

Uptodate on GH/IGF-1 axis actions

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Summary

In December 2010, a workshop was held at "Regina Elena" National Cancer Institute in Rome, Italy, to develop an update on GH/IGF-1 system. The workshop was supported by NoGeRo group and was sponsored by SIE Lazio (Società Italiana di Endocrinologia-Lazio Regional Section) and AME Lazio (Associazione Medici Endocrinologi-Lazio Regional Section). Invited participants included Italian endocrinologists skilled in the field of GH and IGF-I system both in basic and clinical research. In the following two years an extensive review has been structured summarizing the most important points achieved in the discussions during the workshop.

KEY WORDS: GH, IGF-1.

Introduction

GH/IGF-I axis

The GH/IGF-I system comprises of several proteins controlling growth, metabolism and senescence. Once thought to act predominantly through direct effects, it is now clear that the GH/IGF-I system can influence numerous cellular processes via indirect effects on the signaling machinery. From a hierarchical point of view, many growth-promoting actions of this system imply an adequate secretion of GH, a 191 aminoacid single chain polypeptide hormone with 2 disulphide bridges originating from somato (mammo) troph cells located in the lateral sections of the pituitary, where GH is stored in granules of 350-500 nm (1). Five homologous genes exist in the GH locus, a 66 kb region of DNA located in chromosome 17q22-q24. In the circulation, the prevalent GH isoform is of 22 kDa (~73%), while less prevalent is the 20 kDa isoform (~16%) as well as alternative isoforms (~10%). Spontaneous GH secretion is typically pulsatile and peaks every 60-90 min. It increases during the sleep, particularly during the stage 3-4. GH secretion is high in the fetal stage, decreases in the neonatal stage, then spontaneous peaks are more frequent and intense in pediatric age. Subsequently, it is calculated that GH production is 100 mcg/24 hr in the prepubertal stage, 700 mcg/24 hr in adolescents and 400 mcg/24 hr in adults. Elderly individuals harbor the lowest levels of GH and this condition has been termed somatopause. GH yields a 20-40 min half-life if administered intravenously and a 12-24 hr half-life if administered intramuscularly. The regulatory mechanisms of GH secretion are sexually dimorphic, with men having

larger nocturnal GH pulses and relatively smaller pulses at daytime compared to women, who show more continuous and uniform GH pulses. On the other hand, rh-IGF-I administration causes a more potent feedback on spontaneous and GHRH-induced GH secretion in men than women, suggesting in the latter a stronger intervention on hypothalamic GHRH on the negative regulation of GH secretion (2). Several factors are known to influence the secretion of GH. Among these, GHRH and somatostatin act as the chief neuroendocrine stimulator and suppressor, respectively, while the physiological role of ghrelin on GH stimulation needs further elucidation. It is worth mentioning the stimulatory effect of exercise, stress, aminoacids, muscarinic cholinergic and alpha-adrenergic tone, glucocorticoids (acutely), opioid peptides, L-arginine and hypoglycaemia. Oppositely, beta-adrenergic tone, glucocorticoids (chronically), obesity, malnutrition and hyperglycaemia all are negative regulators of GH secretion (3). The peripheral effects of GH include multiple actions on: bone metabolism, by increasing osteoclast differentiation and activity, osteoblast activity, bone mass and endochondral bone formation; linear growth, where GH promotes epiphyseal growth (this is also known as the "dual effector" theory), stimulates the differentiation of prechondrocytes and the local expression of IGF-I, which then increases clonal expansion of chondrocytes; muscle mass, through effects on amino acid transport, nitrogen retention, lean tissue formation and energy expenditure; adipose tissue, by increased lipolysis, inhibited lipoprotein lipase, stimulated hormone sensitive lipase, decreased glucose transport, decreased lipogenesis (4). The cellular actions of GH are mediated by a transmembrane receptor (GHR) of 620 aminoacids that belongs to the type-1 cytokine receptor family; the extracellular domain also works as the circulating GH binding protein, and retains diagnostic significance in the GH-resistance setting (5). In the cell, GH action is mediated by multiple signaling cascades, the most relevant of which is the JAK-STAT pathway (6). Mutations in the GHR gene provoke a condition of GH resistance: over 600 mutations have been identified and all lead to Laron's syndrome, a condition of IGF-I deficiency, short stature and typical somatic features. In addition to the previous, it has become clear that postreceptor mutations, particularly those involving Stat 5-b, can also cause GH resistance. In such settings, treatment with rh-IGF-I is approved as the only effective therapeutic tool to compensate for the hormone loss (6). Once known as the peripheral effector of GH, it is now evident that IGF-I not only is GH-dependently and -independently involved in controlling growth but it also controls cell proliferation. IGF-I is a basic polypeptide of 70 amino acids (M.W. ~ 7600 d), formed by two chains linked by a connecting peptide. The liver produces > 80% of the circulating peptide, however IGF-I is expressed and synthesized in multiple tissues where it acts to govern local cellularity and metabolism via autocrine and paracrine actions. It circulates as a single non-glycosylated chain with two disulfide bridges, and a 50% amino acid sequence homology to insulin explains its ability to bind to insulin receptors and insulin-IGF-1

hybrid receptors, thereby also acting at the metabolic level with insulin-like properties. IGF-I shares ~60% sequence homology to IGF-II. IGF-I levels are influenced by multiple factors, with GH being the most relevant. Among the other contributors, also important are the genetic environment, nutritional state, sex hormones, IGF-BPs, insulin, thyroid hormones, cortisol, immune system and catabolic states (7). IGF-I levels increase gradually from childhood and reach their peak concentration at puberty, then they decrease gradually. In the circulation, IGF-I is associated with ALS and IGF-BP-3 in a ~150kDa complex that works as a reservoir of IGF-I and contributes to regulate its bioactivity and half-life. All components of this ternary complex are regulated by GH. Moreover, IGF-I and IGF-BP-3 can form binary complexes, that are active locally. As mentioned above, recent evidences indicate that GH and IGF-I roles on growth are partly independent. It has been estimated that growth processes in mammals depend for ~34% on a their combined action, for ~14% on GH alone, for ~35% on IGF-I alone and for ~17% on other factors (8). Recent data further suggest that IGF-I can amplify the metabolic actions of GH and contrast its deleterious effects on lipolysis, gluconeogenesis and insulin-resistance (9). Indeed, many cellular actions of IGF-I are proliferative and antiapoptotic in several organs and apparatuses. One central player in the prevention of cell death is the IGF-I receptor (IGF-IR). Transduction of signals through this receptor leads to multiple series of intracellular phosphorylation events and the activation of several signaling pathways. The IGF-IR consists of two disulphide-linked $\alpha\beta$ heterodimers and it is structurally similar to the insulin receptor (10). Both IGF-I and IGF-II bind the IGF-I receptor with high affinity and insulin binds with lower affinity. Upon ligand binding, the receptor has tyrosine kinase activity resulting in autophosphorylation, recruitment of docking proteins such as IRS-1 and subsequent stimulation of many signal transduction pathways, including Ras/MAP (mitogen-activated protein) kinase, PI3 kinase/PKB (protein kinase B) Akt and PI3 kinase/mTOR. These pathways convey mitogenic and metabolic signals and certain effects of insulin and IGFs can be limited by drugs or nutritional conditions that alter AMPK (AMP-activated protein kinase) signalling or mTOR signaling. Mechanisms of IGF system signaling that prevent cell death continue to be identified, suggesting that cells have alternative ways to avert death signals in addition to primary protective pathways. While the IGF-I receptor is virtually ubiquitous in humans, its density appears to differ from one tissue to another. However, IGF-IR activation by IGF-I delivered to the cell by endocrine, autocrine or paracrine modalities, is accompanied with relevant actions on immediate early gene expression, stimulation of myogenesis, apoptosis, cell cycle progression, immune response modulation, induction of enzymes involved in steroidogenesis, and sex steroid production (11). Actions on cell survival involve increased activity of antiapoptotic factors (through modulation of p53, NF- κ B, Bcl-2 expression) and inhibition of proapoptotic factors (through modulation of caspases and Bad) (10). The IGF-II/mannose 6-phosphate (M6P) receptor is

structurally distinct from IGF-I and insulin receptors, it binds IGF-II with high affinity, IGF-I with far lower affinity and does not bind insulin. Its major IGF-related role appears to be IGF-II internalisation and clearance. A soluble form of this receptor which lacks the transmembrane and intracellular domains is found in the circulation. It should be mentioned that IGF-I genotyping has revealed a role for IGF-I mutations in cardiovascular disease, while mutations in the IGF-IR gene may retard intrauterine and subsequent growth in humans (12). The GH/IGF system is thus relevant in many physiological and pathophysiological conditions. The direct involvement of IGF-I in human diseases like GH-related disorders, cancer, atherosclerosis, diabetes, osteoporosis and neuromuscular disorders has prompted extensive research for therapeutic purposes. A better understanding of the system may enable to elaborate on ligand- and tissue-specific IGF-based therapies without interfering with physiological processes. Additionally, their role in the diagnosis and monitoring of diseases should be better clarified in the near future.

IGF-binding proteins (IGFBPs)

The IGF system, besides the two growth factors IGF-I and IGF-II, the cell surface receptors, IGF-IR and IGF-IIR, comprises six specific high-affinity binding proteins (IGFBP-1 to IGFBP-6), as well as the IGFBP proteases, that regulate and propagate IGF actions in several tissues (13). Hepatic IGF-I circulates almost entirely (> 99%) bound to IGFBPs. IGFBP-3, a major IGFBP type in circulation, binds 75 to 90% of circulating IGF-I in a large ternary complex consisting of IGFBP-3, acid-labile subunit (ALS), and IGF (150 kDa). It is still debated whether IGFBP-3 in the liver is positively regulated upon GH stimulation or regulation of circulating IGFBP-3 in human subjects is due to increased formation of the ternary complex induced by GH (14,15). ALS is produced in the liver as a direct effect of GH. The ALS stabilizes the IGF-IGFBP-3 complex, reduces the passage of IGF-I to the extravascular compartment, and extends its half-life (13). More recently it has become clear that IGFBPs 1-6 have intrinsic biological activity (IGF/IGF-IR-independent actions) in addition to their actions to bind IGFs and sequester the active hormone. In particular, IGF/IGF-IR-independent actions of IGFBP-3 (antiproliferative and proapoptotic effects) seem to contribute in improving the pathophysiology of a variety of human diseases, such as cancer, diabetes, and malnutrition (16). Nine other forms of IGFBPs, which have low affinity for IGFs, have been identified due to their high homology in gene sequences with those of IGFBP 1-6 (17). These carrier proteins, whose role is currently under study, were grouped in a family defined as IGFBP related proteins (IGFBP-RP) (17). The binary complexes of 40-50 kDa, carrying 20-30% of IGF-I and II, are composed primarily of IGFBP-2. Plasma concentrations of IGFBP range from 5-50 ng/ml of IGFBP-1 and 100-400 ng/ml of IGFBP-2 to 2-4 mg/ml of IGFBP-3, confirming the greater significance as carrier protein in circulation if this latter BP (17). Although the mech-

anisms by which IGF complexes becomes available for binding with receptors in tissues are not yet fully understood, several studies have highlighted the existence of plasma and tissue specific proteases for IGFBPs, able to facilitate the process of dissociation from IGFBPs (13, 17). Several proteases specific for each IGFBP have been identified so far. Among these, metallo-proteases degrading IGFBP-3 are the best characterized, and are often serine proteases metal- and calcium-dependent (PSA, γ -NGF, plasmin metallo-proteases MMP-1, -2 and -3 are part of this group). Beside the key role in growth and differentiation, there is now compelling evidence to suggest that members of the IGFBP family play important roles in metabolic homeostasis. For example, IGFBP-1 concentrations fluctuate inversely in response to changes in plasma insulin levels, implicating IGFBP-1 in glucose regulation, and fasting levels of IGFBP-1 predict insulin sensitivity at the population level, whereas IGFBP-2 concentrations reflect long-term insulin sensitivity and are reduced in the presence of obesity (18). Accumulating evidence indicates that abnormalities of IGF-I and IGFBP-1 occur in insulin-resistant states and may be significant in the pathophysiology of type 2 diabetes, as well as in the development of cardiovascular disease in metabolic disorders, including diabetes and obesity (19). These evidences are promising for future therapeutic strategies, because, based on the potential vasculoprotective effects of both IGF-I and IGFBP-1, the system represents an interesting and novel therapeutic target in the prevention of cardiovascular disease in type 2 diabetes. Moreover, progress on the metabolic-related function of IGFBPs has indicated the close relation between IGFBPs and the components of the metabolic syndrome. Indeed, abnormal expression of IGFBP has been detected in various states of the metabolic disorders, suggesting that it could be also used as a convenient and sensitive marker of insulin resistance, identification of insulin-resistant individuals at high cardiovascular risk, and may be an earlier marker of the metabolic syndrome (20). Bone metabolism represent another important target since the 6 IGFBPs are expressed by osteoblasts. However the principal actor in this setting seems IGFBP-2, which is required for normal bone formation in mice and seems also involved in bone remodeling (21). Conversely, the role of the other IGFBPs in bone homeostasis is still controversial, although it appears that their fundamental role is to bind and serve as transporters/regulators of IGF-I in this site as well (21, 22). Indeed, as already emphasized, IGFBPs can have both enhancing and inhibiting effects on IGF action, depending on context and posttranslational modifications. The IGF pathway has been shown to be activated in many cancer types (23). IGF binds to two types of receptors, IGF-IR and IGF-IIR, on cell membranes, and activates the tyrosine kinase pathway downstream. Binding of IGFBPs with IGF has been shown to either positively or negatively regulate the binding of IGFs to their receptors, thus directly affecting the IGF signaling pathways. However, several studies have found that IGFBPs

may also function independently, interacting with proteins other than IGFs, may be cleaved, may bind to their own receptor on the membrane (13, 14). Importantly, there is increasing evidence that IGFBP-2, IGFBP-3, and IGFBP-5 are important players in the phenotypes of various cancers (23). Changes in the balance of the components of the IGF system may contribute to the progression of cancer (23, 24). The levels of IGFBPs have been associated with reduced risk for prostate, breast and other cancers (23, 24). Experimental studies have implicated high levels of IGF-I directly and IGFBP-3 inversely in prostate cancer growth, survival, and progression. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, high circulating IGF-1 concentration was positively associated with risk for prostate cancer (25). However, other evidences suggest a much weaker association of IGF-I with prostate cancer development and a stronger antagonistic association of IGFBP-3 with prostate cancer progression (26). Considering the clonal heterogeneity and unpredictable progression pattern of prostate cancer, the role of any single growth factor or its regulator, including IGFBP, as a single determining factor is limited. In breast tumors the abundance of IGFBPs relates to the estrogen receptor status and their production in the breast is controlled by hormones, principally estrogen and progesterone (27). However, also in this setting there conflicting observations between the effects of IGFBPs on the risk of cancer development, in particular IGFBP-3 (27). Indeed, the functional activity of IGFBPs can also be affected by proteolysis, phosphorylation and glycosylation and the implications of these determinants are still unclear so far. In general, the IGFs in certain tissues are present at levels far in excess of that required for maximal receptor stimulation, and the IGFBPs are critical regulators of their cellular actions. Beside this action, IGF-independent BPs effects on cell functions is still controversial. For example, again in breast, locally IGF bioactivity is influenced not only by tissue expression and regulation of IGFs, IGFBPs and IGFBP proteases, but also by other factors delivered from the circulation (23, 24). As prognostic values, only IGFBP-3 may serve as an important factor in evaluating cancer risk, in prognosis and, in the future, as a treatment as well. Although epidemiological studies vary in their conclusions about IGFBP-3 and reduced cancer risk, many population variables and assay problems may contribute to this lack of uniformity. Since IGFBP-3 is a highly effective proapoptotic factor in tumor cells by a variety of mechanisms, therapy directly with exogenous IGFBP-3 or indirectly with hormonal or other up-regulators of IGFBP-3 may be an important mode of cancer therapy in the future (16). Recent evidence suggests that high serum IGFBP-2 could be a risk factor for low-grade prostate cancer (28).

IGF-I system and life span

Several evidences suggest that insulin/IGF-I signalling pathways play a pivotal role in the regulation of

longevity (29-32). This hormonal system originated from a very early common ancestor. In animal models alterations of insulin/IGF-I signalling increase the life expectancy. In the nematode *Caenorhabditis elegans*, the down-regulation of the insulin/IGF-I pathway genes (DAF-2, AGE-1 and AKT) extend survival up to 300% (29). The link between reduced insulin/IGF signalling and longevity was subsequently confirmed in *Drosophila melanogaster* (33). The insulin/IGF-1 pathway became complicated with evolution. Mammals developed two well characterized hormonal systems: insulin and GH/IGF-1, with different metabolic and developmental functions. Rodents with reduced GH and/or IGF-1 signalling showed extended lifespan as compared to control siblings. Importantly, these genetic alterations can keep the animals healthy and protected from several age-related diseases (32, 34, 35). However, in many of these models the long-lived phenotype was exclusively observed for females. In humans there are several contradictory results on this topic (31). Bonafe et al. (36) reported that subjects carrying at least an A allele at IGF-I receptor locus (polymorphism G/A, codon 1013) have low levels of circulating IGF-I and are more represented among long-lived people. A reduced functionality of IGF-I signalling has been subsequently detected in other populations of elderly subjects and centenarians (37, 38). In addition, several epidemiological studies described a positive association between serum IGF-I levels and cancer incidence and mortality (26, 39-41). On the other hand, in the elderly population relative high circulating IGF-I levels have been associated with a decrease in cardiovascular morbidity and mortality (42-44). Besides, Paolisso et al. (45) reported a decrease in serum IGF-I and an increase in IGF-I/IGF binding protein 3 (IGFBP-3) ratio in Italian centenarians compared with aged subjects (70-99 years). This observation indicates an increase of IGF-I bioavailability in centenarians. All these contradictory data are probably related to the complexity of metabolic pathways and the difficulties of evaluating IGF-I bioactivity in humans. Another limit observed in previous studies arises from the comparison of serum IGF-I levels between centenarians and a control group of younger subjects. This comparison induces a significant bias related to the age influence of IGF-I. In fact, IGF-I values rise from birth until puberty then decline with advancing age. Centenarians' offspring, meaning people who have at least one centenarian parent, result to be an emerging model of longevity without the disadvantages observed in the studies of centenarians. These subjects are old people (age range 65-85 years) with a more favourable aging process, when compared with age-matched controls who do not have long-lived parents, and with the following characteristics: a lower risk for all causes of death; a lower prevalence of cardiovascular diseases, stroke, diabetes and cancer; an increased age of onset of age-related diseases (46, 47). Interestingly, it is possible to compare centenarians' offspring with a demographically-matched control group (subjects matched for age, sex, ethnicity, parent year of birth, but born from non long-lived parents)

thus avoiding cohort effects. Suh et al. (38) reported that female centenarians' offspring were smaller and displayed higher IGF-1 levels, indicative of IGF-1 insensitivity, compared with female controls. In addition, an overrepresentation of heterozygotes for mutations in the IGF-1 receptor gene, associated with IGF-1 insensitivity, was found among Ashkenazi Jewish centenarians. Therefore, the down regulation of IGF-1 system observed in centenarians and centenarians' offspring might significantly extend the lifespan by minimising the risk of cancer in these subjects, indicating that IGF-1 pathway is an evolutionarily conserved mechanism of longevity from yeast to humans.

Growth hormone and insulin-like growth factor axis and sport

Many relationships between the growth hormone-insulin-like growth factor (GH-IGF) axis [e.g. hormones (GHRH, Somatostatin, Ghrelin, GH, IGF-1, etc.), GH and IGF binding proteins (GHBP, IGFbps), receptors, proteases, etc.], physical exercise and sport exist. Particularly, we shortly highlight: a) the main characteristics of the GH-IGF-1 axis adaptive responses to acute and chronic exercise-related stress, b) the worldwide use of GH and IGF-1 as performance enhancing substances (i.e. as doping) by healthy athletes, and the anti-doping challenges to detect their abuse, and c) the problem of sport eligibility/participation and therapy in athletes affected by diseases associated to GH and/or IGF-1 hypo- or hyper-secretion/function.

GH/IGF-1 responses to physical exercise

Depending on exercise characteristics, acute sub-maximal and sprint exercises represent a powerful stimulus to human GH secretion, and all molecular GH isoforms increase few minutes after starting a single bout of exercise (48-50). Whereas few studies exist, chronic exercise (e.g. training) has been shown to increase GH pulsatility and/or nocturnal secretion both in healthy athletes trained above lactate threshold (51) or in obese subjects affected by metabolic syndrome (52). GH exerts its specific acute effects directly during and immediately after exercise, but also IGF-1 seems to contribute in mediating the GH signals. In fact, conflicting studies have shown an increase, a decrease or no modifications of serum total or free IGF-1 concentration after acute or chronic exercise (53). These observed discrepancies are probably related to differences in experimental protocols and to the numerous factors influencing the hormones response to exercise (see after). The mechanisms regulating GH response to exercise are yet to be fully elucidated. As also reported in excellent reviews (48, 49), different mechanisms have been proposed to explain the origin of the stimulus for GH response to exercise (e.g. increased blood lactate or hydrogen ion concentration, oxygen demand/availability ratio, afferent signals from muscle metabolic receptors, proprioceptive mechanisms, adrenergic system, changes in core temperature). At

hypothalamus-pituitary level, the reported stimuli may influence GH secretion throughout the combination of release of GHRH, withdrawal of somatostatin and release of a GH releasing peptide (GHRP), such as the putative endogenous GHRP-like ligand Ghrelin. It has been hypothesized that low intensity exercises may induce moderate GH responses through activation of the central cholinergic system, resulting in a reduction in somatostatin release, whereas higher exercise intensities, once hypothalamic somatostatinergic tone is suppressed, further increase GH release by increasing GHRH secretion. The role of the inhibition of somatostatinergic tone in increasing GH secretion after exercise-related stress in humans has been also demonstrated by evaluating the GH response to exercise following pre-treatments with the somatostatin analogue octreotide (54) and with pyridostigmine (55, 56). In addition, the administration of GHRH at the start of an incremental exercise until exhaustion has been shown to have an additive effect on the GH response to exercise, and the co-administration of GHRH and GHRP-2 at the start of exercise further potentiated GH release (57), as did the only administration of GHRP-2 before exercise (58). The characteristics of GH and IGF-1 responses to physical exercise are related and differently influenced by exercise characteristics (e.g. type, intensity and duration, previous training, number and frequency of exercise bouts, muscles involved, time of the day), individual factors (e.g. genetic factors, individual responsiveness, gender, age, fitness status, nutrition), diseases (e.g. GH-deficit, obesity, etc.) and iatrogenic factors (e.g. supplements, doping substances, not prohibited drugs) (49, 59, 60). The biological effects of GH and IGF-1 during and after exercise are related to specific modifications of respective peripheral pathways in different tissues, and to exercise related modifications of the other substances involved in the GH-IGF axis physiology (e.g. mainly IGFBP-1 and IGFBP-3, etc.) (53, 61). The specific role of GH-IGF axis in exercise physiology has not been completely evaluated but many of the GH and IGF-1 related cellular, metabolic and cardiovascular effects (e.g. increased cardiac contractility, stroke volume, lipolysis, free fatty acids availability, uptake and oxidation, fat sensitivity to catecholamines, protein synthesis, sweat secretion, cell proliferation, erythropoiesis, etc.) are of great usefulness in the physiological body's adaptation during and after exercise (i.e. during recovery) (48). Even if many relationships exist we still have to clarify the exact role of the GH-IGF axis in exercise physiology, particularly when local GH-IGF pathways in different tissues are concerned, and the possible positive and/or negative effects of physical exercise-related stress on such hormonal pathways in different physiological (e.g. puberty, aging) or pathological conditions (e.g. GH-deficit, metabolic diseases, osteoporosis).

Doping with GH/IGF-1

GH and IGF-1, and all their respective secretagogues (e.g. GHRH, Ghrelin), are considered prohibited substances by the World Anti-Doping Agency (WADA)

(see at <http://www.wada-ama.org/en/World-Anti-Doping-Program/Sports-and-Anti-Doping-Organizations/International-Standards/Prohibited-List/>) and by all National Anti-Doping Organizations (NADO). GH is one of the most abused prohibited substances between athletes and body builders. However, although GH abuse may increase lean body mass and may improve anaerobic exercise capacity in healthy individuals (62), existing scientific evidences do not support a possible role of GH in enhancing sport performances in athletes (63, 64). In our opinion, a great problem in evaluating the effects of GH/IGF-1 abuse on athletic performance during official experimental protocols (i.e. authorized by an ethical committee) is related to the impossibility to reproduce in healthy athletes the same type of GH abuse really performed by humans. In fact, to enhance each specific type of performance in different sports/physical activities athletes and body builders usually assume together different anabolic substances (e.g. androgenic anabolic steroids, GH, insulin), and other prohibited (e.g. central nervous system stimulants, glucocorticosteroids, etc.) and not prohibited drugs and supplements (e.g. thyroxine, PDE5 inhibitors, amino acids, etc.), often at very high supra-physiological doses and with empirical regimens of administration and associations. Besides the possible GH/IGF-1 abuse related effects and consequences on performance and health (e.g. antibodies anti-GH, nausea, headache, hyperglycaemia, diabetes, hypertension, fluid retention, arthralgia, gigantism, acromegaly, cancer, etc.) (64), it is of great difficulty to know all the possible effects, adverse events and health risks related to multi-substances abuses. In anti-doping, many factors can influence the GH/IGF-1 abuse detection (e.g. similarity between endogenous and exogenous hormones, selection of "reliable markers", type of assays, normal ranges in athletes, used biological fluids, anti-doping timing, assay's reliability), consequently, it is still difficult to find the best and optimal method for detecting doping with such substances (65, 66). Whereas no definitive technologies to detect IGF-1 abuse in athletes exist, two methods are actually used to detect GH abuse: 1) the isoforms method, that was developed to distinguish between the proportions of different human GH isoforms in physiological conditions (i.e. natural mixture of 22-KDa and non-22-KDa isoforms) and those found after recombinant human GH (rhGH: only 22-KDa isoforms) injection (i.e. increase of 22-KDa and decrease of non-22-KDa isoforms), 2) the markers method, that is based on a mathematical calculation performed by using the serum concentrations of specific GH-dependent biological markers (e.g. IGF-1, propeptide of type III pro-collagen) with respect to control values (65, 66). Even if these two methods have a reasonable sensitivity and specificity, some concerns still exist and some new proposed markers (e.g. c-terminal cross-linked telopeptide of type I collagen, haemoglobin α -chain, etc.) or methods (e.g. surface plasmon resonance, mass spectrometry, etc.) to detect GH abuse in athletes should be further evaluated (66-68).

Sport participation in athletes with GH/IGF-1 diseases

Due to the biological effects of GH and IGF-1 in the mechanisms of response to exercise-related stress and on body tissues involved in sport adaptation (e.g. heart, muscles, liver, etc.), sport participation is considered not physiological and not safe in athletes affected by diseases associated to GH and/or IGF-1 deficit or increase, particularly if other hormones deficit (e.g. pituitary adenoma) or serious complications are present. When indicated, athletes affected by GH/IGF-1 deficit or excess had to start the specific treatment before starting a competitive sport activity. According to WADA (see at <http://www.wada-ama.org/en/Science-Medicine/TUE/>) and to respective NADO/Federations regulations, in competitive athletes with GH/IGF-1 deficit it is mandatory to obtain a Therapeutic Use Exemption certificate by the respective NADO or International Sport Federations before starting GH or IGF-1 treatment at substitutive doses and after sport participation. In that Countries where sport eligibility is granted by an external Authority an in deep and sport-specific pre-participation screening is mandatory/indicated (i.e. in Italy is mandatory to obtain the sport eligibility certificate by authorized sport physicians) before starting/re-starting sport participation in treated athletes with GH/IGF-1 diseases (69). Due to rhGH pharmacokinetic, in young and adult athletes affected by GH-deficit further studies are warranted to identify the optimal rhGH administration regimen (i.e. morning or evening administration? single or multiple daily doses?) to guarantee a physiological competition by reproducing also during and immediately after each exercise/competition all the effects of GH on muscles, heart and metabolisms.

GH-IGF-I axis: drug interferences

In normal cells, insulin-like growth factors (IGF-I and IGF-II) and their six high-affinity binding proteins (IGFBPs) contribute to regulation of cell growth, metabolism, and death. IGFBPs modulate IGF activity by reducing IGF bioavailability to bind to the cell surface IGF receptors (IGFRs). Balance between growth factors and IGFBPs is modulated by specific IGFBP proteases. Recent data suggest that IGFBPs may also exert significant IGF-independent actions. Free, unbound IGF-I exerts major actions in carbohydrate, lipid, and protein metabolism through activation of the IGF-IRs. This primary receptor for IGF-I is a heterotetrameric tyrosine kinase membrane receptor. It displays selective, but not exclusively, binding affinity for IGF-I, because IGF-IR can bind both IGF-II and insulin with less affinity ligand. IGF-IR undergoes autophosphorylation and conformational changes that trigger an intracellular signaling cascade through the insulin receptor substrates 1 to 4 (IRS1- IRS4) and Src homology and collagen. These molecules activate the two main downstream signals of IGF-IR: the mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt pathways. IGF-IIR can bind these growth

factors but acts as a signal decoy and does not transduce the signal intracellularly. The last two members of the insulin receptor family are the insulin receptor (IR) and, especially in tumor cells, the hybrid receptors IGF-1R/IR. The hybrid receptors also signal after binding IGF-I or IGF-II, similar to the function of IGF-1R. In normal conditions, both the IGF-1R and insulin receptor (IR) signaling pathways have overlapping functions and complement each other. Differences in the metabolism, availability of the ligand, receptor expression, or pharmacologic manipulations may change the equilibrium in signaling between those two systems. The development of cancer is determined by a combination of environmental factors and genetic predisposition. Recent evidence suggests that dietary and related factors such as physical activity and body size may contribute to malignancy progression through their effects on the serum concentration of IGF-I and its binding proteins. The molecular mechanisms by which the GH-IGF-IGFBP axis is deregulated in malignant cells is complex, and abnormalities at each of the levels have been described in different tumors. Overexpression of the growth factors (IGF-I or IGF-II) or the receptor, by either gene amplification, overexpression of convertases or transcription factors, have been observed in different tumors. The polymorphisms in the genes along the GH/IGF-1 axis could influence hormone levels and cancer risk, especially breast cancer (70, 71). Also, post-translational modifications of the IGF-1R by glycosylation and the potential co-activation of the insulin pathway through their co-expression or through hybrid receptors (IGF-1R/IR) are further potential mechanisms associated to cancer. Modification of the concentration of IGF-BPs (especially IGFBP-1, IGFBP-3, and IGFBP-5) or of the insulin receptor can modify the overall activation of the pathway. Finally, loss of IGF-1R, a negative regulator of IGF signaling that works by as a decoy by binding the growth factor, could drive cells into an IGF-1R - dependent growth. Expression of the IGF-1R has been reported in a broad panel of tumor types. Being so ubiquitous, IGF-1R could play different roles in different tumor types or cellular contexts. Some tumors may be dependent on IGF-1R signaling for survival, and its inhibition might trigger apoptosis and a subsequent cytotoxic effect. This could probably be the mechanism behind the dramatic responses observed in tumors like Ewing sarcomas. Some other tumors may rely on IGF-1R for proliferation, like neuroendocrine tumors. Inhibiting IGF-1R will produce a cell cycle arrest and, thus, a cytostatic effect. Other tumors may have IGF-1R overexpression as a survival mechanism against cytotoxic insults, and combining chemotherapy with an IGF-1R inhibitor may overcome this mechanism of resistance. This could be the case of the observed synergy between chemotherapy or radiotherapy and IGF-1R inhibition, as well as with other targeted therapies like trastuzumab, EGFR inhibitors, and hormone therapies. Recently, discoveries of GH-IGF-IGFBP axis's actions in cancer allowed the design of specific inhibitors that may interrupt the signaling associated with this axis. The ability to manipulate these pathways hold not only significant ther-

apeutic implications but also increase the chance of deeper insight about the role of the axis in carcinogenesis and metastasis. About 25 molecules are currently at different stages of development, including both tyrosine kinase inhibitors and monoclonal antibodies (72, 73). Further approaches are being developed, including peptides, proteins, or antisense oligonucleotides that antagonize IGF-1R. However, these agents have not reached the clinic. Because of compensatory crosstalk between IGF-1R and IR, dual IGF-1R and IR tyrosine kinase inhibitors may have superior anti-tumor activity compared to anti-IGF-1R specific antibodies (74). Hyperglycemia, mild skin toxicities (rash, flushing, pruritus, acne), and fatigue as common toxicities of these drugs. Other observed toxicities, like reduction in CD4+ lymphocytes, thrombocytopenia, and transaminitis, do not seem to be related with the mechanism of action but with specific antibodies. Hyperglycemia seems to be frequent (around 20%) but tolerable, mild to moderate (grades 1 and 2), reversible, and manageable with an oral hypoglycemic drug, such as sulfonylureas. Although still premature, these promising data, consistent with the effect seen with chemotherapy, justify its investigation in cancer types like prostate cancer, advanced head and neck cancer, and locally advanced pancreatic cancer where radiation therapy forms part of the main initial treatment. The estrogen pathway and its relation with IGF has been explored in tamoxifen-resistant breast cancer models. This resistance is in part mediated by IGF-1R/mitogen-activated protein (MAP) kinase signaling pathway and c-Src seems to be one of the critical elements. Thus, the small molecule XL-228, tyrosine kinase inhibitor of both Src and IGF-TK seems a good candidate for development in this setting. Clinical experiments demonstrated no significant modifications in GH response to GHRH after acute or chronic treatment with tamoxifen compared with the basal test. On the contrary, chronic tamoxifen treatment induced a significant decrease in serum IGF-I levels. These data confirmed the inhibitory effect of tamoxifen on IGF-I production, but seem to exclude the possibility that this effect may be due to an inhibition of GH secretion (75). Results of translational research aimed to identify biomarkers associated with sensitivity to IGF-1R/IR inhibitors remains the only way to select the right patients who would benefit of the appropriate single-agent therapy, or the most suitable combination therapy. Mutations in downstream signaling pathways such as PIK3CA, the expression of IGF axis ligand-receptor pairs, markers associated with epithelial-mesenchymal transition, free IGF1 ligand levels, and a host of multi-gene biomarkers are the most promising molecular features that may predict sensitivity to IGF-1R/IR inhibitors.

GH/IGF-1 axis and oncological risk

Many experimental *in vitro* models evidenced the link between GH and IGF system with cancerogenesis processes. In 1950 (76) it was reported that high-dose treatment with extracted GH was able to induce development of lungs, adrenals, ovaries, and breast neoplas-

sia in female rats. Studies with transgenic mice confirmed the role of GH in animal oncogenesis (77); GH was found to stimulate the cellular proliferation of different cancer cell lines, including human leukemic lymphocytes and murine erythroleukemic cells. Another important aspect confirming the effect of GH on carcinogenesis is the GH synthesis in a different number of extrapituitary organs including both normal and neoplastic mammary tissues so a possible local paracrine/autocrine effects independent of or additional to endocrine-mediated IGF-I action could be supposed (78). Furthermore in prostate cancer cell lines, GH is able to increase cell proliferation probably due to the coexpression of pituitary GH as well as GH-R mRNA isoforms; so an autocrine-paracrine pathway seems to be able to stimulate prostate growth (79, 80). Unlike GH, the IGF system modulates the oncogenesis process at different steps (81); once quiescent cells are made competent and the stimulation with IGF-I is sufficient to complete the cell cycle, promote cell proliferation and arrest apoptosis, both in normal and cancer cell lines (82, 83). The effects of IGF-I stimulation is related to the density and functionality of the IGF-IR and this is fundamental for the IGF-I-mediated proliferation (82). A confirmation of IGF-I/IGF-IR involvement in oncogenesis has been shown *in vitro* experiments where knockout of IGF-IR gene was capable of decreasing cell proliferation and increasing apoptosis. Moreover the IGF-I/IGF-IR system seems to further influence tumoral progression promoting different mechanisms such as adhesion and migration of cells, angiogenesis within neoplastic tissues and surrounding areas (82, 84).

In vitro, IGF-I does not seem to promote cellular transformation, rather seems to stimulate proliferation of transformed cell clones and the growth of pre existing tumor tissues. In fact liver-specific IGF-I-deficient mice treated with chronic IGF-I administration are characterized by the presence of processes of growth and metastatic proliferation of colon adenocarcinomas transplanted cells (85). As for GH, also for IGF-1, endocrine and autocrine/paracrine effects in a number of tissues and cell systems are reported. Regulation of cancer growth through an IGF-I/IGF-IR-mediated autocrine/paracrine loop has been reported by the demonstration of IGF-IR mRNA expression in colon carcinoma, more abundant than IGF-I mRNA expression (86), and predominates in samples from colon tumor vs the adjacent normal mucosa, a condition suggestive of paracrine/autocrine modulation (87). *In situ* hybridization studies have evidenced that IGF-I is expressed in the stromal cells and very rarely in the epithelium of breast cancer while IGF-IR has been found on neoplastic breast epithelial cells suggesting the presence of a paracrine interaction (88). IGF-I levels are significantly higher in women with breast cancer (89) and in men with prostate tumors (90), respect to normal population. In the European Prospective Investigation into Cancer and Nutrition (EPIC) study, high circulating IGF-1 concentration was positively associated with risk for prostate cancer (25). In a study of survey on 152 cases of prostate cancers, among nearly 15,000 men subjected, Chan et al. (91) provided evidence of a 2.4-fold greater cancer risk asso-

ciated with IGF-I levels in the upper quartile of the normal range, compared with patients whose IGF-I values were in the lower quartile. Another study conducted by Hankinson et al. (92) reported that the relative risk of breast cancer in premenopausal women with IGF-I levels in the upper tertile of the normal range was 2.3 times higher than in patients with IGF-I in the lower tertile. In particular women below age 50 yr, the highest IGF-I tertile was associated with an increase in breast cancer risk to 4.6 and, after inclusion of IGFBP-3 levels in the multivariate analysis, to 7.3 (92). Ma et al. (93) analyzed IGF-I and IGFBP-3 levels in 193 men diagnosed with colorectal cancer over a 12-yr follow-up, and reported that the relative risk in men having IGF-I levels in the top quintile was 2.5 times greater than in those whose IGF-I was at the bottom quintile. Inversely, the relative risk calculated by IGFBP-3 quintiles was 0.28 for top vs bottom quintile. Another important aspect suggesting the link between the GH/IGF system and human cancers is the demonstration that GHRH-antagonists, GH-antagonist, and somatostatin analogs elicit antineoplastic activity by altering the GH/IGF-I axis at the pituitary level or by inhibiting autocrine/paracrine activity of GHRH, GH, and IGFs (94-97). Moreover, preliminary findings have indicated that the GH-antagonist decreased proliferation, stimulated apoptosis, and reduced metastatic spreading of MCF-7 breast cancer cells (78).

GH/IGF-I axis and heart

GH and IGF-I are potent cardiotropic factors and their involvement is similarly crucial in cardiac diseases. Their role will be here examined relatively to three main settings: the process of cardiac regeneration, the chronic consequences of altered GH secretion and cardiovascular data from the general population. Analysis of these topics will then try to provide reasonable argumentations for their therapeutic use in heart disorders.

Cardiac regeneration mediated by GH/IGF-I

Apoptotic death and necrosis are relevant events in a number of cardiac diseases. Experimental cardiac infarction is associated with an upregulated autocrine IGF-I system which is capable of stimulating DNA replication and mitotic division in viable cardiac myocytes, as confirmed by enhanced proliferating cell nuclear antigen (PCNA) message and transcript (98). IGF-I directly promotes survival and proliferation of resident cardiac stem cells, with consequent improved myocardial regeneration after myocardial infarction (99). This antiapoptotic effect also comprises of indirect actions, as mesenchymal stem cells overexpressing IGF-1 can attenuate infarct by stimulating VEGF expression, which then modulates neovascularization and myogenesis (100). In humans, rhGH increases IGF-I levels in parallel with circulating endothelial progenitor cells (EPC, a sensible predictor of cardiovascular outcomes), these latter exhibiting improved colony forming and migratory capacity, enhanced incorporation into tube-like structures and augmented

eNOS expression (101). IGF-I treatment of human EPCs improved function and attenuated cellular senescence, further suggesting a role for IGF-I in aging EPC. Based on the previous, local IGF-I delivery and genetic manipulation of IGF-I expression seem effective tools to obtain a modulation of cardiac regeneration. These forms of treatment could be repeated over time to reduce progressively tissue scarring and expand the working myocardium.

Cardiovascular consequences of GH and IGF-I excess

Cardiomegaly is a hallmark of visceromegaly associated with acromegaly. It is traditionally thought to progress toward cardiac failure unless GH-excess is removed. Interindividual differences in cardiac involvement likely depend on varying age, familiarity, duration of disease, resistant arterial hypertension (up to 50% of patients are hypertensive), longstanding diabetes mellitus (about 1/3 patients are glucose-intolerant and just as many are diabetic), ventilatory disorders (obstructive sleep apnea affects ~80% of cases), arrhythmia (present in up to 30-40% of patients, being mainly supraventricular and largely conditioned by cardiac hypertrophy and sleep apnea), as well as cardiac valve disease (mitral and aortic regurgitation affects 75% of patients, mostly those with cardiac hypertrophy). The interplay of altered glucose homeostasis, hypertension and aging explain a stepwise increase in the prevalence of cardiac hypertrophy, diastolic and systolic dysfunction (102). Two main goals should be, intuitively, pursued to modify long-term cardiovascular consequences of acromegaly. First, it is crucial to achieve complete control of GH hypersecretion by neurosurgical cure or pharmacological management. Somatostatin analogs are a valid tool to achieve control of cardiovascular complications of acromegaly and recent studies displayed similar global effects of octreotide LAR and lanreotide autogel on left ventricle mass, diastolic and systolic function. The GH-antagonist pegvisomant has also been found to yield significant cardioprotective results in a 18-month analysis (103). Treatment with pegvisomant reduces heart rate and improves rhythm abnormalities in acromegaly (104). Secondly, effective complementary treatment of comorbidities is essential: antihypertensive agents, hypolipemic and antidiabetic compounds, as well as noninvasive mechanical ventilation are all instrumental to achieve full control of cardiopulmonary complications of acromegaly. In this scenario, literature data linking heart valve disease to the use of dopamine-agonist warrant appropriate surveillance in acromegalic patients treated with high-dose cabergoline.

Cardiovascular effects of GH deficiency (GHD)

It is known that hypopituitary patients with GHD bear an increased risk for coronary artery disease and impaired cardiac function leading to a 2-fold greater cardiovascular mortality than the general population.

Childhood- and adulthood-onset GHD reduces left ventricle mass and diameter proportionately to the duration of GHD or to IGF-I levels. This is concomitant to an impairment of cardiac performance at peak exercise and abnormal diastolic filling (105). In large cohorts, cardiac dysfunction occurs proportionately to the severity of GHD, with systolic and diastolic abnormalities affecting 45-78% of GHD patients (106). The so-called hypokinetic syndrome of GHD coexists with other key risk factors, which include visceral adiposity, insulin resistance, atherosclerosis and hypercoagulability. Importantly, patients with GHD following cure of acromegaly or Cushing's disease are more prone to develop hypertension, diabetes mellitus and stroke than those with GHD from any other cause (107). The influence of obesity is equally important as seen in studies on adults with Prader-Willi Syndrome, a monogenic disorder associated with morbid obesity and GHD (108). In populations with classical GHD, GH therapy produces positive cardiac effects depending on patient's medical history, multiplicity of pituitary deficiencies and comorbidities. A metaanalysis reported that GH treatment was associated with a significant increase in LV mass (+10.8 g), septum thickness (+0.28 mm), LV posterior wall (0.98 mm), end-diastole diameter (+1.34 mm), and stroke volume (+10.3 mL), while overall effect sizes were not significant for most indices of diastole (109).

General population studies

Most studies have been so far published on IGF-I, with a role for GH necessitating further evidences. IGF-I is thought to influence cardiovascular disease (CVD) through complex mechanisms, yet results are contrasting. Following a Danish study on ischemic heart disease (IHD) that reported a significant association between the 15-yr risk and low IGF-I and high IGFBP-3 levels (44), the Rancho Bernardo Study confirmed the inverse association between IGF-I levels and IHD mortality in subjects aged over 50 yrs but no association with non-IHD cardiovascular (CVD) mortality (110). A key study by Lamberts' group found IGF-I bioactivity assessed by kinase receptor activation (but not total or free IGF-I levels) to be associated with extended survival and reduced CVD risk in elderly men followed up for > 8 yrs (111). At variance with these data, the NHANES and the Third NHANES Mortality Study carried out in 6226 adults aged 20 yr or older failed to confirm these associations (112). Moreover, there is evidence that a reduced GH-IGF1 signaling increases lifespan in animal models, while a blunted activity in the IGF-I pathway also seems to be associated with longevity in humans (38). In line with this, a population-based study of 642 individuals aged 50-89 yrs found that IGF-I levels in the fourth quartile were independently associated with > 5-fold increased risk of cardiac heart failure (113). The association between IGF-I and mortality is, therefore, not linear and divergences may result from unadjusted confounding phenomena, assay drawbacks or differences in demographic data. These discrepancies are partly recon-

cited by recent evidence showing that both elevated and reduced IGF-I levels may double the risk of CVD mortality, thus that a U-shaped relationship links IGF-I to CVD mortality (114).

Cardiovascular effects of GH or IGF-I administration

In the last 15 years, debated evidence has accumulated on GH or IGF-I utility as therapeutic agents in cardiac failure. Chronic heart failure (CHF) is featured by (primary or secondary) structural and functional cardiac abnormalities that coexist with altered vascular reactivity, this latter being primarily due to endothelium-dependent defects and loss of bioactive endothelial nitric oxide. Increased peripheral vascular resistance further aggravates LV failure. Importantly, GH improves eNOS activity and increases NO availability via IGF-I. Following the description of a GHD patient improving dilated cardiomyopathy with rh-GH treatment (115), several subsequent studies have suggested that rhGH may increase LVEF and decrease systemic vascular resistance in CHF patients. These effects show a rapid onset and depend not exclusively on the improved endothelium-dependent vasodilatation, but also on modifications in loading conditions, myocardial contractility and cardiac morphology. A meta-analysis showed significant overall effects of rhGH on LV walls, end-diastole volume, ejection fraction, systemic vascular resistance, NYHA class, exercise duration and VO_2 max in CHF patients (116). Oppositely, double-blind placebo-controlled studies on rhGH effects failed to show direct effects on cardiac function or structure in CHF patients of different aetiologies (117). Therefore the results are conflicting (118). Scant but relevant are the findings on the therapeutic usefulness of IGF-I. Acute infusion of rhIGF-I in healthy subjects increases cardiac output, heart rate and stroke volume significantly (119). The possibility that IGF-I may be beneficial in cardiac disorders has been explored in CHF patients, and rh-IGF-I yields positive effects on the cardiac index, stroke volume and peripheral resistance (120). However, long-term effects are unknown. It is also interesting to note that, recently, similar results on LVEF, LVM, and exercise capacity were reported after treatment with GH releasing hormone and ghrelin in patients with CHF (121).

GH/IGF-I axis and glucose metabolism

It is well known that diabetes and insulin resistance are the major determinant of metabolic syndrome involving in cardiovascular mortality (122, 123) and that alterations of the GH/IGF-I contribute in determining cardiovascular disease with increased risk for cardiovascular morbidity and mortality both in GH deficiency (GHD) and excess (103). The GHD mediated effects on the cardiovascular function are directly played on the heart and endothelium but also in indirectly manner by increasing all cardiovascular risk factors with particular role made by central adiposity, insulin resist-

ance and finally by glycemic metabolic derangement and metabolic syndrome (124). Visceral obesity is a central feature of metabolic syndrome and furthermore is well known that obese subjects have a functional GH deficiency; in a recent paper Di Somma showed an higher prevalence of metabolic syndrome in GHD obese patient than GH sufficient obese patient suggesting a possible maladaptive role for functional GHD in obesity with increased metabolic risk factors in severe obesity independently from BMI per se (125). Epidemiological perspectives studies confirm the association between low IGF levels and all causes and cardiovascular disease mortality (110) and in prospective observational study IGF-I showed a significant inverse association with subsequent 2-h glucose concentrations independently correlates of IGF-I and risk factors for glucose tolerance providing protective role for IGF-I against development of glucose intolerance (126). Furthermore in large epidemiological cohort study type 2 diabetes showed a curvilinear association with IGFI SDS (127) and in recent cross sectional epidemiological study IGFI is an independent predictor of diabetes (128). Many studies have confirmed that GH therapy in GHD patients reduce visceral fat, improves insulin sensitivity and atherosclerotic profile with in turn reduction in prevalence of cardiovascular risk factor and metabolic syndrome (129). High prevalence of metabolic syndrome in adult GHD patients (respectively 51.8% in USA and 28.6% in Europe) was recently reported in a large series without prevalence difference after GH replacement although baseline MetS status and obesity were strong predictors of Mets after GH treatment (130). Taking metabolic advantage from molecular analogy between insulin and IGFI some study demonstrated either an increase in insulin sensitivity and glycemic tolerance in type 2 diabetes with sever insulin resistance (131) or a reduction in insulin doses in type 1 diabetes using IGFI administration or equimolar IGFI-IGFBP3 complex (132, 133). In acromegalic patient a reduced life expectancy was reported reduced first due to cardiovascular disease (134) with hypertension and glucose intolerance as important independent contributory factors to the vascular morbidity associated with acromegaly (135). Retrospective comparative study reported a significant increase of cardiovascular risk factors in acromegalic patients matched for age and gender from general population with a prevalence of diabetes even 2.9 fold higher (136) while diabetes's prevalence in acromegaly ranges in different study from 19-56% (106). Although somatostatin and somatostatin analogues reduce insulin secretion a recent metaanalysis on somatostatin analogues on glucose homeostasis suggest that modifications of glucose homeostasis induce by SSA may have an overall minor clinical impact (137). Non detrimental SSA therapy's effect on glucose metabolism was confirmed in long term first line SSA therapy without any differences between SSA treated and surgically cured patients with increasing BMI as major predictor of deterioration of glucose tolerance (138). In patients with acromegaly receiving lanreotide as primary treatment only 17% had

a worsening of glucose status (60% had no change, 27% had an improvement) and deterioration was significantly associated with smaller GH decreases during primary lanreotide treatment (139). Only limited data on glucose metabolism during pasireotide (SOM 230) a new multireceptor ligand SSA analogue in acromegaly are now available with significant increase in HbA1c levels after 3 months' therapy (140) according with octreotide and lanreotide 6 and 12 months therapy's studies (141). Data from pegvisomant (a new GH receptor antagonist) therapy in acromegaly demonstrate a significant reduction on HbA1c levels in diabetic and non diabetic patients with a correlation with IGFI reduction. Urbani C et al. found that the prevalence of diabetes or impaired glucose tolerance is higher during SSA than at diagnosis or during pegvisomant (142). Increase in insulin sensitivity after short term therapy (143) by a reduction in overnight endogenous glucose production related to reduced FFA levels was also demonstrated (144) according with data after acute pegvisomant administration using hyperinsulinemic euglycemic clamp in healthy subjects (145). Conflicting data from d3GH receptor polymorphism (a common polymorphic GH receptor variant) impact on metabolic phenotype are emerging in GH deficiency and acromegalic patients. In fact in GHD adults patients the presence of d3GH receptor polymorphism may cause a major sensitivity to negative metabolic GH effect (146). In acromegalic patients was reported a slightly higher prevalence in diabetes mellitus in d3GHR acromegalic patient (147); but on the contrary Montefusco reported a decreased BMI with a preponderance of normal glucose tolerance between d3GHR patients in contrast to fl/IGHR but similar prevalence of overt diabetes mellitus (148). Insulin resistance, impaired glucose tolerance and diabetes mellitus due to acromegaly disease generally improve with the GH/IGFI normalization but no comparative study are available on diabetes's best therapy and we have just suggestions pointed to oral secretagogue hypoglycemic agents and or insulin therapy, particularly new long acting insulin analogues when metabolic disease arise during SSA analogues therapy in responsive patients (149, 150) or Pegvisomant's use in somatostatin analogues resistant patients with coexistent diabetes mellitus (151).

GH/IGF-1 axis and reproduction

Growth hormone (GH) is obligatory for growth and development, but it has also an important role in the reproductive process. It is required for sexual differentiation and pubertal maturation and it participates in gonadal steroidogenesis, gametogenesis and ovulation (152-154). The effects of GH are exerted at three levels: a permissive action on gonadotropins secretion and action, a peripheral direct, or IGF-1 mediated, effect on gonadal development, and a paracrine effect, by locally produced IGF-1 (155). Lobie et al. used immunohistochemistry to localize GH receptor and binding protein and found intense immunoreactivity in the

male reproductive system, including Leydig and Sertoli cells, vas deferens, prostate, ductus epididymis and seminal vesicles (156). This finding suggested that GH might stimulate local IGF-1 production. This parallel what is seen in ovarian tissues, where several authors found a strongly GH-binding activity, GH receptor (GH-R) immunoreactivity and mRNA encoding GH-R (157). The need for local GH and IGF-1 production has been related to the presence of a haematogonadal barrier: even if the gonads are highly vascularized, some cells in the ovary and testis must remain physically separated from systemic circulation since might be antigenic. For this reason some GH actions might mediated by hormones locally produced. Indeed, the entire GH/placental lactogen (GH/PL) gene cluster (chromosome 17), comprising five highly related genes (GH-N, PL-L, PL-A, GH-V and PL-B), is transcribed in the human testes and ovary, with GH-V (the GH variant) being the most active gene transcriptionally (158, 159). In summary, GH presents a large variety of biological actions on reproductive tissues and IGF-1 may mediate some, but not all, of these effects. Nevertheless, the clinical role of systemic and paracrine GH/IGF-1 axes have seldom been explored. Early studies documented that GH is required for normal sexual maturation in mammals: Laron and others showed an association between isolated GH deficiency in boys and delayed puberty, with the frequent clinical observation of micropene (160, 161) or smaller testes. Later on, Laron also showed that in these patients the administration of GH restored normal pubertal development (162). GH has been also implicated in the development and function of wolffian duct-derived structures such as the prostate and seminal vesicles (163). Regarding female reproduction, it was suggested a role of GH as intraovarian modulator and, in a series of *in vitro* studies, it was found that incubation of granulosa cells with insulin and IGF-1 increased their responses to stimulation by FSH (164, 165). So the co-treatment with GH augmented the ovarian response to stimulation by gonadotrophins. Furthermore, some studies found that GH may stimulate particular follicle populations selectively and proliferation of luteinized granulosa cells via an FSH and IGF-1-independent mechanism (166-168). On the basis of these encouraging results and after demonstration in pubertal boys of a clear effect of GH on Leydig cell function and on pubertal maturation of the testis, it was suggested an additional role of GH in spermatogenesis. These studies were mainly performed in the 80s' and 90s', on very small cohort of men, in uncontrolled studies. Nevertheless some of the findings are of interest showing a link between GH deficiency and some alterations of spermatogenesis. Shimonovitz et al. found more than 50% of azoospermic men, as compared to 18% of oligozoospermic men, showed subnormal GH response, as determined by the clonidine test. They proposed that GH may be involved in maturation arrests (169). GH effectively increases sperm concentration and/or motility in males resistant to other forms of fertility treatment in some, but not all, clinical studies with infertile patients (170-172). It has

been proposed that GH affects sperm motility directly, or by actions mediated through Sertoli cells independently of IGF-1 (155). GH actions on spermatogenesis also can be mediated by influences on testosterone synthesis: GH therapy in GH-deficient males augments the testosterone response to human chorionic gonadotropin, increases plasma estradiol levels, and increases the abundance of gonadotropic binding sites (171). The putative steroidogenic action of GH could involve hepatic/local IGF-1 modulation, although *in vitro* studies suggest an IGF-1-independent role for GH in Leydig cell function: Kanzaki and Morris have shown that GH increases androgen synthesis in rat progenitor Leydig cells. GH is thought to exert its effect at initial ratelimiting steps in the steroidogenic synthetic pathway, acting on synthesis of StAR protein (which regulates the translocation of cholesterol to the inner mitochondrial membrane) and 3 β -hydroxysteroid dehydrogenase (3 β HSD) (which converts pregnenolone into progesterone) (173). Also reproductive dysfunctions in some women have been associated with partial GH deficiencies. Clinical studies have shown that GH therapy may be useful in some, but not all, infertile women (174, 175). GH administration to hypogonadotrophic anovulatory women significantly reduces the dosage and duration of hMG treatment required for ovulation induction and increases the percentage of successfully treated patients (175). GH therapy may also improve the success of *in vitro* fertilization techniques by enhancing the hyperovulatory response to hMG (176). Although the optimal therapeutic role of GH in induction or ovulation and spermatogenesis has yet to be totally defined, the reality of its interaction with gonadotrophins has clearly been demonstrated *in vivo* (177). More recently, the availability of human recombinant GH, better definition of GH deficiency, allow a rigorous exploration the potential clinical use the strong connection between physiology of reproduction and growth and development. In addition, systemic and local GH seems to have a complementary autocrine or paracrine actions reflecting a 'fine-tuning' mechanism to regulate gonadal function (178).

GH/IGF-I and bone

Growth hormone (GH) and its mediator insulin-like growth factor I (IGF I) plays an important role in longitudinal bone growth and in acquisition of peak bone mass (PBM) during childhood and adolescence. Studies human and animal models have highlighted the integral effects of GH and IGF-I on skeletal development, linear growth, and the achievement of peak bone mineral density. The acquisition of peak bone mass, occurring at the end of skeletal maturation, is an important determinant of later development of osteoporosis and subsequent fracture risk (179). In addition, to the effects on longitudinal growth after the closure of epiphyseal growth plates, GH and IGF-I are anabolic hormones and have a key role in the regulation of bone modelling and remodelling (180). GH acts

both inducing IGF-I in bone or playing direct effects on skeletal cells (Giustina A et al. 2008). The effects of GH and IGF on skeleton are modulated by interactions between circulating IGF-I and IGF-BPs and the locally produced IGF-I and IGF-BPs. In addition, the skeletal actions of GH/IGF-I axis are regulated by systemic hormones, such as sexual hormones and PTH (180). In particular, IGF-I and PTH have synergistic actions on bone and some effects of the anabolic actions of PTH are mediated by local production of IGF-I, as has been shown *in vitro* and *in vivo* studies both in animals and humans. On the other hand, PTH can induce skeletal IGF-I expression both *in vitro* and *in vivo* (181). Based on the aforementioned findings, it could be argued that diseases affecting GH/IGF-I axis induce skeletal abnormalities. In this section we will discuss skeletal abnormalities occurring in GHD and acromegaly, and the role of GH and IGF-I in the osteoporosis.

GH deficiency and Bone

GHD syndrome is mainly characterized by high fat mass with central adiposity and low lean body mass, dyslipidemia, hypertension, elevated inflammatory markers, insulin resistance, increased intima-media thickness, reduced exercise capacity, impaired quality of life (QoL), low bone mineral density (BMD) and increased risk of fractures (182). As proof of the state of low bone turnover, serum levels of osteocalcin and bone resorption markers are decreased and the same patients report a renal, skeletal, and intestinal cell insensitivity to PTH, leading to a mild state of PTH resistance and increased serum PTH levels (180). In the development of osteopenia a main role is played by GHD as suggested by the reduction of bone mineral density (BMD) both in patient with isolated GHD that in patient with multiple pituitary hormone deficiencies (MPDH) (183). In addition, only patients with very severe or severe GHD have a significant reduction of BMD, associated with abnormalities of bone markers (183). This confirms the importance of GHD in pathogenesis of osteopenia in hypopituitary patients (183). Associated other pituitary hormone deficits and/or replacement therapies have also been proved to be important factors in the pathogenesis of bone loss (180) as well as the age of onset of GHD and the age of the patients (180). Infact, in patients with childhood-onset GHD there is a marked reduction in vertebral BMD (T-scores often between 1 and 2; about one third of the patients have T-scores of 2.5 or less) (180). Other way, the vertebral T score results normal (1 or above) in patients with adult-onset GHD (180). The reduced bone mineral content and density in patients with childhood-onset GHD is probably due to the lack of attainment of bone mass during adolescence and the longer disease duration while the pathogenesis in patients with adult-onset GHD it is less clear (180, 183). The correction for height has demonstrated that short stature is less significant in the determination of a lower BMD observed in childhood-onset GHD, whereas changes in body composition may play a significant

role (180). There is no definitive data demonstrating an increase in the risk of fractures in patient with decreased bone mass for untreated GHD. However, the risk of non-vertebral fractures (prevalently localized at the radius) has been found to be about 3 times more often in untreated GHD patients, suggesting a loss of cortical bone (180). As far as vertebral fractures, recent studies reported an increased incidence of vertebral deformities (180). Other pituitary hormone deficiencies or hormonal replacement therapy do not influence the prevalence of bone fractures while the degree of GHD affects this prevalence (180). In particular, in GHD patients, BMD does not correlate with the prevalence of fractures (180) as well as in other forms of secondary osteoporosis (180). On the other hand, these patients had an increased risk of falls for decreased muscular strength and impaired vision could be contributed to risk of fracture (180). For what concerns the GH replacement therapy it has been observed an initial reduction in BMD due to a stimulation of bone turnover by GH that leads to an increase in remodeling space (182). However long-term GH replacement is associated with an increase of bone mass (182). Jørgensen et al. found an increase of bone markers during the study and an increase in bone mineral content (BMC), BMD and bone mineral area (BMA) in the lumbar spine (L2-L4) and total body measurements after nine months of GH replacement therapy and in the femur (not BMA) measurements after a longer therapy. These different effects in the lumbar spine, total body and femur can be explained probably with the different actions of GH on cortical and trabecular bone. GH replacement therapy increases lumbar (L2-L4) spine and femur neck BMD in younger as well as elderly GHD patients (184). Effects of GHD on bone metabolism and the results after GH replacement therapy are influenced by the sex of patients, too. Many authors have put in evidence that after 18-24 months of GH therapy there was a small increase of BMD in male patients with adult-onset GHD, instead there were not significant evidences of increase in female patient. In a recent study, a 2 yr GH replacement normalizes IGF-I levels, increases bone mass and improves bone turnover both in men and in women with GHD without any difference between the two groups, provided that the dose of GH was modulated on the basis of IGF-I levels. Women receiving oral estrogens should receive a GH dose approximately doubled, as compared to men and women not receiving oral estrogens, to achieve similar effects on bone density and turnover. In particular, GH replacement dose to be successful on bone mass and turnover depend on gender in hypopituitary patients aged below 50 yrs (185).

GH excess and Bone

Gigantism and acromegaly are conditions associated with elevated levels of GH and IGF-I. In these patients the body water and the lean body mass are increased and the body adiposity is decreased (179). As concern the bone metabolism, these patients have an increase

of bone turnover, calcium kinetics and bone histomorphometry (180). Both bone formation and resorption are increased, but the increase of bone resorption markers is higher than the bone formation markers. These difference could be associated to the degree of bone loss observed. Moreover there is a correlation between serum GH and IGF-I levels with bone markers (180). In addition, active acromegaly is associated with increased serum concentrations of PTH, 1,25-dihydroxyvitamin D3, calcium, and phosphorus (180), probably due to the GH stimulation on parathyroid gland (180). The cortical and the trabecular bone have a different sensitivity to GH excess so the skeletal site play an important role in the determination of the effects of GH excess on BMD (180). In fact acromegalic patients have an increased in BMD at the lumbar spine which is rich in trabecular bone, whereas there is an increase in the BMD at the forearm where the cortical bone has a major representation (180). In spite of the variability of data on BMD in acromegaly, due to the different densitometry techniques, the results obtained with peripheral quantitative computerized tomography and bone biopsies have demonstrated different effect of the GH/IGF-I effects on the trabecular and cortical bone (180). In acromegaly other factors could influence bone BMD such as age, gender, and the presence or absence of hypogonadism (180). In fact, it has been observed that the decrease of vertebral BMD is correlated with the increase of the duration of the hypogonadism (180). Even if there is not a large number of studies about the risk of fractures in acromegaly (180) postmenopausal women with active acromegaly have a higher incidence of radiological vertebral deformities than non acromegalic postmenopausal women (186). This can be explained with an association between acromegaly and an increased risk of osteoporotic vertebral fractures (180, 187) which is correlated with the duration of active disease and the serum level of IGF-I, whereas there is no significant relationship between vertebral fractures and BMD values in acromegalic patients (180, 187). As concern the effects of acromegaly's treatment on bone metabolism, an improvement has been observed in fractures and BMD after surgical treatment (transphenoidal pituitary surgery) or pharmacological therapy. However data about treatment and his influence on bone are limited (180). Some authors have demonstrated that therapy with the GH antagonist pegvisomant, a selective antagonist of the GHR, which controls IGF-I secretion lead to a normalization of bone turnover (180). A reduction has been demonstrated in PTH target organ sensitivity and a reduced nocturnal rise in PTH after the normalization of GH/IGF-I levels (180). The hormonal control in postmenopausal women lead to a reduction of risk of radiological vertebral fractures than women with active disease. These improvement is not correlated with BMD value (180). In conclusion, the mechanisms underlying the metabolic bone disease of acromegaly are multifactorial and possibly include an increase in bone resorption secondary to IGF-I excess and to sex hormone deficiency.

Postmenopausal and senile osteoporosis

The occurrence of postmenopausal osteoporosis is about 35% of white women and 19% of white elderly men (180). Among risk factors estrogen deficiency plays a main role both in postmenopausal osteoporosis and in possibly male osteoporosis (180). Secondary hyperparathyroidism, vitamin D deficiency, decreased IGF-I levels and a large number of other factors could lead to the bone loss, probably due to decrease of osteoblastic functions in elderly population (180). A reduction of GH and IGF-I secretion, called "somatopause", has been demonstrated in advancing age. This event could be implicated in the pathogenesis of osteoporosis (180). In fact some studies have observed a reduction of serum IGF-I levels in postmenopausal women which is correlated with BMD. Moreover has been demonstrated that IGF-I promoter polymorphisms have been linked to bone mass (180). Both the serum IGF-I and the IGF-I contained in human cortical bone decrease with age (180). There are different evidences about the effects of rh-GH in postmenopausal female, males with idiopathic osteoporosis and elderly patients. In these patients has been observed an improvement of bone metabolism but the effect of rhGH on BMD is controversial (180). Some authors have observed a positive effect of rhGH treatment on increase in BMD in osteoporotic patients, other authors have demonstrated any beneficial effects although an increase of serum IGF-I levels (180). In a recent meta-analysis has been observed that older people have not any improvement in bone metabolism due to rh-GH treatment. This probably can be explained by the lack of controlled trials and the heterogeneity of the subjects examined (180). Recent studies have demonstrated that treatment with an orally active GH secretagogue (MK677) combined with alendronate can determinate an improvement of BMD only at femoral neck in postmenopausal osteoporosis (180). Recombinant human IGF-I can influence bone metabolism in humans, but there is no skeletal specificity and potential side effects which would limit its possible use in osteoporosis (180).

Obesity and GH/IGF-I axis

It has been shown that nutritional state is an important factor involved in the control of Growth Hormone (GH) secretion. In human obesity, the GH/IGF-I axis is altered at different levels. Basal GH secretion is blunted, with reduced GH half-life, frequency of secretory episodes and daily production rate and it has been reported that an increase in each unit of BMI, at a given age, reduces the daily GH secretion by 6% (188). GH secretion is impaired in response to all stimuli acting at the hypothalamus and to direct stimulation by exogenous GHRH (Growth Hormone Releasing Hormone). The plasma levels of the high affinity GHBP (Growth Hormone Binding Protein) are increased, with a positive correlation between serum levels of this molecule and both BMI and percent body fat mass. The physiological significance of increased GHBP in

obesity is unknown. Increased GHBP levels in obesity may serve to prolong the biological activity of GH, however various studies showed that the clearance of GH is accelerated in obesity. Because circulating levels of GHBP reflect GH receptor density, an alternative explanation might be that the greater density of tissue receptors acts to sequester GH more avidly from the circulation, and this phenomenon might represent tissue adaptation to reduced GH output in obesity. The reduced GH response to GHRH in obese fasting patients seems to be caused by both central and peripheral factors. Among central factors, an impairment of endogenous GHRH tone or an augmented somatostatin release has been hypothesized. It has been also suggested that the defect resides in the pituitary gland, with enhanced somatostatin release being responsible for the blunted GH response. However, many experimental evidences confirmed that both GHRH and somatostatin release are normal in obesity and that there isn't a somatotrope cell insufficiency, since we observe a great GHRH and GH-releasing peptide-6 induced GH release in obese subjects (189). In obesity, GH secretion is blunted in response to other stimuli acting at the hypothalamus (insulin-induced hypoglycemia, arginine, galanin, L-dopa, clonidine) and the pharmacological manipulation of the central neurotransmitter systems are capable of modifying, but not completely restore, the response to these stimuli. Amongst the peripheral factors, the role of the increased bioavailability of IGF-1, measured by IGF-1/IGFBP-3 ratio, and their possible increased negative feedback action on somatotroph cells has been underlined in some studies. However, in obesity, IGF-I plasmatic levels could be normal, low or high, despite high serum levels of the free fraction of IGF-I. Free IGF-I accounts for less than 1% of the total circulating amount of IGF-I and it is believed to be responsible for the bioactivity on target tissue. Two recent study (190, 191) indicated that free IGF-I are significantly increased in overweight and obese patients; therefore, it seems not correct to define obesity a condition of GH resistance or insensitivity. Finally, according to the presence of increased levels of free IGF-I, in obesity IGFBP-1 (Insulin-like Growth Factor Binding Protein-1) and -BP-2 plasma levels are blunted, due to inhibition by insulin, which is generally increased in overweight subjects, whereas IGFBP-3 levels are normal or high. On the other hand, some authors have shown that low IGF-I levels are inversely correlated with abdominal obesity, a condition associated with higher serum concentrations of non-esterified fatty acids (NEFA). The mechanism of this clinical evidence is not well known. We know that adipose tissue is a relevant source of proinflammatory cytokines and recent data suggest that inflammatory mediators may play a role in inducing reduced IGF-I bioactivity (192). The question of whether these mediators play a role in the IGF-I/IGF-binding protein system in obesity requires future study. Insulin and NEFA (or Free Fatty Acids, FFA) levels seem to be the most important peripheral factors involved in the blunted GH levels in obesity. Experimental and clinical data support the ex-

istence of a negative feedback exerted by circulating insulin on GH secretion. Insulin is able to reduce GH release from rat pituitary *in vitro*, suggesting an IGF-I-like effect, and it may be a major determinant in the suppression of GH output in the obese mouse by direct down-regulation of somatotrope function at pituitary level (193). Massive weight loss, inducing a decrease of insulin secretion, seems to be directly involved in the restore of a normal 24 h GH profile (194). The surgical treatment of severe obesity, after stabilization of body weight, decreases body mass index (BMI) and fat mass (FM) while preserving a normal lean body mass (LBM) as well as positively influencing insulin sensitivity. An interesting study in this field showed that in morbid obesity, after biliopancreatic diversion, the decrease in insulin secretion is the starting point for the changes in both body composition changes and somatotrope axis (195). In the same direction, recently, Cornford et al., showed a strong suppressed GH secretion in seven healthy non-obese men, after only 2 weeks of overeating and before any measurable weight gain; this phenomenon was accompanied only by a significant increase in insulin plasma levels, the likely mediator of the reduction in GH secretion (191). The direct infusion of FFA in normal weight subjects completely inhibits GH secretion. Several lines of experimental evidence indicate that high circulating FFA play an important role in the impairment of spontaneous and stimulated GH secretion in obesity, given the improvement of somatotropin release observed in obese patients after the administration of the antilipolytic agent acipimox (196). In such models such as PCO or old normal subjects, where the GH response to GHRH is similar to that in obese subjects, it has been shown how a pharmaceutical decrease in FFA levels can re-establish a normal GH response. However, the majority of these studies indicate that FFA reduction does not directly stimulate GH secretion, but augments the action of other stimuli at hypothalamus-pituitary level. The encouraging effects of GH in the treatment of Growth Hormone deficiency (GHD) syndrome have generated interest in the efficacy of using GH as anti-obesity drug. The rationale of this treatment is firstly the observed lipolytic effect, especially on abdominal body fat, and the concomitant anabolic action related to growth of lean body mass. Conversely, the insulin-resistance, the impaired glucose metabolism, the onset of water retention, edema, carpal tunnel syndrome, arthralgia are the main dose-related side effects of GH therapy. The dose of GH more efficacious in obesity, and without side effects, was in the range of the current dose of GHD treatment, or slightly more low. The studies present in literature showed no significant effects of GH administration on BMI or LBM, independently from duration of treatment. In the majority of cases, a significant reduction in visceral adipose tissue, ranging from 5% to 34% was observed, mainly in the clinical trial of at least 12 weeks of duration (197). In comparison, therapeutic programs, with caloric restriction, diet and physical exercise achieve the same results regarding the fat mass. In conclusion, in obesity a clear de-

crease in GH secretion with normal, high or low IGF-I, but high free IGF-I is observed. This condition is reversible and it seems to originate from peripheral factors, which alter hypothalamic-pituitary function. The restoration of normal GH/IGF-I axis function after weight loss suggests the presence of an acquired defect of metabolic origin. Clinical studies on the efficacy of rhGH as antiobesity drugs does haven't shown results in terms of weight loss significantly larger than those achievable with only diet-therapy. The observed effects of rhGH on body composition changes, with particular reference to reduction of abdominal and total fat mass are encouraging, but long term studies are needed.

GH/IGF-I axis and lymphohematopoietic system

Important evidences show existence of a relationship between neuroendocrine and immune systems. Peptides exert effects in a cytokines like manner on immune, lymphoid and red blood cells. Production and differentiation of forerunners of red blood cells, myeloid and lymphoid cells are under cytokines, peptides and hormones control (198). In respect of hormones' actions, Growth hormone (GH) and its proximal mediator insulin-like growth factor-I (IGF-I) have been shown to play an important role. GH acts directly through interaction on GH receptors (that is a cytokine receptor class 1) or indirectly through the IGF-I share. IGF-I shows both proliferative and anti apoptosis action (joint with receptors of Tyrosine kinase involved in the intracellular signalling of PI3K and anti apoptosis protein Bcl2). The presence of GH- and IGF-I- receptors on B and T lymphoid cells, monocytes macrophages, fibroblasts and neutrophils suggest the possibility of an immune action in association with the principal endocrine activity (199). In the bone marrow and in the thymus IGF-I is capable to promote the survival of forerunners blood cells and to prevent apoptosis (through way of PI3K). GH deficit causes subclinical changes in the chemotaxis and in the phagocytosis of granulocytes and macrophages. Moreover patients affected by GH deficit have a reduction of 50% in the function of natural killer cells, CD3- and CD56+. GH deficiency is often associated with a higher frequency of infectious, autoimmunity disorders and allergy. Furthermore the activity of natural killer cells improves after three-months of replacement therapy with recombinant human GH (rhGH), and normalizes after six-nine month of rh-GH replacement treatment. The functionality of phagocytes and neutrophils improves after a long period of rh-GH therapy too. In humans the important role of GH/IGF-I axis has been underline, also, by *in vivo* study. Patients affected by GH deficiency show many immune changes like reduced functionality of NK, insufficient antibody synthesis, thymus atrophy and changes of immune response mediate by immune cells. GH deficit is associate with increased prevalence of atherosclerosis and, consequently, higher morbidity and mortality for cardiovascular events. Different trials have as-

sessed a possible role of GH deficit and its recombinant replacement therapy on the monocytes's activation. In fact GH caused chemotaxis of monocytes, production of oxygen's radicals by macrophages and manage the TNF α expression. Monocytes play an important role in the development of the atherogenic plaque. In GH deficiency there is a damage in macrophages' functions: in bloodstream the capacity to adhere tightly to endothelial cells is increased by cytokines like IL-6 and TNF- α , markers of monocytes activation at the beginning of atherosclerosis' process. Others studies showed a relationship between GH deficit, anaemia and blood platelet reduction. GH replacement therapy improved concentration of haemoglobin, improved the myeloid activity and also increased secretion of cytokines like TNF α and IL-6. On the other side, in patients affected by high levels of GH, like in acromegaly, a condition of clear polycythemia, reversible by surgical or medical therapy, could be observed. Lastly we can observe a reduction in the function of GH/IGF-I axis in elderly, catabolic conditions, important illness and where there is status of resistance. GH resistance syndrome is characterized by moderate increase of GH levels, high levels of IGFBP-1 and IGFBP-2 and reduction of IGF-I, IGF-II and IGFBP-3. Moreover there is a reduced capacity of IGFBP-3 to work and create complex (more of 90% of IGF-I in bloodstream is bound at complex with IGFBP-3; IGF-I have anabolic effect and this one is higher when is bound in this complex) and so there is further reduction of IGF-I and IGF-II levels in bloodstream and changes in their biological activity. In elderly people changes of GH/IGF-I axis could be responsible for an increase in abdominal adiposity, a greater risk of cardiovascular mortality, and a depressed level of wellness. Immune senescence is associated with wasting thymus and reduction in oligoclonal T cells receptor. Older people are more susceptible of infections and show a reduction of immune answer to vaccines. In the future rhGH therapy could be a new chance to restore thymus functionality, to reduce age related changes and improve immune control against cancer and infections (199). Another important new therapeutic option for GH therapy could be in patients affected by HIV infection. GH/IGF-I axis functionality is important in protein synthesis and to preserve normal muscular mass. GH and IGF-I could increase the retention of nitrogenous and induce protein synthesis (200). Moreover, in some cases, GH/IGF-I function could have an important role in the nutritional status and body composition which are impaired in HIV infected patients. HIV infection produces an unspecific clinical status similar to many others endocrine and non endocrine illness while antiretroviral therapy causes endocrine malfunction too. Moreover in patients with AIDS it could be observed an important cachexia. AIDS-related cachexia is characterized by a reduced muscular mass for changes in metabolism and by a status of resistance to GH (201). IL-6 and TNF- α causes IGF-I resistance, directly related to the gravity of infection phase. There is a relation between GH/IGF-I axis and progression of illness (for example

reduction of number of CD4 lymphoid cells, weight loss and re-allocation of adipose tissue). Moreover patients with reduced rate of CD4 helper show high levels of IGFBP-1, distinctive trait of catabolic status. Moreover TNF- α enhances levels of IGFBP-1 and its liver production. IGFBP-2 is significantly represented in the HIV patients before beginning of AIDS. Patients with AIDS-related cachexia show dramatic reduction of IGF-I, IGF-II and IGFBP-3 levels when more than 10% of body mass is lost. Replacement therapy with rhGH could increase levels of red blood cells and improves physical performance increasing enzymatic oxidative activity of skeletal muscles. rhGH replacement therapy increases IGF-I levels and is responsible for an higher muscular oxidative activity in AIDS-related cachexia. In this respect GH could be a new approach to improve clinical status in patients with HIV infection (202, 203). Also the various processes involved in HIV lipodystrophy result in the suppression of pituitary GH production. The GHRH analog Tesamorelin is the only treatment which is FDA approved for reduction excess abdominal fat in HIV lipodystrophy (204).

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