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END OF TERM PROJECT

RELATIONSHIP BETWEEN INSULIN-LIKE GROWTH FACTOR – I AND BLOOD PRESSURE IN CHILDREN

An observational study

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SUMMARY

“Everyone is trying to accomplish something big,

not realizing that life is made up of little things”

Frank A. Clark

ABSTRACT

Background: Insulin-like growth factor-I (IGF-I) is a relevant hormone in growth regulation. However, dysregulation of IGF-I is also implicated in pathologic states, such as cardiovascular diseases (CVD).

CVDs are the main cause of death globally. Subclinical CVD has its roots in childhood. It has been demonstrated that high blood pressure (BP) in childhood correlates with higher BP in adulthood and the onset of hypertension and metabolic syndrome. Elevated BP is therefore a major modifiable risk factor for CVD.

Justification: Interventions related to CVD prevention and health promotion are needed, especially in paediatric population.

There are conflicting data on published studies defining the association between IGF-I levels and the cardiovascular system. Furthermore, there is a lack of studies reviewing this association in the general paediatric population. Additional knowledge is needed to understand the pathophysiologic mechanisms of IGF-I involved in CVD, which may allow us to perform an early CV risk assessment as well as to apply prevention interventions in those patients at higher risk.

Objectives: The aim of this study is to analyse the association of IGF-I serum concentrations with blood pressure in a population of apparently healthy children. Both cross-sectional associations (studies at baseline and at follow-up) as well as predictive associations (longitudinal analysis) will be sought.

Other associations, as well as possible interactions, between IGF-I and CV risk factors (waist, insulin, insulin resistance, triacylglycerol, high-density lipoprotein cholesterol and carotid intima-media thickness) will be also studied.

Methods: This is an observational prospective, population-based study of a cohort of apparently healthy children in Girona. A target population of 528 children (mean age 8.8 years) were studied; a total of 158 of such children were re-studied after a 4-year period of follow-up (mean age 12.8 years).

Data have been extracted from a database compiled between 2011 and 2017. Data analyses have been performed for all the studied subjects and in subgroups thereof defined by tertiles of serum levels of phosphate and calcium (PxCa product), as higher concentrations of these mineral have been associated with increased risk for CVD.

Results: Basal study (cross-sectional associations at baseline): IGF-I was associated with all the studied variables (age, weight, height, BMI, waist, SBP, DBP, PP, log_{10} HOMA-IR, log_{10} triacylglycerol, HDL-cholesterol, carotid IMT), all p<0.0001. Analyses by PxCa tertiles, showed that the strongest associations between IGF-I and blood pressure parameters (SBP, DBP, PP) were present in children with higher PxCa product.

Follow-up study (cross-sectional associations at follow-up): IGF-I maintained the correlation with all the variables studied in the follow-up study: age, weight, height, SBP, PP, log_{10} HOMA-IR (p<0.0001), BMI (p=0.035), waist (p=0.008), DBP (p=0.001), log_{10}triacylglycerol (p=0.076), HDL-cholesterol (p=0.047) and carotid IMT (p=0.021). Analyses by PxCa tertiles showed that the strongest associations between IGF-I and blood pressure parameters (SBP, DBP, PP) were present in children with higher PxCa product.

Prediction study (longitudinal associations of baseline IGF-I with dependent variables at follow-up): IGF-I is correlated with all the variables studied: age, weight, height, BMI, waist, SBP, PP and log_{10} HOMA-IR (p<0.0001), DBP (p=0.049), log_{10}triacylglycerol (p=0.010), HDL-cholesterol (p=0.020) and carotid IMT (p=0.020). Analyses by PxCa tertiles showed that the strongest associations between baseline IGF-I and blood pressure parameters at follow-up (SBP, DBP, PP) were present in children with higher PxCa product.

In multivariate regression analyses, the strongest cross-sectional and longitudinal associations of IGF-I levels were with SBP and in children with higher PxCa product.

Conclusions: Increasing serum IGF-I levels were found to associate with increasing blood pressure, especially with increasing systolic blood pressure and in children with higher phosphate calcium product.

Keywords: •Insulin-like growth factor-I •Cardiovascular disease •Cardiovascular risk •Blood pressure •Systolic Blood Pressure •Children

ABBREVIATIONS

ALS	Acid-labile subunit
BP	Blood pressure
BP-Pr	Insulin-like growth factor receptor proteases
Ca^{2+}	Calcium
CNS	Central nervous system
CV	Cardiovascular
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
eCRF	Electronic case report form
GH	Growth hormone
GHBP	Growth-hormone-binding protein
GHRH	Growth hormone releasing hormone
GH-S	Growth hormone secretagogues
HDL-C	High density lipoprotein cholesterol
IGFBP	Insulin-like growth factor binding proteins
IGFBP-rP	Insulin-like growth factor binding related proteins
IGF-I	Insulin-like growth factor-I
IGF-II	Insulin-like growth factor-II
IGFII/6MP-IR	Insulin-like growth factor-II/mannose-6-phosphate receptor
IGFI-IR	Insulin-like growth factor receptor
IUGR	Intrauterine growth retardation
IMT	Intimal medial thickness
IR	Insulin receptor
LDL-c	Low density lipoprotein cholesterol
miRNA	Micro-RNA
NO	Nitric oxide
PxCa	Phosphate calcium product
SBP	Systolic blood pressure
SD	Standard deviation
SGA	Small for gestational age
SRIF	Somatotropin-release inhibiting factor
VSM	Vascular smooth muscle
VSMC	Vascular smooth muscle cells

FIGURES

TABLES

1. INTRODUCTION

The essential role of the growth hormone (GH) – insulin-like growth factor (IGF) axis in the regulation of the postnatal growth and body development is well known. However, recent studies have found that dysregulation of the insulin-like growth factor type I (IGF-I) signalling pathways are also implicated in aging and some pathologic states in adults including muscle, neurodegenerative and cardiovascular diseases as well as cancer (1–3).

Cardiovascular diseases (CVD) are the main cause of death globally (4). Blood pressure (BP) is directly associated with cardiovascular risk and systolic hypertension is also a powerful predictor of CVD (5). Moreover, it has been demonstrate that high BP in childhood correlates with higher BP in adulthood and the onset of hypertension and metabolic syndrome in young adulthood (6). Thus, studies of blood pressure regulation in childhood can potentially have a significant impact on future adverse outcomes (7).

1.1 IGF SYSTEM

The IGF family (**Figure 1, Table 1**) encompasses three ligands: insulin, insulin-like growth factor type I (IGF-I) and insulin-like growth factor type II (IGF-II); six IGF-binding proteins (IGFBP-1 to -6), IGFBP-related proteins (IGFBP-rP1 to -rP9), proteases (BP-Pr), an acid-labile subunit (ALS) and three specific receptors: insulin receptor (IR), IGF-I receptor (IGF-I IR) and the IGF-II/mannose-6-phosphate receptor (IGF-II/6MP-IR) (1,2).

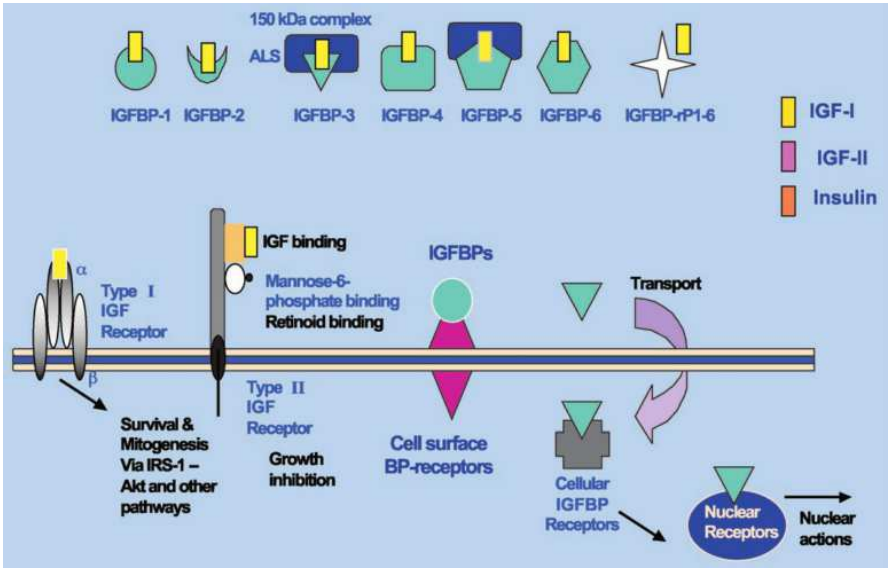

Figure 1. IGF system. All the components of the IGF system (2). *IGF-I, insulin-like growth factor type I; IGF-II, insulin-like growth factor type II; IGBP-1 to 6, IGF-binding proteins; IGFBP-rP, IGFBP-related proteins; ALS, acid-labile subunit;*

Insulin, IGF-I and IGF-II have a high homology at the peptide sequence level (identity with each other is approximately 50%) and also at the function level. Insulin has mainly metabolic

actions, whereas IGF-I and IGF-II have metabolic action (a synergistic role with insulin on post-pandrial hypoglycaemia) as well as mitogenic actions regulating somatic growth and cellular proliferation both in embryological and postnatal states (1).

IGFBPs can potentiate IGF action or exert IGF-independent actions (**Table 1**) which include inhibition of cell growth and induction of apoptosis (8). Unlike insulin, 99% of IGFs are bound by IGFBPs in a high-affinity union which modulate the availability of free IGF-I and IGF-II to the tissues (9). IGFBP-related proteins are low affinity binders (1). Proteases play an important role in modulating levels and actions of IGFs and IGFBPs (2).

Table 1. Components of the IGF system, characteristics and function. IGF-I, insulin-like growth factor-I; IGF-II, insulin-like growth factor-II; IGBP-1 to 6, IGF-binding proteins; IGFBP-rP, IGFBP-related proteins; ALS, acid-labile subunit; IR, insulin receptor; IGF-IR, IGF-I receptor; IGFII/6MP-IR, IGF-II/mannose-6-phosphate receptor; cr, chromosome. (1,8–10)

Insulin	-**Endocrine hormone**: a 51-residue anabolic protein composed by two chains (A and B). -Coded for the short arm of **cr 11 (region p13)**. Secreted by the β-cells in the Islets of Langerhans. -**Metabolic actions**. Regulation of glucose homeostasis: (a) stimulation of glucose uptake from the systemic circulation and (b) suppression of hepatic gluconeogenesis.
IGF-I	-**Endocrine, paracrine and autocrine hormone**. Also known as somatomedin. -Polypeptide of 70aa (7650Da) composed by a single chain with A, B, C and a carboxyterminal D domain. -Coded for the long arm of **cr 12 (region q22-24)**. The gene consists of 6 exons and has 2 promoters. ·Secreted by multiple tissues (most by the liver). The secretory site seems to determine its action. -**Regulated by the GH – IGF-1 axis.** -**Metabolic and mitogenic actions**: (a) cell growth, survival, migration and differentiation, (b) prenatal and postnatal growth, (c) promotion of wound repair, (d) anabolic effects and insulin-like activity.
IGF-II	-**Endocrine, paracrine and autocrine hormone**. Fist characterized as "multiplication-stimulating activity". -Polypeptide of 67aa (7479Da) composed by a single chain with A, B, C and a carboxyterminal D domain. -Coded for the short arm of **cr 11 (region p15-5)**. Secreted by multiple tissues (most by the liver). ·Low levels at birth. Increase during the first week of life and then stay stable during adult life. Decline in old age. -**Regulated nutritionally**. Independent of GH, although it can be affected by IGFBP levels. -**Metabolic and mitogenic actions**: (a) cell growth, survival, migration and differentiation, (b) prenatal and postnatal growth, (c)metabolic effects on adipose tissue, skeletal muscle and liver (insulin-like activity), (d)ovary development.
IGFBP-1	-Coded for cr 7. Binds only a small fraction of circulating IGF-I. -Hepatic production. Regulated by IGF-I and insulin. Diurnal variation.
IGFBP-2	-Coded for cr 2. Binds IGF-II with higher affinity compared to IGF-I -Regulated by GH and nutrition, although the exact mechanisms are unknown. Diurnal variation.
IGFBP-3	-Coded for cr 7. Binds IGF-I and IGF-II, mainly in a high molecular weight (150kDa) ternary complex. - Hepatic production. Regulated by GH state and level and nutritional state. It is the most abundant.
IGFBP-4	-Coded for cr 17. Binds IGF-I and IGF-II with equal affinity. -Regulation unknown (possible influence of PTH in the regulation). Age-dependent variation.
IGFBP-5	- Coded for cr 2. Higher binding affinity for IGF-II compared to IGF-I. -Regulation by glucocorticoids. Related to bone physiology. Age-dependent variation.
IGFBP-6	-Coded for cr 12. Higher binding affinity for IGF-II compared to IGF-I -Regulation unknown. Age-dependent variation.
IGFBP-rP	-Group of peptides (Mac25, CTGF, NovH, WISP-2, L56, ESM-1, others) which bind with low affinity IGFs as well as proteolysed IGFBP fragments that retain their ability to bind IGFs although with lower affinity.
ALS	-Glycoprotein where the 25% of the aminoacids are leucine. Binds carboxyterminal domain of IGFBP-3. -Important role in modulating IGFs and IGFBPs in the circulation.
IR, IGFI-IR	-Cell surface glycoproteins. Tyrosine kinase receptors. Metabolic and mitogenic effects respectively.
IGFII/6MP-IR	-Receptor that acts as a clearance factor for IGF-II by internalizing and degrading cell surface attached IGF-II

Almost 75% of IGFs circulating in serum are carried by IGFBP-3 in a ternary complex consisting of IGF-I or IGF-II, IGFBP-3 and the glycoprotein ALS (smaller amounts are carried by IGFBP-5). Only a 25% is bound in a binary complex, possibly acting as a pericellular reservoir of IGFs. Less than a 1% of the total circulating IGF-I is free (and bioactive) being able to interact with receptors (9,11). So, the formation and integrity of the binding complexes determine the bioavailability of circulating IGFs to tissues and prolong the half-life in plasma (from 8 minutes for free IGFs, 30 minutes for binary complexes to 15-hours-long for ternary complexes), allowing high concentrations of IGFs without the risk of hypoglycaemia (8).

1.1.2 REGULATION OF THE IGF SYSTEM: GH – IGF-I AXIS

The GH – IGF-I axis manifests remarkable dynamic regulation by multiple internal and external cues. Hypothalamic as well as peripheral factors have an important role (**Figure 2**): GH-releasing hormone (GHRH), growth hormone (GH), somatostatin (somatotropin-release inhibiting factor [SRIF]), oestrogen, glucocorticoids, insulin, nutritional factors (ghrelin) as well as age, sleep, metabolic stress, gender, activity level and systemic diseases (1,2,9,12,13).

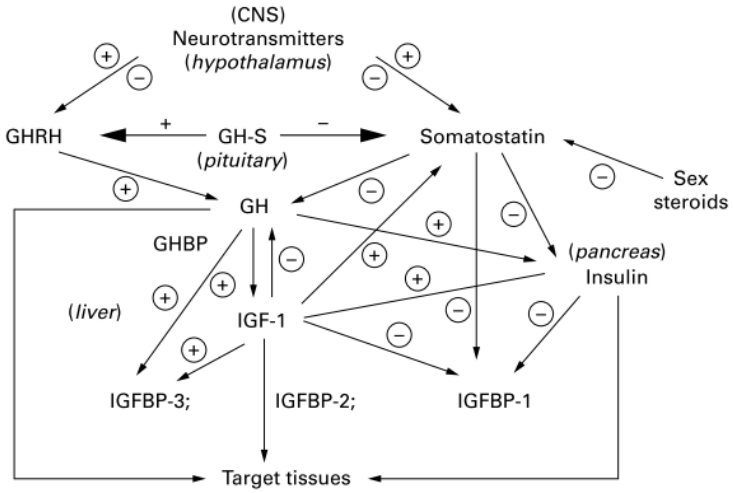

Figure 2. Cascade of the GH – IGF-I axis regulation. *CNS, central nervous system; GH, growth hormone; GHBP, GH binding protein; GH-s, GH secretagogues; IGF-I, insulin-like growth factor-I; IGFBPs, IGF binding proteins; +, stimulation; -, inhibition* (9).

GH is synthesized and secreted by somatotrophs located in the anterior pituitary under the stimulation of the hypothalamic GH-releasing hormone (GHRH), as well as other hypothalamic peptides called GH secretagogues (ghrelin, oestrogen) which act in synergism with GHRH by stimulating GH (9,12). Ghrelin, an octanoylated gastric-derived peptide, induce GHRH and also directly stimulate GH release (12). Somatostatin (SRIF) is also a hypothalamic factor that acts by inhibiting GH secretion and sets basal GH tone, instead of GHRH that is secreted in discrete spikes that elicit GH pulses. Chronic glucocorticoid excess also supresses GH release. IGF-I, the pheripheral target hormone for GH, feeds back to inhibit GH (12).

GH secretion is pulsatile and induces the generation of IGF-I in the liver, which is transported to other tissues acting as an endocrine hormone. Nevertheless, the paracrine production of IGF-I is regulated by both GH and specific trophic factors. In cartilaginous cells, GH is the main controller of the local IGF-I production whereas in uterus oestrogens and not GH stimulate IGF-I expression. Follicle stimulating hormone is a major IGF-I regulator in ovary (14). It is also assumed that IGF-I can act in an autocrine manner as an oncogene (9).

The liver is also the major producer of IGFBP-3 and ALS. IGFBP-3 is produced mainly by hepatic endothelia and Kupffer cells, while ALS and IGF-I are made by hepatocytes. The production of the three components is directly affected by GH (2).

On the other hand, IGF-I inhibits GH secretion acting on the hypothalamus by two feedback mechanisms: (i) inhibiting GH gene expression and (ii) by stimulating the secretion of somatostatin that inhibits the GH production (14).

1.1.3 IGF-BINDING PROTEINS (IGFBP)

The main function of IGFBPs is to bind IGFs with high affinity regulating the bioavailability of IGFs to the IGF-I receptor. The ternary complexes composed of ALS, IGF-I and IGFBP-3 are high molecular complexes of 150-kDa, which, because of its size, are retained in the vascular compartment prolonging the half-life of plasma IGF-I and becoming a reservoir of IGFs (8). IGFs are mobilized from the 150kDa complexes principally by limited proteolysis of IGFBP-3 to a 30kDa fragment that has reduced affinity for IGF-I and IGF dissociation is facilitated. The released IGF may cross the endothelial barrier alone or after associating with other low molecular weight IGFBPs where it can interact with the IGF-I receptor.

The proteases, responsible for limited proteolysis of IGFBP3, have not been fully identified but, matrix metalloproteases 1 and 3 as well as calcium-dependent serine proteases seem to contribute in IGFBP-3 protease activity. Plasma circulating IGFs levels are thought to regulate their own proteolysis (8).

The importance of IGFBP relies also on its capability to act independently from IGF-I regulating growth, apoptosis and metabolism of target cells (**Table 2**).

IGFBP-1	-IGFBP-1 binding to a α5β1 integrin promotes cell migration and wound healing. It also potentiates the mitogenic effect in smooth muscle cells or fibroblasts.
IGFBP-3	-Inhibits growth-stimulation by IGF, insulin and fibroblasts growth factors. Stimulates apoptosis.
IGFBP-5	-Biologically active fragments by limited proteolysis of IGFBP-5 act potentiating IGF-I action. They stimulate DNA and protein synthesis in osteoblast, chondrocytes, fibroblasts and smooth muscle cells.

Table 2. Functions of the IGFBP. IGBP; insulin-like growth factor binding proteins; IGF-I, insulin-like growth factor-I.

1.2 IGF- I

IGF-I is a relevant hormone both in embryological and postnatal states. It is a polypeptide hormone with endocrine, paracrine and autocrine effects, mainly produced by the liver (~75%), under the stimulation of the growth hormone (GH) although almost all tissues are able to secrete IGF-I for autocrine and paracrine purposes (14).

1.2.1 IGF-I GENE

IGF-I is produced by the IGF-I gene, which has been mapped to the long arm of chromosome 12 in humans (12q22-q24.1). The gen consists of at least 6 exons, and transcription results from at least 2 transcription start sites located on exon 1 and exon 2 (15). A common polymorphism in the promoter region of this gene, non-carriers of the 192-bp allele, is associated with decreased (18%) circulating IGF-I levels in adulthood leading to cardiovascular disease (increased risk of ischemic heart disease), type 2 diabetes and variation in growth characteristics (including low birth size and shorter final height in adulthood) (16).

GH promotes IGF-I gene transcription, leading to production of IGF-I microRNAs (miRNAs) and synthesis and secretion of IGF-I (17). miRNAs are small non-coding RNAs of approximately 18-25 nucleotides that regulate gene expression at the post-transcriptional level and have emerged as a new regulator of critical biological processes being involved in disease mechanisms including cancer, cardiovascular and neurodegenerative disease (3).

1.2.2 IGF-I RECEPTOR

The IGF-I receptor is a cell surface glycoprotein member of the tyrosine kinase receptor located on chromosome 15 (15q26.3), involved in the mitogenic effects of IGF-I. Terminal deletions result in decreased IGF receptor binding and intrauterine growth retardation. Thus, due to the homology between IGFs and insulin, IGF-I can also interact with the insulin receptor (Figure 3), although with lower affinity than insulin, explaining the insulin-like activity of IGF-I (the insulin-like activity of IGFs is only 5% that of insulin)(1,14).

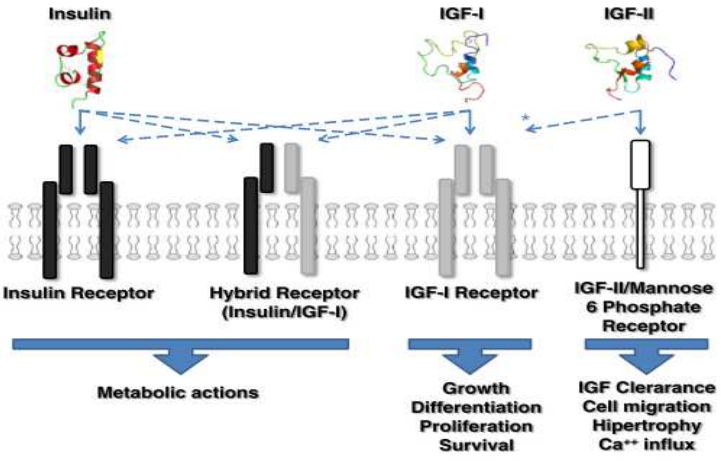

Figure 3. Schematic structures of IGFs and their receptors. Homology between insulin and IGFs allow them to cross-interactions. The hybrid receptors share components from both IR and IGF-IR (14).

1.2.3 IGF-I PHYSIOLOGICAL VARIATIONS

A total daily IGF-I production of approximately 3-10mg/day is estimated on a normal basis, but multiple conditions lead to physiological variations in IGF-I levels.

1.2.3.1 Diurnal variation

A diurnal variation of total IGF-I but most likely a small nocturnal decrease exists. Free IGF-I significantly increases, and IGFBP-3 decreases during night (1).

1.2.3.2 Age variation

Fetal IGF-I seems to play an important role in the regulation of fetal growth. Thus, fetal serum IGF-I levels seem to be associated with intrauterine growth. Then, IGF-I decreases from birth to six months of age with a subsequent increase in late infancy. In very low birth weight infants higher postnatal growth velocity was associated with higher IGF-I and IGFBP-3 levels as well as higher amounts of the lesser phosphorylated forms of IGFBP-1. Therefore, the increase off plasma IGF-I concentrations in low birthweight children may be linked to postnatal catch-up growth that is also linked to metabolic syndrome in adulthood (1).

Circulating GH and IGF-I levels are maximal during peripubertal growth and early adulthood, with maximal levels seen in Tanner stage 3-4 in girls and Tanner stage 4 in boys (1); however, they progressively decline with age (Figure 4). Reduced GH/IGF-I secretion in the elderly is believed to be responsible for or contribute to many symptoms of aging, including loss of muscle mass, increased adiposity, reduced bone mineral density and decline in energy levels. IGF-I is also involved in all the processes of longevity (14).

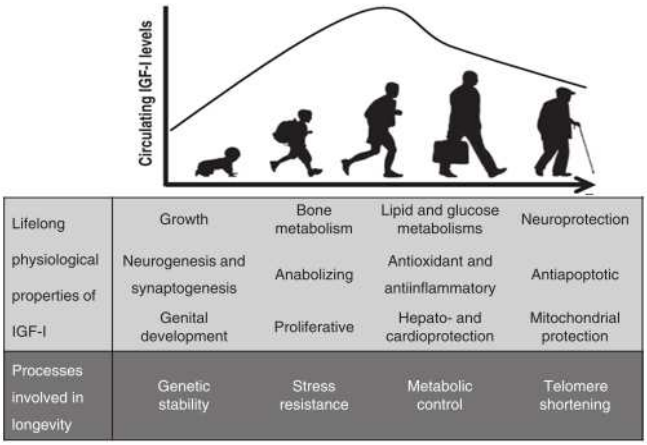

Figure 4. Lifelong beneficial properties of IGF-I. Evolution of IGF-I circulating levels and roles along different stages of human development

1.2.3.3 Sex variation

IGF-I concentrations are a 20% higher in women than in men (12,18) due to the significant gender-differences in sex steroid levels (1), even that *"in the majority of studies no effect of gender on serum IGF-I could be detected in adults*" (1).

1.2.3.4 Disease states

Because GH is the major determinant of hepatic IGF-I synthesis, abnormalities of GH synthesis or action reduce IGF-I levels. Hypocaloric states are associated with GH resistance, IGF-I levels are therefore low with cachexia, malnutrition and sepsis. GH secretion is also reduced in obese individuals, though IGF-I levels may not be suppressed, suggesting a change in the set point for feedback control (12).

1.2.4 IGF-I FUNCTIONS

In physiological conditions, IGF-I is involved in multiple processes (Figure 5), usually unfastened from GH actions as an independent factor: body growth (fetal growth, fetal differentiation and postnatal body growth), central nervous system (CNS) development, liver regeneration, gametogenesis (ovarian folliculogenesis, testicular function), kidney development and function, cardiovascular development and immune modulation (14).

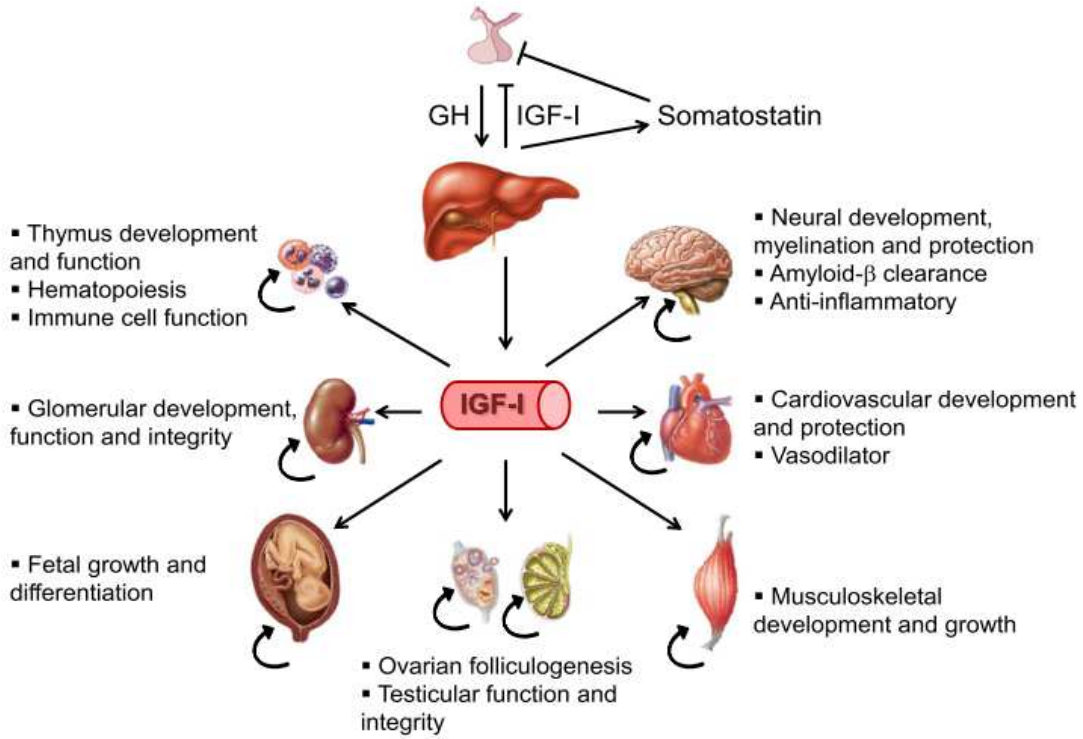

Figure 5. GH – IGF-I axis functions. Pituitary GH interacts with GH receptors in hepatocytes increasing IGF-I secretion for endocrinological purposes in different organs, even the autocrine/paracrine production by these organs (14). *GH, growth hormone; IGF-I, insulin-like growth factor-I.*

1.3 CARDIOVASCULAR DISEASES

The cardiovascular diseases (CVD) are a group of disorders of the heart and blood vessels that include: coronary heart disease (angina and coronary syndrome), cerebrovascular disease, peripheral arterial disease, congenital heart disease, rheumatic heart disease, rheumatic fever, deep vein thrombosis and pulmonary embolism (4).

CVD are the main cause of death globally. About 17.7 million people died from CVD in 2015 (the 31% of all the global deaths), mainly due to coronary heart disease and stroke (4). In Catalonia, CVD were responsible for 27.9% of all deaths in 2015 (30.3% of all deaths in women and 25.5% of all deaths in men)(19).

1.3.1 CARDIOVASCULAR RISK FACTORS

Cardiovascular (CV) risk factors are measurable elements or characteristics that are causally associated with an increased rate of CVD and are also independent and significant predictors of the risk of presenting CVD (5). CVD are usually caused by multiple CV risk factors. The description of the natural history of CVD is shown in Figure 6.

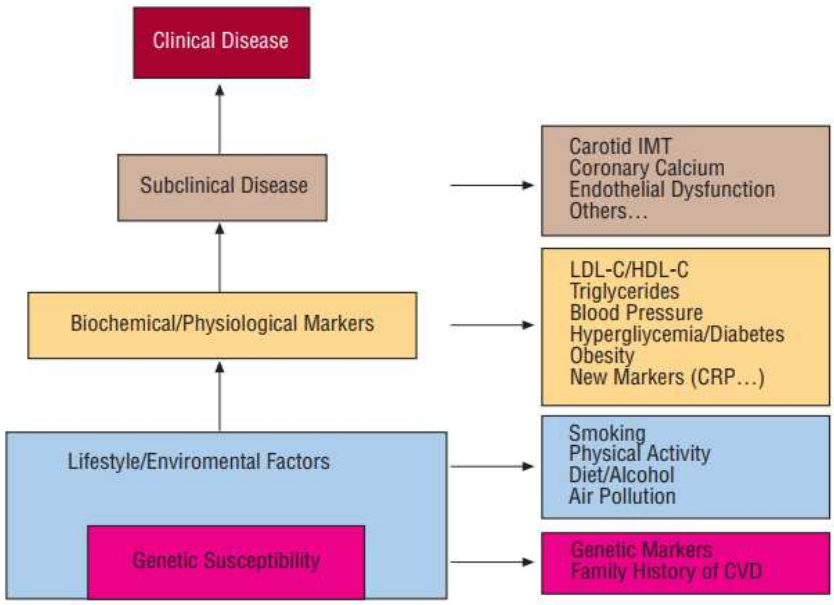

Figure 6. Natural history of cardiovascular diseases and its correspondence with some lifestyle and biochemical/physiological characteristics considered risk factor for these diseases. *Carotid IMT, carotid intima-media thickness; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol* (5).

The metabolic syndrome defines a cluster of CV risk factors that include hypertension, abdominal obesity, unfavourable lipid profile and hyperglycaemia. The metabolic syndrome is driven by peripheral insulin resistance (20,21). Studies show that it is also related to increased fibrinolysis, endothelial dysfunction and subclinical inflammation (Figure 7)(21).

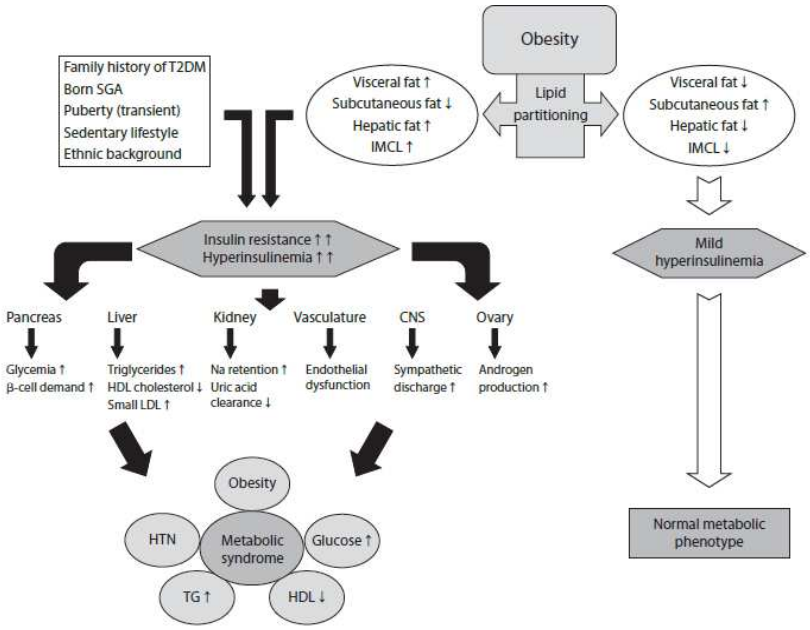

Figure 7. Pathophysiology of the metabolic syndrome in childhood. The major cause of insulin resistance in childhood is obesity. *SGA, small for gestational age; T2DM, type 2 diabetes mellitus ; CNS, central nervous system; HDL, high density lipoproteins; LDL, low density lipoproteins; TG, triglycerides; Na, sodium; HTN, hypertension* (21).

1.3.2 CARDIOVASCULAR DISEASE IN CHILDREN

The prevalence of CVD in children is expected to rise (22,23). As well as other chronic diseases, CVD are the result of complex interactions between genetic and environmental factors over extended periods of time (5). Subclinical CVD has its roots in childhood, where fetal and early-life health may have strong influence on it, increasing the risk of hypertension, stroke and heart diseases in adulthood (24). Elevated BP, and especially hypertension, is a major modifiable risk factor for the atherosclerotic process. Thus, early diagnosis and treatment can potentially have a significant impact on future adverse outcomes (7,24).

"*Cardiovascular events are coming to be regarded as a medical failure rather than the first indication of treatment (Dr W. B. Kannel, Framinhgham Heart Study)"* (5).

1.3.3 BLOOD PRESSURE IN CHILDREN

The prevalence of high blood pressure (BP) among children has risen in recent decades. Elevated BP, especially high systolic blood pressure (SBP), increases the load on the heart and the stresses on arteries accelerating cardiovascular degeneration, atherosclerosis, increases arterial stiffness, collagen synthesis and arterial smooth muscle hyperplasia and hypertrophy (24,25). The undiagnosed and untreated elevated BP and/or hypertension may persist into adulthood and lead to target organ damage such as left ventricular hypertrophy, increased carotid artery intima–media–thickness, vascular changes in the retina and subtle cognitive alterations, among others (7,24).

There are still no data to identify a specific level of BP in childhood that leads to adverse CV outcomes in adulthood (6). However, it is well known that BP is directly associated with CV risk regardless of how labile it is and, even high-normal BP values, are associated with an increased risk of CVD (5). Moreover, it is reported that both systolic and diastolic BP have a continuous, independent, graded and positive association with CV outcomes (5,24).

1.3.3.1 Arterial Stiffness

Arterial stiffness is the ability of arteries to accommodate the stroke volume (**Figure 8**) (26). It is emerging as the most important determinant of increased SBP and pulse pressure and, therefore, the root cause of a host of CV complications: a major contributor to atherosclerotic and small vessel diseases and thus to stroke, myocardial infarction and renal failure (27). So, arterial stiffness is a surrogate marker for CVD (24).

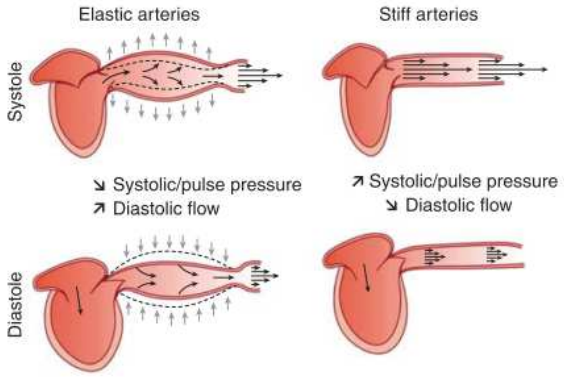

Figure 8. Representation of the role of arterial stiffness in assuring blood flow through the peripheral circulation (26).

The arterial media is a mix of collagen and elastin linked by smooth muscle (27). Hence, the stiffness of elastic arteries increases with:

(i) Smooth muscle tone regulation, affected by nervous activity, hormones, drugs and locally produced vasoactive substances, such as nitric oxide (27).

(ii) Pulsatile mechanical stress generated by the left ventricle over time, causing fatigue and fracture of elastin lamellae in the arterial wall (27).

(iii) High SBP, which increases heart load, arterial stresses and accelerates CV degeneration, such as arteriosclerosis as well as arterial stiffness (24).

(iv) Impaired glucose tolerance, which also enhances nonenzymatic glycation and crosslinking of collagen leading to stiffness (25). Children with metabolic syndrome have shown to have increased arterial stiffness (21).

Systolic, diastolic and pulse pressures depend on the physical properties of large elastic arteries (28). Evidence shows that arterial stiffness represents a cause rather than a consequence of hypertension, despite the fact that elevated BP also causes a direct increase in the arteria diameter and stiffness. The combined deleterious effect of arterial stiffness and elevated BP in the pathogenesis of CVD underlines the importance of its prevention (24).

1.4 RELATIONSHIP BETWEEN IGF-I AND CARDIOVASCULAR DISEASES

The involvement of circulating IGF-I and IGFBP in the pathogenesis of CV disorders have been large investigated (14,15,18,21,22,29–34). Conflicting data exist: on one hand, IGF-I is postulated to protect against endothelial dysfunction, atherosclerotic plaque development, clinical instability and ischemic myocardial damage (21). On the other hand, the relationship of serum IGF-I levels with CVD is not clear as several large prospective cohort studies failed to confirm such an association (21,30).

Low circulating IGF-I levels have been correlated with an increased risk for CVD (coronary artery disease, ischemic heart disease, ischemic stroke and congestive heart failure) in elderly patients (14,21,22,29,31). Similar results are shown in a study in obese adolescents, suggesting that lower IGF-I and IGFPB-I are associated with overall higher CVD risk (22).

Reduced IGF-I, IGF-IR mRNA as well as reduced protein expression in VSMC leads to endothelial dysfunction, apoptosis and impaired endothelial-dependent vascular reactivity, all situations related to the development of CVD (14,15,22). Thus, many of the traditional CV risk factors have also been associated with low serum IGF-I levels. Negative correlations have been reported for IGF-I levels and hyperlipidaemia (mixed dyslipidaemia, hypercholesterolemia, or hypertriglyceridemia), oxidized low-density lipoprotein, abdominal obesity, insulin resistance, diabetes, pro-inflammatory state (elevated C-reactive protein) and pro-thrombotic state (increased plasminogen activator inhibitor-1 and fibrinogen) (14,15,22).

However, chronically overexpression of IGF-I is related to the development of hypertension (15,18). Also patients with acromegaly (a prototypical disease characterized by increased IGF-I levels) typically present hypertension, cardiac hypertrophy, abnormal lipid profiles and insulin resistance, as well as increased incidence of CV mortality (30,33). Studies also demonstrate that subjects with higher IGF-I concentrations have an increased probability of prevalent metabolic syndrome, especially in elderly population (21).

In summary, low levels of IGF-I have been shown to be associated with an increased risk for CVD. However, high levels of IGF-I have also shown similar associations (21,30), suggesting the presence of different physiopathological mechanisms for both low and high IGF-I levels and the development of CVD. Clarifications of these underlying mechanisms are essential for CV risk assessment (21).

1.5 IGF-I ACTIONS ON BLOOD PRESSURE

The vasculature is an insulin and IGF-I sensitive tissue (15). IGF-I and its receptor are expressed in both endothelial cells and vascular smooth muscle cells (VSMC)(14). Although height and weight can account for approximately 40% of the variability of blood pressure in children, IGF-I may also have an important role (35).

IGF-I induces vasodilatation, influencing the regulation of vascular tone, arterial blood pressure and regional blood flow via nitric oxide (NO) (14,32). IGF-I is also implicated in pathophysiologic events related to CVD and arterial hypertension (Table 3) (18). Endocrine IGF-I might be of importance in both the normal physiology of vascular endothelium and in disease states (29). Nevertheless, paracrine and autocrine effects of IGF-I may also have an important role in the regulation of local blood flow (36).

Site of action	Action and potential consequence
Heart	
Myocytes	Induction of hypertrophy and promotion of cell survival that might contribute to development of LVH[a]
Vasculature	
Endothelial cells	Stimulation of NO production that might participate in regulation of BP and regional blood flow
Smooth muscle cells	Stimulation of NO production that might participate in regulation of BP and regional blood flow
	Stimulation of growth that might contribute to remodeling of the vascular wall
Kidney	
Vascular cells	Stimulation of NO production that might determine preglomerular vasodilation and increase of GFR
Mesangial cells	Stimulation of NO production that might determine mesangial relaxation and increase of Kf
	Stimulation of extracellular matrix protein synthesis that might promote glomerular sclerosis
Tubular cells	Activation of Na^+ channels that can facilitate distal Na^+ and fluid reabsorption?
Other	
Skeletal muscle cells	Stimulation of glucose uptake via IGF-IR that leads to diminished secretion of insulin and lowers insulinemia
Fibroblasts	Stimulation of fibrillar collagen synthesis that might facilitate organ and tissue fibrosis

Table 3. Actions of IGF-I of potential relevance to the pathophysiology of cardiovascular disease (18). *LVH, left- ventricular hypertrophy; NO, nitric oxid; BP, blood pressure; GFR, glomerular filtration rate; Kf, ultrafiltration coefficient; IGF-IR, insulin-like growth factor-I receptor.*

The relatively complex mechanisms involved in the vascular functions of IGF-I may explain, at least in part, the conflicting published results regarding the association of IGF-I with BP (33,34,36). It is suggested that IGF-I levels in the general population are associated with changes in BP (33–35,37,38). IGF-I levels in the upper normal range are associated with reduced BP and vascular tone (34). In this line, low IGF-I levels have been postulated to reflect decreased ability of the vascular system to relax and an increased propensity for vasoconstriction and hypertension (38). However, evidence also exists to suggest that chronic overexpression of IGF-I in the vessel wall is associated with increased arterial contractility (37).

This variability on studies may be explained because of the different roles seen in IGF-I action after acute or long-term exposure (15,33,37). Short-term injections of IGF-I appear to function as a vasodilator, decreasing mean BP mediated by its regulation of NO (15,37). However, when IGF-I is chronically overexpressed, an ongoing phenomenon of IGF-I resistance is developed in the vessel–itself. An effect on arterial contractility is reported, which is related to IGF-mediated changes in expression and relative isoform abundance of critical contractile proteins that may account for the enhanced response to contractile stimuli (37).

Thus, the relation between IGF-I and BP would change from negative to positive on a long-term exposure to the growth factor (15,33,37).

1.5.1 IGF-I SERUM LEVELS AND BLOOD PRESSURE

1.5.1.1 Influence of IGF-I ariability on blood pressure

Clarification of the biological role of IGF-I in BP regulation may be obscured by the complex mechanisms involved in maintaining IGF-I tissue bioavailability depending of GH, IGFBP, genetic and environmental modulation, age-related variability as well as endocrine factors (insulin and thyroid hormone increase IGF-I whereas glucocorticoids induce IGF-I resistance) (33). Low IGF-I bioavailability (associated with aging and vascular deterioration), resistance to IGF-I in dysmetabolic states and the complex interplay between IGF-I and other vasoactive hormones could mask the vasoprotective functions of IGF-I (33). Paracrine and autocrine production of IGF-I has also been reported to play an essential role in BP regulation, especially related to adaptative response to the increased BP and in the development of IGF-I resistance (37,39).

Under physiological conditions 90% of all circulating IGF-I is associated with several specific high-affinity binding proteins (35). The association between IGF-I level and hypertension was strength after adjustment for IGFBP-3, the major carrier of IGF-I (38). IGFBP-3 also showed an independent role in the SBP regulation (40).

1.5.1.2 Age-dependent variability of IGF-I and blood pressure

Results suggest a more frequent distribution of young and acromegaly patients in the positive association of IGF-I levels with BP, suggesting a dampened antihypertensive (or increased resistance to) effect of IGF-I in these groups of subjects. On the other hand, a more frequent distribution of older and dysmetabolic patients in the negative association between IGF-I levels and BP has been reported (33).

Dramatic hormonal and physical changes occur during puberty, along with marked growth acceleration (35). The pubertal serum IGF-I levels are in the acromegalic range of adults (41). Elevated BP in acromegalic and perhaps growing adolescents and children may be because of elevated GH levels (33). High GH levels exert a hypertensive action through expansion of the plasma volume by means of sodium-retaining effect on the kidney (33). All this physiological events could explain the positive association of IGF-I regarding high BP in this group of subjects (33).

1.5.1.3 IGF-I and hypertension

The role of circulating IGF-I and IGFBPs in human hypertension is unclear (15). Essential hypertension has been proposed as a growth-related disorder with origins in childhood and manifestations later in adult life (35). Circulating levels of free IGF-I are increased in patients with hypertension (15,18), as well as increased IGF-I to IGFBP-3 ratio and increased IGFBP-1 to IGFBP-3 ratio (15,35).

IGF-I is thought to be an important mediator in the pathophysiological response to increased BP in the vessel wall given its role in the attenuation of both receptor-mediated and voltage-induced vascular tone, predominantly through local direct actions (32,36). This ability is decreased in patients with essential hypertension, suggesting a reduction of endothelium-dependent vasorelaxant actions of IGF-I (18).

1.5.2 IGF-I VASOACTIVE AND PROLIFERATIVE EFFECTS

The vasoactive effects of IGF-I involved in the control of BP and regional blood flow are mainly via NO – dependent mechanisms (15). IGF-I stimulates production of NO both by endothelial cells and vascular smooth muscle cells (VSMC), leading to relaxation of the VSMC and consequent vasodilation (Figure 9) (18,42–44). This basal release of NO is critical for the maintenance of basal vascular tone (43). Either IGF-I and/or endothelial cell-induced NO may also initiate transcription of proteins involved in the regulation of vascular contraction (36).

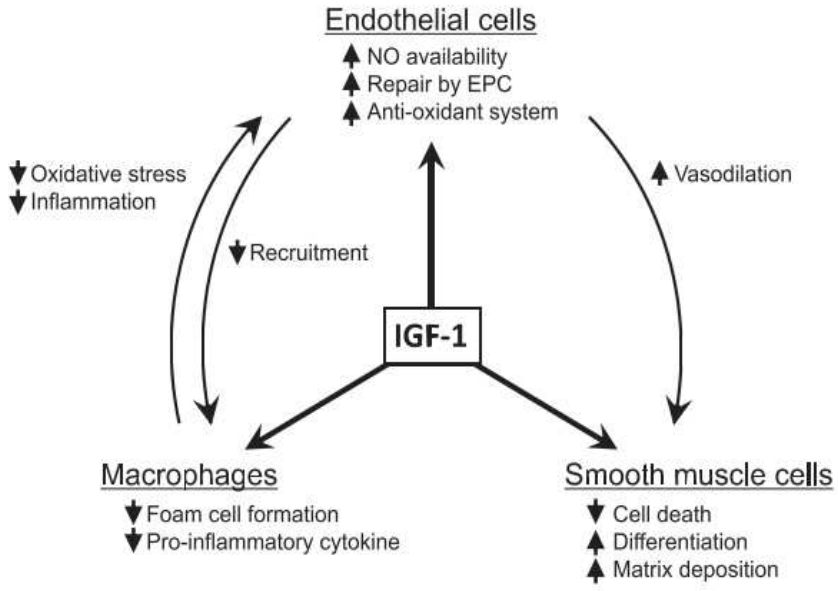

Figure 9. Effects of insulin-like growth factor-I (IGF-I) on blood vessel cells. IGF-I exerts cell type specific effects and also alters cell-cell interactions related to hypertension and atherogenesis (44). *IGF-I, insulin-like growth factor type I; NO, nitric oxide; EPC, endothelial cell progenitors*

Vasoactive effects of the IGF-I are also related to local mechanisms by which IGF-I may decrease vascular reactivity that include: (i) activation of sodium-potassium-ATPase pump, (ii) changes in intracellular calcium, (iii) interactions with α- and β-adrenergic receptors and (iv) release of an endothelium-dependent relaxing factor, among others (36).

The expression of IGF-I, IGF-IR and IGFBPs in blood vessels is regulated by multiple factors including growth factors, cytokines, lipoproteins, reactive oxygen species and hemodynamic forces (15). Moreover, IGF-I may be an important link mediating structurally adaptative growth responses in blood vessels walls (18). There is ample evidence that IGF-I stimulates VSMC growth, developing marked hyperplasia of the arteria media (37).

1.5.2.1 Nitric oxide actions

NO is produced in the cytosol of endothelial cells and diffuses into adjacent VSMC causing an eventual decrease in intracellular calcium (Ca^{2+}) flux. This fall in Ca^{2+} flux causes a decrease in the formation of the calcium – calmodulin myosin light chain kinase complex in VSMC and promotes vasorelaxation (43).

NO also affects vascular cells ranging from the regulation of gene expression to post-translational modification of proteins and protein functions (15,37,43,45,46). Smooth muscle-targeted overexpression of IGF-I results in enhanced vascular contractility, via regulation in higher abundance of contractile protein expression and changes in the relative isoform distribution of critical myofibrillar proteins (15,37).

Endothelial cells also contribute to the maintenance of a non-thrombotic state by secreting vasodilators also via NO-dependent mechanisms (47). NO produced by the endothelium and platelets inhibits adhesion, aggregation and recruitment of platelets to the growing thrombus (43). NO also inhibits VSMC proliferation (15,43,44). However, IGF-I acts as a potent mitogen and antiapoptotic factor for VSMC being responsible for hyperplasia in arteries and veins, with the progressive narrowing of the arterial lumen observed in atherosclerosis and corresponding organ hypertrophy and increased aortic medial area an thickness (15,43,44). For this reason, endothelial dysfunction has a role in a number of physiopathogenic events including atherosclerosis (**Figure 10**), vasculopathy and hypertension (47,48).

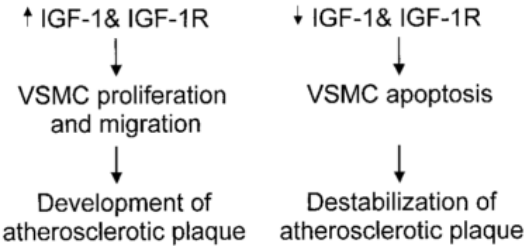

Figure 10. Dual role of IGF-I axis in atherosclerosis. *IGF-I, insulin-like growth factor-I; IGF-IR, insulin-like growth factor-I receptor; VSMC, vascular smooth muscle cells (15).*

1.5.2.2 Calcium, IGF-I and vascular smooth muscle cells

Calcium is an essential regulator of vascular smooth muscle (VSM) contractility and tone (49). VSM myocytes respond to diverse stimuli that signal either contraction or relaxation, altering arterial diameter and tissue blood flow (50,51). Coordinated contraction of VSMC in the blood vessel wall leads to vessel constriction; hence, VSM tone is closely related to systemic BP (49). As previously seen, IGF-I increases endothelial NO production, important on vascular tone regulation for its vasodilatation effects. In addition, IGF-I as well as insulin play an important role in Ca^{2+} regulation(15).

Changes in vessels tone are the result of myosin light chain phosphorylation, a process dynamically controlled by Ca^{2+} influx from the extracellular space (51). Voltage-gated Ca^{2+} channels are the principal conductances that regulate the extracellular Ca^{2+} influx (51). Two type of Ca^{2+} channels are expressed in VSM:

(i) L-type Ca^{2+} channels play an important role in vasoregulation being a therapeutic target for antihypertensive drugs (Ca^{2+} channel blockers).

(ii) T-type Ca^{2+} channels, which are implicated in development and maintenance of myogenic tone (49).

Insulin/IGF-I reduces agonist- and voltage-induced VSMC intracellular Ca^{2+} transients and Ca^{2+}–myosin light chain sensitivity in VSMC, thereby, inducing vascular relaxation (15,30,43). Plasma membrane sodium-potassium-ATPase pump is another important mechanism regulating intracellular Ca^{2+} influx by IGF-I, also attenuating vascular contractility (38,46). Ca^{2+} is also related to VSMC proliferation (52).

1.5.2.3 Other IGF-I regulated-mechanisms involved in blood pressure

In addition to the vasodilatation proprieties of IGF-I, other mechanisms through which IGF-I can be involved directly in the regulation of the BP include:

(i) IGF-I inotropic and growth effects on heart and endothelium (38).

(ii) IGF-I collagen stimulating activity and synergism activity of IGF-I and matrix metalloproteins in the development and maintenance of CVD, high SBP and vascular dysfunction (53).

(iii) Abnormalities of insulin metabolism (25,38,46):

(ii.i) Decreased IGF-I sensitivity contributes to decreased ability of insulin and IGF-I to attenuate vasoconstriction (46).

(ii.ii) Insulin resistance and chronic hyperinsulinemia increases local activity of renin-angiotensin-aldosterone system and

expression of angiotensin II receptors in vascular tissue, thus, leading to arterial wall hypertrophy and fibrosis (25).

(iii) The vasoconstrictor endothelin-1 has also been suggested as a potential link between IGF-I and vascular tone regulation, where IGF-I attenuates endothelin-I-induced contractile responses in SMC (30,44).

1.6 PHOSPATE CALCIUM PRODUCT, IGF-I AND BLOOD PRESSURE

Further to the essential role of calcium is the regulation of vascular tone (49), high levels of phosphate calcium product (PxCa) are also associated with increased risk of CVD including atherosclerosis, heart valve calcification, vascular calcification and arterial stiffness (54–56). An imbalance in calcium-phosphorus homeostasis may be involved in this pathological process, as seen in obese children and adolescents related to PTH elevations or even within a high-normal range, as well as in children with chronic kidney disease (57–59).

High serum calcium phosphate mineral levels may directly promote vascular injury due to the propensity of mineral deposition. Two distinct forms of calcification have been reported, both leading to intimal calcification which is often associated with inflammation and atherosclerosis or medial calcification that occurs in smooth muscle cells (55,60,61):

(i) A passive one, the direct calcium – phosphate precipitation in the vasculature. Calcification varied directly with the calcium concentration and inversely with phosphate concentration, suggesting that calcium may be the most important parameter in this process (38,61,62).

(ii) An active one, which induces the expression of bone-associated genes in VSMC. It is suggested that IGF-I may regulate this process by promoting osteoblastic differentiation of the VSMC which acquire the phenotype of bone-forming (osteoblast-like cells) as well as promoting cellular proliferation and inhibiting apoptosis (61,62).

Nevertheless, other studies suggest that it has never been demonstrated that exceeding the solubility of $CaHPO_4$ in plasma leads to ectopic calcification or that reducing the PxCa has an influence on clinical outcomes in patients (63). This is due, at least in part, to the fact that multiple data come from populations with chronic illnesses. In summary, current data on the role of the PxCa as an independent risk factor for vascular calcification still remain controversial (54,63).

2. JUSTIFICATION

Interventions related to disease prevention and health promotion are the most important, especially in paediatric population, in terms of reduction of future morbidity and mortality.

According to the literature, the prevalence of high blood pressure among children has risen in recent decades, as well as obesity and other cardiovascular risk factors. Considering that cardiovascular disease continue to be the leading cause of mortality in industrialized countries, more effort is required to reduce the burden of these diseases. Primary prevention strategies are those more effective aiming to minimize diseases and associated risk factors in children.

Insulin-like growth factor type I (IGF-I) has been related to several pathologic states, including the development of cardiovascular disease. Several studies have found a correlation between circulating IGF-I levels and increased risk of cardiovascular disease (coronary artery disease, ischemic stroke and congestive heart failure), as well as development of metabolic syndrome and cardiovascular risk factors (hyperlipidaemia, abdominal obesity, insulin resistance, diabetes, pro-inflammatory state and pro-thrombotic state). IGF-I levels have also been related to the regulation of blood pressure (BP) and the development of hypertension.

Conflicting data exist in the results of these studies, with discordances defining the negative or positive association between IGF-I levels and the pathophysiological events regarding the cardiovascular system. This paradox may be due to the diversity of factors involved in IGF-I regulation as well as methodological differences measuring IGF-I.

Moreover, there is not literature reviewing this association in the general paediatric population, and only few studies have reviewed IGF-I correlation in children born small for gestational age, children treated with growth hormone, in obese children and finally in adolescent population. The stronger association between IGF-I and the development of cardiovascular disease has been reported in adult populations, especially in the elderly.

For all these reasons this study could provide new evidence on the role of IGF-I in the cardiovascular system in children. The innovation of this study is to perform an observational, prospective, population-based study in a paediatric population, where no data are still available. Moreover, it will be useful to highlight the potential link between IGF-I and BP and

determine if variations depending on age exist, comparing results with data from adult population studies.

Of further in this field would be to determine which kind of association exists between IGF-I and the blood pressure in paediatric populations. Taking into account the importance of prevention strategies, it may also be appropriate to develop new screening methods in the control and/or prevention of high blood pressure to avoid the development of hypertension as well as other associated risk factors such as atherosclerosis in early stages. Moreover, these studies may reveal newer associations between IGF-I and additional cardiovascular risk factors.

3. HYPOTHESIS

In adults, low levels of IGF-I seem to be related to the development of cardiovascular disease. However, chronic overexpression of IGF-I in the vessel wall is associated with increased arterial contractility and hypertension. Because of conflicting results regarding the association between IGF-I and vascular function, we hypothesized that:

"IGF-I serum concentrations are related to blood pressure in apparently healthy children"

But we cannot a priori establish a direction of such association.

4. OBJECTIVE

To study the association of IGF-I serum concentrations with blood pressure in children. The study will be divided in:

-Associations at baseline

-Associations at follow – up

-Predictive associations

However, other variables and cardiovascular risk factors (waist, insulin resistance, triacylglycerol, high-density lipoprotein cholesterol and carotid intima-media-thickness) will be also studied in order to determine which kind of association exist between them and the IGF-I serum levels, as well as possible interactions (especially with age, gender and BMI).

5. METHODOLOGY

5.1 STUDY DESIGN

This is an observational prospective, population-based study of a cohort of apparently-healthy children in Girona, a region in North-Eastern Spain. The data have been extracted from a database compiled between 2011 and 2017.

5.2 PARTICIPANTS

The population studied are Caucasian healthy children who were consecutively recruited according to inclusion and exclusion criteria between 2011 and 2013 among those seen in primary care setting in Girona.

A target population of 528 children (240 girls and 288 boys) aged between 3 and 15 years were initially studied, achieving only a total of 158 children (76 girls and 82 boys) at a follow-up study 4 years later. For data analysis, the study subjects were also grouped according to their product phosphate calcium (PxCa) values in 3 different groups (PxCa tertiles).

5.2.1 INCLUSION CRITERIA

To be enrolled in this study the subjects have had to be healthy children from the area of Girona who accomplished the following criteria (Table 4):

-**Children of either sex whose parents were both Caucasian**.

Racial variations exist in circulating levels of IGF-I and IGFBP-3 (1). For this reason only children of Caucasian ethnicity were included in order to avoid heterogeneity in the group and limit confounding variables.

-**Children aged between 3 and 15 years of age at the initial study**.

IGF-I values show a wide range of variability during the first years of life (1). Because of this physiological variation and in order to have symmetry in data, only children $\geq$ 3 years old were accepted to participate in our study. As the follow-up study was performed 4 years later than the initial, children no older than 15 years were accepted to avoid having adults in the follow-up study.

-**Height in the mean $\pm$ 2 standard deviation (SD)**, according to growth charts of the 2010 Spanish cross-sectional growth study (Carrascosa Lezcano et al). A height $\geq \pm 2$ SD is considered pathological (see exclusion criteria ii.i and ii.ii).

-**Signed consent by at least one parent or legal guardian**.

5.2.2 EXCLUSION CRITERIA

Children with one or more of the following characteristics (**Table 4**) were directly not accepted to participate in the study because of the possible variation in normal IGF-I levels:

-**An acute disease during the last two weeks prior to recruitment**. It is well known that basal values of IGF-I vary in disease states (1), a situation that can lead to confounding if data are collected during this period.

-**Chronic disease including, but not limited to, chromosomal diseases, endocrinologic, renal, hepatic and/or cardiac aetiology diseases, neoplasia**. Low IGF-I levels were reported in critically ill patients, situation which can lead to confounding data (1). Moreover, the aim of the study is to determine the correlation between IGF-I and BP in apparently healthy population and therefore, patients with chronic illnesses may not be included.

(i) **Chromosomal diseases or presence of major congenital anomalies.** Those patients were not included due to the complexity of their illness.

(ii) **Endocrinologic diseases:**

(ii.i) Short stature (height < 2 SD) or family history of short stature (either parent <p3).

Those patients were excluded because of the relationship of short statures phenotypes with growth hormone deficiency, which courses with low IGF-I levels, as well as with syndromic aetiologies (Turner syndrome, SHOX mutations, etc) (1).

(ii.ii) Patients diagnosed as having gigantism (even though this is a rare pathology), or conditions in which GH excess occurs (64,65) to limit possible confounding derived from excessive IGF-I levels related to these pathologies.

(ii.iii) Syndromic or endocrine diseases.

Pathological situations such as hypothyroidism, Cushing syndrome, structural disorders of the hypothalamus, etc. are associated with weight gain (66). Data show a complex relationship between endocrinologic diseases and/or their treatment and the IGFs and their binding proteins (1). So, these patients were not included to limit confounding variables.

(ii.iv)Patients diagnosed of diabetes and/or treated with insulin

IGF-I levels are decreased in children with newly diagnosis of

insulin–dependent diabetes, with a negative correlation between IGF-I and degree of metabolic control. Insulin treatment normalizes the reduced free IGF-I (1). Due to the interaction of the diabetic state with IGF-I physiological function and levels those patients were excluded.

(ii.v) Early or advanced puberty (puberal signs in 8-9 year-old girls and in 9-10 year-old boys) or delayed puberty (no puberty onset in >13 year-old girls and in ≥ 14 year-old boys).

IGF-I levels increase with increased pubertal maturation. A significant variation with age occurs within each Tanner stage of puberty increasing IGF-I levels with age in the early pubertal stages and a decrease in the late pubertal stages (1). Thus, alterations in the puberty onset are usually linked to other diseases (such as McCune-Albright syndrome, an activating mutation in the G-protein)(1). Because of the wide variability in IGF-I levels associated with this pathological conditions, as well as the complexity of the underlying mechanisms associated, this patients were also excluded.

(iii) **Chronic diseases of renal, hepatic and/or cardiac aetiology.**

Those patients were not included due to the severity of these illnesses, the associated comorbidities as well as the influence in the IGF axis.

(iii.i) Renal disease

Normal or elevated IGF-I levels are found in children with chronic renal failure, despite of their growth-retardation (1).

(iii.ii) Hepatic disease

Children with liver disease (biliary atresia, hepatitis, liver failure) are characterized by growth failure and extremely low circulating IGF-I and IGFBP-3 levels(1).

(iii.iii) Cardiac disease

Any pathological state of the CV system may act as a confounding factor and had been considered exclusion criteria.

(iv) **Tumor process.**

Patients were not included due to the severity of the illnesses.

-**Children born small for gestational age (SGA) or with intrauterine growth retardation (IUGR) defined by birth weight, length or head circumference**

<3rd centile. SGA and IUGR children have significantly lower IGF-I levels during pregnancy. However, higher postnatal growth velocity and higher IGF-I and IGFBP-3 levels are seen in these children may be linked to postnatal catch-up growth consistent with the hypothesis that under-nutrition in utero leads to reprograming of the IGF-I axis and is also associated with the development of metabolic syndrome in young adulthood (1). For this reason and because of the willing of the development of an apparently-healthy children study, these patients were not candidates to be included.

-**Children treated with growth hormone, oestrogen or glucocorticoids**. Because of the influence of this factors and the pathologies treated with them in the GH – IGF-I axis (9). Also **those patients treated with antihypertensive drugs**, for its influence on blood presure. Patients who were treated with those drugs were not accepted in our study to limit confounding variables.

-**Adopted children or conceived through in vitro fertilization**.

To avoid heterogeneity due to racial variations and limiting confounding variables this group of patients were excluded from the study.

-**Subjects who are unwilling or unable in the opinion of the investigator** to undergo all the study procedures.

-**Non accomplishment of one or more of the inclusion criteria**.

INCLUSION CRITERIA	EXCLUSION CRITERIA
-Children of either sex whose parents were both Caucasian. -Children aged between 3 and 15 years of age at the initial study. -Height within the mean ± 2 SD, according to growth charts references. -Signed consent by at least one parent or legal guardian.	-An acute disease during the last two weeks prior to recruitment. -Chronic disease including, but not limited to: i. Chromosomal diseases or presence of major congenital anomalies. ii. Endocrinologic diseases: ii.i Syndromic, endocrine aetiology or family history of short stature ii.ii Patients diagnosed of gigantism or conditions of GH excess ii.iii Syndromic or endocrine diseases. ii.iv Patients diagnosed of diabetes and/or treated with insulin ii.v Early or advanced or delayed puberty. iii. Renal, hepatic and/or cardiac chronic diseases. iv. Tumor process. -Children born small for gestational age (SGA) or with intrauterine growth retardation (IUGR). -Children treated with growth hormone, oestrogen, glucocorticoids, or antihypertensive drugs. -Adopted children or conceived through in vitro fertilization. -Subjects who are unwilling or unable in the opinion of the investigator to undergo all the study procedures. -Non accomplishment of one or more inclusion criteria.

Table 4. **Sample selection.** Summary of the inclusion and exclusion criteria.

5.3 SAMPLING METHOD, SAMPLE SIZE AND STUDY POWER

A consecutive non-probabilistic sampling-method was performed. Children attending well-child check-up visits at the primary care were informed about the study and, if inclusion and exclusion criteria were met, they were referred to the Paediatric Department of the *Hospital Universitari de Girona Doctor Josep Trueta* for recruitment into the study, where examinations were performed and data collection undertaken from January 2011 until December 2013.

Accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, a minimum of 85 subjects were needed to be able to detect a clinically significance correlation coefficient of at least 0.3 between IGF-I and BP. A drop-out rate of 0% was estimated. Sample size has been calculated with GranMo (Version 17.12)(67).

With a sample size of 528 subjects in the basal study and accepting an alpha risk of 0.05, the statistical power of the study was 100% to detect an effect size equal or more than 0.3 for correlation coefficient. Likewise, the statistical power was 96% to detect the same effect size in the follow-up study with a sample size of 158 subjects. Sample power has been calculated with Gpower® (Version 3.1.9.2).

5.4 VARIABLES

5.4.1 INDEPENDENT VARIABLE

The independent variable of our study are the **serum levels of IGF-I**. It is a continuous quantitative variable, expressed in nanograms for decilitre (ng/dL). It has been transformed to its logarithm for the analysis of correlation to improve the symmetry of the variable. Associations have been studied with BP parameters as well as CR risk factors.

5.4.2 OUTCOME VARIABLES

The outcome variables recorded in this study (Table 5) are blood pressure parameters, which include systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse pressure (PP). The description of the outcome variables is the following:

-**Systolic blood pressure** (**SBP**). Continuous quantitative variable, expressed in millimetres of mercury (mmHg). It has been described with the arithmetic mean and the standard deviation. Systolic blood pressure is force exerted on the artery wall when the heart is pumping (contracting) (68). Normal values are < 120 mmHg or < 95th percentile adjusted for age, sex and height (6).

-**Diastolic blood pressure** (**DBP**). Continuous quantitative variable, expressed in millimetres of mercury (mmHg). It has been described with the arithmetic mean and the standard deviation. The force exerted when the hearts relaxes is known as diastolic blood pressure (68). Normal values are < 80 mmHg or <95th percentile adjusted for age, sex and height (6).

-**Pulse pressure** (**PP**). Continuous quantitative variable, expressed in millimetres of mercury (mmHg). It has been described with the arithmetic mean and the standard deviation. Pulse pressure is defined as the difference between SBP and DBP (PP = SBP – DBP), and it has been investigated as CV risk factor (69).

5.4.3 SECONDARY OUTCOME VARIABLES

The secondary outcome variables included in this study are all metabolic parameters, some of them related with the development of CV risk.

-**Weight**. Continuous quantitative variable, expressed in kilograms (Kg). It has been described with the arithmetic mean and the standard deviation.

-**Height**. Continuous quantitative variable, expressed in centimetres (cm). It has been described with the arithmetic mean and the standard deviation.

-**Waist**. Continuous quantitative variable, expressed in centimetres (cm). It has been described with the arithmetic mean and the standard deviation. Waist circumference and abdominal adiposity are associated with hypertension (6).

-**Insulin** (**log_{10}insulin**). Continuous quantitative variable, expressed in mili-international units per litre (mlU/L). It has been described with the arithmetic mean and the standard deviation. It is related to the IGF axis (14).

-**Homeostasis model assessment–estimated insulin resistance** (**log_{10} HOMA-IR**). Continuous quantitative variable, expressed in mili-international units per litre (mlU/L). It is calculated multiplying fasting plasma insulin (FPI) by fasting plasma glucose (FPG) divided by the constant 22.5 (HOMA-IR=[FPIxFPG]/22.5). It has been described with the arithmetic mean and the standard deviation. It is widely used for the estimation of insulin resistance in research (70).

-**Lipid profile.** Lipid profile is defined for total cholesterol, low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c) and triglycerides. Although LDL-c is the principal lipoprotein transporting cholesterol in the blood and is directly associated with CVD, HDL-c and

triglycerides have also been defined as independent risk factors for CVD (5). Moreover, serum triglycerides and serum HDL-c are part of the criteria for the definition of the metabolic syndrome (61). For this reason, our study includes:

·**Triacylglycerol** (**log_{10} triacylglycerol**). Continuous quantitative variable, expressed in milligrams per decilitre (mg/dL). It has been described with the arithmetic mean and the standard deviation.

·**High-density lipoprotein cholesterol** (**HDL-cholesterol**). Continuous quantitative variable, expressed in milligrams per decilitre (mg/dL). It has been described with the arithmetic mean and standard deviation.

-**Carotid intima–media thickness** (**carotid IMT**). Continuous quantitative variable, expressed in millimetres (mm). It has been described with the arithmetic mean and the standard deviation. Evidence in children and young adults suggests that the development of atheromatous plaque begins early in childhood during prepuberal years. Very early vascular modifications can be identified by the measurements of carotid IMT (71).

5.4.4 COVARIATES

Clinical and epidemiological characterization of patients has been performed:

-**Age**. Continuous quantitative variable, expressed in years. It has been collected at the first study and at the follow-up study done 4 years later. It has been described as an arithmetic mean and standard deviation. Age is an important factor because of the physiological variations seen in IGF-I levels according to the stage of development of the subject. Maximal levels are achieved during the peripuberal growth and puberty (14).

-**Gender.** Dichotomus qualitative variable, expressed as male or female. It has been described as an absolute value and a percentage. Gender is also an important covariate because of the role of sexual hormones in the regulation of the GH – IGF-I axis, with higher levels in women (12,14,18). Moreover, the prevalence of high BP is a greater in boys than in girls (6).

-**Body mass index** (**BMI**). Continuous quantitative variable, expressed in kg/m^2. It is calculated through weight (kg) and height (m^2) and described with the arithmetic mean and the standard deviation. Prevalence of hypertension ranges from 3.8% to 24.8% in youth with overweight and obesity. Obesity is also associated with a lack of circadian variability of BP, with the

disappearance of the expected nocturnal BP dip in un to 50% of children with obesity (6), so it can be a confounding factor for our study.

-**Phosphate calcium product (PxCa).** Continuous quantitative variable, expressed in milligrams per decilitre (mg/dL). It has been calculated multiplying serum phosphate values (mg/dL) for serum calcium values (mg/dL). PxCa values, as well as serum phosphate and calcium values, have been described with the arithmetic mean and the standard deviation. These data have been used to divide the population in tertiles of PxCa for the statistical analyses.

-**Insulin-like growth factor binding protein-3 (IGFBP-3)**. Continuous quantitative variable, expressed in nanograms per decilitre (mg/dL). It has been described with the arithmetic mean and the standard deviation. IGFBP-3 is the major carrier of IGF-I and a main regulator of the IGF-I bioavailability in tissues (9). It also showed an independent role in the SBP regulation (38,40).

-**Ratio IGF-I/IGFBP-3.** Continuous quantitative variable, expressed in nanograms per decilitre (mg/dL). It has been described with the arithmetic mean and the standard deviation. Correlation between ratio IGF-I/IGFBP-3 and BP is also studied in order to detect possible differences in this association.

VARIABLES		TYPE	INSTRUMENTATION	UNITS
Independent	Serum IGF-I levels	CQV	Direct immunoradiometric assay (IRMA)	ng/dL
Outcome variables	Systolic blood pressure (SBP)	CQV	Electronic sphygmomanometer (Dinamap Pro100, GE Healthcare, UK)	mmHg
	Diastolic blood pressure (DBP)	CQV		mmHg
	Pulse pressure (PP)	CQV	SPSS calculated variable	mmHg
Secondary outcome variables	Weight	CQV	Calibrated scale (SECA, Germany)	kg
	Height	CQV	Harpendenstadiometer (Holtain Ltd, UK)	cm
	Waist	CQV	Measuring tape	cm
	Glucose	CQV	Hexokinase method (AEROSET c8000)	mg/dL
	Insulin and fasting insulin	CQV	Immunochemiluminiscence (IMMULITE 2000)	mIU/L
	Insulin resistance (HOMA-IR)	CQV	SPSS calculated variable	
	Triacylglycerol	CQV	Glycerol-phosphate oxidase method	mg/dL
	HDL-c	CQV	Accelerator selective detergent method	mg/dL
	Carotid IMT	CQV	High-resolution ultrasonography (MyLabTM25)	cm
Covariates	Age	CQV	Clinical examination	Years
	Gender	DQV	Clinical examination	Female/male
	Body mass index (BMI)	CQV	SPSS calculated variable	kg/m^2
	Phosphate	CQV	Enzymatic colorimetric method	mg/dL
	Calcium	CQV	Enzymatic colorimetric method	mg/dL
	Phosphate calcium product	CQV	SPSS calculated variable	mg/dL
	IGFBP-3	CQV	Immunoassay (ELISA method)	ng/dL
	Ratio IGF-I/IGFBP-3	CVQ	SPSS calculated variable	

Table 5. Summary of the studied variables. *IGF-I, insulin-like growth factor type I; HDL-c, high-density lipoprotein cholesterol; carotid IMT, carotid intima-media thickness; IGFBP-3, IGF binding protein-3; CQV, continuous quantitative variable; DQV, dichotomus qualitative variable; ng/dL, nanograms for decilitre; mmHg, millimetres of mercury; Kg, kilograms; cm, centimeters; mIU/L, mili-international units per litre; mg/dL, milligrams per decilitre; kg/m^2, kilograms per quadrat mete; SPSS, Statistical Package for Social Sciences.*

5.5 DATA COLLECTION AND PROCEDURES

5.5.1 PRE-SELECTION VISIT

Children, as well as their families, who were seen at the primary care and considered to be adequate candidates were informed about the study. Informed consent regarding the participation in the study was given. An appointment one week later was scheduled for the next step. A period of a week was given to all the candidates in order to read all the information about the study (procedures done to their children, treatment of the data, confidentiality, etc.) and to decide about the participation or not.

5.5.2 SELECTION, FIRST-VISIT AND FOLLOW-UP VISIT

At the selection visit, after giving consent (signing the informed consent), the most important features of the clinical history were reviewed with the patient and the family in order to determine if inclusion and exclusion criteria were met.

If the patient was accepted as a participant in the study, clinical, laboratory and ultrasonography assessments were performed. Patients included in the study would be scheduled for a subsequent visit for the follow-up study 4 years later where the same clinical, laboratory and ultrasonography assessments would be performed. An identification code was assigned to all patients included in the study, which remained constant during all the process. This was the identification number in the electronic Case Report Form.

All measurements were performed by well-trained observers, who were unaware of the children's clinical, laboratory and ultrasonography characteristics. All serum samples were obtained between 8:00 and 9:00am under fasting conditions.

5.5.2.1 Clinical assessments

Sociodemographic data (age, birth date, gender and place of residence) as well as medical history, current illnesses and family diseases were assessed during the clinical examination and review of the clinic history.

-**Weight and height**. Subjects were weighed whit a calibrated scale (SECA, Hamburg, Germany) wearing only underwear clothes. Standing height was measured with a Harpenden stadiometer (Holtain Ltd, Crymych, UK), also with the child wearing light clothes without shoes.

-**Waist.** With the patient in the supine position, waist circumference was measured at the umbilical level using a measuring tape.

-**Body mass index (BMI)** was calculated for each patient using weight and height values (BMI = weight [kg] / height^2 [m^2]) by means of the Statistical Package for Social Sciences (SPSS® for Windows version 22.0, IBM Corp, Amonk, NY).

-**Systolic and diastolic blood pressure (SBP, DBP)** were measured using an electronic sphygmomanometer (Dinamap Pro 100, GE Healthcare, Chalfont St. Giles, UK). Blood pressure was measured with the child in decubitus supine position on the right arm, after a 10-minut- rest, in a quiet room at 22ºC. It was assessed twice or three times and the average of two similar measurements was taken as the correct value.

-**Pulse pressure (PP)** was also derived variable (PP = SBP – DBP). Pulse pressure value was also calculated using the SPSS program with SBP and DBP values.

5.5.2.2 Laboratory assessments

Blood samples were obtained in the morning after an overnight fast. All the samples were analysed by the laboratory of the *Hospital Universitari de Girona Doctor Josep Trueta* using the same procedures:

-**IGF-I** was measured by immunoassay with highly specific antibodies for IGF-I by means of the direct immunoradiometric assay (IRMA, Demeditec, Kiel, Germany). Lower detection limit: 4.55ng/mL

-**Glucose** was determined using a quantitative enzymatic assay, the hexokinase method (AEROSET c8000). Lower detection limit: 2.5 mg/dL

-**Insulin and fasting insulin** were measured by immunochemi-luminiscence (insulin-automated assay IMMULITE 2000 System, Diagnostic Products, Los Angeles, CA). Lower detection limit: 0.4 mIU/L

-**Insulin resistance** was estimated by the homeostasis model assessment (HOMA-IR). The values were calculated using glucose and insulin values as follows:

HOMA-IR= fasting insulin (mU/L) x fasting glucose (mM)/22

-**Lipid profile.** Total serum triacylglycerol was measured by glycerol-phosphate oxidase method. High-density lipoprotein cholesterol (HDLc) was measured by accelerator selective detergent method (ARCHITECT, Abbott Laboratories Park, IL). Lower detection limits were 5.0 mg/dL and 2.5 mg/dL respectively.

-**Phosphate and calcium** were measured using a standard colorimetric method using an autoanalyzer (Hitachi, Tokyo, Japan).

-**Phosphate calcium product** was calculated using the phosphate and calcium values obtained in the laboratory assessments.

-**Insulin-like growth factor binding protein-3 (IGFBP-3)** was measured by immunoassay (ELISA method). Lower detection limit: 75.99 ng/mL

-**Ratio IGF-I/IGFBP-3** was calculated using the IGF-I and IGFBP-3 values obtained in the laboratory assessments.

5.5.2.3 Ultrasonography assessments

-**Carotid IMT** was assessed by high-resolution ultrasonography (MyLabTM25, Esaote, Firenze, Italy). A well-trained operator performed all ultrasound measurements with an operator variability <6%, demonstrating a very high repeatability of the method. cIMT measurements were determined from diastolic images obtained using a linear 7.5-12 MHz transducer. cIMT was measured five times, at the level of the right distal common carotid artery, 1cm away from its bifurcation. None of the subjects had signs of atherosclerotic plaques.

5.5.3 DATA MANAGEMENT

Once all the data were collected and the Clinical and Ethical Investigation Committee (CEIC) of the *Hospital Universitari de Girona Doctor Josep Trueta* approved the project, data were registered in an electronic Case Report Form (eCRF).

This database included all the relevant data of the study patients, from both the first determination and the follow-up visit. Homogeneity in data collection was ensured, as only one person intervened in this process. A final review of the eCRF was done to ensure that all the data registration was correct. As previously mentioned, an identification number was created for each participant in the study to maintain data anonymity and confidentiality.

Data from the database was exported to the SPSS® in order to generate derived variables and to perform the data analyses. No identification information was exported, so anonymity and confidentiality were guaranteed. As an investigator, I have only treated data from SPSS® (Statistical Package for Social Sciences for Windows v22.0, IBM Corp, Amonk, NY).

With regard to the follow-up period, an inclusion rate of the 24.24% was reported (from 528 patients at the basal study to 128 at follow-up).

6. STATISTICAL ANALYSES

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS® for Windows version 22.0, IBM Corp, Amonk, NY). P values <0.05 were considered statistically significant. The analysis includes:

(i) **Associations at baseline**, studies are done with basal values (first assessment) of IGF-I and blood pressure (SBP, DBP, PP) as well as other cardiovascular risk factors.

(ii) **Associations at follow – up**, studies are done with the follow-up values (second assessment) of IGF-I, blood pressure (SBP, DBP, PP) and cardiovascular risk factors.

(iii) **Predictive associations**, studies are done with basal values of IGF-I and the follow-up values for blood pressure (SBP, DBP, PP) as well as other cardiovascular risk factors.

6.1 UNIVARIATE ANALYSIS

Results for variables with a normal distribution are expressed with the arithmetic mean and the standard deviation (SD). For those variables without a normal distribution (non-parametric variables), a logarithmically transformation was done to improve symmetry. For categorical variables frequencies and percentages were used.

Baseline characteristics of the population are described using qualitative categorical variables (gender) and quantitative variables with normal distribution (age, weight, height, BMI, waist, SBP, DBP, PP, HDL-cholesterol, insulin, IGF-I, IGFBP-3, ratio IGF-I/IGFBP-3, calcium, phosphate, PxCa and carotid IMT) and mathematical transformed quantitative variables into logarithmic variables for those without a normal distribution (insulin, IGF-I, HOMA-IR, triacylglycerol).

6.2 BIVARIATE ANALYSIS

Cross-sectional differences in continuous variables (age, weight, height, BMI, waist, SBP, DBP, PP, $\log_{10}$ insulin, $\log_{10}$ HOMA-IR, $\log_{10}$ triacylglycerol, HDL-cholesterol and carotid IMT) among tertiles of PxCa were tested using one-way ANOVA. Correlations between continuous variables were studied by Pearson's method in the whole group of subject and in subgroups thereof defined by tertiles of PxCa.

6.3 MULTIVARIATE ANALYSIS

Multiple linear regression analyses was used to study independent associations between IGF-I and SBP, DBP and PP after adjustment for potentially confounding factors (age, sex, BMI). Independent associations were studied in the whole group of subjects and in subroups thereof defined by tertiles of Pxca.

7. RESULTS

As mentioned above, statistical analyses have been performed for all the studied subjects and in subgroups thereof defined by tertiles PxCa due to the important role of calcium in the regulation of the vascular tone and BP that was previously described (32,45,51,60,62). These subgroup analyses have been repeated in the follow-up study and in the prospective study.

7.1 DESCRIPTIVE ANALYSES

7.1.1 BASAL STUDY

A target population of 528 patients was studied. **Table 6** shows the baseline characteristics of the study population in clinical, laboratory and ultrasonography assessments both for the whole group of subject and in subgroups thereof defined by tertiles of PxCa.

	All subjects (n=528)	P x Ca tertil 1 (n=176)	P x Ca tertil 2 (n=176)	P x Ca tertil 3 (n=176)	p lineal
Clinical assessments					
Age (year)	8.81 ± 2.10	9.37 ± 2.12	8.69 ± 1.88	8.36 ± 2.19	<0.0001
Gender (%F)	240 (45.5%)	71 (40.3%)	83 (47.2%)	86 (48.9%)	0.109
Weight (kg)	40.24 ± 16.47	43.71 ± 18.13	38.78 ± 14.83	38.25 ± 15.82	0.002
Height (cm)	135.91 ± 14.82	138.84 ± 15.25	134.98± 13.31	133.93 ± 14.82	0.002
BMI (kg/m^2)	20.85 ± 5.05	21.66 ± 5.34	20.53± 4.78	20.37 ± 4.95	0.016
Waist (cm)	68.95 ± 14.57	71.59 ± 15.14	67.94 ± 13.58	67.33 ± 14.66	0.006
SBP (mmHg)	108.62 ± 10.52	110.90 ± 11.15	108.10 ± 9.86	107.67 ± 10.43	0.031
DBP (mmHg)	62.45 ± 7.89	62.90 ± 8.17	61.85 ± 7.61	62.60 ± 7.87	0.715
PP (mmHg)	46.14 ± 9.33	47.18 ± 9.86	46.17 ± 9.13	45.07 ± 8.90	0.034
Laboratory assessments					
Glucose (mg/dL)	87.36 ± 6.74	87.88± 7.05	87.29 ± 6.25	86.90 ± 6.90	0.175
Insulin (mlU/L)	7.01 ± 6.59	7.12 ± 6.15	6.85 ± 7.41	7.05 ± 6.16	0.926
$\log_{10}$ insulin (mlU/L)	0.99 ± 0.24	1.01 ± 0.24	1.02 ± 0.24	0.93 ± 0.24	0.062
$\log_{10}$ HOMA-IR	0.32 ± 0.25	0.34 ± 0.25	0.34 ± 0.25	0.26 ± 0.25	0.120
$\log_{10}$ triacylglycerol (mg/dL)	1.78 ± 0.20	1.77 ± 0.16	1.80 ± 0.24	1.76 ± 0.18	0.653
HDL-cholesterol (mg/dL)	57.98 ± 17.50	60.98 ± 18.36	53.94 ± 15.61	60.50 ± 18.33	0.992
IGF-I (ng/mL)	216.12± 117.96	211.29± 121.30	202.27± 100.75	234.81± 128.33	0.061
$\log_{10}$ IGF-I (ng/mL)	2.28 ± 0.22	2.27 ± 0.23	2.26 ± 0.19	2.31± 0.23	0.038
IGFBP-3 (ng/mL)	4.46 ± 0.92	4.40 ± 0.93	4.39 ± 0.86	4.58 ± 0.98	0.067
Ratio IGF-I/IGFBP-3	47.03 ± 19.44	46.22 ± 20.18	44.90 ± 16.59	49.99 ± 21.03	0.070
Calcium (mg/dL)	9.93 ± 0.33	9.79 ± 0.32	9.92 ± 0.30	10.06 ± 0.29	<0.0001
Phosphate (mg/dL)	4.86 ± 0.47	4.39± 0.29	4.85 ± 0.18	5.34 ± 0.29	<0.0001
PxCa (mg/dL)	48.27 ± 5.03	42.97 ± 2.84	48.09 ± 1.40	53.73 ± 2.77	<0.0001
Ultrasonography assessments					
Carotid IMT (mm)	0.406 ± 0.007	0.414 ± 0.007	0.402 ± 0.007	0.401 ± 0.008	0.111

Table 6. Descriptive analyses of the population in the basal study. P values are from ANOVA analyses. *F, female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; IGF-I, insulin-like growth factor I; IGFBP-3 IGF binding protein 3; PxCa, phosphate calcium product IMT, intima-media thickness.*

The mean age of the patients was 8.81 years, with a minimum age of 3.22 and maximum age of 15.10 years. The proportion of girls in this study was 45.5%. The results of

anthropometric measurements demonstrate that the majority of the participants were healthy, with a BMI mean of 20.85 kg/m^2 with values that ranged from 12.00 to 40.11 kg/m^2. Means of parameters of metabolism, as well as lipid and ionic profile, were within normal range. The mean of SBP and DBP were also within normal range (108.62/62.45 mmHg with a mean PP of 46.14 mmHg). However, if we analyse maximum and minimum for these variables, we observe a number of low or high values: SBP range from 80 to 143 mmHg, DBP from 41 to 80 mmHg and PP from 20 to 77 mmHg.

Because all subjects were apparently–healthy and unselected from the general population, and the distribution of the BP values in our study was normal, we decided not to exclude those subjects with BP values below of above the normal values for age, gender and height. For this same reason, children with obesity (defined in children as BMI $\geq$ 2 SD, equivalent to a BMI >30 kg/m^2 at 19 years)(72,73) were neither excluded. However, the group of patients with either high BP or obesity represented less than a 5% of the studied population. Similar proportions of high BP and/or obesity were seen in the follow-up study.

The one-way ANOVA analysis, **Table 6**, shows the distribution of the independent factors according to the tertiles of PxCa. Significant association (p value <0.05) were found for age, weight, height, BMI, waist, SBP, PP and IGF-I in the basal study.

7.1.2 FOLLOW-UP STUDY

A target population of 158 patients was studied. **Table 7** shows the clinical, laboratory and ultrasonography assessments in the studied subjects both for general data and after adjustments for tertiles according to PxCa values.

Data for the follow-up study has been collected 4 years later after the basal study. For this reason, the mean age of the patients was 12.77 years, with a minimum age of 7.92 and maximum age of 17.84 years. The proportion of girls in the follow-up study increased to the 48.1%. The results of anthropometric measurements demonstrate that children maintained healthy, with a BMI mean of 22.80 kg/m^2, even that BMI values ranged from 13.60 to 40.80 kg/m^2. Means of the parameters of metabolism, as well as lipid and ionic profile were within normal range. The mean of SBP and DBP have risen as compared to the basal study (114.46/62.43 mmHg and a mean PP of 52.03 mmHg), probably related to children growth (BP increases with higher height and age)(6,35). Again, we did not exclude those subjects with BP values above the lower or upper limit of normality, and thus the ranges for these variables were: SBP from 87 to 149 mmHg, DBP from 42 to 85 mmHg and PP from 29 to 93 mmHg.

	All subjects (n=158)	P x Ca tertil 1 (n=52)	P x Ca tertil 2 (n=53)	P x Ca tertil 3 (n=53)	p lineal
Clinical assessments					
Age (year)	12.77 ± 1.89	13.15 ± 1.78	12.86 ± 1.78	12.31 ± 2.05	0.022
Gender (%F)	76 (48.1%)	25 (48.1%)	23 (43.4%)	28 (52.8%)	0.022
Weight (kg)	57.49± 20.31	59.89 ±22.36	60.69 ± 20.71	51.82 ± 16.59	0.042
Height (cm)	157.44 ± 12.45	157.83 ± 12.80	159.11± 12.04	155.39 ± 12.13	0.309
BMI (kg/m^2)	22.80 ± 6.04	23.39 ± 6.47	23.49± 6.17	21.50 ± 5.35	0.110
Waist (cm)	76.11 ± 15.86	77.51 ± 16.55	78.86 ± 15.39	72.04 ± 15.11	0.074
SBP (mmHg)	114.46 ± 12.20	114.69 ± 12.52	116.28 ± 11.42	112.42 ± 12.56	0.336
DBP (mmHg)	62.43 ± 7.89	62.52 ± 7.78	63.60 ± 8-57	61.17 ± 7.21	0.377
PP (mmHg)	52.03 ± 11.43	52.17± 11.79	52.68 ± 11.80	51.24 ± 11.37	0.678
Laboratory assessments					
Glucose (mg/dL)	86.30 ± 7.22	84.98 ± 7.30	85.85 ± 8.34	88.04 ± 5.52	0.030
Insulin (mlU/L)	11.52 ± 7.04	12.45 ± 8.18	12.10 ± 6.77	10.02 ± 5.91	0.076
$\log_{10}$ insulin (mlU/L)	0.99± 0.24	1.03 ± 0.24	1.01 ± 0.24	0.93 ± 0.24	0.060
$\log_{10}$ HOMA-IR	0.32 ± 0.25	0.35 ± 0.26	0.34 ± 0.25	0.27 ± 0.25	0.140
$\log_{10}$ triacylglycerol (mg/dL)	1.79 ± 0.20	1.79 ± 0.18	1.80 ± 0.24	1.76 ± 0.18	0.532
HDL-cholesterol (mg/dL)	57.26 ± 16.79	58.21 ± 17.36	53.89 ± 15.48	59.70 ± 17.25	0.642
IGF-I (ng/mL)	372.73± 158.26	375.15± 139.20	389.32± 165.22	353.77± 169.30	0.488
$\log_{10}$ IGF-I (ng/mL)	2.26 ± 0.21	2.24 ± 0.19	2.26 ± 0.17	2.29 ± 0.24	0.241
IGFBP-3 (ng/mL)	5.36 ± 0.94	5.34 ± 0.77	5.42 ± 0.96	5.32± 1.09	0.885
Ratio IGF-I/IGFBP-3	68.44 ± 24.21	69.53 ± 22.79	70.38 ± 24.30	65.47 ± 25.64	0.395
Ultrasonography assessments					
Carotid IMT (mm)	0.412 ± 0.004	0.411 ± 0.004	0.410 ± 0.004	0.413 ± 0.004	0.196

Table 7. Descriptive analyses of the population in the basal study. P values are from ANOVA analyses. *F, female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; IGF-I, insulin-like growth factor-I; IGFBP-3 IGF binding protein 3; PxCa, phosphate calcium product IMT, intima-media thickness.*

The one-way ANOVA analysis, Table 7, shows the distribution of the independent factors according to the tertiles of PxCa. In the follow-up study, in contrast to the results obtained in the baseline study, the associations were only maintained for age and weight. New associations with gender and glucose were also found in the follow-up study.

7.2 CORRELATIONS AND MULTIVARIATE ANALYSES

7.2.1 IGF-I AND BLOOD PRESURE AT BASE LINE

Table 8 shows the correlations for logarithmic values of IGF-I ($\log_{10}$ IGF-I) and selected variables of cardiovascular risk studied (age, weight, height, BMI, waist, SBP, DBP, PP, $\log_{10}$ insulin, $\log_{10}$ HOMA-IR, $\log_{10}$ triacylglycerol, HDL-cholesterol, carotid IMT). Table 9 also shows correlations for logarithmic values of IGF-I and the same selected variables according to PxCa tertiles. In the studied subjects as a whole, $\log_{10}$ IGF-I was associated with all the studied variables (all with $p<0.0001$). In the study according to PxCa tertiles, age, weight, height BMI, waist, $\log_{10}$ insulin, $\log_{10}$ HOMA-IR were associated with $\log_{10}$ IGF-I with a p value <0.0001. For $\log_{10}$ triacylglycerol and HDL-cholesterol association was less strong but significant, especially in high tertiles. For carotid IMT the strongest association was seen in tertile 2.

Focusing on SBP, DBP and PP association with IGF-I. Even that in studied subjects as a whole log_{10} IGF-I was associated with all blood pressure variables (all p<0.0001), in the study for PxCa tertiles association with IGF-I became stronger as well as higher was the PxCa tertile, with a p value <0.0001 for all SBP, DBP and PP in tertile 3 but no in tertiles 1 and 2.

log_{10} IGF-I (ng/Ml)	All subjects (n=528)	
	r	p
Clinical assessments		
Age (year)	0.531	<0.0001
Weight (kg)	0.508	<0.0001
Height (cm)	0.604	<0.0001
BMI (kg/m^2)	0.385	<0.0001
Waist (cm)	0.410	<0.0001
SBP (mmHg)	0.322	<0.0001
DBP (mmHg)	0.192	<0.0001
PP (mmHg)	0.200	<0.0001
Laboratory assessments		
log_{10} insulin (mlU/L)	0.439	<0.0001
log_{10} HOMA-IR	0.444	<0.0001
log_{10} triacylglycerol (mg/dL)	0.253	<0.0001
HDL-cholesterol (mg/dL)	-0.152	<0.0001
Ultrasonography assessments		
Carotid IMT (cm)	0.158	<0.0001

Table 8. Bivariate study. Correlation coefficients for IGF-I and selected variables in the studied subjects. Basal study. P and r values are from Pearson correlation analyses. *F, female; IGF-I, insulin-like growth factor-I; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; IMT, intima-media thickness.*

log_{10} IGF-I (ng/Ml)	PxCa tertil 1 (n=176)		PxCa tertil 2 (n=176)		PxCa tertil 3 (n=176)	
	r	p	r	p	r	p
Clinical assessments						
Age (year)	0.602	<0.0001	0.436	<0.0001	0.611	<0.0001
Weight (kg)	0.540	<0.0001	0.491	<0.0001	0.538	<0.0001
Height (cm)	0.650	<0.0001	0.575	<0.0001	0.631	<0.0001
BMI (kg/m^2)	0.424	<0.0001	0.383	<0.0001	0.378	<0.0001
Waist (cm)	0.441	<0.0001	0.396	<0.0001	0.428	<0.0001
SBP (mmHg)	0.228	0.002	0.330	<0.0001	0.448	<0.0001
DBP (mmHg)	0.110	0.148	0.212	0.005	0.263	<0.0001
PP (mmHg)	0.167	0.027	0.175	0.020	0.293	<0.0001
Laboratory assessments						
log_{10} insulin (mlU/L)	0.509	<0.0001	0.405	<0.0001	0.411	<0.0001
log_{10} HOMA-IR	0.518	<0.0001	0.402	<0.0001	0.420	<0.0001
log_{10} triacylglycerol (mg/dL)	0.223	0.003	0.226	0.003	0.306	<0.0001
HDL-cholesterol (mg/dL)	-0.060	0.429	-0.210	0.005	-0.196	0.009
Ultrasonography assessments						
Carotid IMT (cm)	0.177	0.023	0.216	0.005	0.115	0.136

Table 9. Bivariate study. Correlation coefficients for IGF-I and selected variables in the studied subjects classified according to PxCa tertiles. Basal study. *P and r values are from Pearson correlation analyses. F, female; IGF-I, insulin-like growth factor-I; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; IMT, intima-media thickness*

Associations between IGF-I and SBP as well as DBP are graphically depicted in Figures 11 and Figure 12 respectively. Similar associations have been found analysing data for IGFBP-3 serum values as well as for the ratio IGF-I/IGFBP-3 (data not shown).

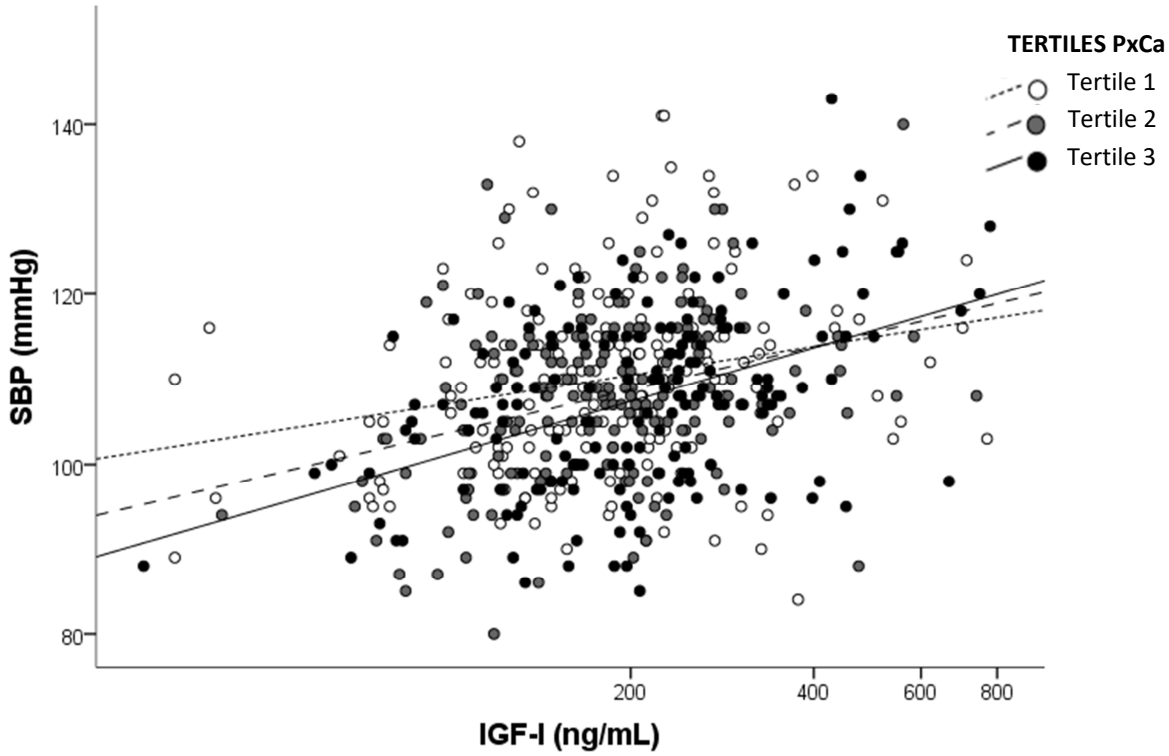

Figure 11. Scatter diagram and linear regression. Correlation between serum values of insulin-like growth factor-I (IGF-I) and systolic blood pressure (SBP). Basal study.Values are represented according to the tertiles of phosphate calcium product.

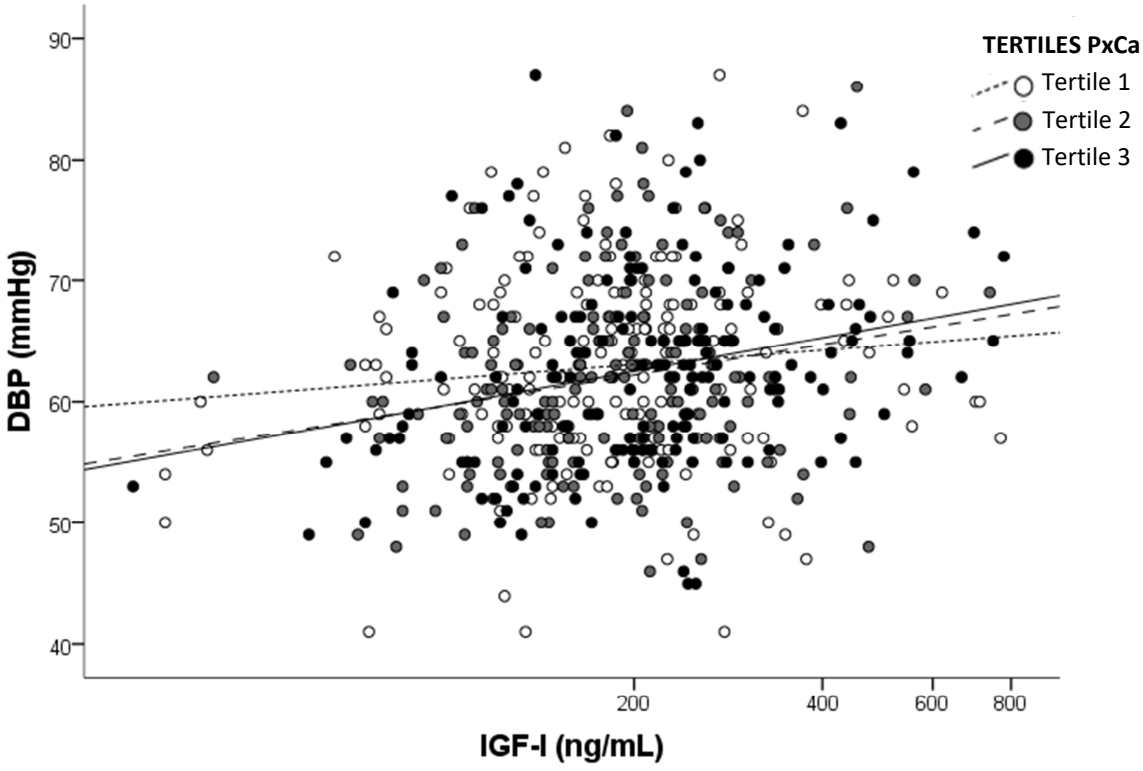

Figure 12. Scatter diagram and linear regression. Correlation between serum values of insulin-like growth factor-I (IGF-I) and diastolic blood pressure (DBP). Basal study. Values are represented according to the tertiles of phosphate calcium product.

In multiple regression analyses adjusted for potentially confounding factors (age, sex, BMI and IGF-I), IGF-I serum concentrations were found to be independent predictor of blood pressure parameters. Association was found for SBP (p=0.029) and PP (p<0.0001). BMI was also an independent predictor of SBP, DBP and PP with a p<0.0001. Age was an important predictor for both SBP and DBP; whereas sex was predictor for DBP and PP (Table 10).

	Beta	Sig	R^2
SBP			
Age	0.184	<0.0001	0.036
Sex	-0.025	0.540	-
BMI	0.330	<0.0001	0.214
IGF-I	0.098	0.029	0.005
Total			0.255
DBP			
Age	0.122	0.011	0.010
Sex	0.152	<0.0001	0.018
BMI	0.283	<0.0001	0.112
IGF-I	-0.027	0.601	-
Total			0.140
PP			
Age	0.091	0.100	-
Sex	-0.177	0.001	0.012
BMI	0.157	<0.0001	0.074
IGF-I	0.181	<0.0001	0.011
Total			0.097

Table 10. Multivariate linear model of blood pressure values as dependents variables. Basal study. *SBP, systolic blood pressure; BMI, body mass index; IGF-I, insulin-like growth factor-I; DBP, diastolic blood pressure; PP, pulse pressure.*

	PxCa tertil 1 (n=176)			PxCa tertil 2 (n=176)			PxCa tertil 3 (n=176)		
	Beta	**Sig**	**r^2**	**Beta**	**Sig**	**r^2**	**Beta**	**Sig**	**r^2**
SBP									
Age	0.115	0.150	-	0.082	0.296	-	0.260	0.003	0.232
Sex	0.030	0.649	-	-0.151	0.027	0.016	-0.005	0.937	-
BMI	0.481	<0.0001	0.226	0.420	<0.0001	0.245	0.177	0.019	0.019
IGF-I	0.030	0.687	-	0.216	0.004	0.019	0.223	0.007	0.032
Total			0.226			0.280			0.283
DBP									
Age	0.060	0.486	-	0.112	0.158	-	0.188	0.022	0.021
Sex	0.207	0.004	0.038	0.063	0.370	-	0.172	0.016	0.019
BMI	0.285	<0.0001	0.073	0.399	<0.0001	0.154	0.271	0.001	0.110
IGF-I	-0.083	0.312	-	0.070	0.355	-	0.037	0.696	-
Total			0.111			0.154			0.150
PP									
Age	0.087	0.313	-	0.088	0.300	-	0.306	<0.0001	0.089
Sex	-0.138	0.056	-	-0.138	0.064	-	-0.089	0.227	-
BMI	0.312	<0.0001	0.092	0.204	0.007	0.036	0.006	0.945	-
IGF-I	0.042	0.596	-	0.114	0.158	-	0.169	0.064	-
Total			0.092			0.036			0.089

Table 11. Multivariate linear model of blood pressure values as dependents variables, adjusted for PxCa tertiles. Basal study. *SBP, systolic blood pressure; BMI, body mass index; IGF-I, insulin-like growth factor-I; DBP, diastolic blood pressure; PP, pulse pressure.*

We repeated the multiple regression analyses with data divided according to PxCa tertiles. Results show that IGF-I was an independent predictor for SBP in groups with higher PxCa product (p=0.004 in tertile 2, p=0.007 in tertile 3). No associations have been found for DBP and PP. BMI remained an important predictor for SBP, DBP as well as PP and in all tertiles (except in tertile 3 for PP where the p value is not significant) (**Table 11**). Independent associations between IGF-I and other CV risk factors were not found (data not shown).

7.2.2 IGF-I AND BLOOD PRESURE AT FOLLOW-UP

Correlations for logarithmic values of IGF-I (log_{10} IGF-I) and selected variables of cardiovascular risk (age, weight, height, BMI, waist, SBP, DBP, PP, log_{10} insulin, log_{10} HOMA-IR, log_{10} triacylglycerol, HDL-cholesterol, carotid IMT) are studied in **Table 12**. Data analysis shows that log_{10} IGF-I maintained the correlation with all the variables studied in the follow-up study. The p value was <0.0001 for age, weight, height, SBP, PP, log_{10} insulin and log_{10} HOMA-IR. Association was lower but still significant for BMI (p=0.035), waist (p=0.008), DBP (p=0.001), log_{10}triacylglycerol (p=0.076), HDL-cholesterol (p=0.047) and carotid IMT (p=0.021).

log_{10} IGF-I (ng/Ml)	All subjects (n=158)	
	r	p
Clinical assessments		
Age (year)	0.430	<0.0001
Weight (kg)	0.310	<0.0001
Height (cm)	0.508	<0.0001
BMI (kg/m^2)	0.168	0.035
Waist (cm)	0.212	0.008
SBP (mmHg)	0.438	<0.0001
DBP (mmHg)	0.267	0.001
PP (mmHg)	0.283	<0.0001
Laboratory assessments		
log_{10} insulin (mlU/L)	0.423	<0.0001
log_{10} HOMA-IR	0.435	<0.0001
log_{10} triacylglycerol (mg/dL)	0.141	0.076
HDL-cholesterol (mg/dL)	-0.158	0.047
Ultrasonography assessments		
Carotid IMT (cm)	0.198	0.021

Table 12 Bivariate study. Correlation coefficients for IGF-I and selected variables in the studied subjects. Follow-up study. P and r values are from Pearson correlation analyses. *F, female; IGF-I, insulin-like growth factor-I;; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; IMT, intima-media thickness.*

As seen in the basal study, when analyses for PxCa tertiles are performed, associations between IGF-I and SBP, DBP and PP become stronger as higher is the PxCa level (**Table 13**). This association is especially important for SBP where p value is <0.0001 in tertile 3. Association with the other variables studied are not so relevant.

log_{10} IGF-I (ng/Ml)	PxCa tertil 1 (n=52)		PxCa tertil 2 (n=53)		PxCa tertil 3 (n=53)	
	r	p	r	p	r	p
Clinical assessments						
Age (year)	0.321	0.021	0.394	0.004	0.508	<0.0001
Weight (kg)	0.207	0.142	0.279	0.043	0.429	0.002
Height (cm)	0.495	<0.0001	0.480	<0.0001	0.536	<0.0001
BMI (kg/m^2)	0.079	0.576	0.125	0.373	0.258	0.063
Waist (cm)	0.169	0.242	0.141	0.316	0.271	0.050
SBP (mmHg)	0.292	0.036	0.371	0.006	0.590	<0.0001
DBP (mmHg)	0.090	0.525	0.279	0.043	0.377	0.005
PP (mmHg)	0.261	0.062	0.157	0.263	0.413	0.002
Laboratory assessments						
log_{10} insulin (mlU/L)	0.386	0.005	0.292	0.034	0.551	<0.0001
log_{10} HOMA-IR	0.403	0.003	0.306	0.026	0.563	<0.0001
log_{10} triacylglycerol (mg/dL)	0.013	0.927	0.129	0.357	0.244	0.078
HDL-cholesterol (mg/dL)	-0.272	0.051	-0.150	0.285	-0.055	0.695
Ultrasonography assessments						
Carotid IMT (cm)	0.144	0.338	0.299	0.051	0.175	0.246

Table 13. Bivariate study. Correlation coefficients for IGF-I and selected variables in the studied subjects classified according to PxCa tertiles. Follow-up study. *P and r values are from Pearson correlation analyses. F, female; IGF-I, insulin-like growth factor-I; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; IMT, intima-media thickness*

Associations between IGF-I and SBP as well as DBP are graphically depicted in Figures 13 and Figure 14 respectively. Similar associations have been found analysing data for IGFBP-3 serum values as well as for the ratio IGF-I/IGFBP-3 (data not shown).

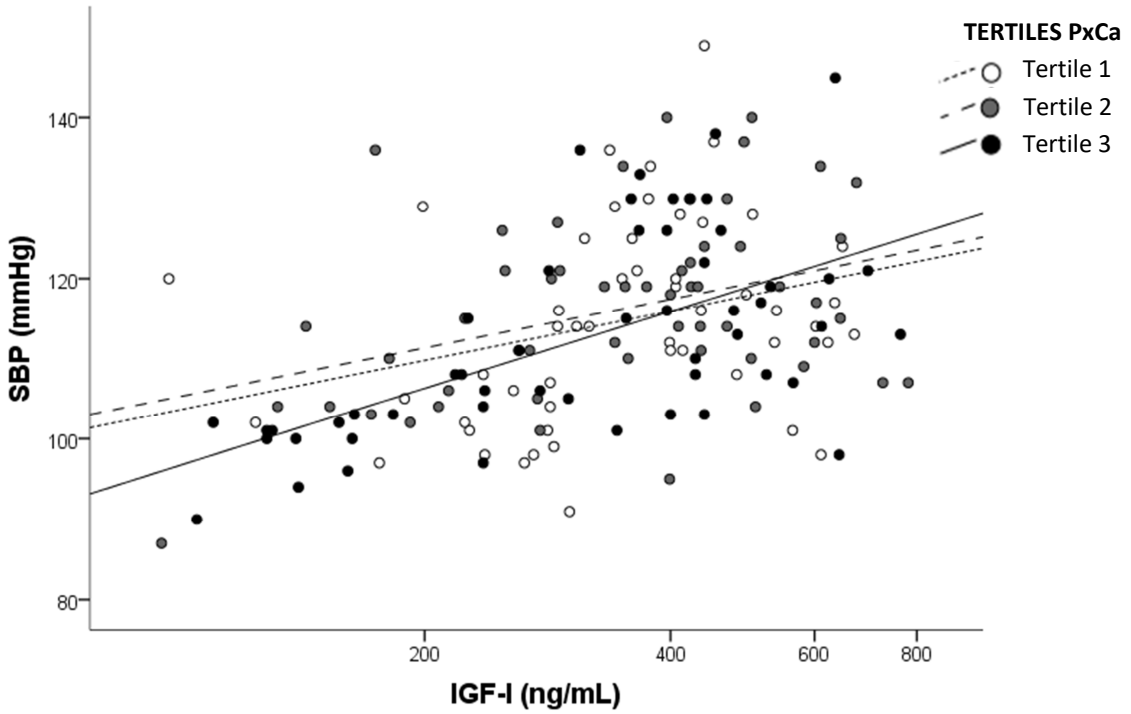

Figure 13. Scatter diagram and linear regression. Correlation between serum values of insulin-like growth factor-I (IGF-I) and systolic blood pressure (SBP). Follow-up study. Values are represented according to the tertiles of phosphate calcium product

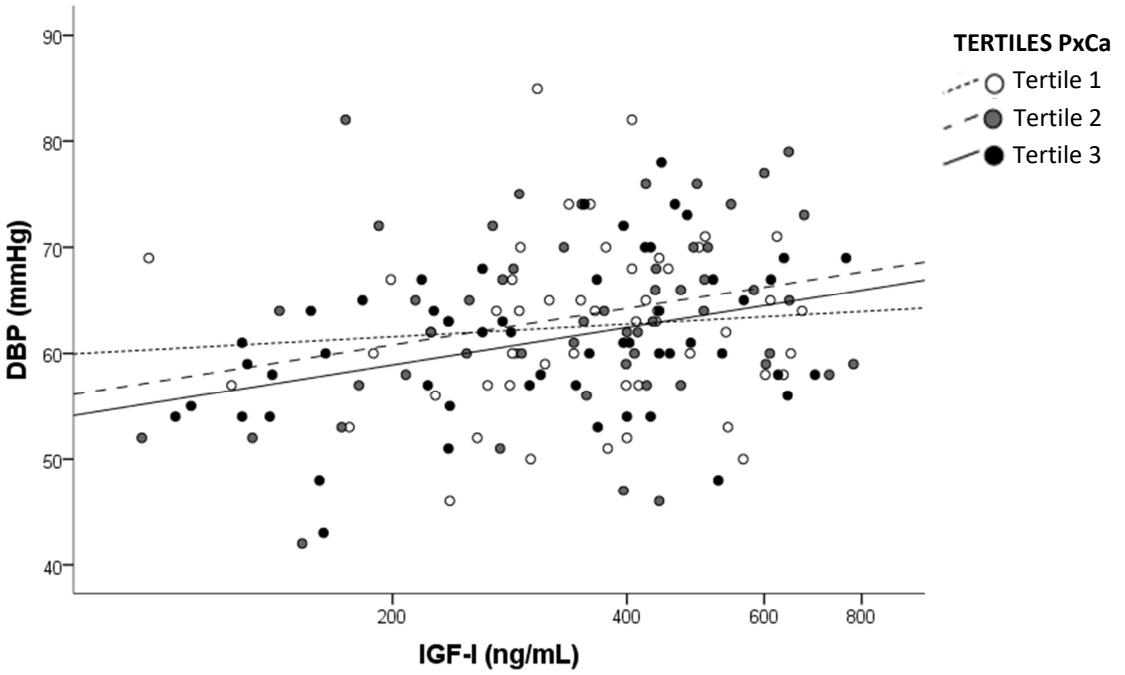

Figure 14. Scatter diagram and linear regression. Correlation between serum values of insulin-like growth factor -I (IGF-I) and diastolic blood pressure (DBP). Follow-up study. Values are represented according to the tertiles of phosphate calcium product

Table 14 shows the multiple regression analyses adjusted for potentially confounding factors (age, sex, BMI and IGF-I) in the follow-up. Values of IGF-I have been found to be an independent predictor for all blood pressure parameters (for SBP p<0.0001, DBP p=0.003 and PP p=0.008). Age, sex and BMI are also important predictor for all SBP, DBP and PP. Similar results were found after adjusting data according PxCa tertiles as we can see in Table 15. Independent associations between IGF-I and other CV risk factors were not found (data not shown).

	Beta	Sig	R^2
SBP			
Age	0.224	0.001	0.037
Sex	-0.132	0.016	0.014
BMI	0.486	<0.0001	0.405
IGF-I	0.284	<0.0001	0.110
Total			0.566
DBP			
Age	0.087	0.354	-
Sex	0.150	0.051	-
BMI	0.217	0.005	0.040
IGF-I	0.230	0.003	0.065
Total			0.105
PP			
Age	0.159	0.049	0.049
Sex	-0.254	<0.0001	0.042
BMI	0.401	<0.0001	0.252
IGF-I	0.194	0.008	0.026
Total			0.369

Table 14. Multivariate linear model of blood pressure values as dependents variables. Follow-up study. *SBP, systolic blood pressure; BMI, body mass index; IGF-I, insulin-like growth factor-I; DBP, diastolic blood pressure; PP, pulse pressure.*

	PxCa tertil 1 (n=52)			PxCa tertil 2 (n=53)			PxCa tertil 3 (n=53)		
	Beta	**Sig**	**r^2**	**Beta**	**Sig**	**r^2**	**Beta**	**Sig**	**r^2**
SBP									
Age	0.198	0.096	-	0.398	0.001	0.127	0.231	0.062	0.379
Sex	-0.140	0.155	-	-0.061	0.551	-	-0.144	0.122	-
BMI	0.675	<0.0001	0.471	0.455	<0.0001	0.358	0.379	0.001	0.098
IGF-I	0.238	0.018	0.047	0.186	0.086	-	0.375	0.001	0.092
Total			0.518			0.485			0.569
DBP									
Age	-0.067	0.646	-	0.356	0.009	0.110	0.085	0.576	-
Sex	0.277	0.029	0.062	0.190	0.150	-	0.096	0.469	-
BMI	0.409	0.002	0.164	-0.043	0.763	-	0.117	0.387	-
IGF-I	0.020	0.877	-	0.164	0.254	-	0.377	0.005	0.126
Total			0.226			0.110			0.126
PP									
Age	0.328	0.015	0.251	0.114	0.385	-	0.342	0.013	0.072
Sex	-0.270	0.021	0.061	-0.211	0.079	-	-0.199	0.079	-
BMI	0.327	0.015	0.051	0.517	<0.0001	0.253	0.348	0.012	0.268
IGF-I	0.196	0.106	-	0.094	0.444	-	0.201	0.125	-
Total			0.363			0.253			0.340

Table 15. Multivariate linear model of blood pressure values as dependents variables adjusted for PxCa tertiles. Follow-up study. *SBP, systolic blood pressure; BMI, body mass index; IGF-I, insulin-like growth factor-I; DBP, diastolic blood pressure; PP, pulse pressure.*

7.2.3 IGF-I AND BLOOD PRESURE. PREDICTIVE STUDY

After combining $\log_{10}$IGF-I values from the basal study with selected variables of cardiovascular risk from the follow-up study (age, weight, height, BMI, waist, SBP, DBP, PP, $\log_{10}$ insulin, $\log_{10}$ HOMA-IR, $\log_{10}$ triacylglycerol, HDL-cholesterol, carotid IMT), the values obtained are summarized in Table 16. $\log_{10}$ IGF-I was associated with all the studied variables with a $p<0.0001$; except DBP, $\log_{10}$triacylglycerol, HDL-cholesterol and carotid IMT ($p<0.05$).

$\log_{10}$ IGF-I (ng/Ml)	All subjects (n=158)	
	r	p
Clinical assessments		
Age (year)	0.536	<0.0001
Weight (kg)	0.472	<0.0001
Height (cm)	0.468	<0.0001
BMI (kg/m^2)	0.421	<0.0001
Waist (cm)	0.411	<0.0001
SBP (mmHg)	0.443	<0.0001
DBP (mmHg)	0.157	0.049
PP (mmHg)	0.365	<0.0001
Laboratory assessments		
$\log_{10}$ insulin (mIU/L)	0.286	<0.0001
$\log_{10}$ HOMA-IR	0.282	<0.0001
$\log_{10}$ triacylglycerol (mg/dL)	0.205	0.010
HDL-cholesterol (mg/dL)	-0.248	0.020
Ultrasonography assessments		
Carotid IMT (cm)	0.190	0.028

Table 16 Bivariate study. Correlation coefficients for IGF-I and selected variables in the studied subjects. Predictive study. P and r values are from Pearson correlation analyses. *F, female; IGF-I, insulin-like growth factor type I; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; IMT, intima-media thickness.*

log_{10} IGF-I (ng/Ml)	PxCa tertil 1 (n=52)		PxCa tertil 2 (n=53)		PxCa tertil 3 (n=53)	
	r	p	r	p	r	p
Clinical assessments						
Age (year)	0.547	<0.0001	0.386	0.004	0.692	<0.0001
Weight (kg)	0.365	0.008	0.552	<0.0001	0.634	<0.0001
Height (cm)	0.494	<0.0001	0.386	0.004	0.556	<0.0001
BMI (kg/m^2)	0.251	0.072	0.539	<0.0001	0.564	<0.0001
Waist (cm)	0.273	0.055	0.466	<0.0001	0.559	<0.0001
SBP (mmHg)	0.254	0.069	0.419	0.002	0.643	<0.0001
DBP (mmHg)	-0.076	0.595	0.143	0.306	0.394	0.004
PP (mmHg)	0.334	0.016	0.302	0.028	0.460	0.001
Laboratory assessments						
log_{10} insulin (mlU/L)	0.118	0.404	0.439	0.001	0.367	0.007
log_{10} HOMA-IR	0.104	0.462	0.405	0.003	0.382	0.005
log_{10} triacylglycerol (mg/dL)	0.074	0.604	0.355	0.009	0.205	0.142
HDL-cholesterol (mg/dL)	-0.1990	0.178	-0.346	0.011	-0.255	0.065
Ultrasonography assessments						
Carotid IMT (cm)	0.293	0.048	0.206	0.185	0.092	0.542

Table 17. Bivariate study. Correlation coefficients for IGF-I and selected variables in the studied subjects classified according to PxCa tertiles. Predictive study. *P and r values are from Pearson correlation analyses. F, female; IGF-I, insulin-like growth factor type I; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; HOMA-IR, homeostasis model assessment insulin resistance; HDL, high-density lipoprotein; IMT, intima-media thickness*

As seen previously, in the study adjusted for PxCa tertiles, association between IGF-I and SBP, DBP and PP became stronger as higher was the PxCa level (Table 17). This association was especially important for SBP where p value reaches a value <0.0001 in tertile 3. Significant p-value was also fund for PP in all tertiles and for DBP, in tertile 3. Association between IGF-I and SBP as well as DBP is graphically depicted in Figures 15 and Figure 16 respectively. Similar associations have been found analysing data for IGFBP-3 serum values as well as for the ratio IGF-I/IGFBP-3 (data not shown).

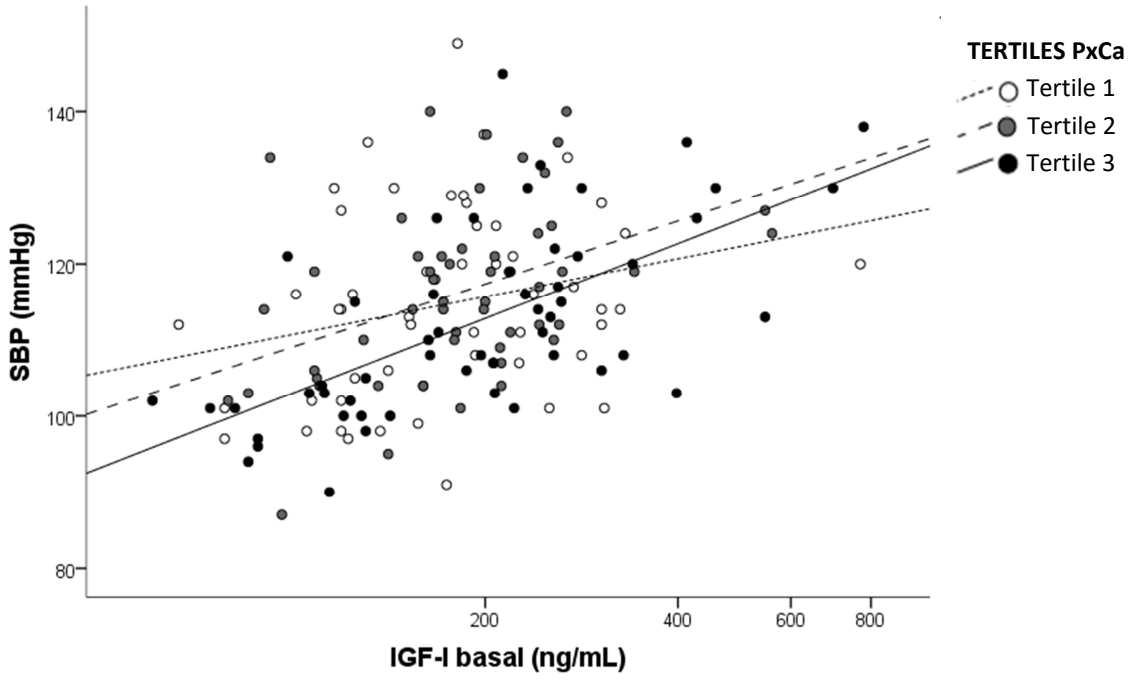

Figure 15. Scatter diagram and linear regression. Correlation between serum values of insulin-like growth factor-I (IGF-I) and systolic blood pressure (SBP). Predictive study. Values are represented according to the tertiles of phosphate calcium product.

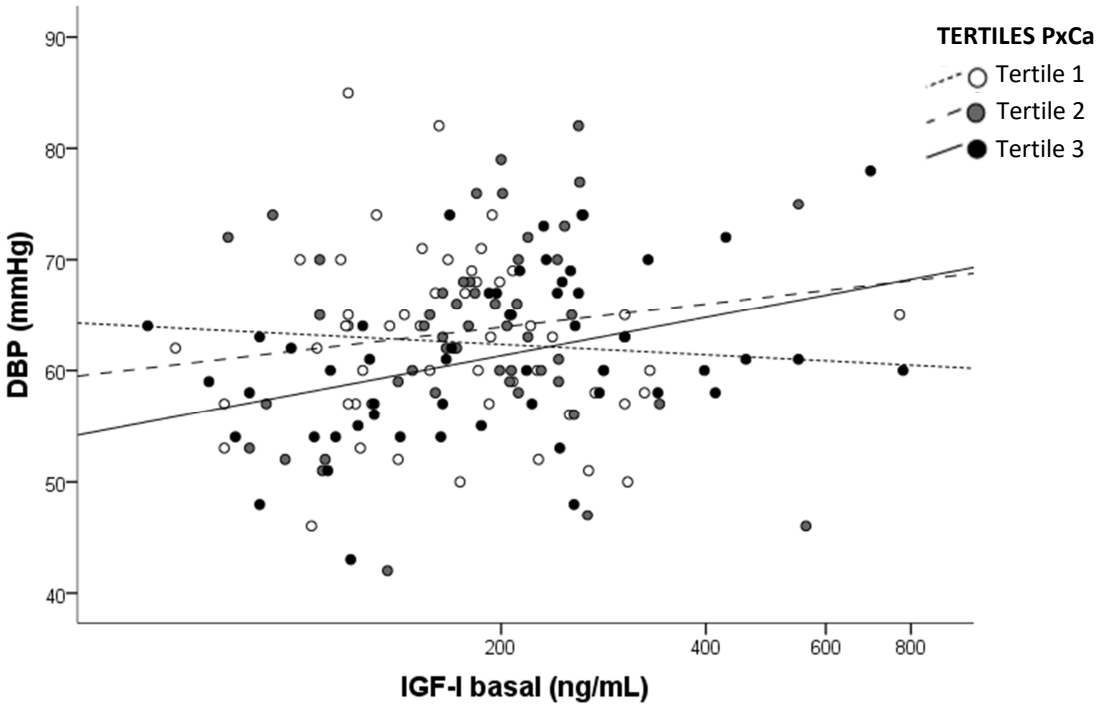

Figure 16. Scatter diagram and linear regression. Correlation between serum values of insulin-like growth factor-I (IGF-I) and diastolic blood pressure (DBP). Predictive study. Values are represented according to the tertiles of phosphate calcium product

Table 18 shows the multiple regression analyses adjusted for potentially confounding factors (age, sex, BMI and IGF-I) combining data from the basal study (values of IGF-I) and the follow-up study (blood pressure values). IGF-I has been found to be an independent predictor in the long term for PP only (p=0.016). Age and BMI were also important predictors for SBP as well as PP; age and sex were independent predictors for DBP and PP.

	Beta	Sig	R^2
SBP			
Age	0.362	<0.0001	0.102
Sex	-0.075	0.185	-
BMI	0.465	<0.0001	0.405
IGF-I	0.079	0.249	-
Total			0.503
DBP			
Age	0.269	0.001	0.061
Sex	0.201	0.009	0.035
BMI	0.161	0.062	-
IGF-I	-0.043	0.646	-
Total			0.096
PP			
Age	0.169	0.039	0.049
Sex	-0.255	<0.0001	0.042
BMI	0.353	<0.0001	0.252
IGF-I	0.178	0.029	0.016
Total			0.359

Table 18. Multivariate linear model of blood pressure values as dependents variables. Predictive study . *SBP, systolic blood pressure; BMI, body mass index; IGF-I, insulin-like growth factor-I; DBP, diastolic BP; PP, pulse pressure.*

After adjusting data according to PxCa tertiles, results show that baseline IGF-I gains importance as an independent predictor for SBP as higher was the PxCa level (p=0.001 in tertile 3). Similar associations were found for DBP. Significant correlations were lost for IGF-I and PP in analyses by PxCa tertiles (**Table 19**). Independent associations between age, sex and BMI for other CV risk factors assessed were not found (data not shown).

	PxCa tertil 1 (n=52)			PxCa tertil 2 (n=53)			PxCa tertil 3 (n=53)		
	Beta	**Sig**	**r^2**	**Beta**	**Sig**	**r^2**	**Beta**	**Sig**	**r^2**
SBP									
Age	0.266	0.022	0.044	0.398	0.001	0.127	0.264	0.065	-
Sex	-0.076	0.447	-	-0.061	0.551	-	-0.206	0.052	-
BMI	0.560	<0.0001	0.471	0.455	<0.0001	0.358	0.348	0.006	0.074
IGF-I	-0.046	0.699	-	0.030	0.807	-	0.447	0.001	0.402
Total			0.515			0.485			0.476
DBP									
Age	-0.067	0.646	-	0.356	0.009	0.110	-0.034	0.853	-
Sex	0.277	0.029	0.062	0.190	0.150	-	0.058	0.669	-
BMI	0.409	0.002	0.164	-0.043	0.763	-	-0.023	0.886	-
IGF-I	-0.184	0.151	-	0.007	0.962	-	0.394	0.004	0.139
Total			0.226			0.110			0.139
PP									
Age	0.328	0.015	0.251	0.114	0.385	-	0.342	0.013	0.072
Sex	-0.270	0.021	0.061	-0.211	0.079	-	-0.199	0.079	-
BMI	0.327	0.015	0.051	0.517	<0.0001	0.253	0.348	0.012	0.268
IGF-I	0.094	0.487	-	0.033	0.820	-	0.058	0.727	-
Total			0.363			0.253			0.340

Table 19. Multivariate linear model of blood pressure values as dependents variables, adjusted for PxCa tertiles. Predictive study. *SBP, systolic blood pressure; BMI, body mass index; IGF-I, insulin-like growth factor-I; DBP, diastolic blood pressure; PP, pulse pressure.*

8. DISCUSSION

The results of this observational prospective population-based study indicate that IGF-I levels are associated with blood pressure in children: the higher serum levels of IGF-I, the higher the blood pressure in these subjects.

The present study appears to be the first to have related serum IGF-I levels to cardiovascular risk factors in an apparently-healthy children. Studies developed in adults reported conflicting data according to the relationship between IGF-I serum levels on the cardiovascular system. However, several studies showed that high IGF-I levels correlated with the development of high blood pressure and hypertension in adults (15,18,33,37,38), supporting our results.

Reviewing studies in adolescent populations, a positive association between IGF-I and BP was found in two studies. The first one, developed in the Bogalusa cohort of children, demonstrated an association between high IGF-I serum levels with both systolic and diastolic BP. However, this relationship only has been found in boys, not in girls, between 11 and 18 years (35). The second one also demonstrated a positive correlation between IGF-I and blood pressure in a mixed population of healthy Danish children and adolescents, as well as a correlation between IGF-I and body mass index (33,35,41). Thus, these similar studies published in adolescents give support to our results.

To the best of our knowledge, this is also the first longitudinal study in children concerning the associations of IGF-I with CVD risk markers. The abovementioned published studies have been developed as cross-sectional studies in adolescent populations. Thus, the innovation of this study is to provide information of the longitudinal associations seen in children between IGF-I and CVD risk markers, which may be useful in terms of prevention of CVD. Longitudinal studies may predict adverse outcomes related to the long - term exposure of high/low IGF-I serum levels on the CV system and their influence on the development of disease.

Chronic overexpression of IGF-I lead to an ongoing phenomenon of IGF-I resistance in the vessels. It is secondary to a lack of response to the IGF-I vasodilatory effects, as well as to a change in the expression and isoforms distributions of contractile proteins leading to the development of high BP or hypertension (15,33,37). This pathophysiological mechanisms is worsen with the hormonal and physical changes during puberty influencing in IGF-I levels, as

well as other changes involving factors such as calcium, abnormalities of insulin metabolism, angiotensin-II, endothelin-1, among others (25,36,44,52).

In our study the strongest association has been reported between IGF-I and SBP. Nevertheless, DBP and PP also correlate to IGF-I levels in a positive association. Barely any differences were observed between cross-sectional associations (basal and follow-up studies). IGF-I was strongly associated to all three BP parameters: SBP, DBP and PP ($p<0.0001$). However, in the longitudinal association (predictive study), SBP and PP were strongly associated with IGF-I ($p<0.0001$), whereas DBP was in the limit of statistical significance ($p=0.049$, $r=0.157$). According to these results, IGF-I strongly associated to SBP as well as PP and, on a long-term exposure, might be a pathophysiological factor related to the development of high SBP. Similar results were found for serum ratio IGF-I/IGFBP-3 values.

SBP is the main physiological marker related to the development of CVD due to its involvement in increasing stresses on arteries, accelerating cardiovascular degeneration, atherosclerosis, and arterial stiffness (7,24,25). The undiagnosed and untreated elevated SBP may persist into adulthood and lead to target organ damage, such as left ventricular hypertrophy, heart failure, stroke, vascular changes in the retina, among others (7,24).

According to the results of the analyses by tertiles PxCa, associations between IGF-I and SBP, DBP and PP became stronger in the highest PxCa tertile. To the best of our knowledge, no information has been published regarding the effect of the PxCa on the regulation of BP in apparently healthy children, and thus we consider theses as being novel results.

The role of calcium in the control of VSMC contractility and vascular tone is well established (49), as well as its involvement in vascular calcification and arterial stiffness (54–56). According to our data, a high phosphate calcium product may play a role in synergism with IGF-I in the pathophysiological mechanisms involved in the development of a high BP. Although the role of PxCa as a risk factor for CV disease has been controversial (54,63), it seems plausible that due to early vascular calcification SBP may be elevated.

After controlling for age, gender and BMI, in multiple regression analysis, blood pressure showed a small but independent relationship with all these variables. BMI has been found as an independent variable for all blood pressure measurements (SBP, DBP and PP) in all the analyses (basal, follow-up and predictive). Similar results have been found for age. Gender was an independent variable for DBP and PP in all the analyses (basal, follow-up and predictive).

These results may be explained because of different causes. Body size is a major determinant of blood pressure in growing children. Moreover, the regulation of circulating levels of IGF-I is complex as it is modified by binding proteins that in turn are under influence and control of GH, oestrogen, glucocorticoids, insulin, nutritional factors, local production of IGF-I, sleep, metabolic stress, gender, activity level, etc. (1,2,9,12,13). This regulation is even more complex in children, for the age-related variability and the high increase of the IGF-I values during prepubertal and pubertal growth. Thus, all these factors may explain, at least in part, the complexity of trying to understand the development of hypertension in early life (33–36).

As regards additional cardiovascular risk factors assessed in this study (waist, insulin, insulin resistance, triacylglycerol, high-density lipoprotein cholesterol and carotid intima-media thickness), positive, but not independent, associations with IGF-I were also found. This is in apparent contradiction with studies in adults, which showed that low circulating IGF-I is associated with an increased cardiovascular risk and development of ischemic heart disease, stroke and atherosclerosis)(14,21,22,29,31). Data from an obese cohort of European Caucasian adolescents, also support an inverse relationship between IGF-I levels and the development of CVD. High IGF-I serum levels are associated to lower CV risk (22).

CV diseases are the result of complex interactions between genetic and environmental factors over extended periods of time. Controversial results in the published studies suggest the presence of different physiopathological mechanisms for both low and high IGF-I levels and the development of CVD. For this reason, further investigation through observational studies with a longer follow-up period is nevertheless needed to support and extend this statement.

In summary, an association between IGF-I serum levels and blood pressure in children exist: the higher the IGF-I values, the higher the blood pressure in children. This phenomenon might be explained because of the development of IGF-I resistance in the vessels in chronic overexpression of IGF-I, leading to a lack of response to the IGF-I vasodilatory effects and to a change in the expression and distribution of contractile proteins. PxCa acts in synergism with IGF-I in this phenomenon, due to the role of calcium in vascular tone regulation, calcification as well as in arterial stiffness. BMI, age and gender act as independent variable for all blood pressure measurements (SBP, DBP and PP). A positive, but not independent, association between IGF-I and other CV risk factors was also found. Further studies are warranted to confirm our results and contribute to the development of paediatric strategies aimed at preventing CV disease in adulthood.

9. CONCLUSIONS

In an apparently-healthy population of children between 3 and 17 years, high serum IGF-I levels were found to associate with a high blood pressure, especially with high systolic blood pressure and in children with higher PxCa values. High phosphate calcium product may act in synergism with IGF-I in the development of a higher blood pressure.

As regards additional cardiovascular risk factors assessed in this study (waist, insulin, insulin resistance, triacylglycerol, high-density lipoprotein cholesterol and carotid intima-media thickness), positive, but not independent, associations with IGF-I were also found. However, due to the complex regulation of the IGF-I and the complex interactions between genetic and environmental factors over extended periods of time in the development of CV disease further investigation through long-term observational studies is needed.

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10. STRENGHT AND LIMITATIONS

This study is representative of the children population of Girona. We consider our data of interest because it is the first study to investigate the correlation between IGF-I and blood pressure in a population-based sample of apparently healthy children.

Being an observational study, a large-size sample could be evaluated, providing a high statistical power to the study. Multiple outcomes could be measured. The hypotheses and results assessed can be used as a first study for future investigations in a field with scarce evidence regarding the clinical role of IGF-I as a risk marker of cardiovascular disease. Furthermore, being an analysis of readily available data, it has been less time consuming and costly, so it has been highly feasible.

Several potential limitations warrant mention. Given that the study design is observational, no control over the variables exists and this only allowed us to assess the correlations between variables. It is possible to have confounding variables, which we may not have been able to adjust for in the multivariate analysis. Based on the results obtained in our study, statistical analysis should be extended in further studies adjusting IGF-I values for age, gender and BMI. In addition, blood pressure values should be adjusted for height. Furthermore, binding proteins should be also analysed in the statistical analyses due to their important role in the regulation of free IGF-I. Differences between methodological techniques measuring IGF-I may cause lack of comparability between studies.

Regarding our results, as the aim of our study was to determine the relationship between IGF-I and blood pressure in children, the total amount of IGF (IGF-I + IGF-II) might have been taken as the main variable, better than IGF-I serum levels alone. Perhaps stronger correlations could have been determined using both IGF-I and IGF-II. However, IGF-II was not analysed in our study, due to the fact that, it is usually neither clinically determined nor used in research as the most important biological marker of IGF system, it is IGF-I.

Furthermore, arterial stiffness is an important matter of research that has not been included in our study. Although it has emerged as the most important determinant of increased SBP and pulse pressure and, therefore, the root cause of a host of CV complications, this parameter has not yet been thoroughly in paediatric population. However, it may well be an important variable to assess in future studies.

Obese as well as hypertensive patients could be excluded from the study and the data could be reanalysed without those subjects. However, this population represented less than 5% of all the subjects studied and none of them represented an outlier in the distribution of IMC and blood pressure. This is the reason why we decided not to exclude them from the analysis.

The power of the longitudinal study was lower than that of the cross-sectional sectional study, as participation in the follow-up visits was only 75.76%. However, ours is also among the first studies to have both cross-sectional and longitudinal clinical data, which allowed us to infer not only associations between clinical variables, but also the direction of such associations and potentially a causal link between them.

11. PROJECT IMPACT ON THE NATIONAL HEALTH SERVICE

Undiagnosed and untreated high blood pressure and, hence, its persistence is related to target organ damage (left ventricular hypertrophy, increased carotid artery thickness, vascular changes in the retina) as well as to an increased stress on arteries accelerating CV degeneration and atherosclerosis (7,24). Prevention, especially in paediatric population, may reduce the cardiovascular diseases, the leading cause of mortality in industrialized countries.

Prevalence of high blood pressure in children has risen in recent decades. Investing time and efforts to avoid this pathological status can reduce future cardiovascular events. Thus, with the knowledge of the pathophysiological factors related to the development of high blood pressure values (such as IGF-I levels, obesity, among others) and with the development of screening programs, future cardiovascular diseases could be prevented.

This study shows that a strong association exist between IGF-I values and blood pressure. The utility of IGF-I serum levels as a screening method should be studied, with the aim to identify those patients at higher risk for cardiovascular disease. Prevention interventions could be applied in order to avoid the development of hypertension and, in a long term, the development of cardiovascular diseases. Consequently, it may reduce the mortality and morbidity related to cardiovascular diseases and, therefore, it could also reduce the national health services resources used to treat this clinical condition and its consequences, as well as the quality of life of the patients.

12. ETHICAL CONSIDERATIONS

The project has been conducted in accordance to the ethical considerations and requirements set out in the international and national standards for epidemiological studies.

This protocol followed the four ethical principles of respect, justice, no maleficence and beneficence, principles established by the World Medical Association in the *Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects* and in the *Declaration of Taipei on Ethical Considerations Regarding Health Databases and Biobanks*. The study had also been submitted to the "*Comitè d'Ètica d'Investigació Clínica (CEIC) de l'Hospital Universitari de Girona Doctor Josep Trueta*", which approval was given in order to allow the development of the study.

In order to ensure the researchers compliance of the principles set out by the national laws, the study must be conducted according to:

> **"*Ley Orgánica 15/1999, de 13 de Diciembre, de Protección de Datos de Carácter Personal*"** as well as the **"*Ley 41/2002, de 14 de noviembre, básica reguladora de la autonomía del paciente y deberes y derechos en materia de información*"**
>
> Laws on data protection, whose goals are to guarantee the confidentiality, privacy, freedom and fundamental rights of physical people involved in the study as well as the confidentiality of their personal information. Treatment, communication and transfer of personal data, as well as security measures for automated files containing personal data is also regulated in this laws. The law 41/2002 also ensures the good practice within the paediatric population, protecting their own rights in terms of autonomy, confidentiality and information to the patient.
>
> **"*Ley 14/2007, de 3 de Julio, de investigación biomédica*"**
>
> Spanish law which comprises all the ethical issues related to the biomedical research in order to ensure the human dignity and the human rights, among others.

All the patients were provided with all needed information documents and the written informed consent. Informed consent had to be signed by the patient or by the child's legal tutor before the start of data recollection procedures. The investigator made sure that the patient understood all information before signing the informed consent. Moreover, this study was without risk seen as it had no changes on the biological, psychological, physiological or social conditions of the patients. Content of the database was encrypted. Patients' identities remained confidential; all data have been treated without identification information.

13. CONFLICTS OF INTEREST

All members of the study declare no conflicts of interest.

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15. REFERENCES

1. Juul A. Serum levels of insulin-like growth factor I and its binding proteins in health and disease. Growth Horm IGF Res [Internet]. 2003 Aug 1 [cited 2017 Nov 23];13(4):113–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12914749
2. Monzavi R, Cohen P. IGFs and IGFBPs: Role in health and disease [Internet]. Vol. 16, Best Practice and Research: Clinical Endocrinology and Metabolism. Elsevier; 2002 [cited 2017 Dec 14]. p. 433–47. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1521690X02902125
3. Jung HJ, Suh Y. Regulation of IGF -1 signaling by microRNAs. Front Genet [Internet]. 2015 [cited 2017 Nov 21];5(JAN):1–13. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4292735/
4. Cardiovascular diseases [Internet]. WHO. World Health Organization; 2017 [cited 2017 Nov 28]. Available from: http://www.who.int/mediacentre/factsheets/fs317/en/
5. O'Donnell CJ, Elosua R. Cardiovascular Risk Factors. Insights From Framingham Heart Study. Rev Española Cardiol (English Ed [Internet]. 2008 Jan 1 [cited 2017 Nov 28];61(3):299–310. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1885585708601188
6. Flynn JT, Kaelber DC, Baker-Smith CM, Blowey D, Carroll AE, Daniels SR, et al. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. Am Acad Pediatr [Internet]. 2017 [cited 2018 Jan 8];140(6). Available from: http://pediatrics.aappublications.org/content/140/6/e20173035
7. Fraporti MI, Scherer Adami F, Dutra Rosolen M. Fatores de risco cardiovascular em crianças. Rev Port Cardiol [Internet]. 2017 Oct [cited 2017 Nov 28];36(10):699–705. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29054491
8. Rechler MM, Clemmons DR. Regulatory actions of insulin-like growth factor-binding proteins [Internet]. Vol. 9, Trends in Endocrinology and Metabolism. Elsevier Current Trends; 1998 [cited 2017 Dec 18]. p. 176–83. Available from: http://www.sciencedirect.com/science/article/pii/S1043276098000472
9. Laron Z. Insulin-like growth factor 1 (IGF-1): a growth hormone. J Clin Pathol Mol Pathol [Internet]. 2001;54(5):311–6. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1187088/
10. Weiss M, Steiner DF, Philipson LH. Insulin Biosynthesis, Secretion, Structure, and Structure-Activity Relationships [Internet]. Endotext. MDText.com, Inc.; 2000 [cited 2017 Dec 14]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25905258

11. Livingstone C, Borai A. Insulin-like growth factor-II: Its role in metabolic and endocrine disease. Clin Endocrinol (Oxf) [Internet]. 2014;80(6):773–81. Available from: https://www.ncbi.nlm.nih.gov/pubmed/24593700
12. Kasper, D. L., Fauci, A. S., Hauser, S. L., Longo, D. L. 1., Jameson, J. L., & Loscalzo J. Harrison's principles of internal medicine (17th editon). In: Shlomo Melmed J Larry Jameson. New York: McGraw Hill Education; 2015. p. 2207–9.
13. Veldhuis JD, Iranmanesh A. Physiological regulation of the human growth hormone (GH)-insulin-like growth factor type I (IGF-I) axis: predominant impact of age, obesity, gonadal function, and sleep. Sleep [Internet]. 1996 Dec [cited 2018 Jan 9];19(10 Suppl):S221-4. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9085516
14. Puche JE, Castilla-Cortázar I. Human conditions of insulin-like growth factor-I (IGF-I) deficiency. J Transl Med [Internet]. 2012 Nov 14 [cited 2017 Nov 23];10:224. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23148873
15. Delafontaine P, Song YH, Li Y. Expression, Regulation, and Function of IGF-1, IGF-1R, and IGF-1 Binding Proteins in Blood Vessels [Internet]. Vol. 24, Arteriosclerosis, Thrombosis, and Vascular Biology. American Heart Association, Inc.; 2004 [cited 2017 Nov 23]. p. 435–44. Available from: http://www.ncbi.nlm.nih.gov/pubmed/14604834
16. van Houten VAA, Mook-Kanamori DO, van Osch-Gevers L, Steegers EAP, Hofman A, Moll HA, et al. A variant of the IGF-I gene is associated with blood pressure but not with left heart dimensions at the age of 2 years: the Generation R Study. Eur J Endocrinol [Internet]. 2008 Sep 1 [cited 2017 Dec 13];159(3):209–16. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18544576
17. Mukherjee A, Alzhanov D, Rotwein P. Defining human insulin-like growth factor I gene regulation. Am J Physiol - Endocrinol Metab [Internet]. 2016 [cited 2017 Aug 21];311(2):E519–29. Available from: http://ajpendo.physiology.org/lookup/doi/10.1152/ajpendo.00212.2016
18. Díez J. Insulin-like growth factor I in essential hypertension. Kidney Int [Internet]. 1999 [cited 2017 Aug 9];55(2):744–59. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9987103
19. Departament de Salut de la Generalitat de Catalunya. Anàlisi de la mortalitat a Catalunya. Bulleti Epidemiol Catalunya [Internet]. 2014 [cited 2017 Nov 28];34:1–15. Available from: http://salutweb.gencat.cat/web/.content/home/el_departament/estadistiques_sanitaries/dades_de_salut_i_serveis_sanitaris/mortalitat/mortalitat_2015.pdf
20. Weiss R. Metabolic syndrome in childhood - Causes and effects [Internet]. Vol. 19,

Endocrine Development. Basel: KARGER; 2010 [cited 2018 Jan 13]. p. 62–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20551669

21. Van Bunderen CC, Oosterwerff MM, Van Schoor NM, Deeg DJH, Lips P, Drent ML. Serum IGF1, metabolic syndrome, and incident cardiovascular disease in older people: A population-based study. Eur J Endocrinol [Internet]. 2013 [cited 2017 Aug 11];168(3):393–401. Available from: https://www.ncbi.nlm.nih.gov/pubmed/23233113
22. Katz LEL, Gralewski KA, Abrams P, Brar PC, Gallagher PR, Lipman TH, et al. Insulin-like growth factor-I and insulin-like growth factor binding protein-1 are related to cardiovascular disease biomarkers in obese adolescents. Pediatr Diabetes [Internet]. 2016 Mar [cited 2017 Nov 23];17(2):77–86. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25491378
23. Levy E, Saenger AK, Steffes MW, Delvin E. Pediatric Obesity and Cardiometabolic Disorders: Risk Factors and Biomarkers. Ejifcc [Internet]. 2017 Mar [cited 2017 Nov 28];28(1):6–24. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28439216
24. Aatola H, Koivistoinen T, Tuominen H, Juonala M, Lehtimäki T, Viikari JSA, et al. Influence of Child and Adult Elevated Blood Pressure on Adult Arterial Stiffness: The Cardiovascular Risk in Young Finns Study. Hypertens (Dallas, Tex 1979) [Internet]. 2017 Sep 1 [cited 2017 Nov 28];70(3):531–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28674036
25. Mikola H, Pahkala K, Niinikoski H, Rönnemaa T, Viikari JSA, Jula A, et al. Cardiometabolic Determinants of Carotid and Aortic Distensibility from Childhood to Early Adulthood. Hypertension [Internet]. 2017 Aug 1 [cited 2017 Nov 28];70(2):452–60. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28652463
26. Briet M, Boutouyrie P, Laurent S, London GM. Arterial stiffness and pulse pressure in CKD and ESRD [Internet]. Vol. 82, Kidney International. Elsevier; 2012 [cited 2018 Jan 13]. p. 388–400. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0085253815555580
27. O'Rourke MF, Staessen JA, Vlachopoulos C, Duprez D, Plante GE. Clinical applications of arterial stiffness; definitions and reference values [Internet]. Vol. 15, American Journal of Hypertension. Oxford University Press; 2002 [cited 2018 Jan 10]. p. 426–44. Available from: https://academic.oup.com/ajh/article-lookup/doi/10.1016/S0895-7061(01)02319-6
28. Paisley AN, Banerjee M, Rezai M, Schofield RE, Balakrishnannair S, Herbert A, et al. Changes in arterial stiffness but not carotid intimal thickness in acromegaly. J Clin Endocrinol Metab [Internet]. 2011 [cited 2017 Aug 9];96(5):1486–92. Available from:

https://www.ncbi.nlm.nih.gov/pubmed/21346071

29. Janssen JAMJL, Stolk RP, Pols HAP, Grobbee DE, Lamberts SWJ. Serum total IGF-I, free IGF-I, and IGFBP-1 levels in an elderly population: Relation to cardiovascular risk factors and disease. Arterioscler Thromb Vasc Biol [Internet]. 1998 Feb 1 [cited 2018 Jan 13];18(2):277–82. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9484994
30. Higashi, Yusuke; Quevedo Henry; Tiwari, Summit; Sukhanov, Wergiy; Shai, Shaw-Yung; Anwar, Asiv; Delafontaine P. The Interaction Between IGF-1, Atherosclerosis and Vscular Aging. NIH Public Acces [Internet]. 2015 [cited 2017 Nov 21];(5):213–23. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4199335/
31. Johnsen SP, Hundborg HH, Sørensen HT, Orskov H, Tjønneland A, Overvad K, et al. Insulin-like growth factor (IGF) I, -II, and IGF binding protein-3 and risk of ischemic stroke. J Clin Endocrinol Metab [Internet]. 2005 [cited 2017 Aug 11];90(11):5937–41. Available from: https://academic.oup.com/jcem/article/90/11/5937/2838395
32. Schut AFC, Janssen JAMJL, Deinum J, Vergeer JM, Hofman A, Lamberts SWJ, et al. Polymorphism in the promoter region of the insulin-like growth factor I gene is related to carotid intima-media thickness and aortic pulse wave velocity in subjects with hypertension. Stroke [Internet]. 2003 [cited 2017 Aug 11];34(7):1623–7. Available from: https://www.ncbi.nlm.nih.gov/pubmed/12791939
33. Schutte AE, Volpe M, Tocci G, Conti E. Revisiting the relationship between blood pressure and insulin-like growth factor-1. Hypertension [Internet]. 2014 [cited 2017 Nov 23];63(5):1070–7. Available from: http://hyper.ahajournals.org/content/63/5/1070
34. Colao A, Di Somma C, Cascella T, Pivonello R, Vitale G, Grasso LFS, et al. Relationships between serum IGF1 levels, blood pressure, and glucose tolerance: An observational, exploratory study in 404 subjects. Eur J Endocrinol [Internet]. 2008 Jul 30 [cited 2017 Nov 23];159(4):389–97. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18603571
35. Jiang X, Srinivasan SR, Dalferes E.R. J, Berenson GS. Plasma insulin-like growth factor 1 distribution and its relation to blood pressure in adolescents. The bogalusa heart study. Am J Hypertens [Internet]. 1997 Jul 1 [cited 2018 Jan 26];10(7 I):714–9. Available from: https://academic.oup.com/ajh/article-lookup/doi/10.1016/S0895-7061(97)00065-4
36. Walsh MF, Barazi M, Pete G, Muniyappa R, Dunbar JC, Sowers JR. Insulin-like growth factor I diminishes in vivo and in vitro vascular contractility: Role of vascular nitric oxide. Endocrinology [Internet]. 1996 May 1 [cited 2017 Dec 13];137(5):1798–803. Available from: https://academic.oup.com/endo/article-lookup/doi/10.1210/endo.137.5.8612517

37. Zhao G, Sutliff RL, Weber CS, Wang J, Lorenz J, Paul RJ, et al. Smooth muscle-targeted overexpression of insulin-like growth factor I results in enhanced vascular contractility. Endocrinology [Internet]. 2001 Feb 1 [cited 2018 Jan 11];142(2):623–32. Available from: https://academic.oup.com/endo/article-lookup/doi/10.1210/endo.142.2.7941

38. Hunt KJ, Lukanova A, Rinaldi S, Lundin E, Norat T, Palmqvist R, et al. A Potential Inverse Association Between Insulin-Like Growth Factor I and Hypertension in a Cross-Sectional Study. Ann Epidemiol [Internet]. 2006 Jul 1 [cited 2017 Dec 13];16(7):563–71. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16431135

39. Tivesten A, Bollano E, Andersson I, Fitzgerald S, Caidahl K, Sjögren K, et al. Liver-derived insulin-like growth factor-I is involved in the regulation of blood pressure in mice. Endocrinology [Internet]. 2002 [cited 2017 Aug 9];143(11):4235–42. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12399417

40. Patel L, Whatmore A, Davies J, Bansal N, Vyas A, Gemmell I, et al. Circulating insulin-like growth factor-binding protein 3 levels, independent of insulin-like growth factor 1, associate with truncal fat and systolic blood pressure in south asian and white european preschool children. Horm Res Paediatr [Internet]. 2014 [cited 2017 Dec 13];81(2):109–17. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24281388

41. Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jørgensen K, et al. Serum insulin-like growth factor-I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab [Internet]. 1994 Mar 1 [cited 2018 Jan 26];78(3):744–52. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem.78.3.8126152

42. Perticone F, Sciacqua A, Perticone M, Laino I, Miceli S, Caré I, et al. Low-plasma insulin-like growth factor-I levels are associated with impaired endothelium-dependent vasodilatation in a cohort of untreated, hypertensive caucasian subjects. J Clin Endocrinol Metab [Internet]. 2008 [cited 2017 Aug 11];93(7):2806–10. Available from: https://www.ncbi.nlm.nih.gov/pubmed/18430768

43. Walford G, Loscalzo J. Nitric oxide in vascular biology [Internet]. Vol. 1, Journal of Thrombosis and Haemostasis. Blackwell Science Inc; 2003 [cited 2018 Jan 17]. p. 2112–8. Available from: http://doi.wiley.com/10.1046/j.1538-7836.2003.00345.x

44. Higashi Y, Sukhanov S, Anwar A, Shai SY, Delafontaine P. Aging, atherosclerosis, and IGF-1. Journals Gerontol - Ser A Biol Sci Med Sci [Internet]. 2012;67 A(6):626–39. Available from: https://www.ncbi.nlm.nih.gov/pubmed/22491965

45. Dominguez LJ, Davidoff AJ, Srinivas PR, Standley PR, Walsh MF, Sowers JR. Effects of metformin on tyrosine kinase activity, glucose transport, and intracellular calcium in rat

vascular smooth muscle. Endocrinology [Internet]. 1996 Jan 1 [cited 2018 Jan 11];137(1):113–21. Available from: https://academic.oup.com/endo/article-lookup/doi/10.1210/endo.137.1.8536601

46. Sowers JR. Effects of insulin and IGF-I on vascular smooth muscle glucose and cation metabolism. Diabetes [Internet]. 1996 Jul [cited 2018 Jan 11];45(3 SUPPL.):S47-51. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8674890

47. Bach LA. Endothelial cells and the IGF system. J Mol Endocrinol [Internet]. 2015 [cited 2017 Nov 21];54(1):R1–13. Available from: https://doi.org/10.1530/JME-14-0215

48. Thum T, Fleissner F, Klink I, Tsikas D, Jakob M, Bauersachs J, et al. Growth Hormone Treatment Improves Markers of Systemic Nitric Oxide Bioavailability via Insulin-Like Growth Factor-I. J Clin Endocrinol Metab [Internet]. 2007 Nov 1 [cited 2018 Jan 11];92(11):4172–9. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2007-0922

49. Brueggemann LI. Low voltage-activated calcium channels in vascular smooth muscle: T-type channels and AVP-stimulated calcium spiking. AJP Hear Circ Physiol [Internet]. 2004 Oct 14 [cited 2017 Dec 13];288(2):H923–35. Available from: http://ajpheart.physiology.org/cgi/doi/10.1152/ajpheart.01126.2003

50. Heald AH, Siddals KW, Fraser W, Taylor W, Kaushal K, Morris J, et al. Low circulating levels of insulin-like growth factor binding protein-1 (IGFBP-1) are closely associated with the presence of macrovascular disease and hypertension in type 2 diabetes. Diabetes [Internet]. 2002;51(8):2629–36. Available from: https://doi.org/10.2337/diabetes.51.8.2629

51. El-Rahman RRA, Harraz OF, Brett SE, Anfinogenova Y, Mufti RE, Goldman D, et al. Identification of L- and T-type Ca2+ channels in rat cerebral arteries: role in myogenic tone development. AJP Hear Circ Physiol [Internet]. 2013 Jan [cited 2018 Jan 17];304(1):H58–71. Available from: http://www.physiology.org/doi/10.1152/ajpheart.00476.2012

52. Simons M, Ariyoshi H, Salzman EW, Rosenberg RD. c-myb affects intracellular calcium handling in vascular smooth muscle cells. Am J Physiol Physiol [Internet]. 1995 Apr [cited 2018 Jan 11];268(4):C856–68. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7733234

53. Sesso R, Franco MCP. Abnormalities in Metalloproteinase Pathways and IGF-I Axis: A Link Between Birth Weight, Hypertension, and Vascular Damage in Childhood. Am J Hypertens [Internet]. 2010 [cited 2017 Aug 9];23(1):6–11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19893495

54. Kwak SM, Kim JS, Choi Y, Chang Y, Kwon MJ, Jung JG, et al. Dietary intake of calcium and phosphorus and serum concentration in relation to the risk of coronary artery calcification in asymptomatic adults. Arterioscler Thromb Vasc Biol [Internet]. 2014 Aug 1 [cited 2018 Jan 11];34(8):1763–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24925973

55. Dhingra R, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB, Gaziano JM, et al. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. Arch Intern Med [Internet]. 2007 May 14 [cited 2018 Jan 11];167(9):879–85. Available from: http://archinte.jamanetwork.com/article.aspx?doi=10.1001/archinte.167.9.879

56. Menon V, Greene T, Pereira AA, Wang X, Beck GJ, Kusek JW, et al. Relationship of Phosphorus and Calcium-Phosphorus Product With Mortality in CKD. Am J Kidney Dis [Internet]. 2005 Sep 1 [cited 2018 Jan 2];46(3):455–63. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0272638605007754

57. Codoñer-Franch P, Tavárez-Alonso S, Simó-Jordá R, Laporta-Martín P, Carratalá-Calvo A, Alonso-Iglesias E. Vitamin D Status is Linked to Biomarkers of Oxidative Stress, Inflammation, and Endothelial Activation in Obese Children. J Pediatr [Internet]. 2012 Nov 1 [cited 2018 Jan 12];161(5):848–54. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22677566

58. Sheth RD, Perez MD, Goldstein SL. Cardiovascular calcifications in pediatric patients receiving maintenance dialysis. Pediatr Nephrol [Internet]. 2003 Aug 1 [cited 2018 Jan 12];18(8):810–3. Available from: http://link.springer.com/10.1007/s00467-003-1156-7

59. Querfeld U. The clinical significance of vascular calcification in young patients with end-stage renal disease [Internet]. Vol. 19, Pediatric Nephrology. Springer-Verlag; 2004 [cited 2018 Jan 12]. p. 478–84. Available from: http://link.springer.com/10.1007/s00467-004-1450-z

60. Wu-Wong JR, Noonan W, Ma J, Dixon D, Nakane M, Bolin AL, et al. Role of phosphorus and vitamin D analogs in the pathogenesis of vascular calcification. J Pharmacol Exp Ther [Internet]. 2006 Jul 1 [cited 2018 Jan 11];318(1):90–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16603671

61. Kim WS, Lee DH, Youn HJ. Calcium-phosphorus product concentration is a risk factor of coronary artery disease in metabolic syndrome. Atherosclerosis [Internet]. 2013 Jul 1 [cited 2018 Jan 2];229(1):253–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23706945

62. Bayes-Genis A, Conover CA, Schwartz RS. The insulin-like growth factor axis: A review of

atherosclerosis and restenosis [Internet]. Vol. 86, Circulation Research. American Heart Association, Inc.; 2000 [cited 2018 Jan 11]. p. 125–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10666406

63. O'Neill WC. The fallacy of the calcium-phosphorus product [Internet]. Vol. 72, Kidney International. Elsevier; 2007 [cited 2018 Jan 11]. p. 792–6. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0085253815527403

64. Eugster E. Gigantism. In: L. J. De Groot, P. Beck-Peccoz, G. Chrousos, K. Dungan, A. Grossman, J. M. Hershman … M. O. Weickert, editor. Endotext. South Dartmouth (MA); 2000. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25905378

65. Rhee N, Jeong K, Yang EM, Kim CJ. Gigantism caused by growth hormone secreting pituitary adenoma. Ann Pediatr Endocrinol Metab [Internet]. 2014 Jun [cited 2018 Jan 27];19(2):96–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25077093

66. Crocker MK, Yanovski JA. Pediatric Obesity: Etiology and Treatment [Internet]. Vol. 38, Endocrinology and Metabolism Clinics of North America. NIH Public Access; 2009 [cited 2018 Jan 23]. p. 525–48. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19717003

67. Marrugat, Jaume; Vila J. GranMo [Internet]. 2012 [cited 2018 Jan 23]. Available from: https://www.imim.cat/ofertadeserveis/software-public/granmo/

68. Westat Inc. Pulse and Blood Pressure Procedures [Internet]. Blood Pressure. 1993 [cited 2018 Jan 21]. Available from: https://wwwn.cdc.gov/nchs/data/nhanes3/manuals/pressure.pdf

69. Pulse pressure - an overview [Internet]. ScienceDirect Topics. [cited 2018 Jan 22]. Available from: https://www.sciencedirect.com/topics/neuroscience/pulse-pressure

70. Qu HQ, Li Q, Rentfro AR, Fisher-Hoch SP, McCormick JB. The definition of insulin resistance using HOMA-IR for americans of mexican descent using machine learning. PLoS One [Internet]. 2011 [cited 2018 Jan 23];6(6):e21041. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21695082

71. Rothermel J, Knop C, Lass N, Toschke C, Wunsch R, Reinehr T. Carotid Intima-Media Thickness in Children Treated with Growth Hormone: A Follow-Up Study over Three Years. Horm Res Paediatr [Internet]. 2017 [cited 2017 Dec 11];87(2):73–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/28068655

72. BMI-for-age (5-19 years). WHO [Internet]. 2015 [cited 2018 Jan 18]; Available from: http://www.who.int/growthref/who2007_bmi_for_age/en/

73. Global Database on Body Mass Index [Internet]. WHO. 2004 [cited 2018 Jan 18]. Available from: http://apps.who.int/bmi/index.jsp?introPage=intro_3.html