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Samenvatting

Leptineresistentie. Pijl H. Ned Tijdschr Klin Chem Labgeneesk 2007; 32: 3-8.
Leptine wordt voornamelijk door adipocyten gemaakt. De plasmaconcentratie van leptine stijgt met toenemen van de vetmassa. Binding van leptine aan receptoren in de hypothalamus en hersenstam coördineert de activiteit van neuronale circuits die de voedselinname remmen en het energieverbruik stimuleren. Leptinedeficiëntie en inactiverende mutaties van de leptinereceptor leiden tot ernstig overgewicht en insulineresistentie bij knaagdieren. Leptinedeficiënte mensen zijn ook morbide adipeus, hetgeen aangeeft dat leptine bij mensen, net als bij knaagdieren, een buitengewoon belangrijke rol speelt in de regulatie van de energiebalans. De plasmaleptineconcentratie is hoog bij de meeste adipeuze patiënten. Kennelijk beïnvloeden die hoge concentraties de energiebalans niet zodanig dat de vetreserve wordt teruggebracht tot 'normaal'. Er is veel bewijs dat hoogvette voeding leidt tot leptine resistentie bij knaagdieren. Er zijn ook aanwijzingen dat adipeuze mensen leptineresistent zijn. Leptineresistentie kan niet alleen de ongeremde groei van vetreserves verklaren, het zou ook ten grondslag kunnen liggen aan een aantal metabole afwijkingen die met adipositas zijn geassocieerd. Dit overzichtsartikel beschrijft de huidige inzichten in de pathogenese en gevolgen van leptinedeficiëntie in knaagdieren en mensen.

Trefwoorden: leptine; adipocyten; insulineresistentie; obesitas

Ned Tijdschr Klin Chem Labgeneesk 2007; 32: 8-12

Adiponectin, role in insulin resistance, atherosclerosis and carcinogenesis

I.M. JAZET and A.E. MEINDERS

Adiponectin is one of the many adipokines secreted by adipocytes. Several isoforms are detectable in the circulation, the HMW isoform is supposed to be the most active one. Two adiponectin receptors have been cloned: Adipo R_1 and Adipo R_2 with a different distribution pattern. Stimulation of these receptors is followed by activation of intracellular signaling molecules like AMP kinase and PPARα. Plasma adiponectin levels are lower in obesity and in men compared to women and are influenced by weight reduction, dietary intake and drugs.
Adiponectin might be the important signal protein from the adipocyte to the vascular wall in the pathogenesis of atherosclerosis. Adiponectin inhibits several processes, which play a role in atherogenesis like smooth muscle cell proliferation and foam cell formation. Adiponectin is positively related to HDL levels. Adiponectin is inversely related to several obesity-associated cancers. Adiponectin inhibits carcinogenesis directly via stimulation of apoptosis and indirectly via inhibition of growth factors like insulin and ILGF-1 and the inhibition of angiogenesis. Adiponectin has anti-diabetic properties. It decreases hepatic glucose output and increases muscular fatty acid oxidation and glucose uptake. Measuring plasma adiponectin levels may be worthwhile in the future for detecting subjects with an increased risk for the development of cancer, atherosclerosis and type 2 diabetes. Mechanisms to increase plasma levels of adiponectin and its action via Adipo R_1 and Adipo R_2 may lead to new therapeutic interventions.

Keywords: adiponectin; adiponectin receptor; obesity; atherogenesis; cancer; diabetes

Adipose tissue can be considered as an organ with various functions (1). In the last decennium it became evident that the adipocyte is secreting several different proteins, also referred to as adipokines (figure 1), that play an important role in cardiovascular integrity, metabolism, inflammation and the development of cancer.
From epidemiological and clinical studies it has become clear that obesity is related to cardiovascular disease, disturbances in carbohydrate and lipid metabolism and several different forms of cancer. This relation is especially true between these diseases and the amount of visceral fat. Visceral fat cells are metabolically the most productive ones, compared with

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subcutaneous fat cells, although the mass of subcutaneous fat is several times greater than that of the visceral fat mass. Many adipokines have been discovered and for most of them a clear (patho) physiological effect has been described.
In general, the amount of adipose tissue is positively related with the production and plasma levels of the adipokines. The only known exception to this rule forms adiponectin. The more adipose tissue the lower the plasma adiponectin level; this finding already suggests that adiponectin might play a protective role in the development of several diseases in contrast to the other adipokines.

Biochemistry
The human adiponectin gene (apM_1) is located on chromosome 3q27, coding for a 244 amino-acid polypeptide. Thus far, only in adipocytes (white and brown) the adiponectin gene encodes a secreted protein. The protein consists of four domains: an amino-terminal signal sequence, a variable region, a collageneous domain and a carboxy-terminal globular domain. Structurally, the molecule is related to C_1q and TNF-α. Posttranslational hydroxylation and glycosylation yields 8 isoforms. These posttranslational modifications give the hormone maximal biological activity. Adiponectin monomers associate to trimers at their globular domains (figure 2). Three or more trimers associate to oligomers at the site of the collageneous domains. These oligomers circulate in the plasma at concentrations of 5-30 μg/ml. (2) Several oligomeric forms circulate: LMW (low molecular weight) oligomers consisting of two trimers (hexamers), MMW (middle molecular weight) oligomers consisting in 4-6 trimers and HMW (high molecular weight) oligomers consisting of 12-18 trimers. LMW adiponectin is the predominant form of adiponectin in the circulation whereas it is suggested that the HMW isoforms are the major source of the active hormone in the circulation. (3)

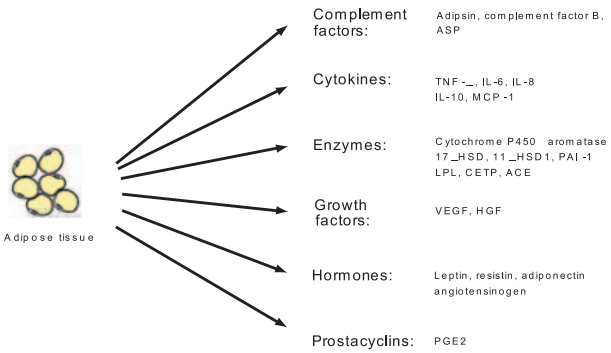

Figure 1. Adipose tissue-derived proteins. Adipose tissue secretes a number of proteins with different functions. ASP, acylation stimulating protein; TNF-α, tumor necrosis factor-alpha; IL, interleukin; MCP-1, monocyte chemoattractant protein-1; 17βHSD, 17β-hydroxysteroid dehydrogenase; 11βHSD1, 11β-hydroxysteroid dehydrogenase; PAI-1, plasminogen activator inhibitor-1; LPL, lipoprotein lipase; CETP, cholesterol ester transfer protein; ACE, angiotensin converting enzyme; VEGF, vascular endothelial growth factor; HGF, hepatic growth factor, PGE2, prostaglandin E2.

Adiponectin has its action via cell-surface receptors. Two receptor forms have been cloned ($AdipoR_1$ and $AdipoR_2$) with different distribution and affinity for circulating isoforms of adiponectin. $AdipoR_1$ is expressed ubiquitously, especially in skeletal muscle and endothelial cells but also in other tissues (4). AdipoR2 is predominantly expressed in liver cells. These receptors are integral membrane proteins, with the N-terminus internal and the C-terminus external. This is in contrast to all other reported G-protein-coupled receptors, in which the C- and N-terminus have the opposite position. The adiponectin receptors are not G-protein coupled but stimulation is followed by activation of signaling molecules like adenosine 5"-monophosphate (AMP)-activated kinase (AMPK) and peroxisome proliferator-activated receptor gamma (PPAR-γ). AdipoR1 and AdipoR2 may form homo- and heteromultimers. T-cadherine, a cell surface receptor located on endothelial and smooth-muscle cells, can also bind HMW-adiponectin. T-cadherin is supposed to be only a binding site, because it has no intracellular domain.
Plasma adiponectin levels are lower in obese compared with lean subjects. Women have higher plasma levels than men. Testosterone decreases plasma adiponectin levels. In women, estrogen levels are inversely related to adiponectin levels. So postmenopausal women have higher adiponectin levels than premenopausal women. Insulin inhibits adiponectin secretion and an inverse relation exists between fasting levels of insulin and adiponectin.
Adiponectin levels increase following weight loss, caloric restriction and low glycemic index diets (5).
In skeletal muscle and liver, adiponectin stimulates glucose utilization and fatty acid oxidation. In skeletal muscle, adiponectin increases tyrosine phosphorylation of the insulin receptor, which may increase insulin sensitivity. It also increases fatty acid oxidation presumably via 5-AMP kinase, thereby decreasing intramyocellular steatosis. In the liver, a

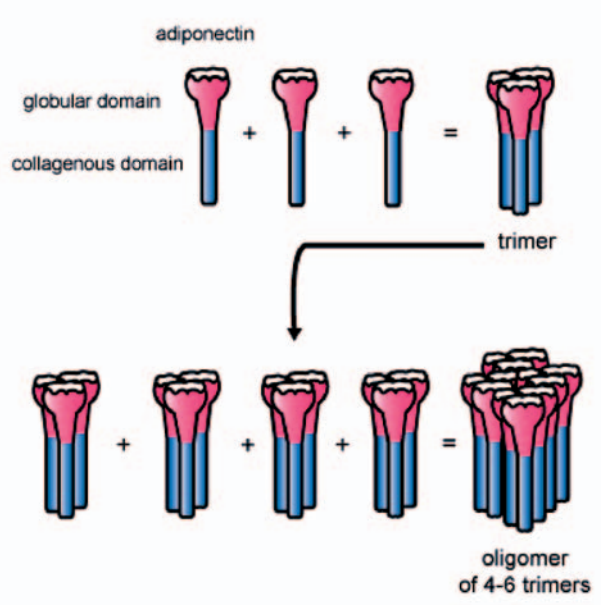

Figure 2. Model for the assembly of adiponectin complexes. (adapted from ref. 2). See text for explanation.

decreased fatty acid influx (by lower expression of the membrane transport protein FAT-CD36) in combination with an increased oxidation results in a reduced hepatic glucose output (by reducing substrate and energy for gluconeogenesis) and VLDL (very low density lipoprotein) synthesis. Moreover, adiponectin inhibits the expression of several gluconeogenic enzymes. In vascular endothelium adiponectin decreases monocyte adhesion, smooth muscle cell proliferation and macrophage-to-foam cell transformation (figure 3). It is an unresolved problem why adiponectin is decreased in case of an increased number of adipocytes. It has been suggested that other adipokines (for instance TNF-α) suppress the production and secretion of adiponectin.

Insulin resistance and diabetes

Adiponectin has anti-diabetic and anti-atherogenic effects and low adiponectin levels contribute to the development of diabetes and the metabolic syndrome. Low adiponectin levels are either genetically determined or the result of environmental factors especially those that lead to (visceral) obesity (figure 4).

Plasma adiponectin levels are influenced by multiple factors like gender, aging, life style and dietary constituents, like the ratio of saturated to unsaturated fat. Adiponectin is an insulin sensitizing adipokine. In the liver, it inhibits the expression of several gluconeogenic enzymes and decreases the rate of endogenous glucose production, resulting in lower fasting plasma glucose levels. In the muscle, adiponectin increases muscle fat oxidation and glucose transport via the AMP kinase pathway.

Single nucleotide polymorphisms (SNP) in the adiponectin gene have been related to decreased plasma adiponectin levels, greater insulin resistance and an increased incidence of type 2 diabetes in several ethnic groups.

Several lines of evidence point to a more active role for HMW adiponectin in relation to insulin resistance and type 2 diabetes. Measuring HMW adiponectin may therefore be of more value than measuring total or LMW adiponectin for predicting insulin resistance and type 2 diabetes.

In animal models (*ob/ob* mice) both Adipo R_1 and Adipo R_2 are decreased in muscle and adipose tissue, making these animals adiponectin resistant. Obesity may therefore be accompanied not only by low adiponectin levels but also by a decreased sensitivity for adiponectin because of a diminished number of adiponectin receptors. In humans as well there are arguments for a decreased expression of adiponectin receptors in case of type 2 diabetes. Since adiponectin receptors are expressed in pancreatic β-cells, insulin secretion might also be influenced by adiponectin. Increasing plasma adiponectin levels, up-regulating adiponectin receptors and stimulation of the adiponectin post-receptor pathway may all be targets for the therapy of insulin resistance, type 2 diabetes and the metabolic syndrome (6). For instance; thiazolidinediones (TZD's) increase plasma adiponectin levels (especially HMW adiponectin) via two mechanisms (mediated by PPAR-γ activation): stimulation of adipocyte differentiation in small adipocytes which produce more adiponectin than large adipocytes and a direct activation of adiponectin gene transcription. This increased adiponectin production and action may be part of the explanation for the insulin sensitizing effects of TZD's. Experimental work demonstrated the up-regulation of adipo R1 and adipo R2 by PPAR-α stimulation. Other molecules might be developed which counteract insulin resistance and type 2 diabetes using the adiponectin system (7).

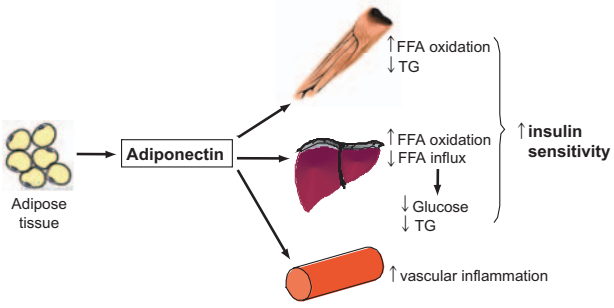

Figure 3. Model for the actions of adiponectin in liver, muscle and vascular wall. See text for explanation.

Cardiovascular disease

Obesity increases the risk for developing atherosclerotic disease and its clinical consequences. This relation is especially true in case of visceral adiposity. To a large extent visceral adiposity is part of the metabolic syndrome, a cluster of cardiovascular risk factors, which contain hypertension, dyslipidaemia (small dense LDL, low HDL, elevated TG), insulin resistance and type 2 diabetes mellitus, a prooxidative, prothrombotic state and increased signs of

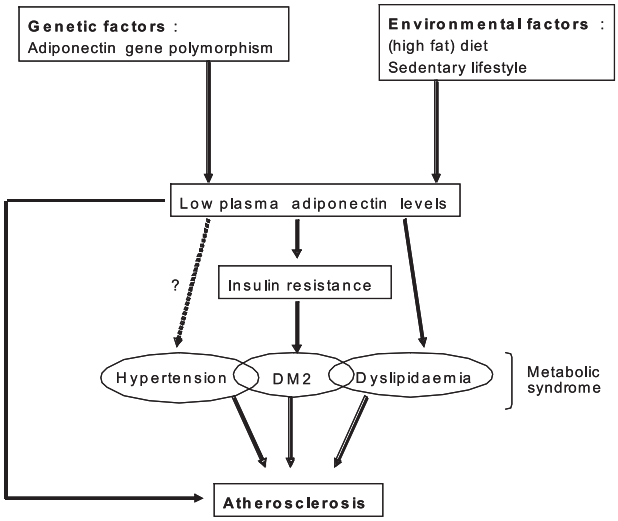

Figure 4. Adiponectin hypothesis for insulin resistance, the metabolic syndrome and atherogenesis. Genetic factors (like SNP 1164T or 276 mutations) and environmental factors that lead to obesity (especially visceral fat accumulation) cause hypoadiponectinemia. Low adiponectin levels play a causal role in the development of insulin resistance, type 2 diabetes and metabolic disease thereby indirectly stimulating atherosclerosis. In addition, low adiponectin levels also have a direct effect on atherosclerosis (see text).

inflammation. A signal from the adipocyte to the vascular wall as a pathofysiological connection for the development of atherosclerosis has long been looked for.
Adiponectin might be one of the candidates because it has been closely linked to endothelial dysfunction. Firstly, adiponectin inhibits smooth muscle cell proliferation and foam cell formation. Secondly, it downregulates the expression of adhesion molecules and thirdly, it increases the expression of matrix metalloproteinase inhibitors. Fourthly, weight-loss is associated with an increase of adiponectin and an improvement in cardiovascular risk factors: decrease in insulin resistance, reduced plasma levels of the fatty acids, C-reactive protein and interleukins 6 and 18 and increased HDL formation. Fifthly, adiponectin also activates PPAR-α followed by an increased production of apoproteins A-I and A-II. In humans, a positive correlation is found between adiponectin and HDL and apo A1 levels. This relation remains significant after correction for BMI.(8)
Low adiponectin levels have been related to the occurrence of coronary heart disease, even after controlling for other risk factors. And in patients with established coronary heart disease, adiponectin levels proved to be lower than in controls. This was also true for diabetic patients with risk factors for cardiovascular disease and without coronary heart disease. However, a direct significant positive correlation between coronary heart disease and plasma adiponectin levels has not yet been definitively established. A recent meta-analysis of seven published prospective reports on adiponectin and CHD (coronary heart disease) in Western populations just failed to be significant (9). It may still be possible that specific isoforms of adiponectin show a stronger relationship with coronary heart disease, but this supposition requires further study.

Cancer and carcinogenesis

Obesity is a risk factor for several cancers, including: endometrial cancer, breast cancer, gastrointestinal cancers (esophagus, colon, rectum and possibly stomach), cancers of the kidney, ovary, cervix uteri, pancreas and liver and possibly prostate. Avoidance of overweight might consequently be accompanied by decreased risk for cancer (10)
Insulin, insulin-like growth factor, steroid hormones and adipokines are endocrine factors which have been associated with carcinogenesis in obesity. Adiponectin is inversely related with the risk of the above mentioned malignancies associated with obesity. The mechanism by which adiponectin exerts its inhibiting effect on carcinogenesis might be indirect as well as direct. Adiponectin increases insulin sensitivity and hereby decreases insulin production and secretion. Indirectly it also decreases insulin like growth factor-I (ILGF-1) production and secretion. Insulin and ILGF-1 promote cellular proliferation, inhibit apoptosis and stimulate the production of vascular endothelial growth factor (VEGF). These processes contribute to carcinogenesis. By lowering insulin and ILGF-1, adiponectin protects against the development of cancer. The protective role of adiponectin as an inhibitor of TNF-α production and action in the development of cancer remains to be elucidated.

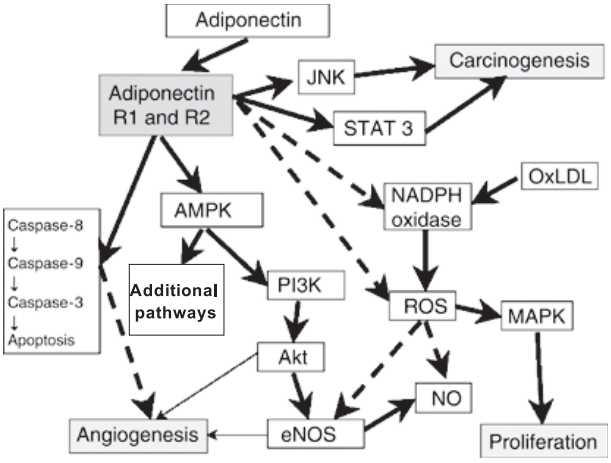

Figure 5. Multiple potential signaling pathways to explain the role of adiponectin in carcinogenesis (adapted from ref. 11). R1 and R2: adiponectin receptor 1 and 2; JNK, c-jun NH2-terminal kinase; STAT-3, signal transducer and activator of transcription-3; NADPH, nicotinamide adenine dinucleotide phosphate; oxLDL, oxidized low-density lipoprotein, AMPK, adenosine 5'-monophosphate (AMP)- activated protein kinase; PI3K, phosphatidylinositol-kinase; ROS, reactive oxygen species; MAPK, mitogen-activated protein kinase; NO, nitric oxide; eNOS, endothelial NO synthase.

Adiponectin directly suppresses the growth of several cell lines in vitro and stimulates apoptosis for instance in preleukaemic cell lines. Adiponectin can also bind to a number of mitogenic growth factors, so that binding of these molecules to the relevant cellular receptors is hampered. Adiponectin might via the AdipoR activate AMP kinase. AMP kinase has several downstream pathways which could be involved in carcinogenesis. AMP kinase inhibits FAS (fatty acid synthase) which is related to colon, breast, prostate and ovarian cancer. It also inhibits mTOR (mammalian homologue of target of rapamycine) which is related to colon, breast, prostate, ovarian, liver and lung cancer. Adiponectin might also via AMP kinase influence the production of eNOS (endothelial NO synthase) followed by inhibition of angiogenesis. Angiogenesis is possibly also inhibited by adiponectin through stimulation of caspase-cascade followed by apoptosis of endothelial cells (figure 5). Altogether, adiponectin might be an important link between adipose tissue and carcinogenesis. Adiponectin plays an inhibitory role in the development of several cancers. Possibly, in the future, low adiponectin levels could be used as a screening tool for the detection of specific cancers. Furthermore, increasing adiponectin levels or up-regulating adiponectin receptors might be beneficial in the prevention of development of cancer (11).

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Samenvatting

Adiponectine, rol in insulineresistentie, atherosclerosis en carcinogenesis. Jazet IM, Meinders AE. Ned Tijdschr Klin Chem Labgeneesk 2007; 32: 8-12.
Adiponectine is één van de vele adipokines, die worden geproduceerd door adipocyten. Er bestaat een aantal isovormen, waaronder een LMW- en een HMW-isovorm. De HMW-isovorm is de meest actieve. Adiponectine bindt aan twee receptoren, Adipo R_1 en Adipo R_2, die een verschillende distributie hebben. Stimulatie van deze receptoren leidt tot activatie van intracellulaire signaaleiwitten zoals AMP-kinase en PPARα. Obesitas gaat gepaard met verlaagde plasma-adiponectinespiegels. Adiponectine remt een aantal processen die betrokken zijn bij de atherogenesis, zoals gladdespiercelproliferatie en de vorming van schuimcellen. Adiponectine is omgekeerd gerelateerd met verschillende vormen kanker. De carcinogenesis wordt geremd door stimulatie van apoptosis, de remming van groeifactoren en het tegengaan van angiogenesis. Adiponectine vermindert de insulineresistentie via een verminderde endogene glucoseproductie in de lever en een toegenomen vetzuurverbranding en glucoseopname door de spier.
Het meten van plasma-adiponectinespiegels draagt in de toekomst wellicht bij aan de opsporing van personen met een verhoogde kans op atherosclerosis, insulineresistentie (en type-2-diabetes) en enkele vormen van kanker. Verhoging van plasma-adiponectinespiegels en stimulatie van Adipo R_1 en Adipo R_2 kunnen in de toekomst een therapeutische rol gaan spelen.
Trefwoorden: adiponectine; adiponectine receptor; obesitas; atherogenese; kanker; diabetes

Ned Tijdschr Klin Chem Labgeneesk 2007; 32: 12-18

Ghreline: van eerste natuurlijke groeihormoon secretagoog tot multifunctioneel peptide

R.M. KIEWIET, M.O. van AKEN, L. SCHEPP, Y.P.M. van der HULST en A.J. van der LELIJ

Ghreline is een eiwit, bestaande uit 28 aminozuren, dat in de maag geproduceerd wordt. In 1999 werd dit hormoon geïdentificeerd als krachtige groeihormoonsecretagoog, een effect dat gemedieerd wordt door de groeihormoonsecretagoog-receptor type 1a (GHS-R1a). Karakteristiek voor de structuur van ghreline is een posttranslationele acylering met een n-octanoylgroep van Ser (3), welke noodzakelijk is voor binding aan de GHS-R1a. Behoudens het groeihormoonstimulerend effect heeft ghreline ook een belangrijke rol bij de energiehomeostase. Ghrelinespiegels zijn verhoogd onder omstandigheden van een negatieve energiebalans, zoals bij vasten en cachexie, en verlaagd in geval van een positieve energiebalans, zoals bij obesitas. Op centraal niveau, in de hypothalamus, stimuleert ghreline de voedselinname en is daarmee vooralsnog het enige in de tractus digestivus geproduceerde hormoon met een orexigeen effect. Vooral de ontdekking van ghreline als regulator van de energiebalans heeft geleid tot een uitgebreid onderzoeksterrein van GHS-R1a-agonisten en -antagonisten.

Trefwoorden: ghreline; groeihormoon; groeihormoonsecretagoog-receptor; orexigeen effect; obesitas

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Groeihormoon (GH) wordt geproduceerd in de hypofysevoorkwab onder invloed van een evenwicht tussen het groeihormoonstimulerende groeihormoon-releasing hormoon (GHRH, geproduceerd in de hypothalamus) en zijn antagonist somatostatine (1). Sinds 1976 zijn er echter meerdere synthetische stoffen met een zeer potente GH-stimulerende werking ontwikkeld. Deze 'growth hormone releasing peptides' (GHRP) vertoonden geen structurele overeen-