

# GROWTH

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## Circadian Rhythms - Genetic Regulation and Clinical Disorders

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### INTRODUCTION

Circadian rhythms are endogenously generated rhythms with a period length of about 24-hours. A biological clock in the hypothalamic suprachiasmatic nuclei is responsible for the generation of circadian rhythms. Notable examples of the circadian rhythms include the sleep-wake cycle and rhythms in hormone production. Abnormalities of the circadian system include biological clock lesions that result in arrhythmic behavior and irregular sleep patterns. Abnormalities of the circadian system also occur when there is desynchronization of environmental clock time with the phase of the "internal milieu" resulting in conditions such as "jet lag". Numerous aspects of human physiology are greatly influenced by the time of day, as is the pathogenesis of illness.

This review summarizes our current knowledge of the organization of the circadian system and the generation and regulation of biological clock function. The role the circadian system plays in human physiology along with the detection and treatment of biological clock disorders is also discussed.

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Robert M. Blizzard, MD  
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## CIRCADIAN SYSTEM ORGANIZATION

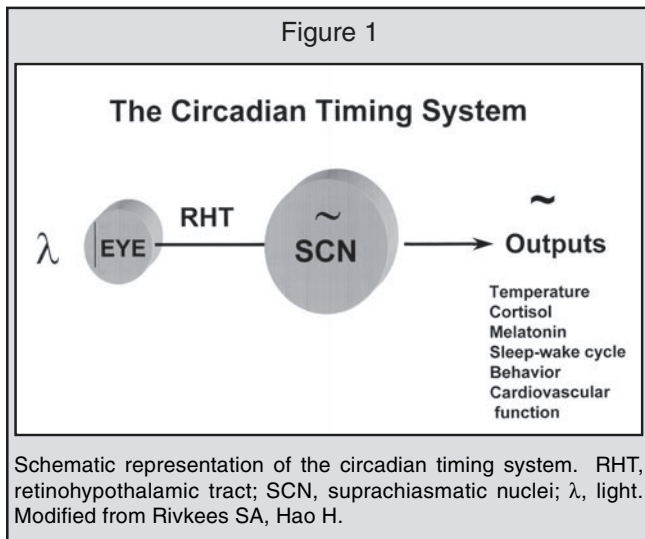
The system responsible for the generation and regulation of circadian rhythms is the circadian timing system. This neural system consists of a biological clock located in the paired suprachiasmatic nuclei (SCN) of the anterior hypothalamus, of an input pathway from the retina, and output pathways from SCN (Figure 1).<sup>1</sup>

Because oscillations of the biological clock are not exactly 24-hours, synchronizing (entraining) the circadian pacemaker each day to the 24-hour light-dark cycle is necessary. Otherwise, clock oscillations will drift (free-run) out of phase with that of the environmental cycle. A direct pathway, the retinohypothalamic tract (RHT), from the retina to the SCN mediates photic entrainment of the SCN.<sup>1</sup> Light is the most potent entraining stimulus (Figure 1).

Two types of photic regulation of circadian phase (types 0 and 1) have been described.<sup>2</sup> In humans, strong (type 0) resetting is observed after very bright light exposure (10,000 lux), and modest (type 1) resetting is observed with ordinary indoor lighting (200 lux). Although cutaneous light has been suggested as influencing circadian function in humans, there is little support for the notion that this or other extraretinal photoreception is important in mammals.<sup>3</sup>

## MOLECULAR BASIS OF CIRCADIAN RHYTHMICITY

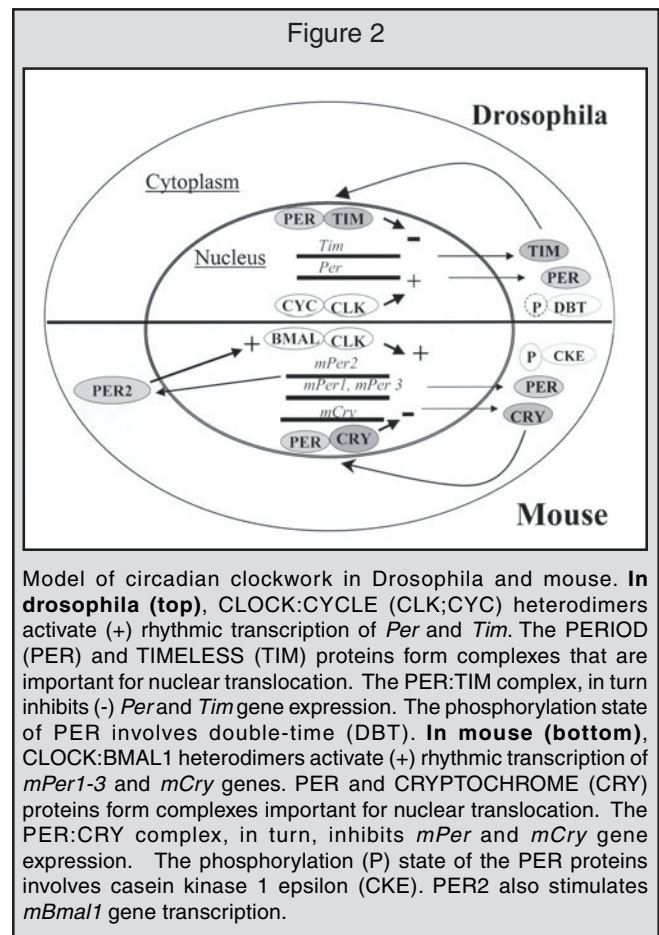
Recent data suggests that the SCN is composed of multiple, single cell circadian oscillators. These oscillate as an ensemble to generate overt rhythms.<sup>4</sup> Gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter, plays an important role in synchronizing the oscillations of individual clock cells.<sup>4</sup>



Considerable progress has been made over the past several years in defining the molecular mechanisms of clock oscillations.<sup>5</sup> In yeast, drosophila, and in mammals, it now appears that the molecular clockwork involves interlocking feedback loops that stimulate or inhibit clock gene expression.<sup>6</sup>

The molecular mechanisms leading to circadian rhythm generation were first detailed in drosophila (Figure 2). In these flies, the circadian feedback loop is generated by the transcriptional regulatory proteins PERIOD (PER) and TIMELESS (TIM) encoded by the *per* and *tim* genes. These are activated in the morning, and their two protein products accumulate in the cytoplasm during the day. In the evening, dimerization of PER and TIM occurs and the complex enters the nucleus. After entering the nuclei, the PER-TIM complex inhibits *per* and *tim* gene expression. In addition to feedback inhibition, the proteins CYCLE (CYC) and CLOCK (CLK) dimerize to stimulate *per* and *tim* gene expression in a rhythmic manner. These processes result in a 24-hour cycle of clock protein oscillations.

In the mammalian clock, several clock genes that are homologous to drosophila clock genes have been recently identified and discovered to play similar roles in clock regulation. Homologous mammalian and



*Drosophila* clock genes are described in Table 1, and their corresponding roles in circadian rhythm generation are illustrated in Figure 2. The rhythmic transcription of *mPer* genes (murine *Pers* 1-3) and *mCry* (Cryptochromes 1 and 2) are driven by the transcriptional activating factors CLOCK and BMAL1, that interact with specific promoter elements. PER and CRY then accumulate in the cytoplasm to form complexes that enter the nucleus. Within the nucleus, CRY will then directly interact with CLOCK and BMAL1 to turn off transcription of the *mPer* and *mCry* genes. As the levels of PER and CRY fall, CLOCK and BMAL1 will dimerize to restart *mPer* and *mCry* transcription restarting the 24-hour cycle.<sup>5</sup>

In addition to PER:CRY feedback inhibition, other processes contribute to the clock mechanisms. For example, PER2 (Figure 2) stimulates BMAL1 expression so that PER and BMAL1 expression are out of phase. Alteration in the phosphorylation status of PER proteins also influences PER stability and cellular localization. In *Drosophila*, the kinase double-time alters PER phosphorylation.<sup>6</sup> In mammals, casein kinase 1 epsilon<sup>7</sup> influences PER phosphorylation. Mutations in each of these kinases alter normal rhythmicity.

Evidence suggests that PER proteins also play a role in the photic regulation of clock phase. Following either photic or glutamatergic stimulation of the SCN, a cascade of calcium-mediated events is triggered, leading to activation of the transcriptional regulator CREB.<sup>4</sup> In turn, CREB binds to cAMP-response-element (CRE) sites within promoter regions to induce the expression of *mPer1* and *mPer2*. Alterations in PER protein expression then play a role in resetting clock phase.

### EXPRESSED RHYTHMICITY IN HUMANS AND OTHER MAMMALS

The rhythmic expression of intrinsic clock genes also drives the expression of clock-output genes, which communicate circadian phase to the rest of the organism.<sup>4</sup> This occurs as E-box elements, which are a binding site for PER, and which are present in promoter regions of other genes.<sup>4</sup>

Mutations in clock genes have been recognized in rodents with abnormal rhythmicity. Very recently, the first mutation of a human clock gene hPER2 has been discovered. This mutation results in the advanced-sleep phase syndrome that is characterized by very early morning awakening.<sup>8,9</sup> As other individuals with abnormal rhythmicity are identified, it is anticipated that additional clock gene mutations will be found.

Table 1  
Homologous Genes in *Drosophila* and Mice that Play a Role in Circadian Clock Regulation

<b>Drosophila</b>	<b>Mouse</b>
period ( <i>per</i> )*	mPeriod1 * mPeriod2 * mPeriod3 *
Timeless ( <i>tim</i> )* Time-out	None mTimeless**
Cryptochromes ( <i>Cry</i> )*	mCry1* mCry2*
clock*	mClock*
cycle*	mBmal1 (MOP3) mBmal2 (MOP9)
double-time*	casein kinases 1 epsilon (TAU)*
*mutation results in arrhythmic behavior **mutation results in embryonic lethal	

Adapted from Reppert and Weaver<sup>1</sup>

Outputs of the circadian system have been widely characterized in human clinical studies. Notable examples include the sleep-wake cycle, daily rhythms in body temperature, and day-night rhythms in cortisol production. Day-night differences in gonadotropin, testosterone, growth hormone and thyrotropin secretion are also recognized.<sup>10</sup> Melatonin production by the pineal gland is also regulated by the SCN, with secretion occurring at night in proportion to the duration of darkness. In seasonal breeding species, changes in the duration of nocturnal melatonin production regulates the activity of the reproductive axis.<sup>11</sup> Melatonin does not appear to influence the human reproductive axis.<sup>12</sup> In humans, the duration of melatonin secretion is related to the length of days. The role of endogenous melatonin secretion in regulating SCN function is also unclear, as pinealectomized animals exhibit normal circadian rhythmicity and normal phase-shifting responses to light.<sup>13</sup>

Day-night differences are recognized for many homeostatic mechanisms such as body temperature, which has a nadir in the early morning hours. Cardiovascular function exhibits diurnal rhythmicity, as

does platelet function.<sup>14</sup> Rhythms in cognitive ability are recognized, and the productivity of shift workers and health care providers varies with the time of day.

There is also increasing recognition that the circadian cycle influences the pathogenesis of many illnesses. Myocardial infarctions and cerebrovascular events occur most commonly in the morning.<sup>14</sup> Croup and certain forms of asthma are associated with evening- hour exacerbations.<sup>15</sup> In some individuals, seizures are related to the time of day. Sudden infant death syndrome (SIDS) has a strong time related component, occurring most frequently in early morning hours.<sup>16</sup> However, we do not know if the circadian system plays a role in SIDS pathogenesis.

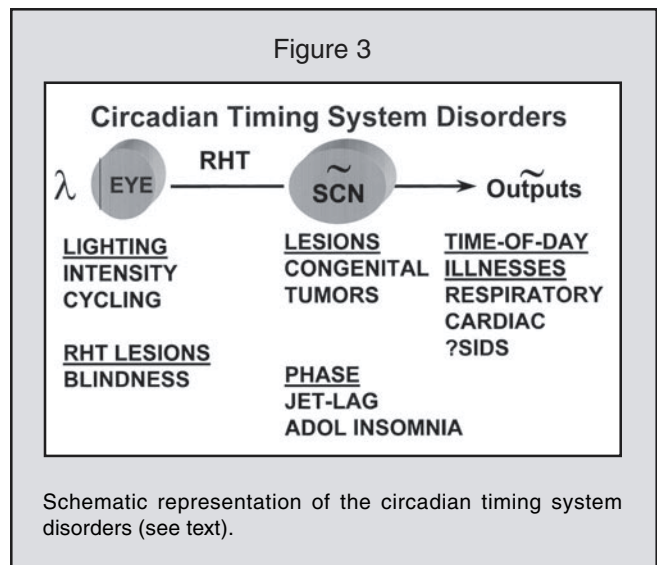
### CIRCADIAN SYSTEM ABNORMALITIES

Since the circadian system exerts potent influences on human behavior and physiology, circadian system disorders will have overt clinical manifestations.<sup>17</sup> Circadian system disorders may be related to abnormal clock function or to abnormal entrainment of the clock (Figure 3).

When more than 90% of the SCN is damaged, arrhythmic behavior may result. Thus, congenital or acquired anterior hypothalamic lesions or tumors may result in the loss of expressed day-night rhythms on sleep-wake disorders.<sup>18</sup> Congenital central system abnormalities may also be associated with clock lesions, as we have discovered arrhythmic activity patterns in a child with septo-optic dysplasia.<sup>19</sup>

Clock disorders include abnormalities in circadian phase, which relate to the timing of expressed rhythmicity (e.g. the onset and offset of sleep-wake cycles) relative to the 24-hour day. Abnormalities of circadian phase occur when the “hands” of the endogenous clock are out of phase with the environmental light-dark cycle. One notable example of this phenomenon is jet lag, which occurs when circadian clock phase does not match that of light-dark cycle after changing time zones.

Another condition in which abnormal phase relationships occur is in delayed-sleep phase insomnia. In this condition that prominently affects adolescents, clock phase is delayed with resultant late sleep-onset and awakening times. Delayed-sleep phase insomnia should be considered when the individual does not fall asleep until after midnight and awakens late in the morning or in the afternoon. This condition becomes exaggerated when the effected individual is allowed to “sleep in” on weekends. Families with abnormally advanced circadian phase have also been described, some with hPER2



mutations, suggesting a strong genetic component for the setting of circadian phase.<sup>8,9</sup>

Entrainment disorders may result from inadequate retinal innervation of the SCN. In blind individuals without intact RHT function, the absence of photic information may result in impaired synchronization of endogenous and environmental phases. The circadian phase of such individuals will free-run, resulting in times when the individuals’ sleep-wake cycles do not correspond with the light-dark cycle. Recent evidence shows that timed melatonin administration may help entrain the circadian phase of blind individuals who do not entrain to the 24-hour day. This helps synchronize sleep-wake cycles with the environmental light-dark cycle.<sup>20</sup> Surprisingly there are blind individuals who have intact retinal innervation of the SCN. In these individuals, environmental lighting will entrain the circadian clock so that endogenous rhythmicity is in phase with the light-dark cycle.<sup>21</sup> Unknown non-photoc factors may also entrain circadian phase in blind individuals, as we have observed sleep-wake cycles in perfect synchrony with the light-dark cycle in individuals with anophthalmia.

Another cause of entrainment abnormalities is related to problems in environmental lighting conditions. If individuals are exposed to constant indoor lighting or darkness, or to low-intensity cycled lighting that is not potent enough to shift the clock (<200 lux), expressed rhythmicity will free-run. This situation can occur in constantly illuminated intensive care units where the patient’s circadian phase will drift from that of care providers. This may result in perceptions of abnormal behavior. The interpretation of time-of-day dependent tests e.g., cortisol levels also will be inaccurate in this setting. Thus, to prevent free-running rhythms, cycled lighting of adequate intensity is needed.

## DETECTING BIOLOGICAL CLOCK DISORDERS

A history of regular sleep and wake times in an individual is reassuring that the biological clock is functioning normally. The lack of regular sleep or awakening time may reflect abnormal clock function. Surprisingly, despite the socially disruptive effects of arrhythmic behavior, clock-related behavioral problems may not be brought to medical attention. Yet upon inquiry, families will give clear histories of abnormal activity patterns.

To assess clock function, diaries of sleep and waking times are useful. If the time the patient awakens and retires to sleep is consistent from day-to-day, this suggests normal clock function. However, if sleep patterns are irregular, or are out of synchrony with those of other family members, clock lesions may be present.

To provide objective assessments of behavior patterns, periods of rest and wakefulness can be assessed using monitors worn on the wrist that collect activity information for extended periods (actigraphy). Analysis of activity patterns collected over 2-3 week periods (actograms) can then be used to determine if there is normal rhythmicity or altered phase-relationships.

## CHRONOTHERAPY

Over the past several years, considerable progress has been made in the treatment of biological rhythm disorders. Light has been recognized to regulate circadian rhythmicity in humans.<sup>2</sup> Exposure to bright light (10,000 lux) during the night is a strong stimulus that produces rapid shifts in circadian phase in humans.<sup>2</sup> Not surprisingly, light therapy is now being considered as a potential therapy for jet lag and other circadian phase disorders.

The concept that bright light resets the circadian clock is also important for night-shift workers. By providing an environment with bright light exposure during work at night and darkness during the daytime when the worker rests, it is possible to shift the endogenous circadian cycle to that of the work schedule.<sup>22</sup> Light therapy is also used in the treatment of certain forms of depression.<sup>23</sup>

Behavioral paradigms can be used to treat circadian-phase disorders. Delayed sleep-phase insomnia can be treated by progressively delaying sleep onset over several days until the patient's sleep-wake cycle is in phase with the desired time of day. Alternatively, imposing regular waking times each morning can help resynchronize circadian phase.

## MELATONIN

Melatonin has received much attention as a "chronotherapeutic". Melatonin is an endogenous indolamine that is produced by the pineal gland at night in proportion to the duration of darkness.<sup>24</sup> In mammals, melatonin exerts its effects through specific high-affinity receptors that include Mel 1a (mel 1) and Mel 1b (mel 2) receptors.<sup>25</sup> These receptors consist of seven transmembrane spanning domains and couple with guanosine nucleotide binding proteins (G proteins).<sup>25</sup> In humans, the melatonin receptors have been identified in the SCN.<sup>26</sup> In non-human primates, melatonin receptors have been identified in the hippocampus, brainstem, thalamus and cerebral cortex.<sup>27</sup>

Melatonin has been touted as a therapy for a variety of conditions ranging from aging to cancer. Yet, as reviewed,<sup>28</sup> most of these claims have little credible scientific support. Melatonin, however, may have legitimate use in treating sleep disorders. Melatonin has well documented hypnotic properties, and is therefore effective in facilitating sleep onset.<sup>29-31</sup> The hypnotic effects of melatonin are most pronounced when melatonin is given in the evening.<sup>32</sup>

It has also been suggested that melatonin can acutely shift circadian phase and may have a role in treating clock disorders such as jet lag.<sup>33</sup> This issue remains controversial. Modest melatonin-induced phase shifts have been detected in some rodent species, but not in others.<sup>34</sup>

In humans, using the onset of melatonin secretion to mark circadian phase, it has been suggested that melatonin induces small shifts in circadian phase.<sup>33,35</sup> However, when primates are studied under rigorous conditions that are very difficult to achieve in humans, no phase shifting effects of melatonin are apparent.<sup>32</sup> These observations suggest that melatonin action in the treatment of jet lag<sup>36,37</sup> may be related to hypnotic effects, rather than phase-shifting properties.

Although melatonin may not acutely shift circadian phase,<sup>32</sup> melatonin administration at the same time each day may entrain free-running circadian phase. In blind individuals, nocturnal melatonin administration has been shown to entrain activity patterns to the 24-hour day.<sup>20,37,38</sup>

## SUMMARY

Increasing evidence show that the circadian system exerts profound effect on human physiology. In parallel with increases in our understanding of the clinical importance of circadian biology, there has been an explosion in our understanding of the genetic

mechanisms that contribute to the workings of the circadian clock. Elucidation of abnormalities of the circadian system has also led to the discovery of new clinical disorders that can now be identified and treated.

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## Letter to the Editor

### Ghrelin-induced obesity

The July issue of *Growth, Genetics & Hormones* (Vol. 17, p 34-35) contains a discussion of the ability of this 28 amino acid peptide to induce body fat accumulation in rodents.

But of great importance to students of human obesity is the observation that the lean weight of these obese animals was probably less, certainly not greater, than that of the controls. This finding puts such ghrelin-treated animals clearly at odds with the human state, for the latter usually have an increase in lean weight, most certainly not a decrement.<sup>1</sup> The only clearly documented exceptions to this rule are patients with the Prader-Willi syndrome<sup>2,3</sup> or Cushing's syndrome. With respect to body composition the human state differs from obesity induced by experimental hypothalamic lesions, from that of the "ob/ob" mouse, and the Zucker rat, all of which are characterized by a subnormal lean weight. Obviously, such animals, and those treated with ghrelin, cannot serve as models for human obesity.

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Gilbert B. Forbes, MD

**Editor's Response:** Dr. Forbes in his talented analytical way has added significantly to the Abstract, Ghrelin: A Gastrointestinal and Hypothalamic Peptide Affecting Hormone Secretion and Fat Metabolism which dealt with studies in rats and not humans. With his astute commentary he reminds us that we should not necessarily project data obtained in rodents to humans. Neither of the Editors commenting on this article were so astute as to mention this most poignant point.

Thanks very much, Dr. Forbes. The Editorial Board eagerly invites each reader to write and comment on pertinent points, ask questions or query us concerning what is published in *Growth, Genetics & Hormones*.

Robert M. Blizzard, MD  
Editor-in-Chief

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## Growth Hormone Treatment Enhances Bone Mineralisation in Children with Chronic Renal Failure (CRF)

Van Dyck et al report on bone mineralisation as determined by Dual Energy X-ray Absorptiometry (DEXA), of the whole body and lumbar spine prior, to and one-year after, the initiation of rhGH therapy in 10 pre-pubertal children with stable CRF. Inclusion criteria for the study included: (1) a height SDS of  $< -2$  SD or a height velocity of  $< 25^{\text{th}}$  percentile for age, (2) absence of growth hormone deficiency, (3) normal thyroid function, and (4) normal PTH levels. DEXA was used to measure total body mineral content (TBMC), lumbar spine bone mineral content (LBMC), total body mineral density (TBMD), and lumbar spine bone mineral density (LBMD), in patients and in a control group of 20 healthy children of similar age. DEXA was performed twice in the CRF patients and in the healthy controls. Body height was measured with a stadiometer and bone age was determined by TW2 method at the start and after one-year of treatment. Data were analyzed using Wilcoxon matched pairs.

Growth hormone treatment (1 unit or 0.3 mg/kg/week given in daily divided doses) was associated with an increase in median height velocity from 5.1 cm/year (3.0-8.8 cm/year) to 10.6 cm/year (8.2-12.7 cm/year). Median creatinine clearance remained unchanged as did calcium, phosphorous, and intact PTH levels. There was, however, a marked change in serum alkaline phosphatase. This is a well-known phenomenon in different groups of patients treated with hGH and reflects osteoblastic activity. At the beginning of the study, the median bone age was delayed 1.9 years and increased 0.8 years over the duration of treatment. The patients' TBMC, TBMD, LBMC, and LBMD increased significantly after one-year of rhGH treatment ( $p < 0.05$  for each – see Table). When compared with height/age match controls, these values were not different at the start of treatment, nor at the end of treatment. Yet BMD, TBMD, and LBMD, significantly improved in patients over one year ( $P < 0.05$ ). When compared with age- matched controls, patients had lower TBMC and LBMC at the

start of treatment and experienced a catch-up of LBMC to values similar to controls over the course of the year.

The authors note that there has been discrepancy in results from previous studies of various parameters of BMD in children with CRF treated with rhGH. They speculate that this might be explained by 2 factors - small sample size and selection bias. In the current study, findings demonstrate significantly improved BMD in children with CRF who are growth retarded. All subjects in the current study were on calcium supplements and their bone mineralisation was adequate for their height at baseline. The authors state that homogeneity of their results is most likely due to the homogeneity of the patients studied, that is pre-pubertal with severe renal disease from early years of life without signs of osteodystrophy. They conclude that rhGH treatment has a beneficial effect on BMC and BMD in pre-pubertal children with CRF. This was the finding of Lanes et al (*Horm Res* 1996;46:263-268).

Van Dyck M, et al. *Eur J Pediatr* 2001;160:359-363.

**Editor's Comment:** At first glance, the results of this short paper might not be appreciated as adding significantly to the information with regard to the effects of rhGH on children with renal disease. It is well known that BMC and BMD prior to puberty are important factors of similar measures in adults. Thus, any improvement which might be gained in the pre-pubertal years, could potentially be realized later in adult life. Indeed, the subjects in the Van Dyck study had indices of bone density comparable to those of height matched children at entry into the study and at the one-year follow up. What is significant is the increased BMC and BMD observed. These studies underline the importance of initiating rhGH therapy in children with CRF even when their absolute height deficiency is modest.

William L. Clarke, MD

Table

Mineralisation parameter	Baseline	After 1 year rhGH	P
TBMC (g)	521 (144-944)	589 (225-1139)	$< 0.01$
TBMD (g/cm <sup>2</sup> )	0.750 (0.672-0.888)	0.775 (0.681-0.995)	$< 0.05$
LBMC (g)	7.5 (3.8-15.7)	10.9 (5.9-18.0)	0.005
LBMD (g/cm <sup>2</sup> )	0.475 (0.281-0.660)	0.525 (0.333-0.660)	$< 0.01$

Adapted from Van Dyck M, et al. *Eur J Pediatr* 2001;160:359-363.

## Adipose Tissue is an Endocrine Gland Secreting Multiple Hormones

*You Are What You Secrete* is a summary and editorial by Sattiel in which he discusses two articles concerning adiponectin.<sup>1</sup> Sattiel emphasizes that our notion of the adipocyte as merely a cargo space for fat has undergone a dramatic change. We now know that adipose tissue is much more complex than previously thought, secreting proteins which include tumor necrosis factor (TNF)- $\alpha$ , leptin, adipsin, resistin and adiponectin known also as Acrp30 or adipoQ. These proteins perform diverse functions but share structural properties of cytokines, and are referred to collectively as “*adipokines*”. Dynamic interactions occur between these proteins and dictate the extent to which insulin is sensed in its target tissues. In an article referred to by Sattiel, Berg et al<sup>2</sup> report that a single injection of adiponectin leads to a 2-3 fold elevation in its circulating levels, which precipitates a transient decrease in basal glucose levels. Similar treatment in ob/ob or streptozotocin - treated mice transiently abolishes hyperglycemia. This relative hypoglycemic effect is not associated with an increase in insulin levels. Moreover, in isolated hepatocytes adiponectin increases the ability of sub-physiological levels of insulin to suppress glucose production. Berg et al propose that adiponectin is a potent insulin enhancer linking adipose tissue and whole body glucose metabolism.

In the article by Yamauchi et al<sup>3</sup> the reversal by adiponectin of insulin resistance associated with both lipotrophy and obesity is described. Yamauchi et al discuss the findings that recent genome-wide scans have mapped a susceptibility locus for type 2 diabetes to chromosome 3q27, where the gene encoding adiponectin is located. This group demonstrated decreased expression of adiponectin and its correlation

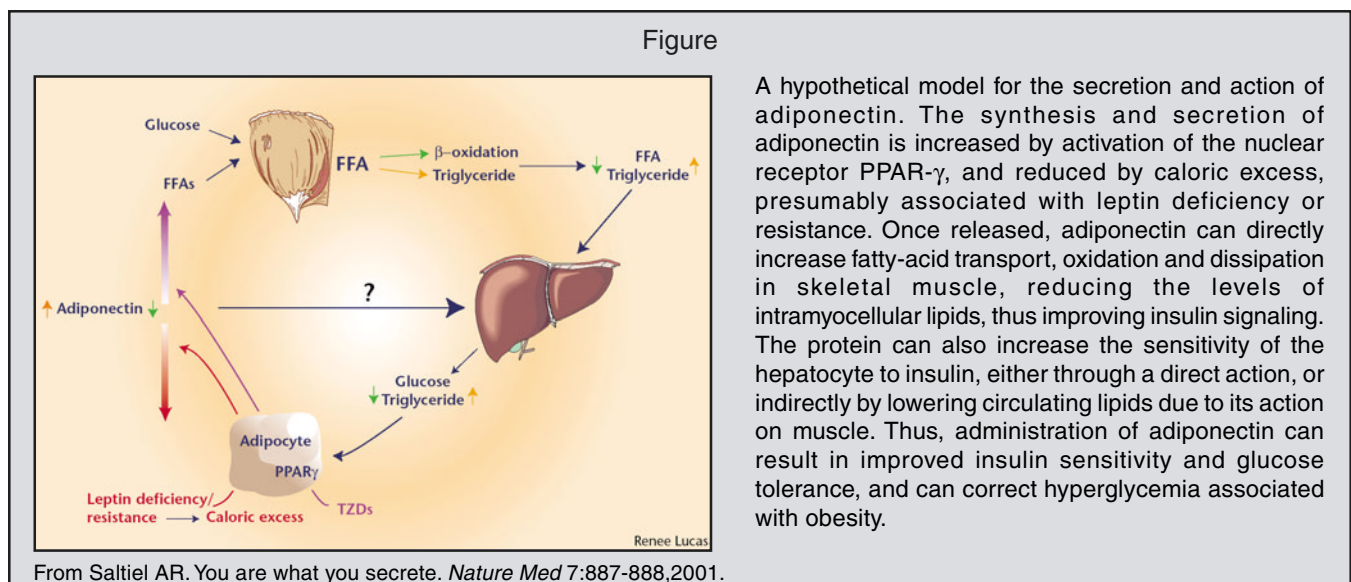
with insulin resistance in mice models of altered insulin sensitivity. Adiponectin decreases insulin resistance in obese mice by decreasing triglyceride content in muscle and liver. Insulin resistance in lipoatrophic mice was completely reversed by the combination of physiological doses of adiponectin and leptin, but only partially by either given alone. Yamauchi et al concluded that decreased adiponectin production is implicated in the development of insulin resistance in mouse models of both obesity and lipoatrophy. Their data also indicate that administration of adiponectin might provide a novel treatment modality for insulin resistance in type 2 diabetes.

### References

1. Sattiel AR. You are what you secrete. *Nature Med* 7:887-888,2001.
2. Berg AH, et al. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nature Med* 7:947-953,2001.
3. Yamauchi T, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nature Med* 7:941-946,2001.

**Editorial Comment:** *Adiponectin is a 247 amino acid protein whose expression in adipose tissue is depressed in obese animals. The plasma concentrations are low in these obese animals and also in obese humans, which is a pattern directly opposite to those of leptin, another adipocyte hormone. As discussed by Yamauchi et al, mice ingesting a high fat diet with increased fat accumulation had low tissue levels of adiponectin mRNA and low serum concentrations. Insulin resistance as reflected by hyperglycemia and hyperinsulinemia occurred.*

Figure



A hypothetical model for the secretion and action of adiponectin. The synthesis and secretion of adiponectin is increased by activation of the nuclear receptor PPAR- $\gamma$ , and reduced by caloric excess, presumably associated with leptin deficiency or resistance. Once released, adiponectin can directly increase fatty-acid transport, oxidation and dissipation in skeletal muscle, reducing the levels of intramyocellular lipids, thus improving insulin signaling. The protein can also increase the sensitivity of the hepatocyte to insulin, either through a direct action, or indirectly by lowering circulating lipids due to its action on muscle. Thus, administration of adiponectin can result in improved insulin sensitivity and glucose tolerance, and can correct hyperglycemia associated with obesity.

*Administration of rosiglitazone, an inhibitor of peroxisome proliferator-activated receptor- $\gamma$  which is an essential element for adipogenesis, increased adiponectin tissue mRNA values and also serum levels. Serum glucose was decreased as were serum levels of insulin.*

*In other mouse models of obesity (e.g. leptin receptor deficiency), administration of adiponectin lowered blood glucose and insulin values. In another mouse model, a lipodystrophic mouse without fat, serum concentrations of adiponectin were undetectable. Hyperglycemia and hyperinsulinemia were present. Administration of adiponectin lowered serum glucose and insulin levels. Both leptin and adiponectin were required in the lipotrophic mice to restore serum glucose and insulin values to normal.*

*In the article by Berg et al, serum glucose concentrations were decreased with the administration of recombinant adiponectin to wild type, leptin deficient, and insulin deficient mice. Berg et al also demonstrated that adiponectin depressed hepatic glucose output in vitro which is thus the second physiological effect that might contribute to enhanced insulin sensitivity. In*

*calorically restricted wild type mice, serum adiponectin concentrations were twice those of freely feeding animals suggesting that this adipokine may be important in prolonging the lives of such animals.*

*Thus, the data in these manuscripts indicate that adiponectin plays a key role in energy metabolism. It enhances insulin sensitivity by lowering serum and tissue triglyceride values, by uncoupling of oxidative phosphorylation in muscle, and by suppressing hepatic glucose output. In addition to the effects on energy metabolism, adiponectin depresses the inflammatory response that accompanies atherogenesis. Indeed, patients with coronary artery disease have lower plasma adiponectin concentrations than do controls. Adiponectin inhibits inflammation in part by suppressing proliferation of myelomonocytic progenitor cells by accelerating apoptosis. The potential utilization of adiponectin as a therapeutic agent for patients with obesity, diabetes mellitus types 1 and 2, hyperlipidemia, and/or atherogenic disorders is clearly enormous. A lead article regarding Adipose Tissue as an Endocrine Gland will appear soon in GGH.*

Allen Root, MD

## Genetic Basis of Stature – Genome-Wide Search for Genes that Influence Normal Adult Height

It is well known that short parents have short children and vice versa, and that variation in normal stature has a strong genetic component. However, despite many decades of interest in the genetics of stature, the relevant genes remain elusive. In fact, the genetics of most common traits and diseases in humans is not well understood. The principal explanation is that the geneticist's primary tool for mapping genes is of only limited power for finding genes that have modest effects, such as those that contribute to common diseases and variable traits such as stature. Recent advances in genomics, however, have made it feasible to apply genome-wide linkage analysis to such entities. Indeed, the group led by Eric Lander has used this approach to identify genetic linkage for adult height.<sup>1</sup>

In total, 2,327 individuals from 483 families were studied. Fifty-eight families resided in the Botnia region of Finland, 183 families were from other areas of Finland, 179 families were from southern Sweden and 63 families were from the Saguenay-Lac-St-Jean region of Quebec. They were originally ascertained to investigate other genetic traits. Males were older than 23.5 years and females older than 21.1 years to exclude individuals still growing. The original genotyping results that were based on average spacing of microsatellite markers from 6.5 cM to 12.5 cM depending on the study population, were reanalyzed using the variance-components method

implemented in the GENE-HUNTER 2 protocol. The method uses nonparametric multipoint approaches to generate LOD scores for chromosomal locations that reflect the likelihood that genotype data being observed is due to linkage relative to the absence of linkage.

Evidence for linkage was detected in four instances. A LOD score of 3.85 was obtained for linkage at chromosome 6q24-25 in Botnia. A score of 3.40 was calculated for a marker located at 7q31.3-36 in Sweden. A LOD score of 3.35 was determined for markers at 12p11.2-q14 in Finland and a score of 3.56 was found in Finland for 13q32-33. The authors note that a companion study also detected linkage at chromosome 7 site.<sup>2</sup>

The authors are optimistic that they have identified chromosomal regions where genes that influence stature reside, especially on chromosome 7. However, they caution that definitive interpretation is difficult in the absence of confirmation of linkage in additional populations. They observe their results were inconsistent across the four study groups, but note that this is typical in linkage studies of common diseases. They discuss possible reasons for the inconsistency including variation in sampling, existence of genetic variation in different populations and statistical fluctuations and false positives due to unknown causes.

## References

1. Hirschhorn NJ et al. Genomewide linkage analysis of stature in multiple populations reveals several regions with evidence of linkage to adult height. *Am J Hum Genet* 69:106-110, 2001.
2. Perola M et al. Quantitative-trait-locus analysis of body-mass index and of stature, by combined analysis of genome scans of five Finnish study groups. *Am J Hum Genet* 69:117-123, 2001.

**Editor's Comment:** An additional comment is pertinent to this topic. Many genes known to influence stature have been identified by searching for disease genes. Examples include genes that harbor mutations that cause chondrodysplasias and many other syndromes associated with short stature. They range from homeobox-containing genes such as *SHOX* to cartilage matrix protein genes, i.e., *COL2A1* to transcription factor and receptor genes such as *SOX9* and *FGFR3*, respectively. Similarly, mutations of *Fibrillin 1* lead to tall stature in the Marfan syndrome. It seems likely that

there are genes that influence stature that are not associated with disease. The approach used here should identify genes in both categories. It will be interesting to see what genes fall into the latter category.

These papers are the first reported genome-wide studies of genetic linkage and stature. They probably represent the tip of the iceberg in terms of what will come as genetic markers become more dense, more populations are studied and analytical approaches become more sophisticated. As noted by Hirschhorn et al, identifying the genetic basis of variation in height raises important ethical issues as the potential for genetic engineering evolves. However, as they point out, a greater understanding of this subject could be beneficial in the contexts of establishing diagnoses and predicting adult stature of "short" children.

William Horton, MD

## Short Stature Homeobox-Containing Gene Deletion: Screening by Fluorescence in Situ Hybridisation in Patients with Short Stature

In an attempt to determine when to screen for *SHOX* gene deletion in subjects with short stature, Müsebeck and colleagues determined the frequency of *SHOX* deletions in 50 children with short stature. All children studied had a height < -2 SDS and 3 of the subjects also had the Madelung deformity (shortening and bowing of the radius with dorsal subluxation of the distal ulna and partial foreleg anomalies). Thirty-five of the 50 subjects had idiopathic short stature (ISS) accompanied by the absence of skeletal, endocrine, or organic symptoms and had no family history of short stature. Twelve subjects had upper limb abnormalities such as cubitus valgus. Two subjects had Léri-Weill dyschondrosteosis, and 3 had a congenital heart defect. Blood was analyzed by FISH process (Fluorescence In Situ Hybridization) for the *SHOX* deletion.

Microdeletions of the *SHOX* gene were not detected in any of the 35 patients with ISS. Of the 12 patients with additional upper limb abnormalities 5 (41.7%) displayed *SHOX* signals on only one sex chromosome. Of the 7 with short stature who displayed *SHOX* signals on 2 sex chromosomes, 3 had Madelung deformity and brachymetacarpia was present in the other 4. Point mutations of course are not picked up in the FISH technique. Molecular genetic methods will possibly detect point mutations in patients such as the 7 referred to above. Three patients with congenital heart defects did not carry *SHOX* deletions.

The authors state that their findings provide important guidelines for selecting patients for *SHOX* analysis. They

state that children with ISS are unlikely to carry such a mutation of the *SHOX* gene. Indeed, other studies have shown the *SHOX* mutation in about 1% of all patients with ISS. The combination of short stature and skeletal abnormalities of the forearm, however, makes the *SHOX* mutation much more probable. The authors caution that a father carrying a *SHOX* mutation on the X chromosome could transmit these mutations to his son because of crossing over between the pseudoautosomal regions of the X and Y chromosomes during paternal meiosis.

Müsebeck J, et al. *Eur J Pediatr* 2001;160:561-565.

**Editor's Comment:** *SHOX* gene deletion determinations have become increasingly popular in endocrine/genetic clinics evaluating children with short stature. Although, the number of subjects studied by Müsebeck et al is relatively small (n=50), their data are convincing. Apparently, *SHOX* gene determinations have little place in the evaluation of the child with ISS and should be reserved for those children who have deformities of the upper extremities even when those are very mild. Hopefully, data can be pooled in the future from numerous centers so that definitive guidelines for evaluation of *SHOX* gene determinations are more clearly defined.

William L. Clarke, MD

## Growth Hormone in Short Children: Beyond Medicine?

The increasing use of rhGH in short children with non-GH deficient (GHD) short stature, whether or not data support the efficacy of such treatment, may lead to its use being perceived as a cosmetic “enhancement”. Drs. Bolt and Mul discuss the merits of the use of rhGH in such children and whether such treatment is “in the medical realm”. Employing a disease-oriented model, rhGH would be administered only to patients with documented GHD or identified abnormal state (e.g., Turner syndrome) to restore health and normal functioning. The authors reject this approach because the differences between normal and abnormal growth and function are often indistinct. On the other hand, they also reject the “client approach” to prescribing of rhGH in which one would administer it “on demand” for any and all types of short stature including familial and idiopathic, because this approach might lead to “medicalization” of many perceived and apparent differences between individuals and make patients of otherwise healthy persons. Bolt and Mul believe the proper goal of medicine is to prevent or relieve suffering, both demonstrable and subjective, and advocate this approach to deciding when the administration of rhGH is or is not warranted. Suffering, while perhaps not always quantifiable, can be perceived by the family and physician. Thus, children with non-GHD short stature may be eligible for treatment with rhGH if s/he demonstrates present suffering or the potential for future suffering. They conclude that because the impact of short stature upon the functional status of normal adults is minor, treatment with rhGH “should take place in a research setting”.

Bolt LLE and Mul D. *Acta Paediatr* 90:69-73,2001.

**Editor’s Comment:** *The suffering individual is anguished, tortured, bitter and sad. However, it may not always be easy to identify the suffering short child.*

*Firstly, the majority of short, otherwise normal children are brought to the office of the pediatric endocrinologist by their parents who are often more concerned about the height of their child than is the child himself. Thus, it is likely that it is the parent who is “suffering” rather than the child. Drs. Bolt and Mul do not address the issue of whether rhGH should be administered to a short child to alleviate parental suffering. Secondly, suffering related to short stature is seldom due exclusively to height, but reflects a constellation of behavioral, learning and social problems. As Macklin<sup>1</sup> points out in a companion commentary, the discomfort of the short-statured child may pale when compared to the physical suffering imposed by the numerous medical procedures that accompany treatment with, and the administration of rhGH. Although the “goal of medicine” involves all of the interrelated components delineated by the authors - disease-oriented, client-related, relief of suffering - this reviewer adheres to the precept that medicine is primarily a science and that medical decision making should be based upon valid scientific data. To date, there are limited and conflicting data relative to the growth promoting efficacy of rhGH therapy of the non-GHD short child and even fewer data concerning any psychosocial benefits of treatment.<sup>2</sup> Thus, I concur with the recommendation of Drs. Bolt and Mul that such treatment be undertaken in the context of a research environment.*

### References

1. Macklin R. Growth hormone in short children: medically appropriate treatment. *Acta Paediatr* 90:5-6,2001.
2. Guyda HJ. Four decades of growth hormone therapy for short children: what have we achieved? *J Clin Endocrinol Metab* 84:4307-4316,1999.

Allen Root, MD

## Extended Life-Span Conferred by Cotransporter Gene Mutations in *Drosophila*

These investigators demonstrate that in the adult fruit fly, *Drosophila melanogaster*, heterozygous inactivating mutations in a newly identified gene *Indy* (for *I’m not dead yet* from the film “Monty Python and the Holy Grail”) double the active, fertile, and fecund life span of this insect. *Indy* encodes a 572 amino acid sodium dicarboxylate cotransporter, a membrane protein that shepherds the uptake and re-uptake of di- and tricarboxylic acid intermediate metabolites (e.g., succinate, citrate) of the Krebs cycle across cell membranes of organs responsible for metabolism and storage of fat, glycogen, and protein (e.g., the liver in

mammals). The investigators suggest that heterozygous loss-of-function mutations in *Indy* decrease the rate of absorption and utilization of metabolites, thus acting functionally to extend life span in a manner similar to that of partial caloric restriction.

Rogina B, et al. *Science* 290:2137-2140, 2000.

**Editor’s Comment:** *Energy restriction has been demonstrated to extend life span in worms, mammals, and insects, but the mechanism(s) by which decreased calories does (do) so have not been identified. It may*

be that caloric restriction down regulates the expression of sodium dicarboxylate cotransporter(s) genes thus decreasing the rate of intracellular metabolism and consequently increasing cellular life. These observations suggest that perhaps some obese subjects possibly have gain-of-function mutations in one or another sodium dicarboxylate cotransporter that enhance intracellular intermediary metabolism leading to accumulation of fat, while other individuals (who can

“eat a tone and never gain an ounce”) may have a variant that impedes metabolism. The data also suggest that it may be possible to modify the activity of these cotransporter molecules chemically - opening a portal for treatment of a group of obese subjects.

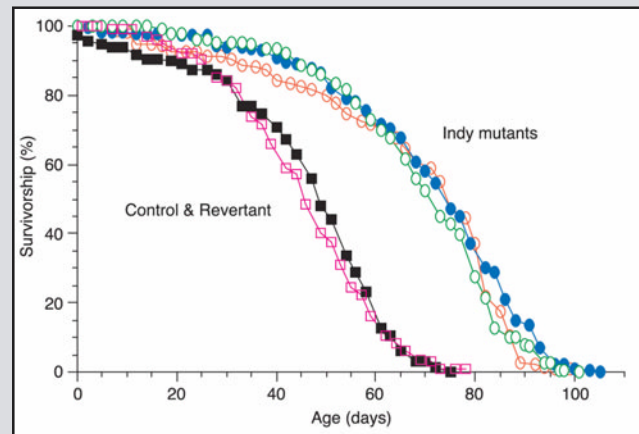
Pennisi E. Old files may hold secrets of aging. *Science* 290:2048, 2000.

Allen Root, MD

Figure

Life-span extension in *Indy* mutants. Survival curves of males heterozygous for three different *Indy* mutations, a precise excision of the P -element from *Indy* 302 (revertant), and an enhancer-trap control are shown. All flies were tested as heterozygotes over a wild-type Canton-S strain. The *Indy* mutants are *Indy*302 (open white circles), *Indy*206 (solid gray circles), and *Indy*159 (strikethrough circles). The excision line (strikethrough squares) is one of four exact excisions (sequence confirmed) of the P element obtained by mobilizing the P element from either the *Indy*302 or *Indy*206 line, using delta 2-3 transposase. The control (solid black squares) is one of four other enhancertrap control lines from the same mutagenesis that generated *Indy*302 and *Indy*206, tested as a heterozygote over Canton-S.

From Rogina B, et al. *Science* 290:2137-2140, 2000.



## Insulin Resistance and Insulin-Like Growth Factors in Children with Intrauterine Growth Retardation

The authors recently proposed that when tissues in utero are chronically depleted of insulin and IGF1, but subsequently exposed after normalization of nutrient supply in postnatal life to increased levels, insulin resistance often develops. Carrying this thesis forward, they postulate that postnatal “catch-up” growth might, therefore, be associated with a higher risk of developing insulin resistance, especially when other risk factors such as genetic predisposition and/or obesity coexist.

To investigate this possibility, 49 children with IUGR (22 boys) with birth weight <10th percentile for gestational age were studied. Children with malformations and/or genetic disorders were excluded. Stature was corrected for mid-parental height. Children were divided into two groups according to their corrected height; specifically, those with corrected height z-score  $\geq 0$  and those  $< 0$ . Insulin resistance was evaluated using OGTT, fasting glucose and insulin levels, and a G/I  $< 6$  to interpret insulin resistance. Thirty-nine percent (19/49) of the children with IUGR had a corrected stature  $> 0$  z-score and 61% had not reached their genetic height, as expressed as MPH z-score. Corrected stature at the age evaluated correlated with birth weight, whereas actual height was related to birth length, MPH

and BMI. Twenty-two percent or 11 of 49 IUGR children had a G/I  $< 6$ . The endocrine variables in children as divided on the basis of G/I  $< 6$  and  $> 6$  are provided in Table 1. All the parameters related to insulin resistance correlated with alanine aminotransferase (ALT) and gamma glutamyltransferase ( $\gamma$ -GT) levels. IGF system parameters were in the normal range and correlated neither with growth nor with insulin sensitivity.

The first aim of the study was to assess the prevalence of insulin resistance in children and adolescents with IUGR. The authors considered that insulin resistance was at a high prevalence since 22% of the children were so classified, and these data are consistent with previous studies reporting impairment in insulin sensitivity in children with IUGR. The second objective was to prove the *catch up growth hypothesis* that catch up growth induces insulin sensitivity. The data in this study suggest that catch up growth is not a risk factor. They further comment that the finding of high prevalence of insulin resistance did not show a significant influence over postnatal growth - is consistent with the intrauterine reprogramming previously postulated by the authors and is consistent with a genetic predispositioning determining both low birth weight and

insulin resistance. The authors also postulate that obesity may be an additional risk factor during childhood. One of the most important findings was the close relationship observed between insulin resistant parameters and liver function tests; this suggests that the liver might be a target organ of the reprogramming process. The authors did not find any indications that the IGF systems (IGF1, IGF1BP-3, etc) are related to the insulin sensitivity status, at least during childhood. The latter data are in accord with those of at least two other authors.

Cianfarani S, et al. *Horm Res* 2001;55(suppl 1):7-10.

**Editorial Comment:** The authors have provided excellent data on a large number of small for gestational age infants. I have not used the term *intrauterine growth retarded children* as in the title of the article, as I believe

that term should be reserved for children who are <3rd percentile. I remain skeptical that one out of every 10 children is *intrauterine growth retarded*, which would be the case if one uses the 10th percentile as cutoff. The article as presented does not indicate to me what percentages of the children born <3rd percentile had insulin resistance. The authors and others are invited to comment to the Editor concerning which criteria are appropriate to use for determination of metabolic alterations in IUGR children, as much confusion now exists among data stated to be that of IUGR.

Regardless of what I consider this limitation, the data are worthwhile and provide interpretations to postulated metabolic alterations in children who are small for gestational age.

Robert M. Blizzard, MD

	G/I <6(n = 11)	G/I >6(n = 38)	p	
Age, years	10.3 ± 3.6	8.9 ± 3.3	n.s.	ALT = alanine aminotransferase
Birth weight, kg	2.16 ± 0.35	2.18 ± 0.38	n.s.	AST = aspartate aminotransferase
Birth length, cm	45.4 ± 2.8	45.8 ± 2.8	n.s.	AUC <sub>ins</sub> = area under the curve of insulin during oral glucose tolerance test
Ponderal index, g/cm <sup>3</sup>	0.002 ± 0.002	0.022 ± 0.004	n.s.	BMI = body mass index
BMI, kg/m <sup>2</sup>	18.5 ± 4.0	16.2 ± 3.9	n.s.	HOMA-β-cell = homeostasis model assessment β-cell function
BMI, z-score	1.0 ± 2.6	-0.29 ± 1.8	n.s.	HOMA-IR = HOMA for insulin resistance
Height, z-score	-1.08 ± 1.29	-1.23 ± 1.3	n.s.	IGF = insuline-like growth factor
MPH, z-score	-1.4 ± 0.6	-0.8 ± 0.9	<0.05	IGFBP = IGF binding protein
Corrected stature, z-score	0.36 ± 1.1	-0.36 ± 1.3	n.s.	IUGR = intrauterine growth retardation
Fasting insulin, mU/l	12.4 ± 9.0	8.2 ± 3.3	n.s.	MPH = midparental height
HOMA-IR	3.5 ± 1.0	1.5 ± 0.8	<0.0001	n.s. = not significant.
HOMA-β-CELL	180 ± 139	43 ± 90	<0.01	
AUC <sub>ins</sub> , mU/l	240 ± 113	164 ± 115	n.s.	
Proinsulin, pM	9.6 ± 11.2	5.0 ± 4.4	n.s.	
IGFBP-1, µg/l	83 ± 59	119 ± 50	<0.05	
IGF-I, z-score	0.41 ± 2.8	0.47 ± 3.0	n.s.	
IGF-II, z-score	0.56 ± 0.7	0.62 ± 0.9	n.s.	
IGFBP-3, z-score	0.35 ± 0.7	0.23 ± 1.3	n.s.	
IGF-I/IGFBP3 ratio	61.2 ± 23	63.5 ± 35	n.s.	
AST, U/l	29.1 ± 8.0	27.4 ± 6.7	n.s.	
ALT, U/l	27.4 ± 17.1	16.3 ± 7.2	n.s.	
γ-GT, U/l	15.7 ± 6.6	11.7 ± 3.8	n.s.	

Adapted from Cianfarani S, et al. *Horm Res* 2001;55 (suppl 1):7-10.

## The Molecular Basis of X-Linked Spondyloepiphyseal Dysplasia Tarda

The gene for X-linked form of spondyloepiphyseal dysplasia tarda has been identified as SEDT, a protein that apparently plays a role in endoplasmic reticulum-to-Golgi transport and involves subcellular localization of normal sedlin constructs. The protein is relatively small with 140 amino acids. It is located in the non-X-inactivated part of Xp22. This suggests that female

carriers express sufficient normal gene to avoid the disease.

The present study looked at 36 unrelated cases and attempted to make phenotype/genotype correlations. Mutations could be found in 30 individuals. The 6 individuals in which mutations were not found either lacked a strong family history or convincing physical

features, and therefore, may represent other diseases. Twenty-one different gene mutations were observed among the 30 cases, and in those cases with several identical mutations, hupetype analysis suggests that they arose separately and, therefore, do not represent a founder effect.

Intrafamilial variation was certainly observed; however, mutations occurring toward the five<sup>1</sup> end of the SEDL gene (mutations in Exons 3 and 4) resulted in kyphosis and scoliosis with severe pain early in life and with more debilitating types of complications. This was observed while mutations in Exons 5 and 6 resulted in milder clinical features.

Mutations were spread throughout the gene, including point mutations, splice alterations, insertions, deletions, and complex rearrangements. The most common type of mutation was a deletion. There was a 10 fold greater occurrence of deletions than would be expected. This may represent slippage during homologous recombination between the Y and X chromosome.

The SEDL phenotype may be explained by reduction in endochondral bone formation in the epiphysis, particularly in the vertebral bodies. A timely switch to up regulate the endogenous expression of a pseudo gene on chromosome 19 might provide gene therapy. The authors are undertaking a study of SEDL mutations in premature osteoarthritis.

Gedeon, AK, et al. *Am J Hum Genet.* 2001;68:1386-1397.

**Editor's Comment:** *When genes are identified for the chondrodysplasias, the possibility of making phenotype/genotype correlations and understanding the basic molecular biology are very enticing. This paper is a lovely demonstration of how a great deal can be learned in rare disorders by large international collaborations. This work hopefully will lead both to a better understanding of disease and to potential therapies.*

Judith G. Hall, OC, MD

## Postnatal Malnutrition and Growth Retardation: An Inevitable Consequence of Current Recommendations in Preterm Infants?

Intake of adequate nutrients in preterm infants is difficult at best, and most often does not accomplish meeting the recommended dietary intakes (RDI). A nutrient deficit therefore accrues, leading to postnatal malnutrition and growth retardation. This study assesses the dietary intake in a prospective single observer design in 105 preterm infants with a body weight of < 1750 grams and a gestational age of < 34 weeks who were admitted to the Neonatal Intensive Care Unit over a 6 month period. Actual intake was subtracted from the recommended energy intake (120 kcal/kg/day) and protein (3 g/kg/day), and nutritional deficits were calculated. Infants were weighed on admission and throughout the hospital stay.

Nutrient intakes meeting current RDI's were rarely achieved during early life. By the end of the first week, cumulative energy and protein deficits were 406 +/- 92 and 335 +/- 86 kcal/kg and 14 +/- 3 and 12 +/- 4 g/kg in infants < 30 and those at > 31 weeks, respectively. By the end of the fifth week, cumulative energy and protein deficits were 813 +/- 542 and 382 +/- 263 kcal/kg and 23 +/- 12 and 13 +/- 15 g/kg. The z scores were -1.14 +/- .6 and -.82 +/- .5 for infants at < 30 and > 31 weeks. Stepwise regression analysis indicated that variation in dietary intake accounted for 45% of the variation in changes in z-score. The authors concluded that preterm infants inevitably accumulate a significant nutrient deficit in the first few weeks of life.

**Editor's Comments:** *This study clearly demonstrated that there is an accumulated nutrient deficit in preterm infants in an NICU setup. It also clearly suggests that the nutritional approach to the care of these infants needs to be re-thought, perhaps with a more aggressive approach, i.e. enteral or parenteral feedings. However, even early parenteral or enteral supplementation might be limited as these infants might not be able to tolerate it. A more aggressive enteral feeding is also hard to attain in the first few days of life, and it could lead to necrotizing enterocolitis or other adverse effects. The long-term consequences of this accumulated nutrient deficit may be important. It is generally assumed that poor growth in the preterm low birth weight infants primarily reflects inadequate nutrient intake, and in this study there was a 45% variation in growth related to such. Nonetheless, despite poor growth during the initial stages of life, most premature infants grow well thereafter and attain a normal height, unless there are other complications. Once the infant matures, the nutrient deficits are recouped and there is nutritional recovery with catch-up growth. However it should be kept in mind that nutrient deficits in early infancy might have other devastating consequences. The data from this study suggest that the clinician is in a quandary and that a more realistic picture regarding the quantity and quality of nutritional care in low birth weight infants needs to be re-thought.*

Embleton NE, et al. *Pediatrics* 107:270-272, 2001.

Fima Lifshitz, MD

## The Land Between Mendelian and Multifactorial Inheritance

Burghes et al discuss the concept that genetic disorders can often be thought of as attributable to Mendelian and/or multifactorial triats. However, we now must consider other possibilities in classifying certain genetic syndromes. One such category has been classified as *triallelic inheritance*. The Bardet-Biedl syndrome, as published by Katsanis et al, is tagged as such. This article prompts a perspective commentary on genetics by Burghes et al.<sup>1</sup>

Although there has been spectacular success in identifying genes responsible for Mendelian inherited disorders, finding *susceptibility* genes involved in multifactorial diseases has been a struggle. How multiple genes interact to give the final phenotype of a multifactorial disease and what we might expect, remains an enigma. The land between Mendelian and multifactorial inheritance is inhabited by genes such as *modifier genes* and *redundant genes* that have many effects on the developing phenotype. Understanding the mode of action of these will help in determining how *susceptibility genes* may interact to give rise to a multifactorial phenomena.

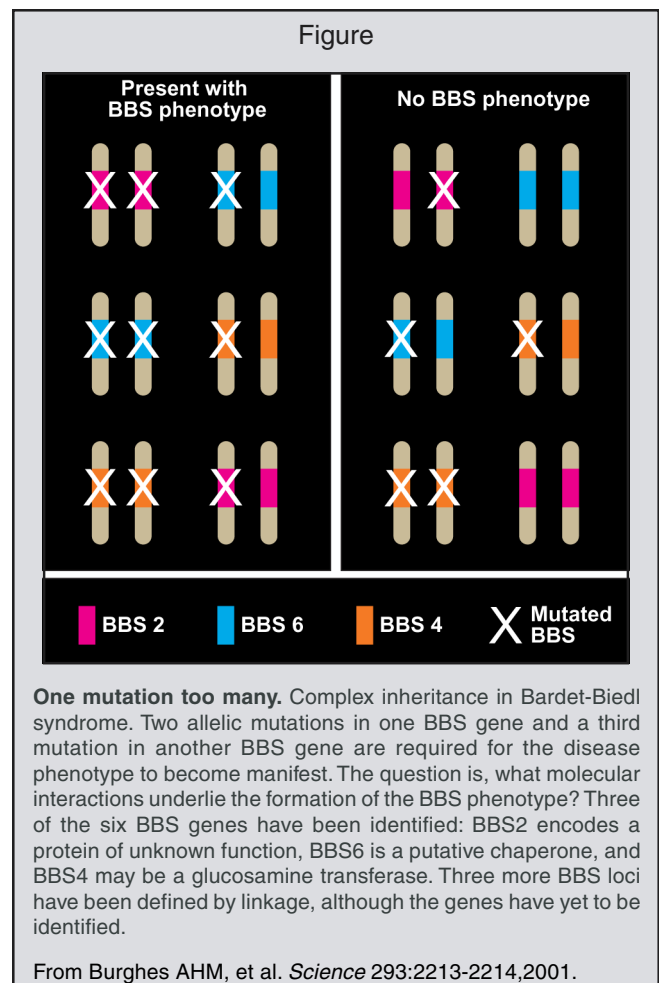
Katsanis et al<sup>2</sup> report that mutations in two genes, rather than one, cause Bardet-Biedl syndrome (BBS). Katsanis points out that six BBS loci exist in humans. Three of these have been identified (BBS2, 4, and 6); the other three have not, as yet. Mutated genes have been identified in BBS2, BBS4, and BBS6 genes. Katsanis et al describe 11 subjects, out of a group of 163, who were genetically characterized with heterozygous or compound heterozygous mutations in BBS2, and three families with normal individuals who had the same two mutated BBS2 alleles. In three pedigrees the affected BBS patient had mutations of both BBS2 alleles and a mutation in one BBS6 allele. In one family the affected BBS patient had a mutation of one BBS2 allele and mutations in two BBS6 alleles. Thus, in four families mutations in three BBS alleles were demonstrated and apparently necessary for expression of the disease phenotype. Katsanis proposed that BBS may not be a single gene recessive disease, but a complex trait requiring three mutant alleles to manifest the phenotype. The phenotype of BBS includes pigmentary retinopathy, polydactyly, obesity, developmental delay, and renal defects. The figure illustrates the complex inheritance in Bardet-Biedl syndrome.

### References

1. Burghes AHM, et al. *Science* 293:2213-2214,2001.
2. Katsanis N, et al. Triallelic inheritance in Bardet-Biedl syndrome, a Mendelian recessive disorder. *Science* 293:2256-2259,2001.

**Editor's Comment:** The concept that mutations of genes on more than two alleles may be necessary for expression of a disorder is at odds with classical Mendelian transmission through dominant or recessive mechanisms, but is not incompatible with our understanding of diseases that appear to require multiple genetic and/or environmental factors for expression (e.g., diabetes mellitus, obesity, spinal muscular atrophy). Inasmuch as the majority of patients with BBS and mutations in BBS2 had normal BBS6, it is likely that these investigators will search for mutations in BBS4 (and BBS1 and 3 when they are identified) in this large group of BBS subjects. Since the phenotype of BBS is consistent despite the genotype, one suspects that the various BBS loci identified will be linked to one another in a metabolic process(es) that when interrupted leads to the disorder. Incidentally BBS6 is also mutated in patients with the McKusick-Kaufman syndrome of congenital heart disease, polydactyly, and transverse vaginal septum leading to hydrometrocolpos in females.

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