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CIRCULATING SCLEROSTIN LEVELS AND BONE TURNOVER IN TYPE 1 AND TYPE 2 DIABETES

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INTRODUCTION

Diabetes and osteoporosis are common and complex disorders with a significant health burden. These disorders can be often associated especially in middle-age and elderly individuals. In fact, a consistent increase in fracture risk has been specifically described in subjects with both type 1 (DM1) or type 2 (DM2) diabetes [66]. Although common age-related conditions (i.e. a decrease in sex hormone or vitamin D levels) or risk factors (i.e. reduced physical activity) may explain at least in part the association between diabetes and osteoporosis, detrimental skeletal effects of glucose toxicity and insulin resistance or deficiency, adipose derived hormones, diabetic complications and pharmacological treatment have been also

postulated [63-65]. However, the pathogenetic mechanisms of impaired skeletal strength in DM1 and/or DM2 remain to be clarified in detail and are only in part reflected by variation in BMD [66, 65]. Of interest, previous experimental and histomorphometry observations often evidenced a condition of low bone turnover and decreased osteoblast activity in both DM1 and DM2 [65, 67-71].

Sclerostin is a secreted Wnt antagonist produced almost exclusively by osteocytes that binds to the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) inhibiting the canonical Wnt/β-catenin signaling pathway and thus osteoblast activity [72]. Its biological importance is underlined by both experimental studies in knockout animals and clinical observations in subjects with sclerosteosis and van Buchem disease, two genetic disorders with impaired sclerostin production and markedly increased bone mass [72]. Consistent with these observations and given the restricted expression pattern of the gene encoding for sclerostin (SOST), neutralizing monoclonal antibodies against sclerostin have been developed and are under investigation as potential novel anabolic therapy for osteoporosis [73-75]. Circulating sclerostin levels can be measured in peripheral blood, increase progressively with age [76, 77], and are negatively regulated by estrogens and PTH in both women and men [78-80]. Remarkably, a recent study also demonstrated that changes in circulating sclerostin levels reflect

changes of similar magnitude in bone marrow plasma sclerostin [79]. Moreover sclerostin levels are increased in long-term immobilized patients and negatively correlate with bone formation markers [81].

The aims of the present study were: 1) to evaluate sclerostin levels in patients with DM1 or DM2 compared with age and sex-matched control subjects; and 2) to analyze the relationship between sclerostin and PTH, 25 OH-vitamin D or bone turnover markers in patients with DM1 and DM2.

To date, however, the exact mechanism behind the bone fragility and fracture in diabetes mellitus is not yet known.

OSTEOPOROSIS

INTRODUCTION

Osteoporosis is a disease of great social impact, in recent years has assumed increasing importance, due to the progressive ageing of the population and the consequent increase of its complications, first of all femoral and vertebral fractures. In fact, osteoporosis is a skeletal disorder characterized by impairment of the resistance to load that causes an increased risk of fracture.

In 1993 the World Health Organization (WHO) defined osteoporosis as a condition marked by decreased bone mass and deterioration of the micro-architecture of bone tissue that leads to greater fragility and an increased risk of fracture (1); in 2001 this definition is reworked from NIH that describes how a skeletal disorder characterized by decreased bone strength predisposing to an increased risk of fracture.(2) The components that characterize the resistance of the skeleton are the amount of bone tissue, measurable as bone density (BMD) through x-ray bone densitometry (DXA, Dual Energy x-ray Absorptiometry), and the quality of the bone that is determined by a series of macro and micro-architectural parameters. The diagnosis of osteoporosis relies on densitometric evaluation; this is compared to the average healthy adult subjects of the same sex (peak bone mass). The unit of measure is represented by the standard deviation (SD) from the peak bone mass (T-score). For each reduction of 1 DS over the reference value of -2.5 is an increase of about 2-2 .4 times the risk of fracture.(3) The values of T-score between -1 and -2.5 are the expression of a condition defined as osteopenia.

CATEGORY	BMD DEFINITION (T-SCORE)
Normal	T-score <-1
Osteopenia	T-score tra -1 e - 2,5
Osteoporosis	T-score >-2,5

The definition of WHO has advantages, such as the simplicity of result standardization and interpretation, emphasizes the need for a diagnosis before the fracture, allows a definition of the disease on the basis of risk factors. However the use of these criteria is burdened by some limits, like the fact that different skeletal sites have different peak bone mass in different times and have a loss of bone mass in different ways depending on the age; are used different technologies and equipment,-reference databases are different and use different averages and standard deviations,-policies are wrongly interpreted as intervention threshold rather than as a diagnostic threshold. (1) the criteria WHO appear therefore of extreme usefulness in clinical practice, but a proper diagnostic evaluation consist in a densitometric data with a clinical examination of the patient and his risk profile.

EPIDEMIOLOGY

Osteoporosis is the most common metabolic disease of the bone. For example, in the United States, it is estimated that about 10 million Americans 50 years of age are osteoporotic > (T-score < -2.5), according to the WHO definition; also about 34 million individuals have a reduced bone mass (T-score < -1) which puts them at high risk of developing osteoporosis.(4) Evidence shows that most women < 50 years old have a normal BMD; at the age of 80 years, 27% is osteopenic while the 70% is affected by osteoporosis. Several

epidemiological studies indicate that there is an inverse relationship between BMD and fractures; as mentioned previously, the risk of osteoporotic fracture increases up to 2.4 times for each reduction of 1 DS. The risk of osteoporotic fracture is 53.2% among women in their fifties, while among men the same age is 20.7%(5). This difference between the two sexes is probably due both to the fact that women have a lower bone mass and a loss of it more rapidly after menopause because men have anatomically greater bone mass and larger bones, which lead to an increase in bone tissue resistance to load.

Most osteoporotic fractures is realized in so-called osteopenic range (T-score between -1 and -2.5); also the sites most frequently affected are those in which there is a large amount of trabecular bone. The frequency increases with age in both sexes and this is due both to a reduction in BMD but also to an increase in the tendency to fall into old age. (6)The femur fractures are the most devastating consequence of osteoporosis because they require the hospitalization of the patient and cause increased morbidity and mortality.

In Europe the people who live farther from the equator seems to have a higher incidence of fractures.(7) Other common osteoporotic fracture in patients is the Colles one(fracture of the distal forearm); the incidence increases before 50 years, presents a plateau between 50 and 60 years and, subsequently, it has only a modest rise related to age. Conversely the frequency of femoral fractures doubles every 5 years from the age of 70 years. These differences could be explained by different way of falling of individuals in relation to age.

There are no accurate epidemiological studies on the frequency of vertebral fractures because only a small percentage of these are clinically evident, while most are asymptomatic or giving few if any symptoms(8); in particular, only one-third of radiological vertebral fractures can lead the patient to physician and less than 10% require

the patient hospitalization(9). Most of these fractures are often diagnosed by chance, or during a radiological examination performed for other reasons. They are associated with a significant increase of long-term morbidity and mortality rate that does not differ much from the secondary to the femoral fractures. The presence of multiple fractures determines a reduction in height (often several centimeters), kyphosis and persistent pain linked to changes in the chest with obvious consequences biomechanics on respiratory dynamics. Vertebral fractures of lumbar associated abdominal symptoms more often such as swelling, early satiety and constipation.(10)

In the table below shows the most common risk factors for osteoporotic invoices.

Genetic and constitutional factors	**Lifestyle and nutritional aspects**
Female sex	Low calcium supply
Age	Smoking
Familiarity	No pregnancy
Caucasian or asiatic race	Alcool or caffeine abuse
Tardive menarche	Vitamine D deficiency
Precox menopause	Immobilitation
	Low BMI (BMI<20)
	Drugs (steroids, eparine, etc)
	Low physical activity

In Italy it is estimated that there are suffering from osteoporosis approximately 3,500,000 women and 1,000,000 men. The "life time risk" to meet for a typical osteoporotic fracture of the wrist, vertebral bodies or proximal femur is around 15% for each specific site and 40% for all sites. In the Italian population over 50 years the number of fractures is higher than 80,000 units per year. Vertebral morphological alterations were found in more than 20% of patients with aged over 65 years old and of both sexes. During the first year

after femoral fracture is mortality of 15-30%, 50% of women presents a significant reduction of self-sufficiency and about 20% of cases require long-term institutionalization.(11)

PATHOPHYSIOLOGY

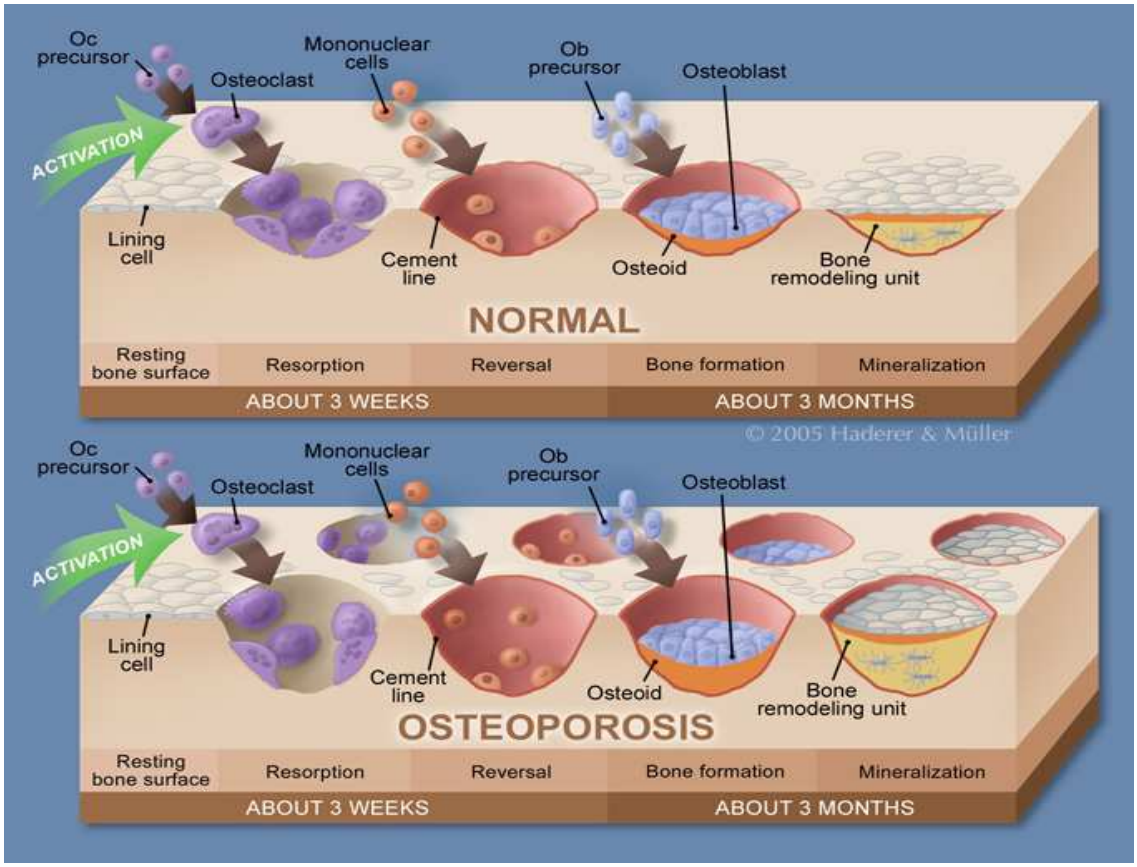
In subjects bone mass is determined by skeletal heritage acquired at the apex of development (peak bone mass) and the amount of bone loss that occurs during aging physiologically.

Bone remodeling is a fundamental process for the renewal of skeletal tissue, during which small volumes of pre-existing bone tissue are removed and replaced by new bone. The main functions of this process of remodelling are two: the first is the prevention of accumulation of micro-fractures due to chronic mechanical load; the second is participation in calcium homeostasis, through the liberation of the mineral in the body's needs.

Bone turnover depends on a number of events that happen sequentially within autonomous functional units called multicellular units (Basic multicellular unit BMU). The unit meets cyclically a process characterized by various stages:

- modification resting State of BMU
- activation of osteoclasts mature accompanied by recruitment of new osteoclasts that adhere to the surface bone
- mineral and organic matrix resorption
- reversale phase that defines the period of removal of absorbed bone followed by training and activating osteoblasts
- recruitment of osteoblasts, which come from the subperiosteal and deposition of osteoid matrix that will meet mineralization
- return to the previous state of rest.

Resorption and subperiosteal are closely related with each other, both in space (occur in the same venue) that over time (the one follows the other necessarily) and this close relationship is called coupling. In normal conditions the BMU are asynchronous, are found in all stages, from the subperiosteal bone resorption; then every single BMU is independent and it is clear that the remodeling mechanisms control is represented by factors originating locally.
Every single BMU loop complete remodeling in times vary from 90 to 145 days, depending on a cortical or trabecular level .



The bone remodelling, therefore, is a complex process that requires a comprehensive monitoring network able to adjust recruitment, proliferation, differentiation and function of cellular components. Experimental evidence has shown that both osteoclasts that osteoblasts derived from bone marrow precursors. Osteoblastic cells are derived from mesenchymal precursors in bone marrow system

and periosteum; they are the elements that form bone tissue actively. Osteoclasts are derived from so-called Colony Forming Unit/Granulocyte monocyte (CFU-GM) from which derives also stem cells of the granulocyte macrophage. The osteocytes ,situated in lacunae within the matrix, are quiescent cells derived osteoblast formation, which are, through their cytoplasmic extensions, baroceptor of bone tissue and are able to adjust, by humoral factors (sclerostina and others), the osteoblastic function. It is believed that the different stages of bone remodeling are all controlled by local factors or autocrine and paracrine activities in microenvironment of BMU products.

The bone resorption by the osteoclasts mediated is the result of two phases which include:

1. formation of osteoclasts from ancestors blood forming tissues
2. the activation of the mature osteoclasts when this comes into contact with mineralized bone.

The most important growth factors in these two stages are cytokines produced by osteoblasts, stromal cells and cells of the immune system. Local factors acting at the stage of activation-reabsorption are listed in the table below.

	CYTOKINES	OSTEOCLASTS FORMATION	BONE RESORTION
A	IL-1	+	+
l	TNF α e β	+	+
s	IL-6	+	+
o	IL-11	+	+
	IL-13	+	+
	M-CSF	+	No effect
d	GM-CSF	+	No effect
u	TGF-β	-	-
r	IFN-γ	-	-
i	IL-4	-	-
	IL-10	-	-
n	IL-18	-	-

g In the cuopling process there are many growth factors that are released from the array during the process of resorption and stimulating proliferation of osteoblastic differentiation.

The main systemic factors involved in the regulation of bone remodeling are the parathyroid hormone (PTH), the 1.25-OH Vitamin D3, the sex hormones (estrogens and androgens), thyroid hormones, glucocorticoids and insulin. At the end of the last century has been identified a new group of proteins belonging to the family of the TNF receptor, activating the nuclear factor kappa B (RANK), and its ligand (RANKL) and osteoprotegerine (OPG) (12), and was clearly established the role of this system in the regulation of osteoclastogenesis and pathophysiology of bone remodeling. The osteoblasts express on its membrane the RANKL that interacts with its receptor (RANK), expressed on precursors of osteoclasts, and promotes the maturation and differentiation in osteoclasts mature; also this link activates osteoclasts and inhibit apoptosis. The osteoprotegerine, however, present as soluble factor in bone produced by osteoblasts and stromal cells, blocking the RANKL and RANK interaction, acting as a receptor for RANKL, and so works by protective factor on bone resorption (13); in particular works by reducing both the activities that the differentiation of osteoclasts and favouring the apoptosis.

The most recent evidence indicate that in animal models, in vivo, the overexpression of OPG or RANKL determines in transgenic mice osteoporosis and osteopetrosis, respectively,. Finally, in organ cultures, ,adding in vitro of OPG, is able to block osteoclastogenesis and bone resorption induced by cytokines such as IL-1, TNF, IL-17,-

11 or by hormones such as (OH) 2 d 3 1.25 and PTH.

More recently it was spotted another way involved in regulating bone metabolism: Wnt/βcatenina pathway. This system represents a physiological response to mechanical load and participates in the process of reconstruction of fractures. The Wnt is a glycoprotein that stimulates different routes of signal through the link with the protein complex, formed by the protein related with 5 of the LDL receptor (LRP5) or LRP6 and one of the 10 Frizzled molecules. (14) The Wnt signal carries 3 main functions on osteoblasts:

-determines the differentiation by osteo/condroprogenitors,

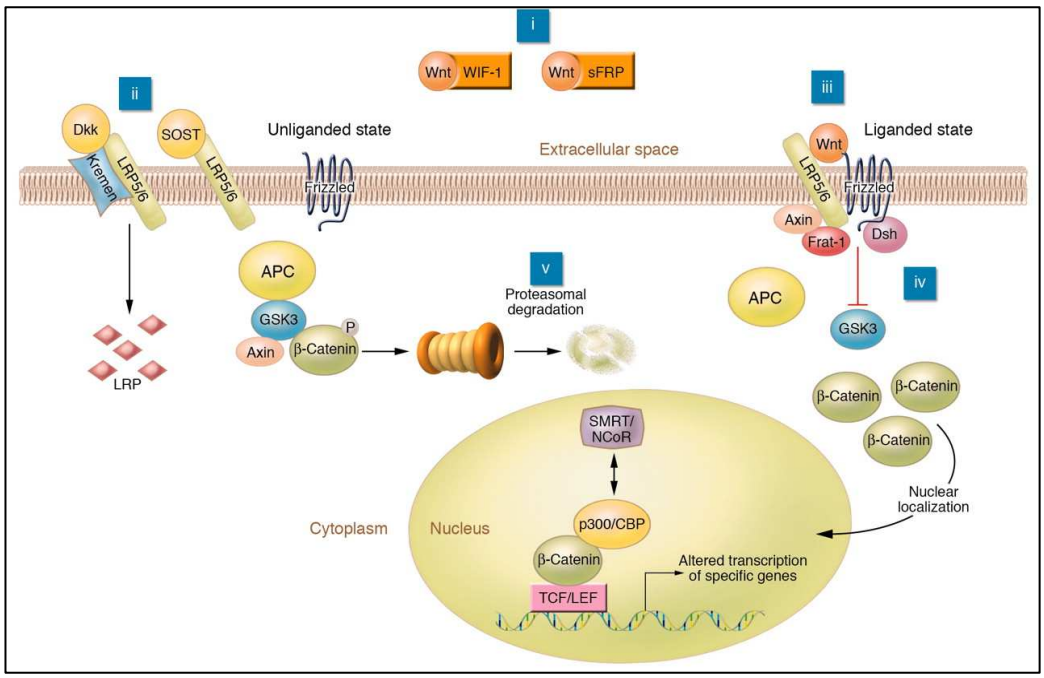
-stimulates osteoblastic proliferation,

-increases the survival of osteoblasts and osteocytes.

The Wnt signal pathway most studied in the osteoblasts is that of canonical Wnt, involving the stabilization of β-catenin and regulating of multiple transcription factors such as TCF (T Cell Factor) and the LEF (Lymphiod Enhancer Factor). In the absence of Wnt, a protein called GSK-3 β (Glycogen synthase Kinase 3 Beta) phosphorylates β-catenin and promotes the protheasomial degradation; in the presence of Wnt, GSK-3 beta was inactivated, the β-catenin is phosphorylated,

bone mass is activated and increased by inducing gene expression for the OPG and then inhibiting osteoblastic maturation. Then the activation of Wnt signal is a stimulus to the osteoblastogenesis, in turn controlled by inhibitory proteins such as DKK1 protein, Sclerostina and the Sfrp1. The first two inhibit Wnt signal pathway through the dissociation of LPR5 by Wnt and Frizzled; the last, however, binds and prevents the Wnt binding to LRP5/Frizzled complex. (15)

The crucial role of this street has emerged from studies that demonstrate how the loss of function of the gene encoding the LRP5 reduces bone mass(16- 19).



ETIOLOGY

There are 2 classes of osteoporosis: the primitive forms and secondary ones.

Primitive forms:

- ✓ Post menopausal osteoporosis
- ✓ Juvenile osteoporosis
- ✓ Senile osteoporosis
- ✓ Male osteoporosis

Secondary forms:

- ✓ Iatrogenic:
  - o Corticosteroids
  - o Cyclosporine
  - o Diuretics
  - o Thyroxine
  - o Anticoagulants
  - o Chemotherapy
  - o Anticonvulsants
  - o GnRH antagonists/Agonists
- ✓ Osteoporosis from alcohol abuse
- ✓ Osteoporosis secondary to endocrine diseases
  - o Hypercortisolism
  - o Hyperthyroidism
  - o Hyperprolactinaemia
  - o Hypogonadism
  - o Diabetes mellitus type 1
  - o Acromegaly
  - o GH deficiency

- ✓ Prolonged immobilization osteoporosis
- ✓ Osteoporosis by hematological diseases:
  - o Diseases myelo and linfo proliferative
  - o myeloma
  - o Thalassemia
  - o Mastocytosis systemic
- ✓ Osteoporosis from gastrointestinal diseases:
  - o chronic hepatic
  - o celiac disease
  - o chronic inflammatory bowel disease
  - o intestinal malabsorption
  - o Gastrectomy
  - o lactose intolerance
  - o pancreatic Insufficiency
- ✓ Osteoporosis by rheumatic disease:
  - o rheumatoid arthritis
  - o LES
  - o Scleroderma
  - o psoriatic arthritis
  - o ankylosing spondylitis
- ✓ Osteoporosis by kidney renal tubular acidosis, renal hypercalciuria, idiopathic chronic renal failure
- ✓ Osteoporosis by metabolic disorders of collagen
- ✓ Osteogenesis imperfect
- ✓ Omocisteinuria disease Ehlers-Danlos syndrome Marfan's disease
- ✓ Osteoporosis by other conditions alcohol abuse
- ✓ COPD
- ✓ Anorexia nervosa
- ✓ Hemochromatosis
- ✓ Cystic fibrosis

- ✓ Organ transplantation
- ✓ Drug
- ✓ Cigarette smoking (20)

CLINICAL MANIFESTATIONS

Osteoporosis is a disease that can remain silent for many years. The symptoms appear only when the reduction of bone mass, if completely asymptomatic, comes at a critical level that accomplish a spontaneous or after minor trauma fracture. The most important symptom is pain. Obviously the symptoms depends on the location of the fracture; the most frequent are those of the vertebrae followed by so-called not-vertebral fractures including femural neck and pretrochanteric, Colles one and other less typical such humeral, tibial, etc. From morphological point of view, vertebral fractures can be classified according to the reduction of one or all three heights of vertebra, there are therefore:

• wedge fracture, reducing the height front
• biconcave fracture, for reducing Central height
• complete fracture (crush), for reduction of all heights .

Vertebral fractures have a dramatic impact on the quality of life of individuals affected with serious worsening of conditions. Most patients complain that sudden-onset pain located posteriorly or radiates prior band; the intensity can be varied, is generally continuous and persistent for months until then a gradual mitigation. More rarely, pain can be associated with symptoms from nerve compression. In addition to pain, vertebral fractures involve both a reduction in height for a large arch kyphosis with reduction of lung volumes, both abdominal protrusion with early sense of satiety and weight loss. It is important to remember that osteoporotic fracture

mortality, especially that of the femur and vertebrae, progressively increases with increasing age. With 5 or more fractures of the spine mortality is more than double compared to the mortality in the absence of fractures.

DIAGNOSIS

The WHO defines osteoporosis according a densitometric criteria using the T-score, which represents the patient's bone density expressed as the number of standard deviations (SD) above or below compared to bone density of young adult (peak bone mass). With the decline of this value (T-score) we can assess whether we are in conditions of osteopenia or osteoporosis. Currently the gold standard for the densitometric evaluation is represented by x-ray bone densitometry (DXA) that enables evaluation of bone mass (BMC) and bone mineral density (BMD); but it is not yet able to discriminate the trabecular bone than cortical. The diagnosis of osteoporosis is, however, a clinical diagnosis, not only a densitometric one; the instrumental assessment must always be accompanied by a thorough clinical examination of the patient and to an assessment of the risk factors listed above. Some blood tests may help direct the diagnosis and in particular certain top-level exams are a valuable aid in the differential diagnosis in respect of most of metabolic diseases characterized as skeleton, osteoporosis, reduction of bone mass.

You will then need to evaluate:

- complete blood count:
- VES
- fractional Proteins
- serum calcium
- phosphorus

- total alkaline phosphatase
- serum creatinine
- calciuria/24 h

In a small number of cases, can be used second-level tests that can help resolve the doubt of diagnosis:

- Ionized Calcium
- serum parathyroid hormone
- TSH
- 25-OH vitamin D serum
- 24 hours Cortisoluria
- free Testosterone in the male
- urinary and serum Immunofixation
- Transglutaminase antibodies
- specific tests for associated diseases

Exist today even the possibility of a more precise evaluation bone turnover by determination of some markers which can be divided into:

-Markers of bone formation alkaline phosphatase isoezima, Osteocalcin, propeptidi of procollagane type I

-Markers of bone resorption as piridoline, desossipiridolina, telopeptidi N or C-terminal of type I collagen. At present, however, these markers cannot be used in routine clinical evaluation, for problems related to the analytical variability and costs still high.

As regards instrumental Diagnostics, DXA, there are other techniques: quantitative tomography (QTC) and ultrasound (QUS). The first allows to measure the volumetric density in g/cm3 and discriminate the trabecular and cortical component; however the DXA is still favorite to QCT for precision scanning times, minor amounts of radiation, lower costs and more stable calibration. Ultrasonographic exam (QUS) is certainly able to predict the risk of osteoporotic

fractures in a way not less than the DXA; this technique seems to reflect the structural features of bone BMD-independent, such as the trabecular connectivity, elasticity, the quality of the matrix; it seems therefore able to provide qualitative information on bone tissue. The combined use of ultrasonographics parameters and risk factors also increases, as BMD, fracture risk prediction. The QUS provides two parameters (speed and attenuation) that are indirect indices of bone mass and structural integrity; is measured mainly on two outlying sites, the phalanges and the calcaneus. An important limitation of QUS is represented by the heterogeneity of equipment that give values not always linkable to each other and therefore the lack of standardized criteria of interpretation. At present, therefore, the DXA is still the recommended methodology for evaluation by BMD, while ultrasound can be used in screening programmes of first level or epidemiological investigations, given the low cost, easy transportability and the absence of radiation. (20)

THERAPY

There are two types of approaches to osteoporosis: a preventive approach that tends to reduce or at least slow the onset of osteoporosis and pharmacological one for osteoporotic patients who have an increased risk of bone fractures.

Prevention is recommended for the entire population at risk and osteoporotic patients and is realised through 3 types of measures:

- evaluation and correction of the risk factors listed above
- control of an adequate intake of calcium and vitamin D
- adequate physical activity.

It is known that the average daily intakes of calcium in the Italian population is insufficient especially in senile age; the following table lists the calcium requirements based on age and particular clinical conditions needs calcium

Needs calcium	**Mg/die**
1-5 years old	800
6-10 years old	600-1200
11-24 years old	1200-1500
25-50 years old	1000
In pregnancy or lactation	1200-1500
postmenopausal women or in estrogenic therapy	1000
50-65 years old men	1000
postmenopausal women without therapy	1500
Men aged over 65 years old	1500

Recommended doses of calcium supplements should be proportionate to the degree of deficiency (typically 500-1000 mg/day). The only calcium supplementation has been shown to be capable of producing modest density increases in subjects with deficient intake or menopause by at least 5 years. (20)

The calcium addition, administered with vitamin D, also reduces the risk of falling apart to fractures.(21-22)

Even vitamin D deficiency is a risk factor for osteoporosis (23-25); its deficiency can be a cause of femur fracture while an adequate intake in the elderly can lead to an increase in BMD(23,26). A study conducted in Italy by Isaiah G et al. in 2003 showed a high prevalence (27%) of vitamin D deficiency (serum < values 5 ng/ml) in older women, uniformly distributed on the Italian territory, has also been seen that the low level of education, smoking, living in Central Italy and low intake of vitamin D leads to an increased risk of fracture. (27).

Vitamin D may be administered in a bolus from 100,000 to 1,200,000 IU in order to restore deposits, followed by a maintenance dose from 800 IU/day in daily doses or firewalls; actually it was seen that these are the minimum dose and that it would be necessary to arrive at 1200 UI/day to ensure the daily requirement. Weekly dose are also possible (4000-14,000 UI), monthly (25,000-50,000 IU), quarterly (50,000-300,000 IU) or annual (600,000 IU or more), thanks to the pharmacokinetics of this vitamin. It has been shown that adequate contributions enhance the therapeutic effects of drugs for osteoporosis. In addition to calcium and vitamin D is necessary for adequate protein to maintain both skeletal muscle system efficient and to reduce the risk of complications after fracture.

About physical activity, it is known that even short periods of immobilization are particularly detrimental for bone mass and it therefore seems important to maintain a minimum level of physical activity. The recommendation therefore is to play a minimum of physical activity such as walking for more than 30 minutes per day.

Pharmacological agents used in the control of osteoporosis act by decreasing the rate of bone resorption, slowing the rate of bone loss or promote bone formation. Since the bone remodeling process is a coupled, medications that prevent absorption decreases the speed of bone formation; Therefore, antiresorptive therapy cannot determine a substantial gain of BMD. The modest increases that are observed during the first year of therapy represents a space reduction of remodeling into a new State of equilibrium; It seems that two years are necessary to achieve a therapy increasing BMD which represent something more than a space reduction of remodeling. (28)

Currently approved drugs for the treatment of osteoporosis include bisphosphonates, parathyroid hormone (PTH), strontium ranelate, hormone replacement therapy (TOS) and selective receptor

modulators of estradiol (Serm); their activity is shown in the following table

ANTIRESORPTIVE DRUGS	- Bisphosphonates - TOS - SERMs
DRUGS THAT INCREASE BONE FORMATION	- PTH
FARMACI MISTI	- Strontium ranelate

Bisphosphonates are synthetic compounds can block osteoclastic activity by binding selectively to the bone surface undergoes remodeling; they are called so because they are characterized by a bisphosphonate link. This class of drugs reduces bone turnover in a dose-dependent and is absorbed only for 0.5-1% from the gastro-intestinal tract. Currently in Europe are used: Etidronate, alendronate, Risedronate, clodronate, ibandronate and zoledronate. Etidronate and clodronate are drugs of second choice who are today less used especially for the low antifracturative effect, emerged during clinical studies. Alendronate and risedronate are aminophosphonates and they can increase bone density and have an extensive documentation of effectiveness for the prevention of vertebral or no-vertebral fractures, whose incidence is reduced by about 40-50% in 3 years; they have also proved effective in reducing the complications related to osteoporosis from corticosteroids. The other aminobisphosphonate, Ibandronate, either for os that injecting proved able to reduce significantly the risk of vertebral fractures; However it appears less active in prevention of femoral fractures. The Zoledronate, an aminobisphosphonate, usable due to ev, proved able to reduce the risk of new fractures clinics administered annually at a dosage of 5 mg; published studies have also shown that this drug can reduce the mortalities in osteoporotic patients.

Side effects are most important for this class of drugs include:

• Esophageal erosions when hired to os

• osteonecrosis of oral bones (osteonecrosis of the jaw-ONJ); regular oral hygiene turns out to be a sufficient measure of effective prevention. However, if you needed invasive dental work should be advised to use local antiseptics and antibiotics in the days before intervention and to 5-6 days later. A brief suspension of bisphosphonates is recommended but is not yet known if this measure to determine effects on risk of this complication.

• Sub-trochanteric Fractures (or stress-fracture); in patients treated with bisphosphonates has been reported the appearance of atypical fractures (transverse) sub-trochanteric femoral, the incidence is low and is linked to the duration of therapy. (20)

Recent studies have refuted this as stating that there is a significant increase in the risk of sub-trochanteric fractures associated with the use of bisphosphonates. epidemiological studies suggest that this type of fractures are most likely caused by osteoporosis rather than by bisphosphonates. (29)

• acute phase response; After the first feeding ev you can highlight an influenza-like framework (flu-like syndrome) lasting 1-3 days with fever and widespread musculoskeletal pain; rarely have been reported cases of musculoskeletal pain of moderate/severe entities that are protracted for several weeks and that have required the interruption of therapy.

The parathyroid hormone (1-84) and the fragment 1-34 (Teriparatide), administered subcutaneously, stimulates osteoblastic activity with an anabolic effect on bone. BMD gains are much higher compared to the results obtained with bisphosphonates, but only at the level of trabecular bone. PTH-bisphosphonates Association determines variations densitometric measurements lower than those obtained with the hormone and doesn't seem to have effects in terms of reducing the risk of fracture. For the high cost, the therapy with

the PTH is reserved for patients at highest risk or "not responsive" to antiresorptive drugs.

The strontium ranelate is effective in reducing the risk of vertebral or not vertebral fractures; strontium is a labile adsorb to the bone hydroxyapatite crystals and appears able to stimulate the subperiosteal new bone while reducing the reabsorption of bone tissue and is ranked among the medications for joint action. It appears able both to stimulate the subperiosteal from osteoblasts that inhibit the reabsorption osteoclastico. Determine density increases, however, appear at least in the first three months, to about 50% greater atomic weight of strontium. However, this type of therapy can cause alterations in Beehive and is associated with a slight increased risk of thrombo-embolism. During post-marketing monitoring were finally reported some cases of syndrome of Dress. (20)

Hormone replacement therapy (TOS) is able to increase the bone mass; Some studies indicate that 17 β-estradiol acts on osteoblasts by reducing the production of IL-6 by stimulating the synthesis of OPG, thus interfering with the recruitment of osteoclastici precursors. (28) the positive effect on fractures and on reducing the risk of colorectal cancer is however offset by the increased risk of breast cancer, stroke, ischemic cerebri and trombo-Embolic events. It has an unfavourable risk/benefit ratio for long-term treatments; This TOS has no indication for therapy or prevention of osteoporosis. The selective receptor modulators (SERMs) are estrogenic compounds, synthetic, mimicking the action of estrogen and bind to its receptors; agonists effects on liver and bone but antagonistic effects breast level and Genitourinary tract. In trade we tamoxifen and raloxifene. Especially the latter prevents bone loss of the early years after menopause and is able to reduce the incidence of fracture. Side effects include an increase of vasomotor phenomena and of cramps in

the lower limbs; Moreover, the TOS, is associated with an increased risk of thrombo-Embolic events for which it is not recommended in patients at risk for DVT. There are two new drugs (Serm) approved by the FDA and the EMEA are the basedoxifene and the lasofoxifene; the first will be used in association with an estrogen for young women at risk of postmenopausal osteoporosis.

It is now available a new drug, denosumab, recently approved by the FDA; It is a monoclonal antibody capable of blocking the RANKL activity and then the process of maturation of osteoclasts. Drug treatment is influenced by risk profile assessment fratturativo. The values of the T-score to DXA were used by who to establish diagnostic thresholds but cannot be used as the threshold for intervention because they do not take account of clinical risk factors listed above; then the estimate of the risk of fracture and the therapeutic threshold must be based on both the risk factors that determine density values.

In a recent publication of the who reported numerous tables was related the risk of fracture (for multiple sites or for the femur) to 10 years with age, BMI (weight in Kg/height in m2), the T-score to the femoral neck and other clinical risk factors. Processing these data is been developed an algorithm called free-use predictive FRAX. (18) the availability of these algorithms has allowed the development of a new instrument of risk estimation of fracture named "Derived Fracture Risk Assessment" or DeFRA. Using the latter guarantees a rational and uniform diagnostic and therapeutic approach of osteoporosis. (20)

RISK INDEX WITH DXA	
Risk of femoral neck fracture = 0,121 age – 0,00045età² –1,512 Tscore – 0,162 Tscore² – 0,0045 Tscore³ –7,538 In rischio di fratture cliniche a 10 anni = (–0,001 eta²/100) + 0,50 età – 0,246 Tscore + 0,032 Tscore² + 0,003 Tscore³ + 0,012 BMI – 1,75	
RISK INDEX WITHOUT DXA	
Probability of femoral neck at 10 years = 0,218 age– 0,008 age³/1000 – 0,082 BMI –9,227 Probability of clinica fractures at 10 years = – 0,003 age³/1000 + 0,099 età – 0,026 BMI –2,925	

Fractures risk Defra	**Femoral fractures**	**Clinical fractures**
Parent fractured hip	1,6	1,2
Current smoking < 10 cigarettes	1,3	1,0
Current smoking < 10 cigarettes	1,9	1,5
Glucocrticoids(>5 mg Prednisone)*	4,5	4,0
Immunosuppressor o hyperthyroidism	1,3	1,3
Glucorticoids<5 mg > 2 mg Prednisone	2,0	1,7
Alcool (>3 unità/die)	1,5	1,2
Previous vertebral or femoral fractures	2,2	2,2
More than one Previous vertebral or femoral fractures	4,0	4,0
Previous fractures not traumatic	1,4	1,4
Rheumatoid arthritis	1,3	1,2

Patients should be eligible for treatment are postmenopausal women and men with more than 50 years that have:-femur Fractures or vertebral-T-score ≤ -2.5 at the head of the femur or at the level of the spine, after appropriate assessment to exclude secondary causes of osteoporosis-T-score between -1 and -2.5 and a risk of fracture, to 10 years for femur ≥ 3% at or for other osteoporosis-related fractures ≥ 20%.

DIABETES

INTRODUCTION

The term diabetes mellitus is a group of metabolic disorders with a state of chronic hyperglycemia, due to a deterioration of the production and utilization of insulin by the body, with repercussions on metabolism of lipids and proteins and carbohydrates.

Diabetes mellitus can be divided, according to an etiological classification, in:

- ✓ Diabetes mellitus type 1 (destruction of cells)
  - ✓ Immun-mediated
  - ✓ Idiopatic
- Diabetes mellitus type 2 (forms with a predominant insulin resistance and relative insulin deficiency and forms with secretory deficiency associated with insulin resistance)
- Other specific types:
  - ✓ genetic defects of cell function
    - Chromosome 12, HNF-1 (MODY 3)
    - Chromosome 7, glucokinase (MODY 2)
    - Chromosome 20, HNF-4 (MODY 1)
    - Chromosome 13, insulin promoter factor-1 (MODY 4)
    - Chromosome 17, HNF-1 (MODY 5)
    - Chromosome 2, NeuroD1 genes (MODY 6)
    - mitochondrial DNA
  - Other forms of genetic defects insulin action:
    - insulin resistance type A
    - Leprechaunism
    - Rabson-Mendenhall syndrome

- lipoatrophic diabetes

- Secondary Diabetes:
  - ✓ Exocrine pancreas disease:
  - ✓ Pancreatitis
  - ✓ Trauma/pancreasectomia
  - ✓ Neoplasm
  - ✓ Cystic fibrosis
  - ✓ Hemochromatosis
  - ✓ pathology pancreatic fibrocalcifica
  - Other Endorine disease:
    - ✓ Acromegaly
    - ✓ Cushing syndrome
    - ✓ Glucagonoma
    - ✓ Pheochromocytoma
    - ✓ Hyperthyroidism
    - ✓ Somatostatinoma
    - ✓ Aldosteronoma
  - Other drug related forms:
    - ✓ Vacor
    - ✓ Pentamidina
    - ✓ Nicotinic Acid
    - ✓ Glucocorticoid
    - ✓ Thyroid hormone
    - ✓ Diazoxide
    - ✓ Beta adrenergic agonists
    - ✓ Thiazides
    - ✓ Dilantin
    - ✓ Peginterferon

- Infections:
  - ✓ Congenital rubella
  - ✓ Cytomegalovirus
- Other rare Forms of diabetes:
  - ✓ Stiff man syndrome
  - ✓ Antibodies anti insulin receptor
- Associated to genetic disease:
  - ✓ Down syndrome
  - ✓ Klinefelter's Syndrome
  - ✓ Turner syndrome
  - ✓ Wolfram Syndrome
  - ✓ Friedreich's Ataxia
  - ✓ Huntington's Korea
  - ✓ Laurence-Moon syndrome-Biedl Dystrophy
  - ✓ Myotonia
  - ✓ Porphyria
  - ✓ Prader-Willi syndrome
- Gestational diabetes mellitus (30)

Type 1 diabetes is caused by destruction of β-cell, idiopathic or autoimmune based, and is characterized by absolute insulin deficiency. There is a variant of this type of diabetes called LADA (Latent Autoimmune Diabets in Adult) and it is an autoimmune form that slowly lead to insulin-dependence; evidence show that approximately 5% of patients initially defined as diabetic type 2 is actually suffering from LADA. Diabetes type 2 is caused by a partial deficiency of insulin secretion, which typically progresses over time but does not lead to a shortage of the hormone and that resides on a condition, often more or less severe, of insulin resistance on the basis of multifactorial .

The MODY (Maturity Onset Diabetes of the Young) is a type of diabetes called monogenic as it is caused by single genetic defects affecting the secretion and/or insulin action; the disease is inherited as an autosomal dominant, in which the condition of hyperglycemia develops before the age of 25 years. Were currently described a dozen different genetic defects that, with different mechanisms lead to an alteration of functional β-cell. The frequency of the different mutations in the Italian population seems to differ from those described in Northern European populations; however, there are no adequate studies of the population.

Secondary forms of diabetes are the consequence of disorders that alter insulin secretion (e.g. chronic pancreatitis or pancreasectomia) or insulin action (such as acromegaly or hypercortisolism) or can depend on chronic use of drugs. Gestational diabetes is caused by functional defects similar to those described for type 2 diabetes and it is diagnosed for the first time in pregnancy and usually resolves after delivery and then recur often after many years with the characteristics of type 2. Remind the metabolic syndrome X, or in the perimeter of which is part of an alteration of carbohydrate metabolism. The metabolic syndrome is characterized by glucose intolerance, insulin resistance, hyperinsulinaemia compensatory and fasting glucose > 100 mg/dl, Dyslipidemia (triglycerides ≥ 150 mg/dl, HDL < 40 mg/dl in men and < women 50), resulting in obesity, hypertension (systolic ≥ 130 mmHg and/or diastolic ≥ 85 mmHg), microalbuminuria, and constitutes an undoubted cardiovascular risk factor. (31)

EPIDEMIOLOGY

The worldwide prevalence of diabetes mellitus has drastically increased over the past two decades, as well as rates of prevalence of impaired fasting glycaemia (IFG). In 2002, in the United States, the prevalence of the type 1 was estimated at 0.19% of the population under the age of 20 years and 8.6% of the population with more than 20 years. There is considerable variability of incidence depending on the geographic area for both type 1 DM for the type 2.

The prevalence of DM also varies within different ethnic groups within membership regions. In 2000, USA, prevalence was 13% in the African American, 10.2% in populations of Hispanic, 15.5% in native American and 7.8% in non-Hispanic white population(32).

In Italy, in the late ' 80 ', the prevalence of diabetes (both type 1 and 2) was around 2.5%. A more recent study, conducted in Piemonte in 2003, highlighted how the prevalence is equivalent to 4.9%. The most recent data collected by ISTAT, from electronic archives of general medicine and some studies based on the consumption of antidiabetics drugs, indicate that the prevalence of the disease has exceeded 5%, indicating that diabetics in Italy are about 3 million. Some studies in Northern Italy, based on random samples of the population aged between 40 and 80 years in which you ran a GLUCOSE TOLERANCE TEST, showed that in the early 90 's of the last century there was a diabetic is not diagnosed every two known diabetics. This relationship, reasonably not unlike nowadays, allows to estimate that the total of diabetic Italians amounts to 4.5 million, of which 1.5 not diagnosed. The prevalence of alterations is very frequent and stands on little percentages less than 10%. It is estimated that in Italy the diabetes mellitus type 2-1 represents 3% of all cases of diabetes and type 2 represent over 90% of cases. The incidence of type 1 diabetes in Italy is around 10-11 per 100,000

persons per year with 3-4 times higher than the national average in Sardegna(33 -34). The risk for this type of pathology is increasing throughout the national territory, as well as internationally, although the causes of this phenomenon have not been yet identified.(31)

PATHOPHYSIOLOGY

Diabetes mellitus type 1 has a multifactorial origin as there are multiple factors that influence the pathogenesis of the disease and especially genetic, environmental and immune deficiencies. With regard to genetic factors, it was seen that there is an alteration of genes HLA class 2 which are divided into groups called DR, DQ and DP. In the Caucasian population, including the Italian, the 90-95% of diabetic patients, compared to 45% of the population of not affected, has antigens class 2 DR3o DR4 or both. Then we can say that all the class 2 (DRB1, DQA1, DQB1) plays a key role in susceptibility to type 1 diabetes. It is important to remember that the presence of a protective allele is sufficient to determine protection from diabetes, while susceptibility requires the presence of all alleles both of locus DR that locus DQ. It was seen that for individuals/DR3 DR4 is a RA (absolute risk) of between 7 and 14%; While the RR (relative risk, i.e. the probability that the subject has Antigen carrier falling ill than to those who do not possess such Antigen) is equal to 20.

Also seem to be very important environmental factors because it was seen that the concordance of homozygous twins does not exceed 50%; environmental factors may be starting factors or factors that convert the preclinical to clinical disease in diabetes. Among the most studied environmental factors include viruses and toxins; of recent interest are dietary factors that cow's milk, gluten but there are no

convincing studies that confirm the latter binding. As for the virus, the ones that seem to come into play are the mumps virus, Cytomegalovirus, enteroviruses, and in particular Coxsakie B4. It seems that the virus acts directly with citolitic effect on β-cells through molecular mimicry.

Some toxins can cause type 1 diabetes mellitus in animals and men; the streptozotocine can cause the disease but the mechanism through which it acts is unclear. Immune factors has very important role finally, in the sense that the diabetes mellitus type 1 is an autoimmune disease with presence of autoantibodies in the blood; more specifically the pathogenesis seems tied to an altered recognition by T cells of autoanthigene presented by APC with formation of autoreactive T cell clones against the β-cells of the pancreas. More specifically, whatever the autoaggression in β cell pancreas, mediated by antibody or cytotoxic T lymphocytes, T helper lymphocytes, however, that put the process in motion. These cells do not recognize soluble antigens, but peptides in a ravine from the molecular structure of major histocompatibility antigens class 2. Usually this process happens at the surface of macrophages and peptides are exogenous origin (for example, proteins of a virus phagocytazed). To explain the Autoimmunity should assume that the process can occur in the same way with endogenous derived peptides. In the first half of the ' 70, it were discovered in fact autoantibodies pancreatic Island (ICA); These precede the disease for many years and, when present, are a high risk of subsequent appearance of the disease, especially in first degree relatives of patients. At the moment there are three target molecules of ICA and namely GAD, tyrosine phosphatase insular IA-2 and the ganglioside GM2-1. After 9 years since the discovery of ICA were identified insulin autoantibodies (IAA); These can appear before clinical onset and are associated with higher risk for first degree relatives of patients. The

IAA have an inverse correlation with age and with duration: most are elevated levels of these antibodies, faster is the progression to disease; Furthermore are present in patients with Autoimmunity β-cell age 10 years and are almost always absent in patients with aged over 15 years.

In the 1980s, in addition, several studies had shown that a high percentage of patients with diabetes mellitus type 1 contained serum autoantibodies that were capable to immunoprecipite a protein specifically insular 64 kD; This protein was later identified as glutamic decarboxylase (GAD). In humans there are two forms that differ in the: a molecular weight of 65 kD and one of 65 kD. The GAD65 is the predominant isoform in the pancreatic islets and is coded by a gene on chromosome 2. Autoantibodies-anti-GAD65 and GAD67 are present in the serum of patients both before and at the time of diagnosis of the disease. Were finally discovered anti-tyrosine-phosphatase insular IA-2 that seem to be highly predictive of future appearance of the disease especially in first degree relatives.

The etiology of Type 2 diabetes has not been clarified today, definitely; certain it has a multifactorial etiology since its development contribute both genetic factors and environmental. It was observed, in fact, a concordance to the disease, including twins, which fluctuates between 70 and 90%, furthermore it has been estimated that a person who has a first degree relative affection has a 40% risk of developing the disease. Among the modifiable risk factors the most important is obesity, especially Central one. The type of diet is among the most important risk factors, given that both the prevalence and incidence of the disease are clearly predominant in the Western world, where the diet is particularly rich in saturated fats, refined sugars and proteins deriving mainly from red meat. The dietary factor often associated with a particularly sedentary lifestyle that helps determine the excess weight.

In addition to the diet-obesity, aging is considered a risk factor, possibly in relation to increased body fat, its distribution, and decline in physical activity that occur in this time of life. The pathophysiologic alterations at the base of diabetes mellitus type 2 are essentially qualitative and quantitative defect of insulin secretion and resistance to the hormone in peripheral tissues, particularly the liver, muscle tissue and adipose tissue. These are the tissues defined "insulin-dependent", because they need the hormone for transport and utilization of glucose within the cells. In fact insulin binds to the extracellular portion of its receptor, by starting the process of autophosphorylation, thanks to the tyrosine kinase activity of the receptor; subsequently, the tyrosine kinase protein catalyzes the phosphorylation of certain cytoplasmic substrates called IRS (insulin receptor substrates, 4 types have been identified): these once phosphorylated can bind other proteins including phosphatidyl-inositol-3-kinase, giving off a cascade of protein-protein interactions involving as last event, the translocation, on the cell surface, of a specific glucose transporter called GLUT-4 (glucose transporter), present on adipose tissue cells and cardiac and skeletal muscle tissue. Other GLUT were identified that are insulin independent and therefore carry within cell glucose based on its extracellular concentration. The GLUT-1 is located in the retina, the GLUT-2 in the pancreatic cells and hepatocytes, the GLUT-3 in retinal cells and the blood-brain barrier, GLUT-5 is the intestinal transporter of fructose. The GLUT-6 is a pseudogene and the identity of GLUT-7 is not yet clear. The GLUT-8 is abundantly expressed in testis and blastocysts and is also present at lower levels in a group of adult tissues, including skeletal muscle. The GLUT-9 is present in the brain and on leukocytes, and in the liver and kidney in another form. The GLUT-10 is present on the hepatic and pancreatic cells while the GLUT-11 is found in the heart and skeletal muscle. There is then the GLUT-12

that, as 4, is located on adipocytes and skeletal muscle, and represent another form of glucose transporter-insulin-dependent. [22-23] Once inside the cell, glucose is phosphorylated to be oxidized in the glycolytic pathway and the Krebs cycle or is stored in the form of glycogen synthetase to enzyme glycogen. Then in DM type 2 molecular defects of insulin resistance can be varied either system load transducer, as the Effector system. Within the transducer system can occur both reductions of the contents of the insulin receptor and its intracellular mediators, both reductions of enzymatic activity. In the Effector system you can check out a reduction of content of GLUT-4, a flaw in the process of translocation on the cell membrane and/or a reduction of its functional activity(20).

Insulin resistance results in various ways to tissue level employees. In the liver, the effect is an increase in the synthesis of glucose from the hepatocytes; It is estimated that the neoglucogenetic hepatic activity increases about 2-3 times in conditions of insulin resistance with fasting glucose of 140mg/dl; this endogenous share accounts for about 5-10% in determining the hyperglycemia. In adipose tissue, in normal conditions, insulin inhibits lipolysis by inhibiting hormone-sensitive lipoprotein lipase. Under condition of insulin resistance the splitting of the stocks of triglycerides in glycerol and free fatty acids increases.

The insulin, in the liver, also stimulates the synthesis of fatty acids through reactions involving the acetyl Co-A-Carboxylase and Coenzyme NADPH. The type of adipose tissue of most responsible for these alterations is the intra-abdominal fat, where the cells have a lower sensitivity to anti-lipolytic effect of insulin and a greater ability to release of free fatty acids. At the level of muscle tissue, in a condition of insulin resistance, a reduction of transport and utilization of glucose in the myocytes is determined; This causes an increase of products of Glycolysis anaerobia, such as lactate and alanine, caused

by the action of free fatty acids mobilised both for the lipolytic effect and for "antilipogenetic" effect caused by insulin resistance with reduced activity of the enzyme acetyl-CoA Carboxylase-Co.

The last factor that contributes to the establishment of Hyperglycemia is inhibition of insulin secretion. In the early stages of the disease, Hyperglycemia, induced by insulin resistance, stimulates insulin secretion and this over time leads to depletion of functional cells whose function is altered by hyperglycemia and by high plasma free fatty acids. The natural history of disease provides a prediabetic phase, i.e. with fasting glycemic values altered (100-125 mg/dl), so if the early alteration is insulin resistance, begins a compensation phase with normoglicemia and hyperinsulinemia, the latter, at the cellular level, causes a decrease in expression of their receptors and increase lipogenesis resulting increase in fat mass. Afetr this phase begins the decrease of insulin secretion and glucose intolerance mainly in the post-prandial time. Finally we come to the overt disease where there are the effects are lipotoxicity, glucotoxicity and hyperglycemia remains constant even fasting(35).

CLINICAL MANIFESTATIONS

Diabetic disease may present with very different forms and this depends on the degree of insulin deficiency (absolute or relative to an excessive increase of controinsular hormones), the degree of insulin resistance, and alteration of organs and tissues. The symptomatology is substantially different between diabetes mellitus type 1 and type 2.

For the Diabetes type 1 the onset occurs in 50% of cases at an age less than 20 years and more frequently during puberty. Among the symptoms by insulin deficiency we have particularly nausea and

vomiting, fatigue, abdominal pain and chest, deep breath, acetonic breath, alteration of consciousness until diabetic ketoacidosic coma; all of these symptoms are due to the excessive production of ketones. The depletion of energy stocks causes muscle weakness, weight loss and reduced growth. Other symptoms caused by hyperglycemia are polyuria and nocturia, loss of fluids and dehydration, polydipsia, skin and mucous membranes dry, energy deficiency, weight loss, asthenia and visual impairment (impairment of refraction)(35).

Frequently there is a curious phenomenon, the regression apparently spontaneous of diabetic state, after the initial period of metabolic imbalance, which lasts some months (so-called "honeymoon"). The explanation of this phenomenon is that the onset of the disease occurs when β cell secretion is very reduced, so that an acute phenomenon that supervening insular production, is able to make insufficient function; regressed the overlapping phenomenon, β-cell function may be appropriate except again deteriorate further and become insufficient in itself(36).

In relation to the symptoms of type 2 diabetes, this begins, in most cases, in a subtle and insidious way and is most often diagnosed by chance during laboratory tests performed for quite other reasons. The disease usually develops very slowly and then it takes a long time before the symptoms related to hyperglycemia and glycosuria manifest themselves. At diagnosis there can be present some mild metabolic signs (polyuria, polydipsia, polifagia) and there are often symptoms related to other tissues such as skin, urinary tract, cardiovascular. Among the conditions that most often push these patients to the doctor there are circulatory disorders linked to atherosclerosis; in diabetics, the vascular atheroscleoric degeneration is more widespread and severe, and appears in earlier times than the average population not suffering from diabetes. Between laboratory tests we can find, besides the hyperglycemia and glycosuria, possibly

an hypertriglyceridemia (VLDL) and hyperuricemia. The following table you can see the main differential features between the diabetes mellitus type 1 and type 2.(35)

AT DIAGNOSIS	DM TYPE 1	DM TYPE 2
Age	<30 years	>30-35 years
Weight	< medium	> medium
Appearance	Acute	Slow
Ketosis	Frequent	Scarse
Metabolic Instability	Frequent	Scarse
Dependent by insulin	+++	++
Sensibility to Sulfonylureas	+/-	++
Sensibility to metformin	+/-	++
Microangiopathy	++++	+
Macroangiopathy	++	+++
Frequency	15%	85%

DIAGNOSIS

According to Italian standards for the treatment of diabetes mellitus 2009-2010, the diagnostic criteria are:

• In the absence of typical symptoms of the disease (polyuria, polydipsia, and weight loss), the diagnosis of diabetes should be placed with the feedback, confirmed in at least two different occasions:

-fasting glucose ≥ 126 mg/dl to (with determination on sampling performed in the morning, around 8 a.m. After at least 8 hours of fasting plasma glucose ≥)

-glycemia ≥ 200 mg/dl 2 hours after oral glucose load (performed with 75 g)

- HbA1c ≥ 6.5% measured only with standardised and calibrated methods according to the IFCC reference system. The result should be reported in mmol/mol and derived units%, as in the following table:

ACTUAL VALUES (DCCT) %	**NEW VALUES (IFCC) Mmol/mol**
4,0	20
5,0	31
6,0	42
7,0	53
8,0	64
9,0	75
10,0	86

In the presence of typical symptoms of the disease, the diagnosis of diabetes can be placed with the acknowledgement, even in only one occasion of random blood glucose values of 200 mg/dl (regardless of relationship to food intake).

For diagnosis and screening, glucose measurement shall be performed on venous plasma, withcare for manipulation of the sample (pre-analytical phase). The use of the glucometer is not recommended, because it generates measurements not standardisable. The glucometer can be used as a pre-screening in

order to identify subjects with values suggestive of diabetes or deserving of a formal screening with blood glucose upon venous plasma laboratory.

It is important to remember that there are conditions that are borderline between normality and diabetes and that must be considered worthy of attention as they identify people at risk of diabetes and cardiovascular disease; to these belong the IFG and IGT, the detection of HbA1c between 6 and 6.49% (only with dosing aligned with the DCCT/UKPDS). The ADA and OMS use the term of impaired glucose tolerance (IGT, Impaired Glucose Tolerance) to indicate a metabolic state where the glucose value 2 hours after oral glucose load for os is 140 and 199 mg/dl. For IGF (Impaired fasting glucose), instead, means an impaired fasting glycaemia, with values between 100 and 125 mg/dl. In patients who present with non-optimal, HbA1c, IFG and/or IGT must be sought the presence of other risk factors for diabetes (obesity, diabetes, family history, etc.) in order to schedule an intervention to reduce the risk of the disease. In these subjects is also advisable to seek the presence of any other cardiovascular risk factors (hypertension, Dyslipidemia, etc.) to define the global cardiovascular risk and establish the appropriate therapeutic measures. In particular, in subjects with IFG, especially in the presence of other risk factors for diabetes, it can be useful to perfom an oral glucose load for better define the metabolic disorder.

Whenever we find ourselves faced with a glucose intolerance, is always good to take into consideration that could be a metabolic syndrome, characterized by insulin resistance, hyperinsulinaemia compensatory, Dyslipidemia, resulting in obesity, hypertension, Endothelial dysfunction, hyperuricemia, increased levels of plasminogen inhibitor type 1 and, as mentioned before, glucose intolerance(31).

In the management of a diabetic patient is of fundamental importance to the degree of compensation in time: the Index considered most reliable currently is the determination of glycated hemoglobin (HbA1c), the protein which accumulates in the process of non-enzymatic glycosylation closely dependent on glycemic values, refer to the last 5-10 weeks, in relation to the average lifespan of erythrocytes. In individuals with diabetes poorly compensated glycated hemoglobin represents the 12-15% of total Hb, whereas in normal subjects is only 4-6%(33)

There is unanimous consensus about the fact that glycated hemoglobin values equal to 6% indicate optimal glycemic compensation(35).

The determination of HbA1c for diabetes control assessment is particularly useful in patients who have extensive daily blood glucose fluctuations, and in particular values of glycated hemoglobin higher than normal were detected in patients with normal fasting blood sugar values but postprandial hyperglycemia(37).

Furthermore, the detection of low levels of HbA1c in patients treated with insulin may suggest the presence of a nocturnal hypoglycaemia and/or asymptomatic.[24] In the table below shows the correlation between the levels of plasma glucose and HbA1c media based on the ADAG study.

HbA1c	**Plasmatic glycaemia (mg/dl)**
6	126 mg/dl
7	154 mg/dl
8	183 mg/dl
9	212 mg/dl
10	240 mg/dl
11	269 mg/dl
12	298 mg/dl

The evaluation of the laboratory also provides an evaluation of fasting lipid profile (total cholesterol, HDL, triglycerides, LDL), liver function indexes and a comprehensive examination of the urine for ketones, proteins and analysis of the sediment. In all subjects should be carried out the test for micro-albuminuria, appearance and progression of diabetic nephropathy, the determination of serum creatinine and the calculation of the rate of Glomerular filtration rate. If there is clinical indication is useful to make a study of thyroid function and a E.C.G. (37).

American Diabetes Association has developed the criteria according to which adult asymptomatic individuals submit to test for the diagnosis of diabetes mellitus.

The criteria are the following:

• Should be subjected to a preliminary examination (blood glucose) all individuals aged over 45 years, in particular those with a BMI of 25 kg/m2. The control should be repeated at intervals of three years.

• A preliminary examination should be considered for younger in overweight (BMI 25 kg/m2) and with the following additional risk factors:

- ✓ sedentary lifestyle
- ✓ First-degree parental with diabetes mellitus type 2
- ✓ Members of a high-risk ethnic population
- ✓ Mothers of a child weighing >4 kg or diagnosis of gestational diabetes
- ✓ Hypertension (140/90)
- ✓ HDL < 35 mg/dl and/or triglycerides > 250 mg/dl
- ✓ Syndrome Polycystic Ovary Syndrome (PCOS)
- ✓ HbA1c ≥ 5.7%, IGT or IFG in a previous test
- ✓ Other clinical conditions associated with insulin resistance (PCOS or achantosis nigricans)
- ✓ History of vascular alterations

To obtain an optimal control of disease and complications is essential to the achievement of certain values as regards the Glycemic and lipid values:

• Glycemic Control:

- HbA1c <7.0% -fasting plasma Glucose = 90-130 mg/dl

-postprandial blood glucose Peak < 180 mg/dl

-blood pressure < 130/80 mmHg

• lipidic profile :

- LDL < 100 mg/dl

- Triglycerides < 150 mg/dl

- HDL > 40 mg/dl in regulating blood sugar values is important to bear in mind some essential points: first, the value of glycosylated hemoglobin is the primary goal for glycemic control (38); in fact randomised controlled, as the DCCT, conducted in patients with diabetes mellitus type 1, and Kumamoto and UKPDS studies, conducted in subjects with type 2 diabetes mellitus, showed how improving Glycemic compensation (average HbA1c values equal to or slightly higher than 7%) is associated with the reduction in the incidence of micro-angiopathic complications (retinopathy, nephropathy and neuropathy). (31)

Glycemic values to achieve however should be individualized depending on the clinical characteristics of the individual patient, we must also consider that a tighter glycemic control can further reduce complications but at the cost of an increased risk of hypoglycemia, therefore, a less intensive glycemic control can be indicated in patients with severe or frequent hypoglycemia, such as the elderly patients. Finally, it is useful to monitor the postprandial blood glucose levels if glycated hemoglobin is not optimal despite the satisfactory values of fasting glucose.

COMPLICATIONS

Hyperglycemia is the cause of alterations that reside at the base of the chronic complications. The glucose in fact tends to link with the aminic groups of proteins such as hemoglobin, using a non-enzymatic reaction, forming irreversible compounds called AGE (advanced glicosilated end-product). This binding causes tissue damage through a direct or an indirect mechanism. Direct action is expressed at the level of extracellular matrix proteins, nucleic acids and nucleoproteins; the indirect mechanism provides interaction with receptors of AGE, places on the cell surface which leads to an increase in oxidative stress level with formation of toxic oxygen radicals and induction of inflammatory response by monocytes, macrophages, T lymphocytes and fibroblasts; the inflammatory response goes finally to stimulate, at a vassal level, the proliferation of smooth muscle cells and inhibit endothelial anticoagulant activity. The final effect translates into a basement membrane thickening and endothelial changes, that lead to obliteration of the vasal lit.

In addition to the production of the AGE the excess glucose causes an increase in the production of sorbitol that accumulates in cells and causes the degeneration by invoking water inside them for osmotic effect. These alterations are the basis of the micro-angiopathyc damage that lead to Glomerulosclerosis, retinopathy, neuropathy. The dysregulation of glycemic metabolism affects finally also on lipid profile: high Glycemic values promote oxidation of low-density lipoprotein (LDL), molecules involved, as is well known, in the genesis of the atheromasyc plack. Thus, oxidized compounds tend to precipitate in the subendothelial tissue where they bind to proteins of the extracellular matrix, which in the case of diabetics have already modified by AGE; This makes the link LDL-matrix more stable thus speeding up the atherosclerotic process. Atherosclerosis is the main

responsible for macro-angiopathic complications of diabetes mellitus such as acute coronary syndrome, vascular disease, peripheral and cerebral vascular disease. From a qualitative point of view atheromasyc lesions of diabetics do not differ from those of non-diabetic subjects; the difference lies mainly in the greater speed with which the lesions form in diabetics(35).

Between acute Metabolic complications is the Diabetic Ketoacidosis, which is established when there is no shortage of insulin and glucagon excess. The lack of insulin, in fact, promotes lipolysis with release of free fatty acids. The latter are transported to the liver where it can be directed towards two metabolic pathways: esterification in triglycerides and oxidation in the mitochondria with formation of ketones (this latter pathway is physiologically active only in fasting or when there is an excess of glucagon). In diabetes mellitus type 1 is an enhanced lipolysis and activating oxidative pathway resulting in an accumulation of ketones which leads to Ketoacidosis; It has as its effect that grow very high levels of hyperglycemia and glycosuria and osmotic diuresis with intense significant dehydration. The Ketoacidosis, if untreated, can progress up to the stage of coma: the patient has a characteristic deep breath and rapid (Kussmaul breathing) and so-called "acetonic"breath; its appearance is deeply dehydrated, with eyeballs sunken, dry and chapped lips. Coma hyperosmolare no ketoacidotic coma, instead, is characteristic of type 2 diabetes mellitus and usually in elderly patients in which metabolic status is compounded by intercurrent events (such as an infection) and the ability to take liquids is altered, so as to make impossible the compensation of losses due to osmotic water diuresis. It follows a severe neurological syndrome caused by intracellular dehydration. The first manifestations are in a confused state, followed by fairly quickly the coma(36).

THERAPY

Italian standards for the treatment of diabetes mellitus 2009-2010 and the American Diabetes Association underline the importance of prevention in this type of pathology. Among the preventive measures relating to the "lifestyle change" appears to be of fundamental importance: avoid overweight and carry out regular aerobic physical activity (20-30 minutes per day or 150 minutes per week). This is a preventive measure and treatment recommended in all patients at risk and must be associated with a change in eating habits, to reduce intake of fats (< to 30% of daily energy intake), particularly with regard to that of saturated fatty acids (which should be less than 10% of the total); should also be increased the supply of vegetable fibres (at least 15 g/1000 kcal).

In agreement with what just said, the italian standards recommend the following composition of an optimal diet:- carbohydrates should be 45-60% of the total kcal; the patient should consume mainly vegetables, legumes, fruits, cereals, preferably integrals, foods of the mediterranean diet-the fibres should be > 40 g/day (or 20 g/1000kcals/die), especially soluble; the patient should eat 5 servings per week of fruit and vegetable or 4 servings a week of legumes. -Proteins should be 10-20% of the total kilocalories. -Fats should be 35% of total kcal; between fats from vegetable seasoning should be Favorites ones. -The salt should be consumed in quantities < 6 g/day and should be avoided preserved under salt foods such as sausage, cheese and canned food. Drug therapy should be implemented in subjects with IGT and obesity, in which the intervention on lifestyle has not produced a weight loss or where the increase of physical activity is not feasible. It is important to remember that evidence shows that the strict blood sugar control reduces the risk of onset and/or progression of retinopathy and nephropathy with a reduction

of risk of onset or worsening of cardiovascular complications. As regards the treatment for diabetes mellitus type 1, the first choice is the basal-bolus (modern replacement insulin therapy is so defined because it tries to reproduce the pancreatic secretion i.e. a basal secretion and spikes during meals to dispose of sugars); This is done with human insulin, analogs and insulin pump. Currently in Italy there are three types of analogues (lispro, aspart, glulisine), two types of slow analog (glargine and detemir), a similar lispro insulin, isofano human adjust and isofano.

Despite a number of trial have shown greater flexibility in the use of similar rapid, the meta-analysis of Cochrane Library, which has included studies published until 2005did not detect differences on glycemic control compared to regular insulin(39).

A recent multi-center trial in Italy has confirmed, in patients with diabetes mellitus type 1, a substantial non-inferiority of basal-bolus with treatment of glargine/human and glargine/lispro for the HbA1c values(7.1%and 6.95) and episodes of severe nocturnal hypoglycaemia. (40)

The reduction of incidents of hypoglycaemia using glargine instead NPH (Neutral Protamine sulfate insulin) as basal insulin has been confirmed in a multicenter Italian study(41).

Type 1 diabetics who, for various reasons, have poor glycemic control and/or recurrent hypoglycemia, the use of therapy with insulin pump (Continuous Subcutaneous Insulin CSII, Infusion) can represent a viable alternative(42,43).

The NICE (National Institute for Clinical Excellence) recommend the use of the CSII which therapeutic option in adults and children >12 years of age with frequent episodes of hypoglycemia or inadequate Glycemic compensation (HbA1c>8.5%)(44).

Thanks to dramatic improvements in technology, the pumps are becoming more manageable and least impact to the patient. In

America are already available so called PATCH-Pump, small tanks of insulin with an integrated needle are applied with particular patches in specific areas of the body; communicate via Bluetooth/wireless with a computer that sets both the speed baseline that meal bolus by administering insulin.(45)

The patch-pump have advantages compared to treatment with the classic pumps, such as ease of use and lower cost, so they seem to be indicated even in type 2 diabetic therapy. (46)

In diabetes mellitus type 2 oral drug therapy should be started when the lifestyle interventions are no longer able to maintain blood sugar control (HbA1c values < 7%). The drug of first choice in obese patients, but also in those of normal weight, is metformin; You must start with low doses to increase over time in order to avoid gastrointestinal intolerance. The efficacy of this drug is dose-dependent, reaching maximum with 2 g/day. It is important to periodically check kidney function because it has been described the appearance of severe lactic acidosis with an estimated incidence in 3 cases per 100,000 patients per year; we must pay particular caution for Glomerular filtrate < 60 ml/min and suspend the Administration if the Glomerular filtrate is reduced further until < 30 ml/min or if we are facing risk of patients with acute renal failure.

In the case of intolerance or contraindication for metformin, or in case of failure with only metformin therapy, you can add one of the following drugs:

-Sulfonylureas and repaglinide allow a fast objective but involve a greater risk of hypoglycemia with possible reduction of compliance;

- Glitazones (agonists of PPAR-γ receptor) enable the maintenance of a good glycemic control in time but inducing water retention and lead to an increased risk of fractures and heart failure.

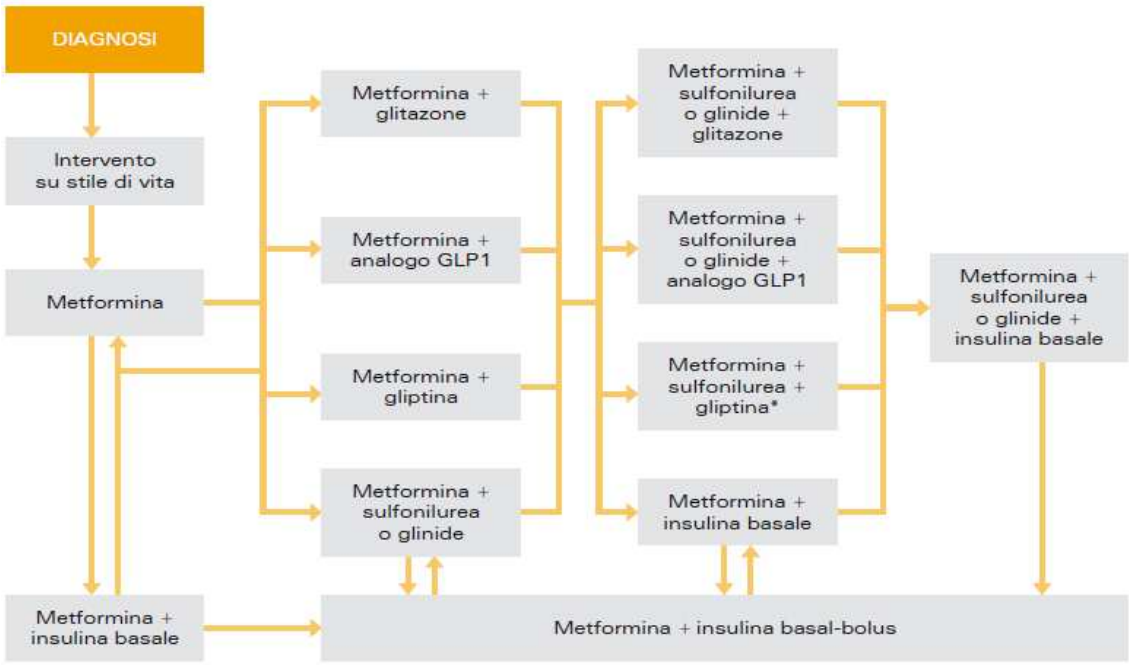
-Incretin (Dipeptil-Peptidase inhibitors IV) and exenatide (analogs of GPL-1) inducing a good blood sugar control, if added to metformin

alone in the absence of iatrogenic hypoglycemia. Exenatide has the advantage of an important weight loss but the drawback of frequent, although temporary, gastrointestinal side effects. The sitagliptin is the only incretin currently authorized by the AIFA to triple therapy with metformin and sulphonylurea.

-Acarbose may represent a viable alternative in patients intolerant to other medications.

Insulin therapy is more effective, but can cause hypoglycemia, weight increase and reduction of compliance; It is important to keep though consider this type of therapy also temporarily. The doses of the drugs may be increased by the metabolic control at frequent intervals (maximum 3-6 months) until reaching the goal.(31).

In the picture below is indicated the flow chart for therapy in type 2 diabetes:



DIABETES AND OSTEOPOROSIS

INTRODUCTION

In the years ' 50, Albright and Regina were able to prove that diabetes was associated with a loss of bone mass due to osteoporosis. (47)

This discovery has received great attention and has been investigated because the presence of osteoporosis, in diabetic patients, can aggravate the morbidity and mortality.

In recent years, in particular, many studies were carried out to try to better understand the effects of diabetic pathology on bone (48 -51); it is known that the States of acute hyperglycemia that chronic, suppress the expression of genes associated with maturation in mouse osteoblastic diabetic models, while increasing the expression of genes such as PPAR which stimulates the differentiation of mesenchymal stem cells in adypocites. (52- 54)

Indeed, the results of recent experimental investigations have shown that, similarly to what happens in other tissues, a State of chronic Hyperglycemia is able to induce non-enzymatic glycosylation and transformation of various proteins in AGE, especially at the level of collagen type 1 (55). Therefore the hyperglycemia and its high oxidative stress, frequently observed in diabetes, would lead to creations of cross-glycosylated links to collagenic chains that constitute the bone matrix, leading to a deterioration of the mineralization of biomechanical properties of the skeleton. The AGEs can also affect bone metabolism by inducing the expression of pro-inflammatory cytokines that promote resorption, i.e."TNF", or inhibiting osteoblastic activity and maturation. (56)

Several studies have shown that insulin exerts an anabolic effect of the skeleton, so that changes in insulin secretion results in a State of

low bone turnover with a considerable reduction of the number of osteoblasts and of their activity(57).

The anabolic action of insulin on bone tissue are at least partly mediated by IGF-1, therefore, a deterioration of the axis GH/IGF-1 has acquired more importance which further inadequate bone formation mechanism in insulin deficiency conditions. (58-59)

In recent studies on adolescent girls suffering from diabetes mellitus type 1 by at least 5 years have been observed low level of IGF-1, high levels of IGFBP-1 and growth hormone (GH) compared with controls, especially in patients with poor metabolic control (60).

In addition, higher levels of blood glucose and principally the duration of the disease were associated with increased bone resorption parameters, while low levels of IGF-1 could be regarded as the greatest predictors of bone strength. It is important to emphasize that these same bone abnormalities have been described in young diabetic patients and with a fairly good glycemic control (HbA1C 8.1%); this media reinforces the concept that the skeleton is a major target organ that reacts quickly to relatively little control metabolic (61).

Iit is important to emphasize that recent evidence has shown that the skeleton can in turn affect carbohydrate metabolism; in particular, it seems that bone proteins such as Osteocalcin (the main protein secreted by osteoblasts) can mediate the secretion of insulin in the pancreas and the expression of adiponectin in adipose tissue cells, suggesting that factors of skeletal derivation might influence the secretion of insulin and glucose tolerance in vivo. (62)

Although common age-related conditions (i.e. a decrease in sex hormone or vitamin D levels) or risk factors (i.e. reduced physical activity) may explain at least in part the association between diabetes and osteoporosis, detrimental skeletal effects of glucose toxicity and insulin resistance or deficiency, adipose derived hormones, diabetic

complications and pharmacological treatment have been also postulated (63-65).

However, the pathogenetic mechanisms of impaired skeletal strength in DM1 and/or DM2 remain to be clarified in detail and are only in part reflected by variation in BMD (66).

Of interest, previous experimental and histomorphometry observations often evidenced a condition of low bone turnover and decreased osteoblast activity in both DM1 and DM2 (65;67 -71).

Sclerostin is a secreted Wnt antagonist produced almost exclusively by osteocytes that binds to the low-density lipoprotein receptor-related proteins 5 and 6 (LRP5 and LRP6) inhibiting the canonical Wnt/β-catenin signaling pathway and thus osteoblast activity (72).

Its biological importance is underlined by both experimental studies in knockout animals and clinical observations in subjects with sclerosteosis and van Buchem disease, two genetic disorders with impaired sclerostin production and markedly increased bone mass (72).

Consistent with these observations and given the restricted expression pattern of the gene encoding for sclerostin (*SOST*), neutralizing monoclonal antibodies against sclerostin have been developed and are under investigation as potential novel anabolic therapy for osteoporosis (73-75).

Circulating sclerostin levels can be measured in peripheral blood, increase progressively with age (76-77), and are negatively regulated by estrogens and PTH in both women and men (78-80). Remarkably, a recent study also demonstrated that changes in circulating sclerostin levels reflect changes of similar magnitude in bone marrow plasma sclerostin (79). Moreover sclerostin levels are increased in long-term immobilized patients and negatively correlate with bone formation markers (81).

PATIENTS AND METHODS

Study Population

A total of 43 consecutive patients with DM1 (age range 24-77 yrs, time since diagnosis 1-52 yrs, mean years since diagnosis 18.5±12.6) and 40 consecutive patients with DM2 (age range 48-79 yrs, time since diagnosis 1-26 yrs, mean years since diagnosis 9.7±7.8) referring to the Diabetes Unit of our Department were included in the study. All patients had normal renal function (as assessed by serum creatinine levels) and no major co morbidities impairing normal daily activity. Age- and sex-matched controls (n=83) were recruited from healthy volunteers (younger cohort, CT1, age range 25-48 yrs) and subjects randomly selected from a population-based study (old cohort, CT2, age range 51-78 yrs). The latter were obtained from an age-stratified, random sampling of elderly men and postmenopausal women (between the ages of 50 and 80 years) in primary care registers of Siena residents, taking part to an epidemiological cohort study [20, 21]. Conversely, CT1 subjects were randomly recruited from the personnel of our Department. All included controls had normal glucose homeostasis as assessed by fasting glucose levels and measurement of glycosylated hemoglobin (HbA1c). Subjects with Paget's disease of bone, primary hyperparathyroidism, congestive heart failure, recent myocardial infarction, multiple myeloma or other neoplasia were excluded from the study. Moreover subjects were also excluded if they received treatment with antiresorptive or anabolic compounds for osteoporosis, previous (>2 months) and current corticosteroid therapy or any other treatment known to affect bone metabolism. All patients with DM1 were on treatment with insulin, while DM2 patients were treated with oral antidiabetic agents alone (n=31) or in

combination with insulin (n=9). Ethics approval for the study was obtained in accordance with local institutional requirements, and written informed consent was obtained from all participants. General and clinical characteristics of patients and controls are reported in Table 1.

Clinical Analysis

At recruitment, height (measured by stadiometer) and weight were recorded from all subjects and body-mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Blood samples were collected in the morning after an overnight fast and stored at -70°C.

Serum concentrations of calcium (corrected for albumin concentration), phosphate, total alkaline phosphatase and creatinine were measured using standard automated laboratory techniques. Levels of serum C-telopeptide of type I collagen (CTX, serum CrossLaps, Immunodiagnostic Systems Ltd, interassay CV<3%), intact osteocalcin (OC, DiaSorin Diagnostics, interassay CV 7.1%) and bone-specific alkaline phosphatase (BALP; Beckman Coulter, Fullerton, CA; with an interassay CV of <7.9%) were measured in serum samples, as markers of bone turnover. Moreover, circulating PTH (DiaSorin, Stillwater, MN; interassay CV<7.3%) and 25-hydroxy vitamin D levels (Diasorin Diagnostics, Saluggia, Italy; sensitivity, 1.5 ng/ml; interassay CV<11%) were evaluated by RIA. Serum sclerostin levels were assessed using a quantitative sandwich enzyme-linked immunosorbent assay from Biomedica (Biomedica Gruppe, Wien, Austria), obtained from Pantec (Pantec Srl, Turin, Italy), with interassay and interassay CVs of 4% and 5.5%, respectively. All assays were run in duplicate after one thaw. At the time of blood sampling, areal bone mineral density (BMD) of the lumbar spine, and

proximal femur was determined by a dual-energy X-ray adsorptiometry (DXA) device (Lunar Prodigy; GE Healthcare, Waukesah, WI).

Statistical Analysis

Data were summarized as means± standard deviations and $P<0.05$ was accepted as the value of significance. Quantitative variables were compared between the case and control groups using analysis of variance (ANOVA) and analysis of covariance (ANCOVA), with Fisher's protected least significant difference post hoc test. Qualitative variables were compared using standard Chi-squared test. Logistic regression analysis was used to assess the independent association between sclerostin and bone turnover markers or calciotropic hormones. The relationship between sclerostin and other variables was evaluated further in DM1, DM2 and control groups using multivariate analysis.

For logistic regression analysis vs. age, controls groups CT1 and CT2 were merged and considered as a single group. As shown in table 1, mean age significantly differed between DM1 or DM2 patients and control groups. This was mainly due to the different age of onset of DM1 and DM2, generally occurring in young and middle-aged individuals, respectively, thus making unlikely to obtain 3 groups of age-matched DM1 or DM2 patients and controls. Since circulating sclerostin as well as bone turnover markers are known to be affected by age (and in part by body weight), as first step differences in sclerostin levels according to diagnosis were tested considering a single control group (CT1+CT2), after adjusting for age and BMI. Then age-matched analyses were performed. Thus, DM2 patients were compared with the age-matched CT2 group. Conversely, the DM1 group comprised 12 subjects aged above 50 yrs (with a

statistical significant difference in age between DM1 and CT1 or CT2) and was split in 2 subgroups, DM1a (n=31, age range 24-49, mean age 37.1±7.2) and DM1b (n=12, age range 50-77, mean age 62.2±8.8) to be compared with CT1 and CT2 groups, respectively.
Analysis was performed using Statistica 5.1 (Statsoft, Tulsa, OK, USA) and SPSS (release 6.1; SPSS, Chicago, IL, USA).

RESULTS

General and clinical characteristics of patients and controls are shown in Table 1. As is evident, all markers of bone turnover and calciotropic hormones were within the normal range in controls, as well as lumbar and femoral BMD (as shown by the Z score levels next to "0"). Consistent with previous observations, BMD at the femoral neck was lower in DM1 but higher in DM2 with respect to control groups CT1 and CT2, respectively. Moreover, a significant reduction of BALP and CTX was observed in DM2 patients with respect to CT2. A similar reduction of CTX was evidenced in DM1 patients vs. CT1, while the reduction in BALP did not reach a statistical significant level. Of interest, in keeping with previous observations [22] 25OHD levels were significantly lower in both DM1 and DM2 patients than in CT1 and CT2 subjects, respectively. Similar results were observed when male and female cohorts were considered separately or when the age-matched DM1 groups (DM1a and DM1b) were considered.
In the overall cohort of subjects, circulating sclerostin levels were higher in males than in females and significantly increased with age and BMI in both genders (Age: $r=0.31$, $p<0.005$ and $r=0.39$, $p<0.001$ in females and males, respectively; BMI: $r=0.34$, $p<0.005$ and $r=0.26$, $p<0.05$ in females and males, respectively). The positive correlation between sclerostin and age was maintained in controls and DM1 but not DM2 patients [Figure 1]. Conversely, the association

between sclerostin and BMI was not significant in controls. Of interest, in DM2 subjects sclerostin levels were positively correlated with years since diagnosis ($r=0.68$; $p<0.001$), while this association was not significant in DM1 patients. Finally, a trend for a positive correlation between sclerostin and HbA1c levels was observed in DM2 patients ($r=0.29$; $p=0.08$). Fasting glucose levels were not significantly associated with sclerostin in the overall group of diabetic patients as well as in DM1 and DM2 cohorts or controls.

In all study groups bone turnover markers were not significantly correlated with serum sclerostin, except that BALP that was negatively associated with sclerostin in control men ($r=-0.60$, $p<0.05$). Moreover, sclerostin levels were higher in DM2 than in controls or DM1 patients, and this difference persisted when adjustments were made for age and BMI [Figure 2a], or when CT1 and CT2 subgroups were considered [Figure 2b and 2c]. Moreover, a similar trend approaching statistical significance ($p=0.06$) was evidenced between DM1a and CT1 groups [Figure 2d].

Consistent with previous clinical and experimental observations, sclerostin levels were negatively correlated with serum PTH in nondiabetic patients ($r=-0.30$, $p<0.01$), independently of age and gender [Figure 3a]. Conversely, an opposite but not-significant trend between PTH and sclerostin was observed in both DM1 ($r=0.26$, $p=0.09$) and DM2 ($r=0.32$, $p=0.07$) groups [Figures 3b and 3c].

Using multivariate analysis we found that age ($\beta= 0.40$; $p<0.001$) and PTH ($\beta= -0.30$; $p<0.01$) were independent predictors of sclerostin in controls, after adjusting for BMI and 25OHD. In diabetic patients we included HbA1C, fasting glucose levels and years since diagnosis as additional factors in multivariate analysis. In DM1 age ($\beta= 0.35$; $p<0.05$) was an independent predictor of sclerostin, while in DM2 the only independent predictor of sclerostin was represented by the years since diagnosis ($\beta= 0.46$; $p<0.005$).

DISCUSSION

Despite several clinical and experimental observations suggested an increased skeletal fragility in both DM1 and DM2 patients, the pathophysiology of reduced bone strength in diabetes remains to be clarified in detail, and might differ at least in part between DM1 and DM2. The results of the present study confirm some previous clinical evidences showing a condition of low bone turnover in both DM1 and DM2 and a reduction of BMD in DM1, with normal or even higher BMD levels in DM2. Moreover, we evidenced for the first time a marked increase in circulating sclerostin levels in patients with DM2, with mean sclerostin concentrations more than 2-fold higher in most DM2 patients than in age and sex matched controls. Such increase in sclerostin levels was comparable or even higher to that observed in immobilized patients (81) and could explain, at least in part, the parallel decrease in bone formation markers observed in our cohort of DM2 patients. In fact, sclerostin is a recently discovered Wnt antagonist that is almost entirely produced by osteocytes and plays a major role in the suppression of bone formation. Together with other factors such as Dickkopf1, sclerostin can bind to LRP5 and LRP6 leading to the inhibition of the wnt/β-catenin signaling pathway in the osteoblast (82).

This in turn leads to reduced osteoblast proliferation, differentiation and life-span (71, 83-84).

Consistent with these experimental data, several clinical observations clearly evidenced a relative increase in bone formation and enhanced bone mass during conditions of impaired sclerostin secretion and/or enhanced wnt/β-catenin signaling, while low bone formation was described with reduced wnt/β-catenin signaling (71, 85, 17-16). Thus our data point toward an increase in sclerostin levels as a potential

cause of the reduction of bone formation in DM2. While a similar reduction in bone formation markers was observed also in DM1 patients, sclerostin did not significantly differ between DM1 and controls. This suggests that partly different mechanisms are implicated in the pathogenesis of skeletal fragility in DM1 and DM2. Indeed, a trend near to statistical significance for higher sclerostin levels was evident in the subgroup of DM1 patients aged below 50 yrs than in the group of age-matched controls. Further studies in larger samples will be required to clarify this issue.

Despite the above observations, we did not detect any association between markers of bone formation or BMD and sclerostin in our cohorts of patients, while a negative association between serum sclerostin and BALP (a marker of bone formation) was observed in the male cohort of controls. Since circulating sclerostin levels has been negatively associated with estrogen but not androgen levels(78,80), it is likely that gender-related differences in sex steroid concentrations may explain the observed differences in the degree of correlation between bone markers and sclerostin in female vs. male controls. Moreover, in elderly women bone turnover markers (including bone formation markers such as BALP or OC) generally increase due to coupling mechanism between osteoclast and osteoblast activity reflecting the age-related increase in bone resorption following the menopause. Thus any potential correlation between sclerostin and bone formation should be more easily detected in younger individuals or elderly men rather than postmenopausal females. Conversely, the lack of association between sclerostin and bone formation markers in DM2 patients could be in part related to the marked increase in sclerostin in this specific cohort, with levels well above the normal range in most patients. This might suggest that above a certain threshold the suppressive effect of sclerostin on bone formation is not linear, likely due to a saturation

point for sclerostin effects on bone cells. However, further studies will be required to clarify more specifically the dose-relationship between circulating sclerostin levels and the suppression of bone formation.

In our DM2 cohort we also evidenced a decrease in the bone resorption marker CTX. While the relationship between sclerostin and osteoclast activity remains to be clarified in detail, other studies identified a possible association between variation in sclerostin levels and bone resorption (76-77).

In keeping with this hypothesis, an increase in bone resorption markers has been observed in patients with impaired sclerostin secretion due to sclerosteosis (86), suggesting that due to compensatory mechanisms a relative increase in bone resorption can be associated with the enhanced bone formation typical of this disorder. Together with additional indications from experimental studies (87), these data suggest that enhanced sclerostin production (at least with levels well above the normal range) might lead to a generalized reduction in bone turnover over a long term. This in turn might impair bone quality more than bone density (i.e. due to persistence of micro-cracks within the bone) explaining the lack of a negative association between sclerostin levels and BMD observed in our DM2 cohort. Indeed, it is now well established that in DM2 patients bone density is generally within the normal range or even increased, while bone fragility is enhanced (66, 65).

As counterpart, recent observations with monoclonal antibodies against sclerostin in animal models or postmenopausal women clearly demonstrated that an acute reduction in sclerostin levels leads to an increase in bone formation and a suppression in bone resorption (73 - 75 88).

Several systemic and local factors have been implicated as possible regulators of sclerostin expression and release by the osteocyte. Among them, PTH has been shown to decrease sclerostin expression

both in vitro and in vivo (89). In fact, PTH suppressed transcription of the SOST gene in vitro (90) and a consistent reduction of sclerostin levels was observed in mice over-expressing a constitutively active PTH receptor 1 variant (87).

Moreover, continuous infusion of PTH to mice markedly decreased SOST expression and sclerostin levels in vertebral bone (91).

A similar even though transient finding was also reported with intermittent PTH injection (90).

These experimental data have been confirmed more recently by different clinical studies. In some cohorts of osteoporotic and non-osteoporotic subjects, serum PTH levels were inversely correlated with circulating sclerostin (78, 92-93), while low sclerostin concentrations were described in patients with primary hyperparathyroidism (94-96). In addition, either intermittent or continuous infusions of PTH 1-34 decreased circulating sclerostin levels in postmenopausal women and healthy men (97,98). Importantly, while a negative association between sclerostin and PTH levels was observed in controls (consistent with the above observations), we did not detect a similar association in our cohort of diabetic subjects. On the contrary, a trend for a positive association between sclerostin and PTH was observed in both DM1 and DM2 patients. This trend was observed despite no major differences in PTH between patients and controls. Indeed, PTH levels were slightly higher in DM1 and DM2 than in controls and this, under normal circumstances, might have led to reduced rather than increased sclerostin levels. Even though further prospective and experimental observations will be required to clarify this issue, our findings suggest that the transcriptional suppression of sclerostin production by PTH may be impaired in diabetes. Indeed, a previous histomorphometric analysis on diabetic and nondiabetic patients with renal osteodistrophy seems to confirm this hypothesis.(99)

In fact, a positive correlation of sclerostin with bone apposition rate and bone formation rate was evidenced only in the nondiabetic group, suggesting that the lower bone formation in the diabetic patients may have arisen in part from a failure of PTH to promote bone mineralization.

The molecular mechanisms leading to the impaired inhibition of sclerostin levels by PTH in both DM1 and DM2 cohorts remain to be demonstrated and might be at least in part mediated by variation in glucose or insulin levels. Of interest, single experimental studies demonstrated that high glucose levels impair the bone cell response to PTH (100) while insulin treatment potentiates the skeletal effects of PTH in streptozocin-induced diabetic rats (101).

This could also explain the positive correlation between sclerostin and HbA1c levels that we observed in DM2 patients. Possibly, the use of different treatments (i.e. insulin or oral antidiabetic agents) might differentially affect sclerostin levels. However, even though in our DM2 cohort we did not detect any major difference in sclerostin levels in relation to the treatment, this hypothesis has to be verified in larger and prospective samples. Moreover, it is known that PTH-induced down regulation of SOST gene is direct, via the intracellular cAMP signaling pathway (90)and the suppression of the myocyte enhancer factor 2 (MEF2)-stimulated SOST bone enhancer activity (102).

Consistent with a potential impairment of PTH action on bone in diabetes, an in vitro observation evidenced that the increase in both cAMP and [Ca2+] levels following PTH was lower in high glucose-treated human osteosarcoma cells than in those treated with normal glucose or high mannitol (100).

Finally, an abnormal regulation of MEF2A and MEF2D proteins has been also described in experimental diabetes (103).

In summary, this study demonstrates an increase in circulating sclerostin in patients with diabetes compared with age-matched controls. While a trend for increased sclerostin levels was observed in DM1, a more significant and consistent increase was observed in DM2. Moreover, the negative correlation between PTH and sclerostin (demonstrated in previous observations) was lost in our cohort of diabetic patients. Further experimental and clinical studies in larger and prospective samples will be required to confirm our data and identify the underlying pathogenetic mechanism. This could be particularly important not only for a better understanding of the causes of skeletal fragility in diabetes but also for its potential therapeutic implications, providing the basis for the use of the monoclonal antibody against sclerostin.

FIGURES and TABLE

Figure 1.

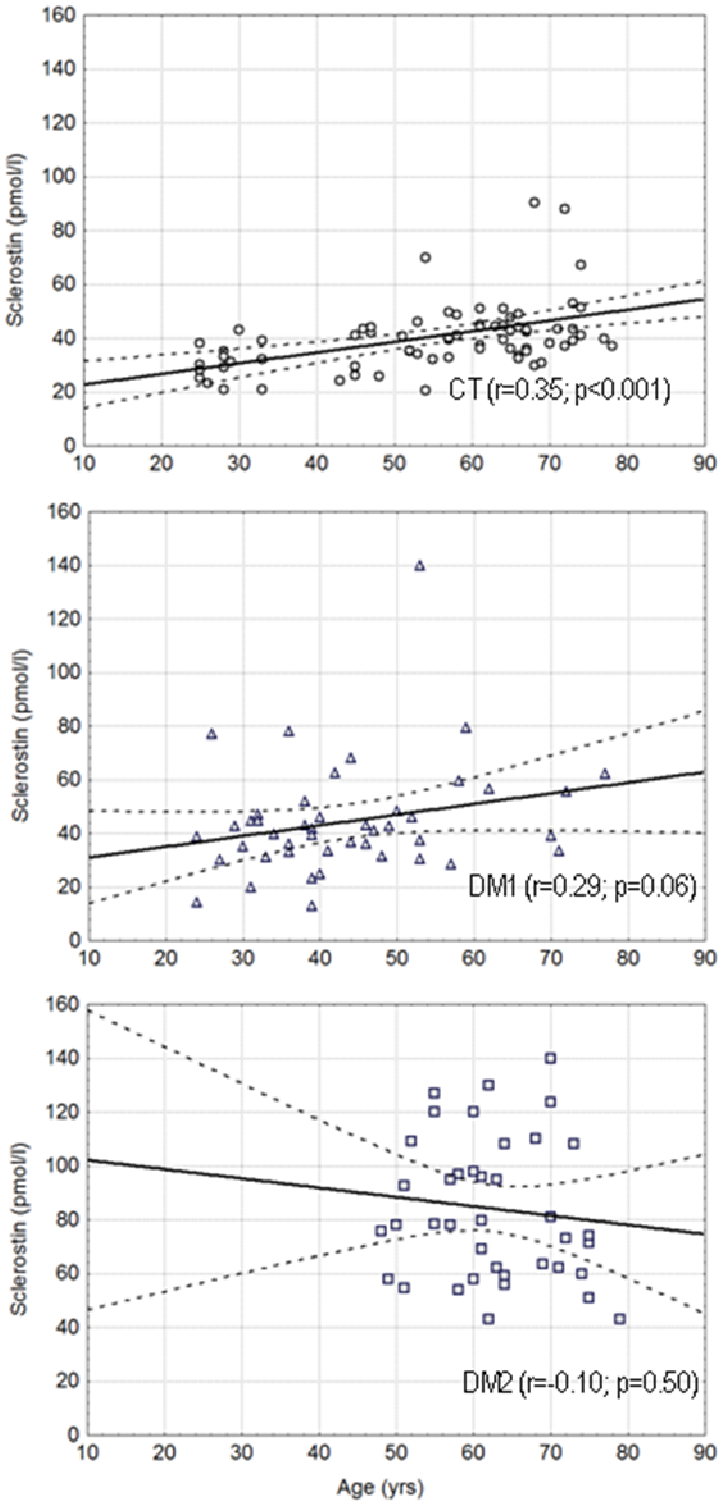


Figure 2.

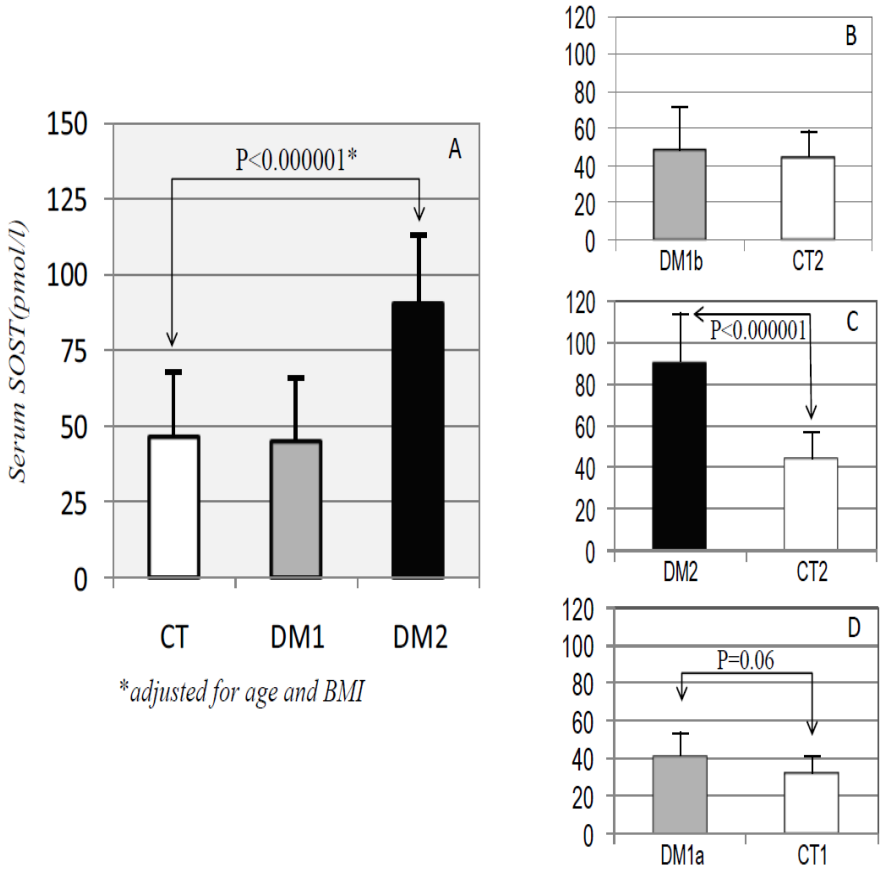

A
P<0.000001*
Serum SOST(pmol/l)
150
125
100
75
50
25
0
CT
DM1
DM2
*adjusted for age and BMI
B
120
100
80
60
40
20
0
DM1b
CT2
C
P<0.000001
DM2
CT2
D
P=0.06
DM1a
CT1

Figure 3.

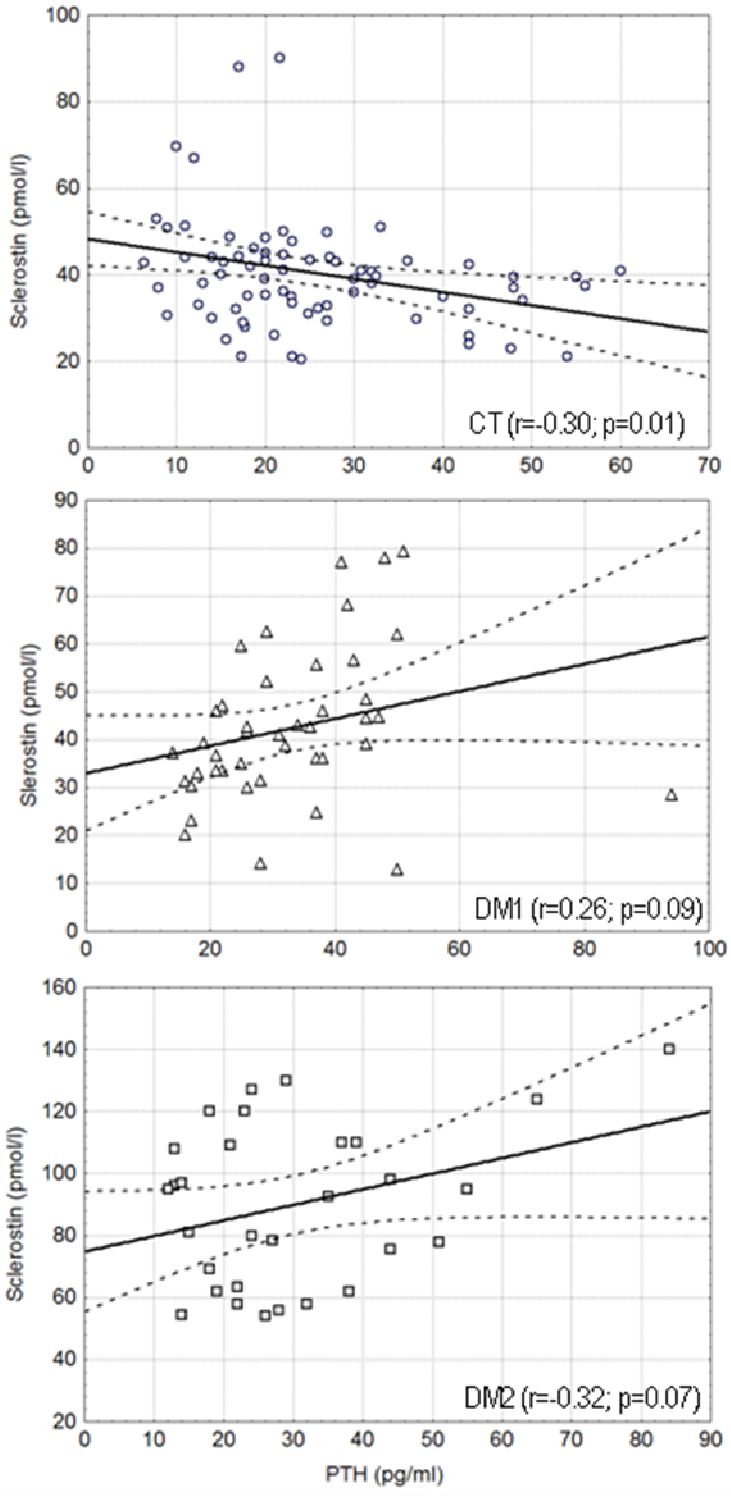

Sclerostin (pmol/l)
CT (r=-0.30; p=0.01)
Sklerostin (pmol/l)
DM1 (r=0.26; p=0.09)
Sclerostin (pmol/l)
DM2 (r=-0.32; p=0.07)
PTH (pg/ml)

Table 1. *Characteristics of Patients and Controls*

	DM1	DM2	CT1	CT2
Subjects (n)	43	40	21	62
Males/females (n)	23/20	20/20	10/11	30/32
Age range (yrs)	24-77	48-79 §	25-48	51-78
BMI	22.9±0.5	26.8±0.6 ***	24.1±0.9	25.7±0.5
LS-BMD (g/cm²)	1.098±0.02 #	1.092±0.03	1.192±0.03	1.052±0.02
-T score	-0.86±0.2 #	-0.87±0.3	-0.12±0.3	-1.20±0.2
-Z score	-0.54±0.2	-0.17±0.2	-0.18±0.3	-0.14±0.1
FN-BMD (g/cm²)	0.902±0.02 ###	0.922±0.03 §	1,055±0.04	0,847±0.02
-T score	-1.03±0.2 ###	-0.78±0.2 §	+0.10±0.3	-1.35±0.1
-Z score	-0.61±0.1 ##	+0.16±0.2 **	+0.12±0.3	-0.17±0.1
BALP (mcg/l)	11.3±0.8	10.9±0.7 §§	12.7±1.6	14.0±0.7
OC (ng/ml)	3.4±0.3	3.6±0.3	4.0±0.4	5.7±0.3
CTX (ng/ml)	0.31±0.03 ###	0.272±0.03 §§§	0.586±0.08	0.626±0.03
25OHD (ng/ml)	16.5±1.3 ###	15.1±2.2 §	34.7±3.8	23.1±2.7
PTH (pg/ml)	33.0±2.2 ##	30.2±3.1	23.3±2.1	25.4±1.8

***P<0.01 DM1 vs DM2:* *§P<0.05 DM2 vs CT2;* *#P<0.05 DM1 vs CT1;*
****P<0.001 DM1 vs DM2:* *§§P<0.01 DM2 vs CT2;* *##P<0.01 DM1 vs CT1;*
§§§P<0.001 DM2 vs CT2; *###P<0.001 DM1 vs CT1:*

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3. Roma 110° Congresso Nazionale della Società Italiana di Medicina Interna 24-27 Ottobre 2009 Comunicazione orale Martini G1, Bucciarelli P2, **Ceccarelli E**1, Martinelli I2,Gennari L1, Bader R2, Galli B1, Franci B1, Campagna MS1,Mannucci PM2, Nuti R1 "Relazione tra iperomocisteinemia e bassa densità ossea nelle donne in post-menopausa".
4. Padova 23° Congresso nazionale Società Nazionale Diabeteologia 9-12 Giugno Poster discussion: **Ceccarelli E**, Merlotti D, Valenti R, Cataldo D, Dotta F, Gennari L, Nuti R IL SESSO MASCHILE COME FATTORE DI RISCHIO PER OSTEOPOROSI NEL DIABETE MELLITO DI TIPO 1

ELENCO PUBBLICAZIONI

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