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Nocturnal Release of Immunoreactive Growth Hormone-Releasing Hormone and Growth Hormone in Normal Children

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ABSTRACT. To evaluate the role of growth hormone-releasing hormone (GHRH) in the physiologic release of growth hormone (GH) we studied the nocturnal secretion of immunoreactive GHRH (ir-GHRH) and its relationship to GH release and various stages of sleep in six prepubertal (three boys) and six pubertal children (two boys) with normal stature. Their ages ranged from 8.1 to 14.9 yr and their bone ages from 6.8 to 14.8 yr. Blood was withdrawn continuously between 2200–0600 h at a constant rate of 5 mL/20 min. The EEG was simultaneously registered. The ir-GHRH and GH data were analyzed by a discrete-pulse detection algorithm (Pulsar). The number of nocturnal ir-GHRH pulses varied from 0–8 (median 7) and the number of GH peaks from 2–6 (median 3). Pubertal children had significantly more ($p < 0.05$) ir-GHRH pulses and the pulse amplitude was higher ($p < 0.05$) than in the prepubertal children. There were no significant differences in the GH parameters between the two groups. The ir-GHRH peaks were not significantly related to any specific sleep stage. The majority of the GH pulses (71%) were associated with slow wave sleep ($p < 0.001$). Two-thirds (69%) of the GHRH peaks preceded closely or coincided with GH pulses ($p < 0.02$). Pubertal subjects had more isolated ir-GHRH peaks than prepubertal children ($p < 0.05$). We conclude that the nocturnal secretion of ir-GHRH is pulsatile and, assuming that the peripheral plasma concentrations of ir-GHRH reflect its release from the hypothalamus, GHRH appears to play a physiologic role in the regulation of GH secretion. The partial dissociation between ir-GHRH and GH pulses suggests that other factors are also involved in the regulation of episodic GH release and/or that some of the circulating ir-GHRH originates from extrahypothalamic sources. Pubertal children have increased ir-GHRH secretion, the importance of which remains to be defined. (*Pediatr Res* 26: 404–409, 1989)

Abbreviations

ir, immunoreactive
GH, growth hormone
GHRH, growth hormone-releasing hormone

Secretory episodes of GH occur within 90–120 min of sleep onset and are associated with slow-wave sleep (1, 2). GHRH specifically stimulates GH release in normal men (3–5) and it has been shown that increases in circulating concentrations of ir-GHRH are followed by GH release in some physiologic situations (6, 7). However, only a little is known about the role of GHRH in the generation of the nocturnal rhythm of GH secretion. Based on observations obtained by intravenous administration of GHRH in the rat it has been suggested that there is a steady-state GHRH and somatostatin release from the hypothalamus and an additional 3- to 4-h rhythmic surge of each peptide (8). This interplay provides for the integration of the ultradian rhythm of GH secretion, as observed in the peripheral circulation. Plotsky and Vale (9) have demonstrated with direct measurements of ir-GHRH and ir-somatostatin in the hypophysial-portal circulation that ir-GHRH is secreted in a pulsatile fashion and that ir-GHRH pulses occur only in the presence of diminished somatostatin release. GHRH infusion studies in man also suggest that GH secretion is regulated by dynamic interaction between GHRH and somatostatin (10, 11). Our study was aimed at evaluating nocturnal variation in the circulating concentrations of ir-GHRH and the possible association between ir-GHRH and GH pulses.

MATERIALS AND METHODS

Subjects. Twelve healthy children (five M and seven F) participated in this study. Their chronologic ages ranged from 8.1 to 14.9 y and their bone ages from 6.8 to 14.8 y (12). The mean relative ht score was +0.01 ± 0.5 SD based on normal growth standards for Finnish children (13). The mean growth velocity before the study was 5.6 ± 0.8 cm/y and 4.9 ± 1.0 after the study. Six of the children were prepubertal and the other six pubertal. Their clinical data are given in Table 1. Among the pubertal subjects the pubertal development (14) of the four girls was at stage 3–4 (B 3 or 4, PH 3 or 4) and their fastest growth period was over at the time of the study, whereas the two boys were at stage 2 (G2, PH 2). Written informed consent was obtained from the subjects and their parents. The study was approved by the Ethical Committee of the Medical Faculty, University of Oulu, Finland and carried out according to the provisions of the Declaration of Helsinki and the ethical guidelines of National Institute of Child Health and Human Development (15).

Study design. The children were admitted to the hospital on the study day. They had a light meal 2 h before the study. A heparinized needle was inserted 30 min before starting the blood collection. Blood was continuously withdrawn between 2200–0600 h through a heparinized catheter at a rate of 5 mL/20 min with a constant withdrawal pump (CORMED, Medina, NY) according to the Cormed-Kowarski method (16). Chilled polypropylene test tubes containing 500 KIU aprotinin (Apronin, Medica, Finland) and 50 μL 10% Na 2-EDTA per mL blood, were changed every 20 min for 8 h. The tubes were kept in ice,

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Table 1. *Clinical data on subjects studied; mean ± SEM*

	Prepubertal children ($n = 6$)	Pubertal children ($n = 6$)
Sex (M/F)	3/3	2/4
Age (yr)	10.8 ± 0.8	14.1 ± 0.3
Bone age (yr)	10.1 ± 0.7	13.2 ± 0.5
Ht (cm)	142.7 ± 5.1	160.5 ± 3.8
Relative ht, SD, score	−0.12 ± 0.5	0.13 ± 0.5
Preceding growth velocity (cm/yr)	5.4 ± 0.2	5.9 ± 1.2
Subsequent growth velocity (cm/yr)	6.4 ± 1.3	3.7 ± 1.3
Growth velocity over the total period	5.8 ± 0.5	4.6 ± 0.8

centrifuged at 1000 × *g* for 15 min within 30 min, and the plasma stored at −70°C. Sleep was recorded continuously with an eight-channel EEG recorder (Mingograph EEG Universal, Siemens-Elema, Solna, Sweden). Silver-silverchloride cup electrodes were fixed to the scalp with collodion in the C3P3 and C4P4 positions along with two reference electrodes at the vertex. Submental electromyogram, eye movements, and heart rate were also recorded on the EEG channels. The EEG recordings were scored as wakefulness, stages 1, 2, 3, 4, and rapid eye movement sleep according to Rechtschaffen and Kales (17). Stages 3 and 4 were considered as slow wave sleep.

Assays. All samples from an individual child were analyzed in the same assay. Plasma ir-GHRH concentrations were measured by RIA as described in detail previously (6). Two mL of plasma were extracted using Sep-pak C 18 cartridges (Waters Inc., Milford MA). The pH of the plasma was adjusted to 4.0 with 0.1% trifluoroacetic acid. The acidified plasma was passed through the cartridge, which was then washed with 10 mL 0.5% triethylamine (pH = 4.0) containing 1% acetic acid and eluted with 3 mL 80% methanol in 0.5% triethylamine. The eluate was evaporated and the residual assayed using a rabbit antiserum specific for the mid portion of GHRH and ^{125}I-GHRH 1-40 as tracer (18). Our HPLC analyses have shown that the major ir-GHRH species in the plasma samples eluates as synthetic human GHRH 1-40 (6). The sensitivity of the assay was 1 pg/tube. The mean recovery of synthetic GHRH 1-44 amide (25 pg/mL) added to the plasma was 76 ± 9% (SD) ($n = 6$) and the results were corrected for recovery. The intra- and interassay coefficients of variation were 11 and 18%, respectively. To verify that the plasma ir-GHRH was stable at +4°C for 20 min 100 pg of synthetic GHRH 1-44 amide was incubated with 5 mL of fresh human blood containing EDTA and Apronin as in test samples for 0 (centrifuged immediately after mixing) and for 20 min at +4°C. After centrifugation the GHRH-RIA was performed as described before.

The recovery of ir-GHRH added to samples centrifuged immediately (0) was 82.5 ± 5.1% ($n = 7$) and that of samples incubated for 20 min at +4° C 84.1 ± 10.3% ($n = 7$; NS).

Plasma GH concentrations were analyzed in duplicate using a commercial RIA kit obtained from Pharmacia, Uppsala, Sweden. Values below the limit of sensitivity, which was 0.5 μg/L, were considered to be 0.5 μg/L. The intra- and interassay coefficients of variation were 7 and 12%, respectively. Plasma levels of IGF-I were determined with a commercial kit (Nichols Institute Diagnostics, San Juan Capistrano, CA). The sensitivity of the assay was 0.02 U/mL, the intraassay coefficient of variation was 5.1% and the interassay variation less than 10%.

Data analysis. Discrete ir-GHRH and GH pulses were identified using Pulsar analysis (19), an objective multipoint, statistically based pulse detection algorithm that removes long-term trends from the series of observations, identifies peaks in the residual series and resolves each peak into overlapping secretory episodes. The SD of the assay is calculated at each point, and the residuals are rescaled in terms of this unit. Pulse criteria were set at a signal to free noise ratio to minimize the occurrence of false positive pulses <5%. Ir-GHRH and GH release were expressed as integrated values per hour for the time period 2200–0600 h.

Table 2. *Characteristics of variations in nocturnal circulating ir-GHRH and GH concentrations in prepubertal and pubertal children; mean ± SEM if not otherwise indicated*

	Prepubertal children ($n = 6$)	Pubertal children ($n = 6$)	Statistics
No. of ir-GHRH peaks, median (range)	6 (0–8)	8 (3–8)	$p < 0.05$
No. of ir-GHRH peaks preceding or coinciding with GH pulses, median (range)	5 (0–6)	5 (3–6)	NS
Basal ir-GHRH (ng/L)	6.9 ± 2.5	11.0 ± 3.3	NS
Peak ir-GHRH (ng/L)	68.0 ± 15.8	71.4 ± 19.1	NS
Integrated ir-GHRH (ng/L)	19.7 ± 4.2	29.5 ± 8.0	NS
Ir-GHRH amplitude (ng/L)	12.6 ± 6.8	32.5 ± 10.9	$p < 0.05$
No. of GH peaks, median (range)	3.5 (2–5)	2.5 (2–6)	NS
Basal GH (μg/L)	0.5	0.5	NS
Peak GH (μg/L)	14.6 ± 2.5	15.5 ± 2.4	NS
Integrated GH (μg/L)	4.6 ± 0.8	5.3 ± 1.2	NS
GH amplitude (μg/L)	7.4 ± 1.2	10.3 ± 1.6	NS

Statistical analysis. The χ^2 test was used to assess the relationship between hormone pulses and sleep stages and the relationship between ir-GHRH and GH peaks. The significance of the difference between two groups was estimated by the Mann-Whitney U test and the correlation between two parameters by Spearman regression analysis. p values <0.05 were considered significant. The results are expressed as mean ± SEM, if not otherwise indicated.

RESULTS

Characteristics of the variations in circulating ir-GHRH and GH concentrations in the study subjects are shown in Table 2. The number of nocturnal ir-GHRH pulses varied from 0–8 (median 7). Pubertal children had significantly more ir-GHRH pulses than prepubertal children ($p < 0.05$). The mean basal ir-GHRH concentration for all subjects was 9.0 ± 2.9 ng/L and the maximal ir-GHRH concentration 69.4 ± 23.3 ng/L. The mean integrated ir-GHRH concentration for all subjects was 24.6 ± 6.6 ng/mL/h. There were no statistically significant differences between the prepubertal and pubertal children in these ir-GHRH parameters. The mean ir-GHRH amplitude was significantly higher in pubertal than in prepubertal children ($p < 0.05$).

The number of GH pulses during the 8-h sampling period ranged from 2 to 6 (median 3). The mean basal GH concentration was 0.5 μg/L and the mean maximal GH concentration was 15.0 ± 2.8 μg/L. The mean integrated GH concentration was 4.9 ± 1.0 μg/L/h and the mean amplitude of GH pulses was 8.8 ± 1.5 μg/L. There were no significant differences in the GH

parameters between the prepubertal and pubertal children (Table 2). The majority of the GH pulses (71%) was associated with slow wave sleep ($p < 0.001$). Ir-GHRH pulses were not significantly associated with any stage of sleep. The majority of the ir-GHRH peaks (69%) closely preceded (interval <20 min) or coincided with the GH pulses ($p < 0.02$). On one-third (31%) of the occasions, no relationship was seen between the ir-GHRH and GH peaks. Pubertal children had more isolated ir-GHRH pulses (range 0–3, median 2.5) than prepubertal children (range 0–2, median 1.5; $p < 0.05$).

A representative pattern of the nocturnal circulating ir-GHRH and GH concentrations in one prepubertal child is shown in Figure 1 and in one pubertal child in Figure 2. No significant correlation was found between the GH and GHRH parameters. Plasma IGF-I concentrations did not correlate with any of the indicators of ir-GHRH or GH secretion.

DISCUSSION

A 24-h sampling with standardized intervals has been used to assess spontaneous GH secretion (20, 21). However, there are also studies suggesting that the GH concentrations during nocturnal sleep can be used as an indicator of GH release instead of the 24-h GH concentrations (22). Inasmuch as sleep is considered to be and is used as a physiologic stimulator of GH (23), we were interested in studying the possible association between nocturnal ir-GHRH and GH pulses in the peripheral circulation. Instead of discrete samples we used a constant withdrawal pump according to the Cormed-Kowarski method (16). With this method it is possible to get an idea of true nocturnal integrated GH and ir-GHRH secretion. Discrete and integrated sampling has been compared in a recent study and no obvious blunting or widening of the peaks using an integrated sampling technique was found (24).

It has been documented previously that children experience both sleep onset difficulty and increased wakefulness after sleep onset resulting in a shorter sleep period and a significantly lower sleep efficiency on their first night in the hospital as compared with subsequent study nights (25). In this study the GH response may have been stronger if the children had spent some time in the hospital before sampling. However, this was not considered ethically justified because the study subjects were normal, healthy children.

According to our present observations the secretion of ir-GHRH seems to be pulsatile, because there were considerable variations in ir-GHRH concentrations in the peripheral circulation in normal children during sleep. This finding is in agreement with the study of Plotsky and Vale (9) on rats *in vivo*. They demonstrated that superimposed upon the tonic release of GHRH and somatostatin from hypothalamus into the hypophyseal portal blood, there are additional rhythmic secretory bursts of GHRH associated with decreased somatostatin secretion.

Administration of GHRH as a bolus produces an acute GH secretory response in man (3, 5). Infusion of GHRH at a constant rate in normal adults produces a persistent effect on GH secretion that is characterized by enhanced pulsatile secretion and an elevation of baseline GH secretion (10, 26). These effects can be observed with infusions as short as a few hours and they persist for as long as 2 wk (27). Accordingly, the infusion studies suggest that GH pulses are a result of GHRH secretion and that a finite amount of GH is released by GHRH (10, 11). In our study, ir-GHRH peaks preceded or coincided with GH peaks in a substantial majority (69%) of the occasions. This observation indicates that GHRH has an active role in sleep-induced pulsatile GH secretion in children, although we could not find any significant association between the quantitative indicators of the release of the two hormones.

ir-GHRH has been detected in the peripheral circulation by ourselves and others (6, 28–32). The source of this circulating ir-GHRH is, however, uncertain. In addition to the CNS, ir-GHRH has been reported in the gastrointestinal tract (31, 33). In rats it

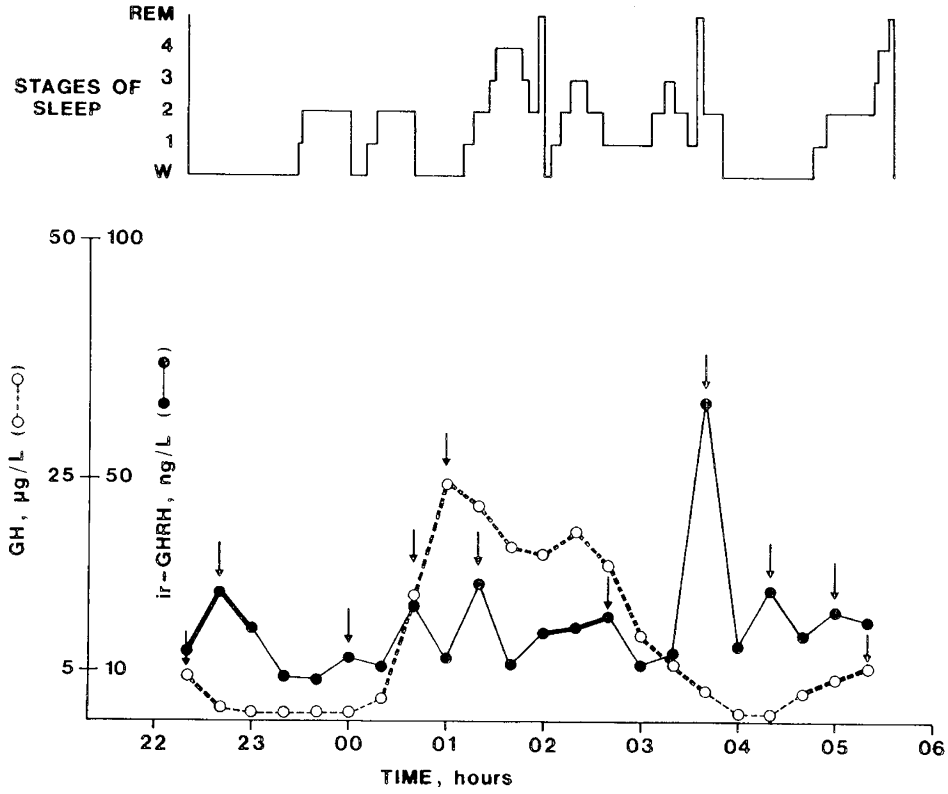

Fig. 1. A representative pattern of nocturnal circulating ir-GHRH and GH concentrations and sleep stages in one prepurbertal child. ↓ represents the maximal peak values. The duration of each pulse is marked with a strengthened line.

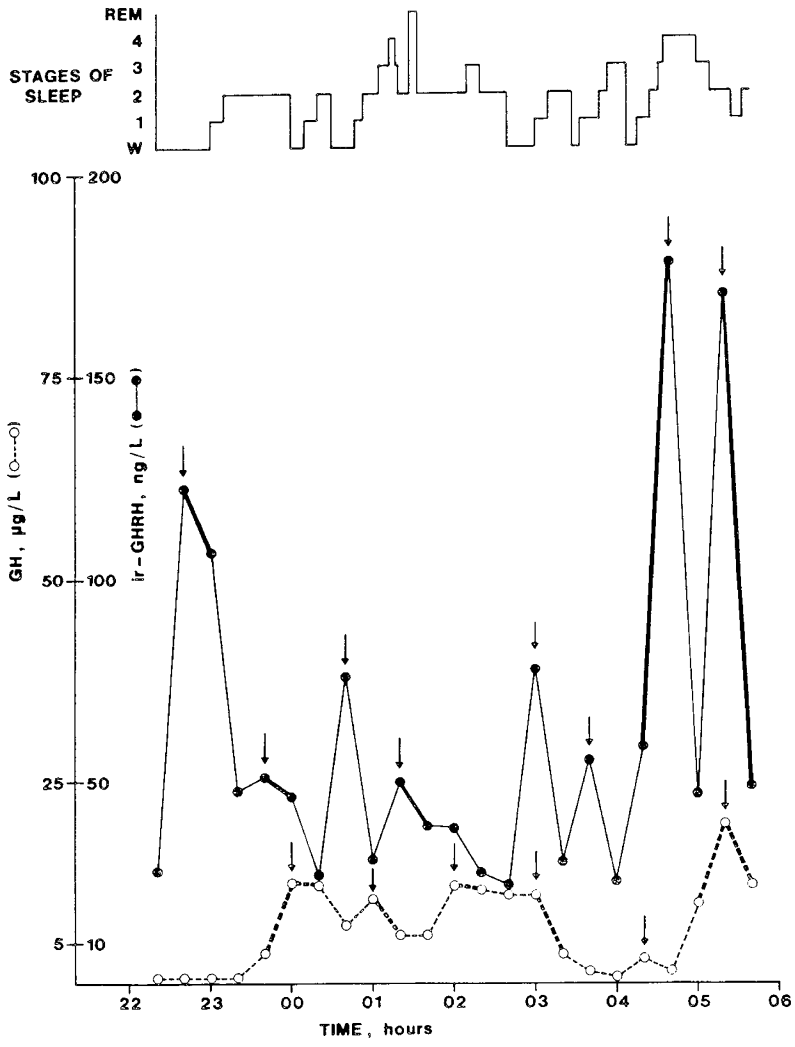

Fig. 2. A representative pattern of nocturnal circulating ir-GHRH and GH concentrations and sleep stages in one pubertal child. ↓ represents the maximal peak values. The duration of each pulse is marked with a strengthened line.

has been shown that the mechanical ablation of GHRH neurones in the medial basal hypothalamus results in a 70% reduction of circulating ir-GHRH levels (34), indicating that the majority of the peripheral immunoreactivity could originate from hypothalamic sources. Changes in circulating ir-GHRH concentrations have been claimed to correlate with the secretion of GH (35, 36), suggesting that peripheral plasma ir-GHRH measurements reflect hypothalamic function. Our results support the view that a considerable proportion of human peripheral ir-GHRH is derived from the hypothalamus, because the nocturnal ir-GHRH and GH peaks showed a close temporal relationship in most of the cases.

In our study about one-third of the ir-GHRH peaks were unrelated to GH peaks, and these isolated GHRH peaks were found in all but one subject. In addition, we also found a small number of separate GH peaks unrelated to ir-GHRH pulses. This partial dissociation between ir-GHRH and GH pulses can be interpreted either as an indicator that some of the circulating ir-GHRH originates from extrahypothalamic sources, or as evidence that other factors are involved in the regulation of episodic GH release. Sopwith *et al.* (37) have also found a dissociation between circulating concentrations of ir-GHRH and GH in normal human subjects. They suggest that an important source of human GHRH is located outside the hypothalamus and that secretion from this source is unrelated to the normal control of pituitary GH release.

Among other factors regulating pituitary GH secretion, somatostatin plays an essential role. Endogenous somatostatin decreases the GH response to GHRH in awake and freely moving rats (38). Vance *et al.* (10, 11) showed partial inhibition of the GH response to continuous GHRH infusion in man, which could either be a direct effect or stimulated by somatostatin. Accordingly, the partial dissociation between the GHRH and GH peaks observed in our study probably reflected both release of extrahypothalamic ir-GHRH and regulatory effects of somatostatin.

Results from previous studies on the effect of puberty on spontaneous GH secretion are conflicting. One group concluded that puberty had no effect on GH secretion (39). Several other investigations, however, have revealed an increased 24-h GH concentration during puberty in both boys and girls (40, 41). We did not find any statistically significant differences in GH secretion between prepubertal and pubertal children, although the pubertal subjects tended to have higher integrated and peak GH concentrations as well as higher GH amplitudes. The lack of significant differences may be due to the small number of subjects studied. In addition, the prepubertal children were relatively old and, although clinically prepubertal, some pubertal hormonal changes may have already been initiated. All but one of the prepubertal children went into puberty during the subsequent year. It has also been documented that the puberty-associated increase in GH concentration results from an increase in the frequency of GH pulses during waking hours, and an increase in the amplitude of GH pulses over 24 h (23). Accordingly, noctur-

nal GH secretion may not provide all information on the characteristics of GH release in puberty.

Our observations demonstrate that the number of nocturnal ir-GHRH pulses and the ir-GHRH amplitude increase in puberty. This is in agreement with the findings of Argente *et al.* (42) who showed that pubertal children have higher basal plasma ir-GHRH levels than prepubertal children. In our study the increased GHRH secretion in puberty was not reflected, however, in any significant differences in GH release. It has been shown previously that the GH response to GHRH infusion is relatively constant during puberty (43). Data on the concentrations of ir-somatostatin in the peripheral circulation before and during puberty are scanty. The divergence between increased ir-GHRH release and unchanged GH secretion in pubertal children can hypothetically be a consequence of enhanced secretion of counteracting somatostatin, an increased release of extrahypothalamic ir-GHRH induced by puberty, or a desensitization of the somatotrophs to GHRH in association with puberty.

Our study shows that the secretion of ir-GHRH is pulsatile, and provided that the peripheral concentrations of ir-GHRH reflect its release from the hypothalamus, GHRH appears to play an active role in nocturnal pulsatile GH secretion in growing children. The partial dissociation between ir-GHRH and GH pulses indicates that other factors are also involved in the regulation of episodic GH release and/or that some of the circulating GHRH is derived from sources outside the CNS. Finally, pubertal children are characterized by an increased number of ir-GHRH pulses and an increased ir-GHRH amplitude.

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Announcements

Abstract Deadline

The American Pediatric Society and The Society for Pediatric Research announce the abstract deadline for the 1990 Annual Meeting (May 7–11, 1990, Anaheim Hilton & Convention Center, Anaheim, CA) has been set as *January 4, 1990.*

For further information contact: 2650 Yale Blvd., S.E., Suite 104, Albuquerque, NM 87106 (505) 764-9099.

Joint PhD-Fellowship Training Program

The University of Chicago Department of Pediatrics announces its unique, new Pediatric Science Training/PhD Program. Trainees wishing to pursue an academic career may simultaneously pursue subspecialty training and a graduate school program leading to the PhD degree in this 5-year program. Training is available in most pediatric subspecialties, and research opportunities are available in diverse fields, including the social and behavioral sciences as well as the biological sciences. Applications are being accepted for 1990 and 1991. This program is funded by the National Institutes of Child Health and Human Development. Address inquiries to Robert L. Rosenfield, MD, Program Director, Pediatric Science Training/PhD Program, Wyler Children's Hospital, 5841 South Maryland, Chicago, IL 60637.

17th Annual Seminar in Pediatric Nephrology

The 17th Annual Seminar in Pediatric Nephrology, "Current Concepts in Diagnosis and Management," will be held at the Diplomat Hotel and Country Club, Hollywood, FL, February 5–10, 1990. *For more information contact* Pearl Seidler, Division Coordinator, Department of Pediatrics, Division of Pediatric Nephrology, University of Miami School of Medicine, P.O. Box 016960, Miami, FL 33101, (305) 549-6726.

Update on Diabetes in Childhood

The International Study Group on Diabetes in Children and Adolescents (ISGD) announces a course on "Update on Diabetes in Childhood," March 17–24, 1990, in Malga, Ciapela, Marmolada, Italy. The course is addressed to pediatricians, practitioners, and endocrinologists dealing with children and adolescents. Directors of the course of Dr. L. Pinelli, University of Verona, Italy, and Prof. Z. Laron, Tel Aviv University, Israel. The scientific program will be held in the mornings and evenings, and during the day winter sports can be enjoyed in the Alps. *For further information contact* Dr. L. Pinelli, Servizio di Diabetologia Pediatrica, Policlinico, I-37134, Verona, Italy; Telephone 0039-45-933667; FAX 0039-45-508222.

Hyperlipidemia in Childhood and the Development of Atherosclerosis

A conference on Hyperlipidemia in Childhood and the Development of Atherosclerosis will be held May 2–4, 1990, at the Hyatt Regency, Bethesda, MD. This conference will examine the role of hyperlipidemia and dyslipidemia in childhood in relation to the development of atherosclerosis. The program will focus initially on morphologic development of the atherosclerotic plaque, pathologic findings in pediatric autopsy series, biochemical correlates, and cellular models of atherosclerosis in the young. Distribution of plasma cholesterol and lipoprotein levels in children will be reviewed, including genetic, dietary, developmental, lifestyle, and pharmacologic influences on serum levels. Tracking of lipid levels over time will be reviewed, as well as international comparisons of lipids in pediatric populations. Cholesterol screening of children will be presented both from high-risk and population-based viewpoints. Pediatric office-based, school-based, and community-wide cholesterol screening activities and interventions will be described. Dietary and pharmacologic therapy of lipid disorders in children will be presented. Abstract deadline is *December 11, 1989.* The abstract, including title, author(s), and affiliation must be typed single space within a 5 × 4-3/8 inch rectangle, and sent to Dr. Christine L. Williams, Preventive Cardiology Center, New York Medical College, Valhalla, New York 10595. *For further information, contact* Conference Department, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, (212) 838-0230.