# What Doctors and Scientists Are Saying About Human Growth Hormone in the Year 2003.

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# 1. DISCLAIMER



Many products are for sale that claim to increase a user's growth hormone level and provide consequent health benefits. I am unfamiliar with all of these products and do not know whether they are safe and effective or not.

Although I hold a Ph.D. in Biological Sciences (Specialty: molecular genetics) from the University of Illinois, I have no medical training at all. Moreover, I have never conducted original research on human growth hormone or any endocrinological subject.

Although I have not deliberately biased this work either to promote or disparage the medical potential of human growth hormone, the literature on this subject is quite large. I am not familiar with all of it, and I may have omitted important research findings accidentally.

This e-document is intended to discuss the scientific background of an important medical topic. I hope that it will leave readers better prepared to ask questions of their doctors and to understand the answers. I hope that it will leave readers better able to ask probing questions of supplement vendors, who ought to be able to provide reasonable support for their advertising claims. I hope that it will enable readers to act as informed citizens since, like all drugs, substances that alter human growth hormone levels in humans are potentially regulated and legislated upon.

I encourage readers to check this web site (xenobiotherapeutics.com) for updates, possible corrections and articles on other health-related topics. This document is also available from other web sites, including http://groups.yahoo.com/group/growth_hormone2.

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(For information how to correspond please see the Afterword at the end of this manuscript.)

## 2. INTRODUCTION

In America, in the year 2003, youthfulness is a national obsession. In the public mind, the advantages of young adulthood (beauty, strength, stamina, sexuality, and good health) greatly outweigh the supposed advantages of age (experience, wisdom, a record of accomplishment, a lifetime of friendships, standing in the community, accumulated wealth and increased earning power). The "baby boom generation" (people born between 1945 and 1955) is approaching retirement with the fear of having accumulated too few assets for that retirement to be comfortable. Many people approaching retirement wonder how they will pay their medical bills, which are sure to mount.

Given the public mood, purported methods to delay or reverse the ageing process inevitably attract attention. Of these, the most discussed is human growth hormone (hGH). Proponents of hGH therapy for ageing, including many hGH vendors hoping to improve their own retirements, argue that it has many benefits. It is claimed to increase lean body mass, reduce body fat, improve physical and mental performance, improve the quality of sleep and reverse the decline of the immune system that occurs with ageing. Detractors pointout that the documented benefits of hGH therapy are usually modest, that many studies have failed to find any benefit, and that hGH has well-documented undesired side effects.

The purpose of this e-book is to describe the benefits and drawbacks of growth hormone therapy, and to offer a glimpse of how growth hormone functions. However, growth hormone does not exist in a vacuum. Instead, it is one of six hormones whose workings are interrelated. The others are Growth Hormone Releasing Hormone (GHRH), somatostatin, ghrelin, and the insulin-like growth factors I and II (IGF-I and IGF-II). These six hormones are together termed the "growth hormone axis," or simply the "axis." This e-book is thus about the growth hormone axis.

In the course of writing this e-book, I consulted between 1000 and 1100 research articles, although I certainly did not read them all in detail. There are many more relevant published articles that I did not consult, very probably including many that I am not aware of.

I have formed the following opinions:

**One.** Despite advertising claims, growth hormone does not reverse the ageing process. Ageing is far more profound than a reduction in hormones,

and reversing it would take much more than restoring those hormones. Hence, the most that growth hormone might do is to postpone or temporarily reverse some of the symptoms.

**Two.** Growth hormone greatly benefits several groups of sick people. Most of the claimed benefits for growth hormone come from studies of these people. Although these people represent a diverse group of illnesses, it is likely that their illnesses all disrupt the normal workings of the growth hormone "axis." Growth hormone therapy restores the axis to its normal function. Hence, the demonstrated benefits of growth hormone therapy in these people may not apply to healthy people.

**Three.** Growth hormone and other members of the growth hormone axis may provide the the following benefits to elderly people (in other words, the purported benefits have at least some support): reduced body fat, increased strength and stamina, improved heart performance, lowered blood pressure, improved blood lipid (cholesterol) profile, increased bone density, improved memory and mental function, increased immune system activity, increased sexual potency, and improved sleep. However, these benefits do not always occur, and may be modest when they do.

**Four.** In some people, growth hormone improves mood and life satisfaction, as well as marital and economic performance. The improvement sometimes lasts indefinitely, and sometimes does not.

**Five.** Growth hormone is sometimes also claimed to improve the vision, remove wrinkles, and restore hair growth and color. There are indeed connections between growth hormone and blood vessels in the retina, between growth hormone and the skin, and between growth hormone and hair. However, in all three cases the connection is complex, and it is not not necessarily true that more growth hormone is better.

**Six.** Growth hormone and other members of the growth hormone axis can have strong adverse consequences, as well as beneficial ones. A few examples are:

People with acromegaly have a large excess of growth hormone. Such people have many physical ailments, and (unless treated) eventually die of heart failure.

Tens of thousands of patients have been treated with growth hormone, and seem not to suffer any statistical increase in cancer. However, evidence suggests that growth hormone and other members of the growth hormone axis can accelerate the growth of many cancers. Cancers in an early state, that could still be stopped by the body's own defenses, might also be accelerated. ( **H5 4** )

Growth hormone and other axis hormones clearly have strong effects on the brain. However, some evidence indicates that high concentrations of growth hormone accelerate mental deterioration in the elderly.

Growth hormone promotes blood vessel growth in the retina of the eye. In diabetics, overgrowth of such vessels causes diabetic retinopathy, which can lead to blindness. A promising therapy is to chemically antagonize the growth hormone.

Growth hormone and other members of the axis induce immune system activity, and hence could be said to "strengthen" the immune system. However, "strengthening" of the immune system is risky; the induced immune system activity may accomplish nothing useful, and may even attack the body, causing autoimmunity.

Elevated levels of growth hormone may reduce rather than increase longevity. Long-lived strains of mice have reduced levels of growth hormone and IGF-I.

**Seven.** Commercially available growth hormone is a protein that is produced with recombinant DNA techniques. As such, it is regulated by the Food and Drug Administration; it is approved as a treatment for several medical conditions but not as a therapy for ageing, and not as an ergogenic aid for sports. Hence, it can be taken legally by only some people and only by a doctor's prescription.

Growth hormone will not enter the body reliably unless injected. Moreover, a year's supply of recombinant growth hormone can cost $10,000 or more. Hence, dietary supplements or sprays which increase a person's growth hormone levels probably do not contain growth hormone itself. Instead, they presumably contain substances that will induce the body to produce more growth hormone.

Dietary supplements, including melatonin and creatine, have been reported to increase growth hormone levels in patients. Therefore, some commercial supplements may indeed increase a user's growth hormone levels.

**Eight.** The best advice on whether to take such supplements will probably come from a physician specializing in Endocrinology. Such a physician should be certified by the American Board of Medical Specialties as qualified to specialize in Internal Medicine, with a subspecialty in Endocrinology.

**Nine.** Growth hormone research is an exciting field that deserves continued financial support. It has helped tens of thousands of people. Although it offers only modest benefits to the healthy elderly, continued research may bring much greater benefits.

The rest of this e-book gives the details.

## 3. A STRING OF SUCCESSES

Growth hormone research has produced a series of medical successes. I begin here for three reasons. First, this subject shows growth hormone research at its best, as having achieved unambiguous good fortune for people. Second, it probably provides the basis for many popular beliefs about the benefits of growth hormone. Third, it sheds light on how the needs of an animal can favor either growth or breakdown of tissues, and how the growth hormone axis helps make that choice. An understanding of how the body's needs change with circumstances, and how axis hormones coordinate multiple changes will be necessary for the full exploitation of the growth hormone axis.

### 3.1 A simple description of the growth hormone system.

A simple intellectual model of the effects and control of growth hormone involves 6 hormones. These are growth hormone itself, GHRH, somatostatin, ghrelin, IGF-I and IGF-II.

**Growth hormone** is also called "somatotropin" and "somatropin." Its main function is to promote the linear growth of children, but its production and its effects persist throughout life.

Circulating growth hormone is produced by the front (anterior) lobe of the pituitary gland. The pituitary gland is located on the underside of the brain. In an adult human, it is the size of a garbanzo bean, and is about three inches back from the bridge of the nose. Growth hormone is one of 9 hormones produced by the two lobes of the pituitary.

The pituitary gland is regulated by the hypothalamus. This is part of the brain, located on the brain's underside, just above the pituitary gland. Hormones secreted by the hypothalamus travel the short distance to the pituitary gland by blood vessels.

**Growth hormone releasing hormone** (GHRH) and **somatostatin** (also called Somatotropin Release Inhibiting Factor or SRIH) are made in the hypothalamus. Growth Hormone Releasing Hormone induces the pituitary gland to release growth hormone into the blood. Somatostatin inhibits this release.

Both the hypothalamus and the pituitary gland are bathed in blood that circulates through the body. Thus they are exposed to hormonal influences that originate elsewhere in the body. One such hormone is **ghrelin** ("growth hormone releasing endogenous ligand - in"). Ghrelin is produced by the

gastrointestinal tract, and is a powerful inducer of growth hormone secretion.

Ghrelin and GHRH act by different mechanisms. Their combined stimulation of growth hormone release from the pituitary gland can be greater than the sum of their solitary effects.

Growth hormone exerts most of its effects by inducing production of two additional hormones in the liver. These are the two insulin-like growth factors, **IGF-I** and **IGF-II**. Both mediate anabolic (i.e. tissue-building) effects of growth hormone, but IGF-I is a much more important mediator after birth. Growth hormone's stimulation of IGF-II is weaker and less certain than its stimulation of IGF-I.

IGF-I acts on the hypothalamus-pituitary system to reduce the production of growth hormone. Thus, IGF-I indirectly limits its own production.

GHRH, ghrelin, somatostatin and growth hormone are all multifunctional hormones, with effects in addition to those mentioned above. These multifunctional effects lead to coordinated behaviors that over evolutionary time have served the welfare of the human lineage.

A final major feature of growth hormone is that its release is pulsatile. It rises and falls at 2- to 3-hour intervals, and is much greater at night during sleep than during the day. This pulsatile secretion is a deeply embedded feature of growth hormone's behavior. It can convey a surprising amount of information to target organs, and is probably important to growth hormone's overall effects.

### 3.2 Growth hormone treatment of growth hormone deficiency in children.

There are many causes of growth hormone deficiency in children and adults. Growth hormone is produced by pituitary gland, under stimulation by the hypothalamus. Genetic defects in the interacting components, or physical defects in the pituitary or the connection between the hypothalamus and the pituitary can be a cause. Frequently, the cause is destruction of pituitary tumors by surgery or radiation, or destruction of other head tumors by radiation.

Irradiation of the pituitary gland to treat tumors is very successful, but nearly always causes delayed loss of pituitary function. The average baseline levels of growth hormone are 50% of normal after 2 years, and 25% of normal after 5 years.

Growth hormone deficiency in children leads to growth failure and other medical problems (see below). Until the mid-1980s, children with growth hormone deficiency were treated with growth hormone extracted from the

pituitary glands of human cadavers. Growth hormone was in very short supply, and this source exposed the patients to transmissable brain diseases. One such disease is Creutzfeldt-Jakob Disease, which is fatal. Patients treated with cadaver-derived growth hormone are still developing Creutzfeldt-Jakob Disease, decades after cessation of treatment.

In the mid-1980s, human growth hormone made from cloned recombinant DNA became available. This reduced the cost of treatment to growth hormone-deficient children, reduced their exposure to dangerous contaminants and greatly expanded the growth hormone supply. It also allowed doctors to seriously consider using growth hormone to treat conditions other than classical childhood growth hormone deficiency.

Children with growth hormone deficiency, or who have growth hormone but cannot respond properly to it, suffer multiple medical problems. These include much reduced height, obesity, low lean body mass and muscular underdevelopment, low bone mineral density and osteoporosis, abnormal smallness of facial bones and extremities, retardation of skeletal maturation, malformations of the skull, abnormalities of brain structure, reduction in mass of the left ventricle of the heart, abnormally high blood cholesterol, insulin resistance, increased risk for cardiovascular disease, impaired kidney function and trace element abnormalities. Their hair often sparse and their voices abnormally high.

Growth hormone is used to treat growth hormone deficiency. Generally, the treatments are well-tolerated, and are effective in increasing height and reversing the other medical stigmata of growth hormone deficiency, although unfortunately the reversal is seldom complete.

Growth hormone has also been reported to accelerate the growth of children with short stature, born small for gestational age. It is usually unclear why these children grow slowly.

Careful analysis of childrens' growth shows that normal prepubertal growth consists of spurts lasting about 8 weeks, separated by periods of very slow growth or stasis. Children with reduced growth, such as in Turner Syndrome and growth hormone deficiency, or intrauterine growth retardation have increased stasis time, and reduced growth spurt amplitude.

Growth hormone treatment of GH-deficient children increases the length and velocity of growth spurts. Their time spent in stasis decreased (in one set of measurements) from 19% to 6% on growth hormone. Their growth patterns during treatment resembled those of normal children.

In a few cases, children make adequate amounts of growth hormone, but

the growth hormone cannot carry out most of its actions, because a defective receptor prevents it from stimulating IGF-I. This disease, called Laron Syndrome, resembles growth hormone deficiency. It is treated by periodic injection of insulin-like growth factor I (IGF-I).

Thus use of recombinant growth hormone to treat a recognizable deficiency is quite successful.

### 3.3 Growth hormone treatment of growth hormone deficiency in adults.

Survivors of childhood cancer, particularly those exposed to irradiation of the head, often have decreased function of the pituitary gland. This includes insufficient production of growth hormone.

The symptoms of adult-onset deficiency or unresponsiveness to growth hormone resemble those of growth hormone inactivity in children, except that normal adult growth is completed. The symptoms of growth hormone deficiency or inactivity in adults include obesity, low lean body mass, reduced bone mineralization and increased risk of fracture, elevated blood cholesterol and triglycerides, insulin resistance, reduced kidney function, defective thermoregulation, decreased ability to sweat, elevated systolic blood pressure and increased risk of cardiovascular disease. There is reduced physical performance and vitality, difficulty in sleeping, constant fatigue, increased anxiety and depression, a greater likelihood of marital problems, and reduced economic performance.

Growth hormone therapy of such adult patients reduces their abdominal fat mass, total serum cholesterol and serum triglycerides. It increases their high-density lipoprotein cholesterol (the so-called "good" cholesterol), lean body mass, cardiac function, exercise capacity, physical strength, kidney function, ability to sweat, vigor, productivity and (very definitely) sense of well-being.

In young adults with child-onset growth hormone deficiency, 10 months of growth hormone treatment increased the mass of the left ventricle of the heart, and the length of the kidney. It was suggested that growth hormone leads to additional maturation of the heart and kidney that had not occurred.

Growth hormone is thought to benefit bone function in growth hormone-deficient adults. However, the issue is clouded by three factors. First, mass is added only after a period of resorption than can last months, and which seems to be a mysterious prerequite for mass addition [**H4 1**]. Second, simply adding mass to a bone does not necessarily improve its function. Third, comparisons are difficult between patients of differing height, weight and muscular development (weight and muscular

development both affect bone development.)

Overall, however, growth hormone treatment of adult growth hormone deficiency is beneficial. As with childhood growth hormone deficiency, replacement of a clear deficiency in adult growth hormone is a medical success.

### 3.4 Growth hormone treatment of childen and adults with kidney failure.

Children with inadequate kidneys grow too slowly. This slow growth continues even after a child receives a kidney transplant. The growth reduction is greatest at the extremities, so that the child grows disproportionately

Children with inadequate kidney function resemble children that are deficient in growth hormone in that they have too little lean mass, too much fat, and weak bones.

Growth hormone therapy accelerates growth of these children. If begun early and continued throughout childhood, it can enable children with deficient kidneys to reach a normal adult height. Equally important, proportional growth is restored, so that extremities are of normal size relative to the head and the trunk.

In these children, growth hormone therapy increases muscle mass and decreases the amount of fat. It is usually (but not always) reported to increase bone mass and mineralisation as well.

Adults on hemodialysis because of kidney failure also suffer from a wastage of muscle and accumulation of fat. They can also be helped by human growth hormone treatment. In one study, 6 months of treatment of adults on hemodialysis increased their lean mass by more than 3 kilograms, and reduced their amount of fat by an equal amount.

Recombinant human growth hormone can also increase nitrogen retention in childen and other patients with kidney failure. Significant results can occur in as little as six days, and can continue thereafter. This increased nitrogen retention is part of growth hormone's activity to reduce or reverse "wastage", the breakdown of lean muscle tissue.

(Interestingly, experimentally induced arthritis in rats also reduces kidney function, and administration of growth hormone ameliorates this effect. Since arthritis is very common among elderly humans, it raises the question of whether reduced kidney function might cause some of the symptoms of old age.)

Growth hormone administration in these patients is generally well-

tolerated. There are some reports of "benign intracranial hypertension", increased fluid pressure in the head.

It is not known exactly why kidney disease causes symptoms that resemble growth hormone deficiency. One piece of evidence is that in some cases, very careful attention to nutrition and aggressive dialysis (exceeding recommended guidelines in effectiveness), can largely restore normal growth. Hence, it seems that failure of the kidneys to do their normal job is the cause, rather than something else (such as a hypothetical chemical distress signal emitted by diseased kidneys).

A second piece of evidence is that kidney disease alters the concentrations of the six "IGF binding" proteins [**H6 9 34**] in serum, with several of the more important IGF binding proteins having elevated concentrations. IGF-I (insulin-like growth hormone type I) is the hormone that mediates most of the growth-related effects of growth hormone [**H6 9**]. It is thought that the increased concentrations of one or more of the IGF binding proteins may decrease the concentration of free, active IGF-I in the blood. Administration of growth hormone is thought to increase the amount of IGF-I in the blood to the point where not all of it can be sequestered by the binding proteins. Thus, normal levels of IGF-I are restored and the symptoms kidney insufficiency disappear.

Adults with kidney disease can tolerate very high levels of both growth hormone and IGF-I, levels that would be toxic under other circumstances. Hence, they appear somewhat insensitive to both hormones.

Treatment kidney insufficiency with growth hormone has been a clear success. However, it seems to be another example where the underlying defect is an effective deficiency in growth hormone.

### 3.5 Growth hormone and Prader-Willi syndrome.

Growth hormone is also beneficial in treating Prader-Willi syndrome.

Prader-Willi syndrome is an extremely debilitating disorder caused by the absence of a segment of the long arm of one of a person's two copies of chromosome 15. The copy of chromosome 15 must be the paternally-contributed one.

Of every million people, about 60 have this syndrome.

Prader-Willi patients are short, with low lean body mass, flaccid muscles, reduced bone density and abnormal facial features. They are usually mentally retarded, and suffer behavioral disorders that include psychosis. They have an excessive appetite which leads to increasing obesity.

They suffer high morbidity and mortality, probably due largely to their obesity.

Prader-Willi patients resemble patients with growth hormone deficiency in that much of the fat accumulation is in the limbs. Most patients have a reduced ability to secrete growth hormone, probably due to malfunction of the hypothalamus, the part of the brain that controls secretion. Levels of IGF-I, the hormone that mediates most of the effects of growth hormone, are also low.

Growth hormone brings clear benefits to Prader-Willi patients. Their height velocity increases. They show increased muscle mass and reduced fat mass. The rate of fat oxidation increases. Their physical strength, agility, respiratory muscle strength and bone density all improve.

There are also reported behavioral benefits.

Unfortunately, although long-term treatment improves these characteristics, it does not normalize them.

Improvements can be maintained over at least 36 months of therapy. Improvements are dose-dependent, with greater improvements coming with higher doses of growth hormone, particularly after the first year of therapy.

Whether growth hormone therapy is beneficial over the very long term is unknown.

### 3.6 Growth hormone and cystic fibrosis.

Cystic fibrosis is a fatal disease that affects perhaps 1 in 2500 people in the Western world. It is caused by mutation in a gene on chromosome 7; the gene encodes a chloride ion transporter.

Cystic fibrosis patients often suffer from malnutrition even when they are very well fed. As result of this malnutrition, and because of the corticosteroid drugs that they are often given, children with cystic fibrosis fall behind in height and weight.

Although the growth hormone levels of cystic fibrosis patients are high, their levels of IGF-I and IGF binding protein 3 (IGFBP-3) are low. IGF-I helps carry out the effects of growth hormone, and IGFBP-3 influences IGF's activity [H6 9 37]. In having low IGF-I levels and disturbed IGFBP levels, as well as malnutrition, cystic fibrosis patients resemble the patients with kidney insufficiency discussed above.

Cystic fibrosis patients also suffer from decreased bone density and increased risk of fractures.

Growth hormone increases the height, weight, lean body mass, muscle strength, bone maturation, exercise tolerance and respiratory function of cystic fibrosis patients. It reduces the amount of fat present. It slows the rate of protein breakdown, improves nitrogen retention and seems to improve the clinical status of the patient, as measured by hospitalizations and courses of

intravenous antibiotics.

Growth hormone is very successful in treating cystic fibrosis patients. However, cystic fibrosis patients resemble other successfully treated patients in having an apparent disruption of the growth hormone axis.

### 3.7 Human growth hormone and Turner Syndrome.

Human growth hormone is also effective in treating some symptoms of Turner Syndrome. Turner Syndrome patients are female, but are missing all or part of one of the two X chromosome copies that females normally have.

Turner Syndrome patients have impaired development of the ovaries, and a number of characteristic physical deformities. They have impaired glucose tolerance, insulin resistance, low exercise capacity and enlarged type IIa muscle fibers. These symptoms indicate diminished oxygen and nutrient supply for metabolic processes, and resemble a pre-diabetic state. They may contribute to the increased mortality seen in Turner Syndrome patients.

A primary characteristic of Turner Syndrome is very short stature. Although growth velocity is nearly normal in the first 3 years of life, it is far below normal during the rest of childhood. In particular, there is no pubertal growth spurt.

Some reports indicate that Turner syndrome patients have reduced bone mineralization and an increased susceptibility to fractures. However, not all reports agree that bone mineralization is reduced in Turner Syndrome patients. Along with poorly functioning ovaries, Turner Syndrome patients have reduced sex hormone secretion; however, the degree of reduction between patients seems to vary. It has been suggested that patients with more normal secretion of sex hormones are spared the bone defects.

Growth hormone therapy increases the height velocity of Turner Syndrome patients. If therapy is begun early in life (ages 2-5 years) and if the doses given are high, Turner syndrome patients have a good chance of reaching normal height. Typically, growth hormone therapy may add 8 centimeters to a patient's final adult height. Growth hormone treatment is well-tolerated by Turner patients has become part of the standard care for Turner Syndrome.

Growth hormone has also been reported to modestly increase the bone mineral density of Turner Syndrome patients.

Several of the Turner Syndrome symptoms mentioned above, including short stature, reduced capacity for exertion and reduced bone density are also seen in patients with growth hormone deficiency. This raises the question of whether Turner Syndrome symptoms are caused by a defect in growth hormone or one of its related hormones.

The answer seems to be yes, but only partly. Turner Syndrome patients have been reported to secrete less growth hormone than do normal women, and the pulsating secretion pattern seen in normal people is disordered.

Growth hormone stimulates production in the liver of a second hormone, insulin-like growth factor I (IGF-I), that mediates most of growth hormone's effects. In Turner Syndrome patients, IGF-I levels are in the low-to-normal range. However, it is suspected that Turner Syndrome patients resist the effects of IGF-I, so that higher than normal amounts of serum IGF-I are needed for normal growth.

The cause of the IGF-I resistance is not clear. However, a blood protein that binds IGF-I, IGF-I binding protein 3 (IGFBP-3) is degraded unusually rapidly in Turner Syndrome patients. The rapid degradation may be due to reduced sex hormone activity. If IGFBP-3 is involved in carrying IGF-I to its proper targets, or in helping IGF-I as a growth stimulator, its impairment might reduce IGF-I's biological effect, even though nearly normal amounts of IGF-I are present.

Growth hormone treatment of Turner Syndrome patients produces higher-than-normal amounts of circulating IGF-I. It has been suggested that this supraphysiological level of IGF-I is necessary to overcome the IGF-I resistance of Turner patients.

Although Turner Syndrome patients share symptoms with patients suffering from growth hormone deficiency, there are differences. It has been reported, for example, that growth hormone does not increase the ratio of lean body mass to fat in Turner Syndrome patients, as it does in growth hormone deficient patients.

At least part of the growth deficiency in Turner Syndrome is due to loss of a gene called SHOX ("short stature homeobox-containing gene"). SHOX is one of the small number of genes with a functional copy on both the X and Y chromosomes. Humans must have two functioning copies to grow normally. People with one inactive SHOX copy are short, but usually respond to growth hormone. This SHOX gene deficiency is not present in other conditions where there is a deficiency in growth hormone or IGF-I.

In any case, doctors and scientists familiar with with Turner Syndrome do not classify it as primarily a defect in growth hormone activity. Hence, here is a case where growth hormone is effective in reversing a serious medical problem that is probably not primarily due to growth hormone deficiency.

### 3.8 Growth hormone and severe burns.

Recombinant growth hormone can save the lives of people with severe burns.

### 3.8.1 The metabolic consequences of severe burns.

Severe burns provoke a powerful hormonal response in humans and other mammals. More than 25 hormones increase or decrease in concentration in the blood. In particular, there are great increases (often nearly 30-fold) in the hormone cortisol, which promotes "wasting."

A burn patient's metabolic rate increases, and the patient expends more energy. At the same time the patient becomes insulin-resistant and accumulates high blood glucose. The patient becomes immunodeficient, and enters a "wasting" state where there is continuing loss of muscle (to meet energy requirements) and bone tissue, but a tendency to accumulate fat. Wasting persists for months after injury and contributes to sickness and death in severely burned patients.

In wasting, the body's ability to assimilate amino acids is greatly reduced. Hence, the ongoing breakdown of muscle protein into amino acids cannot be reversed simply by supplementing the patient's diet with amino acids, or by injecting amino acids into the blood.

### 3.8.2 The benefits of growth hormone therapy for burns

Growth hormone has a number of beneficial effects in patients suffering burn-induced wasting, as well as a few deleterious effects. The benefits are discussed first.

Growth hormone can improve the burn survival of both humans and experimental animals. One small study reported that administration of growth hormone reduced human mortality from severe burns from 44% to 8%. A second study claimed a reduction from 37% to 11%.

Studies with experimentally burned mice indicate that both growth hormone and IGF-I improve survival. However, it was not clear whether the improvement was due to improved wound healing or a strengthened immune system, since growth hormone can stimulate both [H3 10; H4 9].

Burned adults and children have increased amounts of tumor necrosis factor-alpha. Such elevations in TNF-alpha are correlated with multiorgan failure and death. Administration of hGH reportedly lowers TNF-alpha levels after burn injury, at least in children. This has been suggested to contribute to hGH's lowering of burn mortality.

In burned animals, concentrations of both hemoglobin and serum albumin decline. Hemoglobin is a constituent of red blood cells, and is necessary for transport of oxygen to tissues and for removal of carbon dioxide. Albumin is a protein that carries many substances, including many medicines, through the blood. Administration of hGH to burned humans or rats prevents the

declines in hemoglobin and albumin.

Burned humans and animals lose nitrogen, calcium, phosphorus and potassium. Nitrogen loss results from breakdown of proteins in muscle tissue. Calcium and phosphorus loss result from demineralization of bone. The result of these losses can be clinical malnutrition. Administration of growth hormone can limit losses of these nutrients, although reports vary on growth hormone's effectiveness.

In rats, there is a decrease in the antioxidants glutathione and catalase in the 48 hours following a severe burn. As a result, the peroxidation of lipids increases. This is detrimental to recovery because peroxidized lipids can cause further tissue damage. Growth hormone treatment increases tissue glutathione and catalase activities, and prevents lipid peroxidation.

As discussed above, burns cause protein breakdown in tissues, especially muscle. In one study, adults suffering a severe burn lost an average of 8 kilograms (nearly 18 pounds) while in intensive care. Children suffer similar wasting. In most studies with humans and experimental animals, recombinant growth hormone limits wasting and helps increase weight and muscle mass in months or years of recuperation following the burn.

Insulin-like growth hormone-I and insulin have been less studied as post-burn anabolic agents than has growth hormone. However, both seem to mimic the effects of growth hormone. In some reports, the combined effect of IGF-I and growth hormone is greater than the effect of either alone. Similarly, in some reports, the combined effect of insulin and growth hromone exceeds the effect of either alone.

Preservation of muscle tissue has functional benefits. Post-burn grip strength is greater in patients treated with either growth hormone of IGF-I.

Growth hormone also has anabolic effects on organs other than muscle. It also stimulates protein synthesis in the liver and other organs, and stimulates the multiplication of liver cells.

One consequence of severe burns in children is growth delay. Recombinant growth hormone prevents much of this growth loss, particularly in children outside of the so-called "growth spurt" years.

Wound healing is an important part of recovery from burns, both because burns themselves are wounds, and because treatment involves autografts of skin. Donor sites also become wounds which must heal.

Nearly all studies show that growth hormone promotes healing of all wounds including burns. In one study, skin graft donor site healing time was reduced from 14 days to 10 days in burn patients. Examination of healing wounds shows that representative biological components needed to rebuild tissues, such as IGF-I receptors, laminin, types IV and VII collagen and

cytokeratin-14 are all stimulated by growth hormone.

Improved wound healing reduces the risk of infection, and shortens hospital stays. One study estimated that growth hormone therapy of a patient with severe burns over 60% of the total body surface area would cut the hospital stay from 46 days to 32 days. Thus, although recombinant human growth hormone is expensive, it may pay for itself by reducing hospital stays.

Severe burns are often complicated by leakage from the intestines. Part of this is caused by atrophy of the mucosa. Growth hormone and IGF-I heal the mucosa. In one study, IGF-I reduced the incidence of bacterial translocation out of the intestine to the mesenteric lymph node from 89% to 30%.

The reduction in sepsis caused by growth hormone and IGF-I may be due partly to immune system stimulation. Burn patients have reduced immunoglobulin levels and correspondingly less antibody activity than do unburned healthy people. Both growth hormone and IGF-I have been reported to increase immune system activity in animal models. Antibody synthesis, T-lymphocyte proliferation, natural killer cell activity, macrophage activity and interferon-gamma production by mononuclear cells are all increased. Moreover, burned mice are protected by growth hormone treatment from potentially lethal herpes simplex virus infection.

Both children and adults with severe burns suffer from reduced bone mass, termed osteopenia. This is caused by low or absent bone formation, coupled with continued resorption. The end result can be an increase in fractures.

Several causes have been suggested for the bone loss: production by the body of corticosteroids, burn-induced immobilization, magnesium depletion, bone marrow suppression, and a reduction in vitamin D (due in part to avoidance of sunlight be severely burned people to avoid scar hyperpigmentation).

Several reports have indicated that growth hormone can increase the bone mass of severely burned patients, relative to patients who have not received growth hormone. However, other reports describe no such increase, and the matter remains unresolved.

Growth hormone and IGF-I can be used in combination. Although growth hormone causes many of its effects by stimulating the synthesis of IGF-I, growth hormone and IGF-I differ in some of their effects. In particular, growth hormone promotes high blood glucose while IGF-I promotes low blood glucose. This suggests that a combination therapy in the right proportions might reduce the change in blood glucose, and tests confirm this.

The tissue-building effects of growth hormone and IGF-I are greater than the effects of either agent alone. In one study with rats originally weighing 440-470 grams, untreated burned rats lost 24 grams of weight in the 8 weeks after the burn. Burned rats treated with growth hormone gained 49 grams, while burned rats treated with IGF-I gained 11 grams, and burned rats treated with both agents gained 110 grams.

The combined therapy also increased the dry weights of the rats' gastrocnemius (calf) muscles more than either agent did alone, and was more effective in promoting wound healing than either agent was alone.

IGF-I can be delivered in liposome form. (Liposomes are small, hollow spheres made of fat molecules. They can carry drugs, and will fuse with cell membranes, releasing the drugs into target cells.) Very low doses of IGF-I delivered as liposomes stimulated wound healing as effectively as injections of growth hormone and IGF-I, and as effectively as injections of growth hormone and IGF-liposomes.

### 3.8.3 The disadvantages of growth hormone treatment for burns.

Many small trials have seemed to show that recombinant human growth hormone is acceptably safe in the treatment of severe burns and other trauma. However, doctors' faith in hGH dropped after two large European trials showed an unexpected increase in mortality in adult trauma patients given hGH.

Growth hormone's lethality in the critically ill has provoked keen interest. Attention has focussed on hGH's promotion of elevated blood glucose (sugar). The natural regulatory relationship between growth hormone and IGF-I is disrupted in the critically ill, in a way that tends to elevate blood glucose while diminishing growth hormone's tissue-building effects.

Critical illness, including burns, robs growth hormone of its power to induce IGF-I. Normally, growth hormone and IGF-I both counteract wasting.

Growth hormone destroys fat, elevates blood glucose and increases protein synthesis. It causes the first two effects directly, by binding to specialized receptors on cells throughout the body. It causes the third effect, stimulation of protein synthesis, indirectly by boosting the production of IGF-I. IGF-I then stimulates protein synthesis, in muscle and other tissues.

In healthy people, growth hormone and IGF-I regulate each other's concentration in the blood. Growth hormone stimulates the production of IGF-I, while IGF-I inhibits the production of growth hormone. Thus, IGF-I indirectly inhibits its own production. The result is a stable long-term equilibrium.

Burns, as well as other stresses such as liver cirrhosis, major surgery, sepsis (microbial invasion of the tissues) trauma and anorexia nervosa, can change the amounts of growth hormone and IGF-I present in the body. In some cases, such as with anorexia nervosa and cirrhosis, very high concentrations of growth hormone build up in the blood but the blood concentrations of IGF-I remain low. In these cases, growth hormone seems no longer able to stimulate the production of IGF-I; IGF-I has become unlinked from growth hormone. Whether and under what circumstances burns cause a similar unlinking in humans is unresolved, although it is clear that burns reduce IGF-I levels.

When growth hormone loses its ability to stimulate IGF-I, several consequences follow. First, IGF-I levels drop, causing the growth hormone level to reach higher than normal levels, since IGF-I limits the growth hormone level. Second, the elevated growth hormone level will stimulate a rise in blood glucose. Third, the glucose-lowering effect of IGF-I will be reduced, elevating blood glucose levels even further. Fourth, the tissue-building effects of IGF-I will be lessened.

One study has suggested that elevated blood glucose in critically ill patients promotes more frequent infections, particularly by yeast. Patients with elevated blood glucose had an increased failure of skin grafting and increased mortality.

There may be ways to prevent blood glucose levels from rising when hGH is given. One method is to supplement the growth hormone with insulin, which lowers blood glucose. A second method is to supplement the growth hormone with IGF-I. As mentioned above, this often has beneficial effects that exceed the effects of either growth hormone or IGF-I alone.

In addition to its dangerous effects on blood glucose, medical human growth hormone has other undesirable effects in burn patients. These include promotion of "fatty liver", excess blood calcium and a dangerously high metabolic rate.

"Fatty liver" occurs in patients with severe burns, and often swells the liver to twice its normal size. It occurs because of increased breakdown of triglycerides into fatty acids in peripheral tissues. These are not used as fuel, but are instead transported to the liver.

The liver can retransform these fatty acids into triglycerides and send them back to the peripheral tissues. However, in "fatty liver" the liver's capacity for this is overloaded, and the fatty acids accumulate.

A disadvantage of exogenous hGH is that it increases the rate of production of free fatty acids in severely burned patients, in children and

presumably also adults and thus is likely to make the fatty liver of burn victims even worse. This process can be offset by the administration of the beta-adrenergic blocker propanolol, which decreases the release of free fatty acids from adipose tissue without reducing the liver's efficiency in returning the fatty acids to the blood as triglycerides.

Growth hormone treatment elevates blood calcium concentrations. This can be useful in preventing hypocalcemia (too little blood calcium), but more often leads to hypercalcemia (too much blood calcium), especially in people with impaired kidney function.

In one study of severe burn patients, the percentage of patients with hypercalcemia rose from 14% to 43%, and the percentage of patients with severe hypercalcemia rose from 0% to 10%.

Hypercalcemia can become a life-threatening condition. The symptoms are generally worse if hypercalcemia develops quickly. They include malaise, fatigue, headache, decreased alertness, reduced appetite, constipation, nausea, excessive thirst and urination, reduced strength of muscles including the respiratory muscle, altered heart rhythm, increased gastric acid secretion, dehydration, delirium, disorientation, incoherent speech, hallucination, delusion, stupor, and coma.

The metabolic rate in burn patients rises to about 155% of normal. In one study, administration of hGH increased this to about 178% of normal, considered undesirably high.

### 3.8.4 Drug combinations may bring burn therapy progress.

Hope for progress in burn therapy with growth hormone and IGF-I lies in an increased understanding of how the multitude of different hormones and possible drugs interact.

A circumstantial case has been made that many of the changes that occur after a burn injury, including nitrogen loss, and changes in the concentrations of blood proteins that bind IGF-I, are orchestrated by the hormone glucagon. If this proves to be the case, it may be possible to unlink some of these coordinated effects from each other by engineering of artificial glucagon, or by using additional drugs.

There are still surprises in the fields of growth hormone and burn therapy. It had long been assumed that growth hormone causes the secretion of albumin (an important transport protein that carries many medicines) into the blood by activating the liver-specific hormone hepatocyte growth factor, since hepatocyte growth factor also stimulates albumin production. However, actual experimentation has shown that growth hormone suppresses hepatocyte growth factor, and hence must elevate albumin

production some other way. It remains to be seen whether use of growth hormone and hepatocyte growth factor together will be of any benefit.

Researchers in the field are exploring combinations of growth hormone or IGF-I on one hand with beta-blockers, antiglucocorticoids, other growth factors and artificial sex hormones such as oxandrolone on the other.

### 3.9 Growth hormone can reduce wasting in critically ill patients.

Patients that are critically ill with a variety of ailments other than a burn suffer wasting. In this condition, protein is broken down but fat is preserved or even accumulates. Wasting occurs even though the patients are adequately fed (orally or intravenously). Protein synthesis slows or stops, with the result that the protein content of vital tissues is greatly reduced. The longer the critical illness lasts, the greater is the wasting.

Wasting is complex. It originates in the brain, and involves several parts of the brain including the cerebral cortex, the limbic system and the hypothalamus. Many hormones or other signalling molecules are involved. Catecholamines, cortisol, glucagon, the melanocortin family of neuropeptides, tumor necrosis factor-alpha, interleukin 1-beta, interleukin 6 and glucocorticoids increase in amount. Other hormones or signalling molecules that normally build protein within tissues are reduced in amount or prevented from acting; these include insulin, leptin, ghrelin, and several cytokines.

Growth hormone benefits these critically ill patients who have not been burned. Growth hormone partly blocks wasting.

In one investigation, patients given hGH after abdominal (colon) surgery had less loss of limb strength, less loss of work capacity and less loss of lean muscle tissue than did patients given a placebo. As an example, patients given hGH had lost an average of 7.6% of their strength at 10 days after surgery, while patients receiving placebo had lost an average 17.1%.

In another study, patients undergoing abdominal surgery were given either no treatment (placebo), growth hormone for 6 days after surgery, or growth hormone for 6 days both before and after surgery. Growth hormone treatment both pre- and post-operatively had the greatest effect. Growth hormone treatment reduced the postoperative decrease in grip strength by as much as 70%. Post-operative respiratory function and arterial oxygenation were also improved by treatment.

Growth hormone also improves nutrition (i.e. nourishment of tissues) in patients who have not been burned, but who are critically ill. In a study of such patients, hGH reduced nitrogen excretion by an average of 34%.

Potassium and phosporus excretion were reduced 31% and 42%, respectively, by growth hormone treatment.

Hormones that stimulate growth hormone, and drugs that mimic those hormones, might replace growth hormone itself as a tissue-building aid in the critically ill. This has been tested with a mimic of ghrelin, the stomach hormone that stimulates growth hormone production.

In critically ill patients, the ghrelin mimic increased average growth hormone secretion by 4- to 6-fold. This, in turn, caused an average rise of 60% in IGF-I. Thus, the advantages of using growth hormone stimulators, rather than growth hormone itself [**H7 1 2**], may be brought to bear on critical illness.

### 3.10 The effects of axis hormones on skin and wound healing.

Growth hormone affects the skin. Acromegaly (overproduction of growth hormone) is recognizable by skin coarsening and increased elasticity. By contrast, in children with growth hormone deficiency, the skin is thinner and stiffer and less elastic than normal; the thinnest and stiffness are reversible by growth hormone treatment. Growth hormone-deficient rats also have thinner skin, with less collagen, more subcutaneous fat, and smaller sebaceous (oil-producing) glands than normal.

Growth hormone receptors are present in the skin, in rats, rabbits, humans, and presumably other mammals. They are present in sebaceous glands, which secrete skin oil; this probably explains why acromegalics have oily skin. The reduction in growth hormone that occurs during ageing might contribute to the dry skin and skin problems that older people often experience.

Melanocytes are cells in the basal layer of the skin that secrete the skin pigment melanin. IGF-I stimulates melanocytes, and growth hormone also does so in combination with basic fibroblast growth factor. Melanin protects skin from damage by ultraviolet radiation. Such damage can eventually cause skin cancer, including the very dangerous malignant melanoma, and in any case is unsightly and undersirable. This dependence of melanocytes on axis hormones could have unappreciated consequences for ageing and other conditions where axis hormones are diminished.

Growth hormone thickens the skin of normal dogs, by increasing skin collagen. The same effect occurs in mice, but only in males, and depends on male sex hormones.

The capillary density and microcirculation in the skin are reduced in patients with growth hormone deficiency. This may contribute to the

impaired thermoregulation that such patients have. Treatment with growth hormone normalizes these characteristics.

Growth hormone improves wound healing in some cases, but it remains to be determined how effective it is, and under what circumstances. Experiments with animal models have given conflicting results: growth hormone increases wound healing in pigs, but not in horses. One encouraging report is that a combination of growth hormone replacement therapy and topical application of growth hormone temporarily cured ulcers in the feet of a boy genetically predisposed to ulceration.

In rats, administration of hGH for 7 days has been shown to increase the bursting strength of uterine scars from caesarian delivery. Treatment for 7 days with hGH increases the bursting strength of intestinal injuries by about 31%. In irradiated rats (this simulates irradiation for gynecological cancers) hGH treatment increased wound strength by 36%, increased the scar thickness by 41% and reduced the leak rate of the healing intestine from 14.7% to 0%. (Intestinal leakage after irradiation treatment of gynecological cancers causes significant morbidity and mortality.)

### 3.11 Growth hormone therapy in HIV-infected patients.

AIDS-related wasting is the shrinkage of muscle tissue, and comcomitant loss of strength. It is often accompanied by lipodystrophy, the accumulation and maldistribution of fat.

Wasting can impair physical performance, immune function and quality of life. It can rob patients of strength to the point where they cannot get through daily life unassisted. It may increase the number of opportunistic infections and hospitalizations; and since opportunistic infections strongly promote wasting, AIDS-related wasting can become a downward spiral. That spiral will end in death if the patient suffers insupportable malnutrition, or if the patient's body cell mass shrinks beyond the limit necessary to sustain life. Wasting is considered to be a major cause of morbidity and mortality in AIDS.

AIDS-related wasting often accompanies lipodystrophy, in which fat accumulates and is abnormally distributed. AIDS patients suffering lipodystrophy accumulate excess visceral fat and other fat on the body trunk, sometimes including a "buffalo hump".

The preferred method of treating AIDS is with Highly Active Antiretroviral Therapy (HAART). Patients treated with HAART suffer AIDS-related wasting less frequently than did past AIDS patients, but AIDS-related wasting still occurs.

Treatment with hGH and/or IGF-I can reduce AIDS-related wasting and

lipodystrophy. They are usually administered with other countermeasures that include measures to control viral load, improvement of nutritional intake, testosterone therapy in some patients, anabolic steroids and exercise against resistance.

Most studies, including at least two large controlled clinical trials, show that hGH or rhIGF-I counteract AIDS-related wasting. In one study, Swiss AIDS patients were given supraphysiologic (6 mg/day) doses of hGH. By week 12, the gain of lean tissue averaged 4.8 kg (more than 10 pounds) and the loss of fat averaged 1.8 kg (nearly 4 pounds). The changes were evident by week 4 and tended to plateau by week 8.

Another study reported that AIDS patients given 6 mg/day doses of hGH for 12 weeks lost an average of 42% of their visceral fat, and that there were further decreases thereafter. A lower dose of 4 mg every other day reduced visceral fat by 15% after 12 weeks, and there were more decreases thereafter. The higher dose of hGH also significantly reduced subcutaneous fat.

Even patients with severe wasting are greatly helped. Significant gains (> 2 kg) in lean mass and losses (> 0.7 kg)in fat mass have been reported in as little as two weeks in AIDS patients with opportunistic infections.

Over 6 months of treatment HIV-infected men lost an average of 25% of their fat mass (about 4.4 kg) and gained an average of 5.4 kg of lean mass.

Patients infected with HIV, but not suffering from wasting, have normal growth hormone and IGF-I levels. Reports differ as to whether patients with wasting have low growth hormone levels; but in any case, both the effectiveness of growth hormone in stimulating IGF-I and the effectiveness of IGF-I in stimulating muscle and reducing fat may be reduced by AIDS.

One study reported a strong unlinking of growth hormone from its effector hormones IGF-I and IGF-II. AIDS patients who had lost more than 10% of their ideal body mass showed a 55% reduction in serum IGF-I and a 70% reduction in IGF-II. This occurred even though some patients had high levels of growth hormone.

There also were definite abnormalities in two blood proteins that bind IGF-I (and perhaps IGF-II) and probably help them function. The IGF-I-binding protein IGFBP-3 was present in low amounts and appeared to have lost much of its ability to bind IGF-I. The IGF-I- binding protein IGFBP-I was present in high amounts, but in a chemically altered form. Thus, IGF-I (and IGF-II) may be unable to function properly.

These changes persisted over at least 25 months in AIDS patients with

wasting.

Exogenous growth hormone and IGF-I have other metabolic benefits for AIDS patients. First, they improve the nutrition of AIDS patients by promoting the retention of nitrogen and potassium.

Second, AIDS patients often have unfavorable blood lipid profiles with high levels of low density lipoprotein and triglycerides, but low levels of high density lipoprotein. Some, though not all, reports indicate that growth hormone treatment can lower low density lipoprotein and triglyceride levels, and raise high density lipoprotein levels in HIV-infected people.

Growth hormone can strengthen the immune system of AIDS patients. Although most of the body's circulating growth hormone is secreted by the pituitary gland, small amounts of growth hormone are also made by different cell types of the immune system. Hence, growth hormone may be part of the normal communication that occurs between immune system cells.

Recombinant human growth hormone increases both the mass of the thymus gland (where T cell maturation occurs) and the number of circulating naive CD4+ cells. In addition, growth hormone, IGF-I or a combination of the two stimulate a specific immune reaction to the HIV-1 envelope proteins.

In rodents with deliberately damaged immune systems, growth hormone and IGF-I stimulate cell division that can reconstitute the full immune system.

HIV-1 wreaks much of its havoc on the immune system by inducing immune system cells to undergo apoptosis, in other words to commit suicide. The ability of cells to undergo apoptosis is a necessary defense against cancer, but in this case is exploited by a parasite.

Growth hormone and IGF-I, along with drugs such as acetyl-L-carnitine that stimulate IGF-I, reduce HIV-induced apoptosis of immune system cells. Logically, suppression of apoptosis would be predicted to increase the incidence of cancer; but whether this actually occurs is unknown. (In any case, for most AIDS patients, the risk is probably worth it.)

Finally, growth hormone has another effect which may be of value in fighting AIDS: it shifts the immune response away from a "Th2" type response and toward a "Th1" type response. These two responses of the immune system tend to suppress each other, so that one usually dominates. The Th1 response is more effective in eliminating cells that harbor fungal,

viral and other pathogens (although this protection comes at the price of inflammation and tissue damage). In particular, the Th1 reaction includes "delayed type hypersensitivity". This unpleasant response (also triggered by poison oak and poison ivy) is an important defense against intracellular parasites.

The shift between a Th1 and a Th2 response can have great medical consequences. A Th1 response to infection by the Mycobacterium leprae, the bacterium that causes leprosy, leads to the relatively benign condition of tuberculoid leprosy. A Th2 response leads to lepratomous leprosy, which is devastating.

HIV may promote a Th2 response. Immunization of mice with the HIV envelope protein gp120 results in a strong Th2 reaction, which suppresses a possible Th1 reaction. This renders the immune system much less effective against HIV than it might be.

Administration of growth hormone at the time of immunization reverses this, and instead provokes a Th1 reaction. Thus, growth hormone could combat HIV, or other parasites that benefit by biasing the immune system toward a Th2 reaction, by stimulating Th1 instead.

The ability to stimulate a Th1 reaction might improve many vaccines. Hence, growth hormone could be useful against viruses and other parasites generally.

The most serious side effect of hGH treatment of AIDS patients is hyperglycemia, which can sometimes develop into diabetes. One study that followed patients for 6 months concluded that hyperglycemia often receded, leaving patients with nearly normal blood glucose levels.

Several HIV patients have developed cancer while taking part in trials trials of growth hormone, but the relationship of the cancer to the growth hormone treatment is unknown.

Some recipients developed joint pain.

Although there is a consensus that hGH and or rhIGF-I can benefit AIDS patients, there is still confusion about what dosages of which agents should be administered for how long. Not all reports agree that the improvements in body composition are permanent. Higher doses of hGH or rhIGF-I or both tend to be more effective, but also more dangerous.

In summary, growth hormone therapy can greatly benefit HIV-infected patients. The patients who benefit have disturbances in the growth hormone axis. In this respect, they resemble other patients who have been helped by growth hormone therapy.

### 3.12 Suggestions about hGH-responsive medical conditions.

There are a surprisingly large number of influences that can prevent normal function of the growth hormone axis. In at least some cases, such as burn injury, other critical illness and infectious disease, the growth hormone axis is blocked in response to a threat.

A picture is forming of how tissue growth and destruction form a pattern of responses to opportunities and threats. In good times, when food and sleep are plentiful, and there is little disease or stress, tissues grow. In bad times, they are broken down and used up. It behooves us to understand how this process works, because civilization and modern medicine may have changed what is desirable. Consider the example of burn injury, followed by infection.

It may be that the tissue catabolism that occurs in response to a severe burn is the best response to the combination of shock, microbial invasion, hunger, and increased risk from predators that would likely follow in the wild. However, the response may be optimized toward small burns that could actually be survived in the wild; there may be very little evolutionary pressure for a logical response to large-area third-degree burns, because these are probably fatal in the wild, no matter what. The response may be biased toward ensuring survival at the expense of all other considerations and might be biased toward insuring the survival of young adults, near reproductive age, even at the expense of other age groups.

In the modern world, there will be no wolves and no starvation. There will be antibiotics, bed rest, ample fluids, control over ambient temperature, sterile dressings, intravenous feeding and skin grafts. There will be a sophisticated understanding, which may not be built into the body's defenses, of which microbial invaders are life-threatening and which are not. In the future, there may be the possibility of local intervention: to suppress the catabolic response in bone and muscle for example, while allowing it elsewhere. Hence, understanding the response to severe burns, including the role of the growth hormone axis, might allow doctors to optimize that response for individual patients under modern conditions. This might increase our ability to save patients with severe large-area burns, to help the very old and the very young, and to combat non-fatal burn consequences.

The body's response to other types of critical illness might be similarly optimized.

## 4. A Promising Disappointment: Growth Hormone and

## Ageing.

The symptoms of ageing resemble those of growth hormone deficiency. In both cases, there is an increase in fat, a redistribution of fat into the abdomen, a decrease in muscular strength, reduced bone density and bone strength, reduced heart function, elevated low density lipoprotein and triglycerides in the blood, and an impaired immune system.

The amount of growth hormone that people secrete diminishes with age. Reductions in GH secretion range from 50% to 70% in people over 65 years of age, and many older people secrete as little GH as do younger patients with clinical GH deficiency. Moreover, the blood concentration of the growth hormone's effector hormone, insulin-like growth factor-I (IGF-I), decreases in about the same proportion as does the concentration of growth hormone.

As in wasting and other disruptions of the growth hormone axis, in old people the blood concentration of IGF-I depends less on the blood concentration of growth hormone than it does in young people.

In 1990, a researcher named Daniel Rudman pointed out that ageing resembles growth hormone deficiency, and suggested that many of the symptoms of ageing might be reversed by growth hormone treatment. Since then, the effects of growth hormone on the elderly have been assessed many times. So far, documented benefits have been variable and modest. Yet, there is reason to hope that improved understanding of growth hormone and other relevant hormones will increase the benefits.

"Growth hormone treatment" actually includes several possible treatments. One is simply to administer growth hormone. A second is to administer IGF-I or a combination of GH and IGF-I. A third is to administer agents that will stimulate the body's own production of GH. All of these have been tried.

This section of the e-book describes some of the tests that have been done, and the results.

### 4.1 Ageing, bone strength and growth hormone.

An undesirable consequence of human ageing is a loss of bone mass and strength, which increases the risk of fracture. One hope of researchers is that growth hormone treatment of elderly patients with weakened bones will restore bone mass and strength.

Much experimentation on animals makes this idea seem plausible. Transgenic mice expressing the human growth hormone gene have stronger vertebrae (more resistant to compression) than normal mice.

Growth hormone promotes healing of experimental bone wounds and fractures in normal rats and of experimental bone wounds in pigs. In rats,

human growth hormone nearly doubles deposition of material in the wound region. Human growth hormone increases the stability of titanium implants inserted into the tibia of rabbits.

Female rats whose ovaries are removed at 10 months of age, and who are then studied at 18 months of age are experimental models for osteopenia, the loss of bone mass and mineralization. Growth hormone treatment restores lost bone in such rats. However, the restoration occurs at only some bone sites, with sites having the greatest bone loss being most resistant to restoration.

Growth hormone had similar site-dependent effects on aged female rats whose ovaries were still present.

Studies on elderly people usually show no benefit from treatment with growth hormone, but recent evidence suggests that such studies are usually too short (they typically last six months or less). Although such studies reveal ample biochemical evidence that the growth hormone has stimulated both bone deposition and resorption, there is no net gain. In fact, there is typically a loss of bone, followed by restoration to pre-therapy levels. It is not clear how to interpret this activity, but it might be part of a process of bone "remodelling" induced by the growth hormone, in which removal and deposition of bone must occur.

In a study of elderly Japanese women lasting nearly two years (growth hormone was administered for only one year, but the women were followed for an additional 48 weeks), showed that growth hormone did stimulate bone growth over longer periods. After 24 weeks (nearly 6 months) in which bone resorption and deposition both occurred, bone resorption lessened. Net bone deposition then occurred. It continued even after discontinuation of growth hormone therapy, and by 100 weeks after the start of therapy, reached statistically significant amounts.

Thus, growth hormone therapy may initiate complex processes of bone change that take years to complete. Failure to appreciate this may have caused undue pessimism about the benefits of growth hormone therapy on the bones of the elderly.

Bone function is surprisingly difficult to evaluate. First, as discussed above, constructive changes can take months or years in humans, and may involve both deposition and resorption.

Second, an increase in bone growth or deposition of material does not necessarily translate into a healthier bone. One illustration of this is an experiment where the human growth hormone gene was inserted into rats, and expressed in bone-forming cells (osteoblasts). As expected rat femoral (thigh) bones were longer and thicker (a 16% increase in cross-

sectional area). Yet, their strength was only 68% of normal.

Third, it is difficult to make comparisons between people of different heights, weights and muscular strengths. The way that a bone grows depends greatly on the loads that are put on it, with increased loads generally causing increased growth. People of different heights and weights will place different stresses on their bones, and confound attempts to assess additional influences.

Except for brief periods of trauma (such as car accidents or falls), the greatest load on bones is supplied by the skeletal muscles. In fact, it has become common to think of bones are part of a "muscle-bone unit." Thus, influences that strengthen or weaken muscles will indirectly influence bone shape, mass and strength.

An additional complexity of bone development is that multiple influences may act concurrently, and may influence each others' effects. For example, a combination of growth hormone and exercise increases bone strength in aged rats more than either agent does alone.

A similar increase was not observed in a study on aged men: resistance training increased bone strength, but growth hormone did not add to that increase. Instead, growth hormone increased both deposition and resorption. However, this study lasted only 16 weeks. Other evidence (see above) suggests that a longer period of growth hormone adminstration might have stimulated bone growth.

As another example of stimulatory influences that can act together, the combination of parathyroid hormone and growth hormone has a much greater anabolic effect on the bones of female rats with experimentally induced osteopenia than either agent does alone. This may be because they affect different bone-forming tissues (parathyroid hormone stimulates the the "endocortical envelope" while growth hormone stimulates the "periosteal envelope").

It may not be necessary to inject growth hormone or IGF-I into patients in order to stimulate the GH/IGF-I axis. As discussed in Part 6 [**H6**], there exist hormones and drugs that can stimulate the pituitary gland to produce and secrete growth hormone. These agents are much cheaper and easier to administer than are GH or IGF-I, avoid the dangers of supraphysiological GH or IGF-I concentrations, and do not disrupt the pulsatile pattern of GH appearance and disappearance from the blood (See Part 6.2 [**H6_2**]).

The ghrelin-like secretagogues (see Part 6.6 [**H6_6**]) are examples of such drugs. They can stimulate growth in short children, and increase the bone mineral density of rodents.

How could the weak anabolic effect of growth hormone and IGF-I on human bone growth be strengthened, so that it matches or exceeds what has been achieved with rodents?

One part of the answer, as mentioned above, is that it may take much longer in humans than in rodents for growth hormone to stimulate bone growth. However, there are other possible circumstances that could frustrate the anabolic effect of growth hormone.

One such circumstance is the existence of unexpected feedback interactions between the factors that regulate bone mass. Life requires balance, and the body has many restraints to correct imbalances. These restraints can sometimes frustrate medical intervention.

**Growth hormone, vitamin D and bone.** As an example, growth hormone and vitamin D both have anabolic effects on bone, and both are given to people whose bones need strengthening. Very recently, it was discovered that vitamin D reduces expression of the growth hormone gene. Thus one anabolic factor unexpectedly suppresses another.

Another circumstance that can frustrate medical intervention is the existence of multiple requirements, some of them unknown, for a given process to occur. If one or more requirements are not met, the process as a whole either fails or is diminished.

As an example of an unappreciated requirement, it has recently been discovered that the amount of zinc present in the diet of experimental rats is suboptimal, and that adding zinc increases bone growth. Rats fed diets with even less zinc showed bone growth deficiency. Here is a case where an unexpected contraint could prevent growth hormone administration from achieving its full potential. Our present ignorance is such that many other limits on bone growth may have escaped notice.

The real hope for progress in this field lies in ongoing research that will tease apart the regulatory interactions and identify all relevant constraints on bone anabolism. This may finally allow application of a stimulus free of contraints that would frustrate it.

## 4.2 Age, muscle loss (sarcopenia) and growth hormone.

### 4.2.1 A description of age-related sarcopenia

The loss of muscle mass, strength and endurance, that occurs with ageing is termed "sarcopenia." Sarcopenia steadily increases in ageing; observed over long periods the loss of muscle tissue can reach 1% per year.

Clinically significant sarcopenia develops in 10-20% of old people, and the percentage increase with age. Sarcopenia is a major cause of disability

and frailty in the elderly. It brings with it a loss of mobility and independence, an increase in falls and can compromise vital functions such as respiration. It also compromises nutritional reserves, since muscle serves as a nutritional reservior. Sarcopenia is often made worse by simultaneous obesity.

Most of the changes leading to sarcopenia begin by middle age.

In sarcopenia, there is a decline in muscle innervation, and an increase in irregularity of muscle unit firing. In aged muscle, normal muscle fibers are present along with fibers that are abnormally thin and others that are abnormally thick. Aged muscle acquires scleroses (abnormal hardening) and fat deposits. There is a disproportionate atrophy of type IIa (fast-twitch) muscle fibers, and a decrease in synthesis of a key muscle protein, the myosin heavy chain.

### 4.2.2 The causes of age-related sarcopenia.

Sarcopenia is thought to have several causes. One important cause is muscle disuse, caused by a sedentary life, illness or some other immobilizing factor such as arthritis. Other causes include under-nutrition and lack of vitamin D, a loss of nerves that innervate the muscles, and a decrease of anabolic hormones such as testosterone, dehydroepiandrosterone (DHEA), growth hormone, insulin and IGF-I. In addition to reduced circulating IGF-I, made mainly in the liver, there may be a decrease in forms of IGF-I that are made and responded to in the muscle itself.

Although sarcopenia occurs even in the healthy elderly, illness can contribute greatly to it. For one thing, drugs such as corticosteroids, dexamethasone, and cyclosporin can accelerate sarcopenia. Even more importantly, chemical signals (hormones and cytokines) associated with inflammation and disease can cause muscle loss that is never restored. These chemical signals include interleukin-6, tumor necrosis factor-alpha, interleukin-1beta and cortisol.

Finally, DNA damage and other changes may progressively inactivate muscle mitochondria. Mitochondria are the energy factories of cells, where a major intracellular fuel, adenosine-5'-triphosphate (ATP), is made. Mitochondria contain miniature chromosomes, somewhat similar to the chromosomes of bacteria; damage to these (i.e. DNA damage) reduces muscle function.

### 4.2.3 Exercise is the best antidote to sarcopenia

Exercise can delay and even reverse sarcopenia. Programs of weight training consisting of intense exertion against resistance, done typically 2 to

4 times per week and lasting 4 to 6 months, can increase the strength of older adults by one-quarter to nearly one half. One such program, involving men between ages 65 and 77 increased their strength by an average of 40%. Another program involving women of average age 64 increased their thigh strength by an average of 37%. In a study involving elderly men, some muscle groups increased in strength by as much as 84%.

Typically, there are other benefits, such as an increase in the rate of force development. There is both muscular hypertrophy and an increase in the effectiveness with which nervous impulses activate the muscles. In one study involving elderly women, the cross-sectional areas of muscle fiber types I, IIa and IIb increased by 22-36%.

Weight training and the resulting increase in strength reduce the frequency of falls and bone fractures in the elderly. They help maintain independence, and greatly increase the quality of life in older people.

### 4.2.4 Growth hormone may modestly increase muscular strength in the elderly.

In elderly women, ages 70-79, there is an association between knee extensor strength and blood IGF-I concentrations. In elderly women that are deficient in IGF-I, there is a correlation between IGF-I blood concentration and walking speed. Elderly women with low IGF-I levels have greater difficulty in tasks that require mobility.

Heart disease patients with low levels of IGF-I also have reduced quadriceps strength and cross-sectional area. Hence, low levels of IGF-I, the effector hormone of growth hormone, seem to cause deficits in muscular strength, especially in the elderly.

An important medical question is whether supplementation with growth hormone or IGF-I can can replace or augment resistance exercises in the prevention of sarcopenia. The answer seems to be that growth hormone strengthens muscles in the elderly, either with or without resistance training, but that the effects are small.

Whereas all or nearly all studies show that resistance training increases strength, many studies have concluded that growth hormone does not increase strength, either alone or together with resistance training. On the other hand, many other studies do show a modest increase in strength in the elderly after growth hormone treatment. In one study of healthy men over 60 years, growth hormone treatment of 3 months increased muscle mass by an average of 3.3 kg and thigh strength by an average of 14%.

The relative failure of human growth hormone to increase muscular strength in humans is surprising, given its success in rats. Elderly (23

months) female rats were human growth hormone injections for 73 days. The maximum contractile strength of the calf muscles in GH-injected rats increased 23% relative to controls. Mild exercise (walking for one hour per day) added to the GH injections increased calf muscle strength by an additional 18%. (Mild exercise by itself did not influence calf strength.)

It may be that long exposures to growth hormone are required to significantly stimulate muscular strength in humans. Several studies in non-elderly adults who are deficient in growth hormone have shown that strength increases are apparent at 12 months but not at 6 months, or are apparent at 24 months but not at 12 months.

### 4.2.5 Growth hormone increases lean mass, but the benefit is uncertain.

There have been many attempts to dissect the effects of growth hormone on muscle, in hopes of increasing growth hormone's benefits. However, the results so far have not added up to a clear picture of how growth hormone and IGF-I affect muscle.

It is clear that too much growth hormone can harm muscle. In one experiment, rats were injected with a growth-hormone producing tumor that raised their growth hormone levels about 750-fold. The effect on their diaphragm muscles was deleterious.

Humans with acromegaly, i.e. having a pituitary tumor that over-produces growth hormone, have larger than normal muscles. However, muscle strength and performance are not improved.

Growth hormone and IGF-I treatment usually increase a subject's "lean mass" or "fat free mass" and increase nitrogen retention. However, this "lean mass" may consist of retained fluid or connective tissue; it usually is not functional muscle. Similarly, the retained nitrogen is often not retained in muscle proteins.

Muscle fiber size appears to be increased by growth hormone treatment in elderly or GH-deficient adults. However, this can occur even when there is no increase in strength.

Administration of growth hormone to a subject can induce protein synthesis within muscle, and can affect muscle composition by changing the expression of actin and myosin genes. However, it is not clear whether or how this affects muscle performance.

### 4.2.6 Innervation changes may enhance muscle strength.

Muscle size and strength are correlated; hence, it is often assumed that increases in strength require muscle growth. However, experiments show

that muscle strength can increase without growth. Researchers often ascribe such increases to improved innervation of the muscle.

One experiment with elderly men involved training, detraining and then retraining. Men, ages 65-77 were given resistance training for 24 weeks. Their strength increased about 40% during this time, and this increase was accompanied by increases in muscle fiber size.

They then detrained for 12 weeks. During this time their muscle fiber size returned to normal, but they lost only 30% of their strength increase.

They then retrained for 8 weeks. During this time, their strength returned to maximal values but their muscle fiber size increased only slightly. The researchers suggested that much of the strength retention during detraining and reacquisition of lost strength with retraining reflects neural adaptation.

### 4.2.7 Growth hormone resistance

Muscle weakness can result from failure of IGF-I to respond to growth hormone. In one study, strength of the quadricep muscles of the legs was measured in patients with chronic heart failure. As expected, patients with low IGF-I levels had reduced strength (a reduction of 24%) and quadriceps cross-sectional area (a reduction of 12%. However, the levels of growth hormone in these patients were nearly two and one half times their normal values. Thus, IGF-I appeared to have become insensitive to induction by growth hormone.

A number of catabolic hormones, including tumor necrosis factor-alpha, adrenaline, noradrenaline and the cortisol/dehydroepiandrosterone ratio were significantly elevated. Presumably, these elevated hormones contributed to muscle weakness. Whether the elevations caused the unresponsiveness of IGF-I levels to growth hormone is unclear.

Thus, in one condition commonly associated with ageing, IGF-I levels are low because IGF-I synthesis becomes unresponsive to growth hormone level.

One obvious path to growth hormone insensitivity in a given tissue is for the growth hormone receptor frequency to fall. An example of this occurs in the rat hippocampus (part of the brain), where growth hormone can upregulate its own receptor in young male rats, but not in elderly rats. Whether a similar effect occurs in muscle tissue is not known.

### 4.2.8 Exercise induces growth hormone

Since resistance training (certainly) and growth hormone (probably) both increase muscle strength, a logical question is whether resistance training acts by elevating growth hormone levels. The answer could affect future

strategies to coax the greatest muscle-building benefit from the combination of training and drugs. Some studies report that exercise increases growth hormone secretion, while other studies report no such increase. Among the studies reporting an increase, there is agreement that the stimulating effect of exercise on growth hormone diminishes with age.

### 4.2.9 Obesity decreases growth hormone secretion

Obesity often reduces growth hormone secretion. This is particularly true in the elderly, who unfortunately are most likely to be obese. One study of exercise-induced growth hormone stimulation in men showed that lean older men secreted more growth hormone than obese older men in response to exercise, while obese younger men and lean younger men secreted equal amounts of growth hormone in response to exercise.

Thus, control of obesity in the elderly could be a necessary part of strategies to prevent strength loss. [see **H4 3 3**]

### 4.2.10 Locally produced IGF-I may dominate age-related sarcopenia

Although circulating IGF-I originates mainly in the liver, IGF-I is also produced in muscle. Growth hormone can induce this IGF-I, at least in cultured muscle cells; but other stimuli such as exercise also induce it and are probably more important.

A test of weight training effects in young males showed that they made greater gains in arm strength when their legs were also trained. This result (which needs confirmation) suggests that circulating hormones are important in muscle hypertrophy. Growth hormone and IGF-I are good candidates for that role.

On the other hand, it has been suggested more than once that a decline in locally-produced IGF-I is the key to sarcopenia. Drops in circulating growth hormone or IGF-I would thus be less important, and replacement would be correspondingly less effective.

### 4.2.11 GH inducers can strengthen muscles in the elderly.

Hormonal inducers of growth hormone, including Growth Hormone Releasing Hormone and ghrelin [**H6 4**, **H6 6**], have the potential to stimulate muscle growth indirectly with growth hormone as an intermediate. These inducers are more stable, easier to administer and cheaper than growth hormone or IGF-I. In addition, they preserve the natural pulsative pattern of growth hormone secretion -- a pattern that is probably required for full benefit (see Part 6.2 [**H6 2**]).

In one study of elderly men, nightly self-injections of GHRH increased

muscular strength by several measures. This was accompanied by an increase in growth hormone production, although there was no increase in circulating IGF-I.

### 4.2.12 Additional influences on muscle hypertrophy.

Many variables can increase or decrease muscular strength. Attempts to increase the strength of the elderly will have to contend with these, either to circumvent or exploit them.

An important sex steroid precursor, dehydroepiandrosterone (DHEA), was reported to raise IGF-I levels in elderly men and women. This increased muscle strength in the men, but not the women.

Many bodybuilders and other athletes are engaged in an unscientific, but effective effort to promote maximum muscular strength or hypertrophy using illegal anabolic drugs. Many of their methods may eventually be copied in an attempt to strengthen the muscles of the elderly.

One published study of drug-abusing bodybuilders showed that there are unexpected interactions between growth hormone and anabolic steroids. Although growth hormone increases the blood concentration of IGFBP-3, a protein that may affect IGF-I function [H6 9 37], anabolic steroids reduce IGFBP-3.

Dietary restriction has also been reported to reduce IGF-I levels. Hence, attempts to limit fat might also block the building of muscle.

It is unclear why treatment with growth hormone and other members of the growth hormone axis have modest and inconsistent anabolic effects.

One possibility is that its administration is usually non-physiologic and that this compromises its effectiveness. Growth hormone is pulsatile, with peaks and valleys of synthesis occurring about every three hours. Moreover, synthesis is much greater at night than during the daytime, at least in young adults. The body is remarkably stubborn in its preservation of GH pulsatility, and the pulsatility imparts a surprising amount of information to target tissue (see [H6 2]). By this hypothesis, administration of growth hormone in a more physiologic pattern would increase its stimulation of muscle growth; however, attempts to do this have so far produced only modest increases.

A second possibility, mentioned above, [H4 2 7] is that muscle loses its responsiveness to growth hormone as it ages, or as age-related health problems appear. Age-related changes in any of the component of the growth hormone axis could bring this about. In particular, although growth hormone induces IGF-I, IGF-I suppresses its own synthesis. This self-suppressive effect of IGF-I is more powerful than the inducing effect of

growth hormone. An increase in the self-suppression of locally produced IGF-I, perhaps caused by a reduction in some local IGF-I binding protein, could reduce IGF-I levels, and make muscle very resistant to anabolism. In any case, IGF-I levels in muscle do decline with age (a decline of about 25% from age 25 to age 70 in men); in one study, one-third of older men had muscle IGF-I levels below that of any young man tested.

A third possibility is that additional influences are at work which limit the effectiveness of growth hormone. It may be that the muscles of the elderly are undernourished in some regard, even when the person is well-fed. Alternatively, a number of anabolic hormones decline with age, which might limit muscle growth. The declining hormones include serum bioavailable testosterone (T) and estradiol (E2), dehydroepiandrosterone (DHEA) and its sulfate (DHEAS). A rise in another pituitary hormone, luteinizing hormone, seems to reduce muscular strength in elderly men, and increase their difficulties encountered in daily living.

One very powerful influence on muscle growth is the protein myostatin, which inhibits muscle growth. Cattle and mice with defective myostatin genes have hypertrophied muscles, and there are indications that small variations in the human myostatin gene may influence muscular size and strength. Even small variations in myostatin physiology could cause great differences in strength between young and elderly adults; however, so far such differences have not yet been found.

## 4.3 The effect of exercise on growth hormone in the elderly.

### 4.3.1 Exercise stimulates growth hormone.

Exercise, especially heavy exercise, is a major stimulus of growth hormone secretion. This stimulation occurs in both sexes. The biological purpose may be to alter metabolic fuel use (e.g. to raise blood glucose), or to stimulate tissue repair after heavy exercise.

The exercise effect on growth hormone secretion may also affect sleep. Heavy exercise conducted in the afternoon affects the pattern of growth hormone secretion the following night. This presumably affects sleep patterns [H4 10].

Generally, the stimulation is in proportion to the intensity of the exercise, at least in young adults. However, it has also been reported that one exercise bout can prevent stimulation by a second exercise bout, an hour later.

### 4.3.2 Exercise alters multiple components of the growth hormone axis.

Circulating growth hormone consists of multiple similar species, termed

isoforms, that originate from the same gene [H6 8 2]. It is suspected that these isoforms differ somewhat in their function. Heavy exercise alters the ratio of these isoforms. The most prevalent isoform, the form with a molecular mass of 22,000 daltons (22,000 times the mass of a hydrogen atom), forms a smaller proportion of the whole after exercise. A different form, with a molecular mass of 20,000 daltons, forms a larger proportion. Other minor isoforms may also increase proportionally.

The hormones IGF-I and IGF-II are thought to mediate many of the effects of growth hormone. The biological activity of these is in turn potentially affected by at least six types of IGF binding protein. Intense exercise has been reported to strongly affect the concentrations of several IGF binding proteins, although reports disagree as to the changes that occur. (The type of exercise done can affect the results; see below [H4 3 4].)

Although exercise is one of the three main stimuli of growth hormone (along with sleep and hunger), the way in which exercise stimulates growth hormone is still unknown. Exercise may act by inhibiting somatostatin release from the hypothalamus, but this cannot fully explain exercise's GH-releasing effect. Presumably, exercise stimulates GHRH, ghrelin or some other inducer.

### 4.3.3 The effects of age, obesity and physical fitness on the GH axis.

As people age, they usually become more obese and physically less fit. As this happens, their levels of circulating growth hormone and IGF-I drop. Moreover, the secretion of growth hormone in response to exercise diminishes greatly with age.

Because age, obesity, fitness, growth hormone and IGF-I vary together, it is difficult to determine which changes cause the others. The branch of statistics known as "multivariate analysis" has the potential to show which changes are really linked and which merely co-vary due to their association with other variables. Multivariate analysis has been used for this several times, but different authors reach different conclusions.

The following can be said:

Both growth hormone and IGF-I levels fall with age in both sexes.

Growth hormone is an inducer of IGF-I, and growth hormone levels are correlated with IGF-I levels. However, this correlation becomes weaker with age. It is thought that influences other than growth hormone become more important determinants of IGF-I levels as people age.

Women usually have more growth hormone than do men. This is true both for young adults and the elderly.

Young men and women have similar amounts of IGF-I. However, older

women have signficantly less IGF-I than do older men.

Exercise is a potent stimulator of growth hormone secretion. Secretion rises throughout the exercise period and peaks between 0 and 30 minutes (typically 10 minutes) after the exercise has stopped. It declines slowly thereafter.

The stimulation of growth hormone by exercise declines drastically with age. Some studies show a residual effect in elderly people, while others show no effect.

Obesity is inversely correlated with growth hormone levels. Abdominal adiposity, the accumulation of fat around the abdominal viscera, has the strongest inverse correlation with growth hormone secretion. A redistribution of subcutaneous fat into the abdomen is characteristic of ageing.

It is probably true that growth hormone reduces obesity and obesity reduces the secretion of growth hormone; however, this is hard to prove and researchers disagree about how important a block obesity is to growth hormone secretion.

If growth hormone and obesity are mutual antagonists, adiposity may be self-stimulating, with the people most in need of growth hormone unable to make enough. Thus, some additional means to stimulate growth hormone in obese people may be called for.

Physical fitness and obesity are separable. One measure of fitness that is commonly used is respiratory capacity, "VOmax." Researchers disagree about how important fitness is to growth hormone secretion in the elderly. Some believe that fitness is strongly correlated with the ability to secrete growth hormone, while others report that even habitual marathon running or resistance training does not increase growth hormone secretion in the elderly.

There are probably many unexpected influences operating. For example, a report that marathon running did not affect growth hormone, IGF-I or IGF-II, indicated that the IGF-modulating hormones IGFBP-1 and IGFBP-2 were increased. As another example, exercise increases growth hormone levels more rapidly in young obese men than in young lean men; the difference was tentatively attributed to difference rates of body temperature increase. [see **H4 3 6**].

The loss of GH inducibility with age is unexplained, but seems to be deeply embedded in the system. One possibility is that the hormone somatostatin, which inhibits growth hormone release, is more active or more prevalent in older people. However, tests with a drug that inhibits somatostatin show that even a complete inhibition of somatostatin only

partly restores youthful growth hormone secretion to old people.

### 4.3.4 The type of exercise greatly affects its stimulation of growth hormone.

The stimulation by exercise of growth hormone and IGF-I depends on the exercise itself: how intense it is, how long it lasts, and perhaps other factors. The reported variation between different exercise programs is surprisingly large.

One study compared an incremental ergometer cycling exercise lasting 20 minutes with a treadmill-simulated soccer game lasting for 90 minutes and with a long- distance Nordic ski race lasting 3 hours. Serum growth hormone increased more than 15-fold after the cycling exercise, less than 5-fold after the simulated soccer game and less than 3-fold after the Nordic ski race. In general, it appears that greater activation of anaerobic glycolysis and lactate formation increases the amount of GH released.

The three types of exercise also varied markedly in their effects on IGF-I and on the IGF binding proteins IGFBP-1 and IGFBP-3. Presumably, these changes control the hormonal activity of IGF-I in response to different physical challenges.

The significance of these changes is still unclear. However, IGFBP-1 reduces the activity of IGF-I; IGFBP-1 increased in proportion to the duration of the exercise (it rose nearly 12-fold after the 3-hour ski race and by more than 6-fold after the 90-minute simulated soccer game, but only 1.7-fold after the 20-minute cycling exercise). Hence, it was suggested that long periods of exercise stimulates IGFBP-I for the biological purpose of limiting IGF-I's hypoglycemic effects and stimulating glucose uptake by muscle tissue when glycogen stores become depleted.

There is one report from Korea that a type of training called ChunDoSunBup Qi- training is unusually effective in increasing growth hormone levels in the elderly (by more than 7-fold).

Researchers hope that a careful analysis of growth hormone stimulation may provide clues that will allow it to be done more effectively.

### 4.3.5 Amenorrheic women athletes have abnormal growth hormone secretion

Women athletes with very low body fat sometimes experience amenorrhea, the failure to menstruate. One study has shown that amenorrhea is associated with irregularities in growth hormone and secretion. Pulses are nearly twice as frequent (+85%), but last only half as long. The amount of growth hormone secreted per pulse is three times normal, and the half life of

the growth hormone in the blood is increased 60%. The usual peak that appears in response to exercise is reduced by a factor of 4 or 5. Downstream proteins such as blood IGF-I, IGFBP-1 and IGFBP-3 appear not to be affected by amenorrhea.

### 4.3.6 Diet, supplementation and temperature can influence growth hormone secretion.

Growth hormone secretion in response to exercise may be influenced by diet, by supplementation and even by ambient temperature.

Protein-carbohydrate supplements, consumed before and immediately after workouts, have increased the serum levels of growth hormone, IGF-I and insulin in men engaged in heavy resistance training. Melatonin supplementation increased both growth hormone and IGFBP-1 in healthy males engaged in bicycle exercise.

In addition, men engaged in swimming exercise secreted more growth hormone when the pool temperature was 36C than when it was 29C. This increase was associated with a higher body temperature, and occurred in untrained athletes but not in highly trained athletes.

### 4.3.7 Water lost to sweat alters blood levels of growth hormone and related hormones.

The effect of exercise on serum concentrations of hormones is altered by loss of water due to sweating. Losses due to sweating can easily amount to more than half of a liter. This is typically more than 10% of the blood volume and is enough to significantly increase the serum concentrations of growth hormone, IGF-I, IGF-II and IGF-binding proteins.

When exercising athletes drank an amount of water equal to what they lost due to sweating , the total amount of growth hormone that they secreted significantly increased (by nearly 50%).

### 4.3.8 Exercise induces other hormones that could change the effects of growth hormone.

Intense exercise changes the concentrations of hormones other than members of the growth hormone axis. Among the hormones that increase are insulin, testosterone (even in females), estradiol, dehydroepiandrosterone (DHEA), prolactin and cortisol. Most of these hormones are anabolic, or at least not catabolic, but cortisol is catabolic.

Any of these hormones might alter the effects of growth hormone. A complete understanding of exercise's effects, and the chance for optimum manipulation, requires that they be taken into account.

### 4.3.9 The influence of growth hormone on exercise capacity in the elderly.

Too much growth hormone can have deleterious effects on exercise ability. Acromegalics, who produce too much growth hormone, complain of fatigue. They have reduced physical functioning and exercise capacity. Thus, a narrow window for GH/IGF-I levels is required for optimal physical function, and elevated levels might or might not increase the strength and endurance of the elderly.

Although it has proven difficult to raise growth hormone and IGF-I levels in elderly people without growth hormone injections, it is encouraging that much physical improvement is possible in the elderly even without them. One group of men, aged 60-70, ran marathons even though they had no more IGF-I than non-athletic men of the same age. (The marathon runners presumably also had no more growth hormone, although this was not measured in this study.) They had increase regional muscle strength (the quadriceps), and were leaner and lighter than their non-athletic counterparts.

Growth hormone may be of modest benefit to the healthy elderly, so far; but exercise works wonders.

## 4.4 Growth hormone can reduce fat in the elderly.

The proportion of a person's weight that consists of fat increases with age. An ageing person's waist-to-hip ratio, amount of intra-abdominal fat and (to a lesser degree) subcutaneous fat is likely to increase over time.

Such fat contributes to heart disease, cancer and despondence. It increases the danger of falls and fractures in the frail elderly.

Fat accumulation is also a characteristic of growth hormone deficiency. Since a person's blood concentration of growth hormone decreases with age, it has been suggested that the increase in fat that accompanies ageing may be due to this decrease. If so, measures to increase an ageing person's supply of growth hormone might lessen or reverse the accumulation of fat.

Growth hormone treatment reduces body fat in obese rats, and in children and adults with growth hormone deficiency. This is true whether the growth hormone deficiency is the primary defect, or is secondary to some condition such as kidney failure.

Growth hormone does not necessarily decrease fat in everyone. In one short study of young (average age 23 years) power lifters, it had no effect at all.

Attempts to reduce the amount of body fat in ageing people by treatment with recombinant growth hormone usually succeed. Typically, fat is lost

and "lean mass" (not necessarily muscle) replaces it.

In one 6-month study of elderly men (average age, 72 years), growth hormone treatment reduced the abdominal area by an average of 3.9%, and subcutaneous fat by 14%. Growth hormone treatment has been reported to reduce fat in ageing women, in combination with dietary restriction and exercise.

The fat-decreasing effect of growth hormone treatment increases with dose, along with the likelihood of unwanted side effects. The effect may be somewhat less in older people than in younger ones.

Obesity probably decreases growth hormone secretion. Visceral fat, i.e. fat in the abdominal cavity around internal organs, may have the greatest inhibitory effect [see **H4 3 3**].

Obesity actually has multiple influences on growth hormone secretion but their sum is strongly inhibitory. One influence is the production of a protein called "leptin"; leptin stimulates growth hormone production, but its effect is outweighed by the other influences of obesity.

A second influence of obesity is to increase the concentration of free fatty acids in the blood. A third is to increase insulin concentrations; insulin, like IGF-I (insulin-like growth factor-I), suppresses growth hormone secretion. A fourth influence is to unlink IGF-I levels from their dependence on growth hormone, probably by altering the level of IGFBP-1, which inhibits IGF-I; IGF-I levels remain high, and feedback inhibit growth hormone.

Insufficient growth hormone promotes obesity, and obesity may guarantee insufficient growth hormone. Thus, both obesity and leanness may be self-promoting. This possibility has led to a search for medical interventions that could tip the balance toward leanness.

Although fasting normally stimulates growth hormone production, fasting does not increase growth hormone production in obese people, or does so mimimally. Long-term dieting and extensive weight loss do restore growth hormone secretion (unless GH production failure is the primary defect), but this is notoriously difficult and frequently unsuccessful.

In addition, some cases of obesity may result from unsuspected growth hormone deficiency. Some women with large visceral adiposity appear to have disrupted growth hormone secretion. Their growth hormone levels are only one-fourth normal, and the pulsatile regularity of normal growth hormone release is disrupted. Even when these women had lost 40% of their visceral fat through calorie restriction, normal growth hormone secretion was not restored.

One medical intervention is surgical removal of fat, called "lipectomy" or

"liposuction". More research is needed as to whether lipectomy benefits an obese person's blood lipid profile. However, two problems are already apparent. First, without dietary and exercise modification, the removed fat usually returns within a few months. Second, the most dangerous fat is the visceral fat, which lipectomy does not remove. It is even conceivable that by increasing the proportion of fat that is intraabdominal, lipectomy could do harm.

A second intervention is to give patients growth hormone. This is feasible and valuable, but has several drawbacks. It is expensive, requires injection, often has unpleasant side effects, and disrupts the normal pulsatile pattern of growth hormone secretion [see **H5 2** and **H6 2**].

A third intervention would be to decrease the amount of free fatty acids in the blood, presumably by some pharmceutical method. This has been shown to work in principle. The drug acipimox, which prevents the conversion of triglycerides (fat) to free fatty acids greatly enhances the effect of a growth hormone inducer (a ghrelin-like inducer called GHRP-2 [see **H6 6 3** for a discussion of ghrelin mimics]). This particular intervention would be useless in decreasing fat, since it prevents fat breakdown. However, it opens the possibility that some other intervention to decrease the blood concentration of free fatty acids, or to interfere with their effect on growth hormone secretion, might succeed.

This experiment was done with elderly men as the subjects. The fact that growth hormone secretion increased shows that short term pituitary capacity is retained in aged men.

A fourth possible intervention is to increase the hormones that stimulate the pituitary gland to secrete growth hormone. One of these is Growth Hormone Releasing Hormone, which is secreted by the hypothalamus. Unfortunately, visceral fat appears to make the pituitary gland less sensitive to Growth Hormone Releasing Hormone (GHRH).

Another hormone that stimulates the pituitary gland to secrete growth hormone is ghrelin. Ghrelin ("Growth Hormone Releasing Endogenous Ligand-in") [see **H6 6**] is made mainly in the gut, although it is made in other organs as well, such as in the placenta. Its stimulatory effect on growth hormone is often much greater (say four-fold) than that of GHRH.

Ghrelin is the link between hunger and growth hormone. Hunger is one of the major behavioral controls on growth hormone, along with sleep and exercise.

Ghrelin has many biological effects in addition to its stimulation of growth hormone. Several of these would be unwelcome in an effort to lose fat. Ghrelin stimulates hunger, stimulates stomach acid secretion and decreases

fat use by the body. In fact, injection of ghrelin into rodents increases their body fat.

However, drugs might be developed that mimicked ghrelin's stimulation of growth hormone, but not its stimulation of appetite or fat accumulation. As discussed below [H7_3_11] there are good prospects in general for such unlinking of hormonal effects. Moreover, in the case of ghrelin there is already a very rich research history of ghrelin mimics and partial mimics [H6_6]. If there were a clean separation of the activities, and no reduction of the desired activity, such drugs could be powerful inducers of growth hormone and weight loss.

Alternatively, it might be beneficial to reduce ghrelin level or activity. Very obese people sometimes have a gastric bypass operation. This operation greatly reduces their ghrelin levels, and this reduction is thought to be important to the weight loss that follows [H6_6_31]. Ghrelin blockage by drugs or selective inactivation of ghrelin-producing cells in the stomach might reduce fat in the elderly and others.

## 4.5 Growth hormone axis hormones can benefit heart disease.

### 4.5.1 A description of heart disease.

Heart disease is a leading cause of sickness and death in Western societies. In people with heart disease, the flow of oxygenated blood to the body's tissues becomes inadequate to support proper function or even life.

Heart disease can develop in at least two general ways. First, the heart can fail as a pump. Either the muscles may become too weak to pump effectively, or the valves that keep the blood flowing in the right direction may fail, or the heart may undergo some other physical change that prevents it from pumping properly. In addition, the resistance that the heart must work against may become too great for the heart to pump effectively. This can occur when the blood vessels become constricted.

Second, the blood supply to the heart may be reduced or stopped, and the heart muscle may weaken and may be injured or killed. Many influences can increase the chances that a blockage will form in an artery that feeds the heart. One of these is inflammation of blood vessel walls caused by infectious disease, smoking or other chemical insult, or psychological stress. This is particularly true if the smooth muscle in the blood vessel walls is injured and overgrows. A second is an adverse lipid profile [see below H4_5_4], which deposits plaque on arterial walls. A third is an increased tendency toward blood clotting that can be caused by infectious disease, certain types of autoimmunity, or other factors. A fourth is a tendency

toward vasoconstriction of arterial walls. A fifth is high blood pressure, which damages arterial walls and promotes plaque buildup. A sixth is diabetes.

Loss of blood supply to the heart can cause tissue death or other changes that will permanently reduce the heart's pumping capacity. Loss of pumping capacity can further reduce blood supply to the heart. Hence, loss of pumping capacity and loss of blood supply to the heart can increase each other.

Heart disease differs from other other symptoms of ageing, such as loss of muscle strength, in that it is not considered normal. It is not part of healthy ageing.

### 4.5.2 Growth hormone, IGF-I and ghrelin affect cardiovascular health.

Three related hormones of the growth hormone axis play a large role in heart disease, mostly by acting against it. The three hormones are growth hormone itself, insulin-like growth factor-I (IGF-I) and ghrelin. IGF-I is stimulated by growth hormone, and serves as an intermediary between growth hormone and many of its target tissues. Ghrelin is made in the gut, and increases growth hormone secretion by the pituitary gland. The influences of these three hormones on heart disease are discussed below.

### 4.5.3 Growth hormone may improve circulation.

Growth hormone has several short-term and long-term effects on cardiovascular health. One beneficial effect is to decrease resistance to blood flow in the vascular system. The reported experiment involved administration of high doses of growth hormone to young adults for four weeks.

Growth hormone may also increase the diameter of the aorta. Injection of high doses of growth hormone into old female rats for 80 days increased the diameter of the aorta by 5%, resulting in a 10% increase in the cross-sectional area of the aortic lumen (channel). However, the body weight of the rats also increased by an average of 47%; hence, if the two effects are inseparable, the cardiac benefit is uncertain. Nor, is it certain that elderly humans would respond the same way.

### 4.5.4 Growth hormone treatment and lipid profiles.

Growth hormone treatment for one or more years improves the blood lipid profile of adults with growth hormone deficiency. Total cholesterol, low-density lipoprotein cholesterol and triglyceride levels fall. High-density

lipoprotein (the so-called "good" cholesterol) levels rise. Interestingly, the effect increases with patient age.

It is not yet established whether growth hormone can improve lipid profiles in ageing healthy adults. However, it has been reported that high growth hormone levels are statistically associated with favorable lipid profiles in elderly (>65 years) adults, independent of body composition.

Growth hormone may have one adverse effect on blood lipid profiles. GH has been reported to increase (by two-fold) the concentration of the lipoprotein Lp(a), although not all researchers have found this. Lp(a) is a risk factor for heart disease. It may increase cholesterol deposition in arterial wall and promote intra-arterial blood clotting.

IGF-I has the opposite effect; it rapidly lowers Lp(a) levels. Thus, clinical situations in which there are high levels of growth hormone but low levels of IGF-I could be dangerous. Primary growth hormone insensitivity (Laron Syndrome) is one such condition.

### 4.5.5 Growth hormone can mechanically strengthen hearts.

Growth hormone strengthens the left ventricle is rats and humans with growth hormone deficiency. This is accompanied by changes in the synthesis of cardiac proteins, particularly the myosin heavy chain.

### 4.5.6 Growth hormone is diabetogenic.

Growth hormone increases blood sugar, while IGF-I lowers it. Increases in blood sugar can be particular harmful in patients that are overweight. Hence, this effect of growth hormone generally does not benefit the heart.

### 4.5.7 Insulin-like growth factors stimulate heart cells.

IGF-I and IGF-II are stimulated by human growth hormone. However, both are made at a low level even in the absence of hGH.

IGF-I and IGF-II have profound stimulatory effects on heart muscle, as they do on many tissue types. Addition of either IGF-I or IGF-II to cultured rat ventricular cardiomyocytes causes a rapid increase in size, with reaches an average of 57% in two days. This increase in size is accompanied by an increase in protein synthesis.

It is assumed that the purpose of these changes is to strengthen the pumping function of the heart, although this remains unproved. Little is known about the regulation of this process. However, at least in culture, the IGF-I binding protein IGFBP-3 blocks the action of IGF-I.

### 4.5.8 IGF-I elicits nitric oxide, an anti-atherosclerotic substance.

One very important effect of IGF-I is to stimulate production of nitric oxide in the endothelium, a layer of cells that lines the heart cavity and blood vessels. Nitric oxide, in turn, has powerful anti-atherosclerotic effects. First, it dilates blood vessels. Second, it inhibits platelet aggregation and monocyte adhesion (and hence reduces the formation of blood clots). Third, it inhibits the growth of vascular smooth muscle; such growth occurs in response to injury and can narrow or close blood vessels.

As a stimulator of IGF-I, growth hormone plays an important indirect role in ensuring that nitric oxide production by the endothelium is adequate. In growth hormone-deficient patients, nitric oxide-induced vasodilation is impaired. This may contribute to heart failure, since nitric oxide-induced vasodilation is often impaired in patients with heart failure.

Nitric oxide has been reported to inhibit human growth hormone. In this case, nitric oxide was acting as a mediator for interferon-gamma, a signalling molecule associated with disease. Optimal use of IGF-I for cardiovascular health might require preventing this effect.

### 4.5.9 Other influences of IGF-I on heart disease.

IGF-I may regulate cholesterol levels, because people with Laron Syndrome have elevated blood cholesterol. Laron Syndrome is a defect in the growth hormone receptor that prevents growth hormone from increasing IGF-I levels.

IGF-I may also hold the key to a medical mystery. People with low birth weights have an increased chance of developing type II diabetes in later life, and suffering a myocardial infarction. It now appears that low birthweight and a predisposition to type II diabetes and myocardial infarction are both associated with a variant of IGF-I. Hence, some inherited abnormality of IGF-I expression or function might contribute to these maladies.

### 4.5.10 Muscle-specific IGF-I can be important to the health of heart patients.

IGF-I that circulates in the blood is produced mainly by the liver. IGF-I for local consumption is also produced in muscles, both skeletal muscles and the heart. Reductions in this local IGF-I can have profoundly deleterious consequences for muscle size and function.

Chronic heart failure is associated with progressive atrophy of skeletal muscles. Disuse of skeletal muscles and a chronic state of inflammation have both been implicated, but the pathology is mostly unexplained.

Patients with chronic heart failure have serum levels of IGF-I that equal the levels of healthy patients. However, the amounts of IGF-I in skeletal

muscle are reduced by about half. It is plausible that this IGF-I contributes to muscle size, because the amount of IGF-I made is correlated with the cross-sectional area of the muscle. There is a nearly four-fold rise in expression of the IGF-I receptor in skeletal muscle in these patients, suggesting that the muscle is starved of IGF-I.

Local expression of IGF-I can also improve heart function. A mouse model of dilated myocardiopathy was created by genetic engineering. Heart-specific expression of an IGF-I gene was then introduced. IGF-I normalized the heart mass. structure and hemodynamics, and prevented apoptosis. (Apoptosis is a form of cell suicide that is important to the control of cancer, but which can have devastating consequences when it occurs in healthy tissues.) IGF-I induced heart cell proliferation, prevented the cells from assuming a characteristic elongated shape that occurs in heart disease, and restored normal calcium dynamics. Hence, it had strong beneficial effects.

### 4.5.11 Ghrelin may benefit cardiovascular health.

"Ghrelin" is another member of the growth hormone axis that can strongly influence cardiac health. Ghrelin induces the secretion of growth hormone from the pituitary gland. Ghrelin [H6 6] induces feeding, through mechanisms that do not depend on growth hormone, and increases the amount of stored fat in humans and animals.

Although ghrelin's tendency to promote fat is unlikely to benefit cardiac health, ghrelin as also a potent vasodilator. This vasodilation effect is thought to outweigh ghrelin's activity as a fat stimulator. Ghrelin receptors are three- to four-fold more numerous in atherosclerotic blood vessels, suggesting a need for more ghrelin. Although ghrelin might induce vasodilation indirectly (ghrelin -> growth hormone -> IGF-I -> nitric oxide -> vasodilation), its main vasodilator activity has been reported to be independent of growth hormone, IGF-I and nitric oxide.

Ghrelin has been reported to improve the condition of rats with experimentally induced heart failure. In particular, it prevented wasting of the heart. Ghrelin has been suggested to compensate for catabolic (tissue-destructive) processes that occur in heart disease.

Ghrelin decreased arterial pressure and increased left ventricular stroke volume in patients with chronic heart failure.

## 4.6 Growth hormone deficiency may contribute to erectile dysfunction.

According to one study of 80 men, in normal, healthy men during sexual arousal, the concentration of growth hormone increases in the blood, including the blood within the erectile tissue of the penis. The same rise

occurs in men with erectile dysfunction when the dysfunction has a psychological cause. However, in men with erectile dysfunction due to a organic cause, the rise in growth hormone is negligible. Moreover, there is direct evidence for a stimulatory effect of growth hormone on the smooth muscle of the corpus cavernosum, which provides the mechanical force for penile erection. Hence, a decline in growth hormone release may contribute to erectile dysfunction.

## 4.7 The growth hormone axis influences blood glucose.

### 4.7.1 Growth hormone promotes insulin resistance.

Growth hormone causes insulin resistance. As a result, patients' blood glucose levels usually rise. The body usually makes more insulin as result, and the blood typically has both more insulin and more glucose. This is of concern because elevated blood glucose levels can develop into diabetes.

An increase in diabetes mellitus among children who are receiving growth hormone has been reported. However, there is a natural increase in insulin resistance in adolescents, and most authors regard the influence of growth hormone in children as simply inducing or prolonging that phase. Withdrawal of growth hormone reverses the elevated glucose within a few months.

Growth hormone also causes insulin resistance in adults. For the most part, this is tolerated. However, in some cases growth hormone therapy seems to have caused or worsened diabetes in adult patients. In burn patients, who are already insulin-resistant, growth hormone administration may cause raise blood glucose levels and thereby expose the patients to lethal microbial (especially yeast) infections [H3 8 3].

### 4.7.2 Growth hormone contributes to normal diabetes.

Diabetic patients have elevated levels of growth hormone. This seems to result from slower removal of growth hormone from the blood, rather than increased secretion into the blood. Hence raised growth hormone levels may contribute to diabetes even in the absence of growth hormone therapy.

### 4.7.3 Growth hormone, diabetes, obesity and "resistin."

Diabetes is brought on or made worse by obesity. The key failure in type II diabetes (diabetes that does not involve a shortage of insulin) is insulin resistance. Adipocytes (fat cells) secrete a hormone termed "resistin" that acts on skeletal muscle, liver and fat cells themselves, reducing their sensitivity to insulin.

Understanding the behavior of resistin is clearly important to human

health, especially the health of people of middle age or older in affluent societies. In mouse fat cells, at least, resistin in up-regulated by high glucose concentrations. Hence, influences such as growth hormone, which increase glucose levels, might lock a patient into insulin resistance. Hence, although growth hormone might benefit an obese patient by reducing fat, it could harm that same patient by inducing hyperglycemia.

A key to the safe clinical use of growth hormone to treat obesity may depend on finding ways to suppress or thwart resistin. Scientists have identified natural substances that can reduce the blood concentration of resistin. One is insulin; a second is tumor necrosis factor-alpha. A safe drug that suppressed or inactivated resistin without unwanted side effects would clearly be valuable.

Insulin-like growth factor-I, which mediates many of the beneficial effects of growth hormone, lowers blood glucose levels, rather than raising them. Hence, it has been suggested that therapy with IGF-I alone, or a combination of growth hormone and IGF-I, might be safer than therapy with growth hormone alone [Comb].

### 4.7.4 Ghrelin is present in the pancreas, and is probably made there.

The ghrelin hormone is a strong inducer of growth hormone. However, it has other effects, including direct effects on blood sugar. Most of the ghrelin in the body is made in the gut, in response to hunger.

The ghrelin receptor protein, which transmits a signal from ghrelin to cell interiors, is present in the pancreas. Hence, hunger may affect the behavior of the pancreas, and perhaps affect synthesis of insulin by the pancreas.

Ghrelin protein itself can be detected in the pancreas, in the alpha cells. Although ghrelin in the blood that bathes the pancreas may contribute to this, ghrelin also seems to be made in the pancreas, since messenger RNA from the ghrelin gene is detectable in the pancreas. Hence the influence of ghrelin on the pancreas may include both ghrelin that is made locally, and ghrelin that originates in the gut.

### 4.7.5 Ghrelin effect on insulin.

Ghrelin has been reported to effect insulin synthesis (which is made in the pancreas). However, reports disagree on whether ghrelin stimulates or inhibits insulin, with more than one research group on each side of the question.

It has also been reported that ghrelin up-regulates some of the intracellular components that carry the insulin signal to the genes in target tissue, and thus would presumably amplify that signal.

Ghrelin has also been reported to stimulate gluconeogenesis, the manufacture of glucose by the liver.

These hormones form a web of interactions. For its part, insulin reportedly reduces ghrelin concentrations in the blood. Insulin, or the drop in blood sugar caused by insulin, stimulates growth hormone production, probably by inhibiting the release of somatostatin.

### 4.7.6 "Leptin" hormone is very important to growth hormone therapies.

A hormone of great medical importance, and of importance to the biological effects of growth hormone is "leptin." Leptin is secreted by fat cells, and acts to reduce appetite and increase energy expenditure. It probably represents a mechanism by which fat tissue can limit its own growth. Clearly, influences that reduce the effectiveness of leptin might promote obesity, while influences that increase leptin's effectiveness might reduce obesity.

The issue has added importance in modern America due to our ageing population and the popularity of fast food, commercial snack food and soft drinks. Fast food and commercial snack food contain much fat, while soft drinks contain much fructose. Fats and fructose are rich in calories, but are reported not to stimulate the production of either leptin or insulin. (Insulin promotes glucose use, which in turn induces leptin production.) It has been argued that this reduced production of leptin and insulin could contribute to long-term weight gain in consumers of much fat and/or fructose.

Leptin holds great promise for the control of obesity. It (or mimics, or partial mimics) could be used directly as an injectable medicine, or introduced as a product of gene therapy. Introduction of leptin into rat brains reduces food consumption and adiposity. In young rats, it also increases the rate of energy consumption by the generation of heat.

Current research focusses on the web of interactions involving leptin, insulin, free fatty acids, ghrelin, age and hunger [H6 1].

### 4.7.7 Involvement of the hypothalamus and the "arcuate" nucleus.

Appetite and weight are controlled largely by a part of the brain termed the hypothalamus. A portion of the hypothalamus termed the "arcuate" (bow-shaped) nucleus is particularly important. The neurons of the arcuate nucleus respond to many hormones and nutrients, including leptin, insulin, gonadal steroids and glucose.

Leptin is thought to suppress appetite by affecting the activity of neurons in the arcuate nucleus. It reduces the activity of neurons that communicate

via the appetite-stimulating neuropeptide Y, and enhances the activity of appetite-suppressing neurons that communicate via melanocortin.

Ghrelin is a potent stimulator of growth hormone, and is thought to act on the arcuate nucleus. Free fatty acids in the blood can block this stimulation almost completely and glucose in the blood reduces it.

### 4.7.8 Growth hormone, IGF-I and tissue damage.

Growth hormone and IGF-I may participate in some of the harmful consequences of diabetes, as well as in influencing whether a diabetic state develops. First, a circumstantial argument has been made that growth hormone and IGF-I contribute to the kidney damage caused by diabetes.

Second, growth hormone is thought to play a role in diabetic retinopathy, a destructive change to the retina induced by diabetes. Diabetic retinopathy includes overgrowth of the retina by new blood vessels, a process termed "neovascularization." Recent experiments with chickens indicate that an altered form of growth hormone is expressed during normal development of the retina, and presumably contributes to that development. The altered form of growth hormone disappears after hatching. It may be that the combination of tissue injury and elevated levels of growth hormone reactivates processes that ought to have ceased early in life. Alternatively, IGF-I may be the agent that causes neovascularization [H7_3_18].

Somatostatin suppresses growth hormone secretion by the pituitary gland. Somatostatin mimics, such as octreotide, are effective in reducing neovascularization.

Interestingly, children with growth hormone deficiency have reduced retinal vascularization, as measured by vascular branching. This seems not to harm their vision.

### 4.7.9 General prospects for intervention.

Useful drugs often mimic some, but not all, of the effects of natural hormones. This can enable doctors to elicit desired effects without unwanted side effects. One potentially useful example is "hexarelin", which mimics the growth hormone-inducing effect of ghrelin, but not its insulin lowering/glucose-raising effect.

IGF-I therapy has been reported to reduce growth hormone levels in patients with type I diabetes, and thus probably restore insulin sensitivity. This in turn, improved the blood lipid profile.

An anticipated benefit of ongoing research is more effect-specific drugs, mimicking various components of the growth hormone axis.

## 4.8 The growth hormone influence on hair.

There is a strong tendency for people with either growth hormone deficiency or growth hormone insensitivity to have sparse hair, including both head hair and sexual hair. For example, in one study of growth hormone insensitivity involving both sexes, 42% of patients had sparse head hair. Growth hormone deficiency also reduces hair growth in other animals such as rats and dogs. At least in dogs, growth hormone supplementation reverses the loss of hair.

People treated with somatostatin analogs also sometimes lose their hair. This presumably results from the loss of growth hormone secretion that somatostatin causes. It is reversible upon cessation of somatostatin therapy. So far, women seem more vulnerable to this than men, but more data is needed.

The growth hormone receptor is present in human hair follicles, particularly at the base of the follicle. Hence, growth hormone may stimulate follicles.

IGF-I and IGF-II can strongly stimulate cultured hair follicles, with IGF-I being more potent than IGF-II. IGF-I seems to have this effect in vivo, as well.

Cessation of hair growth involves entrance of the follicle into a "catagen", or quiescent, phase. This involves cell suicide (apoptosis), which IGF-I is thought to oppose.

Although growth hormone failed in one case to improve mohair production in goats, IGF-I stimulates hair production in sheep and mice. On the other hand, rising levels of IGF-I in men are strongly associated with greater likelihood of vertex baldness (baldness at the crown of the head).

## 4.9 The growth hormone axis and the immune system.

### 4.9.1 The nervous and immune systems interact.

Although the immune system was once considered to function in isolation, it is now clear that it communicates constantly with the nervous system. This communication occurs both directly and with the endocrine system (including the growth hormone axis) as an intermediary. The direct communication between the nervous and immune systems occurs in both directions: neural cells produce hormones that will influence the immune system, and the immune system produces neurotransmitters. Among the neural products that influence the immune system are substance P, neuropeptide Y, calcitonin gene- related peptide (CGRP), and somatostatin (which inhibits local immune responses). As result of this two-way

communication, the enormous information-processing capacity of the nervous system is harnessed to regulate the response to invading parasites and perhaps cancer.

Several members of the growth hormone axis, including growth hormone, somatostatin and IGF-I, are directly or indirectly under neural control. These hormones also affect the immune system (see below), and hence may be indirect mediators of neural activity on immunity.

### 4.9.2 Stimulation of immune activity may or may not be beneficial.

Several members of the growth hormone axis stimulate immune activity. These include growth hormone, ghrelin and IGF-I. (Somatostatin reduces immune activity). Hence, members of the axis, or their mimics, might be used to boost immune activity in the immunodeficient or the elderly. However, two reservations should be kept in mind.

The first is that it is much harder to determine whether the immune system has gained increased function than it is to make the same judgement about muscle or bone. Observations that the thymus gland has increased in size or that certain immune cell populations have increased in number may suggest, but do not prove, that the immune system has become better at fighting infectious diseases or cancer. Thus, evidence that suggests improved immunological function can be misleading.

The second caveat is that increased immune activity is not necessarily desirable. Although there are few penalties if a muscle is stronger than it need be, an overactive immune system can cause autoimmune disease. In particular, it may be that the decline in immune system activity that occurs with age is a defense against an increased possibility of autoimmunity.

### 4.9.3 The growth hormone receptor resembles immune system hormone receptors.

Growth hormone and another pituitary hormone, prolactin, also affect the immune system (the effects of growth hormone are discussed below). Like all hormones, growth hormone and prolactin exert their effects by binding receptors on the target cells. The receptors of growth hormone and prolactin resemble the receptors of molecules that regularly transmit information between cells of the immune system or deliver information to the immune system from other sources (the interleukins 2, 4, 6, 7, erythropoietin and granulocyte-macrophage colony-stimulating factor). This resemblance very likely indicates shared evolutionary origins, and shared original functions.

### 4.9.4 Growth hormone and IGF-I can alter the appearance of immune organs.

There is much evidence suggesting that growth hormone and related hormones can affect the immune system. One class of evidence is that such hormones can increase the size of immune system organs.

As mentioned in the section on growth hormone and HIV [H3 11], growth hormone causes a marked increase in the mass of the thymus gland in HIV patients. (The thymus gland is located in the chest cavity, just behind the breastbone. It is the place where T-cells mature, after they originate in the bone marrow.) Thus, growth hormone seems to reverse the thymus atrophy that occurs in HIV-infected adults [Imm].

In elderly (9 months) male mice, administration of IGF-I for two weeks caused great changes to immune organs. The spleen nearly doubled in size, while the thymus gland increased by 50%.

### 4.9.5 GH and related hormones cause changes in immune cell populations.

The morphological changes caused by IGF-I in the mice described above were accompanied by increased immune cell activity. The number of lymphocytes in the spleen increased by 2.5-fold, and the number of helper T-lymphocytes (CD4+) doubled. Lymphocyte numbers increased both in the thymus gland and in lymph nodes. The amount of the circulating immunoglobulin class IgG also increased.

Growth hormone also affects the immune system. In immune-deficient mice, growth hormone restored progenitor (CD4+CD8+ lymphocytes) in the thymus gland. Growth hormone stimulates antibody production, increases proliferation of T-lymphocytes, increases the cytotoxic activity of a population of cells called "natural killer" cells (very important in fighting cancer), stimulates priming of macrophages to attack targets, and stimulates neutrophils (which participate in the inflammatory response).

A primary target of growth hormone is the solid tissue of the thymus gland itself, the thymic epithelium. This epithelium provides an environment for T cells to mature. Both physical contact and exchange of chemical signals between the thymic epithelium and T-cells are important for T-cell maturation.

Under some circumstances, growth hormone can enhance the "respiratory burst" of granulocytes. The respiratory burst in a metabolic process that occurs in cells that have ingested microorganisms. The rapid uptake of oxygen produces several different reactive oxygen intermediates (including hydrogen peroxide and hypochlorite ion, i.e. household bleach)

that kill ingested parasites.

Growth hormone improves cell-mediated immune responses and maintains serum antibody concentrations after abdominal surgery in clinically stable patients.

Growth hormone also influences traffic in and out of the thymus gland.

### 4.9.6 Individuals vary in their response to GH and related hormones.

The immune response to growth hormone and related hormones varies between individuals more than is the case with other responses to growth hormone. For example, in one test, growth hormone increased lymphocyte proliferation in 60-70% of children but not the others. Growth hormone enhanced the oxidative burst of granulocytes in only 30-60% of tested children. The effective dose varied from 10-300 nanograms per milliliter, and the necessary incubation time varied from 0 to 90 minutes.

Such differences may be due either to genetic differences between people or to differences in disease history. Alternatively, they might reflect differences in stress, nutrition, exposure to allergens, or any of many variables that could affect the immune system. Whatever the cause, it will have to be understood and dealt with in therapies to increase the immune response using growth hormone and related hormones.

### 4.9.7 Both growth hormone and IGF-I limit burn-induced sepsis.

Sepsis, caused by leakage of intestinal contents, is a frequent and dangerous complication of severe burns. In an experimental mouse model of burn-induced sepsis, both growth hormone and IGF-I reduced the systemic spread of translocated bacteria. This may reflect increased immune system activity, although it could also reflect faster healing.

### 4.9.8 IGF-I may protect immune system cells from apoptosis.

Apoptosis is cellular suicide. It is an important defense against parasites that hide inside cells, where antibodies cannot reach them, and against cancer. It can be induced by a number of stimuli, including attack by a type of immune system cell called a cytotoxic T-lymphocyte, which has a means to recognized infected or abnormal cells. "Natural killer" cells also kill tumor cells by inducing apoptosis.

Apoptosis is a critical part of immunity, but inappropriate apoptosis in whole populations of cells can devastate an organ or organ system. As mentioned above [apop] inappropriate apoptosis contributes to the immune system destruction that occurs in AIDS. IGF-I, and agents that induce IGF-I, suppress this apoptosis, and might protect the immune systems of AIDS

patients.

Growth hormone is secreted by "B" cells, those cells of the immune system that manufacture and secrete circulating antibodies. Other immune system cell types have less of it. There is much diversity between people in the intensity of growth hormone secretion by B cells. The growth hormone receptor protein is also expressed in B cells, although some cells express only growth hormone and not the receptor.

Growth hormone is made in the thymus by thymic epithelial cells, which also have growth hormone receptors. Growth hormone is also made by T cells present in the thymus.

Still other immune system cell types, such as "monocytes" and natural killer cells, have growth hormone receptors and produce growth hormone.

An additional growth hormone-related hormone that appears in many immune cell types is somatostatin. Somatostatin received its name because it is made in the hypothalamus and suppresses the release of somatotropin (growth hormone) by the pituitary gland.

Ghrelin is produced mainly in the stomach, and induces growth hormone secretion by the pituitary gland. Ghrelin and its receptor have been reported in all or nearly all immune system cell types, both differentiated and immature. There are great differences in the expression of these between individuals.

Most circulating IGF-I is produced in the liver, but IGF-I is also produced in the thymus gland. Both thymic epithelial cells and the mobile immune cells that pass through the thymus produce IGF-I. IGF-I is involved in several functions, including proliferation of thymic epithelial cells and their secretion of the hormone thymulin. IGF-I also influences the adhesion that occurs between thymic epithelial cells and mobile cells passing through the thymus.

### 4.9.9 Growth hormone might reverse immunodeficiency in AIDS.

One reason to think that growth hormone might benefit the immune systems of elderly people is that it shows promise in HIV-infected patients. Growth hormone causes a marked increase in the mass of the thymus gland in HIV patients, and increases the number of new helper T lymphocytes, the critical cell population depleted by HIV infection.

### 4.9.10 Growth hormone may restore immune function in aged mammals.

The immune system declines with age. The thymus gland shrinks, and cellular elements in the thymus decline, replaced by fatty tissue. The thymus

undergoes involution, a process thought to be correlated with loss of function.

One mitigating factor may be that the immune system has become "educated" in many elderly people. If there are many circulating strains of, for example, the cold virus and if they do not evolve too quickly, elderly people may be resistant through having been exposed to most of the strains.

Growth hormone treatment of aged rodents tends to restore deficits in immune function. As one example, implantation of GH-secreting cancer cells into 16-month-old rats regenerated normal thymic tissue and reversed the loss of cell-mediated immunity that occurs with ageing.

As a second example, long-term, low-dose growh hormone treatment of aged mice increased their life expectancy, and gave indications of having restored immune function. One of these indications was an increase in production of interleukin-2 to levels comparable to those in young mice.

Interleukin-2 is a powerful immunostimulator, whose medical use is limited by side effects. The problem may be that interleukin-2 is produced, sensed and destroyed within a very short range within the organs of the immune system. Medical science cannot yet administer interleukin-2 in a way that duplicates that localized production. However, even though growth hormone cannot be administered locally either, it might stimulate localized and beneficial interleukin-2 production.

It has been suggested that since thymic involution is complete by middle age, there may be little immunological benefit in giving growth hormone to elderly people. However, the point is not settled.

Use of growth hormone to restore immunological function in the elderly will have to be coordinated with other influences such as exercise, and perhaps nutrition. Strenuous exercise in elderly women (a bicycle test) alters their immune systems in ways suggestive of increased function.

Interestingly, it has also been reported that IGF-I may suppress immune function in elderly men, with higher levels of IGF-I being more suppressive.

### 4.9.11 Exercise influences the immune system.

Exercise has effects on the immune system. Moderate exercise seems to increase immunity. In one experiment on humans, near-daily brisk walking compared with inactivity reduced the number of sick days by half over a 12- to 15-week period. One facet of maintaining the immune systems of elderly people may be to insure that they exercise regularly, and are able to exercise regularly.

By contrast with moderate exercise, prolonged exertion suppresses the immune system. One explanation is that the immune system declines from

lack of micronutrients. People making strenuous efforts to lose weight by a combination of diet and exercise might suffer the ill effects of such an immune system decline.

The decline in immune function that accompanies prolonged strenuous exercise is accompanied by a rise in growth hormone and other hormonse such as cortisol and epinephrine (adrenalin). An understanding of how (and if) these hormones affect the immune system in response to exercise will increase the chances of beneficial intervention.

#### 4.9.12 The growth hormone axis and arthritis.

Elderly people often suffer from arthritis. Arthritis is an inflammatory illness that induces a catabolic state. Arthritis decreases secretion of growth hormone by the pituitary gland, and decreases secretion of IGF-I by the liver. Arthritis might plausibly exacerbate these deficiencies in the elderly.

Administration of growth hormone to arthritic rats alters the disease (it prevents a rise in the protein IGFBP-3 by increasing the rate of IGFBP-3 degradation). It is not known whether treatment with growth hormone or IGF-I could benefit the arthritis itself, but such treatment might reverse growth hormone or IGF-I deficits in old, arthritic individuals.

### 4.10 The growth hormone axis and sleep.

#### 4.10.1 A night of sleep is surprisingly complex.

Human sleep consists of alternating occurrences of rapid-eye-movement sleep (REMS) and non-rapid-eye-movement sleep (NREMS). Slow-wave (i.e. deep or NREMS) sleep is concentrated in the first half of the night, and coincides with a surge of growth hormone. Cortisol secretion is at its lowest during the first half of the night. During the early morning hours, cortisol increases and the amounts of slow-wave sleep and growth hormone are low; sleep is shallower.

#### 4.10.2 Sleep changes occur with ageing and depression.

As people age, both the quantity and quality of sleep shrink. The fraction of sleep time that is spent in slow wave sleep declines from about 19% in men aged 16-25 to 3-4% in men aged 36-50. In men, the amount of time spent in slow wave sleep remains constant after age 50, but the total amount of time spent sleeping declines by about 28 minutes every 10 years. Thus, the elderly sleep less and more shallowly.

The decline in slow wave sleep from early adulthood (age 16-25) to mid-life (ages 36-50) is accompanied by a major decline in nocturnal growth hormone secretion. In later life, growth hormone secretion continued to

decline, but at a slower rate. The amount of growth hormone secretion and the amount of slow wave sleep are correlated, independent of age.

In elderly people, the amount of cortisol present in the evening rises. Cortisol is generally antagonistic to growth hormone in its effects, and is associated with reduced sleep.

Changes in sleep occur in people suffering from depression. These changes resemble those that occur with age. There is a decrease in slow-wave sleep and an increase in shallow sleep. Less growth hormone and more cortisol are produced.

### 4.10.3 The effects of growth hormone releasing hormone (GHRH) on sleep.

Although growth hormone may affect sleep [see below **H4 10 5**], most attention has focussed on growth hormone releasing hormone (GHRH). This peptide hormone is produced by the hypothalamus, and is tranferred to the pituitary gland where it stimulates the secretion of growth hormone. GHRH also stimulates sleep, especially deep sleep; its effects are powerful and unambiguous. This stimulation is independent of its stimulation of growth hormone, although both appear to require intact GHRH receptors. Thus, growth hormone releasing hormone stimulates both sleep and growth hormone, and hence coordinates tissue building with rest.

Mice lacking functional GHRH receptors do sleep, but display less rapid eye movement sleep (REMS) and less non-rapid eye movement sleep (NREMS). The NREMS-promoting activity of GHRH is mediated by the pre-optic region of the brain.

GHRH is effective when given nasally. It stimulates deep sleep during the last third of the night when its is normally rare. It is effective in old people as well as young.

### 4.10.4 Ghrelin and its analogs have a complex effect on sleep.

While GHRH promotes slow-wave sleep, ghrelin and its analogs have been reported in different studies to promote REM sleep and NREM sleep, each at the expense of the other. Ghrelin has also been reported to increase active wakefulness in rats (perhaps because it promotes hunger).

While GHRH reduces cortisol concentration, ghrelin increases it even while also increasing growth hormone. This stimulatory effect on cortisol may be the reason that ghrelin is less powerful in promoting sleep and promotes shallower sleep than GHRH.

Analogs of ghrelin can be administered nasally or orally.

### 4.10.5 Growth hormone effects on sleep are inconsistent.

Growth hormone seems to promote slow wave sleep, because withdrawal of growth hormone from growth hormone-deficient young adult patients reduced the average amount of slow wave sleep per night from 33 minutes to 7.5 minutes. On the other hand, administration of growth hormone to normal humans has been reported to decrease slow wave sleep, even while increasing rapid eye movement sleep.

It has been suggested that one effect of growth hormone is to suppress the synthesis of GHRH, and that this explains why growth hormone can inhibit deep sleep. It has been suggested that growth hormone acts both directly to promote sleep and indirectly to reduce sleep by inhibiting GHRH.

### 4.10.6 The antagonism between GHRH and corticotropin releasing hormone.

Growth hormone releasing hormone and corticotropin releasing hormone (CRH) are both made in the hypothalamus, and both act on the pituitary gland. Stimulation of the pituitary gland by CRH releases adrenocorticotropic hormone (ACTH), which in turn acts on the adrenal glands (located adjacent to the kidneys) to stimulate cortisol production.

Cortisol is a stress hormone which opposes growth hormone in its effects. Whereas growth hormone promotes muscle growth, cortisol promotes muscle breakdown. The amino acid products of this breakdown are transported to the liver, and used to make glucose, which the brain will use as fuel.

The two axes: (GHRH -> GH -> IGF-I) and (CRH -> ACTH -> cortisol) are thought to oppose each other generally.

Sleep is controlled by GHRH and CRH. GHRH promotes sleep, and particularly deep sleep. CRH shifts sleep toward shallow sleep, or banishes sleep altogether. GHRH dominates the first half of sleep, but then diminishes, while the CRH level rises.

It has been reported that a brief administration of cortisol improves sleep. This result is probably due to feedback inhibition of CRH by cortisol.

### 4.10.7 Somatostatin interrupts sleep.

Somatostatin may degrade sleep in the elderly by opposing the action of GHRH. Improvements to sleep in the elderly might involve treatment with GHRH, or might involve treatment with blockers of CRH and/or somatostatin.

### 4.10.8 Exercise influences sleep.

An additional influence on the quantity and quality of sleep may be exercise. One experiment showed that 2 hours of acute heavy resistance exercise in men (from 3-5 p.m.) reduced GH during the first half of the night, but increased it during the second half.

### 4.10.9 Prospects for use of GHRH to improve sleep and combat depression.

The ability to control the effect on sleep of growth hormone and related hormones is desirable for two reasons. The first is that there is a great need for methods to improve sleep, particularly in the elderly. The second is that treatment of patients with growth hormone or related hormones for other reasons, such as a desire to improve their muscle strength, might have the side effect of promoting unwanted sleepiness or interfering with sleep.

The most frequently used drugs to induce sleep are benzodiazepines. These suppress slow wave sleep, rapid eye movement sleep and other features of natural sleep, and hence interfere with sleep's restorative powers. Moreover, they can be addictive. A more natural replacement for them would be very valuable.

Control over the sleep-related effects of growth hormone and related hormones might also open a new door to the treatment of depression. Corticotropin releasing hormone overexpression plays a key role in depression, and might be combatted by GHRH.

A final point is that the elderly might benefit from reduced sleep, particularly reduced deep sleep. Lying for too long in one position can cause blood to pool and clot; the constant shifting of position that occurs in sleep is a way to prevent this. One would think that the chance of forming a dangerous clot would be greater in the elderly; if so, restlessness might have survival value. If this is true, better sleep for the elderly might come at a price, or at least require a method to prevent clotting.

## 4.11 The growth hormone axis and mental function in the elderly.

### 4.11.1 Growth hormone replacement improves mental functioning.

Adults deficient in growth hormone have several psychological problems. They complain of anxiety, depression, low energy, loss of sleep, mental fatigue, dissatisfaction with body image and unhappy moods. Measures of psychological well-being confirm that the presence of these afflictions. Moreover, they show hostility and irritability, are prone to obsession and compulsion, phobias and psychotic symptoms. The marital and socioeconomic performance of adults with growth hormone deficiency is

also reduced.

Growth hormone therapy tends to alleviate these problems, although attempts to assess the consequences of growth hormone therapy are complicated by a major "placebo" effect. (A "placebo" is an inactive mimic of the real treatment, and is given to some members of a drug study to assess the improvement that occurs due to expectation of improvement.)

Growth hormone treatment of GH-deficient adults increases alertness, activity level, memory, motivation, work capacity and endurance. It reduces irritability, the tendency to worry and depression. It makes recipients more extroverted, resulting in better personal relationships and less conflict. People with the lowest perceived quality of life at the start of treatment are the most likely to benefit.

These improvements have socioeconomic consequences. GH-treated patients take fewer days off from work due to reported sickness.

Unfortunately, psychological benefits of growth hormone therapy are not always permanent. One study reported that that some improvements, such as increased energy levels, disappear after a year or two. (This study also reported that the increased self-esteem due to growth hormone treatment did not disappear after even three years.)

Treatment with IGF-I, either alone or in combination with growth hormone, decreased depression and anxiety in post-menopausal obese women. (Growth hormone alone did not have this effect.)

Treatment of GH-deficient adults also improves mental performance, particulary memory. Treated patients also perform better on tests of mental function involving attention, speed and comprehension.

Growth hormone has also been reported to reduce depression in children with Prader-Willi syndrome.

### 4.11.2 Growth hormone, ghrelin and IGF-I act within the brain.

The presence of growth hormone receptors within a tissue is a good indication that the tissue may respond to growth hormone. Growth hormone receptors are present in several parts of the brain. They are present in the choroid plexus, the hippocampus, the hypothalamus and the spinal cord.

The density of growth hormone receptors in various brain regions declines with advancing age. At least in the rat hippocampus, growth hormone up-regulates its own receptor. However, at least in male rats, this effect is also lost with age.

An increase in IGF-I synthesis in a tissue is a good indication of tissue growth. Both growth hormone and a ghrelin mimic upregulate IGF-I in adult male rats. This occurs in the cerebellum (a part of the brain that coordinates

muscle movement), the hippocampus (a part of the brain important for learning and memory), and the hypothalamus.

Remarkably, moderate calorie restriction increases the mean and maximum life span of rodents (mice and rats) by 30-40%. This calorie restriction also reduces the age-related decline in growth hormone secretion, although plasma IGF-I levels decline. Hence, it is possible that some of the effects of calorie restriction involve changed regulation of the growth hormone/IGF-I axis.

The density of very small blood vessels on the surface of the brain decreases with age. This decrease is lessened by moderate calorie restriction. Cerebral blood flow parallels these changes in vasculature; decreases in cerebral blood flow could cause the diminished mental capacity of old age.

### 4.11.3 Reported benefits of axis hormones on mental function in the elderly.

There is a well established decline in cognitive function in ageing adults. Since growth hormone and IGF-I levels also decline, a logical question is whether restoration of these to youthful levels could prevent the decline in mental function.

The spatial reference memory of rats normally deteriorates with age. In one set of experiments with rats, administration of a chemical analog of growth hormone releasing hormone (GHRH) prevented this age-related decrement. GHRH itself has similar effects.

It is unclear whether the GHRH analog itself caused the effect, or whether some downstream hormone in growth hormone sequence was the cause. Growth hormone is one candidate for the cause.

Growth hormone receptors are present in many brain cell types, and growth hormone is probably involved in the normal growth and development of the brain. Growth hormone also seems to be produced after injury to the brain, and to protect the brain tissue from further damage.

In both young adult and ageing male rats, growth hormone upregulates the N-methyl-D-aspartate receptor subunit 2B. This may contribute to growth hormone's enhancement of memory, since induction of NR2B has independently been shown to increase cognitive abilities.

Growth hormone increases the number of tiny blood vessels in the brains of aged animals. These tiny blood vessels are an important short range source of IGF-I for the brain.

IGF-I is another good candidate as the agent that protects against the ravages of age. For one thing, the memory-preserving GHRH analog mentioned above also elevated IGF-I.

IGF-I is suspected to be important in brain function, because blocking IGF-I activity in the brains of young animals impairs both their learning and their reference memory.

IGF-I also protects brain tissue from damage after injury. Its synthesis is induced by brain injury. It acts as both to prevent apoptosis and to promote nerve regeneration. Two breakdown products of IGF-I, the first 3 amino acids, and the first 3 amino acids chemically modified, also have neuroprotective properties. The neuroprotective effect of IGF-I differs from that of growth hormone in its spatio-temporal distribution.

IGF-I also aids the regeneration of peripheral nerves.

The notion that IGF-I protects the brain is strengthened by evidence linking low IGF-I levels to Alzheimer's Disease, a common consequence of ageing. Study of a family having a mutation that predisposes its carriers to Alzheimer's disease showed that only the members with Alzheimer's disease had below-normal IGF-I levels. By contrast, there was no correlation between Alzheimer's Disease status and growth hormone level.

IGFBP-5 [**H6 9 39**] is a blood protein that binds IGF-I and may modify its behavior. IGFBP-5 may also be involved in neural survival, particularly in the hypothalamus.

### 4.11.4 Harm from GH or IGF-I treatment of the elderly.

Studies linking cognitive performance in elderly men to their ability to respond to stimulators of growth hormone and IGF-I produced surprising results. Although increased IGF-I concentration was associated with improved performance, a strong response by growth hormone was actually associated with poorer cognitive performance in elderly men. (Although growth hormone stimulates IGF-I, and thus the concentrations of the two hormones should vary together, this covariation becomes unlinked in the elderly and the hormone concentrations are semi-independent.)

The explanation for these results is not known. However, elevated growth hormone causes elevated blood glucose, while elevated IGF-I does the reverse. Hence, the results may be due to the deleterious consequences of hyperglycemia.

Studies on mice reinforce the suspicion that too much growth hormone can be harmful. Mice with a mutation that decreases growth hormone (and decreases thyroid-stimulating hormone and prolactin as well), are much smaller and live significantly longer than their normal siblings. Unlike normal mice, these dwarf mice do not experience an age-related decline in locomotor activity, nor an age-related decline in memory.

Mice with growth hormone insensitivity also differ from normal mice in

that they keep the ability to retain new information as they age.

### 4.12 Final words: the usefulness of growth hormone in the elderly.

In general, the benefits of growth hormone therapy to reverse the symptoms of ageing are not considered to be worth the risks. Its use is not considered to be justifiable outside of clinical trials. Growth hormone is not approved for use in the elderly.

Two general questions about the use of growth hormone or related hormones to reverse the effects of ageing remain unanswered.

The first question is why the benefits of growth hormone shown to date in the elderly have been so modest.

One possible answer is that growth hormone is fundamentally a mobilizer of the body's reserves, and that in the elderly these reserves no longer exist. People with growth hormone deficiency have developed bones, skeletal muscles, hearts and immune systems; growth hormone merely increases the rate and extent of their development. Moreover, there is a limit to the effectiveness of growth hormone; acromegalics are not supermen or superwomen. So perhaps attempting to cure the evils of ageing with growth hormone is like whipping a horse that is nearly exhausted.

A second possible answer is that we have not identified all of the factors that limit growth of bone, skeletal muscle, hearts and immune systems in the elderly. These tissues depend for their growth on many cooperating factors; it may be that the absence of any one of them can prevent growth. Hence, perhaps we have managed to fill the gas tank with high-octane fuel without understanding that the engine is also out of tune and the clutch is slipping.

The latter explanation offers more hope that the impairments that accompany ageing may someday be reversed or at least delayed.

The second general question is whether reversing some but not all of the effects of ageing will actually benefit patients. There is little doubt that restoring an octogenarian to the physical state of a 25-year-old in every respect would be beneficial. But there are many aspects of ageing that are beyond the reach of growth hormone or any group of hormones: mutation and chromosome rearrangement in cells, the steady shortening of chromosome ends (telomeres) that may limit their replication capacity, the steady loss of neurons from the brain, and deterioration of the vascular system, among others.

It may be that some of the causes of senescence are biologically unavoidable, and that many of the others represent an adaptation to them. By this hypothesis, prevention of these adaptive changes might do more harm

than good.

As one example, an excess of growth hormone in early or mid-adulthood causes diabetes, osteoarthritis, high blood pressure and perhaps cancer. Perhaps even normal levels of growth hormone can promote these maladies to some extent. Since older people are more prone to them, perhaps the safe level of growth hormone is lower in older people.

As a second example, consider sleep. People change positions in their sleep; this change is necessary to avoid pooling and clotting of the blood, with the ensuing danger of thrombosis, heart attack and stroke. Yet the elderly are more prone to these dangers than are young adults; it may be that nighttime wakefulness and reduced amounts of deep sleep in the elderly prevent motionlessness that would be safe in young adults but intolerably dangerous in older people.

Many of the features of ageing can be imagined to be adaptive in this fashion. It might be, for example, that the reduced power of the immune system in ageing offsets an increased tendency toward autoimmunity, arthritis and other examples of inappropriate immune activity. Learning which of the features of ageing can be safely reversed, and under what circumstances, is another major challenge facing growth hormone research.

Modern life has given us new options to solve age-old health problems. Perhaps we could safely increase the amount of time that elderly people spend sleeping soundly if we also improve the quality of the beds that they sleep in, or alter their blood to reduce clotting.

# 5. THE UNPLEASANT SIDE OF GROWTH HORMONE.

## 5.1 The illegal use of growth hormone in sports.

### 5.1.1 Growth hormone is often used to increase athletic performance.

The use of illicit performance-enhancing drugs is widespread in sports. It is not limited to elite athletes; for example, an estimated 1 - 3 million male and female athletes in the United States alone have used anabolic steroids. Although such illicit drug use is a permanent feature of sports, the drugs that are used tend to change.

Recombinant growth hormone historically has been more expensive and harder to obtain than anabolic steroids. However, it has the advantage (discussed below) of being hard to detect. Its use by athletes is common and likely to increase.

### 5.1.2 Indirect methods of increasing growth hormone.

Growth hormone is illegal, expensive, difficult to obtain and (like all

drugs) potentially dangerous. Because of this, athletes often try indirect methods to increase their body's production of growth hormone.

One of these methods is dietary supplementation. Probably, much of what has been tried has not yet been described in the scientific literature; however, it is clear that at least some popular methods to raise growth hormone levels fail. As an example, combined supplementation with the amino acids L-arginine, L-ornithine and L-lysine (each at 2 grams per day, divided into two daily doses) had no effect on growth hormone. On the other hand, ingestion of creatine or melatonin reportedly do increase growth hormone secretion [H7 3 10].

Strenuous exercise increases growth hormone levels, at least in young adults. However, the increase depends on the intensity and duration of the exercise. Short, intense bouts of aerobic exercise induce far more growth hormone than do longer, milder bouts.

### 5.1.3 A scientific assessment of growth hormone's athletic benefits.

Short periods (4 weeks) of growth hormone treatment have been reported to increase heart pumping performance and reduce resistance to blood flow in the vascular system. On the other hand, a six week course of growth hormone given to male power lifters did not increase the strength of either the biceps or the quadriceps muscles.

Another study of power lifters showed that anabolic steroids and dietary restriction can interact with growth hormone supplementation in unexpected ways. Normally, growth hormone treatment increases the concentrations of IGF-I and the IGF-I binding protein IGFBP-3. (IGF-I mediates muscle growth, and IGFBP-3 may alter its activity.) However, anabolic steroids reverse the rise in IGFBP-3 concentrations. When diet is also severely restricted, IGF-I levels may decline rather than rise.

### 5.1.4 The consequences of growth hormone abuse in athletes.

The use of growth hormone as a performance aid is illicit. Moreover, it is often accompanied by other attempts to influence performance or body composition such as anabolic steroids, ephedrine, diuretics, starvation, high-protein diets and thyroid hormone. Hence, it is difficult to assess its side-effects on athletes under conditions that are both realistic and carefully controlled.

Carpal tunnel syndrome is a common complication of growth hormone treatment. At least one male bodybuilder has developed carpal tunnel syndrome related to growth hormone abuse.

One elite bodybuilder experienced shrivelling of the pituitary gland. This

bodybuilder had a long history of self-treatment with growth hormone, testosterone and thyroid hormone. This shrivelling was thought to be due to either to feedback inhibition of growth hormone production by the exogenous growth hormone, or to elevated intracranial pressure caused by the growth hormone, or both.

In yet another elite bodybuilder, a combination of three anabolic steroids, several nonsteroid drugs including human growth hormone, prolonged training and a severely reduced diet led to aggression and other negative moods. (So-called " 'roid rage" is a well-known complication of drug abuse in bodybuilding.)

The side effects of growth hormone treatment are further discussed below. [H5 2 3].

### 5.1.5 Attempts to detect growth hormone use.

Efforts are underway to develop methods to reliably detect self-treatment with growth hormone in athletes. Detection of growth hormone is easy: as little as 2- to 50- billionths of a gram per liter of urine can be detected. However, since growth hormone is produced by the human body, methods must be devised to distinguish between growth hormone produced by the body, and growth hormone that has been administered. The problem is made more difficult by the great variation in growth hormone levels that can occur naturally within a person, and by differences between people.

One promising method is to detect abnormalities in the ratios between the various isoforms of growth hormone. Natural growth hormone is actually a family of closely related proteins, rather than a single protein. These related proteins are termed growth hormone "isoforms." [H6 8 2] The predominant isoform has a molecular mass of 22,000 daltons (22,000 times the mass of a single hydrogen atom). There are several other isoforms, one of which (for example) has a molecular mass of 20,000 daltons. Recombinant growth hormone consists only of the 22,000 dalton type. Administration of the 22,000 dalton isoform increases its concentration while reducing the concentrations of the other isoforms, and hence skewing the ratio between them.

Thus detection of an abnormally high proportion of the 22,000 dalton growth hormone isoform could indicate growth hormone abuse. However, this method is only effective for 24 hours after the recombinant growth hormone treatment.

### 5.1.6 Scientists' and athletes' opinions about growth hormone disagree.

Growth hormone is rumored to be one of the most popular drugs used to enhance athletic performance. Yet scientists who study it believe its benefits on athletic performance to be small or even non-existent. What causes this disagreement, and who is right?

Consider the sport of bodybuilding, where aggressive abuse of anabolic substances is nearly universal, and where the results are most visible:

On one hand, athletes are subject to the placebo effect: the ability of an expectation some benefit, such as an increase in energy, to create that benefit through mental activity alone. Athletes may misjudge their own performance, and may confuse the consequences of one drug with another.

On the other hand, the focus of medicine is conservative; the first rule is to do no harm. Hence, the use of anabolic substances in bodybuilding, which trades future health for a perceived esthetic gain in the present, arouses the medical establishment's disapproval. This disapproval manifests itself in a lack of aggressive experimentation.

Athletes are results-oriented, willing to try combinations of drugs and other aids, and then trade experiences about what has worked and what has not, by word of mouth. Researchers are barred by ethics from such aggressive experimentation on humans, and seldom perform it on animals. The object of medicine is not to amaze, but to heal.

Yet, it is clear from bodybuilding contests, particularly womens' contests, that something the competitors are doing must be effective. Competitors in such contests often achieve muscular development and strength far beyond anything a drug-free competitor could hope for.

Bodybuilding is a deadly sport: a wrecker of health and a truncator of lives. Yet it may be that these competitors, and drug-abusing competitors in other sports, can teach the medical establishment something about muscle growth that can be used to solve medical problems, such as the plight of the frail elderly.

## 5.2 The safety of medical growth hormone therapy.

### 5.2.1 Growth hormone therapy is generally safe.

Growth hormone is usually well-tolerated by patients, particularly when doses are limited. By now there is a large base of medical experience; in England in the year 2000, for example, more than 39,000 prescriptions for human growth hormone were dispensed.

In one study of about 1500 adults deficient in growth hormone, and taking growth hormone therapy, 0.6% discontinued therapy due to side effects over a 3-year period. Some studies have reported no adverse side effects at all.

One technique that can be used to improve the safety of growth hormone

treatment is to use a natural inducer such as Growth Hormone Releasing Hormone (GHRH) or ghrelin. These inducers (called "secretagogues") induce the pituitary gland to secrete growth hormone. Secretagogues preserve the pulsatile pattern of growth hormone release, and do not release unphysiologically large doses of growth hormone into the blood. Thus, they avoid some side effects that growth hormone has.

### 5.2.2 Human populations vary in dose tolerance.

Groups of people differ in their tolerance to growth hormone. Patients with kidney failure have a very high tolerance. Twenty enfeebled patients on hemodialysis received doses of growth hormone 2- to 3-fold higher than doses given to patients who are growth hormone-deficient, but not uremic. The IGF-I levels of these hemodialysis patients rose to equal the IGF-I levels of people with untreated acromegaly. Yet, there were few side effects and no serious ones.

Elderly people are much more sensitive to high doses of growth hormone than are younger people. In studies on elderly people, typically many people drop out due to intolerable side effects. Doses used must be very low, increased very slowly and tailored to the individual needs of each patient.

While young adults and middle-aged subjects can tolerate doses up to four times the replacement dose for 6-12 months, similar doses in the elderly cause side effects in up to half the subjects. The incidence of side effects in the elderly is directly related to the blood concentration of IGF-I, even though this does not rise above youthful levels.

Children on human growth hormone sometimes develop benign intracranial hypertension, which can cause headaches, vomiting and more serious consequences. The incidence of this is about one per 1000 children, including children given growth hormone for any reason, but is several times higher in children with classic growth hormone deficiency.

### 5.2.3 Well-known side effects of growth hormone treatment.

Among the well-known side effects of growth hormone treatment are carpal tunnel syndrome, joint pain, elevated blood glucose (hyperglycemia), fatigue and several symptoms caused by excess water and salt retention. Among these are edema, and benign intracranial hypertension which can cause headache and vomiting. In some cases, elevated blood sugar has led to type II diabetes.

These symptoms are usually curable by reduction of the administered dose of growth hormone (or in some cases, IGF-I).

### 5.2.4 Additional side effects of growth hormone treatment.

As mentioned above [H3 8 3], growth hormone treatment increases mortality in critically ill patients. Also as mentioned above [H4 11 4], a higher responsiveness of growth hormone to inducing agents has been linked to reduced cognitive ability in ageing men. The first of these ills, and perhaps the second, may follow from the rise in blood glucose that growth hormone increase causes.

Growth hormone excess may reduce blood concentrations of C-reactive protein (a protein with anti-microbial activity), and seems to have caused one case of pituitary gland shrivelling in a bodybuilder. Growth hormone treatment reduces thyroid gland function in some people. In mice, elevated growth hormone may reduce the expression of several genes that control oxidation damage in the liver and kidney, and perhaps thereby increase the frequency of liver tumors, kidney failure and early death in these animals.

Elevated levels of growth hormone and IGF-I affect the prostate gland in males. They cause overgrowth of the prostate gland, calcifications, nodules, cysts and vesicle inflammation. Elevated blood levels of IGF-I may be correlated with increased risk of prostate cancer, although some studies dispute this.

Growth hormone excess can cause abnormal breast development (gynecomastia) in men. In critically ill burn and surgical patients, it greatly increases the blood concentration of calcium; this in turn can produce calcium stones in the kidney and other symptoms [calc]. Growth hormone caused one case of reversible Bell's palsy (paralysis of one side of the face). Extended growth hormone excess can cause coarsening of facial features and of fingers, toes and limbs.

At least one case has been reported of localized lipoatrophy in the region of growth hormone injection. Subcutaneous fat in the region of the injection was destroyed, having an unsightly result. This was thought to result from growth hormone's lipolytic effect.

In addition to the above, therapy with growth hormone inducers may affect appetite and sleep. GHRH, an inducer of growth hormone, is also a sleep inducer. Ghrelin, another inducer of growth hormone, stimulates hunger. Additional possible adverse effects of growth hormone therapy are discussed below in the "acromegaly", "cancer" [H5 4] and "contamination" [H5 5] sections.

## 5.3 Growth hormone excess: acromegaly as a medical condition.

An additional benefit of our increased understanding of how growth

hormone acts is improved treatment of acromegaly and pituitary gigantism.

When chronic growth hormone excess develops before the end of adolescence, the patient suffers pituitary gigantism; people with pituitary gigantism can reach heights of 7 or even 8 feet. When chronic growth hormone excess develops after the end of adolescence, height does not increase, but the patient develops acromegaly.

Symptoms of acromegaly develop slowly, but are eventually debilitating and can become fatal. Acromegalic patients develop enlarged facial features, enlarged jaw, enlarged frontal bone of the skull, widely spaced teeth, enlarged bones of the extremities, enlarged lips and nose, and thickening of the skin and soft tissues of the face.

Other symptoms include hoarseness, enlarged sebaceous glands, sleep apnea, joint pain, carpal tunnel syndrome, vomiting, easy fatigue, weakness, impaired respiratory capacity, sweating, hypertension (particularly intracranial hypertension), elevated blood glucose and insulin resistance, visual impairment, severe headaches, and cardiovascular disease.

The cardiac impairments of acromegalic patients include structural abnormalities and unproductive enlargement of the heart. In untreated disease, cardiac performance slowly but inexorably deteriorates and heart failure eventually develops.

### 5.3.1 Cause and treatment of acromegaly.

Acromegaly is usually caused by a benign pituitary tumor that secretes excessive amounts of growth hormone. Surgical resection of the tumor is the indicated treatment, but complete resection of the tumor is not always possible.

Alternative treatment is with drugs. One class of drugs mimics somatostatin, the inhibitor of growth hormone release by the pituitary gland. In principle, somatostatin itself could be used; but somatostatin breaks down inside the body in a matter of minutes. Mimics of somatostatin such as octreotide, lanreotide and newer and even more powerful drugs are used to suppress growth hormone secretion by the tumor. These drugs remain in the system much longer than somatostatin.

Unfortunately, octreotide at least has serious side effects. It raises blood glucose levels, lowers insulin levels and lowers glucose tolerance. It also interferes with the secretion of several digestive tract hormones.

A second type of drug does not prevent excess growth hormone synthesis, but interferes with growth hormone's biological activity by blocking the growth hormone receptor. "Pegvisomant" is a representative of this drug class. Pegvisomant binds the growth hormone receptor in competition with

growth hormone, but does not activate that receptor. The drug prevents a high concentration of growth hormone from stimulating a high concentration of IGF-I. Whether the drug also interferes with effects of elevated growth hormone that do not involve IGF-I is being investigated.

### 5.4 Growth hormone and cancer.

One fear that researchers have expressed about growth hormone therapy is that growth hormone or its related hormones might promote cancer. Part of the reason is simply that growth hormone and its related hormones sometimes promote cell proliferation; and the combination of ongoing DNA damage that occurs in all tissues and cell proliferation can produce cancer-causing genetic imbalances. However, there is also specific evidence of involvement of growth hormone and related hormones in several cancer types.

#### 5.4.1 Skin cancer.

The growth hormone receptor is expressed in normal skin, but seems to be overexpressed in some abnormal skin conditions, including some cancers. There is particularly suggestive evidence for this in squamous cell cancers, histiocytomas and malignant melanoma, especially metastasized melanoma. It was suggested that growth hormone may induce IGF-I production in these tumors, which then acts in an autocrine (cellular self-stimulation) fashion.

#### 5.4.2 Prostate cancer.

It has been reported that in men, the concentration of IGF-I in the serum correlates with the frequency of prostate cancer, although this is controversial. Another examination of 46 men with acromegaly (elevated growth hormone) found no increase in prostate cancer as such, but found a predisposition to calcifications, nodules, cysts and vesicle inflammation.

Growth hormone receptors are expressed in both normal and cancerous prostate tissue. When growth hormone was added to cultured prostate cancer cells, it more than doubled their rate of proliferation. Human growth hormone also stimulated a line of cells representing non-cancerous overgrowth of the prostate.

#### 5.4.3 Lung cancer.

Lung cancer cells produce IGF-II and the receptor IGF-IR, which is activated by IGF-II. IGF-II and IGF-IR together further stimulate proliferation of the lung cancer cells. Reduction of expression of these two genes with "antisense oligonucleotides" reduced cancer growth by up to

80%.

Studies of a specific non small-cell lung cancer showed that IGF-I stimulated its growth. Expression of the IGF-I binding protein IGFBP-3 within the cells prevented this.

### 5.4.4 Growth hormone, IGF-I, IGF-II and breast cancer.

In breast cancers, self-produced growth hormone represses a proliferation-opposing gene called the placental transforming growth factor gene. As a result, the cells' capacity for apoptosis (a form of cell suicide that controls cancer) is weakened. In addition, the cells gain an increased ability to proceed past cell cycle "checkpoints."

Normally growing cells are restrained by cell cycle checkpoints that prevent proliferation until necessary conditions are met, such as the repair of DNA damage or the receipt of a growth-inducing signal from outside the cell. Expression of IGF-I can help mammary cells override a checkpoint early in the cell cycle, and is indispensable for progression past a late checkpoint.

IGF-II also stimulates breast cancer proliferation.

### 5.4.5 Growth hormone, IGF-I and colon cancer.

Reduced levels of growth hormone and IGF-I are associated with reduced risk of colon cancer in normal human populations. Moreover, people suffering from acromegaly (in which there is increased growth hormone and IGF-I) have an increase in polyps of the colon; these polyps can become cancerous.

In colon cancer cells that are on their way to escaping control, IGF-I suppresses a gene that codes "peroxisome proliferator activated receptor gamma" (PPARgamma). PPARgamma is expressed in the mucosal tissue of the colon, where it inhibits growth and cell differentiation. IGF-I also stimulates expression of Tumor Growth Factor alpha, which in turn stimulates cell division.

### 5.4.6 Growth hormone and liver cancer.

Growth hormone stimulates production of IGF-I and probably IGF-II in the liver. Much of this is secreted into the blood and enters the general circulation. Whether IGF-I and IGF-II also act locally within the liver is not known.

As mentioned above [**liver**], chronic growth hormone excess in mice compromises antioxidant mechanisms in the mouse liver. This may contribute to an excess of liver cancer in these animals.

The liver is the organ where the "cytochrome P450" genes are expressed. The cytochrome P450 genes are critical parts of the body's ability to increase the water-solubility of toxins, as a precondition of removing them. In the process of solubilizing foreign substances they often alter their cancer-causing potential. Sometimes the chemical change reduces the agent's cancer causing potential, but the cancer-causing potential can also be increased. When the cancer-causing potential of a foreign substance is increased, the liver is affected disproportionately. Growth hormone alters the expression of at least two cytochrome P450 genes, and hence has the potential to alter the effect of foreign substances on liver cancer risk. However, the exact effect on such risk is not yet known.

One hepatic ademoma, which was successfully treated, developed in a Turner Syndrome patient after 3 years of growth hormone therapy. The adenoma was attributed to the therapy.

### 5.4.7 IGF-II and meningioma invasiveness.

The meninges are membranes that surround the brain and spinal cord. Meningiomas are cancers of the meninges. Meningiomas vary from benign to malignantly invasive. The malignant meningiomas are dangerous because of their location to vital central nervous tissue.

Meningioma behavior depends largely on IGF-II. Increasing amounts of IGF-II are correlated with increasing invasiveness. The IGF-binding protein IGFBP-5 is inversely correlated with invasiveness, while another IGF-binding protein, IGFBP-6, may be positively correlated with invasiveness.

IGF-II is rarely if ever suggested to have possible therapeutic use. Hence, IGF-II elevations would become important only if IGF-II were induced by growth hormone, or some other drug. Growth hormone is likely, but not certain, to stimulate IGF-II synthesis.

### 5.4.8 IGF-II, Wilms tumor and neuroectodermal cancers.

IGF-II is thought to play a critical role in the development of Wilm's tumor (a childhood cancer of the kidney) and other childhood cancers. IGF-II is the major autocrine factor in neuroectodermal (mainly childhood) tumors; "autocrine" means that the tumor both secretes IGF-II and is stimulated by it. IGF-II may promote resistance in the tumor to retinoic acid, which normally inhibits neuroblastoma.

### 5.4.9 IGF-I, IGF-II and cancer generally.

Cancer reactivates cell behaviors that were appropriate to embryogenesis and fetal development, but which are inappropriate later. IGF-I and IGF-II

are important stimulators of embryonic and fetal development. Hence, overexpression of IGF-I or IGF-II induces cancer-like cell behavior.

Both IGF-I and IGF-II have anti-apoptotic activity. This has been shown in pre-adipocytes (cells destined to become fat cells), and lymphocytes, as well as in breast cancer cells, as mentioned above [H5 4 4].

Experiment with mouse embryonic fibroblasts show that when these cells detach from their matrix, they undergo apoptosis. This spontaneous cell death is an important safeguard to prevent cells of a given type from invading and colonizing inappropriate organs. It also serves to limit the spread (metastasis) of cancers.

Provided that a second gene, phospholipase C-gamma1 is also expressed, IGF-I can prevent this detachment-induced apoptosis. Thus, in a cancer that expressed phospho C-gamma1, IGF-I could undermine a crucial defense against spread of that cancer throughout the body.

Observations on mice and rats suggest that there is an optimal, and rather low, level of IGF-I for longevity. Rats with a greatly reduced amount of growth hormone and IGF-I had a lifespan 5-10% shorter than normal rats, primarily because of increased cancer. However, rats with an intermediate low level of GH and IGF-I lived 7-10% longer than normal rats.

### 5.4.10 Growth hormone may sensitize some leukemia cells to drugs.

Interestingly, there is one contrary report that when the human growth hormone gene is transferred to a human myeloid leukemia line (U937, a frequently investigated cell line), that the cells were sensitized to an apoptotic signal mediated by anticancer drugs. This occurred both when the cells were tested as cultured cells, and when they were transferred to mice. Hence, the role of growth hormone-related genes in cancer cells may be complex.

### 5.4.11 Growth hormone treatment seems not to stimulate cancer.

Acceleration of cancer growth could be a devastating event, particularly in the early stages of a cancer's development, when it might still be contained by the body's defenses. As discussed above, there are ominous indications that growth hormone, either directly or by stimulating IGF-I and IGF-II might accelerate cancer. Despite this, there is no statistical evidence that it actually does so.

Tens of thousands of people have have been treated with recombinant growth hormone. Some have developed cancer, but no more than would be expected in people not treated with growth hormone. Nor is there an excess of any particular kind of cancer. Most gratifying, people who survived

childhood cancer and who have growth hormone deficiencies due to the cancer treatment, do not (as a rule) see their cancers return.

### 5.4.12 Growth hormone and special subpopulations.

It remains possible that special groups of people might have vulnerabilities to growth hormone treatment that most people do not have. An example that has aroused some concern is the fact that Turner Syndrome patients have many more pigmented naevi (moles of the skin) than do normal people. A high count of acquired pigmented naevi is a major risk factor for malignant melanoma. Hence, Turner Syndrome patients might be at risk for malignant melanoma, or might be at risk if treated with an anabolic agent such as growth hormone.

A survey of the medical literature done by the authors of that study indicated that Turner Syndrome patients seem not to be at risk. However, this is an example of the surprises that genetic variation can hold.

### 5.4.13 Cancer and GHRH, ghrelin and somatostatin.

Secretion of growth hormone by the pituitary gland is stimulated by both Growth Hormone Releasing Hormone (GHRH) and ghrelin (growth hormone releasing endogenous ligand). GHRH is made by the hypothalamus and ghrelin is made by the gastrointestinal tract.

GHRH and ghrelin, and their respective receptors, are made in smaller quantities in many tissues other than the hypothalamus and gastrointestinal tract. GHRH and its receptor are made in ovary, testis and digestive tract.

GHRH receptors have been reported in prostate, pancreatic, ovarian, breast, endometrial and small cell lung cancer lines. (The endometrium is the lining of the uterus that sloughs off during menstruation.)

Ghrelin and/or its receptor are also expressed in many normal tissues outside the gastrointestinal tract and the pituitary gland (such as thyroid tissue, prostate tissue and the immune system), and are expressed in many cancers. Among the cancers expressing ghrelin are prostate cancers, a thyroid cancer, pituitary adenomas, gastrointestinal, pancreatic and lung cancers.

In some cases, such as some prostate cancers, ghrelin and its receptor are both expressed, and the ghrelin stimulates further tumor growth. Thus ghrelin has autocrine activity in these cancers.

The involvement of GHRH and ghrelin in cancer behavior is important for several reasons. First, GHRH and ghrelin have been proposed as superior alternatives to growth hormone itself for raising growth hormone levels. The advantages of these compounds and compounds that mimic their activity is

that they are inexpensive, easily administered (e.g. via a nasal spray or orally), do not raise growth hormone above physiological levels, and preserve the pulsatile nature of growth hormone appearance in the blood. Growth hormone itself has none of these advantages. However, it is possible that treatment with GHRH or ghrelin or their mimics might accelerate cancer growth.

A second reason that involvement of GHRH and ghrelin is important is that inactive or toxic analogs of them can be used to retard cancer growth. Extensive research has produced stable inactive analogs of GHRH that bind the GHRH receptor much more strongly than GHRH itself does. It is hoped that similar mimics will be found for ghrelin.

Several tumor types shrink when treated with GHRH analogs. These include bone, lung, prostate and kidney cancers. Other cancers hoped to be vulnerable to GHRH analogs include breast, pancreatic, colon cancers and glioblastomas.

A third reason that involvement of GHRH and ghrelin is important is that both have the potential to for paraneoplastic activity. Tumors that secrete significant amounts of bioactive substances are paraneoplastic, i.e., they have an effect beyond the immediate effect of the neoplasm (tumor) itself. Tumors might produce large amounts of GHRH or ghrelin, with their accompanying effects. (An example of paraneoplastic effects is discussed below.) [**paraneo**]

An additional related hormone is somatostatin. Somatostatin is made by the hypothalamus, and transported to the pituitary gland, where it blocks the release of growth hormone. Somatostatin is also produced in normal tissues outside the hypothalamus and some tumors carry somatostatin receptors.

Molecular mimics of somatostatin can reduce or block growth of some tumors. Mimics that are radioactively labelled have been used to locate tumors. Mimics that are radioactively labelled or conjugated to a toxin have been used to kill tumor cells.

In patients with advanced bowel cancer, where there is a bowel blockage, somatostatin has been used as a palliative to reduce gastrointestinal secretions. This can reduce nausea, vomiting and pain.

### 5.4.14 IGF-II and paraneoplastic effects.

Some cancers (e.g. Wilms tumor and soft tissue sarcomas) secrete abnormally large variants of IGF-II. It is not known whether this represents abnormal secretion of normal IGF-II precursors, or whether the proteins themselves are abnormal.

These abnormally large IGF-II variants cause hypoglycemia [**paraneo**].

### 5.4.15 Conclusions about the axis hormones and cancer.

In summary, there are reasons to fear that growth hormone and its related hormones might promote cancer, but there is no evidence that they actually do so.

As with any drug that has been in widespread use for less than 20 years, remains possible that recombinant human growth hormone and related hormones cause cancer in a small number of people who have escaped statistical detection, or that some people with an unusual genetic composition are vulnerable, or that the high doses used illicitly to enhance athletic performance are dangerous, or that adverse consequences appear only after several decades.

## 5.5. Degraded or non-human growth hormone might provoke autoimmunity.

There is an additional danger in purported "human growth hormone" obtained illicitly via the black market: the growth hormone might be chemically modified, or might be derived from a non-human species. Chemically modified growth hormone or non-human growth hormone could induce an immune response in a user that could spread to include the user's own growth hormone and even to the user's own bodily tissues.

Fake drugs are flooding the USA and seem to be a problem worldwide. It is certainly possible that black market hGH could be chemically modified. This could happen if the hGH came from a batch rejected by the manufacturer, or if the hGH were stored too long or in the wrong way. Alternatively, the purported hGH might in fact be cow or pig growth hormone. (Non-primate growth hormone does not function in humans, but a black market vendor might not know this.)

The human immune system has evolved not only to neutralize foreign proteins, but also to attack and kill cells that contain foreign proteins. This occurs even if the foreign protein never appears on the cell surface.

Proteins that resemble human proteins, but are slightly modified are particularly dangerous because they can lead to autoimmunity. This is a well documented phenomenon because it often occurs in a medical setting. The first step is "molecular mimicry"; the second step may be autoimmunity.

Molecular mimicry is an ancient strategy of microbial parasites. Potentially immunogenic proteins in the parasites evolve to resemble host proteins. This shields them temporarily from attack; however, usually the host immune system eventually responds.

The specificity of the immune system is not perfect, and such an immune response can widen to include the hosts own proteins, a process termed "cross-reaction." The results can be serious or devastating. Both Lyme disease-induced arthritis and the cardiovascular morbidity that results from Chagas disease are caused this way.

Since human growth hormone is strongly immunogenic in rats and rabbits, it seems likely that the growth hormone of non-primate animals would be immunogenic in at least some humans. If the growth hormone taken were distinct enough to provoke an immune response, yet similar enough to provoke a cross-reaction with human growth hormone, the results could be autoimmunity. Antibodies against the user's own growth hormone could develop, and there might be an attack against those tissues of the user that either make or consume growth hormone. Since, as discussed above, many cell types make and receive growth hormone, the results could be devastating.

# 6. A GLIMPSE AT THE WORKINGS OF THE SYSTEM.

## 6.1 The growth hormone axis has more than six components.

Near the beginning of this e-book [H3 1], the six hormones of the growth hormone axis were listed: growth hormone releasing hormone (GHRH), ghrelin, somatostatin, growth hormone, IGF-I and IGF-II.

The axis actually contains a number of additional components. Some of these components exist in more than one form. The axis contains at least the following:

Six hormones:
- Growth hormone releasing hormone (GHRH)
- Ghrelin
- Somatostatin (also called somatotropin release inhibiting hormone or SRIH)
- Growth hormone. (There several variants of growth hormone).
- IGF-I (There may be multiple variants of IGF-I)
- IGF-II

A number of hormone receptors:
- GHRH receptor
- Ghrelin receptors (at least two types)
- Somatostatin receptor (At least 6 types are known).
- Growth hormone receptor.
- IGF-I/IGF-II receptors. (At least 5 types are known).

A number of hormone-binding proteins:
Growth hormone binding protein (a blood component).
Six different blood proteins that bind IGFs.
Acid-labile subunit.

Hence, at least 25 proteins participate in the function of the growth hormone axis. Deficiency of, genetic variation of, or autoimmunity to any of them could affect the functioning of the system overall.

This section of the e-book summarizes what is known about how these components fit together.

## 6.2 Growth hormone pulsatility.

### 6.2.1 GH is nocturnally biased and pulsatile.

Growth hormone secretion is not constant during the day. Instead, it is both nocturnally biased and pulsatile.

Nighttime secretion of growth hormone is much greater than daytime secretion, especially in young adults. Secretion is highest during the first half of a typical eight-hour sleep period.

Growth hormone secretion is pulsatile in all species studied. In humans, bursts of growth hormone secretion occur every two to three hours. Between bursts, growth hormone falls to very low levels.

### 6.2.2 Pulsatility is important to growth hormone function.

Growth hormone is a more effective stimulator of growth and of IGF-I when provided in a pulsatile fashion, as opposed to a constant infusion. If only a single administration of growth hormone is given daily to growth hormone-deficient patients, it is most effective when given near bedtime, and thus mimics the largest growth hormone pulse of the daily cycle.

There seems to be an expectation of growth hormone pulsatility built into at least some target cells. Growth hormone administration to cultured muscle cells causes them to secrete IGF-I, which then stimulates muscle tissue growth. In one set of experiments, growth hormone applied as a 10-minute pulse followed by an 8-hour interpulse interval increased expression of the IGF-I gene more than did continuous exposure. After exposure to growth hormone, the cultured muscle cells became refractory to it for a period that lasted four to six hours. After six hours, maximal stimulation was again possible.

### 6.2.3 Growth hormone pulsatility can convey much information.

The cytochrome P450 genes are a family of genes active in the livers of mammals. They are important in protection from toxic biochemicals, which in nature enter the body chiefly through food. There are about 50 such genes in humans, and at least 50 in rats.

Male and female rats express different P450 genes. Remarkably, male and female patterns of P450 gene expression are controlled by patterns of growth hormone secretion into the blood.

Male rats have pulses of growth hormone secretion into the blood every 3.5 to 4 hours. These pulses are separated by interpulse periods devoid of detectable growth hormone. By contrast, growth hormone is continuously present in the blood of female rats.

Male rats with their pituitary glands removed can be manipulated to recreate masculine, feminine, intermediate or otherwise altered patterns of growth hormone presence. Experiments where this was done have shown that expression of P450 genes depends on several different features of the growth hormone cycle, and that the controlling information varies from gene to gene.

At least one masculine gene that is not expressed when there is no growth hormone, is restored to full expression if the growth hormone peaks are just 2.5-5% of normal amplitude. Another masculine gene requires 25% amplitude. Still others require nearly normal amplitude.

Other genes sense the length of the growth hormone-devoid interpulse period. Still others recognize the mean circulating growth hormone concentrations, rather than the pulse amplitudes. Some genes recognize a combination of signals. The authors of the study concluded that growth hormone pulsatility contains multiple signals to both induce and repress P450 genes.

It is not known whether any human tissues respond to subtle features of the pulsatile growth hormone pattern. However, medical researchers believe that the safest and most effective growth hormone therapy will mimic the natural pulsatile pattern in humans.

### 6.2.4 Pulsatility is deeply embedded.

The causes of growth hormone pulsatility have been hard to pin down. There may be more than one cause.

The pulse cycle of secretion is entrainable and disruptable by exogenous growth hormone. When growth hormone is given to rats at intervals of 3 hours, approximately in phase with their endogenous growth hormone pulses, the endogenous and exogenous growth hormone

peaks become entrained. However, when the exogenous peaks are repeated more frequently the regular endogenous pulsatility disappears. Thus growth hormone must to some degree control its own synthesis via a feedback mechanism.

For many years, the main cause of growth hormone pulsatility was thought to be a feedback relationship between growth hormone releasing hormone (GHRH) and somatostatin. GHRH stimulates growth hormone secretion from the pituitary gland, while somatostatin inhibits it.

GHRH and somatostatin vary 180 degrees out of phase with each other; when GHRH levels are high, somatostatin levels are low, and vice versa. There are connections between the GHRH-producing neurons and the somatostatin-producing neurons, which suggests direct influence between them. GHRH inhibits the release of somatostatin. Growth hormone and its downstream hormone IGF-I promote somatostatin release and inhibit the release of GHRH.

These observations have led most researchers to suspect that growth hormone is under an oscillating system of feedback regulation where GHRH stimulates a strong pulse of growth hormone, which then acts to prevent further such pulses for several hours.

Such a feedback regulation system may in fact exist, but pulsatility persists even when GHRH or somatostatin are removed from the system. In healthy men, blockage of somatostatin with an inhibitor increases the amplitude of growth hormone pulses by a factor of two or three, but does not change their frequency.

Experiments with people who lack a functional GHRH receptor, and thus cannot respond to GHRH, show that pulsatility persists here also. Growth hormone secretion persists in the pituitary gland, although at a very low level. Pulsatile secretion was maintained, although the timing of the pulses was a bit ragged, and the nighttime increase of growth hormone secretion was maintained also. Hence, the pituitary tissue itself may be programmed to pulsatile secretion.

The main lesson is that pulsatility is very deeply embedded, probably for good biological reasons.

### 6.2.5 Secretagogues and growth hormone pulsatility.

GHRH and ghrelin, as well as drugs that mimic them, stimulate growth hormone secretion, and hence are collectively termed growth hormone "secretagogues." The secretion induced by growth hormone secretagogues maintains its pulsatile pattern, even when GHRH and ghrelin secretagogues are used together. This is one reason for the great medical interest in them.

GHRH and ghrelin also have pulsatile patterns of appearance and disappearance, although the variations are smaller and less regular than those of growth hormone. In both cases, pulsatile administration has a stronger effect in promoting naturalistic growth hormone secretion and/or growth than does continuous infusion.

GHRH has a stronger effect when given in the evening than in the morning. Thus, in this case also, it is advantageous to follow the natural rhythm.

Besides promoting growth hormone secretion, GHRH strongly stimulates slow wave sleep. Pulsatile GHRH promotes slow wave sleep, while continuous infusion is not effective.

### 6.2.6 IGF-I is not pulsatile.

Although IGF-I synthesis is stimulated by growth hormone, IGF-I concentrations are not pulsatile. Morever, IGF-I concentrations have little diurnal variation.

## 6.3 Similarities and differences between males and females.

The body is an integrated system. In order to understand and manipulate the growth hormone axis productively, the influences of other hormones will have to be taken into account. A very important influence on the body plan and body composition is biological sexuality.

Male and female rats differ greatly with regard to growth hormone appearance in the blood, and the effects that growth hormone has. In humans, the difference between the sexes is smaller.

Women in general have more frequent pulses of growth hormone secretion, and their amplitude is larger. Their basal levels of growth hormone secretion are larger. Moreover, their response to exercise and to ghrelin mimics is greater than mens' response.

Under some circumstances, men seem to respond more strongly to growth hormone stimulation. In elderly men and women, growth hormone treatment increased the level of IGF-I more in men. In one study, six months of growth hormone treatment reduced abdominal and subcutaneous fat in elderly men but not elderly women. On the other hand, fat suppresses growth hormone secretion in both sexes about equally. There may also be slight differences between the sexes with respect to the improvements that growth hormone causes in elderly (older than 65 years) people.

Growth hormone increases lean body mass in growth hormone-deficient men more than it does in growth hormone-deficient women. However, it improves heart function about equally in both.

It has been claimed that age-related reduction in growth hormone secretion occurs differently in men and women. In men, GHRH production decreases, which causes much of the drop in growth hormone secretion. In women, GHRH production does not decrease.

In both humans and rats (where it can be more aggressively studied) ghrelin secretion is the same in both sexes.

### 6.3.1 Mutual influence between growth hormone and testosterone.

Testosterone is sometimes reported to boost growth hormone secretion in men. It has been reported to increase both growth hormone and IGF-I in older (60-82 yr) men after 3 weeks of treatment. Chemical castration of older men with prostate cancer does not reduce their growth hormone levels still further, so perhaps testosterone levels in older men are too low to have any effect on growth hormone.

In boys, testosterone nearly doubled the growth hormone response to a ghrelin mimic, hexarelin. The induction of male puberty with androgens or the administration of testosterone to men who are deficient in it also causes a surge in growth hormone.

It has been suggested that deficiencies in growth hormone and testosterone in men may magnify each other over time. A deficiency in growth hormone may accentuate failure of the Leydig cells of the testicles to produce testosterone, and testosterone deficiency may contribute to a fall in growth hormone and IGF-I.

On the other hand, acromegalics, with their enormous surfeit of growth hormone, usually have a deficit of testosterone and dihydrotestosterone.

### 6.3.2 Estrogen and estradiol can boost growth hormone response in women.

Post-menopausal women have low levels of growth hormone and IGF-I. Hormone replacement therapy with estrogen or estradiol can increase those levels and perhaps also levels of IGF-II. Moreover, estrogen therapy and estradiol increase the response to ghrelin mimics.

Estrogens also increase the response of growth hormone to stimulation by secretagogues at puberty.

The effects of estrogen are complex. For example, one duty of estrogen seems to be to stabilize IGFBP-3, a blood protein that binds IGF-I and presumably modifies its activity.

### 6.3.3 The influence of estradiol on growth hormone in men.

Testosterone increases growth hormone concentrations in at least some

men. Testosterone can be converted to estradiol by a biological process called "aromatization," although other androgens such as dihydrotestosterone cannot be aromatized. It is thought that estradiol is the actual stimulator of growth hormone in men.

Inhibition of estradiol production in young men (aged 15-22 yr) for 10 weeks was found not to change body composition or strength, but to reduce IGF-I concentration by 18%. However, estradiol influences are complex; infusion of estradiol into boys at early puberty decreased the amount of IGF-I by 20%. These same experiments showed that while overall growth hormone concentrations in these boys remained constant, the biological activity of the growth hormone decreased by two-thirds.

Whether estradiol could raise growth hormone concentrations in elderly men is not known, although there is no correlation between estradiol levels on one hand and growth hormone and IGF-I on the other.

Exercise might provide some of the testosterone that could increase growth hormone activity in older men. Comparison of a group of Masters runners (ages 60-70 years) with minimally exercising men of comparable ages showed that the athletic men had about 27% more testosterone. The extra testosterone was not free in the blood, but was complexed with Sex Hormone Binding Globulin.

Men who do not produce enough testosterone become fatter and lose both muscle tissue and strength. These changes are normally reversed by testosterone supplementation; however, some men cannot tolerate testosterone supplements because of, for example, sensitivity of the liver. For these men, the adverse changes can be prevented by treatment with growth hormone of IGF-I.

### 6.3.4 Combined therapy with testosterone and growth hormone or IGF-I.

Compared with healthy young men, 80% of healthy old men are both growth hormone-deficient and testosterone-deficient. This trend toward deficiency in both hormones is greater in old men who are chronically institutionalized.

In boys who are deficient in both growth hormone and testosterone, synergistic effects have been reported: both the growth-promoting and androgenic effects were magnified. In some males, testosterone increased growth hormone production and growth hormone improved androgen availability to the tissues.

Growth hormone and sex steroids have similar effects on many body tissues. Both growth hormone and estrogens stimulate IGF-I production in

osteoblasts. Both growth hormone and androgens increase IGF-I production in skeletal muscle. Hence, it is possible that the optimal treatment to limit the ravages of ageing will include both growth hormone and appropriate sex hormones (estrogens for women and androgens for men.)

A weak androgen that might be appropriate for use in women is dehydroepiandrosterone (DHEA), including its main circulating form, DHEA-sulfate. In older women, serum DHEA sulfate is directly correlated with bone density. DHEA acts against obesity in both rodents and humans. It increases IGF-I in both men and women (those tested were of age 50-65 yr).

### 6.3.5 Smoking and other unappreciated influences.

The relationship between sex hormones and hormones of the GH axis is not simple, and experimental results do not always agree. One possible reason for this is the existence of significant influences that were not understood or taken into account when the experiments were done. One such influence is cigarette smoking, which lowers free IGF-I levels in post-menopausal women.

## 6.4 Growth Hormone Releasing Hormone (GHRH)

### 6.4.1 GHRH promotes deep sleep.

As mentioned in the section on sleep [H4 10], GHRH promotes slow wave sleep. This stimulation is independent of its effect on growth hormone. Simultaneous stimulation of growth hormone and deep sleep coordinates anabolic activity with favorable circumstances.

Although GHRH loses much of its ability to stimulate growth hormone production in the elderly, it apparently does not lose its ability to promote deep sleep. This fact may someday be a great boon to the elderly.

GHRH has been reported to increase "rapid-eye-movement" sleep, as well as slow-wave sleep. However, this effect may be mediated by growth hormone.

### 6.4.2 GHRH and mental ability.

Spatial memory declines with age. Studies with rodents suggest that GHRH treatment reduces this decline, perhaps by inducing IGF-I in the brain.

### 6.4.3 GHRH pulsatility can cause growth hormone pulsatility.

Female rats normally have a more continuous growth hormone secretory pattern than male rats. This sex-specific pattern controls P450 gene activity in the liver [P450]. Intravenous injection of GHRH pulses at 3-hour

intervals to female rats, induces a male-type plasma growth hormone pattern, with growth hormone pulses following each GHRH infusion, and low growth hormone levels between the pulses.

It is not known whether subtle changes in growth hormone pulsatility have important genetic consequences in humans, as they do in rats. If so, timed GHRH release might control those consequences.

### 6.4.4 Natural and artificial inhibitors of GHRH.

Consumption of large amounts of glucose (e.g. 100 grams) can inhibit GHRH. A rise in free fatty acids can almost abolish a response.

At least in cultured anterior pituitary cells, interferon-gamma inhibits GHRH's stimulation of growth hormone. Interferon-gamma is produced in response to infectious disease, and generally induces a catabolic state. Nitric oxide (NO) is involved in this inhibition of GHRH. As a substrate for NO synthase, arginine increases the inhibition of GHRH under these circumstances.

### 6.4.5 GHRH and ghrelin synergistically increase growth hormone release.

GHRH and ghrelin can be synergistic in their release of growth hormone from the pituitary: their combined effect can exceed the sum of their individual effects. Although ghrelin is generally a more powerful stimulator of growth hormone release than GHRH is, ghrelin's effect depends on the presence of GHRH. Expression of GHS-R (the ghrelin receptor) is stimulated by GHRH.

Interestingly, although most ghrelin in the body is made by the gastrointestinal tract, small amounts of ghrelin are made in the pituitary gland itself (at least in rats). GHRH up-regulates ghrelin expression in the pituitary by 1.9-fold.

The unexpected presence of ghrelin synthesis in the pituitary gland itself is representative of a frequent feature of the growth hormone axis. Circulating hormone is made by some specialized organ devoted to that task, but small amounts of the hormone are made in many other tissues as well. Presumably, these small amounts of hormone are made for localized consumption.

### 6.4.6 The causes of reduced growth hormone secretion in the elderly.

It is still unclear why growth hormone secretion diminishes in the elderly. In theory, reduced performance of any part of the system could be the cause. GHRH from the hypothalamus and ghrelin from the gastrointestinal tract

stimulate growth hormone release from the pituitary gland. Somatostatin from the hypothalamus inhibits it.

In theory, a reduction in GHRH or ghrelin secretion could cause the drop, as could an increase in somatostatin secretion. Reduced sensitivity to GHRH or ghrelin, or increased sensitivity to somatostatin could also be the cause. Alternatively, the cause could be a reduced ability of the pituitary either to make or secrete growth hormone. In fact, there is experimental support for several of these explanations, but none completely satisfies.

Sustained treatment of older adults with GHRH can restore their ability to secrete youthful amounts of growth hormone in response to a provocative dose of GHRH. Treatment with GHRH plus a ghrelin mimic can also restore the ability of older adults to secrete youthful amounts of growth hormone.

Treatment of older men with GHRH increased their average nocturnal growth hormone release, reduced their total blood cholesterol and modestly increased their physical strength.

### 6.4.7 The growth hormone peak shifts as subject age increases.

The physiology of the growth hormone changes that occur with ageing are still being explored. One change whose significance is unknown is the shift in the growth hormone peak that often occurs with age. In young adults, most of the growth hormone secretion occurs at night. In most older adults nighttime growth hormone secretion does not exceed daytime secretion.

### 6.4.8 The advantages and disadvantages of therapy with GHRH.

Treatment with GHRH has several potential advantages over treatment with growth hormone. First, growth hormone actually consists of a spectrum of different molecular variants. The differences in function between them are still mysterious, but there probably are differences. Commercial growth hormone consists of only the most abundant of these, the 22-kilodalton variant. GHRH presumably induces the full set in the natural proportions.

Second, GHRH administration preserves the diurnal and 3-hour pulsatile rhythms of growth hormone secretions.

Third, GHRH treatment preserves the feedback mechanisms that prevent growth hormone levels from reaching supraphysiological levels. Although an excess of GHRH can produce growth hormone excess [H6 4 9] it is much more difficult to induce growth hormone excess with GHRH than with growth hormone itself.

In addition, GHRH is a smaller molecule than growth hormone (although GHRH is 44 amino acids in length, nearly all of the function is present in the

first 29 amino acids). It is cheaper to make, more stable, and can be administered nasally.

A disadvantage of GHRH is its short in vivo half-life, which necessitates frequent administration. This obstacle may eventually be overcome through implantable subcutaneous pellets that slowly release GHRH, or by development of chemical mimics that degrade more slowly.

Extensive research as been done on GHRH, and many molecular analogs have been developed, that are both more potent and more stable. The more powerful analogs are typically between 40 and 220 times as powerful as GHRH.

GHRH or its analogs can be used in most situations where growth hormone itself would be effective. Most children with growth hormone deficiency actually have a deficiency in GHRH production, for example. Only a few examples are known of defects in the GHRH receptor.

In addition, GHRH or its analogs and ghrelin or its analogs can be given together for a synergistic effect.

### 6.4.9 GHRH overproduction can cause acromegaly.

The defenses against over-stimulation of the pituitary gland by GHRH are not perfect. Tumors of the hypothalamus can cause acromegaly by overproduction of GHRH and hence growth hormone. Since GHRH is expressed at a low level in a number of non-pituitary tissues, tumors of these tissues can also overproduce GHRH. In particular, pancreatic tumors that overproduce GHRH have caused several cases of acromegaly.

### 6.4.10 Further investigation of GHRH therapy.

Research into GHRH therapy is continuing. It has been reported that GHRH injections are more effective when given in the evening than in the morning, and that injections better preserve the natural rhythm of growth hormone secretion than does continuous infusion.

Potent blockers of GHRH function have also been developed, which will be useful in research and in treating some cases of acromegaly (overproduction of growth hormone).

Women engaged in strenuous athletics sometimes develop a failure to menstruate (amenorrhea). This is associated with a disordered secretion of growth hormone, which is thought to include reduced GHRH output.

Another area of research is the need for growth hormone in development of the human fetus. Growth hormone seems to be needed for proper fetal development, but can mostly be supplied by the mother.

It is unclear why children deficient in growth hormone grow nearly

normally for their first year. A related question is why a few rare children grow to above average height even though deficient in growth hormone. It has been suggested that part of the explanation for the normal growth of growth hormone deficient children during their first year is that they may also make too little somatostatin, the brain chemical that reduces growth hormone secretion. However, the mystery is far from solved. When it is solved, scientists may learn significantly more about how to provide the best possible hormonal environment for people in need of growth hormone therapy.

### 6.4.11 GHRH expression outside the hypothalamus.

GHRH follows the example of many members of the growth hormone axis in that while much GHRH is secreted by a dominant organ, in this case the hypothalamus, it is also made locally by other tissues. GHRH and its receptor are expressed in several prostate cancer lines. Blockers of GHRH activity inhibit the growth of these prostate cancers and their production of IGF-II both in cell culture and in living animals. This suggests the presence of an intact GHRH autocrine pathway in these cancers that is necessary for their proliferation.

GHRH messenger RNA, and presumably GHRH itself, have also been reported in a glioblastoma cancer cell line.

### 6.4.12 Multiple isoforms of the GHRH receptor.

Proteins often exist in multiple isoforms. Protein isoforms are variants of the same protein that differ somewhat in their amino acid sequence.

Protein isoforms can be produced in several ways. First, they can arise from similar but distinct genes. Second, they can be produced by "alternative splicing", a kind of editing of the messenger RNA transcript that carries information from the protein-coding gene to the protein-synthesis machinery. Third, they can be produced by alternative modifications to a given protein after it has been made.

Protein isoforms can differ in the function that they perform, in the efficiency with which they perform that function, in the location where they reside, in the length of time that they persist before degradation, or in other characteristics. Although there are undoubtedly cases where different isoforms of the same protein are completely interchangeable, it is presumed that distinct isoforms exist generally to perform distinct but related functions.

Pigs, rats and humans have all have more than one isoform of the GHRH receptor, and humans have at least three. In pigs, the two known isoforms

differ in the strength with which they bind GHRH and in the strength the of intracellular signal that they generate. In rats, the two known isoforms both bind GHRH, but only one has been shown to generate an intracellular signal.

## 6.5 Cortistatin: another piece of the regulatory puzzle.

A complete understanding of growth hormone function and regulation will be necessary for the best possible medical intervention. Gaining this understanding will require identifying all of the significant influences on the growth hormone axis. One probable influence receiving attention is cortistatin-14.

Cortistatin-14 is a small (14 amino acid) peptide hormone made in mostly in the cerebral cortex and hippocampus. It is very similar in its amino acid sequence to somatostatin. It binds all somatostatin receptors, but also binds the ghrelin receptor.

Cortistatin mimics somatostatin in some ways. It inhibits growth hormone secretion and the growth hormone response to GHRH. Both cortistatin and somatostatin block ghrelin's stimulation of growth hormone, but oddly do not block ghrelin's stimulation of the pituitary hormones prolactin or adrenocorticotropic hormone, or the body's synthesis of cortisol. Neither hormone blocks ghrelin's stimulation of blood glucose levels.

Ghrelin, somatostatin and cortitropin-14 all reduce insulin secretion by acting on the islets of Langerhans in the pancreas.

## 6.6 Ghrelin and its mimics.

### 6.6.1 The history of growth hormone secretagogues.

The earliest peptide stimulators of growth hormone were discovered by accident, even before GHRH was discovered. Opioid peptides are endogenous pain relievers; derivatives of these opioid peptides were found to stimulate the secretion of growth hormone. This action is independent of any opioid activity that they have. These peptides and non-peptidyl compounds that mimic them are called growth hormone "secretagogues". The term "growth hormone secretagogue" is sometimes extended to include GHRH and its mimics, but the term usually refers to this new class of inducers. They are also called "growth hormone releasing peptides" (GHRPs). GHRH and GHRPs both induce growth hormone secretion by the pituitary, but act on different receptors, which transmit their signals by different means.

The GHRP receptor was eventually isolated and characterized. However, decades passed without any clue as to the true nature of the biological inducer that bound and activated the secretagogue receptor. Hence, an

enormous amount of work was done on artificial compounds, both peptidyl and non-peptidyl, that bound and stimulated the GHRP receptor; this was done without knowledge of the true ligand (i.e. the natural molecule that bound and stimulated the GHRP receptor.)

Finally, in 1999, a Japanese group described ghrelin, the first known natural ligand of the GHRP receptor. "Ghrelin" stands for "growth hormone releasing endogenous ligand -in". One other small peptide, cortistatin-14, also binds the GHRP receptor. Whether other ligands of the GHRP receptor exist is not known.

In this discussion, for simplicity, I assume that ghrelin is the natural ligand of the GHRP receptor. I refer to it as the ghrelin receptor, and the GHRP secretagogues as ghrelin mimics.

### 6.6.2 Ghrelin chemical characteristics.

Ghrelin is a short peptide, a chain of amino acids. In humans the mature peptide is 28 amino acids is length, although this is a fragment of a progenitor peptide containing 117 amino acids. The third amino acid residue, a serine, is chemically modified by the attachment of an octanoyl group. This modification is required for it to bind the ghrelin receptor and initiate a signal.

In solution, ghrelin is a random coil, i.e. a protein with no fixed structure. Experiments with cultured cells suggest that the minimum active fragment consists of the first five amino acids.

### 6.6.3 Characteristics of ghrelin mimics.

Ghrelin has three drawbacks as a potential drug. First, it is ephemeral, with a half-life of perhaps 30 minutes after injection. Second, it has multiple effects. Third, it must be injected if it is to be effective and predictable. Hence, there is much interest in ghrelin mimics that are more stable, that can be administered orally or nasally, and that might induce only a desired subset of ghrelin's effects.

Because of ghrelin's odd history (see above) many chemical mimics of ghrelin have been discovered and characterized. Now that both ghrelin and one ghrelin receptor have been characterized, there is an opportunity to systematically explore the requirements for binding to and activating ghrelin receptors. Although much work has been done to this end, clear understanding is still elusive.

One problem is that results with model systems have sometimes been misleading. Experiments with cultured cells have suggested that the minimum effective portion of ghrelin consists of the first 4-5 amino acids.

However, other experiments indicate that these truncated derivatives are not effective in vivo.

A second serious complication is that some of the compounds that supposedly mimic ghrelin, may actually act by inducing ghrelin. It is not yet clear whether these compounds act only by inducing ghrelin or whether they are both ghrelin inducers and ghrelin mimics.

When these complications are resolved and when the entire set of ghrelin receptors (however many members it may contain) is discovered and characterized, informed rational design of ghrelin mimics will become possible.

There are three categories of ghrelin mimics: peptides, modified peptides and non-peptides. The non-peptides are usually less effective than the others, but have the great advantages of being stable, consistent in their effects, and orally administrable.

One promising ghrelin mimic, MK-0677, induces growth hormone, which in turn induces IGF-I. Two weeks of daily dosing of healthy, elderly men and women with 25 mg of MK-0677 per day caused an IGF-I elevation to twice normal. A dose of 10 mg MK-0677 per day caused an elevation of about 40% over normal. Hence, there seems to be a 4% rise in IGF-I levels for every mg/day of MK-0677, and desired elevations of IGF-I may be regulable.

Another ghrelin mimic, NN703, is in phase II clinical trials.

### 6.6.4 Hopes for partial ghrelin mimicry.

At least one ghrelin mimic has been characterized that has only part of ghrelin's activity. Like ghrelin, it binds receptors in the hypothalamus; but unlike ghrelin, it does not bind receptors in heart tissue. Whether or not this particular mimic proves useful, its existence is a hopeful sign that mimics that induce subsets of ghrelin's activities may be devised.

Ghrelin is made mainly in the stomach, but exerts powerful effects on the brain. Hence, it must cross the blood-brain barrier. Transport of ghrelin across this barrier is complex, and it appears that there are separate systems to transport ghrelin from blood to brain and from brain to blood. Sequence differences can selectively change the affinity of ghrelin for these transporters. Such changes might be used to concentrate ghrelin in one or the other compartment, and thus limit ghrelin's effects.

### 6.6.5 Ghrelin is coded by a single highly-conserved gene.

Genetic analysis indicates that ghrelin is encoded by a single gene in humans, chimps, pigs, cows, rats and mice. This gene is highly conserved,

suggesting its importance.

Even in bullfrogs, a species distantly related to mammals, ghrelin resembles mammalian ghrelin in its structure and activity. (However, bullfrog ghrelin is only minimally effective in rats and vice-versa.)

### 6.6.6 Ghrelin receptors.

GHRH and ghrelin activate different, albeit related receptors. Both receptors belong to the superfamily of G-protein-activating receptors with seven regions that cross the cell membrane. GHRH activates the cyclic AMP/protein kinase A intracellular messenger system while ghrelin activates the phospholipase C/protein kinase C system. There appears to be some cross-stimulation between pathways, and both have the ultimate effect of inducing calcium inflow into the target cells.

The ghrelin receptor exists in at least two isoforms: GHS-R 1a and 1b. Form 1b may be non-functional, in which case it may inhibit ghrelin by sequestering it. In addition, other receptors of unknown function, which are clearly related to the ghrelin receptor have been described in humans.

A homolog to the human ghrelin receptor was isolated from pufferfish. This receptor was 58% identical to the human ghrelin receptor, and was activated by two different ghrelin mimics. This suggests that the ghrelin receptor has been conserved for at least 400 million years. This, in turn, suggests that ghrelin and its receptor are of fundamental importance to the physiology of vertebrates.

### 6.6.7 Ghrelin is subject to saturation and desensitization.

Contradictory reports on the effects of ghrelin and ghrelin mimics have occasionally appeared. One reason may be that the tissues that respond to ghrelin may become desensitized. Higher doses of ghrelin or a ghrelin mimic, or more frequent administration may not increase the effect, and may in fact do just the opposite. Ignorance of this could lead to differing conclusions about whether increased ghrelin levels have desired consequences.

In one case it was reported that multiple subcutaneous injections of the ghrelin mimic hexarelin induced growth hormone in humans, but that two daily injections of a given amount were just as effective as three injections of that same amount.

Experiments done on rats showed that administration of ghrelin or either of two ghrelin mimics into the brains of rats inhibited the secretion of stomach acid. For each of the three peptides, there was an optimal concentration, with higher concentrations being less effective.

Pulsed (every 3h) infusion of the ghrelin mimic GHRP-6 induced ongoing growth hormone secretion in rats that were growth hormone-deficient. However, continuous infusion of GHRP-6 stimulated growth hormone transiently, but then lost its effectiveness.

There is evidence that desensitization results from a rise in somatostatin, which has an inhibitory effect. However, cultured cells of the rat anterior pituitary gland are also desensitized when pulsed every hour, but not when pulsed every three hours. Under these conditions, somatostatin was probably not present, but the desensitization occurred nevertheless; hence, there may be an additional mechanism of desensitization.

Some experiments on continuous infusion of ghrelin mimics have not produced desensitization, but have instead produced a sustained, pulsatile secretion of growth hormone. Hence, a full understanding of desensitization remains to be achieved.

### 6.6.8 Ghrelin secretion in different species.

Use of animals allows aggressive, well-controlled experiments that could not easily be done in humans, and which can be extremely informative. However, the medical value of the information will be reduced if the animals are poor models for humans. Hence, it is important to verify that experimental animals are indeed good models or, failing that, to study the experimental animal that most resembles humans.

In the past, it seemed as though experimental animals might differ from each other and from humans in the details of ghrelin secretion by the stomach. However, careful experimentation on humans, dogs and rats has shown that ghrelin is secreted by similar cell types in all three species. Differences between the species seem confined to the size of the secretory granules: the diameter of ghrelin secretory granules in dogs is nearly twice that in humans, with rats located in between.

### 6.6.9 The major source of ghrelin is the stomach.

Ghrelin was first isolated from rat stomach, and stomach is the main source of circulating ghrelin. Plasma ghrelin in totally gastrectomized patients (patients in which the stomach has been removed) is reduced to 20-35% of those in normal controls.

Ghrelin is produced throughout the gastrointestinal tract, but mainly in the stomach. A gradient of ghrelin production occurs in the gastrointestinal tract with the highest ghrelin production in the acid-producing tissue of the stomach, and the lowest in the colon.

In rats, ghrelin is not detectable in the fetal stomach, but increases

progressively after birth. Plasma ghrelin levels increase in parallel.

It has been argued that ghrelin-producing cells in the gastro-intestinal tract actually consist of two types: open and closed. Their distribution is not uniform: the open cells increase in the direction from the stomach to the lower intestine, contrary to what is observed for the entire population of ghrelin-producing cells. This opens the possibility that the two cell types may be under different regulation.

### 6.6.10 Ghrelin is expressed at lower levels in non-gastrointestinal tissues.

Although most circulating ghrelin is made in the stomach, ghrelin is expressed in many organs outside the gastrointestinal tract. Thus, it conforms to the usual pattern of the growth hormone axis, where one organ produces the most of the circulating hormone, but smaller amounts are produced in many tissues.

Ghrelin is expressed in the pancreas, in a distinct subpopulation of cells (about 3% of the total in rats). Ghrelin is expressed in the fetal pancreas, and its expression precedes by far the expression in the stomach. The pancreas secretes insulin, and in fact ghrelin affects insulin production [H6 6 20].

Ghrelin is expressed in the placentas of humans and rats. It is also expressed in the thyroid gland.

Ghrelin receptors (type 1a) are expressed in the prostate gland, although ghrelin itself seems not to be. Ghrelin receptors are expressed in cardiovascular tissue, and apparently mediate ghrelin's potent ability to dilate blood vessels.

Ghrelin is expressed in rat testes. It is expressed only when Leydig cells (the cells that secrete testosterone) are present, and thus may be secreted by them. The ghrelin receptor is still present when the Leydig cells are removed, however. Ghrelin seems to repress testosterone expression and the expression of several testicular proteins. At least in mice, a testis-specific ghrelin isoform is expressed. Although it is coded by the ghrelin gene, it is longer than ghrelin and considerably different.

Although the pituitary gland is a target for circulating ghrelin produced elsewhere, it also produces a small amount of ghrelin. The biological consequences of this production are not known.

Altogether, the ghrelin receptor type 1a has been observed in the pituitary and thyroid and adrenal glands, and in the pancreas, spleen and myocardium. Ghrelin itself is made in the stomach, other parts of the gut, adrenal gland, atrium, breast, buccal mucosa, colon, esophagus, Fallopian tube, fat tissue, gall bladder, ileum, kidney, liver, lung, lymphocytes and lymph nodes,

muscle, myocardium, ovary, pancreas, pituitary gland, placenta, prostate, skin, spleen, testis, thyroid gland and veins. Indeed, it appears everywhere or nearly everywhere it is searched for.

### 6.6.11 Ghrelin expression in tumors.

Ghrelin and its receptor are sometimes expressed in tumors. This has at least two potential medical consequences. First, tumors that overproduce ghrelin could have profound metabolic consequences. Second, there may be cases where tumors are self-stimulating because they both produce and respond to ghrelin. Such autocrine behavior often occurs with growth factors in cancer.

In one survey, ghrelin was expressed in about three-quarters of pancreatic tumors, while the ghrelin receptors GHS-1a and GHS-1b were present in one-quarter and one-half of the tumors, respectively. Ghrelin is also expressed in a large proportion of gastric tumors (76%) in one small survey), and about half of the lung endocrine tumor examined. Ghrelin is also present in normal and cancerous pituitary tissue.

Ghrelin mimics bind both normal and cancerous human thyroid tissue. They have been reported to inhibit the growth of thyroid-derived cancerous cell lines.

Autocrine activity of ghrelin is suspected in prostate cancer. Normal prostate tissue expresses the ghrelin receptor GHS-R1a, but not the 1b isoform or ghrelin. By contrast, 4 prostate cancer lines reportedly express ghrelin and probably the GHS-R1b receptor isoform.

One prostate cancer line showed increased growth (33% above normal) in response to ghrelin. This implies a potential tumor-promoting role for ghrelin in prostate tissue.

### 6.6.12 Ghrelin effect on the pituitary gland.

Ghrelin and its mimics stimulate the release of growth hormone from the pituitary gland. It acts synergistically with GHRH: their combined effect is often much greater than the sum of their individual effects. Ghrelin has been reported to inhibit release of somatostatin, which would contribute to its induction of growth hormone.

Adiposity tends to be self-sustaining. The reason is that growth hormone is needed for lipolysis (fat burning), but adiposity blocks the release of growth hormone. Ghrelin or ghrelin mimics have been suggested as a possible means to increase the production of growth hormone even in the presence of adiposity. An obstacle to this is that ghrelin itself induces adiposity; but if this effect could be blocked, ghrelin might become a valuable inducer of

weight loss.

### 6.6.13 Ghrelin stimulates small amounts of prolactin, ACTH and cortisol.

Although the major activity of ghrelin and its mimics on the pituitary gland is to stimulate release of growth hormone, they also cause the release of minor amounts of prolactin and adrenocorticotropic hormone. The adrenocorticotropic hormone in turn causes the adrenal glands to secrete cortisol, a stress hormone.

### 6.6.14 Ghrelin stimulates hunger by acting on the hypothalamus.

Ghrelin and its mimics greatly stimulate appetite, feeding and weight gain. During fasting ghrelin levels rise; injection of these levels of ghrelin stimulates eating in rats.

In an experiment with mice, injection of 3, 10 or 30 micrograms of ghrelin per mouse increased food intake to 1.8-, 2.6- and 3.6-fold, respectively, over mice that did not receive ghrelin. Hence, the food intake response increases as the ghrelin dose increases, and can rise to several times normal. Other reports indicate that ghrelin can stimulate food intake in rats to more than four times normal.

Ghrelin also increases both appetite and food intake in humans (these can be measured separately). One experiment with ghrelin injection showed an average increase of 28% in energy consumption.

It makes sense that people with too much ghrelin might be prone to overeating and obesity. One such person has been mentioned in the scientific literature (although, interestingly, this person had no symptoms of growth hormone excess).

Ghrelin also increases the rate of emptying of the stomach and small intestine in some experimental animals. This effect may or may not occur in humans.

Ghrelin has been reported to act on many areas of the brain. These include several parts of the hypothalamus: the arcuate nucleus, the paraventricular nucleus, the dorsomedial nucleus, and the lateral hypothalamus. They also include two regions of the brainstem: the nucleus of the tractus solitarius and the area postrema.

Of these, the best understood is ghrelin's action on the arcuate nucleus of the hypothalamus. This region is thought to control hunger and eating. Genetically engineered rats with reduced expression of the ghrelin receptor in the arcuate nucleus had a lower body weight and less fat tissue than did normal rats. Female rats of this engineered strain also had reduced secretion

of growth hormone, and consequently reduced levels of circulating IGF-I.

Two populations of neurons within the arcuate nucleus seem to be important for hunger and adiposity. The first of these can be identified by the fact that they contain and release a neurotransmitter called neuropeptide Y. These have been reported to be sensitive to a wide variety of nutrients and hormones.

The effect of ghrelin and a ghrelin mimic on hunger in rats is blocked by preadministration of an antagonist to the neuropeptide Y receptor. Ghrelin induces expression of an important regulatory gene, called "Fos", in the neuropeptide Y-producing cells that it activates.

Neurons that do not contain neuropeptide Y may also be involved in the weight gain that ghrelin causes. Mice completely lacking neuropeptide Y are viable, and respond to ghrelin with increased adiposity. It is thought that a second group of neurons, expressing agouti-related protein mediate this effect. When agouti-related protein is chemically blocked in mice lacking neuropeptide Y, ghrelin no longer stimulates weight gain.

A hormone called leptin, secreted by fat cells, inhibits hunger and weight gain. Leptin binds many of the same neurons that ghrelin does, but reduces their activity instead of stimulating it.

Non-functional ghrelin mimics, which occupied ghrelin receptors in the hypothalamus and prevented them from being stimulated by ghrelin could be powerful aids in dieting and weight loss.

### 6.6.15 Ghrelin may affect sleep.

Ghrelin and its mimics are clearly less promising than GHRH and its mimics for sleep induction. Ghrelin mimics have been reported to increase sleep in humans, particularly the type of sleep involving rapid eye movement. However, they have been reported to do just the opposite in mice; this may have been because the mice were awake and eating or searching for food.

### 6.6.16 Ghrelin effects on the gastrointestinal tract.

Ghrelin can stimulate gastric acid secretion, but inhibits gastric acid secretion when injected into the brain. It may do this by inducing somatostatin.

Ghrelin reportedly stimulates gastrin. Gastrin is the collective name for a group of stomach hormones that induce the stomach lining to secrete hydrochoric acid.

Ghrelin stimulates gastrointestinal motility. It accelerates emptying of the stomach and small intestine of rats. Reports differ as to whether it

accelerates emptying of the stomach in humans.

### 6.6.17 Ghrelin may affect obesity other than by stimulating appetite.

The effect of ghrelin and its mimics in stimulating appetite are well established. Opinion is divided as to whether ghrelin and its mimics also affect fat metabolism, independent of food intake. Some researchers believe that ghrelin reduces fat breakdown and use, while others believe that ghrelin accelerates fat breakdown and tends to increase lean body mass.

Confusion of this sort is most likely in experimental systems where multiple variables are operating and researchers have not yet agreed on standard sets of experimental conditions. Ghrelin increases appetite and growth hormone secretion; growth hormone destroys fat. Ghrelin may also influence or be influenced by leptin, the hormone secreted by fat cells that limits obesity [H6 6 25 H6 10]. Finally, ghrelin may have effects of its own on fat metabolism. These various influences on fat have not been clearly sorted yet.

### 6.6.18 Ghrelin can benefit the heart.

Aside from its stimulation of weight gain, ghrelin has effects on the heart that appear mainly beneficial. It is a potent vasodilator and acts without increasing heart rate; hence, it can decrease the circulatory resistance that the heart must overcome and can presumably increase the flow of blood to the heart. It has been reported that people with atherosclerosis have a three- to four-fold increased concentration of ghrelin receptors in their blood vessels; this is thought to partly compensate for the atherosclerosis.

Ghrelin is an ephemeral hormone and vasodilation/vasoconstriction is a prompt response; hence, rapid reductions in ghrelin concentration might be dangerous to people at risk for heart attack. Since large, nourishing meals trigger a reduction in ghrelin concentration, such meals might be particularly dangerous to at-risk people (in addition to any adverse effects the meal might have on circulating lipids and the propensity of the blood to clot).

Ghrelin and its mimics benefit the heart itself. They can prevent degeneration of the heart's efficiency in heart disease. However, the mechanism is still unclear. The benefit may come from improved circulation and nourishment of the heart, or through the stimulation of growth hormone, which is also beneficial. On the other hand, one ghrelin mimic binds the cardiac protein CD36, and has been argued to act by that mechanism.

### 6.6.19 Ghrelin and blood pressure during pregnancy.

Ghrelin levels differ between nonpregnant women, normal pregnant

women and women with pregnancy-induced hypertension. Ghrelin might influence blood pressure in normal pregnancy or in pregnancy complicated by high blood pressure. Clearly, any medical scheme to use ghrelin or its mimics to increase infant birth weights would have to consider this. In fact, any use of ghrelin or a ghrelin mimic in pregnant women, or any genetic therapy that permanently alters ghrelin levels in a woman who might later become pregnant, need consider this.

### 6.6.20 Ghrelin may influence insulin secretion and activity.

Ghrelin binds pancreatic cells. It has been reported both to increase and reduce insulin production by the pancreas. These contradictory reports might be caused by multiple opposing effects of ghrelin on insulin, with the importance of the effects varying according to circumstances. Ghrelin, growth hormone, insulin, pancreatic somatostatin and the insulin-like growth factors interact in complex ways.

Insulin and ghrelin both up-regulate several liver enzymes, suggesting shared function. However, unlike insulin, ghrelin also upregulates gluconeogenesis, the conversion of amino acids to glucose.

### 6.6.21 Ghrelin's effect on early development.

In human fetuses, ghrelin is present in the developing gut, pancreas and lung from gestational week 10 onward. Ghrelin treatment of pregnant rats increases the birth weight of newborn rats. Moreover, ghrelin treatment of newborn rats advances their development.

It is conceivable that ghrelin or its mimics might be used to counter low birthweight. Such use could occur either before or after birth.

### 6.6.22 Additional ghrelin effects.

Ghrelin and at least one of its mimics transiently reduce the core body temperature of rats when injected into their brains near the hypothalamus and pituitary gland. This could result either from an effect on the hypothalamus, which regulates body temperature, or might result from ghrelin's effect as a vasodilator.

On the other hand, ghrelin seems to have no role in the induction of growth hormone that occurs with exercise. Neither does it seem to be involved in controlling the short-term pulsatile secretion of growth hormone.

### 6.6.23 Food, hunger and ghrelin secretion.

Food and hunger are the main regulators of ghrelin secretion. Ghrelin levels do not vary greatly in the fed state, but increase in response to fasting,

and develop a diurnal rhythm. Ghrelin levels peak at about 2 a.m. This diurnal rhythm of ghrelin induces a similar diurnal rhythm in growth hormone secretion.

Ghrelin levels drop after a meal. In experiments with humans that had fasted overnight and then taken a standard meal, food decreased blood ghrelin concentrations by 28%. Very similar results were obtained with rats.

Fasting induces ghrelin secretion in both rats and people. Fasting for 12 hours increased ghrelin levels in human subjects by 31%. Ghrelin levels in people with eating disorders are generally higher than average. In patients with anorexia nervous, ghrelin levels were more than double their usual value (+108%). In recovering anorectics, ghrelin levels tend to fall.

People with bulimia nervosa also have elevated ghrelin levels, even though their body composition does not differ from normal. It was suggested that bingeing and purging may increase overall ghrelin levels.

Long-term dieting, to reduce obesity, also increases ghrelin levels. Average increases of between 12-24% have been reported.

The expectation of food may increase ghrelin levels. In humans fed scheduled meals, ghrelin levels nearly doubled immediately before a meal, and fell to trough levels after the meal. A similar pre-feeding surge occurs in sheep fed scheduled meals.

The signals that control ghrelin secretion may be complex. Pseudo-feeding in sheep lowers ghrelin levels, but only temporarily, before they rebound to higher levels.

### 6.6.24 The control of ghrelin by fat and glucose.

A complex system has evolved to maintain energy balance in humans and other mammals. However, since under natural circumstances an energy deficit is the greatest threat to survival, this system is biased toward weight gain. Hence, people in comfortable circumstances tend to become obese.

Obesity is an enormously important degrader of health, especially in the Western world. It promotes heart disease, cancer, type II diabetes and many other evils.

Obesity reduces the secretion of growth hormone. Obese people have lower ghrelin levels than do lean people. However, an comparison of lean and obese people showed that while eating decreased blood ghrelin by 40% in the lean people, it did not lower blood ghrelin levels in the obese people. Thus, eating may not diminish hunger in obese people.

Obesity decreases the ability of ghrelin mimics to stimulate growth hormone release. It appears that free fatty acids in the blood cause this decrease. Blockage of triglyceride (fat) degradation to free fatty acids (with

the drug acipimox) increased the release of growth hormone by a ghrelin mimic in elderly men.

In rats, dietary fat greatly diminishes ghrelin secretion, as does glucose (whether eaten or injected). Thus, food which is likely to promote obesity tends to limit further food consumption. If dietary supplements existed that could mimic fat's suppressive effect on ghrelin without themselves promoting obesity, they might be very valuable.

### 6.6.25 The effect of leptin on ghrelin secretion.

Leptin is secreted by fat cells, and reduces further weight gain. Leptin and ghrelin are antagonistic hormones: each tends to counter the effects of the other. The effects of targetted leptin gene implantation into the brains of rats was assessed. Increased leptin expression in the brain lowered leptin levels in the blood. This, in turn, increased ghrelin levels in young, but not old, rats.

Thus, circulating leptin may reduce ghrelin levels in some cases.

### 6.6.26 Growth hormone may affect ghrelin levels.

Cooperating hormones often follow a pattern where one hormone induces the second, but the second hormone inhibits expression of the first. Such an arrangement promotes the stable expression of both. By contrast, if both hormones stimulated each other's expression, the expression of both would tend to spiral upwards indefinitely, limited only by the biosynthetic capacity of the body. Alternatively, if the two hormones suppressed each other's synthesis, one would tend to dominate and suppress the other unless other influences intervened.

One might expect that since ghrelin induces growth hormone, growth hormone would likely suppress ghrelin, in order to maintain the expression of both at physiological levels. This has in fact been reported in humans, rat and mouse, but is not always observed.

### 6.6.27 The effects of age on ghrelin expression and effectiveness.

Ageing can affect ghrelin secretion and the responsiveness of the body to ghrelin. One question is whether elderly people differ from the rest of the population.

In mice, ghrelin levels were low at the 18th day of gestation, but increased rapidly after birth and reached a peak at the 21st day after birth. The ghrelin levels then declined to 75% of the peak at day 60 after birth, to 67% of the peak at 6 months, and to only 5% of the peak at 19 months.

It has been reported that ghrelin levels rise in humans as they age.

However, the growth hormone-releasing potency of ghrelin declines with age; this has been attributed to reduced activity of GHRH with age and/or to increased synthesis of somatostatin in the hypothalamus. If ghrelin retains it appetite-stimulating properties, but loses its growth hormone-stimulating properties as people age, the rise in ghrelin with age could explain the tendency toward obesity with age.

Under some circumstances this loss of potency of ghrelin and mimics can be overcome. In one study with post-menopausal women continuous infusion of a ghrelin mimic increased growth hormone secretion by seven-fold. When estrogen supplementation was added, the increase rose to nearly nine-fold. Neither pulse frequency nor pulse duration was affected, but the growth hormone levels in both the troughs and peaks rose.

In another study involving elderly subjects (ages 66-81 yr), a mixture of arginine, GHRH and a ghrelin mimic boosted levels of growth hormone secretion to levels that equalled or exceeded the highest levels obtainable in young subjects (ages 24-28 yr).

A second question is whether children differ from adults. In general, ghrelin levels in normal children were similar to those in normal adults.

A third question is whether and how ghrelin levels change in the very young, either before or after birth. In fact, ghrelin levels do change in the very young in a tissue-specific fashion.

Ghrelin-secreting cells are very frequent in the pancreas of the human fetus from mid-gestation through the early post-natal period. They are less frequent in the adult pancreas.

Ghrelin expression in the rat pituitary gland peaks prenatally (at day 18 of gestation), and declines postnatally.

Ghrelin protein is present in the endocrine cells of the human fetal lung in decreasing amounts from embryonic to late fetal periods. Its expression is maintained in lungs of newborns and children under 2 years, but is nearly absent from lungs of adults.

Although ghrelin expression is greatest in the stomach, ghrelin expression in the stomach begins comparatively late. In rats, gastric ghrelin expression was lowest at day 9, and reached a stable level of expression at day 40 in both female and male rats. The increase in female rats was more gradual than in the males.

The effects of ghrelin on infants will have to be considered if ghrelin or ghrelin mimics are given to women who are pregnant, or if ghrelin-producing gene therapy is given to women who may later become pregnant. In addition, ghrelin or its mimics might have prenatal medical uses, such as increasing birth weights.

### 6.6.28 GHRH and somatostatin influence ghrelin's release of growth hormone.

GHRH and ghrelin (or ghrelin mimics) can have a synergistic effect on release of growth hormone from the pituitary: the effect of the two together considerably exceeds the sum of their individual effects. However, the relationship between GHRH and ghrelin seems asymmetric; GHRH is thought to be necessary for ghrelin to exert an effect, but the reverse is not true.

GHRH stimulates both the ghrelin receptor and ghrelin expression itself in the anterior pituitary gland.

Somatostatin can completely block the growth hormone-releasing activity of GHRH in the anterior pituitary gland. However, its ability to block the activity of ghrelin and ghrelin mimics is only partial.

### 6.6.29 Ghrelin is under multiple biological controls.

It is clear from studies on identical twins that ghrelin levels are controlled genetically. The details are unclear, but learning the genetic and non-genetic influences on ghrelin secretion could be of enormous help in the management of heart disease.

In addition to the influences discussed above, ghrelin secretion is influenced by thyroid function, melatonin, insulin and adenosine. Medical control over ghrelin levels and ghrelin effects will require an understanding of how these influences act, and why it is advantageous for them to act as they do.

Hyperactivity of the thyroid decreases ghrelin secretion by the stomach. Abnormally low thyroid activity increases it.

Melatonin was reported to decrease ghrelin secretion in rats.

Insulin also seems to decrease ghrelin secretion. However, the magnitude of the decrease is variable (one study of eight human subjects showed that suppression ranged between 19% and 64%) and is not always observed.

Adenosine tends to enhance ghrelin's effects.

### 6.6.30 The influence of gender and sex hormones.

In humans, gender seems not to influence ghrelin secretion, although differences have been reported in other species.

Humans do show gender differences in the effect that ghrelin or ghrelin mimics have on growth hormone release. There are many reports that women show a greater response than do men.

Both testosterone and estrogens increase the stimulation of growth

hormone by ghrelin mimics. This increase is at least a partial cause of the adolescent growth spurt. The activity of testosterone appears to occur when it is converted to estradiol. The higher concentration of estradiol in women may cause their greater responsiveness to ghrelin mimics.

#### 6.6.31 The effect of stomach conditions on ghrelin levels.

Since most circulating ghrelin is made by the stomach, conditions within the stomach might influence circulating ghrelin levels.

An enormous amount of research remains to be done. However, it is already clear that gastrin does not control ghrelin secretion in the stomach. Gastrin is a group name for several stomach hormones that stimulate hydrochloric acid secretion.

Gastric bypass surgery greatly lowers ghrelin levels in the blood. One study showed that despite a 36 percent weight loss after gastric bypass in gastric-bypass patients, ghrelin secretion was 72% lower than in matched controls. The normal, meal-related fluctuations and diurnal rhythm of the ghrelin concentrations were absent after gastric bypass.

The discovery that this is so has changed opinions about why gastric bypass is as effective as it is. Gastric bypass has brutal consequences. Perhaps some gentler procedure to destroy or inactivate the ghrelin-secreting cells of the stomach might substitute for gastric bypass.

The interactions between ghrelin and stomach ulcers or between ghrelin and many other abnormal stomach conditions are unknown. There is much of importance to be learned.

### 6.7 Somatostatin and its mimics.

#### 6.7.1 Somatostatin inhibits growth hormone.

Somatostatin is a peptide hormone made in the hypothalamus, as well as in other parts of the body. It consists of 14 amino acids, and is excised from a larger precursor protein. Somatostatin acts upon the anterior pituitary gland to prevent the release of growth hormone.

Somatostatin acts on pituitary cells by preventing increases in "cyclic adenosine monophosphate." The result is that while GHRH can still bind its receptor, news of this never reaches the cell nucleus.

Somatostatin also increases the charge difference between the inside and outside of pituitary cells and inhibits the uptake of calcium. These actions prevent the release of growth hormone.

Growth hormone secretion from the anterior pituitary gland occurs in pulses. When somatostatin is chemically blocked, the amount of growth hormone secreted in each pulse increases, but the frequency and duration of

pulses does not change. Moreover, a small amount of growth hormone is secreted between pulses; this is not affected by somatostatin.

### 6.7.2 Somatostatin and pulsatility.

Growth hormone release is pulsatile. As discussed above [H6 2] this pulsatility is important for growth hormone function and seems to be enforced by more than one mechanism.

One of the mechanisms to enforce pulsatile release of growth hormone may be feedback inhibition of growth hormone of its own release. Growth hormone is thought to increase the secretion of somatostatin by the hypothalamus and reduce the secretion of GHRH. If this is correct, there must be a lag period before the suppression sets in, so that growth hormone does not suppress its own synthesis immediately.

There is data in rats and sheep indicating that the growth hormone inducer GHRH and somatostatin are induced out of phase, so that peaks of GHRH correspond to troughs of somatostatin, and vice versa.

Part of the enforcement of pulsatility may come from interconnections between the neurons that secrete GHRH (growth hormone releasing hormone) and the neurons that secrete somatostatin. A subpopulation of GHRH-releasing neurons is innervated by somatostatin-releasing neurons, and is inhibited by somatostatin from releasing GHRH. A subpopulation of somatostatin-releasing neurons is innervated by GHRH-releasing neurons, and is inhibited by GHRH from releasing somatostatin. This has been termed the "ultrashort feedback loop." It may help enforce the out-of-phase relationship between GHRH and somatostatin.

On the other hand, suppression of somatostatin release with pyridostigmine does not suppress pulsatility. Hence, it may be that the pituitary cells themselves are programmed toward pulsatile release.

The pituitary gland can become desensitized to ghrelin mimics if continuously exposed to them. Somatostatin elevation occurs along with this desensitization, and may cause all or part of it. (By contrast, there is no desensitization if the ghrelin mimic is administered in pulses every three hours. Instead there is ongoing secretion of growth hormone.)

### 6.7.3 Somatostatin and ageing.

Growth hormone synthesis declines with age in humans. A rise in somatostatin occurs concurrently, and it might be thought that this rise causes the drop in growth hormone synthesis. However, suppression of somatostatin secretion with pyridostigmine only partly restores growth hormone secretion in elderly men.

### 6.7.4 Somatostatin receptors.

Somatostatin is secreted by many tissues other than the hypothalamus and has actions throughout the body (see below). Its effects are complex.

The complexity of somatostatin action is mediated by a multitude of receptors. There are six known somatostatin receptor types: sst1, sst2A, sst2B, sst3, sst4 and sst5. The functional receptor may be a dimer at least under some circumstances. The different receptor proteins can form either homodimers (two subunits of the same type) or heterodimers (two subunits of different classes). In addition, the receptor proteins can form hybrids with receptor types that are not one of the six sst classes.

### 6.7.5 Somatostatin mimics and antagonists.

A number of drugs have been developed either to mimic or to block the action of somatostatin. The mimics generally have three potential advantages: they are much more long-lived in the body, and they usually activate only a subset of the somatostatin receptors, and in some cases they bind their receptors much more strongly than does somatostatin.

The more widely-used somatostatin mimics bind only to sst2 and sst5. New mimics, SOM230 and KE108, bind all somatostatin receptor classes.

Somatostatin analogs may be used to prevent the growth of some cancers (see below), by activating somatostatin receptors on their cell surfaces. As an alternative, the analogs can be linked to a toxic molecule or a radioactive atom, and kill the cells that take them up. It is also possible to use labelled somatostatin analogs to locate tumors that carry somatostatin receptors (see below).

Somatostatin antagonists have also been developed. However, this research is less developed than the search for somatostatin mimics.

One risk inherent in mimics and antagonists of somatostatin is that, as foreign molecules, they may provoke an antibody response that could neutralize them or even induce anaphylactic shock. One antibody response to octreotide and lanreotide, which are somatostatin mimics. has been reported.

### 6.7.6 Somatostatin and cancer.

Somatostatin receptors have been found on many cancer types. These include lymphoblastic leukemia cells, malignant lymphomas, tumors of the thymus, pituitary tumors, and tumors of the pancreas. It is presumed that the somatostatin receptors were present in the cell type from which the cancer developed.

Sst 2 and sst 5 are very prominently represented in such cancers. Hence, somatostatin analogs that bind sst 2 and sst5 will bind somatostatin receptors in these cancers.

As discussed above [soma], somatostatin analogs can be used against cancers that carry somatostatin receptors. They can be used to inhibit the cancer by somatostatin-like activity, or can be used to deliver a toxic or lethally radioactive anticancer agent. Neuroendocrine tumors, tumors of the thymus, as well as Hodgkin and non-Hodgkin lymphomas have been inhibited with somatostatin analogs.

Somatostatin analogs can also be used to locate cancers, and deduce their stage by "painting" them with a detectable agent. The location and prognosis of Hodgkin and non-Hodgkin lymphomas has been assessed by painting with somatostatin analogs.

### 6.7.7 Additional influences on somatostatin release.

Ongoing research is aimed at learning how different biological conditions and drugs affect somatostatin release. Pyridostigmine is a powerful suppressor of somatostatin release from the hypothalamus, and thus promotes growth hormone release from the pituitary gland. Orally administered melatonin has been suggested to have the same effects in weaker form. Arginine also can stimulate growth hormone release by the pituitary, perhaps by inhibiting somatostatin release. The neurotransmitter GABA (gamma amino butyric acid) also inhibits somatostatin release.

Agents that increase somatostatin release include the neurotransmitter dopamine and the hormone corticotropin releasing hormone. Large doses of interleukin-1, a result of bacterial infection, also stimulate somatostatin.

### 6.7.8 Other somatostatin activities.

Somatostatin inhibits the stimulation of growth hormone by ghrelin, but the inhibition is only partial. Growth hormone stimulation by ghrelin in the presence of somatostatin is greater than growth hormone stimulation by GHRH without somatostatin.

Somatostatin has many activities throughout the body. It inhibits the secretion of glucagon, insulin, gastrin, secretin and inhibits the secretion of gastric acid by the stomach. It also plays a role in neurotransmission.

### 6.7.9 Somatostatin affects the immune system.

Somatostatin receptors are present on resting lymphocytes, activated lymphocytes and monocyte/macrophage cells. Somatostatin, acting on its receptors, inhibits more than 90% of the secretion of interleukin-8 and

interleukin-1-beta from two intestinal epithelial cell lines. This occurred whether the secretion was spontaneous or induced by the bacterium Salmonella.

### 6.7.10 Somatostatin and diabetes-induced blindness.

People with diabetes may suffer diabetic retinopathy. This condition, which can lead to blindness, involves growth of new blood vessels into the retina (neovascularization).

The usual therapy is laser ablation of the new blood vessels. However, somatostatin analogs also reduce neovascularization in retinopathy. It appears that somatostatin activates a receptor in retinal cells, and inhibits pro-survival signalling pathways in the cells that would otherwise develop into new blood vessels.

It is hoped that somatostatin analogs or delivery strategies will be developed that can directly inhibit neovascularization without directly reducing growth hormone secretion by the pituitary gland.

### 6.7.11 Somatostatin and sleep.

Somatostatin is a modest disrupter of sleep. In young people, it has no apparent effect, but it disrupts the sleep of older people. This is probably because young people sleep better than old people do, and somatostatin is not a strong enough sleep disrupter to to affect their sleep.

## 6.8 The molecular biology of growth hormone.

### 6.8.1 The growth hormone gene family.

The gene that encodes human growth hormone is one member of a family of five genes. These five genes are very similar and are clustered together on the long arm of chromosome 17. There are two growth hormone genes and three "placental lactogen" genes.

One growth hormone gene, GH-V, is expressed only in pregnant women. The other growth hormone gene, GH-N, encodes all of the growth hormone variants that are made in non-pregnant people.

The different growth hormone variants are made as final-length proteins; there is no prohormone that is degraded to liberate the mature hormone, as happens with the insulin-like growth factors (IGF-I and IGF-II).

The human growth hormone gene GH-N encodes several different growth hormone variants. Other genes of the five-member family also do this, creating quite a variety of proteins.

### 6.8.2 There are multiple growth hormone isoforms.

The growth hormone gene is "transcribed" to produce molecule of mRNA (messenger ribonucleic acid). This mRNA is transported to the ribosomes where it is "translated" to form a protein. Before the mRNA molecule reaches the ribosomes, it is edited to remove various portions. Since there are multiple different ways for the mRNA to be edited, there are multiple different proteins that can be produced. Such editing is called "alternative splicing", because the editing involves splicing out of parts of the mRNA molecule.

mRNA from the growth hormone gene can be edited several different ways. Hence, several related growth hormone proteins can be produced. These are called growth hormone "isoforms."

The most abundant growth hormone isoform weighs about 22,000 times as much as a hydrogen atom, or 22 kilodaltons (22 kDa). The next most abundant isoform weighs 20 kilodaltons (20 kDa). There are additional growth hormone isoforms.

As mentioned above, the placentally expressed genes also encode multiple isoforms.

### 6.8.3 The two major growth hormone isoforms.

The 22 kDa and 20 kDa growth hormone isoforms have many similarities. Both mediate the main biological effects of growth hormone, such as stimulating osteoblasts, the cells that form bone.

Both isoforms can form dimers in the blood. Two 22 kDa proteins can pair, as can two 20 kDa proteins. Whether a 22 kDa protein can pair with a 20 kDa protein is not known.

There are some differences between the two isoforms. In one set of experiments, a human breast cancer line was stimulated by the 22 kDa isoform, but inhibited by the 20 kDa isoform.

It was also reported that the 22 kDa isoform has a much greater effect in stimulating the breakdown of fat than does the 20 kDa isoform (12.5-fold greater), although this point is controversial. The larger isoform is also more prone to raise blood glucose (1.7-fold greater).

Both isoforms stimulate water retention. However, the 22 kDa isoform is more potent.

Both isoforms can form dimers. However, dimerization greatly reduces the biological activity of the 22 kDa form, but does not change the biological activity of the 20 kDa form.

The 20 kDa isoform is less-influenced by the growth hormone binding protein (GHBP [H6 8 9]). Whereas the GHBP interferes with the 22 kDa isoform's suppression of leptin secretion and stimulation of fat breakdown, it

interferes much less with these activities in of the 20 kDa isoform.

### 6.8.4 Control over the isoform ratio.

Growth hormone limits it own synthesis, either directly or by inducing IGF-I. The 22 kDa and 20 kDa isoforms suppress not only their own synthesis, but each other's. Thus administration of just one isoform (presumably the 22 kDa isoform) can drive the other isoforms below their normal range.

This fact has encouraged people who hope to detect illicit growth hormone use by athletes. However, it might also complicate the medical use of growth hormone by promoting unphysiologic ratios of the various growth hormone isoforms, or causing shortages of isoforms that were not administered.

One approach to understanding the roles played by multiple growth hormone isoforms is to look for changes that occur with gender, age or physiological state.

Although women have more growth hormone than do men, the 22:20 ratio is the same in both sexes. Although total amounts of growth hormone change with age, the 22:20 ratio seems not to. Moreover, most stimuli that affect growth hormone production do not change the ratio.

Exercise causes a spike of growth hormone secretion. Following this spike, the proportion of non-22kDa isoforms increases because they persist longer.

Pituitary tumors secrete an oversupply of growth hormone. One unanswered question is whether these tumors ever secrete skewed ratios of growth hormone isoforms. If so, an understanding of the effects of this might improve medical care of acromegaly and at the same time elucidate the role of growth hormone isoforms in normal physiology.

### 6.8.5 The human growth hormone receptor.

The growth hormone receptor can also be exist as one of several possible isoforms. These are derived from a single gene by alternative splicing. In order to transmit a signal, the growth hormone receptor must dimerize after binding growth hormone; in other words, two copies of the growth hormone receptor must physically associate in the membrane of the target cell.

### 6.8.6 The distribution of receptor isoforms.

Tissues differ in their mix of long and short growth hormone receptor isoforms, although both long and short isoforms are present in most or all tissues. Generally, the long isoform is more prevalent than the others. There is more than one type of short isoform, but the functional differences, if any, between them are unknown.

### 6.8.7 The short hGH receptor isoforms may modulate GH sensitivity.

The short hGH receptor isoforms differ from the long isoform in that they appear to be non-functional. Moreover, they can prevent the long isoforms from functioning also. The accepted explanation is that the short isoforms can form dimers with the long isoform, and that these dimers are also inactive.

It has been suggested that a purpose of the short isoforms is to reduce the activity of the long isoforms. Production and destruction of short growth hormone isoforms is one method by which cells could control their own sensitivity to growth hormone. This method of reducing growth hormone sensitivity seems unnecessarily complex, since it would be simpler to reduce the concentration of full-length receptors. However, such apparently unnecessary complexity is common in molecular biology.

An unanswered question is whether reductions in growth hormone sensitivity that occur during ageing or for other reasons might be caused by increases in non-functional growth hormone receptor isoforms in the target tissues. If so, and if the causes could be discovered and controlled, more might be done to stimulate anabolism (tissue construction) when necessary, and perhaps in a tissue-specific fashion.

### 6.8.8 Other examples of growth hormone receptor complexity.

Proteins are often modified by the attachment of sugars or other molecules to them. The attachment of sugars to a protein is called "glycosylation." Growth hormone receptor molecules are extensively and variably glycosylated. The significance of this is under investigation.

### 6.8.9 Growth hormone binding protein.

Growth hormone in the blood is often complexed with a protein called "Growth Hormone Binding Protein" (GHBP). Growth Hormone Binding Protein is coded by the same gene as is the growth hormone receptor; however, it consist of only the extracellular part of the receptor.

Growth Hormone binding protein is present in other mammalian species such as rodents. In rats and mice, the unneeded parts of the GH receptor protein are removed by alternative splicing of the messenger RNA. In rabbits and humans, they are simply chopped off of the growth hormone receptor protein(s).

GHBP prolongs the lifespan of growth hormone in the blood. By analogy with the proteins that bind IGF-I [H6 9], it may also reduce growth hormone's biological activity. In any case, its biological activity is not

obvious.

Defects in GHBP presence or behavior might lead to subtle medical problems that would be hard to explain or even recognize.

One of the short isoforms of the growth hormone receptor protein, which lacks 97.5% of the full-length intracellular domain, is especially prone to the proteolysis that releases GHBP. This short isoform can produce large amounts of GHBP.

Two isoforms of GHBP are now known. One contains a segment of protein called "exon 3" or "E3". The other lacks this segment.

The levels of the two isoforms are not correlated. Women have more of the isoform that lacks E3 than men do.

As the isoform that contains E3 rises in abundance, so do risk factors for cardiovascular disease, such as adiposity, elevated blood pressure and elevated blood triglycerides. Hence, the isoform that contains E3 may exacerbate, ameliorate or at least predict heart disease.

The liver seems to be the major source of GHBP in blood serum. The GHBP levels in female rats exceed those in males, and are even higher in pregnant females.

### 6.8.10 GHRH receptor isoforms.

Growth hormone releasing hormone (GHRH) stimulates the release of growth hormone from the pituitary gland. GHRH has its own receptor in the pituitary. An interesting point is that distinct isoforms of this receptor also exist in experimental animals and probably in humans. These differ in the strength with which they respond to GHRH. It is not known whether they also differ in the pattern of the growth hormone isoforms that they induce.

## 6.9 IGF-I and IGF-II

### 6.9.1 IGF-I and IGF-II are related to insulin

The effects of growth hormone are largely mediated by insulin-like growth factor I (IGF-I) and insulin-like growth factor II (IGF-II). As their names imply, these growth factors are proteins that resemble the insulin protein. The IGF-I, IGF-II and insulin genes are almost certainly descended from a common ancestral gene, although the functions of the proteins they code has diverged.

The protein family contains at least one more member: "relaxin", which relaxes the muscles of pregnant women during childbirth. The beta subunit of 7S nerve growth factor may be a fifth member of this family.

IGF-I is 70 amino acids in length, while IGF-II is 67 amino acids in length.

Like insulin, both are derived from longer proteins that have been trimmed.

### 6.9.2 IGF-I & II gene locations.

The IGF-I and IGF-II genes are each present only once. IGF-II is located on the short arm of chromosome 11, next to the insulin gene. Since most gene duplication events leave the two descendent genes as neighbors where the source gene had existed this juxtaposition may have existed since they originated as separate genes.

The IGF-I gene is located on chromosome 12.

### 6.9.3 IGF-I & II have both general and local activities.

IGF-I and IGF-II follow the typical pattern of the growth hormone axis, in that most of the circulating hormones are made by one organ (the liver), but smaller amounts are made and consumed locally by many tissues.

(When a hormone is made in one organ and exerts its effects within another it is said to have "endocrine" activity. When a hormone exerts its effects near the cells that synthesized it, it is said to have "paracrine" activity. When a hormone exerts its effects on the same cells that made it, it is said to have "autocrine" activity.) IGF-I and IGF-II have endocrine activity and probably either paracrine or autocrine activity.

Although IGF-I and (to some extent) IGF-II are stimulated by growth hormone, the forms that are produced and consumed locally are probably also more subject to local regulation than are the IGF-I and IGF-II produced in the liver.

Although most of the circulating IGF-I is made in the liver, some of it originates elsewhere. When a trick of genetic engineering was used to selectively eliminate IGF-I expression in the livers of mice, while allowing it to continue in other tissues, circulating IGF-I was reduced by 75%.

### 6.9.4 IGF-I is growth hormone's main mediator.

In mammals that are past infancy, IGF-I is the intermediate between growth hormone and most of its effects (see below). However, growth hormone has some effects that are independent of IGF-I. Growth hormone raises blood glucose levels without IGF-I as an intermediate.

Transgenic mice that overexpress IGF-I have increased brain size, while trangenic mice that overexpress growth hormone have increased heart and liver size.

### 6.9.5 The effect of IGF-I on physical performance.

Muscle hypertrophy has been linked to IGF-I.

In one set of experiments, rabbit muscle was induced to undergo rapid size increase (hypertrophy) by experimental stretching. Although only one type of IGF-I was present in resting (unstretched) muscles, two types were expressed in stretched muscles.

It was suggested that this novel IGF-I type controlled the muscle growth in response to stretching. An altered type of IGF-I is also induced in human muscles in response to physical activity.

Studies on IGF-I and human muscle function often involve people with compromised muscle function. One such group is cardiac patients.

A study of cardiac patients concluded that deficiency in IGF-I is associated with disability. Cardiac patients with reduced IGF-I had reduced quadriceps strength (-24%) and reduced quadriceps cross-sectional area (-13%), relative to cardiac patients with high IGF-I.

Another well-studied group of people are the elderly. Reports differ as to whether levels of IGF-I are correlated with muscle function in the elderly. Some studies find no association between IGF-I and measure of muscle strength, physical performance or mobility among the elderly. Other studies find that elevated levels of IGF-I are associated with increased strength, and that declines in IGF-I are associated with decreased mobility.

One revealing measurement showed a correlation between IGF-I levels and walking speed in elderly women (ages 70-79). However, this correlation existed only in women with low levels of IGF-I. Hence, IGF-I may be important in the elderly only when it drops below a threshold of sufficiency.

### 6.9.6 Effects of IGF-I on growth and development.

IGF-I is needed for normal development. A strain of mice was created by genetic engineering that lack the IGF-I gene. Such mice are viable, but at birth they are 35% smaller than normal. They grow slowly and do not attain puberty. Adult mice that lack the IGF-I gene are 1/3 the weight and 2/3 the length of normal mice.

Injection of high doses of growth hormone does not stimulate growth of IGF-I deficient mice, even though it greatly stimulated the growth of normal mice (30% greater body weight and 12% increased length at day 56 after birth.) Growth hormone did stimulate liver growth in IGF-I deficient mice.

On the other hand, one report describes a genetic engineering trick that was used to selectively eliminate IGF-I expression in the livers of mice. Although circulating IGF-I was reduced to only 25% of normal, the mice grew normally.

IGF-I deficiency is present in humans with Laron Syndrome. Such people have defective growth hormone receptors, and thus a deficiency in IGF-I,

although not an absolute absence. Laron Syndrome patients have a number of brain and skull abnormalities, have other symptoms resembling growth hormone deficiency.

Low birthweight in humans is associated with a later risk of type II diabetes (insulin resistance) and heart attack. These are also associated with a variant of the IGF-I gene that presumably alters IGF-I behavior somewhat. Hence, a slight change in the function of the IGF-I gene may cause health problems.

A study of adult women, mostly past menopause showed that both IGF-I and IGF-II are correlated positively to height. IGF-II was also correlated positively with weight.

IGF-I is present in large amounts in the ovary and is needed for development of ovarian follicles. Defects of either the IGF-I gene or one type of IGF-I receptor will prevent follicle development.

### 6.9.7 Effect of IGF-I on adiposity.

Studies of elderly women indicate that IGF-I, either alone or in combination with growth hormone, can promote fat loss. In one study involving obese, postmenopausal women, a combination of diet, exercise, growth hormone and IGF-I for 12 weeks caused an average weight loss of 8.4 kilograms (about 18.5 pounds). The combination of growth hormone and IGF-I was effective in accelerating the effects of diet and exercise.

### 6.9.8 IGF-I and cancer promotion.

Epidemiological studies suggest a link between IGF-I and some cancers. One study concluded that a genetic polymorphism that causes less growth hormone to be secreted, and thus reduces IGF-I levels, also reduces colon cancer.

IGF-I appears to activate an early stage of colon cancer. IGF-I induces tumor growth factor alpha (TGFalpha). The inappropriate expression of TGFalpha in growth arrest stimulates malignant progression in early-stage human colon cancer cells.

Other studies have indicated that IGF-I levels are high in men that are prone to prostate cancer. A study of men with acromegaly, i.e. overproduction of growth hormone, found no increase in prostate cancer, but showed that growth hormone and IGF-I cause prostate overgrowth and other prostate disorders [H5 4 2].

IGF-I has the general effect of opposing cell suicide ("apoptosis"). The ability of the immune system to induce abnormal cells undergo apoptosis is an important defense against both viral infection and cancer. However,

inappropriate apoptosis of healthy tissue can be devastating.

Some cells, such as mouse embryonic fibroblasts, undergo apoptosis when they detach from the extracellular matrix. This may be a defense against the inappropriate mixing of tissues and against the spread of cancer. IGF-I prevents detachment-induced suicide of these cells [**matrix**].

IGF-I, along with IGF-II, also protects developing fat cells (adipocytes) from apoptosis. Clearly, the proper control of IGF-I and IGF-II expression is important.

In AIDS patients, HIV infection causes massive, inappropriate apoptosis of the CD4+ T-helper lymphocyte population. This critically important immune system cell population is the most important target of HIV. Treatment of HIV patients with the biochemical acetyl-L-carnitine increases circulating IGF-I and reduces apoptosis of the CD4+ population [**H3 11**] .

### 6.9.9 The effects of IGF-I on cardiovascular health.

IGF-I has a number of direct and indirect effects on cardiovascular health. Most of these effects appear beneficial.

In mice, IGF-I reduces dilated cardiomyopathy, the pathological expansion of the heart in heart disease. IGF-I normalizes heart mass, shape, blood flow, and calcium signalling. It prevents the apoptosis of heart tissue that occurs in dilated myopathy, and prevents abnormal elongation of the heart cells.

Both IGF-I and IGF-II induce cell expansion and protein synthesis in cultured rat heart cells. These heart cells increased in size by 57% when exposed to optimal concentrations of either IGF-I or IGF-II. Protein synthesis at least doubled in response to either IGF, and several contraction-related proteins were up-regulated.

IGF-I also lowers blood levels of the lipoprotein Lp(a). Its effect is contrary to that of growth hormone, which increases Lp(a). Elevated Lp(a) is a risk factor for coronary artery disease, and the only known Lp(a)-lowering medicines, niacin and neomycin, often have intolerable side effects. Hence, IGF-I might be a useful Lp(a)-lowering medicine.

Experiments on baboons suggest that IGF-I can cause as much as a 30% lowering of Lp(a) levels within just 2 hours, and that the effects last at least one week.

IGF-I also induces vasodilation, by inducing the synthesis of nitric oxide. Nitric oxide inhibits platelet aggregation and monocyte adhesion, and prevents vascular smooth muscle growth. All of these activities reduce obstructions to blood flow; hence, IGF-I is anti-atherosclerotic.

On the other hand, IGF-I may raise blood triglyceride levels in some people. The effects of IGF-I on elderly men and women were tested. IGF-I

raised blood triglycerides in the men. High blood triglycerides are a risk factor for heart disease.

### 6.9.10 Effects of IGF-I on the nervous system.

IGF-I is needed for proper development of the nervous system, in some cases heals the nervous system, and in some cases seems to prevent nervous degeneration.

As mentioned above [Laron], patients with insensitivity to growth hormone (Laron Syndrome) have a number of brain abnormalities.

IGF-I is important to brain recovery from injury. Following brain injury, IGF-I is produced within microglia, a type of brain cell. The IGF-I stimulates nerve growth and prevents neural cell suicide in response to the stress. Degradation products of IGF-I also have neuroprotective properties.

IGF-I also seems to protect against neurodegeneration not associated with an obvious injury. Although elevated growth hormone is associated with a steeper decline in mental performance in ageing men, elevated IGF-I reduces the age-associated mental decline.

A family in Sweden that was predisposed to Alzheimer Disease was studied. Members of this family carried a characteristic mutation in the amyloid precursor protein that is involved in Alzheimer Disease. Some carriers developed Alzheimer Disease and some did not; those that did, had reduced levels of IGF-I.

### 6.9.11 Effect of IGF-I on longevity.

Although medical experiments seem to suggest that high IGF-I levels are mainly beneficial, experiments with mice suggest otherwise.

In one research project, two mouse strains recently derived from wild mice were studied. These mice were smaller than laboratory mice, reached sexual maturity more slowly, lived longer and had less IGF-I.

Direct intervention to manipulate IGF-I levels supports the association between moderate IGF-I levels and a long life span. Dwarf mice, which have a genetic change that reduces their IGF-I levels, live longer than their normal counterparts.

A trick of genetic engineering was used to construct rats with either greatly reduced growth hormone expression, or moderately reduced expression. The life span of the rats with greatly reduced growth hormone expression was 5-10% shorter than the life span of normal rats, mainly due to death from cancer. However, the life span of moderately deficient rats was 7-10% longer than that of normal rats.

### 6.9.12 Other effects of IGF-I on healing.

IGF-I may also aid intestinal healing. Animals studies suggest that IGF-I, stimulated through the growth hormone axis, is important in the reconstitution of intestinal epithelial integrity following injury to the intestinal mucosa.

### 6.9.13 Splice variants of IGF-I.

As mentioned above, there is only one IGF-I gene. However, more than one IGF-I protein is made. The explanation involves "alternative splicing" [discussed above, **H6 8 2**].

Genes are transcribed to create messenger RNA molecules. Each messenger RNA molecule then moves to the ribosomes, where it is translated to create one or more identical protein molecules.

Before the messenger RNA reaches the ribosomes, it is usually edited (spliced) to remove segments, often including internal segments. If an RNA molecule can be edited to create more than one final mRNA, then more than one type of protein may be produced. This is called "alternative splicing."

Alternative splicing allows the body to produce multiple related but individually specialized proteins to perform distinct functions.

Messenger RNA from the IGF-I gene is alternatively spliced. At least four distinct final messenger RNA products have been identified, and new ones are still being discovered.

One class of IGF-I messenger RNA transcripts is made in the liver. This class is stimulated by growth hormone, and is believed to encode the circulating IGF-I. As mentioned above [**H6 9 5**], another splice variant is made in muscle in response to exercise or stretching.

### 6.9.14 The family of IGF-I receptors.

IGF-I and IGF-II stimulate target cells by activating proteins on their cell surfaces. These proteins are termed "receptors." Binding of the hormone to the receptor causes the receptor to transmit a signal into the interior of the target cell. The signals are mediated by intracellular molecules that are termed "second messengers." Generation of these second messengers can have many possible consequences; but typically, they cause gene activation within the target cell. In any case, the target cell generally reacts in some way that benefits the entire organism.

The value of this arrangement is that when it is no longer advantageous for the target cell to react to the hormone, it can make itself insensitive simply by down-regulating expression of the receptor. One event that can trigger such down-regulation is differentiation of one cell type into another.

A number of different receptors for IGF-I are known. The existence of multiple receptors increases the possible complexity of the response to IGF-I. The existence of multiple receptors may also allow drugs to be designed that active only a subset of the receptors, perhaps even only one type of receptor, and thus allow physicians to narrow the effects of an IGF-I mimic or antagonist.

Receptors to members of the insulin protein family often respond to more than one of those members. The biological advantages of this oddity are not clear.

The five known insulin family receptor types are:

-- The IGF-I receptor (IGF-I-R).
-- The IGF-II/mannose-6-phosphate receptor (IGF-II/M-6-P).
-- The insulin receptor.
-- The insulin receptor-related receptor.
-- The hybrid IGF-I/insulin receptor.

There may be subtypes within these basic types.

IGF-I and IGF-II both activate the IGF-I receptor. The IGF-II/mannose-6-phosphate receptor is unusual in that it may lack signalling activity, and instead function mainly by scavenging and removing IGF-II.

Much of the ongoing research on the human growth hormone axis involves unravelling the subtleties of receptor function. As an example, the insulin receptor actually exists as two variants, IR-A and IR-B. Both can form hybrid receptors with IGF-I receptor subunits. However, it was recently discovered that while hybrid receptors that include IR-A can bind IGF-I, IGF-II and insulin with high affinity, hybrid receptors that include IR-B bind only IGF-I with high affinity and do not bind insulin at all. This seems to suggest that a shift in the proportions of IR-A and IR-B that are made could have profound effects on the proliferation and migration of the target cells. However, deducing the details will take years of effort.

Another active area of research involves deducing the link between IGF receptor abnormalities and hitherto unexplainable medical problems. It was recently discovered, for example, that children with retarded intrauterine growth often have an IGF receptor (IGF-R) protein with reduced affinity for IGF-I.

An understanding of this cause of intrauterine growth retardation might allow its remedy by genetic counseling and elective abortion, or by development of IGF-I mimics to effectively bind and activate the altered IGF-I receptor.

### 6.9.15 Regulatory interactions between growth hormone and IGF-I.

Growth hormone stimulates the production of IGF-I, particularly in the liver which produces most of the circulating IGF-I.

People who are deficient in growth hormone or are insensitive to growth hormone have reduced circulating IGF-I levels. Moreover, administration of growth hormone to young, experienced male weight lifters increased their circulating IGF-I.

Growth hormone also stimulates IGF-I in cultured muscle progenitor cells (myoblasts). However, the presence of IGF-I itself prevents this stimulation. Hence, IGF-I limits it own synthesis.

Inducers of growth hormone also indirectly stimulate IGF-I, at least under some circumstances. This has been reported for ghrelin and ghrelin mimics.

Growth hormone does not induce IGF-I under all circumstances. Growth hormone did not raise free IGF-I levels in patients totally dependent on intravenous feeding, for example. Also, there are many reports that IGF-I becomes independent or partly independent of growth hormone in the elderly [H6 9 17]. In addition, newborns secrete high levels of growth hormone, but low levels of IGF-I.

IGF-I not only limits its own synthesis directly (see above), but acts on both the pituitary gland and the hypothalamus, to limit the secretion of growth hormone.

The anabolic effects of growth hormone and IGF-I are often synergistic.

### 6.9.16 IGF-I levels vary with age.

During human fetal development, expression of IGF-I is low, and restricted to a subset of tissues (derived from the "mesenchyme"). During this stage, IGF-II expression is relatively high. After birth, IGF-II activity becomes constant and IGF-I expression becomes much more widespread and extensive.

In healthy humans, IGF-I levels are low at birth, rise during childhood, peak at puberty, and decline thereafter as age increases. IGF-I levels in particular decline after age 40. Even among people older than 70 years, the decline of IGF-I with age continues.

IGF-I levels may not decline in all tissues. Studies on rats showed no decline in IGF-I with age in the kidney and testis.

In women, both growth hormone and circulating IGF-I levels fall after menopause. This may be because estradiol, which stimulates IGF-I, drops after menopause.

### 6.9.17 Growth hormone and IGF-I levels in the very young and in the elderly.

In infants, IGF-I levels are thought to be independent of growth hormone.

Growth hormone stimulates IGF-I in young adults.

A key question is to what degree growth hormone stimulates IGF-I production in older people. If growth hormone fails to raise IGF-I levels in older people, most of the hoped-for benefits of growth hormone in older people will be lost.

There are many reports that growth hormone and IGF-I levels are not well-correlated in adults over age 40. In particular, normal or near-normal IGF-I levels can coexist with growth hormone deficiency.

On the other hand, some reports suggest that traces of a correlation remain. Moreover, most studies suggest that growth hormone can raise IGF-I in old people. In one study, the levels of IGF-I in older humans averaged 53% of the normal level for young adults. When the older humans were treated with human growth hormone, their levels rose to an average 91% of the normal young adult level.

The explanation may be that a baseline level of blood IGF-I persists, even when there is no effective stimulation by growth hormone, and that this is the usual situation in old people. According to this explanation, the ability of growth hormone to stimulate blood IGF-I persists.

### 6.9.18 Effect of exercise on IGF-I in young and elderly.

Exercise does not increase IGF-I levels in elderly people, although it does have this effect in young adults. However, exercise has great benefits in older people, even without an increase in IGF-I.

### 6.9.19 IGF-I and muscle degeneration in the elderly.

Progressive muscle weakness and loss of muscle tissue is an important medical problem in the elderly. Although, growth hormone therapy is sometimes effective in increasing strength, it is unclear why the strength gains are not greater.

One theory is that a decline in locally-produced IGF-I within skeletal muscles causes much of the loss of strength. This local IGF-I is thought not to be greatly influenced by circulating growth hormone or IGF-I [H4 2 10].

### 6.9.20 IGF-I is unlinked from typical effects.

It is not clear that IGF-I increases would have the same benefits in the

elderly that they do in younger people. A recent report from the Framingham Heart Study indicates that in older people (ages 72-94), low levels of IGF-I did not show the expected associations with increased body fat and low lean mass. Nor were they correlated with physical strength (grip strength) or overall health.

These results did not assess interventionist strategies to increase IGF-I. The possible benefits of raising IGF-I, either in the blood or in selected organs, remain unclear.

### 6.9.21 IGF-I effects on the ageing immune system.

Experiments on mice suggest that that IGF-I can bolster the immune systems of aged animals. A 7-day or 14-day course of IGF-I treatment of 9-month-old retired male breeder mice caused great changes to the organs of the immune system. The spleen nearly doubled in size after 7 days of treatment; the number lymphocytes (immune system cells) in the spleen grew by 2.5-fold, and were more prone to proliferate in response to an inducing signal. The thymus gland grew by more than 50% and the number of lymphocytes in the thymus doubled. The number of lymphocytes in lymph nodes also increased. The critically important "helper" T lymphocyte population doubled in size. The basal level of antibody synthesis (at least of the "IgG" class) increased.

Increased activity of immune system organs and cells suggests an increase in function. However a more active immune system is not necessarily a better immune system, and it is unclear whether increasing immune activity with IGF-I in elderly humans would confer benefits. It is even possible that if IGF-I were found to confer other benefits to elderly people, that the immune system stimulation of IGF-I would have to be selectively suppressed [H4 9 2].

### 6.9.22 Side effects of IGF-I in the elderly.

Although low doses of exogenous IGF-I are well-tolerated in the elderly, higher doses can cause unpleasant side effects. These include hypoglycemia, headache, fatigue, joint pain, water retention and (in one case) reversible facial paralysis (Bell's palsy).

One study suggested that elderly women are more sensitive to IGF-I's side effects than are young women.

### 6.9.23 Adverse conditions that reduce the amount or effect of IGF-I.

Some physical conditions reduce the amount of IGF-I present, or reduce the effect that IGF-I has, or both. One such condition is kidney insufficiency.

Children with chronic renal failure, before or after kidney transplant, have abnormalities of IGF-I. In one study, the amount of IGF-I in the blood appeared to be normal, but the biological activity of IGF-I was low. One consequence was abnormally low growth rate.

In other studies of patients with kidney failure, the IGF-I level is at the upper end of the normal range, even though IGF-I biological activity is subnormal. Treatment of such patients with growth hormone raises IGF-I to levels seen in patients with acromegaly, and restores normal IGF-I activity (which resulted, for example, in a gain in lean mass, a loss of fat and an increase in grip strength). In addition, the nutritional status of the patients seems to improve: levels of the important blood protein albumin rise to normal. The treatment is well-tolerated.

The cause of the reduced biological activity of the IGF-I is not known. It might include unnoticed chemical changes to the IGF-I, an increase in blood proteins that can bind and sequester IGF-I (see below), reduced IGF-I receptor numbers or effectiveness in the target tissues, or more than one of these possibilities.

One study suggested that there may be two causes of low IGF-I function in children with kidney failure. The first is insensitivity to growth hormone, so that less IGF-I is produced. The second is insensitivity to IGF-I.

The insensitivity to IGF-I may be due to proteins in the blood that sequester IGF-I and reduce its biological activity. There are at least six blood proteins that strongly bind IGF-I, and probably both protect it and reduce its biological activity [**H6 9 34**]. These are termed the IGFBPs (insulin-like growth factor binding proteins). In children with kidney failure there was an elevation of four of these, IGFBP-1, 2, 3 and 6. IGFBP-6, in particular, was elevated 16-fold.

When the body enters a "catabolic" state, it may become resistant to IGF-I. Although tissue maintenance and growth ("anabolism") are normally desirable, during an emergency the body may desist from them and instead live on accumulated reserves. This breaking down of accumulated reserves, including muscle tissue, is termed "catabolism."

IGF-I promotes anabolism. Under catabolic conditions, its effects are impeded, although exogenous IGF-I can overcome catabolic conditions.

One condition that is strongly catabolic is Acquired Immune Deficiency Syndrome (AIDS). AIDS patients suffer AIDS-associated wasting, which is promoted largely by the opportunistic infections that develop. One study found that AIDS patients who had lost more than 10% of their body weight had a 55% reduction in IGF-I and a 70% reduction in IGF-II compared to healthy HIV-negative subjects. IGF-I levels were depressed, in some

patients, despite high serum levels of growth hormone.

Attempts have been made to overcome AIDS-related wasting by injection of IGF-I. So far the attempts have been unsuccessful. This may be because IGF-I inhibits growth hormone synthesis, which in turn down-regulates the IGF-binding protein IGFBP-3, which is necessary for IGF-I to function. It may be that a combination therapy of growth hormone and IGF-I will be more successful. It might also be beneficial to include IGF-II.

Another catabolic state that reduces synthesis of IGF-I in the liver is arthritis (at least in rats, when it is experimentally induced). This point is important because most elderly humans suffer from arthritis. It might be that ageing, and the relative insensitivity to growth hormone and IGF-I, might result from unexpectedly widespread catabolism.

The relationship between arthritis and IGF-I may be complex. Experimental arthritis damages the kidneys of rats. This raises, rather than lowers, IGF-I in the kidneys. Hence, the relationship between catabolism and tissue-specific IGF-I may not be obvious.

An additional catabolic state that causes wasting of skeletal muscles is chronic heart failure. One study reported that patients with severe chronic heart failure had a normal serum level of IGF-I, but that their skeletal muscle IGF-I was reduced by an average of 52%. The reduction in muscle IGF-I was correlated with a reduction in muscle cross-sectional area.

The chronic heart failure patients with severe muscle wasting showed signs of peripheral growth hormone resistance, as indicated by elevated growth hormone levels and reduced IGF-I levels.

### 6.9.24 IGF-I and exercise.

IGF-I is often stimulated by intense physical exercise, although this effect disappears in older people. Types of exercise differ in the effects that they have on the levels of IGF-I and related proteins.

IGF-I is strongly induced by short periods of intense exercise; however, lengthening of the period of activity reverses this. Long periods of physical activity strongly reduce IGF-I.

The IGF-binding protein IGFBP-1 also responds to exercise duration. As duration increases, the IGFBP-1 level rises.

The likely explanation for this combined response of IGF-I and IGFBP-1 to exercise of long duration is that it protects the body from glucose deprivation. IGF-I is strongly hypoglycemic, promoting glucose uptake by muscles. Although this could be beneficial during a short burst of strenuous activity, it would not be beneficial if allowed to continue. Thus, as exercise time lengthens, IGF-I falls and a protein that sequesters IGF-I (IGFBP-1)

rises.

Growth hormone and IGFBP-3 (another protein that binds IGF-I) also respond to exercise in ways that depend on the intensity and length.

Naturalistic methods of raising growth hormone and IGF-I levels (as opposed to simply giving a patient an injection) are favored by doctors as being safer and less likely to omit something important. However, if naturalistic methods of inducing growth hormone and IGF-I are to be used effectively, the complex effects of age, gender, exercise type and many other factors (see below) will have to be understood and taken into account.

Even though exercise does not alter IGF-I levels in the elderly, it does alter levels of proteins that bind IGF-I. Exercise in healthy older women did not affect IGF-I levels, but caused levels of IGFBP-I to drop by 21% to 47%; the greater drop occurred with more intense exercise. IGFBP-1 levels remained depressed for at least one hour after exercise.

Exercise caused increases in IGFBP-2 and IGFBP-3 in these older women. The increases persisted after the activity.

The significance of this is that exercise might increase the bioactivity of IGF-I in older people, even though it does not increase the total IGF-I amount. This important point remains to be settled.

### 6.9.25 Additional influences on IGF-I levels.

Several factors, in addition to those discussed above, influence IGF-I levels.

One important influence is heredity. Studies with elderly twins have shown that about 60% of the IGF-I differences between people can be explained by heredity.

A second influence is nutrition. Of the variation in IGF-I levels between elderly people that is not attributable to heredity, much is due to differences in nutrition. Poor nutrition is associated with low IGF-I levels in the elderly. Moreover, in younger men, consumption of protein/carbohydrate supplements can increase the response of IGF-I to exercise. Conversely, athletes who diet may unintentionally reduce their levels of IGF-I, even when they are abusing anabolic steroids.

A third influence on IGF-I levels is a person's level of sex steroids. IGF-I levels are lower in postmenopausal women than in premenopausal women. Moreover, there is a direct correlation between sex steroids in women and IGF-I levels, as well as between sex steroids and IGF-II levels.

A possible fourth influence on IGF-I levels is temperature. Baby pigs grow faster when raised at 21 degrees Celsius, as opposed to 32 degrees. However, the expression of growth hormone receptor in the liver, and the

expression of IGF-I and IGF-II by the liver are greater in piglets grown at the higher temperature.

The greater expression of IGF-I and IGF-II in the livers of piglets that are growing more slowly (at the higher temperature) may be a compensatory mechanism. It is the type of complication that could lead unwary investigators to incorrectly report a lack of positive correlation between IGF-I levels and growth rate.

Finally, it has been reported that postmenopausal women who smoke have lower free IGF-I levels than postmenopausal women who do not smoke.

Different research groups sometimes report conflicting results. Many of these discrepancies are probably due to unrecognized influences that were not known and therefore not taken into account when the research was done. These may include the above influences, and perhaps others still unknown.

### 6.9.26 IGF-II also affects human development.

Less research is done on IGF-II than on IGF-I, and less is known about it. Growth hormone stimulates IGF-II production, but perhaps not under all circumstances, and may be less important for IGF-II stimulation than for IGF-I stimulation.

IGF-II seems to be very important to development before birth. After birth, IGF-II levels do not vary much.

Despite this, IGF-II is expressed in children and adults and seems to have an effect. In one study of women, most of whom were past menopause, both height and weight were correlated positively with IGF-II levels.

### 6.9.27 IGF-II in human cancers.

"Autocrine" activity occurs when cells secrete factors that stimulate their own growth. Although autocrine arrangements may seem intuitively to be unnecessarily complex, they are very common. Perhaps their advantage is that they enable masses of cells to grow in a coordinated fashion.

A disadvantage of autocrine arrangements is that overexpression of the growth factor or its receptor can lead to a runaway situation where cells stimulate their own proliferation without limit. This is, or can become, cancer.

IGF-II seems to be an autocrine stimulator of many cancers. This has been suggested for many adrenocortical cancers, at least one glioblastoma, at least one neuroblastoma, and is clearly the case for many lung cancers. It is also the case for Wilms tumor generally.

IGF-II is also expressed in meningiomas, cancers of the tissue that

surrounds the brain. In meningiomas, there is a clear correlation between the level of IGF-II expression and cancer invasiveness.

Cancers that overexpress IGF-II often secrete IGF-II in an abnormal form. The IGF-II proteins secreted by cancers are often much larger than normal IGF-II and are termed "big" IGF-II. Since IGF-II is derived by protein processing from larger precursors, big IGF-II may simply correspond to unprocessed precursors; however, the point has not been settled.

Although IGF-II has autocrine activity in many cancers, the gross oversecretion of IGF-II can also affect tissues far beyond the cancer. (Such effects are termed "paraneoplastic" effects.) Since IGF-II resembles insulin in promoting glucose uptake, one common paraneoplastic effect of tumors that produce IGF-II is profound hypoglycemia. The "big" IGF-II molecules secreted by cancers bind IGFBPs (proteins that bind both IGF-II and IGF-I) poorly. Thus, they are more likely to be free in the blood, and probably have increased biological activity as result.

IGF-II may also have suppressive effects on some cancers. It would not be surprising if some provision exists to prevent the kind of runaway autostimulation by IGF-II described above. In fact, IGF-II binding to the mannose-6-phosphate/IGF-II receptor in cancers that express the receptor seems to restrain cancer growth. This has been shown directly in breast cancer cells and is probably also true in lung cancers. Loss of the mannose-6-phosphate/IGF-II receptor by mutation in developing cancer cells is a significant adverse event.

### 6.9.28 Effects of IGF-II on the heart.

Both IGF-I and IGF-II cause hypertrophy of cultured heart cells. Optimal concentrations of either IGF-I or IGF-II increased the size of cultured rat heart cells by 57% within two days. Both caused protein synthesis to at least double.

### 6.9.29 IGF-II receptors.

As mentioned above, IGF-I and IGF-II both bind the IGF-I receptor. Also as mentioned above the IGF-I receptors forms hybrids with subunits of the insulin receptor. The type of insulin receptor, IR-A or IR-B, determines the specficity of the hybrid receptor for IGF-I, IGF-II and insulin.

Finally, as mentioned above, the mannose-6-phosphate/IGF-II receptor seems to restrict cell growth upon binding of IGF-II.

### 6.9.30 Mutual influence between growth hormone and IGF-II.

Direct analysis of the human IGF-II gene suggests that growth hormone

increases its transcription. Growth hormone has been reported to increase IGF-II levels in elderly patients with growth hormone deficiency, in children with short stature of unknown cause and in postmenopausal women. Acromegalics (people with an excess of growth hormone) have also been reported to have elevated serum IGF-II levels.

Human granulosa cells (which form the wall of the ovarian follicle) secrete IGF-II when provoked with growth hormone. It is also true that IGF-II can largely substitute for growth hormone in rats in which the pituitary gland has been removed. IGF-II duplicates growth hormone's effects on growth, cartilage development and nitrogen retention.

Despite all of this, the stimulation of IGF-II by growth hormone is relatively weak, and does not occur in all tissues (e.g. bone tissue of acromegalics has less IGF-II than normal).

It has been reported that, unlike IGF-I, IGF-II does not inhibit growth hormone production.

### 6.9.31 IGF-II expression throughout the life cycle.

IGF-II is expressed before birth at very high levels in many tissues. Gene targetting experiments on mice have shown that animals without IGF-II are markedly growth-deficient at birth, although they grow at a rate comparable to normal animals after birth.

In rats, IGF-II declines after birth in most tissues. In humans, IGF-II expression continues at a constant level, but its function is largely unknown. During late adulthood in humans, IGF-II declines along with IGF-I.

### 6.9.32 IGF-II and catabolic states.

What little we know about IGF-II suggests that the same influences that reduce IGF-I amounts or function have the similar effects on IGF-II. In patients with kidney insufficiency, IGF-II levels are elevated (as IGF-I levels sometimes are), suggesting a reduction in IGF-II effectiveness (as occurs with IGF-I).

AIDS-associated wasting reduces IGF-II levels, even more than it reduces IGF-I levels. In one study, AIDS patients who had lost more than 10% of their body weight had a 55% reduction in IGF-I and a 70% reduction in IGF-II compared to healthy HIV-negative subjects.

### 6.9.33 Additional influences on IGF-II levels.

As befits a hormone that is present in large amounts prenatally, IGF-II is induced by placental lactogen. Placental lactogen is a close relative of growth hormone that is secreted by the placenta.

As mentioned above, experiments with piglets show that although elevated temperature reduces growth, it increases IGF-I and IGF-II [IGF heat].

IGF-II levels decline in post-menopausal women. IGF-II levels in post-menopausal women are correlated with levels of estradiol and other sex steroids, suggesting that sex steroids boost IGF-II levels.

### 6.9.34 IGF binding proteins (IGFBPs)

The activities of IGF-I and IGF-II are modulated by IGF binding proteins (IGFBPs). These bind IGF-I and IGF-II with high affinity. Six IGFBPs are known. They share 40-60% sequence identity overall, and hence show clear evidence of descent from a common ancestor protein.

The IGFBPs modulate the activity of IGF-I and IGF-II. They seem to both protect the IGFs from degradation and sequester them so that they have less biological activity. They may alter the circumstance under which the IGFs can act, and may also have biological activities of their own. IGFBPs have been suggested to control IGF transport, efflux from vascular compartments and association with cell surface receptors.

The IGFBPs vary in both the tissue and stage of development that they are synthesized in. IGFBP-2, for example predominates in fetal serum. The main circulating IGFBPs in adult life are IGFBP-1 and IGFBP-3.

IGFBP-1 is the only IGFBP whose levels change markedly (5-fold) with fasting and feeding. It seems mainly to inhibit IGF-I activity.

IGFBP-3 is the main IGFBP in adult serum. It is induced by growth hormone. It binds most of the circulating IGF-I as part of a complex that includes another protein, the "acid-labile subunit."

Both IGF-I and IGF-II bind these IGFBPs, and can compete for them. Large doses of exogenous IGF-I can drastically lower the blood concentration of IGF-II. This is presumably because IGF-I displaces IGF-II from circulating IGFBPs, and thus exposes the IGF-II to degradation.

Reduction of one IGF by the other is a complication that could frustrate medical use of IGF-I or IGF-II. Indeed, it is possible that successful medical use of IGF-I or IGF-II will require control over IGFBPs.

When IGFBP function is better understood, it may become possible to design artificial IGFBPs that enhance desired IGF activities while blocking undesired activities. As with most protein-based medicines, some strategy would have to be devised to circumvent the immune system.

### 6.9.35 IGFBP-1

IGFBP-1 sequesters IGF-I and inhibits its activity. IGFBP-1 levels rise during catabolic states. IGFBP-1 levels are usually elevated in patients who

are admitted to the intensive care wards of hospitals, but may fall in response to nutritional support.

IGFBP-I levels are high in people with kidney insufficiency; in children, this rise in IGFBP-1 is associated with reduced height. Growth hormone treatment reduces IGFBP-1 levels in people with kidney failure by at least half; this may explain its anabolic effects in those patients.

Patients totally dependent on intravenous feeding often lose nitrogen. Recovery of the ability to retain nitrogen occurs only in patients who decrease their IGFBP-I and increase their free IGF-I.

AIDS patients suffering from AIDS-related wasting have elevated levels of IGFBP-I. In one study, AIDS patients who had lost more than 10% of their ideal body mass had serum IGFBP-I levels that were increased 3.75-fold relative to normal subjects.

Exercise also affects IGFBP-1. A study of healthy older women showed that exercise increased growth hormone levels by 1.3- to 2.6-fold. This had no effect on circulating IGF-I levels, but caused a drop of between 21%-41% (depending on the intensity of the exercise) in IGFBP-1 levels. The drop persisted for at least one hour. Thus, exercise may increase total free IGF-I in the elderly, and thus stimulate IGF-I's biological effects.

Older (50-78 yr) men who are frequent marathon runners have greatly increased levels of IGFBP-I, although their total IGF-I and free IGF-I levels are normal. Thus one suspects that being in excellent physical condition, and being capable of very long periods of strenuous aerobic exercise might depend in some way on elevated IGFBP-1 levels. (The purpose might be to prevent excessive muscle hypertrophy, since great muscle hypertrophy would be a mixed blessing at best to a marathon runner or someone with a similar pattern of exercise).

Reduced IGFBP-1 levels are associated with thickening of the carotid artery wall in type II diabetics.

Although circulating IGF-I levels are not pulsatile, as growth hormone is, IGFBP-1 levels show a diurnal rhythm. Thus, IGFBP-I levels rise at night and free IGF-I levels fall.

### 6.9.36 IGFBP-2

IGFBP-2 can reduce the activity of IGF-I. While IGF-I overexpression in mice increases their size, concurrent overexpression of IGFBP-2 partly reverses this. Overexpression of IGFBP-2 in mice with normal levels of IGF-I reduces their size also.

IGFBP-2 increases during critical illness, and is elevated in children with chronic kidney failure. In such children, the concentration of IGFBP-2 in the

blood correlates negatively with height.

IGFBP-2 is not induced by growth hormone, but in one group of patients totally dependent on intravenous feeding, growth hormone reduced its concentration.

In elderly men (50-78 yr) who habitually ran marathons, IGFBP-2 levels were elevated by 45% over the average. In healthy older women, intense exercise increased IGFBP-2 levels by about 30%.

It has been argued that IGFBP-2 may not inhibit IGF-II.

Mimics of IGF-I are sometimes more potent than IGF-I itself. One set of mimics had the same amino acid sequence as IGF-I, except that the glutamine residue at position 3 was replaced by glycine or arginine. These were more potent than IGF-I. Both mimic bound very poorly to IGFBP-2 but almost as well as IGF-I to the IGF-I receptor. It was proposed that reduced binding to IGFBPs caused the increased biological activity of these mimics.

## 6.9.37 IGFBP-3

### 6.9.37.1 IGFBP-3 is stimulated by growth hormone

IGFBP-3 is the main IGFBP present in the serum after birth. IGFBP-3 is considered to be growth hormone-dependent. In people with growth hormone deficiency or growth hormone insensitivity (a defect in the growth hormone receptor protein), IGFBP-3 levels are abnormally low. Growth hormone elevates IGFBP-3 levels in liver, muscle and skin.

Many studies have concluded that growth hormone increases IGFBP-3 levels under a variety of circumstances. This includes normal young adulthood, kidney failure, other illness and ageing. However, it is occasionally reported that growth hormone has no effect.

By contrast, IGF-I increases IGFBP-3 levels in serum, but its effect on IGFBP-3 production by individual organs is complex, and not always stimulatory.

In children with chronic kidney failure, growth hormone increases the levels of IGF-I, IGFBP-3 and the acid-labile subunit. These three components are part of the "ternary complex" (see below) that IGFBP-3 functions in. The increase in "catch-up" growth caused by growth hormone in these children with short stature correlates with the rise in each of the components of the ternary complex.

It is not clear whether IGFBP-3 facilitates or inhibits IGF-I. The reason is that while growth hormone elevates both IGF-I and IGFBP-3, the elevation of IGF-I is usually greater. Hence, while it might be that the complex that includes IGF-I and IGFBP-3 is the active species, it could be that free IGF-I is the active species.

#### 6.9.37.2 IGFBP-3 forms a ternary complex.

IGF-I, IGFBP-3 and another protein called the "acid-labile subunit" (ALS) combine to form a ternary complex. Most of the IGF-I that circulates in the blood is present in IGF-I/IGFBP-3/ALS complexes. Apparently, IGF-II can replace IGF-I in such a ternary complex.

Conditions that cause growth hormone resistance usually reduce levels of the acid-labile subunit (ALS). Diabetes, burn injury and AIDS-related wasting all do this. Since IGF-I mediates most of growth hormone's effects, if the IGF-I/IGFBP-3/ALS ternary complex is necessary for IGF-I function, this drop in ALS might cause what appears to be resistance to growth hormone. If so, the key to reversing growth hormone resistance might be to restore ALS levels.

In the case of diabetes and burn injury, insulin can partly restore ALS levels. In the case of AIDS-related wasting, the IGFBP-3 ternary complex can only be restored by addition of purified IGFBP-3 and ALS to the patients' serum.

#### 6.9.37.3 Glycosylation and proteolysis of IGFBP-3

IGFBP-3 exists in more than one form. It can be glycosylated or not glycosylated. It is subject to proteolysis, and one of the breakdown products, the 29-kDa fragment, may also have biological activity.

Proteolysis (breakdown) of IGFBP-3 seems to be an important regulator of its concentration. In experimental arthritis in rats IGFBP-3 proteolysis is abnormally low; in human Turner Syndrome, it is abnormally high. In both cases, hormone therapies to treat the condition restore a normal rate of IGFBP-3 breakdown.

#### 6.9.37.4 IGFBP-3 changes with age and medical conditions.

IGFBP-3 levels change with age. Before 10 months, IGFBP-3 levels are very low. They increase at 10 months, coincident with the onset of childhood growth.

IGFBP-3 declines during late adulthood, but the decline may not be as rapid as the decline in IGF-I. Hence, the IGF-I/IGFBP-3 ratio may also decline.

Several medical conditions have been reported to affect IGFBP-3 levels. Both arthritis and kidney insufficiency have been reported to elevate the levels. AIDS-related wasting lowers IGFBP-3 levels, and Turner Syndrome increases IGFBP-3 breakdown.

A high proportion of children with short stature of unexplained cause have

elevated levels of IGFBP-3. This could sequester IGF-I.

#### 6.9.37.5 Other influences on IGFBP-3.

Gender seems not to influence IGFBP-3 levels. In healthy older women, intense exercise increased IGFBP-3 levels by almost half (48%). One study has indicated that while illicit supplementation with growth hormone in young power lifters increased IGFBP-3 levels, that anabolic steroid use reversed this.

#### 6.9.37.6 IGFBP-3 effects.

The demonstrated effects of IGFBP-3 are to oppose IGF-I activity. IGFBP-3 blocks the hypertrophic effects of IGF-I on cultured rat heart cells. It also blocks the stimulatory effects of IGF-I on a lung cancer line, both in culture and when the cancer is grown in mice. There is also suggestive (but not conclusive) evidence that a 6-fold overproduction of IGFBP-3 may be the cause of Seckel Syndrome, a human syndrome characterized by very short stature.

### 6.9.38 IGFBP-4.

IGFBP-4 increases during critical illness, and is high in children with kidney failure. Its level is normally considered to be growth hormone-independent, but treatment of children who had chronic kidney failure with growth hormone for one year increased their IGFBP-4 levels by an average 26%.

IGFBP-4 levels gradually decline in older adults.

Direct evidence from transgenic mice suggests that IGFBP-4 opposes the effects of IGF-I. IGFBP-4 is normally degraded by proteases. An engineered protease-resistant variant, that binds IGF-I as well as does wild type IGFBP-4, was inserted into the genetic material of a strain of mice.

The inserted gene was expressed in aorta, bladder, stomach and intestinal smooth muscle. All of these organs were smaller or lighter than normal. Thus, elevated IGFBP-4 levels opposed IGF-I action in these organs.

### 6.9.39 IGFBP-5

#### 6.9.39.1 IGFBP-5 is most conserved.

Of the six known IGFBPs, IGFBP-5 is the most well-conserved between species. This suggests that it is part of processes that are relatively unchangeable by mutation over evolutionary time. Such processes are likely to be indispensable and complex. Evolutionary change is greatly slowed when multiple components of a biological system must change in unison for

the system to function well.

#### 6.9.39.2 IGFBP-5 is GH dependent, and complexes with ALS.

IGFBP-5 resembles IGFBP-3 in that it is stimulated by growth hormone, and forms complexes with IGF-I or IGF-II and the acid-labile subunit. Twelve months treatment with growth hormone of children with chronic renal failure increased their IGFBP-5 levels an average of 49%.

#### 6.9.39.3 Correlations between IGFBP-5 and other components.

In children with kidney failure, that were treated with human growth hormone, IGFBP-5 levels were significantly correlated with the growth rate. IGFBP-5 levels also were significantly correlated with levels of IGF-I, IGF-II, IGFBP-3 and the acid-labile subunit. It was argued that IGFBP-5 may function in the acceleration of growth by growth hormone treatment in children with kidney failure.

#### 6.9.39.4 IGFBP-5 regulates bone, kidney, breast tissue.

IGFBP-5 regulates physiological process in bone kidney and mammary gland. IGFBP-5 is thought to have actions that are independent of IGF-I, and is located in the nuclei of cells in some tissues.

#### 6.9.39.5 IGFBP-5 and cancer.

IGFBP-5 is very important to the control of some cancer types. One of these is meningiomas, tumors of the tissues that surround the brain (meninges). IGFBP-5 levels were highest in the most benign meningiomas. Since IGF-II activity appears well-correlated with invasiveness in meningiomas, IGFBP-5 may limit the activity of IGF-II.

### 6.9.40 IGFBP-6.

Children with kidney insufficiency have symptoms of inadequate IGF-I levels, but total IGF-I levels are actually high. This has prompted suggestions that kidney failure may cause sequestration of IGF-I, perhaps by IGFBPs.

IGFBP-6 is elevated 16-fold in children with kidney failure. Hence, IGFBP-6 may sequester IGF-I in this disease.

IGFBP-6 is also elevated in critical illness, similar to other IGFBPs. However, IGFBP-6 is unusual in that it may promote rather than inhibit some cancers. A study of meningiomas, tumors of the tissues that surround the brain, showed that IGFBP-6 levels were highest in the group that aggressively invaded the brain.

### 6.9.41 The likely target proteins of IGF-I.

Beyond the six IGFBPs that bind IGF-I with high affinity, additional proteins bind it with low affinity. These proteins are probably not modulators of IGF-I activity, but instead are probably the target proteins on which IGF-I acts. Examples include connective tissue growth factor, and several other proteins.

## 6.10 Leptin.

One additional hormone, leptin, is discussed here. Leptin is not a primary control protein of growth hormone, as are GHRH, ghrelin and somatostatin. Nor is leptin an effector of growth hormone, as are IGF-I and IGF-II. Instead, leptin is a critical hormone in the control of adiposity, and interacts with members of the growth hormone axis. Its importance to this discussion springs from its importance to one of the main goals of growth hormone therapy: to control adiposity.

Leptin is a protein that is secreted mainly by white fat cells, although small amounts are produced by the anterior pituitary gland. It acts on the hypothalamus to reduce appetite, and to reduce fat by other mechanisms, such as promoting the burning of fat to create heat. Thus, it is a mechanism that fat tissue uses to limit its own growth.

Leptin has multiple effects. It appears to stimulate bone growth, for example, both by stimulating osteoblast proliferation and reducing osteoblast apoptosis. It also stimulates collagen synthesis and bone mineralization.

### 6.10.1 Leptin acts on the hypothalamus.

Leptin acts on that part of the brain known as the hypothalamus. It communicates the body's nutritional status to hypothalamic areas that govern appetite, energy expenditure and body weight.

The leptin receptor has at least six isoforms. The largest form of the receptor is highly expressed in the hypothalamus. Shorter forms with truncated intracellular regions are also present.

Leptin inhibits appetite by reducing activity in neurons that contain the neurotransmitters "neuropeptide Y", "agouti-related peptide" and/or pro-opiomelanocortin. Many of these neurons are located in the arcuate nucleus of the hypothalamus.

Leptin's action on the hypothalamus is the basis of a genetic therapy to promote weight loss. The therapy succeeds in rats, but has not been tested on humans.

The therapy involves injection into the brain of a virus that has been

modified to express the leptin protein. Expression of the leptin protein in or near the hypothalamus suppressed postpubertal weight gain in rats by suppressing fat accumulation. Reports disagree as to whether appetite is also suppressed.

There are several mechanisms that the body might use to reduce fat accumulation without reducing appetite. One such mechanism that is activated by the genetic leptin therapy is non-shivering thermogenesis. This is accomplished by increased expression of "uncoupling protein" in brown fat. Uncoupling protein causes fat to be consumed to create heat. (Thermogenesis by shivering is another possibility, but it is not stimulated by leptin.)

Production of leptin near the hypothalamus could avoid the need to raise the level of leptin generally throughout the body. Leptin has bad effects as well as good ones. One such bad effect is to impair vascular function, perhaps by reducing arterial distensibility.

### 6.10.2 Adipose tissue affects growth hormone via leptin.

Adipose tissue affects growth hormone secretion by at least two signals: free fatty acids and leptins. Increases in adipose tissue increase free fatty acids in the blood, which strongly reduce growth hormone secretion. Leptin has complex effects on growth hormone secretion (see below).

### 6.10.3 Connections between leptin and the GH axis.

Over the long term (months or years), increases in growth hormone reduce leptin levels, while reductions in growth hormone increase leptin levels. Thus, acromegalics have little leptin, while people with growth hormone deficiency have much.

Growth hormone decreases body fat; body fat increases leptin. Hence, the long-term effect of growth hormone on leptin is attributed largely to changes in fat. Some researchers believe that this completely explains the long-term effect of growth hormone on leptin, while others suspect additional connections.

Exploration of the relationship between growth hormone and leptin has focussed on short term interactions. In some cases, humans or animals are injected with growth hormone or leptin and the results are assessed within hours or days, before significant changes in body fat can occur. In other cases, tissues such as fat or pituitary gland are removed from experimental animals, disaggregated into cells, and cultured; the effects of growth hormone or leptin on these isolated tissues are then assessed.

Increases in leptin almost always increase growth hormone. Decreases in

leptin decrease growth hormone. Leptin may act by stimulating the production of nitric oxide, which in turn stimulates the release of growth hormone.

The short-term effect of growth hormone on leptin is less clear. Reports that growth hormone increases, decreases or does not change leptin levels appear with similar frequencies. Part of the explanation may be that there is a "rebound" effect: growth hormone treatment reportedly causes an initial rise in leptin concentration (measured at 24 hours after treatment), followed by a drop that can last a week or more.

Leptin levels have been linked to levels of blood IGF-I, fat tissue-specific IGF-I, and the IGF-binding proteins IGFBP-1, and IGFBP-3. However, there is no consistent pattern.

Leptin has also been reported to modify the behavior of the growth hormone secretagogues GHRH and ghrelin. However, in this case also, there is no consistent pattern. Leptin and somatostatin appear to suppress each other.

There is a strong positive correlation between leptin and the growth hormone binding protein GHBP, which circulates in the blood. Both rise as the amount of fat rises, but it has been suggested that the influence between them may be direct, rather than through fat. [**H6 8 9**]

In addition to the hormones that interact with leptin, there are environmental influences. Smoking, for example, reportedly raises leptin levels in men.

### 6.10.4 Influences on leptin.

Since adipose cells secrete leptin, fat accumulation increases leptin levels. On the other hand, fasting reduces leptin secretion, even in people who are overweight.

Women (and females of other species such as mice) generally have more leptin than their male counterparts. Typically, women have about twice as much leptin as men. Researchers disagree as to whether this can be explained by the presence of more fat in women.

An interesting medical problem is the relationship between the modern diet of the western world and efforts to control weight, with or without use of growth hormone. The modern diet increasingly consists of fast food and soft drinks; the fast food is rich in fat, and the soft drinks are rich in fructose.

Leptin is central to any effort to control obesity. Expression of the leptin gene and leptin secretion are increased by insulin-stimulated glucose use. Although they add many calories, dietary fat and fructose reportedly do not

stimulate insulin secretion and leptin production. Thus (it has been argued), the modern diet may promote overeating and obesity by its content, as well as by its flavor and its marketing. If further research confirms this argument, it could lead to changes in recommended diets or even to regulations altering the production of fast food and soft drinks.

Ghrelin, the stimulator of growth hormone secretion that is made by the stomach, opposes leptin. Leptin-responsive neurons in the hypothalamus respond to ghrelin. Whereas leptin reduces electrical activity in these neurons, ghrelin stimulates it. It is thought that leptin is reducing hunger while ghrelin is stimulating it, in these experiments.

### 6.11 Unresolved molecular biological questions.

An enormous amount of work remains to be done, so that the full medical benefits of growth hormone axis manipulation can be realized. Four molecular biological puzzles are of particular interest.

#### 6.11.1 The puzzle of growth hormone resistance.

Some conditions cause the body to resist the effects of growth hormone. The blood level of IGF-I drops, due either to decreased IGF-I production or increased IGF-I removal from the blood, or both. Because there is less IGF-I to inhibit the production of growth hormone, growth hormone concentration rises.

Conditions that cause growth hormone resistance include critical illness, HIV infection (even without opportunistic infection or liver damage), sepsis, trauma, burns, cancer, and renal or liver failure.

As mentioned above [H6 9 37 2], a decline in acid-labile subunit is implicated in growth hormone resistance. However, the problem is far from solved.

Treatment to elevate IGF-I levels can include administration of growth hormone or IGF-I itself. However, growth hormone treatment of critically ill patients increases mortality, perhaps because growth hormone elevates blood glucose and thus promotes opportunistic infections.

An understanding of how growth hormone resistance develops might lead to more effective methods of countering it.

#### 6.11.2 The puzzle of growth without growth hormone.

In some cases, patients with a deficit of growth hormone grow to normal or above-normal heights. This condition is called "growth hormone independence." A study of one such patient showed that other stigmata of

growth hormone deficiency were present: delayed bone maturation and increased adiposity with central fat deposition.

Normal growth and bone cell proliferation in growth hormone independence are not mediated by growth hormone, insulin, leptin or IGF-II, and probably not by IGF-I. The involvement of an unknown growth factor or mechanism has been suggested.

Growth without growth hormone is typically seen in children recovering from brain tumors that have arisen near the pituitary gland. If it could be understood, it might be used in the treatment of childhood renal failure, Turner Syndrome and other conditions where the single most important concern is greatly reduced final adult stature.

### 6.11.3 The question of prenatal growth hormone disorders.

One unanswered question is to what extent axis abnormalities cause birth defects or poor health in infancy. Growth hormone receptors appear prenatally as early as 8.5 weeks in liver, kidney, skeletal muscle and vascular tissue, and appear later in the small and large intestines, hair follicles, sebaceous glands and skin. Moreover, ghrelin is also expressed prenatally in pancreas, gut and lung. Hence, axis disorders may present many opportunities for medical trouble.

### 6.11.4 Exploration of unusual contexts.

The most desirable expression pattern of axis hormones differs between patients. Understanding how the best expression pattern changes with the context is a major challenge. In particular, doctors need to know what is most desirable in patients who are not normal and not likely to become so.

One way to explore how the axis hormones can act harmoniously under unusual circumstances is to examine groups of healthy people whose axis expression and physical makeup are very unusual. For this reason, the axis behavior of three groups of pygmies has been analyzed.

Pygmies are aboriginal peoples who are very short. Attention has been focussed on three groups of pygmies: a group in Africa, a group in Papua/New Guinea and a group in the Philippines. The pygmies of Africa are unrelated to the other two groups.

Studies of pygmies from all three areas show decreased levels of Growth Hormone Binding Protein. Further study of the Philippine Negrito pygmy population showed reduced IGF-I, IGFBP-3 and acid labile subunit, but increased IGFBP-2. (IGFBP-2 binds IGF-I and reduces its activity.) There was no change in the levels of growth hormone or IGF-II.

Further study of these pygmy populations may reveal how large changes in

axis expression can occur with a healthy result.

# 7. PROSPECTS FOR THE FUTURE.

Research is underway to improve our ability to manipulate the growth hormone axis. The goals are to increase effectiveness, and to reduce unwanted side effects, inconvenience and cost.

## 7.1 Improvements in growth hormone administration.

### 7.1.1 Carrier matrixes.

Several new methods to continuously administer growth hormone or related agents are under development. The goal is to reduce the number of injections that a patient must take. The strategy is to inject growth hormone or a related agent subcutaneously, in a form where it will be released continuously and predictably over a period of weeks, months or longer. The active agent must be confined by some carrier substance that will slowly release it; usually, the carrier itself slowly disintegrates.

Carbonate hydroxyapatite gels, hyaluronic acid gels and other gels have been tested, and can be relied on to release somatostatin mimics for at least 28 days. Cross-linked high-amylose starch and poly(lactic-co-glycolic acid) microspheres are also being considered.

### 7.1.2 The advantages of secretagogues.

Growth hormone itself has several disadvantages as a medical agent. It is very expensive ($10,000-$15,000 per year), difficult to store, and must be injected. Injection is unlikely to mimic either the natural pulsatility of growth hormone nor the natural array of growth hormone isoforms, and may expose patients to supraphysiological growth hormone levels and their attendant side effects. Continuous infusion of growth hormone can reduce the need to inject supraphysiological amounts of growth hormone, but disrupts growth hormone pulsatility even more than single injections do.

These disadvantages might be overcome by induction of a patient's own growth hormone using GHRH-related or ghrelin-related secretagogues. Secretagogues are chemically simpler, less expensive to manufacture and store, and can be given orally or nasally. They are also more compatible with the slow-release technologies discussed above. Secretagogues induce bursts of growth hormone that are naturally pulsatile, which are much less likely to reach supraphysiological concentrations, and which presumably mimic the normal spectrum of growth hormone isoforms (although this has not always been checked).

### 7.2 Improved axis hormone mimics.

Growth hormone, its regulators (GHRH, ghrelin and somatostatin) and its effectors (IGF-I, IGF-II) are all proteins. Although the proteins themselves can be used as medical agents, mimics may be more advantageous. Mimics can be more powerful (or less powerful, if the aim is block rather than enhance activity), more stable during storage, easier to administer and longer-lived in the body.

Analogs of GHRH have been developed that are between 40 and 220 times as powerful. Powerful antagonists of GHRH have also been developed: these antagonists occupy GHRH receptors, but do not activate them, and prevent GHRH from doing so. Such antagonists inhibit several important cancers including: bone, lung, prostate and kidney cancers.

Mimics are of three basic types: novel proteins with a sequence related to the protein being mimicked, chemically modified proteins, and non-proteins. Proteins and chemically modified proteins are typically more powerful, but harder to deliver and more variable in their biological effects. Non-protein mimics are less powerful, but can often be given orally and are more predictably taken up. In addition, non-protein mimics are easier to engineer in ways that will modify their activity.

Research is underway to exploit growth hormone and other hormones of the growth hormone axis, and their mimics, for medical and veterinary purposes. Although growth hormone from domesticated animals is inactive in humans, other mammals share the main features of the growth hormone axis with us. Thus, research on the growth hormone axis is potentially influenced by industrial desires for better meat, milk, leather and wool production.

The relevance of growth hormone research to industrial animal husbandry will probably have several consequences. It is likely to increase private financial support for growth hormone research, and thus may provide medical benefits to the public without public expenditure. It is likely to stimulate government-funded research. It is intended to, and may well, increase the productivity of agribusiness and thus of Western economies generally.

It may also skew research toward goals that benefit powerful interests more than society generally, and might delay the publication of important results for commercial reasons. Since profit rather than animal welfare is the main point of industrial veterinary medicine, it may become a powerful new source of animal suffering. Finally, since chemical mimics of hormones of the growth hormone axis could remain in the meat or milk of animals that consume them, it has the potential to harm public health.

## 7.3 The benefits of continued research.

Continuing research offers the best hope of manipulating the growth hormone axis to produce medical benefits. Such research will allow enhancement of the known benefits, a reduction in the unwanted side effects, and discovery of new possible benefits.

One special desire is that growth hormone will be transformed from a marginally helpful curiosity, as far as the healthy aged are concerned, to a set of potent medicines that can block the ravages of ageing.

### 7.3.1 The discovery of treatment conflicts.

One benefit of continuing research is the discovery of unexpected conflicts between promising therapies. As an example, [see D] growth hormone promotes bone mineralization in people who are growth hormone-deficient. Since secretagogues have many advantages over growth hormone itself, it makes sense to use secretagogue therapy to increase expression of the growth hormone gene, instead of growth hormone therapy. Since vitamin D also promotes bone mineralization, it would seem to make sense to combine vitamin D with growth hormone secretagogues. However, recent research has shown that vitamin D inhibits expression of the growth hormone gene.

Several strategies might be used to circumvent this conflict. Perhaps some analog of vitamin D could be found that stimulates bone growth, but does not inhibit growth hormone secretion (see below). Perhaps some means of restricting vitamin D to skeletal tissue might be found (see below). Perhaps growth hormone itself must be given to patients when vitamin D is also given.

Unexpected conflicts of this sort may explain some of the failures of growth hormone to do more to reverse the symptoms of ageing. Continued research is the only reliable method of discovering and circumventing such conflicts.

### 7.3.2 The engineering of hormone mimics.

A major goal of modern pharmacology is to devise drugs that mimic natural compounds, but improve upon them in some way. The drug may be more potent, or longer lived in the body, or may induce only a subset of the natural compound's effects. Alternatively, they may have no effects of their own, but may prevent the natural compound from acting by occupying the receptors it would bind.

A ghrelin mimic has been created that is more stable in the body than ghrelin is. The third amino acid of ghrelin is linked to n-octanoic acid; this

modification is necessary for activity. The ester bond that connects the octanoic acid residue to the ghrelin peptide chain can be replaced with more stable chemical bonds without compromising ghrelin's biological activity.

IGF-I mimics have been devised that have increased biological activity. In these mimics, the third amino acid is changed from glutamic acid to glycine or arginine. These changes greatly reduce binding to IGFBP-2. Perhaps the reduced IGFBP-binding activity of the modified IGF-I causes its increased activity.

An analog of growth hormone called "pegvisomant" is effective in treating acromegaly. Pegvisomant is a genetically engineered mimic of growth hormone that binds the growth hormone receptor, but prevents its dimerization. This prevents the receptor from transmitting a signal into its parent cell. Pegvisomant treatment normalizes IGF-I levels in patients with acromegaly.

The six IGF binding proteins (IGFBPs) are good potential targets for engineering, once their functions are understood in detail. They sequester and protect IGF-I and (probably) IGF-II. However, they probably also modify IGF activities in ways that could be productively mimicked.

A barrier to molecular engineering of the type discussed above is the immune system. Large complex proteins and other macromolecules usually evoke an immune response when they are foreign. Very probably many of the hormone mimics discussed above would induce an immune response that would neutralize their activities and could perhaps harm the patient.

There has been much progress recently in understanding how parasitic microbes evade the immune systems of humans and other mammals. This progress has stimulated hope that some of the parasites' methods could be adapted to protect complex medicines.

There are many tricks that parasites use to evade the immune system. However, one of the simplest and most promising is that parasites can repeatedly change the antigenic characteristics of proteins while maintaining those proteins' functions. Hence, it might be possible to design a set of similar complex medicines, each having the same function, but differing antigenically. As immunity developed to one member of the set, a new one could be substituted. (For more on this subject, see the e-article on xenobiotherapy at www.xenobiotherapeutics.com). [See below **H7 3 14**].

### 7.3.3 Improved strategies for drug discovery.

New methods are available to create and check sequence variants of a given protein for improved receptor-binding characteristics. One such

method, the screening of "bacteriophage display libraries" allows millions or even billions of different variants to be tested. Billions of bacteriophage, many bearing different variants of the protein in question, are screened for binding that is more powerful, less sensitive to interference or more selective.

### 7.3.4 Targetted delivery of drugs to tissues.

Members of the growth hormone axis, especially growth hormone itself and IGF-I, can have adverse side effects when administered in high doses. Yet high doses may be needed to obtain maximum therapeutic benefit. One possible solution is to concentrate the growth hormone or IGF-I in the tissue or tissues where it is needed, while reducing its concentration overall.

One method to do this employs liposomes, small hollow spheres that can contain drugs, and release those drugs when taken up by a cell. Experiments with scalded rats showed that small doses of IGF-I, delivered in the form of liposomes and administered locally, are as effective in promoting burn healing as are higher doses of growth hormone plus IGF-I.

A second method to concentrate drugs within a given area is to alter them so that they have a greatly shortened life span, and then administer them locally. As the drugs drift away from their target tissue, their concentration will fall. Some long-term method of releasing the drugs at the desired site is needed. As mentioned above [H7 1 1], experiments with subcutaneous implants of matrix-bound drugs shows promise, and in the future, xenobiotherapeutic methods may be possible [H7 3 14].

### 7.3.5 Stimulation of growth hormone production by non-pituitary tissue.

Growth hormone is produced by tissues other than the pituitary, and it might be possible to stimulate such tissues to produce enough growth hormone to overcome a deficit. Such experiments have succeeded in dogs.

Progestins are hormones that prepare the uterus for egg implantation and pregnancy. Artificial progestins are used as contraceptives.

Progestins stimulate the mammary glands of dogs, in both sexes, to produce growth hormone. In contrast to growth hormone secreted by the pituitary, the induced growth hormone secretion is not pulsatile.

Progestins were used to elevate the growth hormone levels of dogs. One tested dog developed mild acromegaly. There were other side effects of mild to moderate severity.

### 7.3.6 Tissue engineering by genetic therapy.

The majority of growth hormone in the circulation is produced by just one small gland, the pituitary gland. Much of the control over the pituitary gland resides in one small brain region, the hypothalamus. These facts make growth hormone a good candidate for genetic therapy.

Rats were genetically engineered to have reduced expression of the ghrelin receptor specifically in the arcuate nucleus of the hypothalamus. These rats had reduced appetite, weight and adiposity. Although similar engineering of the germ line could not be done with humans, viruses might be used to deliver antisense RNA or ribozymes (techniques to prevent expression of a specific gene) to suppress the ghrelin receptor gene in the hypothalamus.

Alternatively, engineered viruses have been used to deliver the leptin gene to specific areas of the brain in rats. This reduced the rats' appetite and adiposity. A similar therapy might be useful in humans.

Alternatively, hormone-producing cells might be delivered by skin graft. Cultured skin cells which were then grafted into mice were used to express human growth hormone in mice. Presumably, other members of the growth hormone axis, which might be more useful medically, could also be expressed.

Injection of naked plasmid DNA into muscle is an effective method of gene expression, provided that the plasmid carries a useful gene driven by a strong gene expression promoter. One such plasmid, expressing the human growth hormone gene, was shown to increase proliferation of spleen cells in mice. Presumably, other members of the growth hormone axis could also be expressed this way.

### 7.3.7 Growth hormone and physical therapy.

It may be that efforts to use members of the growth hormone axis to reverse the symptoms of ageing would benefit from more sophistication. Most experiments to measure the effect of growth hormone on elderly humans or animals simply administer the hormone, perhaps along with exercise or perhaps not, and then perform simple tests of grip strength, knee extensor strength, soleus muscle strength, swimming endurance (in rats) or something similar. Yet it is clear from experience with people who have lost muscle function that expert physical therapy can make an enormous difference toward regain of function. Perhaps an expert combination of physical therapy and growth hormone would produce better results.

### 7.3.8 More aggressive experimentation.

Expanding knowledge about the growth hormone axis may also allow more aggressive, goal-oriented experimentation. There is unmistakeable

evidence from the sports of bodybuilding and power lifting that anabolic supplementation can be extraordinarily successful, provided that one is willing to try many drugs and combinations of drugs and dietary supplements, and to take serious risks. In the past, such experiments would have been unjustifiable even in animals because of the low likelihood of interpretable results; but perhaps increasing knowledge will allow informative experiments under a greater variety of conditions.

### 7.3.9 Variability between people.

Experiments on the effect of growth hormone on the immune systems of children have shown enormous variability. This variability is present in both the response of the immune system cells to growth hormone, but also the presence of growth hormone receptor in those cells.

It is not clear whether this variability is genetic, or reflects something else, such as nutritional status or disease history. However, it may have to be taken into account if growth hormone is to be used optimally. Perhaps therapy will have to be tailored to individual patients.

### 7.3.10 Improvements in growth hormone-related supplementation.

Supplementation can increase a person's secretion of growth hormone. Oral supplements purporting to do this are sold. Continued research is likely to reveal combinations of supplements that can be given orally, nasally or by injection that will greatly elevate growth hormone concentrations in people who would benefit. Some of these may be for sale without a doctor's prescription, while others are likely to require one.

Infusion of the amino acid arginine into a subject can enhance the secretion of growth hormone. Oral feeding of arginine can have the same effect if the doses are high, e.g. 9 grams per day. The combination of GHRH, ghrelin and arginine can increase growth hormone secretion in old people to levels that are high even by youthful standards.

Arginine may act by serving as a substrate for NO synthase, which produces nitric oxide. Although arginine and nitric oxide generally stimulate growth hormone, under some circumstances, arginine and the nitric oxide it gives rise to can strongly inhibit the secretion of growth hormone. Hence, the use of arginine requires knowledge and caution.

Smaller oral doses of arginine in the range of 2-3 grams of per day have been reported not to stimulate growth hormone secretion, and even to inhibit it. The amino acids lysine, ornithine and tyrosine are also ineffective when taken orally in this dose range.

Melatonin given orally in the range of 1 gram enhances production of

growth hormone. Ingestion of 20 grams of creatine also raised growth hormone secretion in healthy male subjects; the increase averaged 83%, but with large differences between subjects.

Ginseng has been reported not to induce growth hormone or IGF-I.

The drug acipimox prevents the breakdown of fats to form free fatty acids. Treatment of elderly men with acipimox increased their response to GHRH by 2.8-fold. Prevention of fat breakdown would neutralize one of the great benefits of growth hormone treatment of the elderly. However, if some other means could be found to remove fat, e.g. liposuction, acipimox could be a valuable enhancer of GHRH. Alternatively, some means to remove free fatty acids, rather than prevent their formation from fat, could be used to enhance the release of growth hormone by GHRH.

(ANY supplementation designed to alter person's metabolism, whether requiring a prescription or not, should be done under the care of a qualified professional. The best medical advice is likely to come from a Board-Certified Doctor qualified to practice in Endocrinology. [H8])

### 7.3.11 Unlinking of hormonal activities.

Hormones and other signalling molecules frequently interact with different types of receptors. Although one might think that the same features of a hormone would be required for all of its interactions, this is often not the case. Instead, hormones and other signaling molecules often carry multiple distinct signaling features that can be separated. This separability of signaling features in signaling molecules may increase evolutionary flexibility.

The phenomenon of alternative splicing of messenger RNA, discussed above [H6 8 2], exists mainly to promote selective unlinking of protein features. However, alternative splicing usually keeps or removes relatively large protein segments, and separability of signaling features can occur on a much shorter scale.

The possibility of unlinking of biological signals offers great potential medical advantages. It can allow doctors to induce just some of the effects of a given hormone, while suppressing the others. Moreover, in some cases it can also allow doctors to block the effects of a given hormone by producing mimics that occupy the hormone receptor, but do not activate it.

### 7.3.12 Unlinking of signaling features in ghrelin, as an example.

Ghrelin is a short hormone, only 28 amino acids in length. Yet extensive unlinking of effects has been demonstrated or is suspected in ghrelin. For one thing, messenger RNA from the ghrelin gene is alternatively spliced to

encode both ghrelin and a related protein that is restricted to the testis (at least in mice). Moreover, ghrelin mimics sometimes have only a subset of ghrelin's effects.

Ghrelin transport in and out of the brain depends on its sequence in a way that seems to be separable from ghrelin activity per se.

The ghrelin mimic hexarelin induces growth hormone, as ghrelin does, but lack's ghrelin's ability to decrease blood insulin levels and increase blood glucose. Although some ghrelin mimics bind both the hypothalamus and heart tissues, at least one mimic does not bind heart tissues.

At least one ghrelin mimic is known that binds the ghrelin receptor but has no growth hormone-releasing activity. This compound might occupy the ghrelin receptor and prevent binding by active hormone, thus blocking its activity.

The natural neuropeptide cortistatin, which is only 14 amino acids in length, also binds the ghrelin receptor. However, it does not induce growth hormone, and does not induce the prolactin or the hormone ACTH, as ghrelin does.

Testing of modified ghrelin indicates that in cultured cells, all of ghrelin's activity is contained within the first 4 amino acid residues, and even part of this functions mainly as spacer. (These short derivatives of ghrelin are inactive in living animals, for unknown reasons). Hence, even short proteins such as ghrelin are long enough to contain multiple distinct signals.

### 7.3.13 Unlinking of features in other axis hormones.

Somatostatin binds six different receptor types. Somatostatin analogs bind only a subset of these.

Leptin is a protein produced by fat cells that limits fat accumulation. Change of position 128 of human leptin from arginine to glutamine does not change leptin's ability to bind its receptor, but destroys its activity.

It is likely that with further research, many opportunities to unlink signaling features of the axis hormones, and their auxiliary proteins such as the IGFBPs, will be found.

### 7.3.14 "Xenobiotherapy" for spatial segregation of medicines in the body.

Molecular genetics has progressed to the point where it may soon become possible to use engineered microbes as medical agents. Living microbes might be genetically engineered either to release drugs when and where needed, or to act as drugs themselves. Microbes might be engineered to recognize and attack cancer cells or HIV-infected cells, or to sequester and

degrade the amyloid plaque that produces Alzheimer Disease. (I suggest the term "xenobiotherapy" to denote use of engineered proteins and microbes for medical purposes.)

Two problems have limited such use in the past. The first is that the microbes were too complicated and mysterious to engineer successfully, and engineering techniques were too feeble. The second is that such agents are very likely to provoke an immune response that will neutralize them and might harm or even kill the patient.

Despite the obstacles, microbes have at times been used as medicines. One example is the use of viruses that infect bacteria (bacteriophage), to control bacterial infections. Medical bacteriophage are receiving renewed attention in the Western world, as the threat of antibiotic-resistant bacterial infections increases.

Engineered microbes have many potential advantages as medical agents. They can have enormously greater specificity than other medicines, which might be exploited to selectively kill cancer cells or HIV-infected cells (for example). They may be easy to manufacture in bulk, since they are self-replicating. They also have the potential to restrict themselves to specific regions of the body, while avoiding others.

The most serious drawback of xenobiotherapeutic agents is that they might themselves become the source of new diseases. If this drawback can be overcome through effective containment methods, or if the risk turns out to be low, engineered microbes are likely to be widely used as medicines in the coming century.

It will also be necessary for xenobiotherapeutic agents to evade the patient's immune system. It seems likely that methods will be found, because we have many human and animal pathogens to imitate, all of which evade the immune system at least temporarily. Most if not all human disease parasites except opportunistic ones have effective methods to paralyze or otherwise thwart components of the immune system. Many of these methods could probably be exploited for xenobiotherapy.

One of the most promising methods of immune system evasion is to repeatedly change the immunological characteristics of immunity-provoking proteins without changing their functional characteristics. A number of humans parasites, including the influenza virus, do this; learning exactly how they do it is the focus of ongoing research.

(For more on the potential engineering and use of xenobiotherapeutic agents, see the e-pamphlet on xenobiotherapy at www.xenobiotherapeutics.com).

The advantage of xenobiotherapy most relevant to growth hormone is the

ability to restrict xenobio agents to specific regions of the body. If short-lived medicines were used, this could allow a continuous supply of useful medicines to be delivered to selected sites, but kept away from other sites.

As an example of how localized delivery of proteins can succeed, leptin protein has been successfully delivered to specific areas of rat brains. This had the desired effect of reducing the rats' weights through decreased appetite and the consumption of fat to produce heat. The method of delivery was a virus engineered to express the leptin protein.

Expression of the leptin gene specifically in the brain overcame two therapeutic problems. The first was that there was no need to load the blood with large amounts of leptin, which can be harmful [**lept**]. The second was that there was no need to move leptin across the blood-brain barrier, which becomes increasingly difficult with age.

### 7.3.15 Localized expression of IGF-I and growth hormone.

There are several medical situations where localized expression of growth hormone or IGF-I might be useful.

Muscle hypertrophy and wasting are thought to be greatly influenced by production of IGF-I within the muscle itself. Increased expression of this "local" IGF-I has been suggested as a potential therapy for the skeletal muscle wastage that can accompany heart disease.

The selective expression of IGF-I in the hearts of mice having diseased and enlarged hearts, greatly improves their heart condition. Thus, selective delivery of IGF-I to the hearts of heart patients might also be valuable.

Growth hormone, IGF-I and degradation products of IGF-I are strongly protective of brain neurons after brain injury. The patterns of protection afforded by growth hormone and IGF-I differ, so IGF-I is not simply acting as a mediator of growth hormone. Delivery of growth hormone or IGF-I specifically to the brain or to parts of the brain following a stroke or other injury could have benefits.

### 7.3.16 Localized expression of IGFBP-3

IGF-I is not always beneficial. IGF-I promotes the growth and spread of lung and other cancers. This is an important reason that localized expression of IGF-I might be desirable: to avoid promoting cancer elsewhere.

It also appears that localized inhibition of IGF-I could also be valuable. IGFBP-3 binds IGF-I, and probably reduces its biological activity. IGFBP-3 can prevent IGF-I from stimulating cultured lung cancer cells. Moreover, IGFBP-3 therapy can initiate massive destruction of the lung cancer in live mice.

The IGFBP-3 protein was expressed in mice via a virus, but other biological vectors such as engineered bacteria could probably also be used. Localized expression of IGFBP-3 or other IGF binding proteins might be used to forestall or prevent cancer in specific organs, such as the lungs of reformed smokers.

### 7.3.17 Transfer of growth hormone into cancers.

Xenobio agents might be use to identify and kill cancers directly. However, if the ability of a xenobio agent to identify cancer cells were imperfect, it might still be used to erase the increased resistance to chemotherapy that cancer cells often develop.

Provided that the xenobio agent could be restricted to a specific region, it might infect a mixture of healthy and cancerous cells with some construct that erased the cancer cells' advantage.

One gene that might differentially sensitize cancer cells is the growth hormone gene. It has been reported that transfer of the human growth hormone gene into cancers can make them more vulnerable to chemotherapy.

### 7.3.18 Localized expression of somatostatin.

Another member of the growth hormone axis for which localized expression could be very valuable is somatostatin. Somatostatin suppresses many different processes throughout the body, and might be used, for example, to specifically suppress autoimmune disease.

One interesting possible use for locally expressed somatostatin is to prevent diabetes-induced blindness. Diabetes causes neovascularization of the retina. The usual treatment is laser ablation of the aggressive blood vessels; however, somatostatin analogs also inhibit the blood vessel growth. Xenobio agents could be used to release somatostatin, or analogs, in the region of the retina.

### 7.3.19 Delivery of preformed drugs with xenobio agents.

Although xenobio agents might in many cases synthesize the drugs that they deliver, they might also be pre-loaded with synthetic drugs. One example of pre-loaded drugs that might be delivered with xenobio agents is inhibitors of nitric oxide synthase.

Interferon gamma, which is a response to infectious disease, inhibits growth hormone secretion by the anterior pituitary gland. Nitric oxide is involved in this inhibition. Delivery of nitric oxide inhibitors to the pituitary gland might prevent conditions which cause prolonged elevation of

interferon gamma from causing growth hormone deficiency.

## 8. THE DECISION TO USE GROWTH HORMONE.

Most people who would like to experiment with growth hormone cannot do so legally, at least in the United States. Growth hormone is a drug, and as such, is regulated by the Food and Drug Administration (FDA).

Drugs must undergo an exacting process of approval by the FDA, and are often restricted as to the conditions that they can be prescribed for. To my knowledge, growth hormone has been approved to treat only the following conditions: childhood growth hormone deficiency, adult growth hormone deficiency, childhood kidney insufficiency, Prader-Willi Syndrome, Turner Syndrome, HIV-induced wasting and small birth relative to gestational age. In any case, growth hormone therapy requires a doctor's prescription.

Some of the major vendors of human growth hormone are listed below. Their trade names for human growth hormone are given in parentheses.

Genentech (Nutropin)
Eli Lilly (Humatrope)
NovoNordisk (Norditropin)
Pharmacia (Genotropin)
Serono (Serostim or Saizen).

Growth hormone is injected, subcutaneously or into muscle. It is not given as a dietary supplement or as a spray. Hence, edible supplements and sprays that purport to raise a user's growth hormone levels presumably do so by increasing the user's growth hormone production.

Substances that are claimed to raise a user's production of growth hormone can be sold as food supplements. The Dietary Supplement Health And Education Act of 1994 (DSHEA94) amended the Federal Food, Drug, and Cosmetic Act of 1938 to change the way in which dietary supplements are regulated and labeled. The stringency and severity of regulation are much greater for drugs than for dietary supplements.

DSHEA94 is controversial. It has enthusiastic defenders, and bitter critics who charge that it prevents the FDA from protecting the public. Anyone planning to regularly consume supplements that purport to raise growth hormone levels would do well to study DSHEA94 and any following legislation to decide for themselves whether it offers adequate regulatory oversight. Much information about DSHEA94 (for, against and neutral) is available for free on the Internet.

It seems clear that some dietary supplements can indeed raise growth hormone levels [H7 3 10]. Melatonin, creatine and arginine (in large doses) have all been reported to be effective. Hence, commercial supplements may work. However, it is not clear that such supplements are always effective, or always safe.

The best advice is likely to come from a Board Certified Endocrinologist. This is a person certified by the American Board of Medical Specialties as qualified to specialize in Internal Medicine with a subspecialty of Endocrinology. Such a doctor is most likely to know whether human growth hormone has been approved for treatment of a given patient, and whether specific commercial products are legal, safe and effective. It is perfectly permissible, and wise, to consult more than one doctor. The price of consulting a Board Certified Endocrinologist is probably less than the price of supplements consumed for months or years.

It is perfectly permissible to ask any supplement vendor for evidence that its products are safe and effective. I personally would place the greatest weight on published research in reputable journals, conducted by researchers who had no personal stake in the findings.

Unless it is very new, a good journal is likely to be on the library shelves of at least some major universities or other public institutions that conduct scientific research. One can learn whether a given university campus has a given journal either by phone or via the Internet.

Federal funding agencies, such as the National Institutes of Health and the National Science Foundation, require that scientists whose research they support disclose any financial interest they may have in the results of their research. Moreover, some states and universities may have additional disclosure requirements, even if the research is not federally funded. One may learn of such disclosures by asking the university.

## 9. CONCLUSIONS.

The demonstrated benefits of human growth hormone may be a bit disappointing to someone who is familiar with only the hype. However, successful pharmacology includes a long process of refinement. Useful drugs usually begin their existence as compounds that show modest benefits and nasty side effects. Experimentation ensues where the chemical structure of the drug, the mode and schedule of delivery and the dosage are repeatedly modified and tested in the hope of maximizing the benefits and minimizing the harm. The target

population is also studied to learn how different population subgroups respond to the drug, and drug administration can be optimized for each subgroup.

Human growth hormone, along with its inducers and antagonists, is near the beginning of that process. Moreover, axis hormones and their mimics differ from other drugs (such as penicillin) in that beneficial interactions between axis hormones and other influences on body composition (such as diet, exercise and other drugs) may be possible. Hence, there is reason to hope that the effectiveness and safety of growth hormone therapy will increase.

## 10. AFTERWORD

CONTACT US!

We would love to hear from you. One purpose of this manuscript is to assess the market for health-related writing that is aimed at the general public, but grounded in the scientific literature.

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-Was it clear?
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(This manuscript contains many internal hyperlinks. Most are functional; a few are not, for reasons that we have not been able to cure. We know about

them.)

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# 11. INDEX OF SELECTED TERMS.

This index is intended to supplement the Table of Contents, rather than to replace it. It locates terms that occur only a few times in the text, and which the reader might otherwise miss. It lists only those occurrences where new information is imparted.

Many terms, such as "growth hormone" or "IGF-I" or "sleep" are not listed because large sections of the book are devoted to them or because they are mentioned in many places. For these, see the Table of Contents, or use the Adobe Acrobat search function.

cytochrome P450 H6_2_3 P450
dehydroepiandrosterone (DHEA) DHEA DHEA1 DHEA2 DHEA3
depression H4_10_2 H4_10_9 H4_11_1 dep1
diabetic retinopathy ret1 ret2 ret3
Dietary Supplement Health and Education Act of 1994 DSHEA1
erectile dysfunction H4_6
estradiol H6_3_2 H6_3_3 estr1 estr2 estr3
fake drugs, including growth hormone fake1
fatty liver (see liver)
frailty frail frail2
fructose fruct
gastric bypass byp1 byp2
gigantism (see acromegaly)
glucagon gluca1
growth hormone independence H6_11_2
growth hormone insensitivity Laron
growth hormone resistance H4_2_7 H6_11_1 GHres1
growth spurt(s) spurt1
hair H4_8 hair1
hepatocyte growth factor HGF1
hypothalamus H4_7_7 H6_6_14 H6_10_1 hthal1
hypercalcemia hcalc1
impotence (see erectile dysfunction)
infection, opportunistic opp1 opp2 opp3
intracranial hypertension H5_2_3 htens1 htens2 htens3
kidney maturation kmat1
Laron Syndrome (see growth hormone insensitivity)
lipid profile H4_5_4
lipodystrophy lipdys1
lipoprotein(a) lp_a lp_a1
liposome(s) liposm1 liposm2
liposuction lsuct1
liver (fatty liver) fliv
luteinizing hormone lutein1
melatonin mel1 mel2 mel3
mitochondria mito
multivariate analysis mvari1