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The Endocrine Function of Adipose Tissue

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INTRODUCTION

The traditional view of the adipocyte as a passive receptacle for storage and combustion of triacylglycerol is undergoing rapid change. It is now recognized that a variety of adipocyte and adipose stromal cell derived proteins act both locally and distally through autocrine/paracrine and endocrine effects to regulate fat cell differentiation, and sense and adjust systemic energy balance.¹ These adipokines are molecules that were previously identified to be derived from immune cells, while others, cytokines produced by adipocytes, were known to be involved in hemostasis, inflammatory response, vasoregulation, and steroid metabolism (Figure 1). Many of these proteins increase as fat mass accumulates and, thus contribute to the multiple morbidities of obesity. Increased activity of three of these, tumor necrosis factor, interleukin 6, and resistin, play a role in the development of the insulin resistance present in obesity. In contrast, other adipokines, like adiponectin and leptin, are insulin sparing through stimulatory effects on the beta oxidation of fatty acids in skeletal muscle.

The concept of "lipotoxicity" postulates that the accumulation of excess lipids in hepatocytes and skeletal muscle cells interferes with insulin signaling,² and the increased lipolytic activity of visceral fat contributes to this process by shunting fatty acids through the portal vein to the liver. Local overproduction of glucocorticoids in visceral fat ("Cushing's disease of the omentum") is also pathogenic. Increased activity of 11 hydroxysteroid dehydrogenase (11 HSD-1) raises adipose tissue cortisol levels, adversely partitioning fat into visceral sites and stimulating release of metabolically harmful adipokines.² Many of these adipokines also act centrally. Leptin, tumor necrosis factor (TNF) and interleukin (IL-6) enter the hypothalamus where they affect sympathetic tone, feeding behavior, thermogenesis, reproduction, and the activity of various hypothalamic-pituitary axes. Adipocyte

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Letter from the Editor:

The lead article in this issue covers a very current topic, one which pediatric endocrinologists may not be thoroughly familiar or are just beginning to incorporate into their sphere of interest (outline of article at www.gghjournal.com). However, it is a subject about which we all will be hearing a great deal more in the near future as pediatric endocrinologists become more involved in the care of obese patients. The epidemic of obesity is confronting our profession more than ever. Consequently, most readers of *Growth Genetics and Hormones* will benefit from having this article as a source for reference to broaden their knowledge about The Endocrine Function of Adipose Tissue. To serve this purpose the presentation of this article by necessity was very inclusive and written as an introduction to, and compilation about, the existence and known function of the many hormones outlined in the text. Dr. Diamond is to be commended for undertaking a difficult task and achieving the intended goal.

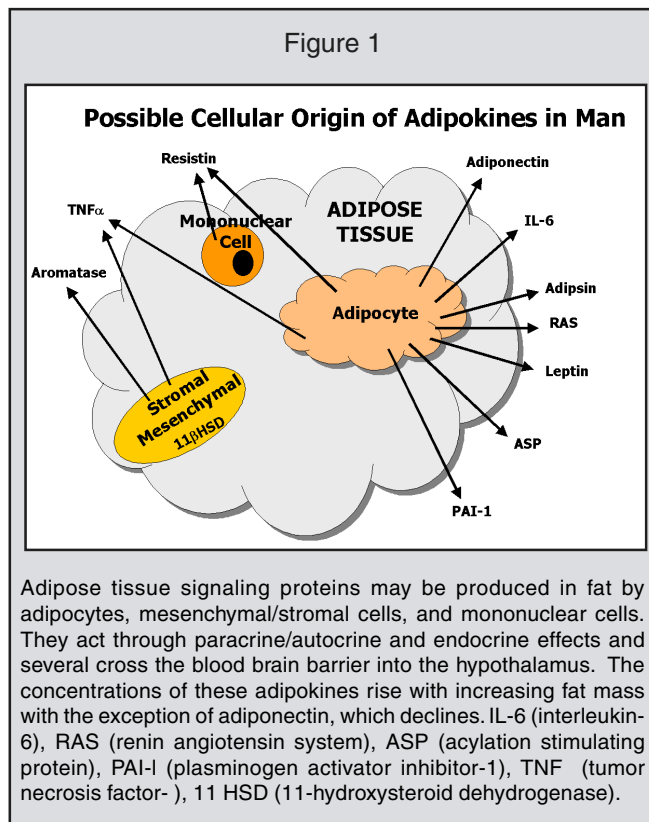
For the Editorial Board
Robert M. Blizzard, MD
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differentiation is controlled by the nuclear transcription factor, peroxisome proliferator activated receptor (PPAR)(Figure 2).³ As energy surplus develops, adipocyte differentiation and lipid accumulation are inhibited through feedback loops of adipocyte-derived factors such as TNF, angiotensinogen (AGT), and resistin (for resistance to insulin). When energy deficit occurs, there is a decline in other adipocyte secreted proteins, such as adiponectin and leptin, and there is activation of trophic proteins such as acylation stimulating protein (ASP) and angiotensin II (AngII). These signal a drive to adipocyte formation and renewed triglyceride accumulation. Insulin is central to this process, promoting lipogenesis and energy storage. The development of insulin resistance which is concomitant with excessive accumulation of body fat may signify a physiologic counter regulation activated to maintain energy homeostasis of the adipocyte. As body fat accumulates beyond that needed for energy balance, and as adipose tissue is chronically exposed to excess dietary fatty acids and glucose, there are further maladaptive responses of adipokines, which result in insulin resistance, inflammation, hypertension, and endothelial disease.

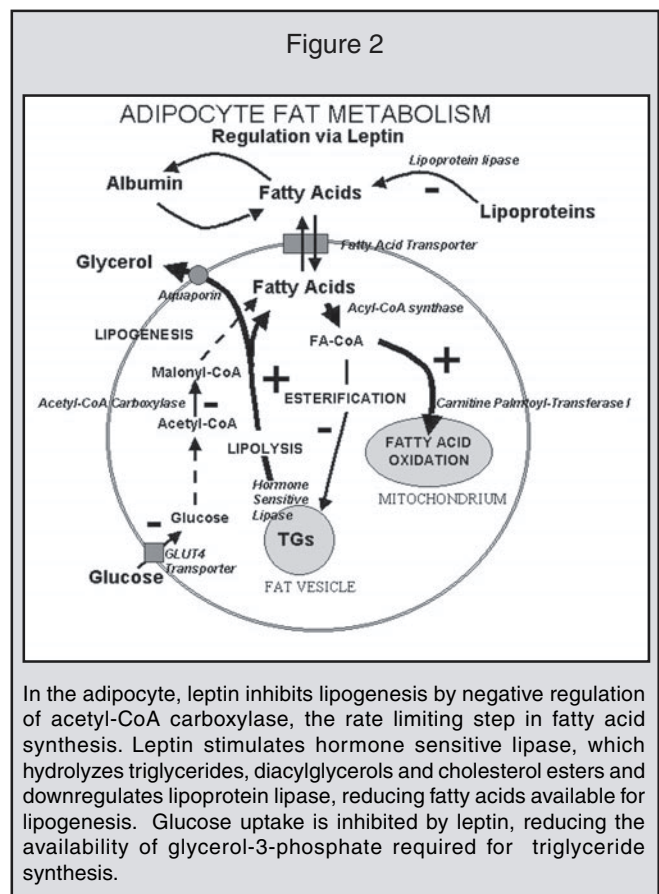
A review of the function and regulation of adipokines is made in this paper to facilitate the understanding by which obesity may contribute to the pathogenesis of the complications of this disease and of the alterations associated with this condition.



ADIPOKINES ASSOCIATED WITH INSULIN SENSITIVITY

Adiponectin

Adiponectin [Adipocyte complement-related protein (ACRP)], a soluble defense collagen, which is a circulating matrix-like protein, is expressed abundantly and exclusively in white adipose tissue.⁴ Adiponectin appears to be an endogenous anti-inflammatory and anti-atherogenic factor that is protective against insulin resistance and macroangiopathy.⁵ Its serum concentrations are reduced in obese mice and humans and rise following weight loss. This suggests that adiponectin plays a negative feedback role in fat storage.⁶ Levels are lower in men compared to women and in individuals with obesity, type II diabetes, and coronary artery disease as compared to healthy subjects.⁷ Its concentrations correlate with the insulin sensitivity state and with steady state plasma glucose, and rise in response to insulin. The protein is not an insulin sensitizer, however, but protects insulin action by accelerating beta oxidation of free fatty acids in skeletal muscle.⁸ Intravenous administration of the "fat burning" c-terminal globular region of AdipoQ, the mouse homologue of adiponectin, reduces circulating free fatty acids and diet induced weight gain and corrects both hyperglycemia and hyperinsulinemia in genetically obese animals.⁹ Hypoadiponectinemia may also



contribute to the insulin resistance of lipoatrophic animals, explaining the apparent paradox of glucose intolerance in both obese and fat depleted models. Adiponectin is highly regulated during adipocyte differentiation and may mediate some of the insulin-sensitizing effects of thiazolidinedione (TZD) binding to PPAR. Clinically, treatment of insulin resistant human subjects with TZDs significantly increases plasma adiponectin concentrations without affecting body weight. Additionally, adiponectin suppresses phagocytic activity, macrophage release of $TNF\alpha$, and transformation of macrophages to foam cells in vitro. It also is deposited in vascular smooth muscle to protect vessel walls and thereby modulates the disease risks of coronary artery disease.¹⁰

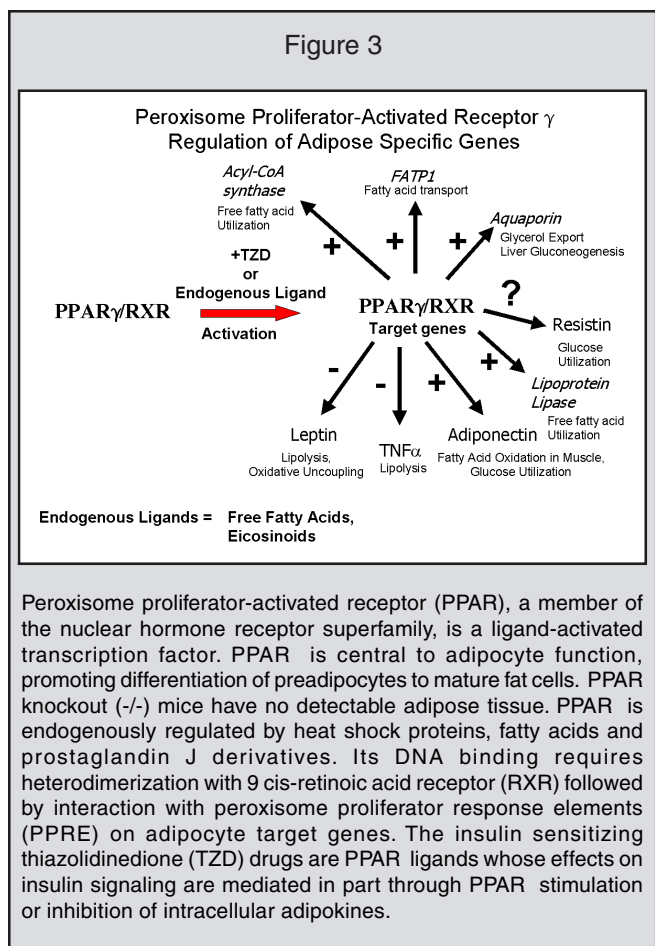
Leptin

Leptin is a 16 kDa adipocyte-derived cytokine synthesized and released from fat cells in response to changes in energy stores and in systemic energy balance. Leptin's primary physiologic function is the defense of body fat. Declining levels in adipose tissue and serum signal the presence of energy deficit to the brain. Leptin circulates partially bound to plasma proteins and enters the CNS by diffusion through capillary junctures in the median eminence and by saturable receptor transport in the choroid plexus. In the hypothalamus leptin binds to long receptor isoforms which stimulate anorexigenic and inhibit orexigenic peptides.^{11,12} Leptin also increases sympathetic nervous system activity and energy expenditure.¹³ Adipocyte levels of leptin mRNA and protein correlate closely with both circulating leptin values and total body fat.

Leptin's lipolytic role in adipocyte metabolism is shown in Figure 3. Leptin reduces the levels of intracellular lipid in skeletal muscle, liver and pancreatic beta cells, thereby improving insulin sensitivity. In muscle this insulin sensitizing effect is achieved through inhibition of malonyl CoA, permitting increased transport of fatty acids into mitochondria for beta oxidation. These changes are partially mediated by central sympathetic activation of adrenergic receptors.²

Leptin synthesis is both constitutive and hormonally controlled. It is influenced by the state of energy reserve, and it is modulated by the sympathetic nervous system through an inhibitory feedback loop. Both adipocyte size and location dictate leptin production, although the mechanism(s) of these paracrine/autocrine modulated effects remain largely undefined. Larger fat cells contain more leptin than smaller ones and subcutaneous fat releases more leptin than visceral fat.^{14,15} Several experimental findings suggest that glucose is an important regulator of adipocyte leptin release.¹⁶ In cultured rat adipocytes, glucose inhibitors block leptin synthesis. In man, glucose infusion attenuates the rapid

Figure 3



fasting decline of leptin. The hexosamine biosynthetic pathway into which 2-3% of cellular glucose uptake enters may mediate this link. Exposure of isolated subcutaneous adipocytes to UDP-N-acetylglucosamine (an end product of hexosamine biosynthesis) increases leptin release. Its inhibition reduces glucose-stimulated leptin release and ob gene expression. UDP-N-acetylglucosamine levels in human subcutaneous adipose tissue correlate significantly with both body mass index (BMI) and serum leptin levels.¹⁷

Insulin stimulates the secretion of leptin when administered to human subjects for several days. In adipocytes from rat white adipose tissue, leptin is present in the endoplasmic reticulum in the absence of insulin, whereas it localizes into the plasma membrane following insulin treatment.¹⁸ Glucocorticoids, whose effects may be primarily permissive, induce leptin synthesis in vitro and in vivo, with greater responsiveness in obese as compared to lean individuals.^{19,20} Females produce more leptin than males when matched for age, weight and body fat. This is probably related to gender differences in fat depots and to the leptin-suppressive effects of testosterone. At birth, the leptin concentrations in umbilical cord blood from girls are double those present in boys.²¹ Pulsatile

leptin secretion correlates with female sex hormones. However, there are conflicting data regarding the influence of ovarian sex steroids on leptin release.^{22,23} Other controlling factors are listed in the addendum.²⁴⁻²⁶

The prevailing evidence of the physiologic role of leptin suggests that it is an anti-obesity hormone, but this concept must be reconciled with the inability of high endogenous leptin levels to prevent most obesity. It appears that in the majority of cases there may be leptin resistance mediated by inhibition of leptin signaling, thereby altering the dominant role of this hormone as a signal to switch between fed and fasted states.

ADIPOKINES ASSOCIATED WITH INSULIN RESISTANCE

Resistin

Resistin is a 12.5 kDa cysteine-rich adipocyte secreted protein which was identified during the screening for genes induced during adipocyte differentiation. This adipokine is down regulated by TZDs. It also is known as Fizz3 (for found in inflammatory zones). Worthy to note is that resistin is one of a family of similar molecules present in fat. Resistin administered to wild type animals induces insulin resistance, but in the obese-insulin resistant mouse it restores normal insulin sensitivity.²⁷ In morbidly obese humans, resistin mRNA from adipose tissue samples is increased as compared to that in lean controls.²⁸ However, a number of clinical and experimental observations suggest that resistin may not be the long sought major link between human obesity and insulin resistance.²⁹

Tumor Necrosing Factor

TNF α is a multi-potential cytokine with diverse immunologic functions. Initially it was described as a cause of tumor necrosis in septic animals and was associated with cachexia-inducing states, such as cancer and infection.³⁰ In obese humans TNF α and its receptors (TNFR1 and TNFR2) are synthesized and secreted in increased amounts by adipocytes and stromovascular cells. Their autocrine effects contribute to the insulin resistance of obesity and diabetes;³¹ TNF α inhibits insulin action by down regulating GLUT4 mRNA in fat and muscle. It also reduces insulin receptor autophosphorylation and phosphorylation by decreasing insulin receptor substrate-1. Circulating free fatty acids (FFA) increase from the lipolytic effects of TNFR1.³² TNF α induces lipolysis which is blocked by PPAR ligands in insulin resistant animals.³³ In man, TNF α concentrations decline with weight loss and treatment with TZDs. The administration of TNF α causes hyperinsulinemia without hypoglycemia.³⁴

TNF α also has important effects on the hypothalamus. In rats, intravenous or intracerebroventricular injection of

TNF α stimulates ACTH secretion through eicosanoid cyclooxygenase mediated release of CRH and inhibits secretion of TSH.³⁵ Thus, TNF appears to have a net effect in prevention of obesity through the inhibition of lipogenesis and increased lipolysis with facilitation of adipocyte death via apoptosis.

Interleukin-6

In man, ~30% of circulating IL-6 originates from adipose tissue.³⁶ Concentrations are higher in visceral fat as compared to subcutaneous fat. They increase with obesity and are stimulated by TNF and IL-1.³⁷ Elevated levels are associated with increased risk of coronary artery disease, athero-sclerosis, and unstable angina.³⁸ Acting on the liver, IL-6 is a primary stimulant of acute phase reactants, such as C-reactive protein, fibrinogen and haptoglobin, thus contributing to a hypercoagulable state. Importantly, IL-6 also promotes the release of endothelial adhesion molecules³⁹ and adversely affects insulin sensitivity by inhibiting GLUT-4, hepatic glycogenesis, and lipoprotein lipase. The resultant lipolysis increases non-esterified free fatty acids (NEFA) which impedes nitric oxide mediated endothelial vasodilation.⁴⁰

IL-6 receptors are present in the hypothalamus where IL-6 stimulates thermogenesis and satiety by increasing prostaglandin synthesis and release of corticotrophin releasing hormone (CRH).⁴¹ It remains to be determined whether IL-6 is a link between obesity and thromboembolic complications.

ADIPOCYTE PROTEINS AND LIPID METABOLISM

Adipsin

Adipsin (ADIPocyte-trypSIN) is a 24-kDa adipocyte secreted protease with close homology to human complement D. This protease is required for the synthesis of acylation stimulating protein (ASP) (*vide infra*), which is described below and which is an important mediator of lipogenesis. Although adipsin concentrations are reduced in rodent models of obesity, paradoxically they are increased in humans with excess adiposity;⁴² for example in obese Pima Indians serum adipsin levels are 45% higher than in non-obese Pimas or other controls. In subjects with anorexia nervosa the adipsin levels are low and rise during refeeding. Insulin stimulated adipsin release is mediated by ADP-ribosylation factor 6 (ARF6) which acts on endocytotic and recycling pathways in the adipocyte; therefore being an important protein in fat metabolism.⁴³ Adrenalectomy of ob/ob mice raises circulating adipsin levels; and corticosterone replacement reverses these changes. Adipsin secretion also is stimulated in animals by sympathomimetic agents, but not by cold stress.⁴⁴

Acylation Stimulating Protein (ASP)

ASP is a 76-amino acid protein that stimulates fatty acid uptake and esterification into triglycerides. Retinoic acid (transported as retinyl ester by transthyretin and chylomicrons) stimulates the C3 gene leading to increased postprandial production of ASP.⁴⁵ Up to a quarter of patients with coronary artery disease have elevated concentrations of ASP. Hyperapobeta-lipoproteinemia, a familial dyslipidemia characterized by increased hepatic release of LDL and VLDL, may result from impaired adipose tissue actions of ASP.⁴⁶ In the ASP-knockout mouse, postprandial triglyceride clearance is delayed and weight gain decreased. Like insulin and additive to it, ASP promotes movement of glucose transporter vesicles in cell membranes in adipose tissue and muscle by activation of the diacylglycerol/protein kinase C pathway.⁴⁷ This provides glucose substrate for glycerol-3-phosphate synthesis of fatty acids and triglycerides. Thus a deficit of ASP results in increased post prandial fatty acids and decreased weight gain and triglyceride synthesis.

Aquaporin Adipose (AQPap)

AQPap is an adipose specific glycerol channel gene abundantly and exclusively expressed in white adipose tissue. AQPap regulates glucose homeostasis by controlling the flux of glycerol into hepatic gluconeogenesis. In wild-type mice, AQPap expression increases during fasting, and declines with refeeding. This takes place through insulin action at the AQPap promoter's negative insulin response element (IRE).⁴⁸ AQPap is increased in adipose tissue from TZD treated mice and reduced in PPAR +/- heterozygous knock-out rodents.

ADIPOKINES & HEMOSTASIS

Plasminogen Activator Inhibitor-1 (PAI-1)

PAI-1, which is synthesized in the liver and in adipose tissue regulates thrombus formation by inhibiting the activity of tissue-type plasminogen activator, an anti-clotting factor. PAI-1 concentrations in serum increase in proportion to visceral adiposity and are entrained by adipocyte size and lipid content.⁴⁹ Omental tissue explants secrete significantly more PAI-1 than subcutaneous tissue from the same subject.⁵⁰ Increased PAI-1 levels are found in patients with coronary artery disease and following myocardial infarction, while levels decline with caloric restriction, exercise, weight loss, and treatment with metformin.⁵¹

THE ADIPOCYTE RENIN-ANGIOTENSIN SYSTEM (RAS)

A renin-angiotensin system (RAS) located in the intra adipose tissue regulates fat cell mass and energy stores through paracrine/autocrine effects on adipocyte

differentiation and lipid storage. Angiotensinogen (AGT), renin, angiotensin-converting enzyme (ACE), angiotensin II (AngII) and its receptors (ATI, AT2), and the non-renin-angiotensin enzymes chymase, cathepsins D and G, and tonin, are all expressed by adipose tissue.⁵² Plasma AGT, renin activity and ACE correlate positively with body mass index while adipose tissue AGT expression correlates significantly with waist-to-hip ratio in man.⁵³ Adipose tissue AngII controls terminal differentiation of preadipocytes to adipocytes through the action of prostacyclin (PGI₂) and regulates adipose tissue blood supply. Adipose tissue AGT also influences adipocyte vascular resistance, but negatively regulates fat mass by decreasing lipogenesis. Ang II and AGT receptors are found in higher concentrations in visceral fat as compared to subcutaneous adipose tissue in both lean and obese individuals.⁵⁴ Glucocorticoids in the presence of insulin, and beta-adrenergic stimulation, and nutritional changes modulate adipocyte AGT gene expression.⁵⁵ In man, the role of the adipocyte RAS in the relationship between obesity and hypertension remains to be further defined.⁵⁶

ADIPOSE AROMATASE AND INTRAADIPOSE GLUCOCORTICOIDS

Aromatase

Sex steroids are not synthesized de novo in fat, but are formed by the action of stromal enzymes on adrenally derived precursors. In human adipose tissue aromatase activity is principally expressed in mesenchymal cells of undifferentiated preadipocyte phenotype.⁵⁷ P450arom, a heme protein product of the CYP 19 gene, converts androstenedione to estrone. Estrogen production in fat rises as body weight increases and as subjects age.⁵⁸ Importantly, adipose tissue-derived estrogens partition fat to subcutaneous and breast tissues, while androgens promote central or visceral fat accumulation.⁵⁹ Aromatase activity varies significantly by region, with greater expression in adipose tissue from buttocks and thighs compared to that from abdomen and breasts.⁶⁰ In vitro, aromatase expression is stimulated by glucocorticoids in the presence of serum, and by class I cytokines. TNF increases aromatase expression in adipose stromal cells exposed to dexamethasone; leptin has little effect.⁶¹ In the aromatase deficient ArKO mouse which lacks a functional Cyp 19 gene, there is a progressive accumulation of intra-abdominal fat and reduced lean body mass.⁶²

11- HYDROXYSTEROID DEHYDROGENASE

11-hydroxysteroid dehydrogenase (11 HSD-1), which regenerates metabolically active cortisol from cortisone in man and corticosterone from 11 dehydrocorticosterone in mice, is increased in adipose tissue from obese

subjects. Adipose tissue corticosterone was overproduced by 30% in a transgenic (Tg) mouse that modestly over expresses 11 HSD in all its adipose tissues. The Tg male animals disproportionately accumulated visceral fat in adipocytes which were three times the size of those of control animals. The mice became hyperphagic, hyperglycemic, and hyperinsulinemic, had reduced levels of adiponectin and uncoupling protein-I, and had increased concentrations of leptin, TNF, angiotensinogen, lipoprotein lipase, and portal free fatty acids. This clinical and biochemical pattern mimics the human "metabolic syndrome".⁶³ In humans thiazolidinediones significantly reduce 11 HSD-I mRNA in vitro and in vivo, and preferentially reduce visceral fat.⁶⁴

OTHER ADIPOCYTE PROTEINS

Metallothionein is an adipocyte secreted low molecular weight metal binding and stress response protein which may function to protect fatty acids from oxidative damage.⁶⁵ The metallothionein genes (MT-I, MT-2) are expressed in adipocytes early in their differentiation process. In vitro, MT-I transcription is stimulated by dexamethasone, forskolin and bromo-cAMP, and to lesser extent by insulin and leptin. Fasting-induced adipose factor (FIAP), a circulating fibrinogen-angiopoietin-related protein, is an adipocyte derived protein which increases during caloric deprivation and interacts with PPAR.⁶⁶ Lipoprotein lipase, cholesteryl ester transferase, apolipoprotein E, and retinol binding protein are other adipocyte proteins important for lipid metabolism which are under study.

CONCLUSION

The mechanisms by which obesity contributes to insulin resistance, hypertension, and endothelial disease are among the most important scientific questions facing medical investigators today. Research into the function and regulation of adipocyte signaling proteins, adipocyte differentiation, and the control of fat partitioning will likely result in further insight into these mechanisms and the discovery of targeted therapies for treatment of obesity and obesity related diseases.

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Addendum (re Leptin)

Many regulatory sites for leptin are found within the ob gene promoter, including cyclic AMP and glucocorticoid response elements, as well as loci for CCATT/enhancer and SP-1 binding.^{24,25} Thiazolidenediones reduce leptin mRNA in adipocyte 3T3-L1 cells through negative PPAR effect at the leptin promoter.²⁶ Peripheral leptin administration activates suppression of cytokine signaling-3 (SOCS-3) which is co-expressed in hypothalamic nuclei with long-form leptin receptors. Increased SOCS-3 expression in vitro has been shown to blunt leptin receptor signal transduction by inhibiting JAK activity. SH2-containing phosphatase 2 (SHP-2) also blocks STAT-3 mediated leptin transcription. Moreover leptin is negatively regulated by the sympathetic nervous system via beta-2 and beta-3 catecholaminergic input at the adipocyte. The increased sympathetic enervation in visceral fat may thus partly explain its reduced leptin content compared to subcutaneous fat tissue. Infusion of isoprenaline or epinephrine in man acutely suppresses leptin release, as does cold exposure. Growth hormone, thyroid hormone, and melatonin have also been shown to decrease leptin secretion.

Abstracts from the Literature

Celiac Disease in Children with Autoimmune Thyroid Disease

This study was designed to test for the presence of celiac disease among children with autoimmune thyroid disease (ATD). Ninety patients (78 females) ages 1.8 to 17.3 years with ATD were studied; 20 of them had Graves' disease, and 16 had other associated conditions i.e. alopecia (4), vitiligo (2), juvenile rheumatoid arthritis (2), autoimmune hepatitis (2), Down's syndrome (1) and other miscellaneous autoimmune alterations (5). Screening for IgA antiendomysium antibodies (EMA) and HLA typing for Class I and II DQA1 and DQA2 heterodimers were done. There were 7 patients with positive EMA; an intestinal biopsy in these patients revealed intestinal villi alterations, with partial or total atrophy, crypt hyperplasia and intraepithelial lymphocytes. Clinically, one of the celiac disease patients had iron deficiency, one had diarrhea, and one had short stature, while the others were asymptomatic. A significant positive correlation was present for celiac-susceptible heterodimers in the patients with celiac disease. The authors concluded that screening for celiac disease should be done on all patients with ATD.

Larizza D, et al. *J Pediatr* 2001;139:738-740.

Editor's Comments: *This report is one more in the recent literature documenting the presence of celiac disease among patients with endocrinopathies. The prevalence of celiac disease in patients with ATD was 7.7% which is higher than that observed in other studies of adults with ATD, and of course much higher than the 1% reported in normal populations.¹⁻³ In Vol 17 No 2 of Growth Genetics & Hormones, I abstracted and commented upon the article describing the presence of celiac disease in 4.6% of children with type I diabetes.⁴ Celiac disease was a significant factor in the development of hypoglycemia complicating the course of the diabetic illness. The presence of celiac disease in the patients in this study, as well as those in other reports, was without clinical evidence of malabsorption and the patients were largely asymptomatic.*

Nonetheless, it has been suggested that the presence of unidentified celiac disease could play a role in the development of autoimmune disorders, and the prompt diagnosis and treatment of this disease could prevent the onset of other alterations.⁵ The availability of an accurate, sensitive and specific test (IgA antiendomysium antibodies) to screen for celiac disease should not be overlooked by Pediatric Endocrinologists who in my opinion should test all patients with autoimmune endocrine disorders regularly for antibodies reflecting the presence of celiac disease.

Fima Lifshitz, MD

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The Role of Fetal-Maternal Microchimerism in Autoimmune Disease

Over the last 4 or 5 years, more and more diseases are described in which fetal cells are found at the site of autoimmune maternal disease and more recently maternal cells are being found at the site of newborn destructive ("graft-versus-host") diseases. Many diseases including systemic sclerosis and fetal dermatomyositis have now been attributed to fetal-maternal microchimerism. The report by Klinschar et al adds to the evidence that Hashimoto's thyroiditis includes fetal microchimerism in the fetal thyroid gland. These authors took thyroid gland specimens, extracted DNA, and then used Y probes to look for evidence of male cells in the maternal thyroids. They specifically used thyroid glands from women who had male children, and found evidence of male microchimerism in half the specimens. Among the controls (nodular goiter), only 1/25 specimens had evidence of Y chromosome microchimerism.

The importance of this observation is related to the question of whether the fetal cells can be a cause of autoimmune diseases since there is an excess of thyroid autoimmune disorders in females. The molecular

techniques presently used look for Y DNA probes in females and female cells in males. The new molecular techniques allow this sort of recognition. It seems likely that all of us carry some maternal stem cells and that women who have been pregnant carry fetal cells, which can respond to damage and stress. What is not clear is whether the fetal cells are the cause of auto immunity or simply represent a stem cell response to injury.

Klinschar M, et al. *J Clin Endocrinol Metab* 2001;86:2494-2498.

Editor's Comment: *It will be important to look at multiple tissues for fetal cells. It appears that pregnancies which have been complicated are more likely to have fetal cells in circulation. Thus, pre-eclampsia and aneuploidy are known to have increased trafficking between mother and fetus. In addition, loss of co-twins can predispose to microchimerism. Keep your eyes open for more work in this area since it is highly likely that additional papers will try to discriminate the source of the cells, and determine the time at which they would have migrated to specific tissues.*

Judith G. Hall, OC, MD

Table

Number of children, sons, and daughters in Hashimoto patients with and without detectable microchimerism

Patient no.	No. of children	No. of daughters	No. of sons	Microchimerism
1	4	2	2	Yes
2	1	0	1	Yes
3	3	1	2	Yes
4	2	1	1	Yes
5	2	1	1	Yes
6	2	1	1	Yes
7	4	1	3	Yes
Mean	2.57	1	1.57	
9	1	0	1	No
10	2	1	1	No
11	1	0	1	No
12	1	0	1	No
13	0	0	0	No
14	0	0	0	No
Mean	0.83	0.17	0.67	
P value	0.009	0.013	0.035	

Patients with microchimerism have significantly more children (sons and daughters) than patients without microchimerism, whereas no differences were found between the latter patients and controls.

Adapted from Klinschar M, et al. *J Clin Endocrinol Metab* 2001;86:2494-2498.

Mutations in *PTPN11*, Encoding the Protein Tyrosine Phosphatase SHP-2 Cause Noonan Syndrome

In approximately 50% of subjects with Noonan syndrome (NS is mapped to chromosome 12q24.1) the investigators identified mutations in the 15 exon gene (*PTPN11*) encoding the non-receptor protein [tyrosine phosphatase (PTP) - SHP-2]. This protein has two SH2 (Src homology docking) domains and a long enzymatic domain with the sites interacting to achieve an active or inactive state of function. Diverse missense mutations were found in the third exon encoding the amino-terminal SH2 (Src homology) domain and in three exons (7,8,13) encoding the PTP domain that apparently rendered the protein constitutively active. SHP-2 is a component of several intracellular signal transduction systems involved in embryonic development that modulate cell division, differentiation, and migration, including that mediated by the epidermal growth factor receptor. The latter pathway is important in the formation of the cardiac semilunar valves. The mutations associated with NS are in conserved amino acid sites in which the alteration leads to conformational changes that "lock" the protein in its enzymatically active state. The down-stream pathways that are affected by this "positive" change in enzyme activity have yet to be identified.

Tartaglia M, et al. *Nat Genet* 2001;29:465-468.

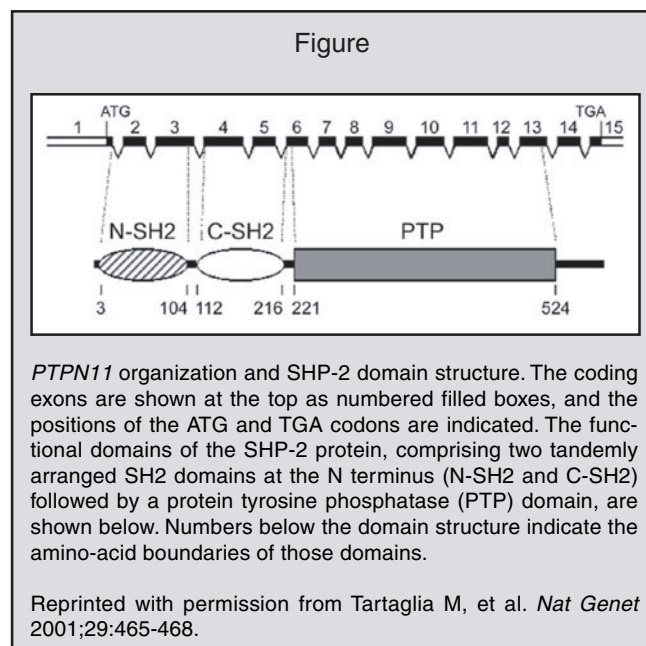
Editor's Comment: Noonan syndrome (OMIM 163950) is characterized by "Turner-like" facial features, short stature, webbed neck, cubitus valgus, pulmonic stenosis (rather than coarctation of the aorta which is frequent in Turner syndrome), developmental delay, and bleeding diathesis. Since the Noonan phenotype is genetically heterogeneous, other genetic errors may exist, including mutations in the non-coding regions of *PTPN11* that were not determined in the present report. The short stature and many of the skeletal abnormalities found in patients with Leri-Weill dyschondrosteosis and Turner

syndrome (TS) have been attributed to haploinsufficiency of *SHOX* (chromosome Xpter-p22.32) either due to its deletion or to loss-of-function missense or nonsense mutations.^{1,2} Given the visual similarity of the NS and TS phenotype, it will be of interest to determine if the proteins regulated by *PTPN11* and *SHOX* interact. Might the product of *SHOX* be an inhibitor of SHP-2 generation or activity?

Allen W. Root, MD

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Mothers with Congenital Adrenal Hyperplasia (CAH) and their Children: Outcome of Pregnancy, Birth and Childhood

The authors examined the gestational history of 122 women with 21-hydroxylase deficient CAH which was confirmed by genotyping in the majority. These women were born after 1948, followed in the investigators' clinic (University Children's Hospital, Munich) and were over 20 years of age at the time of study. Eighteen of the 122 women (15%) had delivered 31 children. The diagnosis of the 18 mothers was as follows: salt-losing, 1 of 48 total (2%); simple virilizing, 12 of 64 total (19%), and non-classical, 5 of 10 total (50%). The woman with

salt-losing CAH had two miscarriages before delivering her child. One woman with non-classical CAH had two tubal pregnancies.

Conception occurred between 18-36 years (mean 28 years). The pregnancies were uneventful with the women receiving hydrocortisone, prednisone, prednisolone, or dexamethasone during gestation. Sixteen pregnancies required cesarean sections, primarily in women not having nonclassical CAH. Five of the 31 offspring were <10th percentile for gestational

age. One developed an intracerebral hemorrhage. An additional patient was microcephalic at birth. None of the 18 female offspring had malformation of the external genitalia. Follow-up of the 31 offspring, 6 of whom were less than 5 years of age, 7 of whom were between 5-10 years, and 18 who were older than 10 years of age at the time of evaluation, revealed that all were growing, maturing, and developing normally.

Krone N, et al. *Clin Endocrinol* 2001;55:523-529.

First Editor's Comment: *These data are encouraging in that women with simple virilizing and non-classical CAH are often able to conceive and deliver healthy children, thus confirming previous reports. More data on the degree of adrenal suppression during pregnancy, and knowing post-natal neonatal adrenal function would have been of interest.*

That only one of 48 women with salt-losing CAH had an infant illustrates the difficulties still encountered in the management of many of these patients. As Krone et al discuss, the relative infertility of women with CAH may be due to hormonal (hyperandrogenism), anatomic (inadequate reconstruction of the vagina), or psychosocial factors (behavioral masculinization, low marriage rate, and/or sexual preference). It is anticipated that prenatal detection and treatment of females with CAH and establishing neonatal screening programs for this disorder will change substantially the "natural history" of pregnancy in females with CAH.

Regarding surgical reconstruction of the external genitalia in the virilized female, while clitoroplasty may be appropriate in the neonatal period, vaginoplasty

should be deferred until the peri menarchal period, as earlier reconstructive surgery is usually inadequate.¹ In 39 adolescent phenotypic females (20 with CAH) (mean age at examination 15 years) who underwent vaginal surgery in infancy at a median age of 10 months, Creighton et al found that approximately 60% had a good or satisfactory cosmetic appearance of the external genitalia, but almost all required further surgery to permit use of tampons during menses and, presumably, sexual relations in adulthood.

Allen W. Root, MD

Second Editor's Comment: *Much is being discussed and written in 2002 regarding surgery on the genitalia of patients with enlarged clitorises. The current recommendation of many surgeons and pediatric endocrinologists is that surgery on the clitoris be delayed in most cases in the newborn period. For more details the reader is referred to references 1, 2, and 3 below. A lead article concerning the dilemmas of gender assignment and surgery will be published soon in GGH to provide up-to-date considerations for you our reader.*

Robert M. Blizzard, MD

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3. Ramecroft L and Members of the Working Party of the British Association of Pediatric Surgeons on the Surgical Management of Ambiguous Genitalia. Available from: <http://www.baps.org.uk/documents/Intersex%20statement.htm>.

Growth Hormone Improves Clinical Status in Prepubertal Children with Cystic Fibrosis: Results of a Randomized Controlled Trial

Hardin and colleagues studied the effects of recombinant GH (0.3 mg/kg/wk) in 10 children with cystic fibrosis (CF) (ages 7-12, Tanner stage I) as compared to a control group of 9 similar children. All children recruited for the study were $\leq 10^{\text{th}}$ percentile for both height and weight and had adequate caloric intake as determined on 2 evaluations. Only one had an abnormal growth hormone stimulation test. Children were excluded from the study if they had been hospitalized within 6 weeks or had been treated with systemic or oral steroids within 6 weeks. Evaluations were made of pulmonary functions including forced vital capacity (FVC) and forced expiratory volume at 1 second (FEV₁). In addition, peak expiratory pressure (PEP) and peak inspiratory pressure (PIP) were measured. Resting energy expenditure, was determined using indirect calorimetry, and lean body mass was determined by

whole body dual energy x-ray absorptiometry. Studies were made at baseline and every 3 months. Data were collected with regard to the number of hospitalizations and antibiotic therapy. All data for both the treatment group and the control group were similar at baseline.

The height and weight Z scores were significantly greater in the treatment group after one year than in the control group; furthermore the treatment group had a significant increase in lean body mass. Additionally, at 12 months the treatment group had a significant improvement in percent FVC, PIP, and PEP. There was no significant change in percent FEV₁. The GH treated group had a significant decrease in the number of hospitalizations, although outpatient antibiotic therapy was not different between the two groups. There was no significant change in resting energy expenditure or nutritional intake during the study and carbohydrate

intolerance did not develop in either group. The advancement in bone age over the 12 months was not different between the two groups.

The authors conclude that growth hormone therapy is of significant benefit to pre-pubertal children with CF in terms of their height, weight, body composition, pulmonary function, and number of hospitalizations.

Hardin DS, et al. *J Pediatr* 2001;139:636-642.

First Editor's Comment: *This study by Hardin and associates is the first randomized, controlled trial of growth hormone therapy in children with cystic fibrosis. The findings are highly significant, although they have only been collected for a single year. Many questions remain unresolved. It would be important for studies to be undertaken to determine whether or not the change in lean body mass was due to an improved use of ingested calories and protein as suggested by the authors. In addition, the long-term benefits of treatment need to be evaluated, and the optimal dose needs to be determined. Furthermore, it will be important to follow these children to determine whether or not they are at increased risk for glucose intolerance over time. Hardin and associates have provided the preliminary data necessary to undertake a much larger scale study of the use of growth hormone in these children.*

William L. Clarke, MD

Second Editor's Comment: *Growth Hormone treatment in patients with cystic fibrosis has been shown*

to be of benefit in various short-term trials.^{1,2} However this is the first randomized controlled trial of GH treatment in patients with this disease. Growth hormone resulted in improved clinical status and increased growth. In CF, malnutrition develops as a result of unfavorable energy balance caused by a combination of poor intake, malabsorption of nutrients, chronic pulmonary disease and increased energy expenditures. Malnutrition adversely affects the course of the disease as well as the survival of the patients. Therefore any means to improve the anabolic state of CF patients may be of benefit. In this study GH treatment also improved the quality of life. Nonetheless, detrimental effects of GH treatment could occur in patients with CF, as diabetes is prevalent among this population.³ Although in this study no patient developed this problem, the data cannot be extended to other patients or to those who would undergo a longer-term treatment. It should also be kept in mind that improvements in growth and nutrition status of CF patients may be accomplished with aggressive nutritional supplementation without GH treatment.⁴

Fima Lifshitz, MD

References

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3. Lang S, et al. *BMJ* 1995;311:655-659.
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Intake of Vitamin D and Risk of Type I Diabetes: A Birth-Cohort Study

To ascertain whether vitamin D supplementation or vitamin D deficiency in infancy could affect the development of type I diabetes, a birth-cohort study was done in Oulu and Lapland, Finland. All infants born in 1996 were studied (n = 12,055). Data were collected on vitamin D supplementation and on the presence of suspected rickets during the first year of life. The primary outcome measured was the diagnosis of type I diabetes by the end of 1997 (30 year follow-up). Of the 10,366 children included in the analysis, 81 were diagnosed with type I diabetes. Vitamin D supplementation was associated with a decreased frequency of this disease. Children who took the recommended 2000 IU of vitamin D on a daily basis had a rate ratio of 0.22 of developing the disease, as compared with those who received no vitamin D. The rate ratio in those who received a lesser amount of vitamin D supplementation was 0.12. Children suspected of having rickets during the first year of life had a rate ratio of 3.0 as compared with those without

such diagnosis. The authors concluded that vitamin D supplementation was associated with a reduced risk of type I diabetes.

Hypponen E, et al. *Lancet* 2001;358:1500-1503.

First Editor's Comments: *This is a very provocative study implicating the deficiency of one hormone (vitamin D) on the development of another hormone deficiency (insulin). The mechanisms of such association were thought to be related to the triggering of an immune process resulting from the lack of vitamin D. This is consistent with data from animal studies, and with the observation that cod liver oil supplementation during pregnancy is associated with a reduced rate of type I diabetes in the offspring.¹ The Eurodiab study also showed that vitamin D supplementation in early childhood may prevent this disease.² However, only 0.3% of infants in the Eurodiab study were not given*

vitamin D during the first year of life, thus the comparative population was rather small. The increased prevalence of this disease (3x) among children in this Finnish study, who were suspected of having rickets, is impressive. However the data are not very compelling since there was no radiologic or biochemical evidence of rickets presented.

The infants who took 2000 IU of vitamin D as a daily supplement had a 78% lower risk of developing diabetes. This dose of vitamin D, however, is high and not recommended by most authorities. (The Committee of Nutrition of the American Academy of Pediatrics, among others, state that an adequate intake of this vitamin is 200 IU per day.) Others have recommended dosages ranging from 400 to 1000u per day,³ where there may be lack of sunlight exposure, particularly during the long winter months in the northern hemisphere. Although there is no single recommendation for the amount of vitamin D supplemented, exposure to the sun usually will satisfy the requirements to prevent rickets and vitamin D deficiency. As little as 1 minimal erythemal dose (MED) of sunlight is equivalent to ingesting about 10,000 IU of vitamin D. Simple exposure of hands and face two or three times per week provides a third to a half of the MED (about 5 minutes for fair-skinned people) is more than adequate. Moreover, sunlight is without risk of hypervitaminosis D which may occur when large amounts of vitamin D supplements are ingested. Thus, caution should be exercised to the possible temptation of increasing vitamin D supplementation in an attempt to prevent type I diabetes. Further studies are needed

to assess if there are other factors to ascertain why there is a high prevalence of type I diabetes among populations who also are exposed to insufficient sunlight such as found in Finland.

Fima Lifshitz, MD

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Second Editor's Comment: In the early 19th Century, cod liver oil was given to prevent rickets. The classical role of vitamin D in the prevention of rickets is to assist absorption of calcium and phosphate. Vitamin D also appears to play a role in preventing some cancers and autoimmune diseases. Ideally, in a study such as the one reported here, evaluation would include plasma 25(OH) D or 1,25(OH) 2D₃ concentrations. When sun exposure is limited, as in northern Finland, supplementation or dietary intake is an important source of vitamin D. Breast milk does not contain enough vitamin D to cover an infant's needs. The role of vitamin D in the pathogenesis of type 1 diabetes certainly deserves follow-up. If vitamin D does impair the immune system functioning in infancy, there may be other long-term effects. Interesting as well, Finland has the highest incidence of type 1 diabetes in the world.

Judith G. Hall, OC, MD

Beneficial Effects of Intensive Therapy of Diabetes during Adolescence: Outcomes after the Conclusion of the Diabetes Control and Complications Trial (DCCT)

The DCCT, in 1994, reported the results of intensive diabetes therapy of adolescents (age 13-17 years at the time of enrollment into the study). Those results demonstrated a significant reduction in the risk for the development, and progression of retinopathy and microalbuminuria. Since that time, subjects from both the intensive and conventional therapy groups have been offered the opportunity to participate in the epidemiologic study of diabetes interventions and complications (EDIC). EDIC is a long-term observational study of the DCCT cohort. In this manuscript the DCCT/EDIC research group presents their latest findings. Of the original 195 adolescents, 175 agreed to participate in the EDIC study. At the end of the DCCT all subjects returned to their health care providers in the community for continuing diabetes care, and all conventionally treated subjects were offered instruction in the use of

intensive therapy. Approximately 50% of the subjects continued to receive their care at a DCCT/EDIC site. Subjects were seen on a yearly basis for determination of HbA1c and the recording of severe hypoglycemic episodes. Retinopathy was assessed by stereoscopic fundus photography at year 4, and classified according to the criteria described in the DCCT trial. A 3-step or more progression was classified as significant. Renal function was determined every other year by measurement of albumin excretion.

At year 4, 1/3 of the subjects who were originally randomized to conventional therapy continued to use 1 or 2 injections a day. The rest switched to multiple daily injections or insulin pump therapy. Ninety percent of former intensive therapy subjects continued to use multiple daily insulin injections or pump therapy. Total insulin doses and frequency of blood glucose monitoring

were similar between the 2 treatment groups. The difference in HbA1c between treatment groups was highly significant at the closeout of the DCCT, but by the end of the first year of the EDIC study there were no significant differences in HbA1c levels between the two groups. This was the result of both an increase in HbA1c by the intensive therapy group, and a decrease by the conventionally controlled group. These HbA1c values remained stable over the next 3 years (8.38% vs. 8.45%, intensive vs. conventional). In addition, the relative risk of severe hypoglycemia for patients in the former intensive treatment group was < 1, which was a decrease from the rates during the DCCT, and an increase in hypoglycemic occurrence for the conventionally controlled group. There was no difference in body weight, BMI, or percentage of subjects overweight at year 4 of the EDIC study.

After 4 years of follow-up in the EDIC study, 65% of the original conventionally treated patients showed a 3-step or more progression in retinopathy as compared with 32% of the former intensive group patients. This represents an odds ratio reduction of 74% for those having been in the intensive control group. Thus, the benefits of intensive therapy persisted for an additional 4 years in a significant number despite increased levels of glucose control. Similar findings were observed for the progression of nephrological disease. There was an 85% reduction in the adjusted odds ratio for developing albuminuria in the former intensive treated patients vs. the former conventionally controlled group. Thus, the benefits of previous intensive therapy continued for another 4 years with regard to renal function.

The authors state that these results demonstrate conclusively that the benefits of intensive therapy outweigh any associated risks of hypoglycemia and weight gain, and persist for at least four years. In addition, the data suggest that less than optimal glycemic control during the early years of diabetes (in adolescence) has a long lasting, detrimental effect on

the development of complications even after better glycemic control is established. Thus the recommendation is that intensive therapy be the standard of care for adolescents with type I diabetes mellitus. The DCCT/EDIC study is planned to continue for at least 10 years.

The Diabetes Control and Complications Trial/Epidemiology of Diabetes of Interventions and Complications research group: *J Pediatr* 2001;139:804-812.

Editor's Comment: *The results of the DCCT/EDIC at year 4 in adolescents are not different from those presented for the entire group (New Engl J Med 2000;342:381-389). The findings are important and have significant implications for the treatment of adolescents starting at diagnosis, and perhaps pre-adolescent children with type I diabetes mellitus. Some have assumed that the intensive therapy achieved by the DCCT research group, while important in reducing complications, might not be a reasonable and cost-effective treatment regimen for all adolescents with diabetes. These data prove otherwise. Intensive therapy initiated early in the course of diabetes has prolonged and long-lasting effects of reducing the risks of microvascular complications. Alternatively, diabetes management resulting in poor glucose control during the early adolescent years may be associated with an increased risk of microvascular complications, even after intensive therapy and a reduction in HbA1c has been achieved. Thus, these data support the initiation of intensive diabetes therapy designed to achieve near normal glucose control as early as possible in newly diagnosed adolescents. This must be the standard of care. Patients, their parents, and third-party payers must be educated to understand, demand, and compensate for such treatment.*

William L. Clarke, MD

Growth in Human Immunodeficiency Virus Type 1-Infected Children Treated with Protease Inhibitors

About 33% of children infected with HIV have impaired growth. The extent of such impairment may be regarded as a clinical criterion predicting progression to AIDS. The addition of protease inhibitors (PIs) has been demonstrated to frequently reduce plasma HIV RNA levels, to increase CD4 lymphocyte numbers, and to improve the general condition of children and adults with HIV retrovirus type I infections.

Steiner et al present data on the long-term (72 week) impact of PI treatment on growth of infected children.

Data are reported on 44 children between the ages of 0-17 years with confirmed infection. They were observed for 72 weeks prior to starting PI treatment. Nintonavir or nelfinavir were added to the previous treatment of two nucleoside analogue reverse transcriptase inhibitors. Growth, HIV-1 RNA plasma levels, and CD4 lymphocyte counts were determined at 0, 24, 48, and 72 weeks of treatment. Heights were reported in SD scores as determined from normal aged and gender individuals. Data from 44 children were analyzed in 3 age groups [6

children <3 years of age (group I), 23 children 3-10 years of age (group II), and 15 children >10 years of age (group III)]. All had completed 72 weeks of PI treatment. Multiple regression analyses were used to determine the relationship between parameters of growth and variables such as CD4 cell count and CDC HIV-1 categories. Children in group I were more frequently in the severe CDC clinical category "C" and had higher plasma HIV-1 RNA levels at baseline than those in groups II and III.

By 24 weeks of treatment, there was a significant decrease in mean plasma HIV-1 levels in children of group I vs. those in groups II and III. Twenty-seven of the 44 children showed a sustained reduction of HIV 1 RNA levels. In the 72 weeks before the initiation of PI therapy the differences between Δ -Z scores at 24 week intervals indicated progressive growth retardation which was reversed with a significant increase in growth during the 72 weeks after the PIs were added. This increase was biphasic with a greater increase between weeks 0-24, and a second increase between 48-72 weeks. The greatest increase in growth was in the 6 children in group I, all of whom had significant growth retardation at baseline and in the 4 significantly retarded children in group II. The 19 other children in group II and all 15 in group III had growth rates maintained within 1 SDS of the mean. Growth while receiving PIs was negatively correlated with growth during the preceding period, and positively correlated with an increase in CD4 cells. No correlation was seen between the decrease in plasma HIV-1 levels. Thus, age categories and CDC clinical categories were significantly associated with catch-up growth, but multiple regression analysis revealed that only growth during the preceding period and the age

category were significantly associated with growth during PI therapy.

The authors note that previous studies have shown that stunting has been correlated with higher plasma HIV-1 RNA levels. Of note, the older children in the cohort were not as severely stunted as the younger children, and did not have as significant a growth response to PI therapy. The authors speculate that these findings may be the result of the older children having a slower progression of HIV infection than the younger children, since they survived infancy in the era prior to aggressive therapy. In addition, the authors point out that others have attributed stunting in HIV infected children to sub-clinical hypothyroidism, low IGF-1, or proteolysis of IGF BP3. The authors did not measure these hormone levels.

Steiner F, et al. *Eur J Pediatr* 2001;160: 611-616.

Editor's Comment: *The data reported in this paper by Steiner, et al are important from two aspects. First, treatment with a protease inhibitor can improve growth rates in young HIV infected children. Secondly, those with the greatest catch-up growth are those who are the most stunted initially. Such information is similar to that which has been shown for treatment of nearly every chronic disease of childhood. Unfortunately the authors did not determine biochemical markers of growth, including IGF-1 and IGF BP3. They suggest this be done in future studies. These data might have been useful in helping decide which children could benefit the most from such therapy. The data presented, however, are clinically useful.*

William L. Clarke, MD

Paternal Contribution to Aneuploidy

The relationship of maternal age to chromosomal abnormalities is well established; however, there have been conflicting data with regard to paternal contribution. Of potential pertinence is that 10 – 30% of autosomal trisomies arise during paternal meiosis, 100% of XYYs and 50% of XXYs are paternal in origin, and 80% of Turner syndrome patients are missing the paternal X. Also, an increase in paternal age is associated with the development of uniparental disomy 15, and trisomy 18 is seen with increased paternal age. To further study the relationship of paternal age to diploidy and disomy of sperm, the authors of this paper screened human sperm using four-colour FISH probes. Chromosomes 6, 21, X, and Y were examined to determine the incidence of disomy in sperm related to paternal age where the normal usual sperm are haploid.

Almost 200,000 sperm were examined from 18 healthy donors, ages 24 to 74. The investigators found a significant increase in the level of autosomal disomy and a marginally significant increase in sex chromosome disomy with increasing male age. Significant individual variation was observed. The increase in disomy ranged from 0.3 to 17% for each 10-year period. This suggests that older men have a tendency to show synaptic abnormalities perhaps related to the deterioration of testicular environment with advancing age.

Bosch M, et al. *Euro J Hum Gen* 2001;9:533-538.

Editor's Comment: *There is a growing interest in paternal contributions to congenital anomalies, both potential teratogens and the effect of aging itself. Although triploids are not usually viable, it is interesting*

that paternal age would seem to lead to an increased contribution to triploid conceptions. This could also play some role in triploid-diploid mixaploid individuals. This article is an excellent review of current knowledge pertaining to diploidy, aneuploidy, and disomy in the

sperm of males of various ages and in various chromosomally determined clinical conditions.

Judith G. Hall, OC, MD

New Syndrome of Hyperinsulinism and Hyperammonemia

Although there are many causes of hypoglycemia, a new syndrome associating hyperinsulinism with hyperammonemia was recently described (Zammarachi, et al. *Metabolism* 1996;45:957; Weinzimer, et al. *J Pediatr* 1997;130:661; Stanley, et al. *N Eng J Med* 1998;338:1352). This syndrome is identical or closely related to the leucine-sensitive hypoglycemia syndrome and is congenital in origin. Clinical manifestations are usually observed in neonates and/or infants. The diagnosis of patients with HSS is crucial as therapy differs radically, medical and not surgical, from that of other hyperinsulinemic patients. A positive response to diazoxide- and/or leucine-free diet is usually observed. All but one of the 12 patients in the article by De Lonlay had at least a partial response to diazoxide.

Genetically all 12 cases studied seem to be new mutations, as they occurred sporadically without family histories. This mutation results in a gain of function in the glutamate dehydrogenase gene (GLUD1). It also results in a decreased inhibitory effect of guanosine triphosphate on the enzyme. It has been suggested that the elevated oxidation of glutamate to α -ketoglutarate stimulates insulin secretion by increasing the ATP/ADP ratio in the pancreatic Beta cell, although this is unproven. All 12 patients studied had mutations located within or outside the GTP binding site, without

any correlation between phenotype and genotype. The mutations in the GLUD1 gene are found in exons 6, 10, 11, and 12, which includes the antenna region of the enzyme and the GDP binding domain.

In a review of hyperinsulinemic patients by the authors in their institution over the past 20 years, plasma ammonia concentrations were measured in 71 (45 neonates and 26 infants) and hyperammonemia was found in 12 of the 71. The incidence of this type of hypoglycemia is significant. The authors conclude that ammonia concentrations should be measured in every patient investigated for hyperinsulinism and that, conversely, hypoglycemia should be looked for in all patients with unexplained hyperammonemia.

De Lonlay P, et al. *Pediatr Res* 2001;50:353-357.

Editor's Comment: *Heterogeneity is the name of the game, and molecular techniques allow us to recognize many of the reasons for heterogeneity. Within heterogeneity, many new biochemical pathways and mechanisms of disease are being identified. As in the case of this syndrome, different types of therapy become most appropriate.*

Judith G. Hall, OC, MD

15 Years After Chernobyl: New Evidence of Thyroid Cancer

A striking increase in childhood thyroid cancer was reported after the Chernobyl accident. Because proper dosimetry was not done at the time, the exact amount of exposure to children was not clear. The children who attended school within a 150 km radius of Chernobyl have been carefully screened over the ensuing 14 years. The nuclear power plant accident happened on April 26, 1986. One case of thyroid cancer was recorded per 2,409 children born between April 27, 1986 and December 31, 1986, (intrauterine exposure). A much higher rate, with 31 thyroid cancers among 9,720 children (ages 1 day – 4 years), was seen in those born in the 4 years prior to the accident. Over 20,000 children have been followed and repeatedly examined using ultrasound, as well as measurements of TSH, free thyroxine and thyroid peroxidase antibodies. An increase in thyroid cancer has not been seen in children

born since 1987 (post Chernobyl conceived). All of the cancers were papillary adenocarcinomas.

Shibata Y, et al. *Lancet* 2001;358:1965-1966.

Editor's Comment: *The conclusion of this follow-up study is that children at a young age and probably up until 10 years of age are at particularly high risk for developing thyroid cancer after exposure to radioactive fallout. Hopefully, there will never be another Chernobyl. If there is, careful dosimetry to know the amount of exposure and the rapidity of decay will be important. However, it is clear that children, particularly young children, are at the greatest risk and need to be followed carefully.*

Judith G. Hall, OC, MD

Letter to the Editor

I read the commentaries and the review of the paper by Zucchini et al on SCA final height after growth hormone treatment from *Arch Dis Child*, in the October 2001 issue of *GGH*. I would like to add the following points.

As the reviewer states, the treatment had begun late (approximately 10.8 years). What the reviewer does not state clearly is that the GH dose was too low. I calculated the dose to be about 0.22 mg/kg/week. The FDA approved GH for SGA at a recommended dose of 0.48 mg/kg/week. It is not surprising therefore that less than 50% of the recommended dose gives disappointing results. de Zegher et al presented near final height at the joint meeting in Montreal and the robust height SDS gains appeared to be sustained.

In essence then, the disappointing results of the Zucchini paper can be summarized as "too little, too late". That conclusion did not come across in the comments.

Paul Saenger, MD

First Editor's Comments: We appreciated the remarks of Dr. Saenger with regard to the abstract of the article by Zucchini, etc, *Arch Dis Child* 2001 84:340. Although, as Dr. Saenger pointed out, the dosage of GH used in the study was significantly less than that approved by the FDA for treating short SGA children, the children treated in this study were classified as growth hormone deficient based on stimulation tests. Thus one might argue that the magnitude of the difference between recommended and actual GH dose was not as different for these GH deficient children as it might have been had they been GH sufficient. Indeed, the presentation by de Zegher in Montreal last summer was very encouraging. Long-term studies, treating SGA children from an early age, at the recommended dose are necessary to answer the question of the overall benefit on adult height of GH treatment of SGA children.

William L. Clarke, MD

Second Editor's Comment: The Reviewer thanks Dr. Saenger for his helpful comments about the manuscript of Zucchini et al¹ concerning the effect of rhGH in short children born small for gestational age (SGA). The dose of rhGH utilized by these investigators (calculated to be 0.27 mg/kg/week) was indeed less than that employed by de Zegher et al^{2,3} (ranging between 0.23 and 0.7 mg/kg/week). In addition, these investigators began treatment with rhGH between 2-8 years of age, thus affording longer treatment periods. The adult heights of their patients have not been reported as yet, although through 6 years of therapy there was an increase in height of +2 SDS. However, treatment with high doses of rhGH resulted in insulin resistance that may not be completely reversible⁴ and in high levels of IGF-I during treatment.⁵

Even if rhGH is able to increase adult stature to a statistically significant extent, there are no data indicating that greater height is meaningful in terms of improved psychosocial well-being, educational attainment, or economic success. Given the potential hazards of insulin resistance, elevated levels of IGF-I (if only temporary), and lack of documented enhancement in the quality of life (QoL), treatment of SCA children with rhGH, particularly at the dose that has been approved by the FDA, seems hazardous to this reviewer and should only be employed in an investigative setting until its statural and QoL efficacies and safety have been well documented.

Allen W. Root, MD

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