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Faculty of Medicine

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THE ASSOCIATION BETWEEN PTH AND IGFBP-1 LEVELS AND THEIR INFLUENCE ON MORTALITY IN ELDERLY WOMEN IN A 10 YEAR PROSPECTIVE FOLLOW-UP STUDY

Diploma Thesis

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ABSTRACT

Key words: Parathyroid hormone, Insulin-Like Growth Factor Binding Protein 1, Cardiovascular Diseases, Mortality.

Introduction: The aim of this research was to define and study some of the direct and indirect interactions between the influencing factors of calcium homeostasis system and metabolic syndrome. We analysed possible correlations between PTH, IGFBP-1 and some markers of metabolic syndrome, then we applied analysis with Cox proportional hazard regression to study the joint impact of PTH and IGFBP-1 on mortality from cardiovascular or ischaemic heart diseases, which are known to be closely related to metabolic syndrome.

Materials and methods: This is longitudinal, population based, prospective cohort 10 years follow-up study. Population represents women, born between year 1920 and 1930, living in the area of Bagarmossen and being able to visit a primary healthcare centre. Study population consisted of 351 participants, 339 of whom had both PTH and IGFBP-1 measurements at inclusion.

Results: We have found no significant correlation between serum levels of PTH and IGFBP-1 at inclusion and weak significant correlation between levels of PTH and fasting blood glucose and BMI. We have also found 203% increase in relative risk of cardiovascular diseases related death for every one SD step increase in levels of IGFBP-1 in participants with high PTH level, $p<0.001$, adjusted for age and levels of IGF-1. The increase in risk is relevant in patients with high PTH, but not in patients with low or normal PTH, HR = 1.20, p= 0.355, adjusted for age and IGF-1 levels. All patients were then divided in three tertiles by their IGFBP-1 levels. In patients in lowest or middle tertiles of levels of IGFBP-1 the difference made by normal or high levels of PTH was insignificant, but in the highest tertile of IGFBP-1 levels the relative risk was significantly higher by 286% in patients with high PTH, p=0.005. The group with the highest relative risk of cardiovascular diseases-related death of all is the group of patients of the highest tertile of IGFBP-1 levels who also had the high PTH.

Discussion: In our group of patients, the levels of IGFBP-1 only made the difference regarding the risk of CVD-related mortality if the PTH was also high, as well as the levels of PTH only made the difference for the patients in the highest IGFBP-1 tertile group. The combination of having the high level of PTH and high level of IGFBP-1 possesses the highest risk for CVD-related death.

Conclusions: Our findings show previously undescribed indirect relation between PTH and IGFBP-1 regarding significant increase in mortality from cardiovascular diseases.

KOPSAVILKUMS

Atslēgas vārdi: Paratiroīdais hormons, Insulīnam Līdzīgā Augšanas Faktora Saistošais Proteīns-1, Kardiovaskulārās Slimības, Mirstība.

Ievads: Šī pētījuma mērķis bija definēt un pētīt tiešu un netiešu mijiedarbību starp faktoriem, kas ietekmē kalcija homeostāzes sistēmu un metabolā sindroma attīstību. Mēs analizējām iespējamās korelācijas starp PTH un IGFBP-1 un dažiem metabolā sindroma marķieriem, tad pielietojām Koksa regresijas analīzi, lai izpētīt kopējo PTH un IGFBP-1 ietekmi uz mirstību kardiovaskulāro slimību dēļ, kuriem ir zināma saikne ar metabolo sindromu.

Materiāli un metodes: Šis ir longitudināls, uz populāciju balstīts prospektīvs kohortas pētījums ar 10 gadu ilgu pēcpārbaudes periodu. Populāciju pārstāv sievietes, dzimušas 1920. - 1930. gados, kas dzīvo Bargamossenas apvidū un ir spējīgas apmeklēt veselības aprūpes centru. Pētījuma populācija sastāvēja no 351 dalībnieces, no kurām 339 tika veikti gan PTH gan IGFBP-1 mērījumi pētījuma sākumā.

Rezultāti: Statistiski nozīmīga korelācija starp PTH un IGFBP-1 līmeņiem pēc sākotnējiem mērījumiem netika konstatēta, tika atrasta vāja nozīmīga korelācija starp PTH līmeni un asins glikozes līmeni pirms ēšanas, kā arī ar ķermeņa masas indeksu. Mēs konstatējām 203% lielu ar kardiovaskulārām slimībām saistītās nāves relatīvā riska pieaugumu pacientiem ar augstu PTH līmeni par katru IGFBP-1 pieaugumu par vienu SD, $p<0.0001$, pielāgojot modeli pacientu vecumam un IGF-1 līmenim. Riska pieaugums ir nozīmīgs pacientiem ar augstu PTH, bet ne pacientiem ar normālu PTH līmeni, $HR = 1.20$, $p = 0.355$, modelis pielāgots vecumam un IGF-1 līmenim. Visi pacienti tika sadalīti trīs trešdaļās pēc IGFBP-1 līmeņa. Pacientiem, kuru IGFBP-1 līmenis ir zemākajā vai vidējā trešdaļā PTH līmenis neietekmēja mirstību, savukārt pacientiem augšējā trešdaļā pēc IGFBP-1 līmeņa un augstu PTH līmeni ar kardiovaskulārām slimībām saistītās mirstības relatīvais risks bija paaugstināts par 286% salīdzinājumā ar grupu ar normālu PTH līmeni, $p=0.005$. Grupa ar vislielāko relatīvo risku attiecībā pret visām citām grupām ir pacientu grupa, kuru IGFBP-1 līmenis ir augšējā trešdaļā un kuriem ir augsts PTH līmenis.

Diskusija: Mūsu pacientu grupā IGFBP-1 līmenis bija nozīmīgs attiecībā uz ar kardiovaskulārām slimībām saistītās nāves risku tikai gadījumā, ja PTH līmenis arī bija augsts, kā arī PTH līmenis bija nozīmīgs tikai pacientiem augšējā trešdaļā pēc IGFBP-1 līmeņa. Augsta IGFBP-1 un PTH līmeņa kombinācija ir saistāma ar vislielāko ar kardiovaskulārām slimībām saistītās nāves risku.

Secinājumi: Mūsu iegūtie rezultāti norāda uz iepriekš neaprakstīto netiešo saikni starp PTH un IGFBP-1 attiecībā uz nozīmīgo ar kardiovaskulārām slimībām saistītās nāves riska pieaugumu.

INTRODUCTION

This study focuses on the problem of the complexity and interconnections of the human endocrine system, its various subsystems, and the ways they may be related in order to provide the integral homeostasis in the human body.

The human endocrine system has been traditionally studied as a conglomerate of several subsystems that regulate various aspects of homeostasis in the human body. Each subsystem consists or multiple hormones, receptors, binding proteins and other regulating factors that provide stimulatory and inhibitory effects necessary for a close regulation of the body homeostasis. One of such systems that will be revised in the background section of this study is a calcium metabolism regulation system. It implements a complex mechanism that provides adequate calcium absorption, storage and regulates plasma calcium levels necessary for other metabolic processes.

However, the focus of this study is on possible relations and interactions between calcium balance system and glucose metabolism system. Some existing research indicates that various endocrine systems do not function separately, but rather influence each other and some elements may possibly have clinically significant functions in several metabolic systems. A review article by C. Huang et al. (2013) indicates the existing relation of parathyroid hormone, which is typically attributed to the calcium homeostasis system, to metabolic syndrome, which is mainly described as a glucose metabolism system disorder. It shows that levels of parathyroid hormone are predictive of cardiovascular mortality. This study suggests that further investigation regarding the role of the parathyroid hormone in metabolic syndrome development. A study by P. Jehle et al. (2003) on relations between insulin-like growth factor-1, insulin-like growth factor binding protein-1 and bone metabolism, and their possible role in osteoporosis also highlights the importance of interactions between seemingly unrelated systems. The data provided by this study strongly suggest an existing functional connection between the insulin-like growth factor family and bone metabolism, that is known to be directly related to calcium homeostasis system. An article by H. Salminen et al. (2008) shows the inverse relation between insulin-like growth factor-1 and bone mass density, while the relation between insulin-like growth factor binding protein-1 values and bone mass density is positive. A study by J. Reis et al. (2007) suggests metabolic syndrome as a possible link between the systems. It concludes that elevated levels of parathyroid hormone suggest an increased risk of metabolic syndrome in older men.

The aim of this research was to define and study some of the direct and indirect statistical relations between the influencing factors of calcium homeostasis system and metabolic syndrome. This aim was being realised by statistical analysis of the clinical data available from a 10-year long prospective cohort study project called Primary Health Care and Osteoporosis (PRIMOS), that was performed in Stockholm, Sweden between 1999 and 2010.

Two approaches were used in this research – one was establishing possible correlation between various parameters at inclusion, and the other is a complex Cox regression analysis of data over the period of time, the outcome being cardiovascular diseases related mortality. Parameters analysed for correlation were chosen on the basis of their clinical relevance to calcium metabolism system or metabolic syndrome as well as scientific suggestions found in the above mentioned studies. They included parathyroid hormone, insulin-like growth factor-1, insulin-like growth factor binding protein-1, body mass index and prevalence of diabetes mellitus at inclusion.

The objectives of this study were the following:

1. To study the correlations between the levels of parathyroid hormone and body mass index, fasting blood glucose levels and levels of insulin-like growth factor binding protein-1 at inclusion.

2. To establish difference in relative risk of cardiovascular diseases related death in patients with different levels of parathyroid hormone and different levels of insulin-like growth factor binding protein-1, adjusted for levels of insulin-like growth factor-1 levels:

    2.1. Differences in relative risk of cardiovascular diseases related death in patients with high levels of parathyroid hormone depending on levels of insulin-like growth factor binding protein-1;

    2.2. Differences in relative risk of cardiovascular diseases related death in patients with normal levels of parathyroid hormone levels depending on levels of insulin-like growth factor binding protein-1;

2.3. Differences in relative risk of cardiovascular diseases related death in patients with given levels of insulin-like growth factor binding protein-1 depending on their levels of parathyroid hormone being elevated or not elevated.

3. To compare all groups of patients and define the group with highest relative risk of cardiovascular diseases-related death.

   3.1. To compare all patients with high levels of PTH, with the group of the highest tertile of IGFBP-1 levels taken as a reference regarding the relative risk of CVD-related mortality.

   3.2. To compare the patients within the group of the highest tertile of IGFBP-1 levels with group with high PTH levels taken as a reference regarding the relative risk of CVD-related mortality.

   3.3. To compare group of patients of middle tertile of IGFBP-1 levels to the group of the highest tertile of IGFBP-1 levels taken as a reference regarding the relative risk of CVD-related mortality.

   3.4. To compare group of patients of lowest tertile of IGFBP-1 levels to the group of the highest tertile of IGFBP-1 levels taken as a reference regarding the relative risk of CVD-related mortality.

LITERATURE REVIEW

Parathyroid hormone

Main functions of parathyroid hormone

Parathyroid hormone (PTH) is a hormone secreted by chief cells of parathyroid glands. It has a molecular mass of 9500 Da. It is being produced by cleavage from the pre-hormone containing 115 amino acids to pro-hormone built of 90 amino acids to the mature hormone (Brewer et al. 1972).

The traditionally known role of the parathyroid hormone is the calcium homeostasis regulation. It acts to increase serum levels of ionic calcium, by activating the parathyroid hormone receptor 1 or 2. Parathyroid hormone receptor 1 is abundantly present is kidney and bone tissue, and parathyroid hormone receptor 2 is found in pancreas tissues, placenta, testis and the nervous system. The half-life of the parathyroid hormone is 4 minutes (Bieglmayer et al. 2002). Accordingly to the commonly used research data the average level of the parathyroid hormone is between eight to 51 pg/mL (Longo et al. 2011).

Regulation of parathyroid hormone secretion

The secretion of parathyroid hormone is closely regulated within the organism. The main factor contributing the parathyroid hormone levels is serum ionised Ca^{2+} levels. The control is being provided via negative feedback mechanism (Brown 2015). On the cellular level this is being achieved by calcium binding receptors located on the parathyroid hormone secreting cell surface. These receptors are being activated in response to elevated calcium levels. Receptor binding then results in activation of the Gq G-protein coupled cascade, which involves the phospholipase C membrane-associated enzyme cleaving activity. This causes phosphatidylinositol 4,5-biphosphate (PIP2) to release intracellular messengers inositol triphosphate and diacylglycerol.

The result of this cascade process is the release of the Ca^{2+} ions from the intracellular storage, which in turn causes the influx of the extracellular calcium into the cell cytoplasmic environment. The rising levels of intracellular calcium ions have the inhibiting effect on parathyroid hormone release from the intracellular storage. This mechanism of inhibition is the reverse of what most secreting cells are implementing, where high intracellular ionized calcium levels have the stimulating effect on secretion process (Mannstadt & Kronenberg 2015).

While in contrast to the usual mechanism in most cells, the increase in intracellular calcium ions concentration causes the inhibition of secretion in parathyroid hormone secreting cells, the concentration of magnesium plays the opposite role in stimulus-secretion coupling. Mild decrease in magnesium levels intensifies the kidney resorption in response to the PTH activity (Agus 1999).

Another factor contributing to the regulation of the parathyroid hormone level is serum phosphate level. Phosphate binds to the calcium forming calcium phosphate salt that cannot bind to the calcium sensing receptors thus decreasing the intracellular calcium level and increasing the release of parathyroid hormone (Costanzo 2007).

So the main factors stimulating the release and activity of the parathyroid hormone are decrease in the serum calcium concentration, mild decrease in the serum magnesium concentration and increase in serum phosphate level, whereas factors that inhibit the release of the parathyroid hormone are increase of the serum calcium level, severe hypomagnesemia and calcitriol hormone activity (Costanzo 2007).

Parathyroid hormone family

The parathyroid hormone belongs to the parathyroid hormone family, which also includes parathyroid hormone-related protein (PTH-rP) that has a significant role in epithelial-mesenchymal interactions in formation of the mammary glands as well as performs a signalling molecule in bone resorption process in teeth eruption. It also has been linked to certain types of cancer and is known to be occasionally secreted by breast cancer and some types of lung cancer cells. Its action involves autocrine, paracrine and endocrine regulation mechanisms (McCauley & Martin 2012).

Parathyroid hormone has multiple widespread sites of binding is many organs and tissues, so it’s mechanism of action is complex and intertwined with actions of other hormone systems, building a mechanism of maintaining homeostasis that is still not entirely understood.

Calcium homeostasis regulation

The role of the parathyroid hormone in calcium homeostasis regulation

Calcium metabolism within the organism is a system of processes that ensures the slight variation of plasma ionized calcium level only within a small range of values and its careful regulation to avoid the states of hyper- or hypocalcemia. Plasma calcium concentration plays an important role for many cellular processes. Parathyroid hormone is being released when serum ionized calcium levels are falling low and prevents the state of hypocalcemia. When calcium levels rise above the optimal, thyroid gland releases the hormone calcitonin that acts in reverse to the parathyroid hormone and thus prevents the state of hypercalcemia (Brini et al. 2013).

As is shown on the Figure 1, parathyroid hormone has multiple sites of action in order to maintain calcium balance. It binds to the receptors in kidney tissues thus increasing ionized calcium reabsorption, as well as binds to the receptors in bone tissues, increasing calcium resorption from mineral deposits.

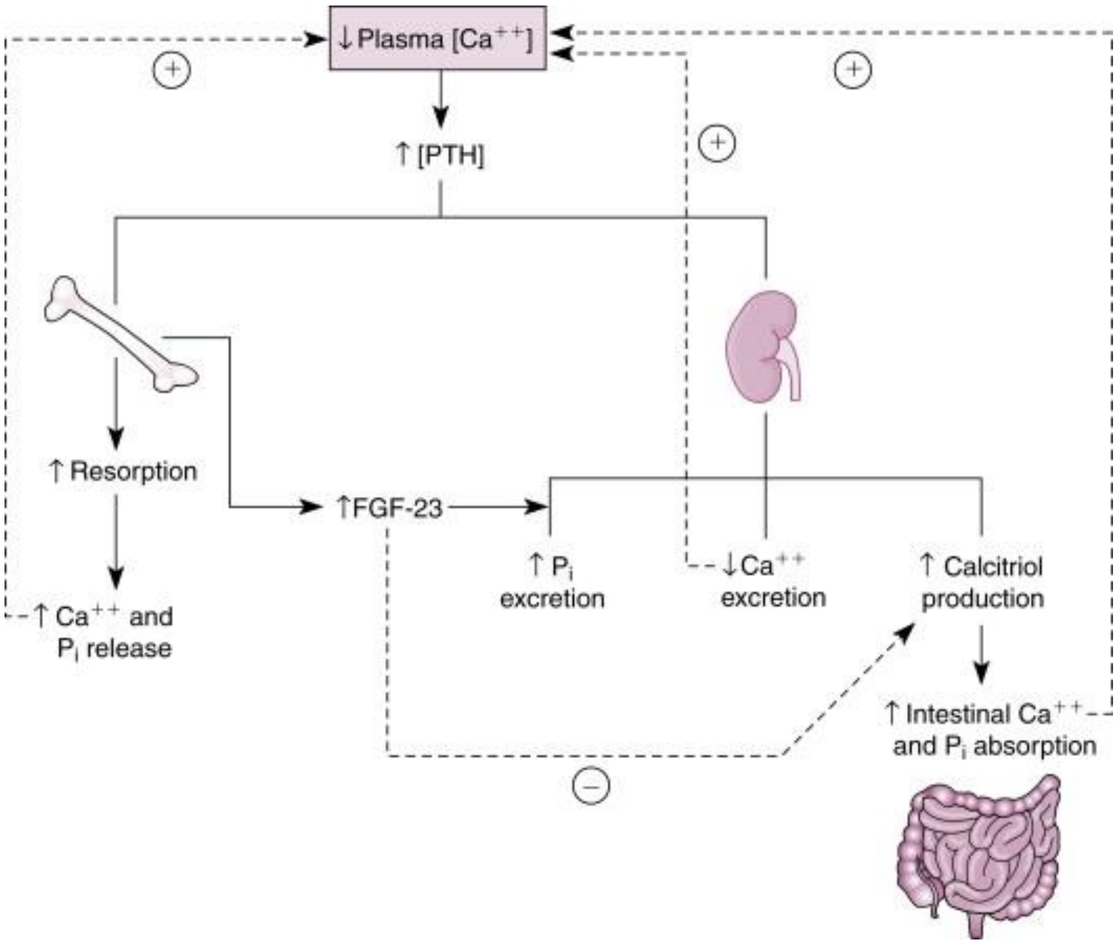


Figure 1. Overview of the major hormones regulating plasma concentration of Ca^{++} [Ca^{++}]. *Dashed lines* indicate negative feedback. *FGF-23*, Fibroblast growth factor 23; P_i, inorganic phosphate; *PTH*, parathyroid hormone (Koeppen & Stanton 2013).

Normal range for total calcium concentration in the body is 2.2-2.6mmol/L (9-10.5mg/dL), however the most clinically used is the ionized plazma calcium value. The normal valua range for the ionized plazma calcuim is 1.3-1.5mmol/L (4.5-4.6 mg/dL). The biologic effect is provided by the ionized calcium, so this measurement is more clinically valuable. The amount of total calcium depends on the plasma albumin levels. Approximately 35 to 50 percent of total calcium is protein-bound, around five to 10 percent forms organic acids and phosphate salts. The remaining 50-60 percent is ionized (Diem 1971).

The role of vitamin D in calcium homeostasis regulation

Vitamin D is an important factor in calcium homeostasis maintenance mechanism. It works in close relation to the parathyroid hormone. Vitamin D acts to enhance the reabsorption of not only calcium, but also magnesium, iron, phosphate and zinc. The main source of vitamin D in the organism is dermal synthesesis in activated by exposure to the UV radiation. The synthesised form is biolgically inactive and it undergoes series of enzymatic reactions in order to become active. The enzymatic conversion, specifically, hydroxylation takes place in kidney and liver tissues. Evidence suggest that the negative feedback mechanism prevents overproduction of inactive and active forms of vitamin D (Wolf 2004).

Cholecalciferol (also known as vitamin D_3) and ergocalciferol (known as vitamin D_2) can be ingested with food or vitamin supplements. Cholecalciferol is then modified in liver to calcidiol, also known as 25-hydroxycholecalciferol or 25-hydrovitamin D_3 or 25(OH)D_3. Ergocalciferol is modified to 25-hydroxyergocalciferol, also known as 25-hydroxyvitamin D_2 or 25(OH)D_2 (DeLuca et al. 1971). Some part of calcidiol, the product of cholecalciferol modification is then converted to calcitriol, which is the biologically active form of vitamin D that has a direct role in calcium balance regulation. Calcitriol also takes part in phosphate level regulation, affects bone metabolism, immunity, and neuromuscular functions (Hewison 2011; Bolland et al. 2014).

The biological activity of the vitamin D is very broad. The vitamin D-binding receptors are located in the nuclei of the expressing cells. The binding activated a transription factor that regulates gene expression in the target cells. Vitamin D receptors are expressed in wide variety of organs – brain, heart, breast, prostate, skin. In bone tissue vitamin D plays an important role in both bone resoption and remodelation. The lack of vitamin D often results in reduced bone mass and bone density. Vitamin D also has an effect on the immune system by influencing cell proliferation and differentiation in some classes of white blood cells (Watkins et al. 2015). As is shown on the Figure 2, vitamin D acts in close relation to the parathyroid system to ensure only minute variations in the calcium levels in healthy humans.

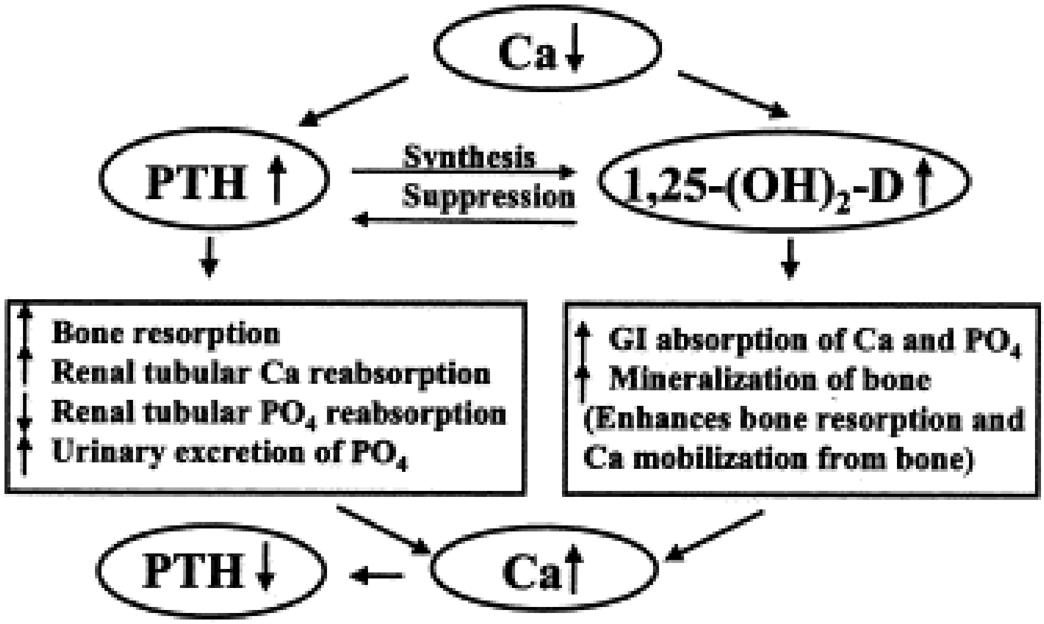


Figure 2. Schematic picture of the regulation of bone turnover and calcium (Ca) homeostasis by the active form of vitamin D (1,25-$(OH)_2$-D_3) and parathyroid hormone (PTH) (Berglund et al. 2000).

Insulin-like growth factor family

Insulin-like growth factor axis

Insulin-like growth factors are a set of proteins with a significant structural similarity to the insulin. Insulin-like growth factor take part in a complex system of communications that cells implement in order to maintain internal homeostasis and communicate with the physiological environment. The system is often named an IGF axis. It includes two insulin-like growth factor receptors named IGF1R and IGF2R, two types of insulin-like growth factors, IGF-1 and IGF-2 that are ligands for the receptors, a family of six insulin-like growth factor binding proteins, IGFBP-1 to IGFBP-6 and the protease enzymes (Jørgensen et al. 1993; Arai et al. 2009).

Insulin-like growth factor axis works in close relation to the growth hormone and it stimulated by it. Insulin-like growth factor-1 is secreted in liver tissues in response to the growth factor stimulation. It promotes cell proliferation and inhibits apoptosis. This is important in normal physiological metabolic processes, but also plays an important role in pathogenesis or various cancerous diseases. Insulin-like growth factor-2 is known to be important in early development; however insulin-like growth factor-1 is more important to achieve maximal growth. Insulin-like growth factor-2 also is important in development of certain organs, as brain, kidney and liver.

Insulin-like growth factor-1 has highly variable values depending on multiple factors – time of day, sex, stress, workout status and many other factors. Insulin-like growth factor-1 has an important role in development of the neural structures, myelination and neurogenesis as well as neuroprotection after the damage (Gunnell et al. 2005). The ability to control the apoptosis is the basis of the influence of the insulin-like growth factor-1 on the cochlear development. According to some data, the insulin-like growth factor-1 deficiency leads to the hearing loss (Welch & Dawes 2007).

Insulin-like growth factor-1

It is a protein that bears distinct similarity to the insulin structure. It is also named somatomedin C and is encoded by the insulin-like growth factor-1 gene (Höppener et al. 1985). Arguably the most important biological function of the insulin-like growth factor-1 is providing hormonal basis of the growing and developmental process. A synthetic analogue has been developed named mecasermin that is used to treat growth failure (Keating 2008). Insulin-like growth factor-1 is produced mainly as an endocrine hormone in liver and in some other target tissues for purposes of autocrine or paracrine regulation. Multiple factors affect its production – growth hormone has a stimulating effect, whereas malnutrition, lack of sensitive receptors or growth hormone insensitivity have inhibiting effect. One of the factors influencing levels of insulin-like growth factor-1 is dietary protein intake. Regardless of total calorie consumption protein intake increases levels of insulin-like growth factor-1. The median level of insulin-like growth factor-1 also varies throughout lifetime. The peak level is during puberty growth period and it reaches its lowest in older individuals (Levine et al. 2014).

The mechanism of action of insulin-like growth factor-1 is provided by a specific receptor, insulin-like growth factor-1 receptor (IGF1R). When the hormone binds to the receptor, tyrosine kinase activates the intracellular signalling pathway, that promotes cell proliferation and growth and inhibits the process of apoptosis. Insulin-like growth factor-1 is the most important mediator of the growth hormone activity. Growth hormone is being synthesized in the anterior part of the pituitary gland, it stimulates the secretion of the insulin-like growth factor-1 in liver tissues, which in turn promotes growth of the body organs and cell proliferation (Yakar et al. 2002).

Insulin-like growth factor binds to six subtypes of insulin-like growth factor binding proteins – IGFBP1, IGFBP2, IGFBP3, IGFBP4, IGFBP5, IGFBP6, IGFBP7. Approximately 98% of insulin-like growth factor is bound to proteins. Some of the binding proteins have inhibitory effect on insulin-like growth factor activity, as they bind it with greater affinity than the receptors do, thus decreasing levels of circulating insulin-like growth factor. However, as the name suggests, insulin-like growth factor-1 structurally resembles insulin molecule, and therefore is capable of binding to the insulin receptor at affinity approximately 0.1 that of insulin (Kiselyov et al. 2009).

Insulin-like growth factor-1 has a substantial clinical significance, as it binds the receptors in most cells of the organism and takes part in metabolic processes in every part of the body and controls an important function of growth and proliferation. It is a part of an intricately complex system of hormones, their receptors and binding proteins, that ensures growth, electrolyte balance and controls many other metabolic functions. Therefore insulin-like growth factor has been linked to many metabolic and growth diseases.

Some types of dwarfism (growth retardation) are characterized by inability to produce or respond to insulin-like growth factor-1. Acromegaly disease is caused by abnormalities of the anterior part of the pituitary gland, most commonly, a growth hormone secreting adenoma. This results in abnormally high levels of growth hormone that in turn increases the level of insulin-like growth factor-1. The disease manifests as characteristic anatomical and metabolic changes (Giustina et al. 2014). Patients with severe insulin-like growth factor-1 deficiency can be treated by substitution therapy with a synthetic analogue. It is currently being produced by a recombinant bacteria and yeast and used as a growth failure treatment (Rosenbloom 2007). Some studies found a potential beneficial effect of therapeutic administration of insulin-like growth factor-1 in some peripheral neuropathies as a neurotrophic agent on reversal of degeneration of the spinal cord motor neuron axons (Lewis et al. 1993). Some animal model data suggest that insulin-like growth factor-1 might be used in combination with erythropoietin for a stroke therapy with both behavioural and cellular function improvements (Fletcher et al. 2009).

Insulin-like growth factor binding proteins (IGFBPs)

The insulin-like growth factor binding protein is a family of related proteins that are carriers for the insulin-like growth factor-1. Around 98% of the insulin-like growth factor-1 is bound to the insulin-like growth factor binding protein, majority of which being IGFBP3. The important mechanism of regulation of the insulin-like growth factor-1 is its binding to the IGFBP in the liver that allows the continuous stimulation of the growth hormone to produce insulin-like growth factor-1. It is thought that the purpose of IGFBPs might be to prolong the lifespan of the circulating insulin-like growth factor-1 by binding it in all tissues (Stewart et al. 1993). Some IGFPBs are thought to either enhance or diminish the biological effect of the insulin-like growth factors. All six IGFBP subtypes share about 50% of structural similarity (Clemmons et al. 1995). Despite prominent structural similarity, some features of the different IGFBPs are different – the exact configuration, the chromosomal location of the encoding gene, heparin binding domains, preference for binding either insulin-like growth factor one or two and certain enzymatic differences in glycosylation and phosphorylation. These differences provide different characteristics of IGFBPs and their interaction with the cellular membrane (Hwa et al. 1999).

IGFBP1, also known as the placental protein 12. It binds both insulin-like growth factors one and two thus prolonging their half-life and altering their interaction with cellular membranes. The most abundant insulin-like growth factor binding protein, the IGFBP3 is the main carrier of the insulin-like growth factors one and two. It affects the binding to the cell receptors and also enters the cell nucleus and binds to the nuclear receptors. In many cell types IGFBP3 has an antiproliferative effect by blocking binding of the insulin-like growth factors that promote growth and proliferation.

IGFBP3 levels are regulated by the growth hormone and it therefore elevated in acromegaly patients and are low in growth hormone deficient individuals (Olivecrona et al. 1999). Insulin-like growth factor binding protein-4 (IGFBP4) binds both insulin-like growth factors and circulates in plasma in both glycosylated and non-glycosylated forms. It has been linked to reducing some cancer cell activity in vitro, however the data about its clinical relevance in therapy of human cancers is inconclusive (Durai et al. 2007). IGFBP5 and IGFBP6 also are known to bind and carry the insulin-like growth factors. IGFBP7 has less affinity to the insulin-like growth factor than the other six subtypes of binding proteins and has a cell adhesion promoting effect (Oh et al. 1996). It is currently studied in relation to the hepatocellular carcinoma as a potentially tumour growth inhibiting factor (Chen et al. 2013).

Insulin-like growth factor receptors

Insulin-like growth factor receptors are transmembrane protein complexes that belong to the tyrosine kinase receptor class. They bind insulin-like growth factors one and two, mediating their effect intracellularly. Binding to the insulin-like growth factor causes addition of the phosphate group on some tyrosine amino acid compounds on certain intracellular proteins that in turn activates the intracellular signalling cascades (LeRoith et al. 1995). The signalling pathway induced by the insulin-like growth factor receptor activation plays an important physiological role in development of the mammary glands during pregnancy and lactation. Some data suggest its role in development of some cancer types due to its anti-apoptotic effect that helps cancerous cells to survive and to develop resistance against certain chemotherapeutic medicines or radiotherapy (Warshamana-Greene et al. 2005).

Insulin-like growth factor-2 receptor is also called a cation-independent mannose-6-phosphate receptor. It is present both intracellularly and on the cell surface. On the surface it binds to the insulin-like growth factor-2, whereas in the intracellular environment it binds mannose-6-phosphate-tagged proteins (Laureys et al. 1988). After binding the IGF-2 on the cell surface, the receptors accumulate and make vesicles coated by a protein clathin that provides a mechanism for formation and internalization of vesicles (Ghosh & Kornfeld 2004).

Metabolic syndrome

Metabolic syndrome definition, diagnostic criteria and prevalence

Metabolic syndrome is a complex of several medical conditions that includes abdominal obesity, hypertension, elevated fasting blood glucose, blood triglycerides and low high-density lipoprotein levels (Kaur 2014).

Several organisations provided certain diagnostic criteria for the metabolic syndrome. The definition developed by the International Diabetes Federation in 2006 is the following: central obesity (defined as waist circumference with ethnicity specific values, if body mass index is above $30kg/m^2$, central obesity can me assumed without waist circumference measurment) and two of the following:

- Elevated triglycerides to >150mg/dL (1.7 mmol/L), or consumption of specific medication to treat dyslipidaemia;
- Reduced high-density-lipoprotein cholesterol: <40mg/dL (1.03 mmol/L) in men or <50 mg/dL (1.29 mmol/L) in women, or consumption of specific medication to treat dyslipidaemia;

- Elevated blood pressure: systolic brachial blood pressure >130mm Hg or diastolic brachial blood pressure >85 mm Hg, or consumption of specific medication to treat previously diagnosed hypertension;

- Elevated fasting plasma glucose >100mg/dL (5.6 mmol/L) or previously diagnosed type 2 diabetes. However, if fasting blood glucose is above 100mg/dL (5.6 mmol/L) it does not necessarily define the presence of the metabolic syndrome, but rather indicates the necessity of performing an oral glucose tolerance test (Alberti et al. 2006).

Worldwide prevalence of the metabolic syndrome varies from 10 to 84%, affecting approximately 25% of world population (Desroches & Lamarche 2007). The prevalence of the metabolic syndrome in a particular region depends on multiple risk factors including higher socioeconomic status, increased body mass index, sedentary lifestyle, smoking and dietary habits (Cameron et al. 2004).

Pathogenesis of the metabolic syndrome

The pathogenesis of the metabolic syndrome compounds is complex and remains under investigation. It is thought that multiple lifestyle factors have major contribution as well as stress, genetic predisposition, disrupted sleep pattern, excessive alcohol intake and intake of some medications. Multiple biomarkers for metabolic syndrome are already discovered, among them are markers of systemic inflammation, that are often increased among metabolic syndrome patients. Inflammation markers include C-reactive protein, interleukin-6 (IL-6), tumour necrosis factor-alfa (TNFα), plasminogen activator inhibitor-1 (PAI-1) and others (Lau et al. 2005). This inflammatory reaction might be related to the state of obesity. It causes progressive adipocyte enlargement, reduction in blood supply and facilitates the development of hypoxia and local necrosis that causes the release of inflammatory mediators (Jacobs et al. 2009).

The mechanism of stress influence on the metabolic syndrome is thought to be related to the dysfunction hypothalamic-pituitary-adrenal axis, that causes an increase in circulating cortisol level that elevated blood glucose level and disrupts insulin regulation mechanism that affects adipose tissue and might contribute to central adiposity, dyslipidaemia and high blood pressure and osteoporosis (Tsigos & Chrousos 2002). The dysfunctional hypothalamic-pituitary-adrenal axis might be one of the contributing factors linking metabolic syndrome to cardiovascular disease and to the calcium homeostasis system. Metabolic syndrome is closely associated to various endocrine diseases – hyperuricemia, non-alcoholic fatty liver disease, polycystic ovary disease in women, erectile dysfunction in men and acanthosis nigricans, that suggests a complex mechanism of cooperation between different hormonal systems maintaining integral homeostasis of the organism environment (Kaur 2014).

The relation between metabolic syndrome and cardiovascular diseases

Metabolic syndrome is known to have very prominent relation to the various cardiovascular diseases, primarily atherosclerosis, thromboses, ischaemic heart disease, chronic heart failure and cerebral stroke (Kaur 2014). Multiple studies show the close association between metabolic syndrome and various cardiovascular conditions. The cardiovascular conditions are generally considered being an outcome of chronic metabolic syndrome condition (Sung et al. 2015; Moller & Kaufman 2005; Moreira et al. 2014; Ma & Zhu 2013).

The relation between parathyroid hormone, insulin-like growth factor family and metabolic syndrome

The relation between parathyroid hormone and metabolic syndrome

Both parathyroid hormone and insulin-like growth factor binding protein-1 are known to be related to metabolic syndrome. Majority of relevant studies found positive statistical associations between hyperparathyroidism, cardiovascular diseases and metabolic syndrome (Huang et al. 2013). Some studies found parathyroid hormone being an independent predictor of development of metabolic syndrome in different populations. The role of vitamin D, however, still remains undetermined. Recent data shows that high parathyroid hormone serum levels increase risk of metabolic syndrome, although the exact mechanism of parathyroid hormone influence is still debated. (George et al. 2013; Reis et al. 2007; Hjelmesaeth et al. 2009). Previously parathyroid hormone was viewed only as a part of the calcium homeostasis regulation system and was mainly studied in context of osteoporosis, bone mineralization and renal dysfunction. Some studies indicate the important role parathyroid hormone has in bone remodelling and turnover, as well as plasma calcium regulation (Parfitt 1976; Aleksyniene et al. 2009; Weinman & Lederer 2012; Carrivick et al. 2015).

The relation between insulin-like growth factor family and metabolic syndrome

Metabolic syndrome components – hypertension, atherosclerosis, ventricular hypertrophy and type 2 diabetes have established relation to growth factor system components. One of the most commonly studied are insulin-like growth factor-1 and insulin-like growth factor binding protein family. Some studies show that IGFBP-1 plays an important role in pathogenesis of obesity and insulin resistance and may be a marker for metabolic syndrome components (Ruan & Lai 2010). Some data show an inverse correlation between IFGBP-1, body mass index and insulin level (Lemne & Brismar 1998). Another study concludes that IGFBP-1 is closely related to macrovascular disease and hypertension in type 2 diabetes patients (Heald et al. 2001). Insulin-like growth factor-1 is also associated to metabolic syndrome components: deficiency of this factor is associated with increased risk of atherosclerosis and cardiovascular diseases (Arai et al. 2009; Hyppönen et al. 2008). However, despite the name, IGFBP-1 is not a major regulating factor of IGF-1 activity, as 80% of circulating IGF-1 is bound by IGFBP-3, creating an inactive complex (Binoux 1995).

The relations between calcium homeostasis system, insulin-like growth factor family and metabolic syndrome

Multiple known factors, such as vitamin D, PTH-related peptide and insulin-like growth factor system components have been suggested as possible links between calcium balance system and glucose metabolism. (Sciacqua et al. 2014; Ding et al. 2014; Bellan et al. 2014; Legakis & Mantouridis 2009; Jehle et al. 2000; Jehle et al. 2003; Salminen et al. 2008b). However, the mechanism and clinical relevance of this relation often remains unclear (Huang et al. 2013).

Some studies also have shown the relation between elevated parathyroid hormone serum levels and all-cause mortality, especially of cardiovascular diseases (Fitzpatrick et al. 2008; Schierbeck et al. 2011; Bansal et al. 2014). However, despite some evidence about relations between calcium balance and glucose metabolism systems, their possible interaction and influence on mortality of cardiovascular diseases remains largely unknown and future it vitro and in vivo biochemical and clinical studies are needed. A review article on association of plasma parathyroid hormone levels with metabolic syndrome and risk for cardiovascular disease states that the present evidence is inconsistent (Huang et al. 2013). Insulin-like growth factor-1 binding protein is one factors associated with glucose metabolism and metabolic syndrome. It has also been previously studied in relation to hyperparathyroidism (Salminen et al. 2008b; Legakis & Mantouridis 2009; Jehle et al. 2003; Jehle et al. 2000).

MATERIALS AND METHODS

Study population

The present longitudinal, population-based, cohort follow-up study was part of a larger project called Primary Health Care and Osteoporosis Study (PRIMOS). It was initiated in 1999–2001 and the patients were followed up for a period of ten years. The criteria for inclusion were: born between 1920 and 1930, living in the area of Bagarmossen, being of female sex and being able to visit the primary healthcare centre (PHCC) in Bagarmossen. The formation of our study population in seen on the Figure 3.

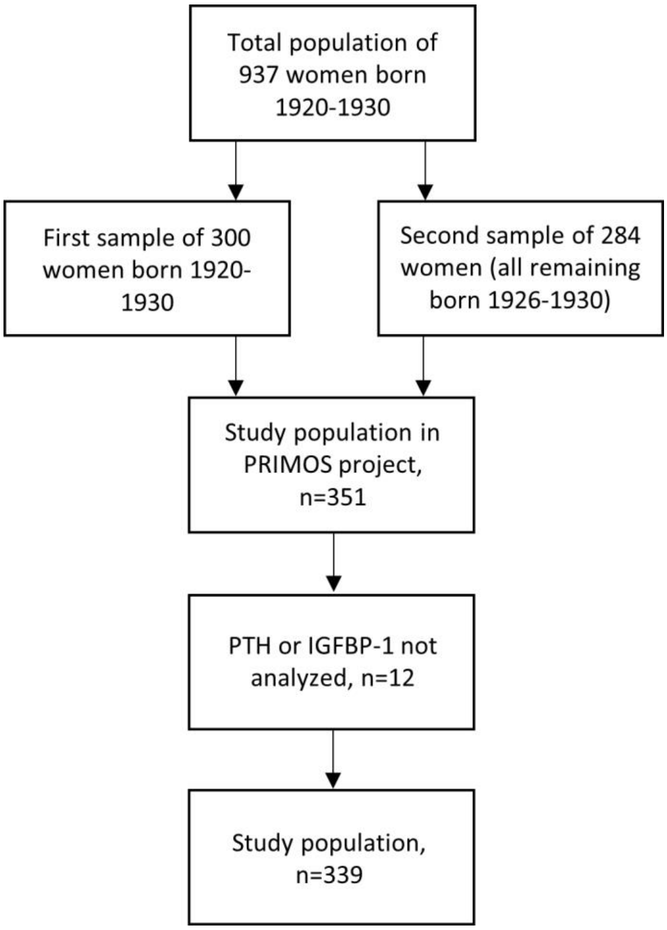


Figure 3. The formation of the study population for current research. N=number of participants.

Of the total population of 937 women, 584 were invited by letter to participate in the study and 351 chose to participate. Most common reasons for not participating were either frail health status or not being mobile enough to visit the PHCC.
Of the participants 87.5% were of Swedish descent, 3.1% from Finland, 2.6% from Norway, 0.6% from Denmark and about 6 % from countries outside of Scandinavia. The women were

all examined by the same physician at the PHCC, beginning in March 1999 and ending in February 2001. In 2010, register data were collected from the Swedish Cause of Death Register about mortality until 31 December 2009. Since this register is complete, no participant was lost during follow-up. No participants were excluded on later stages of the study either. In 11 participants PTH was not analysed and in 11 participants IGFBP-1 was not analysed, 10 patients of both groups overlap, so 339 participants are analysed regarding PTH and IGFBP-1. The general Swedish population of women with same age distribution as our study population in years 2001 to 2010 would have had 100 deaths. In our study population there were only 73 deaths which means that the death rate in our population was only 73% of the expected in the Swedish population.

Biochemical analyses

At baseline fasting blood samples for biochemical analyses were drawn between 8am and 10am, after 12 hours of fasting. Serum calcium was measured by an endpoint method with a normal range of 2.15–2.50 mmol/L for adults. Serum samples for PTH, 25(OH)D and glucose were frozen and stored in a freezer at –74 until analysed. Serum levels of intact PTH were determined by an electrochemiluminescense immunoassay (ECLIA) method and the normal laboratory reference range was 10–65 ng/L. IGF-I was determined in serum by RIA after separation of IGFs from IGFBPs by acid ethanol extraction and cryoprecipitation. IGFBP-1 concentrations in serum were determined by RIA according to the method of Póvo et al (Póvoa et al. 1984). Serum 25(OH)D was measured with the Nicholas Advantage® 25-Hydroxyvitamin assay (Nichols Institute Diagnostics), which uses a chemiluminescence system. The laboratory's total coefficient of variation (CV%) varied from 10.4–11.5 at the levels of 25-Hydroxyvitamin of 70–80 nmol/L. Estimated glomerular filtration rate (GFR) was calculated by using the Cockcroft-Gault formula.

Outcomes

Follow-up was through the Swedish Cause of Death Registry. The outcome was death from cardiovascular disease or death from any cause and the study time was set from the date of inclusion until 31 December 2009. Cardiovascular mortality was defined using the International Classification of Diseases by ICD10 codes I00–I99 (cardiovascular diseases, CVD), since it has been used in other studies (Hagström et al. 2009; Michaëlsson et al. 2010).

Both main cause of death and contributing causes were included. The accuracy of classification of causes of death in the Swedish Cause of Death Registry is high (Tunstall-Pedoe et al. 1994).

Statistical analyses

The participants were divided into two groups: parathyroid hormone <65 ng/L and parathyroid hormone ≥65 ng/L, which were then divided further regards the levels of insulin-like growth factor-1 binding protein in one standard deviation large groups. Regarding IGFBP-1, we first divided participants by one standard deviation of IGFBP-1 levels to calculate the increase in mortality risk in patients with high PTH. These groups were compared regarding cardiovascular mortality.

Then, for a comparative analysis, we divided the participants in six groups. First in two groups by not high or high levels of PTH, in three further groups by tertiles of levels of IGFBP-1, getting six groups in total. We then compared these groups regarding risk of CVD related mortality. Table 3 provides the description of the groups analysed.

Baseline characteristics are presented as mean, median, standard deviation, frequencies and percentages for continuous respective categorical data, or as stated otherwise. Comparisons of continuous data between groups at baseline were made using two-tailed t-test and Mann-Whitney test when normality assumption was not met. For dichotomous variables the chi-square test was used and Fisher's exact test when any expected cell frequency was less than 5. The defined cardiovascular risk factors were age, BMI, hypertension, diabetes mellitus, previous stroke, present CVD, cigarette smoking, physical activity, outdoor activity, medications (including diuretics) and fasting blood glucose. Cox regression was employed for multivariate analysis. The proportional hazards assumption and goodness-of-fit of each model were explored using Wald chi-square, −2 log likelihood statistic, graphical methods, Schoenfeld residuals and time-varying covariates.

SAS 9.2, LISREL 8.8 and Stata 13 were used to perform all analyses, and a 5% significance level was used to reject the null hypothesis.

RESULTS

Baseline characteristics

Descriptive baseline characteristics of the study population (n=340) divided by PTH category are presented in Table 1 (found in Appendix 1). Higher levels of PTH (PTH ≥65 ng/L) were significantly associated with use of loop diuretics and lower serum 25(OH)D levels. Those with high PTH levels were also older and has slightly higher BMI, but no significant differences between groups were noted regarding diabetes mellitus, cigarette smoking, plasma calcium ion level, GFR and daily calcium intake. Levels of IGF-1 and IGFBP-1 did not differ significantly between both groups.

Follow-up outcomes

During the ten-year follow-up there were 73 (21%) deaths among 351 participants, of which 54 were related to CVD (ICD10 codes I00–I99).

Correlation analysis results

1. We found small significant correlation between the PTH and the BMI at inclusion, Spearman's rho= 0.1907 (p= 0.0004). Table 2 illustrates the correlation coefficients for the level of PTH and various factors related to either metabolic syndrome or calcium balance system.
2. We found small significant correlation between the PTH and the fasting glucose levels at inclusion, Spearman's rho= 0.2143 (p=0.0001).
3. We have not found any significant correlation between serum levels of PTH and IGFBP-1 at inclusion, Spearman's rho= -0.0122 (p=0.8227). Figure 4 is illustrating the scatter of PTH and IGFBP-1 with no apparent linearity or significant correlation. The overall scatter of levels of IGFBP-1 is shown in the Figure 5.

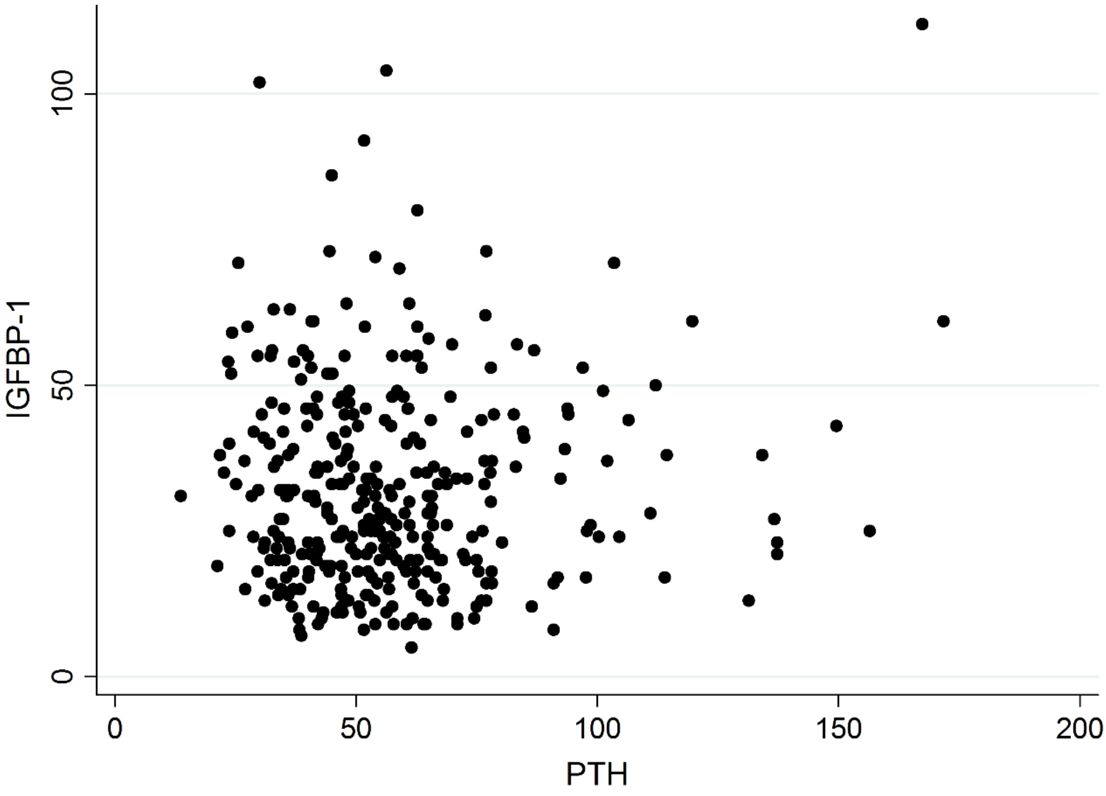


Figure 4. The plot of levels of PTH and IGFBP-1 at inclusion showing no significant correlation.

Table 2 Spearman's correlation coefficients for PTH and other factors of interest.

	PTH
BMI	0.1907 (p=0.0004)
IGFBP-1	-0.0122 (p=0.8227)
Fasting glucose	0.2143 (p=0.0001)
25-OH Vitamin D	-0.2064 (p=0.0001)
Total daily calcium intake	-0.1569 (p=0.0038)

Multivariate analysis results

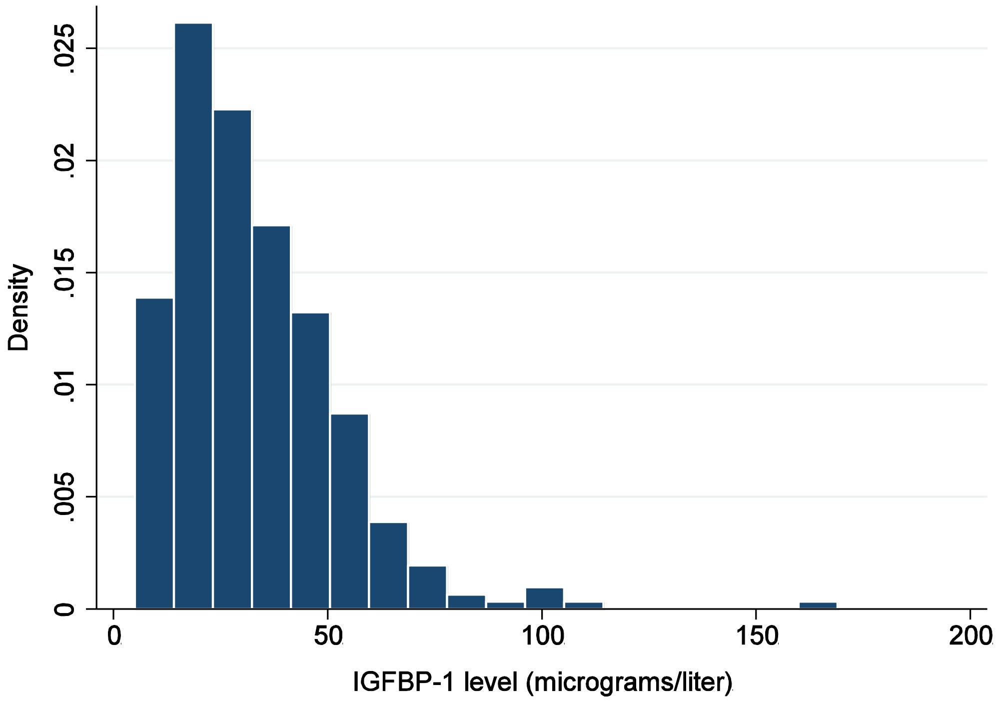
Multivariate Cox regression analysis with linear combinations of estimators shows following results:

1. We found 203% increase in relative risk of cardiovascular diseases related death (HR=3.03) for every one SD step increase in IGFBP-1 ($p<0.001$) in participants with high PTH levels. We have adjusted our model for age of the patients and for IGF-1 levels.

2. This HR was non-significant in the group with normal PTH levels, HR = 1.20, p= 0.355.

3. Comparing individuals with high PTH versus participants with normal PTH we found insignificant results in lowest and middle tertile IFGBP-1 level groups, but a significant increase in risk by 286% in highest IGFBP-1 tertile group, p=0.005. Table 4 depicts the hazard ratio in three groups.

Figure 5. Histogram of IGFBP-1 levels.



4. Comparison of all six groups regarding the relative risk of CVD-related death. Multivariate Cox regression analysis comparing the groups described in Table 3 regarding cardiovascular disease-related mortality, adjusted for age and levels of IGF-1. The sequence of comparison is the following: Groups D and E to F, then group C to F, then group B to F and then group A to F. The results of the analysis are presented below.

Table 3. Division of the participants into six groups regarding PTH and IGFBP-1 levels.

	IGFBP-1 lowest tertile	IGFBP-1 second tertile	IGFBP-1 highest tertile
PTH normal (<65 ng/L)	A	B	C
PTH high (≥65 ng/L)	D	E	F

4.1. Comparison of all groups with high PTH, highest IGFBP-1 tertile group (group F) taken as a reference. The HR for middle tertile group when compared to high tertile group (E to F) is 0.30 (p=0.038). The HR for lowest tertile group when compared to high tertile group (D to F) is 0.35 (p=0.045).

4.2. Comparison of highest IGFBP-1 tertile group with normal PTH to the highest IGFBP-1 tertile group with high PTH (C to F). The risk of CDV-related death is 2.86 times higher (186% increase in risk) in the latter group (p=0.005).

4.3. Comparison of middle IGFBP-1 tertile group with non-high PTH to the highest IGFBP-1 tertile group with high PTH (B to F). The risk of CVD-related death is 16.7 times higher in the latter group (p=0.000).

4.4. Comparison of the lowest IGFBP-1 tertile group with non-high PTH to the highest IGFBP-1 tertile group with the high PTH (A to F). The risk of CVD-related death is 3.85 times higher (285% increase in risk) in the latter group (p=0.001).

Table 4. HR for CVD-related mortality in all tertiles of IGFBP-1 depending on PTH-levels. Results were adjusted for age and IGF-1 levels.

	HR for PTH>65 compared to PTH≤65
Lowest tertile of IGFBP-1 levels	1.17 (0.39–3.50), p=0.772
Middle tertile of IGFBP-1 levels	4.67 (0.94–23.29), p=0.060
Highest tertile of IGFBP-1 levels	2.86 (1.37–5.99), p=0.005

DISCUSSION

The main finding of this study is the increase in hazard ratio for cardiovascular diseases-related mortality in patients in the highest tertile of IGFBP-1 levels and high PTH levels in comparison to all other groups. The most significant increase by 16.7 times was seen in comparison to lowest IGFBP-1 tertile group with normal PTH levels. In patients with high PTH the increase in relative risk by 203% was found for every one SD increase in IGFBP-1 levels.

The correlation analysis results of this study show that at inclusion there is small significant correlation between parathyroid hormone levels and body mass index or fasting glucose levels, which are considered typical markers of metabolic disorder. Most importantly for further complex analysis, there is also no correlation between levels of parathyroid hormone and insulin-like growth factor binding protein-1. These findings are not unexpected, as some clinical studies suggest such lack of direct relation, for example, study by J. Díez et al. (2006) has found no significant correlation between levels of parathyroid hormone and insulin-like growth factor binding protein-1 in patients with primary hyperparathyroidism.

Multiple studies, however, suggest some indirect relations between parathyroid hormone and metabolic syndrome. A study H. Salminen et al. (2008) indicates significant inverse relation between bone mineral density and levels of insulin-like growth factor binding protein-1 as well as significant positive relation between levels of insulin-like growth factor-1 and bone mineral density. Research on cell cultures, for example, a study by H. Kaji et al. (1997), show the role of insulin-like growth factor-1 in formation of the osteoclasts.

The regression analysis data show increase in relative risk of cardiovascular diseases-related death in patients with high parathyroid hormone levels for each one standard deviation increase in levels of insulin-like growth hormone binding protein-1. These results are unexpected given lack of existing data about statistical relations between the two factors as well as lack of significant correlation that we found during this study, but are in agreement with the hypothesis of complex indirect relations between calcium balance system and metabolic syndrome factors. Another interesting finding is lack of difference made by insulin-like growth factor binding protein-1 levels when parathyroid hormone levels are normal. All these complex statistical findings mean that when analysed together, both parathyroid hormone and insulin-like growth factor binding protein have certain influence on cardiovascular diseases related mortality, however, their joint influence is far more prominent, than influence of each one of them on their own. As we have compared all other groups to the highest IGFBP-1 group with high PTH, we found that the relative risk of cardiovascular diseases related death is the highest in this group. This means that the combination of having the high level of PTH and high level of IGFBP-1 possesses the highest risk for CVD-related death. We cannot detect any significant difference made by insulin-like growth factor binding protein-1 levels in patients with normal levels of parathyroid hormone. So we can conclude, that in our group of patients, the levels of IGFBP-1 only made the difference regarding the risk of CVD-related mortality if the PTH was also high, as well as the levels of PTH only made the difference only for the patients in the highest IGFBP-1 tertile group.

Some research show different aspects of relation between calcium balance and glucose metabolism systems. Study on insulin-like growth factor system components and hyperparathyroidism by P. Jehle et al. (2000) indicates higher levels of IGFBP-1, -2, -3 and -6 in patients with primary and secondary HPTH, when total IGF-1 levels are not or moderately increased. J. Díez et al. (2006) in their study on IGFBP-1 response to oral glucose in primary HPTH patients conclude that IFGBP-1 does not seem to be directly involved in HPTH associated changes in carbohydrate metabolism Although direct link between IGFBP-1 and PTH has not been established, number of studies show the relation of either PTH or IGFBP-1 to metabolic syndrome. Researches by K. Gokulakrishnan et al. (2012) and B. Yeap et al. (2010) show strong negative association of IFGBP-1 levels and components of metabolic syndrome. Several studies on relation of PTH to metabolic syndrome show that patients with high PTH levels possess increased risk for metabolic syndrome and thus PTH can be seen as an independent predictor (Huang et al. 2013; Hjelmesaeth et al. 2009; George et al. 2013; Reis et al. 2007). Our findings show previously undescribed indirect relation between PTH and IGFBP-1 regarding significant increase in mortality from cardiovascular diseases, and are in line with data presented in other research that some indirect relations between the calcium homeostasis and metabolic syndrome components might be present.

In contrary to expected, our results linked increase in IGFBP-1 levels to higher risk of death from cardiovascular events. Previous research show negative correlation between IGFBP-1 levels and increased risk for metabolic syndrome (Gokulakrishnan et al. 2012; Yeap et al. 2010). This might be explained by inverse correlation between IGFBP-1 levels and peripheral insulin levels that is shown to be consistent both in morning hours and total 24h period of sampling (Fowelin et al. 1994; Cotterill et al. 1993; Kalme et al. 2003). However, study by J. Fowelin et al. (1994) indicates that in patients with insulin-dependent diabetes mellitus levels of IGFBP-1 remained increased even in when peripheral insulin levels where increased as well. Soma research indicates IGFBP-1 as a predictor for mortality independent of IGF-1 (Nolte et al. 2015). Thus it is debatable whether higher mortality due to cardiovascular diseases in our participants with both high PTH and increased IGFBP-1 levels might be related to existing subclinical glucose metabolism changes, or calcium balance system dysregulation, or both. Also it is possible that along with insulin, PTH might have certain role in regulation of IGFBP-1 level, or both of them could have an indirect effect on other product that contributes to mortality from cardiovascular diseases. In order to evaluate this hypothesis further research is necessary.

Strengths of our study include population based approach, the long follow-up and no dropouts, as well as accuracy of the laboratory testing – drawing blood samples at the same time of day and same fasting time, minimising error due to daytime variability of sample parameters. All samples were drawn at fasting and at the same time of day. IGF-1 and IGFBP-1 samples were frozen and analysed at the same time for all subjects using high precision RIA method. Limitations that must be considered are the restricted number of subjects included in this study. Larger cohort studies are needed to verify our results. The women who participated in this study were healthier than the general population since the general mortality in our population was 73% of mortality in the same population of non-participating women in Sweden. Non-participants had a poorer health status and were not mobile enough to take part in the study. Since this study was conducted in relatively elderly healthy women with similar background and ethnicity, the generalisation of these findings and results to other populations (other ethnicities, men and younger individuals) is limited.

DISKUSIJA

Galvenā šī pētījuma atradne ir riska attiecības pieaugums attiecībā uz ar kardiovaskulārām slimībām saistīto mirstību pacientiem augstākajā trešdaļā pēc IGFBP-1 līmeņa un ar augstu PTH līmeni salīdzinot ar visām citām grupām. Vislielākais pieaugums 16.7 reizes tika novērots salīdzinot ar grupu zemākajā trešdaļa pēc IGFBP-1 līmeņa un normālu PTH līmeni. Pacientiem ar augstu PTH līmeni tika novērots 203% liels relatīvā riska pieaugums ar katru SD lielu pieaugumu IGFBP-1 līmenī.

Korelācijas analīzes rezultāti parāda, ka pēc sākotnējiem mērījumiem ir neliela statistiski nozīmīga korelācija starp PTH līmeni un ķermeņa masas indeksu vai preprandiālo glikozes līmeni asinīs, kas tiek uzskatīti par tipiskiem metabolā sindroma marķieriem. Visnozīmīgākā atradne turpmākai analīzei ir tas, ka nozīmīga korelācija starp PTH un IGFBP-1 līmeņiem netika atrasta. Šāda atradne nav negaidīta, jo daži klīniski pētījumi norāda uz to, ka tiešā saikne nepastāv, piemēram vienā no veiktajiem pētījumiem netika atrasta nozīmīga korelācija starp PTH un IGFBP-1 līmeņiem pacientiem ar primāro hiperparatireoidismu (J. Díez et al. 2006).

Tomēr vairāki pētījumi norāda uz iespējamām netiešām saiknēm starp PTH un metabolo sindromu. Viens no pētījumiem norāda uz nozīmīgu inverso korelāciju starp kaulu masas blīvumu un IGFBP-1 līmeni, kā arī uz nozīmīgu pozitīvo saikni starp IGF-1 un kaulu masas blīvumu (H. Salminen et al. 2008). Šūnu kultūru pētījumi parāda IGF-1 lomu osteoklastu veidošānā (H. Kaji et al. 1997).

Regresijas analīzes dati parāda ar kardiovaskulārām slimībām saistītās nāves relatīvā riska pieaugumu pacientiem ar augstu PTH līmeni par 203% par katru SD lielu pieaugumu IGFBP-1 līmenī. Šādi rezultāti ir negaidīti, jo līdz šim brīdim nav pieejami citu pētījumu dati par statistisko saikni starp abiem faktoriem, kā arī šī pētījuma laikā starp PTH un IGFBP-1 līmeņiem netika atrasta nozīmīga korelācija. Tomēr šī atradne atbalsta hipotēzi par iespējamo kompleksās mijiedarbības pastāvēšanu starp kalcija homeostāzes sistēmu un metabolā sindroma faktoriem. Cita interesanta atradne ir IGFBP-1 līmeņa nenozīmīgums gadījumos, ja PTH līmenis ir normāls. Analizējot šīs sarežģītas statistiskas atradnes kopumā var secināt, ka PTH un IGFBP-1 līmeņiem ir noteikta ietekme uz ar kardiovaskulārām slimībām saistīto mirstību, kā to parāda citu pētījumu dati, tomēr to kopējā ietekme ir ievērojamāka, nekā ietekme, ko šie faktori rada katrs atsevišķi. Salīdzinot visas pacientu grupas ar grupu kuras IGFBP-1 līmenis ir augstākā trešdaļā un kurai ir augsts PTH līmenis, mēs secinājām, ka ar kardiovaskulārām slimībām saistītais risks šajā grupā ir vislielākais. Tas nozīmē, ka augsta

IGFBP-1 un augsta PTH līmeņu kombinācija pakļauj pacientu visaugstākam nāves riskam, salīdzinājumā ar visām citām kombinācijām. Nozīmīga IGFBP-1 līmeņa loma pacientiem ar normālu PTH līmeni netika atrasta. Tādēļ mēs varam secināt, ka mūsu pacientu starpā IGFBP-1 līmenis nozīmīgi ietekmēja ar kardiovaskulārām slimībām saistīto mirstību tikai pacientiem, kuru PTH līmenis arī bija augsts. Savukārt, PTH līmenis nozīmīgi ietekmēja ar kardiovaskulārām slimībām saistīto mirstību tikai pacientiem, kuru IGFBP-1 līmenis bija augšējā trešdaļā.

Daži pētījumi norāda uz dažādiem saiknes aspektiem starp kalcija homeostāzi un glikozes metabolisma sistēmām. Pētījums par IGF sistēmas komponentiem un hiperparatireoidismu norāda uz augstākiem IGFBP-1, -2, -3 un -6 līmeņiem pacientiem ar primāro un sekundāro hiperparatireoidismu, kad IGF-1 līmenis nav ievērojami paaugstināts (P. Jehle et al. 2000). Pētījums par IFGBP-1 atbildi uz orālo glikozi primārā hiperparatireoidisma pacientiem secina, ka IGFBP-1 nav tiešā veidā iesaistīts ar hiperparatireoidismu saistītām izmaiņām ogļhidrātu metabolismā (J. Díez et al. (2006). Dati par tiešo saikni starp IGFBP-1 un PTH nav pieejami literatūrā, tomēr vairāki pētījumi norāda uz PTH vai IGFBP-1 saikni ar metabolo sindromu. Daži pētījumi norāda uz spēcīgu negatīvo saikni starp IGFBP-1 līmeni un metabolā sindroma komponentiem (K. Gokulakrishnan et al. 2012; B. Yeap et al. 2010). Pētījumi par PTH saikni ar metabolo sindromu parāda, ka pacientiem ar augstu PTH līmeni pastāv paaugstināts metabolā sindroma risks, tādēļ PTH var uzskatīt par neatkarīgo rādītāju (Huang et al. 2013; Hjelmesaeth et al. 2009; George et al. 2013; Reis et al. 2007). Mūsu rezultāti parāda iepriekš neaprakstītu netiešo saikni starp PTH un IGFBP-1 attiecībā uz nozīmīgu ar kardiovaskulārām slimībām saistītās nāves riska pieaugumu, kas atbalsta citos pētījumos iegūtos datus par iespējamo netiešo saikni starp kalcija homeostāzes sistēmu un metabolā sindroma komponentiem.

Pretēji paredzamam rezultātam, mūsu iegūtie dati saista IFGBP-1 līmeņa pieaugumu ar augstāku ar kardiovaskulārām slimībām saistītās nāves risku. Iepriekš veiktie pētījumi norāda uz negatīvo korelāciju starp IGFBP-1 līmeni un paaugstināto metabolā sindroma risku (Gokulakrishnan et al. 2012; Yeap et al. 2010). Iespējamais skaidrojums šim faktam ir inversā korelācija starp IGFBP-1 un perifērā insulīna līmeņiem. Literatūrā ir norādes uz tās pastāvīgumu gan rīta stundās ņemtos paraugos gan 24 stundu laika perioda paraugos. (Fowelin et al. 1994; Cotterill et al. 1993; Kalme et al. 2003). Tomēr pēc viena pētījuma datiem, insulīna atkarīgajiem cukura diabēta pacientiem IGFBP-1 līmenis palika paaugstināts arī kad perifērā insulīna līmenis bija paaugstināts (J. Fowelin et al. 1994). Daži pētījumi norāda, ka IGFBP-1 ir neatkarīgs no IGF-1 mirstības rādītājs (Nolte et al. 2015). Vai augstāka ar kardiovaskulārām slimībām saistītā mirstība mūsu pētījuma dalībniekiem ar augstu PTH un IGFBP-1 līmeni ir saistīta ar esošām subklīniskām izmaiņām glikozes metabolismā vai kalcija regulācijā, vai abās sistēmās, ir diskutabls jautājums. Pastāv iespēja, ka parathormonam, līdzīgi insulīnam, var būt noteikta loma IGFBP-1 līmeņa regulācijā, vai iespējams, ka gan PTH gan IGFBP-1 netiešā veidā ietekmē citus faktorus, kas ietekmē ar kardiovaskulārām slimībām saistīto mirstību. Šīs hipotēzes izvērtēšanai ir nepieciešami turpmākie pētījumi.

Šī pētījuma priekšrocības ir uz populāciju balstīta pieeja, ilgstošs pēcpārbaudes periods un visu sākotnēji iekļauto dalībnieku iekļaušana pēcpārbaudē, jo neviens dalībnieks netika izslēgts, kā arī laboratorisko testu precizitāte – asins paraugu ņemšana vienādā dienas laikā, vienāds badošanās laiks, kas minimizē kļūdas dēļ parametru diennakts variabilitātes. Visas analīzes tika ņemtas pirms ēšanas vienā un tajā pašā dienas laikā. IGF-1 un IGFBP-1 analīzes tika sasaldētas un analizētas vienlaicīgi visiem dalībniekiem, izmantojot augstas precizitātes RIA metodi. Pētījuma ierobežojums, kas ir jāņem vērā ir salīdzinoši neliels iekļauto dalībnieku skaits. Lielāki kohortu pētījumi ir nepieciešami lai apstiprināt mūsu iegūtos rezultātus. Sievietēm, kas tika iekļautas pētījumā bija labāks vispārējs veselības stāvoklis nekā populācijā, jo vispārēja mirstība mūsu populācijā sastāda 73% no mirstības tajā pašā vispārējā sieviešu populācijā Zviedrijā. Pētījumā neiekļautām sievietēm bija sliktāks vispārējās veselības stāvoklis un viņas nespēja pietiekoši pārvietoties, lai piedalīties šajā pētījumā. Šis pētījums tika veikts relatīvi veselu vecāka gadagājuma sieviešu starpā ar līdzīgu etnisko piederību, tādēļ mūsu rezultātu vispārinājums uz citām populācijām (citas etniskas piederības cilvēki, jaunāki cilvēki) ir ierobežots.

CONCLUSIONS

1. We have found no significant correlation between serum levels of PTH and IGFBP-1 at inclusion and weak significant correlation between levels of PTH and body mass index, as well as between levels of PTH and levels of blood fasting glucose.
2. We found 203% increase in relative risk of cardiovascular diseases related death for every one SD step increase in levels of IGFBP-1 in participants with high PTH level, adjusted for age and levels of IGF-1.
3. The increase in risk is relevant in patients with high levels of PTH, but not in patients with normal levels of PTH, adjusted for age and IGF-1 levels.
4. In patients in lowest or middle tertiles of levels of IGFBP-1 the difference made by normal or high levels of PTH was insignificant, but in the highest tertile of IGFBP-1 levels the relative risk was significantly higher by 286% in patients with high PTH.
5. The group with the highest relative risk of cardiovascular diseases-related death of all is the group of patients of the highest tertile of IGFBP-1 levels who also have the high PTH.

SECINĀJUMI

1. Mēs neatradām nozīmīgu korelāciju starp seruma PTH un IGFBP-1 līmeņiem sākotnējās analīzēs un vāju nozīmīgu korelāciju starp PTH līmeni un ķermeņa masas indeksu, kā arī ar preprandiālās asins glikozes līmeni.
2. Tika konstatēts 203% augsts ar kardiovaskulārām slimībām saistītās nāves relatīvā riska pieaugums pacientiem ar augstu PTH līmeni par katru SD lielu IGFBP-1 pieaugumu, modelis tika pielāgots pacientu vecumam un IGF-1 līmenim.
3. Riska pieaugums ir nozīmīgs pacientiem ar augstu PTH līmeni, bet ne pacientiem ar normālu PTH līmeni, modelis tika pielāgots pacientu vecumam un IGF-1 līmenim.
4. Pacientiem zemākajā vai vidējā IGFBP-1 līmeņu trešdaļā PTH ietekme uz mirstību nav nozīmīga, bet augstākās IGFBP-1 līmeņu trešdaļas pacientiem ir novērots 286% liels relatīvā riska pieaugums, ja arī PTH līmenis ir augsts.
5. Pacientu grupa ar augstāko ar kardiovaskulārām slimībām saistītās nāves relatīvo risku, salīdzinājumā ar pārējām grupām ir augstākās IGFBP-1 līmeņa trešdaļas grupa ar augstu PTH līmeni.

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Conflict of interest

None to declare.

Compliance with ethical standards

PRIMOS study was approved by the Regional Ethical Board in Stockholm. Informed consent was collected from all participants before enrolment at baseline.

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APPENDIX

Appendix 1

Table 1. Baseline characteristics of the population (n=340) by PTH levels.

	PTH ≤65 (ng/L)	**PTH >65 (ng/L)**	**P-value**
Subjects, n (%)	250 (74)	90 (26)	
Age (y), mean (SD)	73 (2.1)	74 (2.6)	<0.01
BMI kg/m^2, mean (SD)	26.5 (4.1)	28.4 (5.0)	<0.01
Diabetes mellitus, n (%)	19 (8)	10 (11)	0.31
IHD[1] at inclusion	20 (8)	14 (16)	0.03
Cigarette smoking, n (%)	43 (17)	10 (11)	0.17
Loop diuretic treatment, n (%)	11 (4)	19 (21)	<0.001
Serum 25-(OH)D, nmol/L, mean (SD)	96.9 (35.1)	83.3 (27.5)	<0.001
Plasma calcium ion, mmol/L, mean (SD)	1.22 (0.03)	1.22 (0.05)	0.92
GFR[2], mL/min (SD)	58.1 (11.4)	57.5 (14.5)	0.70
Calcium intake, mg/day, mean (SD)	637 (332)	575 (323)	0.25
IGF-1, ng/mL, mean (SD)	121 (40)	115 (34)	0.38
IGFBP-1, ng/mL, mean (SD)	32.4 (17.6)	34.8 (23.7)	0.67

PTH= Parathyroid hormone. BMI= Body mass index. CVD=cardiovascular disease. IHD=ischaemic heart disease. 25-(OH)D = 25-hydroxyvitamin D. GFR=glomerular filtration rate.

[1]Defined as ICD10 codes I20–I25 obtained from medical records

[2]Calculated using Cockroft-Gault equation

Mean values are used for continuous variables with a normal distribution, median values for skewed variables and n (%) for categorical variables. P<0.05 was considered statistically significant. Normal plasma PTH was defined as <65 ng/L and PTH ≥65 ng/L reflected secondary hyperparathyroidism.

Appendix 2

List of abbreviations

BMI	Body mass index
CVD	Cardiovascular disease
CV%	Coefficient of variation
ECLIA	Electrochemiluminescense immunoassay
FGF-23	Fibroblast growth factor 23
GFR	Glomerular filtration rate
HPTH	Hyperparathyroidism
ICD	International Classification of Diseases
IGF axis	Insulin-like growth factor axis
IGF-1	Insulin-like growth factor-1
IGF-2	Insulin-like growth factor-2
IGFBP-1	Insulin-like growth factor binding protein-1
IGFBP-2	Insulin-like growth factor binding protein-2
IGFBP-3	Insulin-like growth factor binding protein-3
IGFBP-4	Insulin-like growth factor binding protein-4
IGFBP-5	Insulin-like growth factor binding protein-5
IGFBP-6	Insulin-like growth factor binding protein-6
IGFBP-7	Insulin-like growth factor binding protein-7
IGF1R	Insulin-like growth factor-1 receptor
IGF2R	Insulin-like growth factor-2 receptor
IHD	Ischaemic heart disease
IL-6	Interleukin-6
P_i	Inorganic phosphate
PAI-1	Plasminogen activator inhibitor-1

PHCC	Primary healthcare centre
PIP2	Phosphatidylinositol 4,5-biphosphate
PRIMOS	Primary Health Care and Osteoporosis Study
PTH	Parathyroid hormone
PTH-rP	Parathyroid hormone-related protein
RIA	Radioimmunoassay
SD	Standard deviation
TNFα	Tumour necrosis factor-alfa
UV radiation	Ultraviolet radiation
$25(OH)D_2$	25-hydroxyvitamin D_2
$25(OH)D_3$	25-hydroxycholecalciferol or 25-hydrovitamin D_3