demographic characteristics, BMI SDS, and insulin daily dose (Table 1). Of the 55 case subjects, 33 (60%) had transient MA (defined as MA present for 1 year with normoalbuminuria the following year) and 22 (40%) developed persistent MA (defined as MA present for ≥2 consecutive years).

Table 1—*Characteristics of case (MA^+) and control (MA^-) subjects*

	MA^-	MA^+
Sample size (F)	55 (35)	55 (35)
Age of diagnosis of diabetes	10.3 (6.1–13.1)	10.0 (6.2–12.4)
Age at onset of MA in case subjects	15.6 (13.1–19.2)	15.6 (12.1–18.4)
Duration diabetes at onset of MA in case subjects	5.3 (3.0–9.0)	5.3 (3.0–9.1)
Age at end of follow-up	18.8 (14.8–22.9)	18.9 (14.0–23.9)
Total period of follow-up	9.5 (5.0–12.6)	9.3 (4.7–12.8)
Insulin dose (units · kg^{-1} · day^{-1})	0.9 ± 0.2	0.9 ± 0.3
BMI SDS	0.7 ± 0.9	0.7 ± 1.0

Data are median (range) or means ± SE unless otherwise noted.

Data for case subjects (MA^+) and control subjects (MA^-)

HbA_{1c}. HbA_{1c} levels were higher in the MA^+ compared with the MA^- group (10.8 ± 1.8 vs. 9.9 ± 1.3%, $P < 0.001$), and when female subjects were considered separately (10.9 ± 2.0 vs. 9.8 ± 1.8%, $P < 0.001$) to male subjects (10.6 ± 1.4 vs. 9.7 ± 1.3, $P = 0.005$).

Total IGF-I. Total IGF-I levels were lower in the MA^+ compared with the MA^- group (1.2 ± 0.6 vs. 1.4 ± 0.6 mU/l, $P = 0.001$), and when female subjects (1.2 ± 0.6 vs. 1.4 ± 0.6 mU/l, $P = 0.03$) were considered separately to male subjects (1.1 ± 0.6 vs. 1.3 ± 0.6 mU/l, $P = 0.006$).

Free IGF-I. Free IGF-I levels were lower in the MA^+ compared with the MA^- group (1,678 ± 745 vs. 1,887 ± 906 ng/l, $P = 0.004$). In female subjects, the difference between the groups was highly significant (1,767 ± 634 vs. 2010 ± 881 ng/l, $P = 0.002$) (Fig. 1*A* and *B*), but this was not the case in males (1,552 ± 946 vs. 1,668 ± 930 ng/l, $P = 0.77$). In females with similar HbA_{1c} levels, free IGF-I levels were consistently lower in those with MA compared with those without (Fig. 1*C*).

IGFBP-I. No significant differences in IGFBP-I levels were found

Testosterone, SHBG, and FAI. In female subjects, testosterone levels (median [interquartile range]) were higher in the MA^+ compared with the MA^- group (1.3 [0.9–1.6] vs. 1.1 [0.7–1.3] mmol/l, $P = 0.04$), as was the FAI (2.4 [1.3–4.0] vs. 2.0 [1.0–3.2], $P = 0.03$) (Fig. 1*D* and *E*). At similar HbA_{1c} levels, FAI was consistently higher in those with MA compared with those without MA (Fig. 1*F*). In male subjects, no differences were found. No relationship was seen with daily insulin dose and SHBG levels for either sex (nonfasting samples).

Proportional contribution of hormonal covariates and HbA_{1c} to probability of developing MA

Independent of puberty, the probability of having MA was associated with lower free IGF-I levels ($P = 0.01$, Exp[B] = 0.999, 95% CI 0.998–1.0; i.e., a 10% increase in probability with a reduction in free IGF-I levels by 100 ng/l), with higher testosterone SDS ($P < 0.001$, Exp[B] = 3.9, 95% CI 2.1–7.3; i.e., by a factor of 3.9 for a unit rise), and with poor glycemic control ($P < 0.001$, Exp[B] = 3.5, 95% CI 2.1–5.8; i.e., by a factor of 3.5 for a 1% increase in mean HbA_{1c} levels). When the sexes were considered separately, the findings for female subjects were similar to when both sexes were considered together, but for male subjects, the probability of having MA was associated with poor glycemic control only ($P = 0.04$).

Differences between those with transient and persistent MA

Of the 35 female case subjects, 11 developed persistent MA, whereas 24 had transient MA. Of the 20 male subjects, 11 developed persistent MA, whereas 9 had transient MA. This sex difference was not significant ($\chi^2 = 2.9$, $P = 0.09$). Comparison of hormonal variables are described in Table 2. Urinary ACR plotted against years relative to onset of MA is displayed in Fig. 2. For female subjects only, in the persistent MA group compared with the transient and control groups, free IGF-I levels were lower (1,519 ± 752 vs. 1,874 ± 1,063 vs. 2010 ± 881 ng/l, $P = 0.02$), whereas HbA_{1c} levels were higher (11.7 ± 2.6 vs. 10.4 ± 1.9 vs. 9.8 ± 1.8%, $P < 0.001$). For male subjects only, in the persistent MA group compared with the transient and control groups, total IGF-I levels were lower (1.0 ± 0.4 vs. 1.1 ± 0.6 vs. 1.3 ± 0.6 mU/l, $P = 0.007$), whereas HbA_{1c} levels were higher

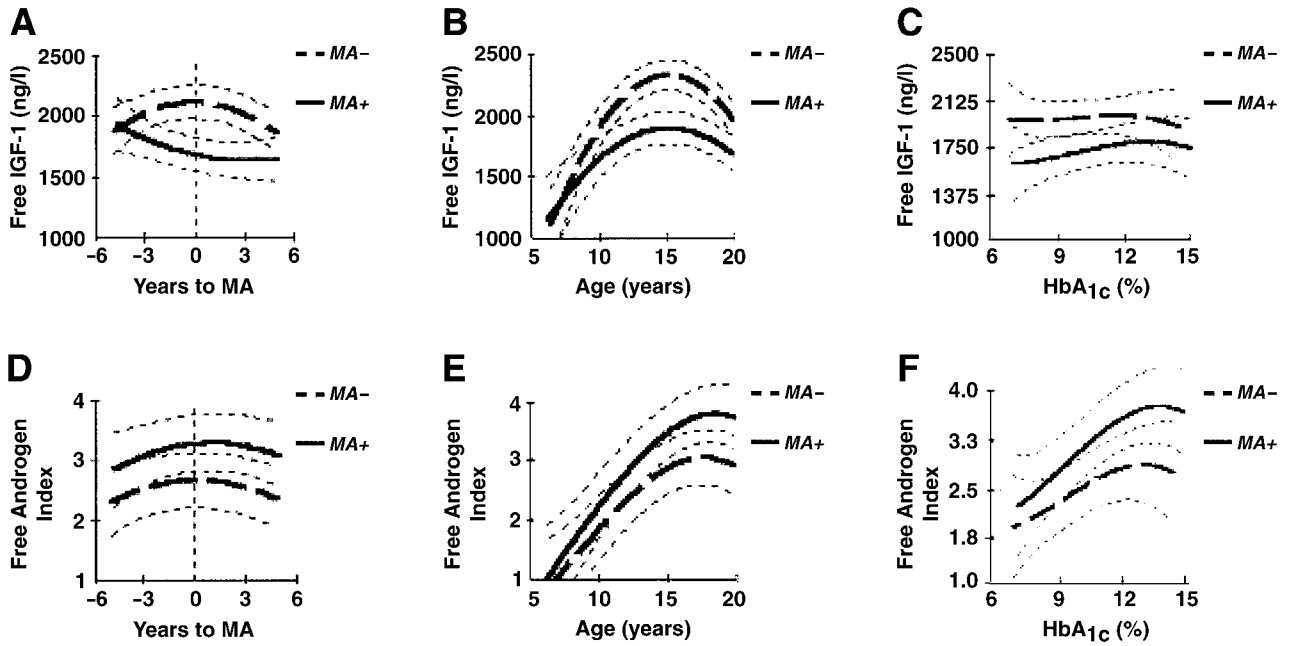

Figure 1—*Free IGF-I in female case subjects (MA^+) and control subjects (MA^-) across years relative to appearance of MS, time 0 denoting first appearance of MA* (A)*, across age* (B)*, and across corresponding HbA_{1c} values* (C)*. FAI levels in case subjects (MA^+) and control subjects (MA^-) are also shown across years relative to appearance of MA, time 0 denoting first appearance of MA* (D)*, across age* (E)*, and across corresponding HbA_{1c} values* (F) *(derived from multilevel modeling, dotted lines representing ± SE).*

(11.2 ± 2.1 vs. 9.8 ± 1.1 vs. 9.7 ± 1.3%, $P = 0.004$). No other differences were found.

CONCLUSIONS — There is little longitudinal data on the natural history of MA in children with type 1 diabetes. Data from ORPS has shown that the cumulative probability of developing MA after 9 years' diabetes duration is 40% (3). Follow-up of the cohort shows that MA persists in 40% of these subjects, with persistence being unequivocally related to poor glycemic control. However, the difference in HbA_{1c} between those with transient MA and control subjects was not significant, and the increasing prevalence of MA during puberty cannot be entirely explained by HbA_{1c}. Puberty and sex are also independent risk factors for the development of MA (3). Pubertal onset is associated with an acceleration in the urine albumin excretion rate compared with the years during prepuberty, and this may be predictive of MA (20), and female subjects are at a twofold greater risk of MA compared with male subjects (3). This marked sexual dimorphism is also apparent in other areas at puberty, as evidenced by the increased risk in adolescent female subjects for developing retinopathy (7), obesity (5), and attenuated growth (6). These data suggest hormonal changes during puberty may effect the development of MA in susceptible individuals.

In female subjects and, to a lesser extent in male subjects, those with MA had lower total and free IGF-I levels than their control subjects, and this could by implication relate to variation in GH levels. In type 1 diabetes, relative portal insulinopenia (caused by failure to administer insulin directly into the portal vein) results in impaired hepatic generation of total and

Table 2—*Summary of hormonal variables for those with transient MA and persistent MA and their matched normoalbuminuric control subjects (MA^-)*

	MA^-	Transient MA	Persistent MA	ANOVA
n	55	33	22	
HbA_{1c} (%)	9.9 ± 1.3	10.3 ± 1.3	11.8 ± 2.1	<0.001
ACR at diagnosis of MA (mg/mmol)	0.9 (0.6–1.1)	3.6 (1.4–5.9)	7.3 (4.2–11.3)	<0.001
Total IGF-I (mU/l)	1.4 ± 0.6	1.3 ± 0.6	1.1 ± 0.5	0.002
Free IGF-I (ng/l)	1,886.7 ± 906.1	1,907.3 ± 793.4	1,370.9 ± 567.9	<0.001
IGFBP-I (ng/l)	47.3 (29.9–81.7)	44.6 (33.4–75.6)	60.6 (39.3–95.2)	N/S
Testosterone SDS	0.2 (−0.5 to 1.0)	0.4 (−0.4 to 1.0)	0.2 (−0.6 to 0.8)	N/S
SHBG (nmol/l)	53.0 (36.0–80.1)	64.0 (40.5–100)	53.2 (38.4–65.9)	N/S
FAI SDS	−0.02 (−0.5 to 0.5)	0.01 (−0.7 to 0.3)	0.3 (−0.8 to 0.4)	N/S

Data are mean ± SD or median (interquartile range). ACR at diagnosis of MA are the geometric mean (interquartile range) of the urinary ACR.

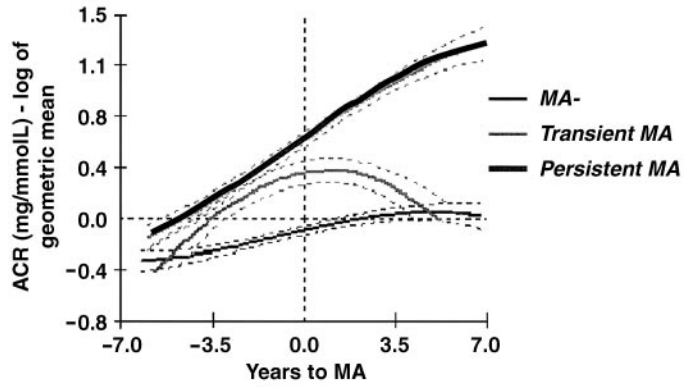

Figure 2—*Urine ACR in case subjects with persistent and transient MA and their matched normoalbuminuric control subjects (MA$^-$) across years relative to the onset of MA, time 0 denoting first appearance of MA. ACR is expressed as the log of the geometric mean of three consecutive early-morning first-void samples (derived from multilevel modeling, dotted lines representing the mean ± SE).*

free IGF-I (9), leading to a lack of negative feedback drive for GH hypersecretion, over and above that seen in normal puberty (10). Thus, circulating GH levels are increased while circulating IGF-I levels remain low, and these changes may be more apparent in female than in male subjects (10). The integrity of GH pathways in tissues other than the liver are thought to remain intact, and both GH hypersecretion and local paracrine IGF-I production have been implicated in the pathophysiology of diabetic nephropathy. In animal models, GH increases renal blood flow (12), renal expression of IGF-I, and renal size (21), with IGF-I expression within the kidney correlating directly with diabetic nephropathy–like changes (22). Selective GH blockade leads to reduced renal expression of IGF-I, renal size, GFR, and urine albumin excretion (22). In humans, GH hypersecretion and local paracrine IGF-I generation have been associated with the development of diabetic nephropathy (11) and retinopathy (23,24) in cross-sectional studies. Acromegaly is associated with increases in GFR and urinary albumin excretion (25), whereas specific GH blockade leads to normalization of these factors (25). However, to date no renal physiology data exist on such intervention in type 1 diabetic subjects.

Our findings of raised androgens in those with MA, particularly in female subjects, confirms findings in previous cross-sectional studies (13). It is probable, but unproven, that the elevated androgens in female subjects derive from the ovary, and that ovarian hyperandrogenism is a principal feature of PCOS. The relationship between ovarian hyperandrogenism and polycystic morphologic changes of the ovary is much debated, but in one study of adolescent girls with type 1 diabetes, up to 50% had ovarian changes characteristic of PCOS, in contrast to 20–30% in the general population (5).

Ovarian hyperandrogenism in PCOS is related to insulin resistance and peripheral hyperinsulinemia (26), although elevated GH and reduced IGF-I levels could also theoretically affect ovarian function (27). The GH-associated increase in insulin resistance during puberty in type 1 diabetes may be the principal cause of the ovarian hyperandrogenism. However, other genetic variation in factors leading to insulin resistance may also be important because insulin resistance can predate the appearance of MA (28), with familial clustering of diabetic nephropathy occurring in association with changes in insulin resistance (29).

We currently have no direct measures of insulin resistance in our case-control series. Insulin doses were similar in case and control subjects, yet HbA_{1c} was higher in case subjects. This might indicate underinsulinization rather than insulin resistance, and poor control might lead to abnormalities of both the GH–IGF-I axis and MA. However, free IGF-I levels were lower and FAI higher in those with MA, independent of HbA_{1c} (Fig. 1*C* and *F*), suggesting that these hormone changes and any associated increase in GH levels may influence the expression of MA in genetically susceptible individuals as they move through puberty. Thus, background variability in common genetic polymorphisms, such as in the IGF-I gene and the androgen receptor gene, may contribute to MA risk; however, this has yet to be determined. The role of changes in IGF-I levels and FAI in the pathogenesis of MA cannot be further elucidated by these studies. However, the differences in the degree of abnormality seen in those with persistent and transient MA may be predicted by lower HbA_{1c} (20) and less profound abnormalities of the GH–IGF-I and ovarian axes.

In summary, the development of MA at puberty may reflect not only poor glycemic control but also changes in the GH–IGF-I axis and ovarian function. Changes in pubertal hormonal variables differ in those with MA, particularly in female patients, and these differences may relate to disease progression. Aggressive insulin therapy has been shown to reduce diabetic complication risk (2). However, this may confer increased weight gain and hypoglycemia (2) and predispose to the detrimental effects of peripheral hyperinsulinemia, such as the development of ovarian hyperandrogenism. Increasing the insulin dose to overcome insulin resistance may lead to further weight gain (2) and, in female patients, a cycle of insulin omission to lose weight (30). This pattern of insulin misuse has been linked to the increased risk of diabetic complications in adolescent girls during puberty (31). Our observations suggest alternative therapy directed at underlying mechanisms, such as insulin sensitizers, IGF-I therapy, and anti-androgen therapy, might also be explored in attempts to reduce progression of microvascular complications in high-risk individuals during puberty.

Acknowledgments— This study was supported by the Novo Nordisk Center for Research in Growth and Regeneration, Aarhus University, Denmark (Danish Health Research Council Grant 9700592); the Institute of Experimental Clinical Research, University of Aarhus, Denmark; and the Hørslev Foundation. R.A. is a Novo Nordisk U.K. Research Foundation fellow. The ORPS is funded by the British Diabetic Association.

We acknowledge the Juvenile Diabetes Research Foundation; the study field workers; the laboratory assistance of Angie Watts and Dot Harris; the Barts-Oxford Study field workers; and the pediatricians, physicians, and diabetes nurse specialists in the Oxford region.

In addition, We are indebted to Joan Hansen for skilled technical assistance.

APPENDIX

Members of the ORPS Steering Committee are D.B. Dunger, R.N. Dalton, J. Fuller, E.A.M. Gale, H. Keen, M. Murphy, H.A.W. Niel, C.J. Schultz, R.J. Young, and T. Konopelska-Bahu.

Members of the ORPS are R.A.F. Bell and A. Taylor, Horton General Hospital, Banbury, U.K.; A. Mukhtar, B.P. O'Malley, B.R. Silk, and E.H. Smith, Kettering District Hospital, Kettering, U.K.; R.D.M. Scott, King Edward VII Hospital; F.M. Ackland, C.J. Fox, and N.K. Griffin, Northampton General Hospital, Northampton, U.K.; N. Mann, H. Simpson, P. Cove Smith, and M. Pollitzer, Royal Berkshire Hospital, Reading, U.K.; R.S. Brown and A.H. Knight, Stoke Mandeville Hospital, Aylesbury, U.K.; J.M. Cowen and J.C. Pearce, Wexham Park Hospital, Slough, U.K.

References

1. Viberti GC, Jarrett RJ, Keen H: Microalbuminuria as prediction of nephropathy in diabetics (Letter). *Lancet* 2: 611, 1982
2. Diabetes Control and Complications Trial Research Group: Effect of intensive diabetes treatment on the development and progression of long-term complications in adolescents with insulin-dependent diabetes mellitus: Diabetes Control and Complications Trial. *J Pediatr* 125:177–188, 1994
3. Schultz CJ, Konopelska-Bahu T, Dalton RN, Carroll TA, Stratton I, Gale EA, Neil A, Dunger DB: Microalbuminuria prevalence varies with age, sex, and puberty in children with type 1 diabetes followed from diagnosis in a longitudinal study: Oxford Regional Prospective Study Group. *Diabetes Care* 22:495–502, 1999
4. Andersen AR, Christiansen JS, Andersen JK, Kreiner S, Deckert T: Diabetic nephropathy in type 1 (insulin-dependent) diabetes: an epidemiological study. *Diabetologia* 25:496–501, 1983
5. Adcock CJ, Perry LA, Lindsell DR, Taylor AM, Holly JM, Jones J, Dunger DB: Menstrual irregularities are more common in adolescents with type 1 diabetes: association with poor glycaemic control and weight gain. *Diabet Med* 11:465–470, 1994
6. Ahmed ML, Connors MH, Drayer NM, Jones JS, Dunger DB: Pubertal growth in IDDM is determined by HbA_{1c} levels, sex, and bone age. *Diabetes Care* 21: 831–835, 1998
7. Yokoyama H, Uchigata Y, Otani T, Aoki K, Maruyama A, Maruyama H, Hori S, Matsuura N, Omori Y: Development of proliferative retinopathy in Japanese patients with IDDM: Tokyo Women's Medical College Epidemiologic Study. *Diabetes Res Clin Pract* 24:113–119, 1994
8. Salardi S, Cacciari E, Pascucci MG, Giambiasi E, Tacconi M, Tazzari R, Cicognani A, Boriani F, Puglioli R, Mantovani W, et al.: Microalbuminuria in diabetic children and adolescents. Relationship with puberty and growth hormone. *Acta Paediatr Scand* 79: 437–443, 1990
9. Clayton KL, Holly JM, Carlsson LM, Jones J, Cheetham TD, Taylor AM, Dunger DB: Loss of the normal relationships between growth hormone, growth hormone-binding protein and insulin-like growth factor-I in adolescents with insulin-dependent diabetes mellitus. *Clin Endocrinol (Oxf)* 41:517–524, 1994
10. Edge JA, Dunger DB, Matthews DR, Gilbert JP, Smith CP: Increased overnight growth hormone concentrations in diabetic compared with normal adolescents. *J Clin Endocrinol Metab* 71:1356–1362, 1990
11. Cummings EA, Sochett EB, Dekker MG, Lawson ML, Daneman D: Contribution of growth hormone and IGF-I to early diabetic nephropathy in type 1 diabetes. *Diabetes* 47:1341–1346, 1998
12. Hirschberg R: Effects of growth hormone and IGF-I on glomerular ultrafiltration in growth hormone-deficient rats. *Regul Pept* 48:241–250, 1993
13. Rudberg S, Persson B: Indications of low sex hormone binding globulin (SHBG) in young females with type 1 diabetes, and an independent association to microalbuminuria. *Diabet Med* 12:816–822, 1995
14. Cole TJ: The LMS method for constructing normalized growth standards. *Eur J Clin Nutr* 44:45–60, 1990
15. Davis JE, McDonald JM, Jarett L: A high-performance liquid chromatography method for hemoglobin A1c. *Diabetes* 27: 102–107, 1978
16. Frystyk J, Skjaerbaek C, Dinesen B, Orskov H: Free insulin-like growth factors (IGF-I and IGF-II) in human serum. *FEBS Lett* 348:185–191, 1994
17. Morrell DJ, Ray KP, Holder AT, Taylor AM, Blows JA, Hill DJ, Wallis M, Preece MA: Somatomedin C/insulin-like growth factor I: simplified purification procedure and biological activities of the purified growth factor. *J Endocrinol* 110:151–158, 1986
18. Westwood M, Gibson JM, Davies AJ, Young RJ, White A: The phosphorylation pattern of insulin-like growth factor-binding protein-1 in normal plasma is different from that in amniotic fluid and changes during pregnancy. *J Clin Endocrinol Metab* 79:1735–1741, 1994
19. Goldstein H, Healy MJ, Rasbash J: Multilevel time series models with applications to repeated measures data. *Stat Med* 13: 1643–1655, 1994
20. Schultz CJ, Neil HA, Dalton RN, Dunger DB; Oxforn Regional Prospective Study Group: Risk of nephropathy can be detected before the onset of microalbuminuria during the early years after diagnosis of type 1 diabetes. *Diabetes Care* 23: 1811–1815, 2000
21. Miller SB, Rotwein P, Bortz JD, Bechtel PJ, Hansen VA, Rogers SA, Hammerman MR: Renal expression of IGF I in hypersomatotropic states. *Am J Physiol* 259:F251–F257, 1990
22. Flyvbjerg A: Putative pathophysiological role of growth factors and cytokines in experimental diabetic kidney disease. *Diabetologia* 43:1205–1223, 2000
23. Alzaid AA, Dinneen SF, Melton LJ 3rd, Rizza RA: The role of growth hormone in the development of diabetic retinopathy. *Diabetes Care* 17:531–534, 1994
24. Feldmann B, Jehle PM, Mohan S, Lang GE, Lang GK, Brueckel J, Boehm BO: Diabetic retinopathy is associated with decreased serum levels of free IGF-I and changes of IGF-binding proteins. *Growth Horm IGF Res* 10:53–59, 2000
25. Hoogenberg K, Sluiter WJ, Dullaart RP: Effect of growth hormone and insulin-like growth factor I on urinary albumin excretion: studies in acromegaly and growth hormone deficiency. *Acta Endocrinol (Copenh)* 129:151–157, 1993
26. Dunaif A, Thomas A: Current concepts in the polycystic ovary syndrome. *Annu Rev Med* 52:401–419, 2001
27. Cara JF: Insulin-like growth factors, insulin-like growth factor binding proteins and ovarian androgen production. *Horm Res* 42:49–54, 1994
28. Ekstrand AV, Groop PH, Gronhagen-Riska C: Insulin resistance precedes microalbuminuria in patients with insulin-dependent diabetes mellitus. *Nephrol Dial Transplant* 13:3079–3083, 1998
29. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease: evidence for genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320: 1161–1165, 1989
30. Bryden KS, Neil A, Mayou RA, Peveler RC, Fairburn CG, Dunger DB: Eating habits, body weight, and insulin misuse: a longitudinal study of teenagers and young adults with type 1 diabetes. *Diabetes Care* 22:1956–1960, 1999
31. Bryden KS, Peveler RC, Stein A, Neil A, Mayou RA, Dunger DB: Clinical and psychological course of diabetes from adolescence to young adulthood: a longitudinal cohort study. *Diabetes Care* 24:1536–1540, 2001