

## GROWTH HORMONE THERAPY IN CHRONIC KIDNEY DISEASE

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### **INTRODUCTION**

In the last few years, there has been a shift in emphasis on the medical management of children with chronic kidney disease (CKD) from strategic attempts to preserve renal survival to optimizing global biological potential, and thereby maximizing quality of life. Early diagnosis and prompt treatment have become the cornerstones of modern care. Thus, in addition to measures like anemia

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#### **From The Editor's Desk**

This issue contains 9 printed and 8 e-abstracts of important papers in the field with editorial comments. Thus, we have doubled the content of *GGH* with the introduction of e-abstracts. This feature also allowed the publication of longer abstracts, data, and comments. Please take advantage of this added feature. I welcome your comments regarding the on-line and print aspects of the journal. Your feedback is important as we continue to grow and strive to serve your needs. The lead article by Drs. Bamgbola and Kaskel on Growth Hormone Therapy in Chronic Kidney Disease is a very comprehensive review of the pathophysiology of the disease as it pertains to growth hormone. It includes provocative ideas about possible future direction for treatment with growth hormone and insulin-like growth factor. I am sure you will enjoy it and save it as a reference source.

The expanded journal and all of its content is carefully prepared by the editorial board and all the lead articles are reviewed to comply with the high scientific standards of a peer review journal. *GGH* is also in compliance with the code of conduct for medical publishers on the internet (Health on the Net Foundation [www.hon.ch](http://www.hon.ch)).

Respectfully,  
Fima Lifshitz, MD

control and improved nutritional intake, there is increasing use of recombinant human growth hormone (rhGH).

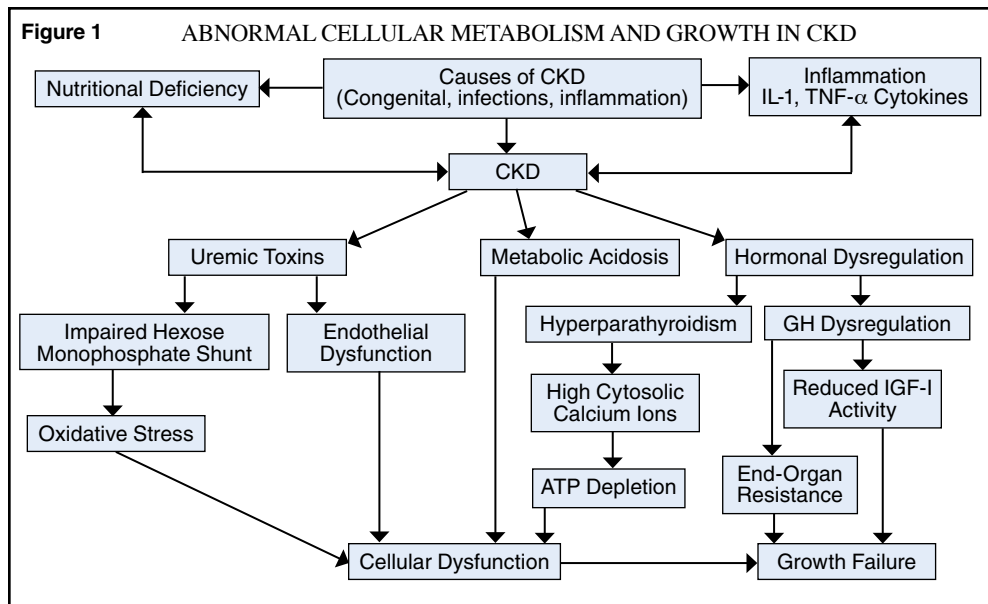
Although the FDA-approved indication for use of rhGH in CKD is growth failure, there are other clinically significant metabolic effects of the hormone. In this review, we shall highlight the potential benefits of rhGH therapy in CKD, including its positive impact on cellular growth and metabolism, immune regulation, and energy homeostasis. The roles of rhGH in modulation of psychosocial function, sleep physiology, and bone metabolism in children with CKD will also be discussed.

#### **GROWTH FAILURE**

More than 50% of adults with childhood-onset CKD attain final heights that are below the third percentile.<sup>1</sup> The burden of growth retardation in patients with renal disease is enormous, resulting not only in physical handicaps but also the potential for psychological and social distress.

CKD, whether caused by congenital anomalies, chronic infection, immune disorders, or connective tissue diseases, may be associated with nutritional deficiency and

growth retardation (Figure 1). Conversely, consequences of renal disease such as metabolic acidosis, endocrinopathy, chronic anemia, persistent micro-inflammation, recurrent infection, and cardiac dysfunction may also result in growth failure. Inadequate dietary intake (often less than 80% of RDA) and defective protein metabolism are common features of CKD. However, increased food intake does not necessarily translate into a healthy nutritional outcome, and it often leads to greater adiposity rather than musculo-skeletal growth.



Furthermore, metabolic acidosis, which is a common outcome of CKD, accelerates protein degradation by activation of the ubiquitin-proteasome pathway, stimulation of branched-chain keto-acid-dehydrogenase, and promotion of end-organ resistance to anabolic effects of GH.<sup>2</sup> In addition, steroid therapy, often used as an anti-inflammatory agent in some kidney diseases, or for immune suppression following renal transplantation, may not only impair GH release but also increase end-organ resistance. In this regard, there is a positive correlation between the cumulative dose of steroids and adult-height deficit in pediatric allograft recipients. Treatment with steroids may inhibit GH synthesis by stimulation of (hypothalamic) somatostatin production. Consequently, by acting on multiple receptor-sites of the pituitary gland, GH-releasing peptide-2 (a GH secretagogue) has the therapeutic potential of bypassing the inhibitory effect of somatostatin.<sup>3</sup> Similarly, the use of rhGH alone or in combination with insulin-like growth factor (IGF)-I promotes musculo-skeletal growth, essentially by attenuating the inhibitory effect of steroids on protein synthesis.<sup>4</sup>

Whereas somatic growth at an early age is predominantly determined by factors such as birth size and adequate nutritional status, functional availability of GH is essential during childhood, and gonadotropin is a necessary adjunct for post-pubertal maturation.<sup>1</sup> Consequently, provisions of an optimal metabolic and nutritional milieu are often sufficient for growth in children with CKD who are less than 2 years of age, while use of rhGH is commonly required in older children.

## GH/IGF AXIS

Although the pulsatile release of GH is blunted in uremia, the total amount of GH secretion from the pituitary gland is often increased.<sup>5</sup> IGF-I and -II are derived from both hepatic cells and local tissues (of target organs) in response to a

primary activation of the GH receptor (GHR).<sup>6,7</sup> Despite the higher plasma level of circulating GH,<sup>8</sup> there is less synthesis of IGF-I due to end-organ resistance.<sup>9</sup>

Factors that contribute to GH tissue resistance in CKD include hyperparathyroidism, metabolic acidosis, and pro-inflammatory cytokines.<sup>9-12</sup> The mechanism of the end-organ resistance is inhibition of calcium-mediated intracellular signaling and impaired transcription of GHR-mRNA. Thus, GH activation of growth plates in uremic animals results in reduced local synthesis of IGF-I, impaired chondrocyte replication, and therefore retarded skeletal growth.<sup>13</sup>

The physiologic functions of GH are mediated by 2 different but complementary mechanisms: GH directly activates target organs while its indirect effects are mediated through IGF-I.<sup>7</sup> While GH increases the hepatic production rate of glucose and glycerol (an index of lipolysis), IGF-I acts in concert with insulin to increase peripheral glucose uptake and to reduce protein breakdown.<sup>14</sup>

IGF-I is a small, single-chain peptide belonging to the same family of genes as IGF-II and pro-insulin,<sup>15</sup> and its free bioactive form accounts for 1% of total plasma concentration.<sup>7,16</sup> IGF-I has a very short half-life (20 minutes), rapidly losing its metabolic function in the absence of a carrier binding-protein (IGFBP).<sup>6,7</sup> The most abundant of the 6 IGF-binding proteins (IGFBP-1 to -6) is IGFBP-3; it binds to circulating IGF-I and acid labile-sub-unit (ALS) as a 150 kDa ternary complex, thereby protecting it from premature degradation.<sup>7,16</sup>

IGF-I receptors are heterotetramers comprised of 2 alpha and 2 beta sub-units attached by disulfide bridges. IGF-I ligand binds to the extracellular alpha sub-unit which in turn induces the transmembrane beta unit,

resulting in an autoactivation of tyrosine kinase and phosphorylation of an intracellular tyrosine residue.<sup>15</sup> Interaction between insulin receptor substrates (IRS-1 and -2) and the receptor-tyrosine residue evokes a signal transduction thereby activating the downstream MAP-3 kinase (and protein kinase-B) pathways.<sup>15</sup> The 2 pathways mediate protein synthesis, cellular growth, cell motility, and inhibition of apoptosis.

IGFBP-3, by sharing a similar molecular structure, competitively inhibits IGF-I receptors.<sup>15</sup> However, the receptor molecules have stronger affinity for the IGF-I ligand. Consequently, there is a regulated but slow release of the plasma IGF-I from its carrier proteins at the designated target tissue. In uremic plasma, IGFBP-3 peptides are more rapidly degraded into smaller fragments. The smaller molecules of IGFBP-3 have less avidity for IGF-I and are often poorly excreted by the diseased kidneys. The reduced renal clearance of the relatively inefficient IGFBP-3 fragments and retention of inhibitory binding proteins, including IGFBP-1, -2, -4, and -6, substantially reduce the bioavailability of IGF-I.<sup>16,17</sup>

#### **Future Directions for GH/IGF-I Treatment**

Despite end-organ resistance to GH in uremia, exogenous administration of rhGH accelerates skeletal growth by increasing the molar ratio of IGF-I to IGFBP-3. However, CKD patients often require dose levels of rhGH 2 to 3 times higher than doses administered to GH-deficient subjects.<sup>7</sup> In addition, combined therapy with rhGH and rhIGF-I results in a greater than additive effect, or synergistic interaction, in CKD patients.<sup>6</sup>

Given the prevalent organ resistance to GH in CKD, therapeutic approaches that increase functional availability of IGF-I may be more effective than the simple administration of rhGH as is currently practiced.<sup>6,7</sup> These measures may include the use of exogenous IGFBP-3 to replace the inhibitory smaller fragments and IGF-I analogs to displace endogenous IGF-I from its binding proteins.<sup>6,7</sup> While the binding protein may prolong the half-life of IGF-I, IGF-I analogs may increase the effective concentration of the bioactive free IGF-I. Therapeutic administration of combined IGF-I and IGFBP-3 complexes have been successfully used to enhance positive nitrogen balance in burn patients.<sup>6</sup>

Furthermore, synthetic GH-releasing peptide (GHRP) and its endogenous equivalent, ghrelin, may be available for oral administration in the near future.<sup>7</sup> These GH secretagogues are more potent than the conventional GH releasing hormone (GHRH) in stimulating a pulsatile release of GH. They act on specific receptors of the anterior pituitary gland, thereby restoring its normal physiologic characteristics. These include capacity for feedback regulation and a greater than 6-fold increase in IGF-I synthesis.<sup>6</sup> This therapeutic approach has been introduced into clinical practice with the combined use

of GHRP and thyroid-releasing hormone to reactivate pulsatile pituitary secretion of GH and thyroid-stimulating hormone, thereby preventing protein catabolism and muscle wasting in protracted critical illness.<sup>18</sup>

#### **Delayed Puberty, Hypogonadism, and rhGH**

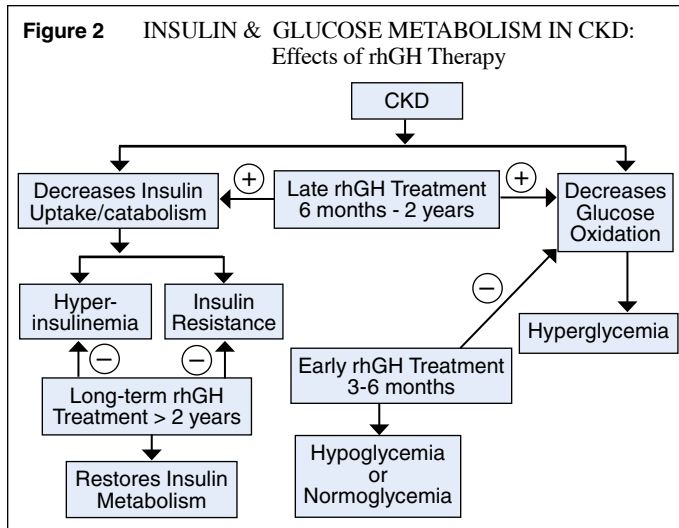
There is a complex interaction among GH, IGF-I, and sex steroids in maximizing growth potential and body composition and in promoting sexual and reproductive capacities in human subjects.<sup>19</sup> Although the mechanism is unknown, the increase in pituitary GH synthesis during mid-puberty in boys is preceded by an increase in plasma testosterone. Similarly, the GH/IGF-I axis is activated by small increases in plasma estrogen in girls at the onset of puberty. GH and IGF-I influence reproductive function directly by modulation of gametogenesis and indirectly by enhancing steroidogenesis. Achievement of critical body weight is associated with pubertal onset, suggesting that somatic effects of rhGH treatment may play a role in the attainment of spontaneous puberty.<sup>20,21</sup>

The common findings of hypogonadism and delayed puberty in CKD are characterized by a loss of the normal pulsatile hypothalamic release of gonadotropin-releasing hormone (GnRH).<sup>22</sup> Puberty may be delayed for up to 2 years, while peak height velocity is often less than 50% of normal in CKD patients. There is a low expression of GHR in a GHR gene knockout-mouse model, similar to the findings in human CKD subjects. These mice have delayed maturation of seminal vesicles, spermatids, and testes, with a poor testicular response to leutinizing hormone, supporting a role for rhGH in induction of pubertal maturation.<sup>23</sup> The use of rhGH/IGF-I administered with GnRH analog (experimental hypogonadism) in men has been shown to preserve protein synthesis and lipid oxidation compared with controls, indicating an independent effect of the combined regimen in the maintenance of fat-free mass.<sup>24</sup> Similarly, combined therapy with rhGH and testosterone synergistically promotes muscle IGF-I gene expression, whole body protein anabolism, bone turnover, physical performance, and sexual function.<sup>25,26</sup>

#### **METABOLIC CHANGES AND rhGH THERAPY**

##### **Insulin and Glucose Metabolism**

Insulin and glucose metabolism in CKD (Figure 2) is characterized by reduced activity of glycolytic enzymes with a consequent decrease in glycolysis, glycogen synthesis, and storage. In uremic rats, there is 25% to 45% reduction in hepatic gluconeogenesis and glucose formation rate from fructose and pyruvates.<sup>9</sup> Similarly, due to a defective intracellular (post-receptor) signaling there is impairment of hepatic insulin metabolism in uremic rats. In addition, although pancreatic insulin secretion is reduced, its renal degradation is substantially compromised in CKD. The resultant hyperinsulinemia stimulates plasminogen activator inhibitor,



reduces fibrinolysis and, therefore, promotes vascular thrombus formation.

### rhGH Therapy and Glucose Metabolism

In the early phase of rhGH therapy, insulin-like effects (including hypoglycemia and protein synthesis) predominate and serve to overcome the uremic-induced insulin resistance (Figure 2). This effect is due to a cross-affinity of IGF-I with insulin receptors leading to an increased glucose uptake and cellular oxidation.<sup>27</sup> On the other hand, with long-term rhGH administration, there is impairment of insulin-mediated glucose uptake, increased lipid oxidation, and formation of insulin-resistant (glycolytic type II) muscle fibers.<sup>28</sup> Consequently, hyperglycemia ensues with an increase in glycosylated hemoglobin. In general, restoration of normal glucose tolerance has been shown to occur within 2 years of starting rhGH therapy.<sup>29,30</sup> These paradoxical effects of rhGH may result from functional and structural diversities of its fragments. For example, GH fragment 1-15 is endowed with insulin-like effects, whereas GH fragment 177-191 possesses anti-insulin properties, and the 20K-GH variant promotes cellular growth.<sup>31</sup>

### Protein Metabolism in CKD

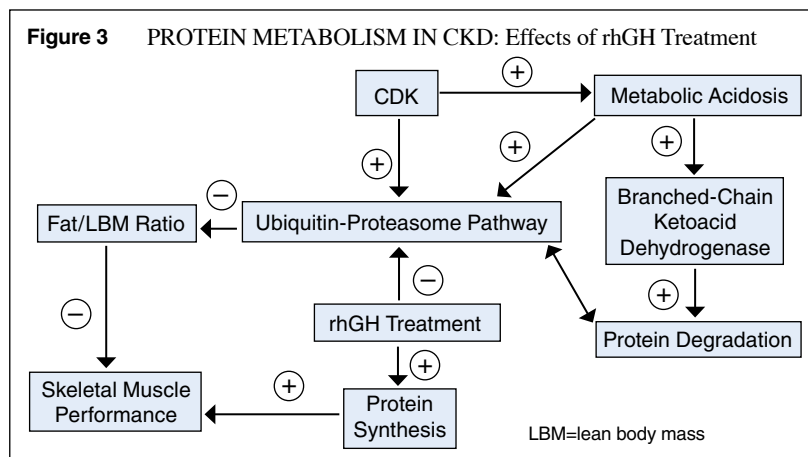
Although hepatic synthesis of total serum protein is often preserved in CKD subjects, production of specific proteins such as IGF-I and apolipoprotein A1 are commonly reduced.<sup>9</sup> Similarly, there is a 30% to 40% reduction in enzymatic activity of the urea cycle, with a down-regulation of ureagenesis and accumulation of nitrogenous substances, including middle molecule toxins (poorly dialyzed, larger-sized uremic molecules) such as advanced glycation end products, and  $\beta$ 2-microglobulin.<sup>9</sup>

As previously stated, metabolic acidosis and uremic-induced inflammation cause protein degradation by activation of ubiquitin-proteasome pathway, induction of

branched-chain ketoacid dehydrogenase, and promotion of end-organ resistance to insulin and GH/IGF-I (Figure 3). The physiologic impact of activated uncoupling proteins (UCP polymorphism) on mitochondrial oxidative phosphorylation is substantial and may account for up to 20% of basal energy expenditure.<sup>32</sup> Tumor-necrosis factor (TNF)- $\alpha$  cytokine, often elevated in CKD, promotes negative nitrogen balance by up-regulating UCP-2 and -3 genes in skeletal muscles of experimental rats.<sup>33</sup>

### rhGH Therapy on Protein Metabolism

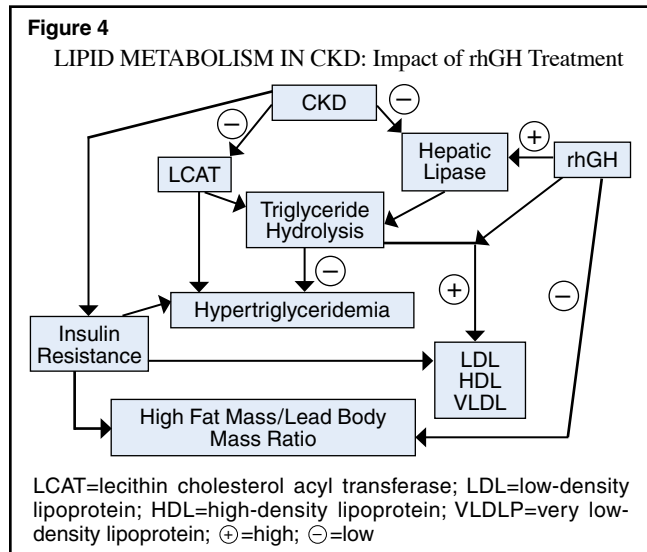
Treatment with rhGH increases protein synthesis, not only by stimulating uptake of amino acid, but also by promoting intracellular peptide assembly.<sup>34</sup> Protein degradation is prevented by inhibition of lysosomal and ATP-ubiquitin-proteasome pathways. Thus, the net effect of rhGH therapy in CKD is an efficient use of dietary branched-chain amino acids with improved skeletal muscle performance.<sup>35,36</sup> Consequently, administration of rhGH therapy after long-term mechanical ventilation has been shown to result in improved respiratory muscular strength, reduction in ventilator settings, and successful extubation in post-surgical patients.<sup>37</sup> Similarly, combined use of GH/IGF-I as an adjunct to total parenteral nutrition results in a net positive protein balance in critically ill patients.<sup>38</sup> On the other hand, in a multi-institutional, randomized, controlled trial of critically ill adults, the use of high dose rhGH resulted in longer length of hospitalization and a higher mortality rate.<sup>39</sup>



### Lipid Metabolism in CKD

CKD subjects exhibit a reduction of lecithin-cholesterol acyl transferase (LCAT) enzyme, down-regulation of apo-A1 genes, and inhibition of hepatic lipase activity.<sup>9</sup> (Figure 4) Consequently, there is impaired hydrolysis of triglycerides (TG) in high-density lipoprotein (HDL), very low-density lipoprotein (VLDL), and intermediate-density lipoprotein (IDL), resulting in hypertriglyceridemia. Plasma low-density lipoprotein (LDL) has been shown to be elevated due to a down-regulation of its receptor function.<sup>9</sup> In addition, insulin resistance may promote dyslipidemia and pro-coagulant activity in CKD.<sup>40</sup> The pattern of lipid profiles in CKD patients are strikingly similar to findings

in metabolic syndrome. Both clinical syndromes share other characteristics such as hypertension, altered body composition, low-grade persistent inflammation, and hyperinsulinemia with a common outcome of premature cardiovascular (CV) disease.<sup>41</sup>



### rhGH Therapy and Lipid Metabolism

In general, rhGH therapy improves lipid profiles by decreasing LDL and apo-B while increasing HDL.<sup>40</sup> By induction of lipoprotein lipase and stimulation of LDL receptor, rhGH attenuates the characteristic increase in VLDL-TG in CKD.<sup>40</sup> In addition, rhGH reduces visceral adiposity, increases lean body mass, and restores normal body composition in CKD.<sup>42</sup> However, it is yet to be seen if these favorable metabolic and biological changes will translate into a better long-term CV outcome in CKD. On the other hand, GH therapy may increase lipoprotein (a), an independent CV disease risk factor.<sup>40</sup> While it shares a common lipid fraction with LDL, lipoprotein (a) clearance is not influenced by the GH-induction of LDL-receptor activity.<sup>40</sup> Nevertheless, the clinical significance of the modest yet notable increase in lipoprotein (a) during rhGH treatment on CV health is not known.

### FOOD INTAKE AND ENERGY HOMEOSTASIS

Uremia promotes excessive transport of tryptophan across the blood-brain barrier and consequently increases neuronal synthesis of serotonin, an endogenous anorectic compound.<sup>43</sup> Adequate food intake may be further compromised in uremic patients by an accumulation of cholecystokinin, TNF- $\alpha$ , interleukin (IL)-1, leptin, and middle molecule toxins (eg, beta (2)-microglobulin, advanced glycation end products).

### Ghrelin and rhGH in CKD

Ghrelin, an endogenous ligand for GH secretagogue-receptor, is principally secreted by pancreatic alpha-like cells (designated Gr cells) from the stomach fundus, in

response to changes in nutritional status.<sup>44</sup> In addition to a potent pituitary stimulation for GH secretion, ghrelin increases food intake by activating agouti-related peptides and neuropeptide Y within the hypothalamus.<sup>45</sup> Experimental use of ghrelin in human subjects was shown to increase food intake, energy consumption, and visual analog scores for appetite.<sup>46</sup> Although the physiological consequence is unknown, there is often accumulation of biologically active (acylated polypeptide) and inactive (desacyl) ghrelin in CKD subjects because of impaired renal clearance. It may be speculated that ghrelin retention constitutes an adaptive mechanism to promote caloric intake in chronic uremia. Perhaps ghrelin's failure to correct the calorie deficiency state arises from the prevailing end-organ resistance to its orexigenic (appetite-stimulating) effects. Similarly, its role in promoting appetite may be physiologically counteracted by the anorexic forces from excessive accumulation of leptin, serotonin, and cytokines in CKD. It has yet to be determined whether the use of ghrelin as an adjunct to rhGH might be beneficial in overcoming anorexia in chronic uremia.<sup>45</sup>

It has been suggested that there may be a negative feedback control of ghrelin by the GH/IGF-I axis. Thus, a short-term rhGH induction of IGF-I causes a proportionate reduction in ghrelin with no alteration in plasma adiponectin.<sup>47</sup> On the other hand, a reduction in body fat mass from long-term use of rhGH may contribute to an increase in circulating levels of ghrelin and adiponectin.<sup>47</sup> The confounding effect of impaired filtration and/or catabolism of ghrelin in renal failure on the purported ghrelin-GH/IGF-I feedback axis is not known.

### Leptin and rhGH in CKD

Hyperleptinemia is a common finding in renal failure, and may result from decreased renal clearance, increased secretion from adipose tissue, and hyperinsulinemia. Leptin is a potent endogenous anorexic agent; its effect may be modulated by rhGH therapy. Thus, administration of rhGH in the Zucker obese rat (which is characterized by leptin and insulin resistance) induces lipolysis and down-regulates leptin gene expression in visceral fat mass.<sup>48</sup> However, as previously stated, the appetite-promoting effect of rhGH may be overcome by persistent hyperleptinemia in CKD subjects. Recent discovery of leptin receptor isoforms in multiple organs suggests that leptin is an important mediator of other unknown biological functions.<sup>49</sup> Therefore, further studies are required in defining the roles of leptin in the modulation of metabolic and nutritional derangements in uremic syndrome.

### SLEEP DEFECTS AND rhGH

About 50% to 70% of adults with end-stage kidney disease suffer from sleep apnea, insomnia, daytime somnolence, and restless leg syndrome.<sup>50</sup> In CKD the

high prevalence of sleep disorders may be confounded by co-morbidities of obesity and depression. However, there is often a strong positive correlation between blood urea nitrogen and indices of sleep dysfunction in patients with kidney failure.<sup>50</sup> Potential complications of sleep defects in uremia may include resistant hypertension, autonomic dysfunctions, and left ventricular hypertrophy.<sup>51</sup> Corroborating the role of uremic burden in sleep dysfunction is the remarkable improvement in symptoms with the administration of daily nocturnal hemodialysis. To date, there are no studies in humans on the therapeutic role of rhGH on sleep defects in CKD, although rapid eye movement (REM) sleep is restored by rhGH, and non-REM sleep is modulated by GHRH in GH-deficient (transgenic) animal models.<sup>52</sup> Similarly, use of ghrelin, a GH secretagogue, results in a preponderance of the more physiological pattern of slow and delta waves that occur during sleep.

Although there are case reports of sudden deaths from obstructive sleep apnea attributed to the use of rhGH in patients with Prader-Willi syndrome, scientific analysis has failed to confirm these assumptions.<sup>53-55</sup> On the contrary, there is potential for beneficial effects on respiratory physiology because of the favorable effects of rhGH on inspiratory drives, ventilatory muscle functions, respiratory quotients and resting energy expenditure.<sup>56-58</sup>

## IMMUNE FUNCTION

CKD is characterized by a persistent micro-inflammatory state with increased circulating levels of IL-1, IL-6, and TNF- $\alpha$  cytokines. Negative nitrogen balance may result from the reduced hepatic syntheses of albumin and apolipoprotein; however, increased release of fibrinogen and amyloid precursors by the liver may enhance vascular thrombogenicity.<sup>9</sup>

Immune deficiency in CKD results from a direct inhibition of uremic toxins and/or altered metabolic activities of immunological cells, including neutrophils, lymphocytes, and macrophages. One subset of T-helper cells, Th-1, is the effector of cell-mediated immunity and recruits new Th-1 cells by producing interferon-gamma while inhibiting Th-2 induced cellular differentiation.<sup>59</sup> The other subset of T-helper cells, Th-2, secretes inhibitory IL-4 and IL-10 cytokines and consequently attenuates the self-perpetuation of Th-1 cells. Uremia shifts the delicate regulatory balance between Th-1 and Th-2 cellular pathways in favor of the latter, thereby causing a depression of cell-mediated immunity.<sup>59</sup> In addition, the impaired expression of B7-2 (co-stimulatory) molecules on the surface of antigen-presenting cells may weaken activation of effector T cells.<sup>60</sup>

The capacity for B-cell antibody production and superoxide generation by polymorphonuclear leukocytes

are also reduced in a uremic milieu.<sup>9</sup> The defect may be due to elevated cytosolic Ca<sup>2+</sup> resulting in poor ATP generation (impaired mitochondrial oxidative phosphorylation) and may be reversed by calcium-channel blockers.<sup>9</sup> Increase in neutrophil apoptosis is in part mediated by the Fas-Fas-L pathway in CKD; there is a positive correlation between Fas-mediated apoptosis and creatinine clearance in plasma obtained from uremic subjects.<sup>61</sup>

## rhGH Impact on Immune Dysfunction

GH stimulates T-cell cytotoxicity and releases superoxide anion from inflammatory cells. CD4 and NK-cell activities were shown to be restored in GH-deficient adults treated with rhGH, while phagocytic function was normalized.<sup>62</sup> In addition, rhGH was shown to prevent apoptosis of immunologic cells by inactivating the pro-apoptotic Fas-FADD pathway and increasing the anti-apoptotic expression of Bcl-2. The overall physiological impact was a down-regulation of Caspase 3, an intracellular effector of apoptosis.<sup>63</sup>

GH is a member of the cytokine super-family and has a similar structure to granulocyte colony-stimulating factor.<sup>64</sup> GHRs, which bind to GH, are found on a number of immunological cell surfaces. Use of rhGH in severe sepsis may exacerbate the ongoing inflammatory process by cross-activation with other cytokine-receptors and, thereby result in a higher fatality rate.<sup>65</sup> In a rat model of bacterial sepsis, increased expression of suppressors of cytokine signaling (SOCS)-1 and -3 inhibited intracellular signaling of GHR, resulting in a poor generation of IGF-I.<sup>66</sup> Thus, a relative IGF-I deficiency may contribute to the impairment of glomerular filtration rate that may result from septicemia. Although in normal circumstances IGF-I increases renal perfusion, its administration in a rat model of ischemic renal failure results in higher mortality, apparently by evoking adverse inflammatory processes.<sup>67</sup>

The pro-inflammatory activity of rhGH was initially postulated to be a potential cause of allograft rejection. However, clinical evidence suggests otherwise, and the safety and efficacy of rhGH was recently demonstrated in renal transplantation.<sup>68</sup> In pediatric renal allograft recipients, rhGH has also been shown to prevent steroid-induced protein catabolism, maintain skeletal mass, and improve linear growth rate. In addition, postoperative administration of rhGH in rats with small bowel transplant restores morphology of allograft mucosa and promotes a net positive nitrogen balance.<sup>69</sup> Furthermore, the perioperative use of rhGH in immunocompromised rats enhances surgical wound healing.<sup>70</sup> Given that post-transplant use of the immunosuppressant sirolimus may cause a delay in wound healing because of its antifibrotic property, a study of the role of rhGH in this regard may provide useful information.

## BONE MINERAL CONTENT AND rhGH

Within a few weeks of initiation of rhGH therapy, the molecule interacts with the bone-forming unit by increasing the biochemical markers of bone formation and resorption. In general, short-term (3–6 months) rhGH therapy may reduce or maintain bone mineral density, while treatment of GH-deficient adults for 2 years results in a sustained increase in mineralization.<sup>71</sup> On the other hand, the common use of high-dose calcium and calcitriol in CKD subjects for the treatment of hyperphosphatemia may result in suboptimal skeletal response to rhGH. Calcium-containing phosphate binders and vitamin D inhibit chondrocyte proliferation and delay mineralization, thereby causing adynamic bone disease.<sup>72</sup> Resistance to GH effects is manifested by low expression of IGF-I protein and decreased bone morphogenetic protein-7 staining, despite an increase in GH concentration and higher density of GHR.<sup>72</sup> It may therefore be prudent to avoid calcium-containing phosphate-binders and ensure appropriate vitamin D doses in CKD subjects receiving rhGH.<sup>73</sup>

There is evidence to suggest that GH may play a modulatory role in the musculo-skeletal effects of parathyroid hormone. Administration of rhGH to GH-deficient subjects improves end-organ responsiveness with a decrease in urinary calcium excretion, increased tubular phosphate reabsorption, and increased markers of bone turnover (type I collagen C-telopeptide and pro-collagen type I amino-terminal propeptide).<sup>74</sup>

## QUALITY OF LIFE

Psychometric analysis and physical assessment of renal patients reveals a high prevalence of reactive depression, reduced physical performance, and cognitive deficits. However, psychosocial support, physical exercise, and anemia control may ameliorate many of these deficits. Administration of rhGH may also play a positive role as replacement therapy in GH-deficient adults; rhGH has been shown to improve quality-of-life indices.<sup>75</sup> Similarly, rhGH improves linear growth and physical agility, and reduces psychosocial burden in children with Prader-Willi syndrome.<sup>76,77</sup> Confounding variables such as anemia in CKD make studying the psychosocial impact of rhGH a difficult exercise.

## CONCLUSIONS & SPECULATION

This review describes and highlights the potential therapeutic impact of rhGH in CKD patients. In the absence of kidney transplantation, it is important to restore the profound metabolic and physiological defects arising from renal insufficiency. In many instances, studies in GH-deficient models have demonstrated the beneficial effects of rhGH therapy beyond the longitudinal

skeletal growth for which rhGH is commonly indicated. Additional problems in CKD patients for whom rhGH may play a significant role include modulation of nutritional inadequacies, altered body composition, immune dysregulation, and impaired sexual development and/or reproductive capacity. However, given the differences in their pathogenesis, it may be overly simplistic to project similar benefits of rhGH therapy to all the clinical settings of growth failure in CKD.

The multifaceted physiological effects of rhGH should still be taken into consideration in future studies of renal patients. Efforts must be made to broaden the scope of outcome measures to include cellular growth, cellular metabolism and function, neurocognitive development, psychosocial impact, sleep physiology, energy homeostasis, and anemia control. The beneficial role of rhGH in uremic cardiomyopathy, bone disease, anemia management, body composition, hospitalization requirements, and vascular diseases should also be examined. Co-morbidities are common in CKD and, therefore, multiple pharmacological agents are often needed to treat the disease. The physiological outcome of the combined use of erythropoietin, steroids, vitamin D, carnitine supplements, and other nutritional supplements with rhGH requires further study. Experimental studies in animals suggest a favorable role for rhGH in surgical wound healing; studies are therefore needed to examine the role of rhGH in ameliorating delayed wound-healing that may characterize the use of sirolimus after surgical transplantation.

Furthermore, the role of ghrelin (a recently discovered endogenous GH secretagogue) in CKD requires critical evaluation. Relevant questions for future studies are numerous. What is the role of ghrelin in food intake behavior in CKD patients? What are the metabolic effects of uremia on the capacity of Gr cells to produce ghrelin? What is the effect of uremia on the pituitary GH secretagogue receptor? What is the therapeutic impact of oral administration of ghrelin as a sole agent and/or combined therapy with rhGH/rhIGF-I, GH releasing peptides, exogenous IGFBP-3, and IGF-I analogs? What are the relationships between ghrelin, leptin, cytokines, and UCP polymorphism in the regulation of food intake, energy balance, and body composition in CKD?

Finally, the essence of this review is to inform the scientific community of the need for operational research endeavors concerning the metabolic impacts of rhGH therapy. Therefore, efforts must be made to critically assess the risk and benefit of the continued use of rhGH beyond the traditional end-point of linear skeletal growth in children with CKD. Hopefully, an improved understanding of the roles of rhGH in restoring physiological disturbances in CKD will provide added value to the treatment of such patients throughout their lives.

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## ABSTRACTS FROM THE LITERATURE

### Hypothalamic Amenorrhea and Leptin

The authors assessed the effects of leptin treatment in 8 patients with hypothalamic amenorrhea, compared with 6 patients who were not treated. All 14 patients had secondary amenorrhea for 6 months or longer, coincident with increased exercise or low body weight and were otherwise healthy without acne, hirsutism or LH/FSH, TSH, and prolactin alterations. Basal and follow-up assessments in a clinical research center included comprehensive endocrine, body composition, metabolic rate analyses, bone densitometry and pelvic ultrasonography. The patients treated with leptin (r-metHuLeptin) received 0.08 mg/kg/day subcutaneously for 2 to 3 months, with 40% of the dose given at 8:00 AM and 60% given at 8:00 PM. If patients ovulated the study was terminated at 2 months. If no ovulation occurred the dose was increase to 0.2 mg/kg/day for a third month. Leptin treatment increased mean LH levels and LH pulse frequency after 2 weeks of treatment and increased maximal follicular diameter, the number of dominant follicles, ovarian volume and estradiol levels over the study period. Three patients had ovulatory menstrual cycles; 2 had preovulatory follicular development and withdrawal bleeding during treatment. Leptin treatment significantly increased levels of free T<sub>3</sub>, free T<sub>4</sub>, IGF-I, IGFBP-3, bone alkaline phosphatase and osteocalcin but not cortisol, corticotropin, nor urinary N-telopeptide. Untreated control patients did not have any significant changes in any of these variables. Body weight did not change in the control patients; however it decreased slightly among the treated ones, owing to a small decrease in body fat without changes in lean body mass. No significant changes in metabolic rates or food intake occurred. The authors concluded that the relative leptin deficiency in women with hypothalamic amenorrhea is improved with leptin treatment. This results in improved reproductive, thyroid, growth hormone axis and markers

of bone formation, suggesting that leptin is required for normal reproductive and neuroendocrine function.

Welt CK, Chan JL, Bullen J, et al. Recombinant human leptin in women with hypothalamic amenorrhea. *N Engl J Med* 2004;351:987-97.

**Editor's Comment:** *Hypothalamic amenorrhea, also called functional amenorrhea, is frequently seen in women who are athletic, underweight and/or stressed. It is usually preceded by irregular menses, weight loss or increase in physical activity and it is considered to be the result of energy deficiency. In non-athletic women of normal weight it may be associated with psychosocial stress also related to subtle deficits in calorie and macronutrient intake. The central energy-related hormone, leptin, is the common factor underlying the pathogenesis of this entity. The study by Welt et al adds data substantiating the importance of leptin in mediating the neuroendocrine abnormalities of hypothalamic amenorrhea, a leptin deficiency condition. They demonstrated an improvement with leptin treatment, without other medications to induce menstruation, while the patients maintained their usual dietary intake, exercise habits and lifestyle. However, let's not tread into new expensive treatments without correction of nutrient deficiencies or without first attempting to modify the dietary intake to meet all the energy and nutrient needs of the patient. The accompanying editorial by Ahima<sup>1</sup> addresses the distinguishing features of this condition from anorexia nervosa, as well as an erudite explanation of the pathophysiology of the disease as it relates to body fat, leptin and hypothalamic amenorrhea.*

Fima Lifshitz, MD

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### Statin Therapy in Hypercholesterolemic Children

Wiegman and associates report findings of a 2-year randomized placebo-controlled efficacy and safety trial of pravastatin for the treatment of familial hypercholesterolemia in children ages 8 to 18 years. Two hundred fourteen children (100 boys), mean age 13.0 years, were studied. Inclusion criteria were: one parent with a definite clinical or molecular diagnosis of familial hypercholesterolemia; at least 3 months on a fat-restricted diet (<30% of total calories from fat, 10% saturated fat); 2 fasting LDL-C levels of at least 155mg/dL; no current drug treatment or use of plant sterols.

The primary efficacy variable was change from baseline of carotid intima-media thickness (IMT) as measured by ultrasound. Blood samples were measured for total cholesterol, HDL-C, LDL-C and triglycerides at 3–6 month intervals over the 2-year study. In addition, ALT, AST, and CPK were measured for safety reasons, and levels of sex steroids, gonadotropins, cortisol, and TSH were determined to survey for potential side effects of the drug on growth and sexual development. Height and weight were measured and Tanner staging was performed at baseline, 1, and 2 years.

Baseline characteristics were similar in both groups. The mean carotid IMT was attenuated after 2 years of treatment, while there was a trend towards an increase in the placebo group. The overall change between the 2 groups was statistically significant. LDL-C levels were reduced in the treatment group, while HDL-C, triglyceride and lipoprotein(a) levels remained unchanged. All hormone levels (corticotropin, cortisol, LH, FSH, DHEA-S, TSH, estradiol, testosterone) were similar in both groups at 2 years. Height and weight increased similarly in both groups, as did stages of sexual development. AST, ALT, and CPK levels were also similar, although one child in the placebo group had an asymptomatic but marked increase in CPK, which returned to normal.

The authors point out that this is the first long-term safety and efficacy trial of a 3-hydroxy-3 methylglutaryl coenzyme A reductase inhibitor (statin) in children with familial hypercholesterolemia. The drug was both effective and well tolerated with minimal observable side effects. There were no effects on growth or sexual development. Despite these encouraging findings, they caution that even longer studies are needed to establish

the safety of this class of drugs.

Wiegman A, Hutten B, de Groot E, et al. Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized controlled trial. *JAMA* 2004;292: 331-7.

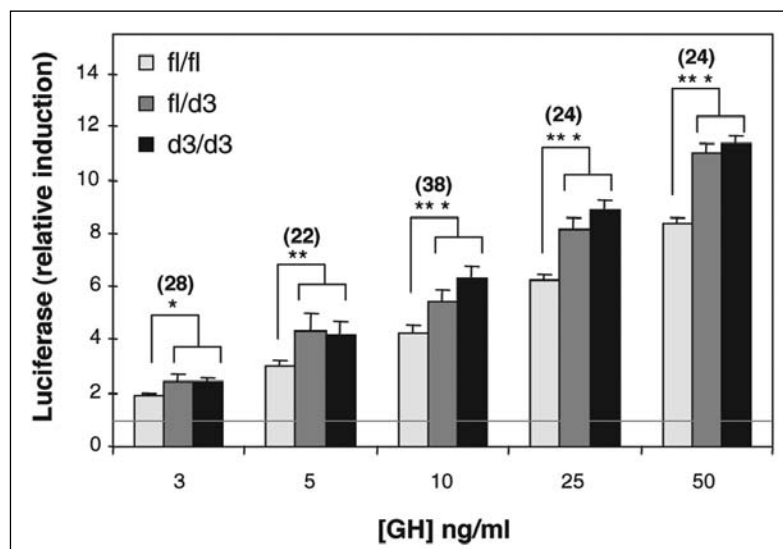
**Editor's Comment:** This is a welcomed study. Although the efficacy of statins in reducing LDL-C has been reported in several studies, this safety study is reassuring. More and more frequently pediatric endocrinologists are faced with younger and younger children with obesity and hypercholesterolemia, or diabetes and hypercholesterolemia, and need to recommend effective and safe therapy. Diet and exercise, unfortunately, are rarely practiced with sufficient adherence to be considered effective and realistic treatment options. Furthermore, resins are poorly tolerated in this age group. The use of statins is therefore an obvious therapeutic choice, but information regarding their long-term safety and side effects has been lacking. We would encourage these authors to continue their study of these children with the anticipation that further data will establish safety over an even longer time period.

William L. Clarke, MD

## Growth Hormone Receptor and Responsiveness to Growth Hormone

Intrigued by the clinical observation that the linear growth response to a similar dose of growth hormone (GH) in GH deficient (GHD) and in non-GHD children with idiopathic short stature (ISS) or children with intrauterine growth retardation (IUGR) varied substantially, the investigators correlated the biological effectiveness of recombinant human GH (rhGH) with 2 known isoforms of the GH receptor (GHR). The human gene GHR consists of 9

coding exons with exons 3–7 encoding the extracellular domain of 246 amino acids; there is one full-length isoform of GHR and a second isoform in which the 22 amino acid sequence coded by exon 3 is omitted by alternative splicing during transcription (d3-GHR). In prepubertal children with ISS or IUGR (defined by short birth length), the frequency of the d3-GHR isoform was comparable to that of normal subjects. The GHR genotype (GHR/GHR, GHR/d3-GHR, d3-GHR/d3-GHR) did not affect basal growth rate. When treated with rhGH, subjects with at least one d3-GHR isoform grew more rapidly in response to a standard dose of rhGH (0.36 or 0.23 mg/kg/week in 2 separate trials) than did those with the GHR/GHR genotype during the first 2 years of therapy. There was no difference in growth response to rhGH between children with 1 or 2 d3-GHR alleles or between those with ISS or IUGR. Expression of the GHR and d3-GHR isoforms in HEK fibroblasts *in vitro* demonstrated that in response to hGH the transcriptional activity of the luciferase reporter gene was ~30% greater in cells with d3-GHR than GHR. The authors concluded that analysis of the GHR genotype may permit more appropriate individualization of rhGH dosage (pharmacogenetic dose selection) in clinical conditions in which administration of rhGH is appropriate.



*In vitro* bioactivity of full-length GHR and d3-GHR. HEK 293 cells transiently expressing full-length GHR, d3-GHR or both were stimulated by increasing concentrations of GH for 8 h. Relative induction of LHRE-luciferase reporter gene is expressed relative to unstimulated cells (value of 1, horizontal line).

Number of experiments in ( ), \*P < 0.005, \*\* P < 0.0005, \*\*\*P < 0.00001

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Dos Santos C, Essioux L, Teinturier C, Tauber M, Goffin V, Bougnères P. A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone. *Nat Genet* 2004. 36:720-4.

**Editor's Comment:** The mechanism(s) by which the shorter d3-GHR transmits a more potent signal in response to ligand bind than does the full-length GHR is not known. The 22 amino acid sequence of exon 3 is not near the interface of ligand and receptor, and the mechanism by which its loss leads to increased receptor activity is unknown at present. It does not affect hGH/GHR binding or internalization. The d3-GHR polymorphism might permit more rapid propagation of signal to the intracellular signal transduction systems that mediate the cellular responses to hGH. In this regard,

it would be of interest to study the dynamics of this system in cells expressing either the full-length or shortened GHR isoforms. The report also raises the question that if a polymorphism that increases responsiveness to hGH exists, might there not also be a subtle polymorphism that mildly depresses GHR transduction of the hGH signal? Might this be another pathway through which the "genetic" regulation of growth and adult stature is mediated?

Allen W. Root, MD

## Therapeutic RNAi for Genetic Skeletal Disease?

RNA interference (RNAi) is a gene silencing phenomenon first identified in the nematode, *C. elegans*, but was subsequently found to occur in higher organisms including humans. It probably evolved as an ancient defense mechanism for cells to fend off mobile genetic elements, such as RNA viruses and transposons, but today it has been implicated in a growing number of cellular processes.

As discussed by Stevenson, RNAi involves sequence specific degradation of target RNAs triggered by the formation of double stranded RNA (dsRNA). When it occurs naturally, long dsRNA is processed to short interfering RNAs (siRNAs) 21-24 bases in length by a dsRNA-specific endonuclease named Dicer (Figure). They are incorporated into a nuclease complex referred to as the RNA-induced silencing complex or RISC. Unwinding of the siRNAs activates and directs RISC to the target RNAs, which are cleaved and degraded. The complementarity between the siRNA and the target RNA determines the sequence specificity of RNAi.

An important advance in the RNAi field was the discovery that exogenous synthetic siRNAs or endogenously synthesized siRNAs driven by viral vectors could be incorporated into RISC and induce sequence-specific degradation of target RNAs. This created an extremely powerful tool for scientists to "knock down" expression of genes of interest simply by adding synthetic RNA duplexes to the medium of cultured cells, introducing viral vectors that express siRNAs into cells or even generating transgenic animals that synthesize siRNAs.

RNAi is much more complex than outlined here, and there are many technical difficulties that complicate the use of RNAi to knock-down gene expression in experimental systems. Nevertheless, RNAi has stimulated considerable interest in the pharmaceutical/biotech industry as a potential therapeutic agent for human disease. The best examples to date have to do with treatment of infectious diseases, such as those caused by HIV, hepatitis viruses and poliovirus, as well as cancers that are mediated in part by overactive oncogenes. In the case of viral infections, interfering RNAs could be targeted to viral transcripts required for viral replication or survival. In the second case, using RNAi to silence expression of BCR-ABL, the fusion gene that results from the Philadelphia chromosome translocation in chronic

myelogenous leukemia or mutated RAS oncogenes that drive several types of cancer would be appealing.

Receiving less attention to date, but of probably at least as much interest to readers of *GGH*, is the potential use of RNAi to knock down expression of mutant alleles in dominantly inherited genetic disease. In concept, siRNAs could be tailored to distinguish mutant from normal (wild type) alleles and block only mutant allele expression. This could convert a dominant negative disorder, ie, a disorder in which the product of the mutant allele interferes with the function of the normal (wild type) allele product, to a disorder that results from haploinsufficiency or functional loss of one allele. For families in which both forms occur, manifestations are usually milder in the form resulting from haploinsufficiency, ie, osteogenesis imperfecta type I – haploinsufficiency vs osteogenesis type II – dominant negative. Thus, there is potential benefit from this therapeutic strategy.

Despite the excitement and promise of therapeutic RNAi, there are many obstacles, the greatest of which is delivery. Systemically delivered siRNAs face degradation by nucleases, and the use of viral vectors to target organs of interest is still in its infancy. A recent publication by Soutscheck and colleagues provides evidence that chemically modified siRNAs can successfully knock down endogenous genes in living mice. More specifically, they targeted expression of the gene encoding apoprotein B (*apoB*) in the mouse liver and jejunum where it is known to be expressed at high levels with 2 siRNAs known to silence *apoB* in cultured cells. They modified the *apoB* siRNAs by chemically stabilizing their backbone and also by adding cholesterol to their 3' end. The modified siRNAs were then compared to unmodified *apoB* siRNAs and other controls.

The results showed that the cholesterol-conjugated *apoB* siRNAs were significantly more stable in serum than their unconjugated counterparts. When administered intravenously, one of the conjugated *apoB* siRNAs was very effective at lowering *apoB* mRNA and *apoB* protein levels, as well as total cholesterol and LDL cholesterol. They observed no evidence of "off-target" effects, that is, effects attributed to silencing of genes other than *apoB* or other obvious complications from the injections. The authors concluded that exogenously administered chemically

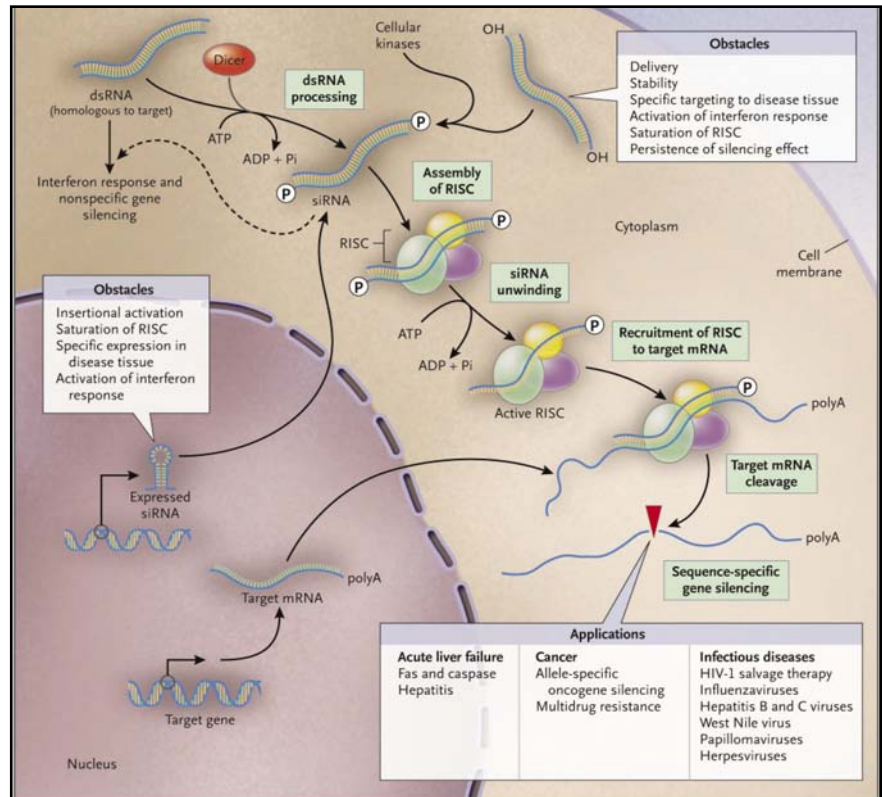
modified siRNAs can potentially be used to silence expression of endogenous genes involved in human disease.

Stevenson M: Therapeutic potential of RNA interference. *N Engl J Med* 2004;351:1772-7.

Soutschek J, Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 2004;432:173-8.

**Editor's Comment:** RNAi has had a major impact on science since its relatively recent discovery. It is still not entirely clear how it works and there remain concerns about specificity and the so-called off target effects on genes other than specifically targeted genes. Nevertheless, it has great promise as a means to treat not only cancer and infectious diseases, but genetic diseases in which mutant alleles differing from their normal alleles by only a single base can be specifically targeted. It will probably be years before such treatment becomes realistic for humans, but the success of substantially knocking down *apoB* expression by systemically administering chemically modified *apoB* siRNAs in mice is very encouraging. One note of caution is that the growing skeleton may be difficult to target because the cartilaginous growth plate is relatively avascular compared to most tissues such as liver and gut.

William A. Horton, MD



Mechanism of Gene Silencing by RNA Interference. The double-stranded RNA (dsRNA) is processed and assembled into the RNA-induced silencing complex (RISC) and subsequently incorporated into target mRNA for the sequence-specific gene silencing application.

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## Variation in Expression in Human Genes

Medical genetics textbooks typically distinguish between continuous and discontinuous variation in (clinical) phenotype. The latter can often be traced to a single change in the DNA sequence of a gene, ie, a "mutation" that serves as the basis of classic mendelian disorders. The genetic basis of continuously variable traits, such as height or blood pressure, is more difficult to explain. Variation in baseline expression of genes represents a mechanism that could contribute to continuous phenotypic variability. It is known to exhibit familial aggregation suggesting that it is heritable, but the tools to study the genetics of variation in human gene expression have only recently made it feasible to explore this notion. Morley and colleagues now document the existence of regulators of baseline gene expression.

The investigators utilized microarray technology to measure expression levels of genes, which they refer to as "gene expression phenotypes," in immortalized B cells from members of 94 Center d'Etude du Polymorphisme Humain

(CEPH) Utah families. Starting with approximately 8500 genes active in these cells, they found 3554 genes that showed greater variation of expression between individuals than between replicates from the same individual. They then carried out genome-wide linkage analysis using single nucleotide polymorphisms to identify the genetic determinants of this variation. The results showed that variation in expression of 984 genes was genetically linked to one or more regions of the genome.

They assumed that regions linked to expression levels were regulatory regions or "regulators". They examined the spatial relationship of the regulators to the 142 "target" genes that exhibited the strongest evidence for linkage. Twenty seven (19%) mapped to within 5 Mb of the target gene; they considered these to be *cis*-acting regulators because of their relatively close proximity to the coding sequence of the target gene. One hundred ten (77.5%) mapped further away and were designated *trans*-acting regulators. Both *cis*- and *trans*-acting regulators were

found for 5 (3.5%) of the variably expressed genes. Many of these genes (164/984, or 16%) had multiple regulators of expression.

In addition to genomic regions containing regulators that influence single expression phenotypes *in cis* or *in trans*, the authors also found genomic regions that contained transcriptional regulators of multiple expression phenotypes. To further characterize these regulators, they divided the genome into 5 Mb windows and searched for regulatory "hotspots" within these windows. Two hotspots were detected, one of which mapped to chromosome 14 (14q32) and the other to chromosome 20 (20q13). Further analysis showed that these 2 regulatory hotspots influence expression of 31 of the 984 target genes under investigation. The authors suggest that their existence provides evidence for master regulators of baseline gene expression in humans.

Finally, they asked if differential expression of target gene alleles could be explained by *cis*-acting regulators. Analysis of individuals in whom alleles could be distinguished by single nucleotide polymorphisms showed that some of the variable expression could be attributed to the influence of the *cis*-acting regulators.

Morley M, Molony CM, Weber TM, et al. Genetic analysis of genome-wide variation in human gene expression. *Nature* 2004;430:743-7.

Cox NJ. An expression of interest. *Nature* 2004;430:733-4.

**Editor's Comment:** This paper reminds us that the level of expression is an important aspect of gene action. Reduced or increased gene expression can influence quantitative traits, such as height. One can also envision a situation in which a mutation in a trans-acting regulator could cause disease by decreasing or increasing expression of its target gene(s). Take osteogenesis imperfecta type I for example; it typically results from mutations that cause transcripts from a mutant COL1A1 allele to terminate prematurely or undergo nonsense-mediated mRNA decay, functionally inactivating one of the 2 COL1A1 alleles. It is conceivable that a loss of function mutation of a trans-acting regulator of this locus could produce a similar adverse effect on type I collagen synthesis, especially if it were homozygous. Of note, such a mutation would not show linkage to the COL1A1 locus. There are several limitations of this investigation as noted by the authors and an accompanying news and views article. For instance, mRNA levels are only one determinant of the level of protein encoded by a given gene. Gene expression differs in different tissues, at different developmental stages and in response to physiologic and pathologic factors that are probably not reflected in immortalized B cells.

William A. Horton, MD

## IGF-I Receptor Signaling: Mechanisms of Growth Stimulation

Wu and colleagues used 2 cell models to study the effects of insulin-like growth factor-I receptor (IGF-IR) signaling via insulin receptor substrate (IRS)-1 on the upstream binding factor 1 (UBF1), a regulator of ribosomal RNA (rRNA) synthesis. 32D cells (myeloid cells dependent on interleukin-3 (IL-3) for growth)

express neither IRS-1 nor IRS-2. In complement, mouse embryo fibroblasts (MEFs) express IRS-1 but have a targeted disruption of the IGF-IR gene (R<sup>-</sup> cells).

Apoptosis normally takes place in 32D cells upon removal of IL-3. 32D cells expressing IGF-IR (32D IGF-IR cells) continue growing for 48 hours after IL-3 is replaced

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with IGF-I, and then undergo granulocyte differentiation. 32D IGF-IR cells ectopically expressing IRS-1 grow indefinitely without differentiation. R<sup>-</sup> cells were also used to develop sister cells for comparison. R<sup>-</sup>/T cells express the SV40 large T antigen, while R<sup>+</sup> cells have the IGF-IR reintroduced. IRS-1 is mostly nuclear in IGF-I-stimulated R<sup>+</sup> cells and in R<sup>-</sup>/T cells, but cytoplasmic in the parental R<sup>-</sup> cells.

Using these 2 systems, the authors showed that IGF-I increased transcription from the rDNA promoter (ie, activated UBF1) in a time course compatible with nuclear translocation of IRS-1. Since UBF1 activation generally occurs via phosphorylation, additional experiments showed that UBF1 phosphorylation, mainly in the C terminus, was IGF-I stimulated and IRS-1 dependent. Beyond that, UBF1 regulation in the 2 cell models differed. In the myeloid cells deprived of IL-3, 32D IGF-IR/IRS-1 cells died without IGF-I, but maintained high levels of UBF1 protein when stimulated with IGF-I. The 32D IGF-IR cells (ie, without IRS-1) had high UBF1 protein levels, which dropped at 48 hours (ie, while the cells were still growing exponentially and not yet showing any morphologic signs of differentiation) and completely disappeared by the time the cells were differentiated into granulocytes. The drop in UBF1 protein was due to both decreased synthesis and increased degradation, though UBF1 mRNA levels remained unchanged. In the MEFs, cells that do not differentiate, UBF1 protein levels were stable after IGF-I treatment in both R<sup>+</sup> and R<sup>-</sup> cells. Thus, the authors concluded that IGF-IR/IRS-1 signaling regulates UBF1 activity, and hence the rDNA promoter, through phosphorylation and in some cells, through changes in protein level. UBF1 protein loss may

be related to the differentiation process, which tends to involve nucleolar dissolution.

Wu A, Tu X, Prisco M, Basergo R. Regulation of upstream binding factor I activity by IGF-I receptor signaling. *J Biol Chem* 2005; 280:2863-72.

**Editor's Comment:** *IGF signaling through the IGF-IR is understood to stimulate cellular survival and proliferation, and at the systemic level, growth. IGF-IR is a tyrosine kinase that is activated by ligand binding. Phosphorylation of tyrosine residues in IGF-IR recruits adaptor molecules like IRS-1 that then start kinase cascades, most notably the PI3 kinase/Akt pathways and the MAP kinase pathway (for reviews, see References 1-2). The paper by Wu et al adds another mechanism whereby IGF-IR signaling stimulates growth: activation of UBF1 through nuclear translocated IRS-1 and presumably PI3 kinase. UBF1 regulates RNA polymerase I activity at the rDNA promoter, thereby regulating the rate of ribosome biogenesis. Because ribosomes are required for protein synthesis, proliferating cells invest much energy in ribosome generation (reviewed in Reference 3). Without concomitant synthesis, proliferating cells would only become progressively smaller. Thus, growth involves increasing numbers of cells with maintenance of proper cell size, and IGF-IR is involved in regulating both these processes.*

Adda Grimberg, MD

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## Long-term Effects of Estrogen Treatment on Fertility in Tall Girls

Venn and colleagues identified from medical records 1248 Australian women who had been assessed and/or treated with estrogens (3mg DES or 150µg ethinyl estradiol daily) for tall stature during the years 1959 to 1993, to assess the effects of this treatment on long-term fertility. A group of 184 self-referrals (members of Tall Girls Inc an Australian advocacy group) were included in the study. To be included subjects had to have had a bone age determination at the time of assessment. Subjects were invited to complete a written questionnaire and computer-assisted telephone interview. The interview included questions regarding reproductive history including whether or not they had ever seen a doctor due to difficulty becoming pregnant, whether they had ever tried unsuccessfully for more than 12 months to become pregnant, and whether or not they had ever taken fertility drugs as treatment for infertility. The time to pregnancy was analyzed for each month of attempting pregnancy. Data from the medical records included age at menarche, treatment type, duration of treatment, and first and last assessment of estimated

mature height by Bailey and Pinneau method.

The final sample size included 618 women (75% of the treated and 95% of the untreated). The mean age of these women was 39.8 years (treated) and 37.7 years (untreated). Both groups were similar in terms of marital status and highest level of education. Self-reported current height was greater in the treated women (179.0cm vs 176.8cm). Both groups were similar in terms of history of smoking, oral contraceptive use, age of first sexual intercourse and lifetime number of male sexual partners. There were no differences between the women treated with DES or ethinyl estradiol on any parameter. Women who had been treated with estrogen were more likely to report problems with fertility. When the data were adjusted for age, the women who had been treated were less likely to have ever been pregnant and to have ever had a live birth. Treated women were more likely to have tried unsuccessfully for 12 months to become pregnant, to have seen a doctor because of difficulty becoming pregnant, and to have taken fertility drugs. Height was not related to fertility problems and the differences between

the 2 groups remained when the self-referred women were excluded from the analysis. A significant, but weak duration of treatment effect was observed.

The authors state that the data were not sufficient to establish a pathophysiological cause for the reduced fertility. They also state that the likelihood of ever becoming pregnant and having a live birth, although statistically reduced for women who had been treated for tall stature, was only slightly lower than that for untreated women and that newer treatments for infertility may reduce that difference.

Venn A, Bruinsma F, Werther G, et al. Oestrogen treatment to reduce the adult height of tall girls: long-term effects on fertility. *Lancet* 2004;364:1513-8.

**Editor's Comment:** Clearly there has been a significant drop in the number of girls seeking treatment to reduce

mature height potential over the past 20 years. However, the authors note that a recent survey of pediatric endocrinologists in the United States reveals that 23% have treated such girls over the past 5 years. Thus, although the absolute number of girls seeking treatment is low, such treatment is still being sought and is available. The current study, although not the first to show the possibility of adverse reproductive effects of estrogen treatment for tall stature, is perhaps the largest long-term follow-up to date. The information is interesting and important. Pediatric endocrinologists need to be able to discuss these facts with each family seeking to reduce their daughter's mature height potential. It is reassuring that no obvious safety concerns were identified through these interviews and chart data.

William L. Clarke, MD

## Micropenis: Long-term Follow-up

These authors report the long-term outcomes of 46,XY males with micropenis, but no other genital deformity, identified and treated intermittently with androgens or hCG during infancy, childhood and/or adolescence. Lee and Houk determined adult stretched penile length (SPL) and social adjustment in 20 patients with SPL  $<-2$  SD of normal at initial examination: 11 had hypogonadotropism and 3 primary testicular failure; in 6 patients no cause of the micropenis was identified. SPL increased in all subjects; adult SPL was  $>-2$  SD of the adult mean in 14 subjects and between  $-2.5$  and  $-2$  SD in 4; 2 patients had adult SPL  $<-2.5$  SD of the mean. Among these 20 patients and another 2 with micropenis first evaluated as adults, 21/22 were heterosexual; 8 were/had been involved in long-term heterosexual relationships. Relative to age-matched control subjects, those with micropenis (N=12 studied) had comparable findings in regard to heterosexual dating and sexual functioning, male friendships, education, employment, sports/leisure activities; none had a psychiatric illness. Despite normal adult SPL, 5 primarily obese patients stated that their penises were small. The investigators concluded that in adult men who had micropenis as children/adolescents: 1) 90% had adult SPL within the broad range of normal; 2) there was "reasonable social adjustment," no psychological pathology, and gender-appropriate sexual functioning.

Husmann evaluated adult SPL in 20 men with micropenis (here defined as SPL  $<-2.5$  SD of normal) diagnosed and treated during infancy in whom SPL did not increase appreciably despite multiple courses of testosterone. Five patients had a mutation in the androgen receptor, 6 had hypogonadotropism, and 9 had no known cause of the micropenis. Mean pretreatment SPL was  $-3$  SD (range  $-5.5$  to  $-2.6$ ) for age/race and mean adult SPL was  $-3.4$  SD (range  $-5.9$  to  $-2.2$ ).

All patients considered their penises to be small, and 5 had undergone (unsatisfactory) surgery to enlarge their penises; 19/20 were heterosexual; 12/20 men were sexually active, but 4 were incapable of vaginal penetration; 5 patients had mental illnesses requiring professional therapy. Despite these findings, Husmann concluded that these patients accept a male gender identity and many engage in a "satisfying heterosexual relationship."

Lee PA, Houk CP. Outcome studies among men with micropenis. *J Pediatr Endocrinol Metab* 2004. 17:1043-53.

Husmann DA. The androgen insensitive micropenis: Long-term follow-up into adulthood. *J Pediatr Endocrinol Metab* 2004.17:1037-41.

**Editor's Comment:** In the report of Lee and Houk, in 5/20 patients (1 hypogonadotropic subject, 1 with primary testicular failure, and 2 with "idiopathic" micropenis) SPL SD score did not appreciably increase between diagnosis and adulthood, but these subjects are not specifically discussed further, and their psychosocial status is unknown. It would have been of interest if Husmann had also reported his experience with the outcome of patients with micropenis responsive to testosterone. These data are reassuring in that they further demonstrate that there is no basis to consider sex reversal in the 46,XY male with micropenis as their gender identity is firmly masculine. Furthermore, with current surgical procedures for penile reconstruction, the opportunity for satisfactory penile enlargement has improved substantially.<sup>1</sup>

Allen W. Root, MD

## Reference

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## Growth Hormone Sensitivity in Obesity

These authors sought to explore the observation that insulin-like growth factor (IGF)-I levels remain normal in obesity despite reduced growth hormone (GH) levels. Ninety-one healthy adults (mean age about 50; range 21-82 years) were subdivided by body mass index (BMI) and gender; there were 19 normal weight men, 23 normal weight women, 15 obese men and 34 obese women (obesity defined as BMI > 30). Fat mass and percent body fat were measured by bioimpedance. GH sensitivity was assessed by an IGF-I generation test, with IGF-I levels measured before and 24 hours after a single, standard 7mg dose (21IU) of GH. The increment in IGF-I was greater in obese than normal-weight equivalents, negatively correlated with baseline IGF-I concentration, positively correlated with GH binding protein (GHBP) level, and seen in both men and women (pre- and post-menopausal). GHBP concentrations were higher in obesity, and also correlated with BMI, fat mass and percent body fat. The authors concluded that their study provides evidence of increased GH sensitivity in obesity. The fact they used a single, standard GH dose makes the result cleaner than earlier studies that employed a weight-based GH dosing scheme; IGF-I levels were higher in obese subjects, but in those studies, the obese subjects also received a greater GH dose. Because GHBP is the extracellular domain of the GH receptor (GHR), it is sometimes used as an indirect measure of GHR number. The finding of a positive association between GHBP level, markers of obesity and IGF-I increment led the authors to hypothesize that the enhanced GH sensitivity of obesity may be due to increased GHR density, itself resulting from the lower GH levels. Because the data are all associative, further studies are needed to test this hypothesis.

Gleeson HK, Lissett CA, Shalet SM. IGF-I response to a single bolus of growth hormone is increased in obesity. *J Clin Endocrinol Metab* 2005;90:1061-7.

**Editor's Comment:** *This paper clearly showed increased*

*hepatic sensitivity to GH in obesity, at least in terms of IGF-I generation, which helps to explain the discordance between the low GH but normal IGF-I levels seen in obesity. The pediatric correlate of this adult study is the enhanced growth frequently experienced by obese children who continue growing despite GH deficiency (classically, craniopharyngioma patients who develop hypothalamic obesity and GH deficiency); the growth without GH phenomenon is reviewed in Reference 1. Proposed mechanisms include hyperinsulinism-stimulated growth, decreased IGFBP-1 levels resulting in increased bioavailable (free) IGF-I, and increased growth plate stimulation by sex steroids (increased aromatization by the greater adipose mass). An interesting finding came from studies of a model of endochondral ossification, the chondrocyte population of the skeletal growth centers in the mouse mandibular condyle. The growth center chondrocytes expressed leptin receptors and when stimulated by leptin, increased expression of IGF-I receptor, increased both proliferation and differentiation processes, and had larger growth plate growth.<sup>2</sup> Furthermore, when mice were calorie-restricted by 40%, circulating IGF-I levels dropped by 70% and tibial growth decreased by 5%; leptin treatment corrected the growth deficit despite further reductions in circulating IGF-I levels.<sup>3</sup> Thus, the growth-promoting consequences of obesity are multi-factorial, and it will be interesting to see if enhanced hepatic GH sensitivity, perhaps due to increased GHR density, also plays a role in the growth of obese children.*

Adda Grimberg, MD

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