Role of calcium metabolism in the pathogenesis of osteoporosis and colorectal cancer

Doctoral dissertation

Krisztián Bácsi MD

Semmelweis University

Pharmaceutical Sciences of Ph.D. Studies

Tutors:	Péter Lakatos MD, PhD, DSc
	Gábor Speer MD, PhD
Opponents:	Károly Cseh MD, PhD, DSc
	Miklós Tóth MD, PhD
Chairman of committee:	Lídia Sréter MD, PhD, DSc
Members of committee:	Gyula Poór MD, PhD, DSc
	Gábor Kovács MD, PhD

Budapest

2008

Table of contents

1 Abbreviations……... 4
2 Introduction…….. 7
2.1 Role of calcium intake in different diseases……...…….. 7
2.1.1 Role of calcium intake in osteoporosis……...…….. 7
2.1.2 Importance and epidemiology of osteoporosis……. 8
2.1.3 Role of calcium intake in colorectal cancer……. 9
2.1.4 Importance and epidemiology of colorectal cancer……..... 10
2.1.5 Role of calcium intake in other diseases…….. 10
2.2 LCT 13910 C/T polymorphism and lactose intolerance…….. 11
2.2.1 LCT 13910 C/T polymorphism in osteoporosis and colorectal cancer…… 12
2.3 Calcium-sensing receptor function, an outlook…… 13
2.3.1 Calcium-sensing receptor on osteoblasts and osteoclasts…… 13
2.3.2 Calcium-sensing receptor on colon cells……..…… 14
2.3.3 CaSR A986S polymorphism…… 15
2.4 Dehydroepiandrosterone sulphate and dehydroepiandrosterone……..... 16
2.4.1 Role of DHEA(S), estrogens and androgens in osteoporosis…… 18
2.4.2 Role of DHEA(S), estrogens and androgens in colorectal cancer……. 20
2.4.3 Role of DHEA(S) in other diseases…….. 22
2.4.4 Role of DHEA(S) in intracrinology…….. 23
2.5 CYP3A7*1C polymorphism and DHEA(S)…… 23
2.6 Relation between serum calcium and DHEAS level……..... 24
2.7 Diagnosis of osteoporosis……. 25
2.8 Diagnosis of colorectal cancer……..... 26
3 Objectives……..... 27
4 Patients and Methods……..... 29
4.1 Patients…..……... 29
4.2 Methods…… 30
4.2.1 Genetic analysis of CaSR A986S G/T and LCT 13910 C/T polymorphisms... 30
4.2.2 Genetic analysis of CYP3A7*1C polymorphism……. 31
4.2.3 Bone densitometry……. 31
4.2.4 Biochemical measurements……... 31
4.2.5 Cell cultures…….. 32
4.2.6 Determination of viable cells……. 32
4.2.7 Measurement of alkaline phosphatase activity on culture plates…….. 33
4.2.8 Gene expression study…….. 33
4.2.9 Statistical analysis……..... 34
5 Results…….. 36
5.1 Effects of LCT13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of osteoporosis ..……...…… 36
5.1.1 Basic clinical characteristics and laboratory parameters……..... 36
5.1.2 LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphism distributions……... 36
5.1.3 LCT 13910 C/T genotype, risk factors for osteoporosis and laboratory parameters……... 36
5.1.4 CaSR A986S genotype, risk factors for osteoporosis and laboratory parameters……... 37
5.1.5 CYP3A7*1C genotype, risk factors for osteoporosis and laboratory parameters……... 39

5.1.6 LCT 13910 C/T genotype and BMD……. 40
5.1.7 CaSR A986S genotype and BMD……. 42
5.1.8 CYP3A7*1C genotype and BMD……. 42
5.1.9 Serum calcium, DHEAS level risk factor for osteoporosis and BMD…….. 42
5.2 Effects of LCT13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of colorectal cancer……... 48
5.2.1 Laboratory and tumor parameters……..... 48
5.2.2 LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphism distributions…… 48
5.2.3 LCT 13910 C/T genotype and laboratory parameters……..... 49
5.2.4 CaSR A986S genotype and laboratory parameters……..... 49
5.2.5 CYP3A7*1C genotype and laboratory parameters……... 50
5.2.5 LCT 13910 C/T polymorphism and tumor parameters……. 50
5.2.6 CaSR A986S polymorphism and tumor parameters……..... 50
5.2.7 CYP3A7*1C polymorphism and tumor parameters…….. 51
5.2.8 Serum calcium level and tumor parameters……..... 51
5.3 Effects of calcium supplementation on the proliferation, ALP activity and protein expression of MC3T3-E1 osteoblast cells…….. 56
5.3.1 Proliferation assay……..... 56
5.3.2 Alkaline phosphatase activity…… 56
5.3.3 Gene expressions……... 56
6 Discussion……. 58
6.1 Effects of LCT13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of osteoporosis ……. 58
6.2 Effects of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of colorectal cancer……..……..…..... 61
6.3 Effects of calcium supplementation on the proliferation, ALP activity and protein expression of MC3T3-E1 osteoblast cells…….. 63
7 Conclusion…… 64
7.1 Role of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms and calcium intake in the pathogenesis of osteoporosis..... 64
7.2 Role of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of colorectal cancer..……...…….. 66
8 Summary……... 68
9 Összefoglalás…… 70
10 References…….. 72
11 Original papers……... 98
12 Acknowledgements…… 102

1. Abbreviations

ACF	aberrant crypt foci
ACTH	adrenocorticotropin hormone
AFP	alpha1-fetoprotein
AJCC	American Joint Committee on Cancer
ALP	alkaline phosphatase
ANOVA	analysis of variance
ANCOVA	analysis of covariance
BGLAP	bone gamma carboxyglutamate protein (osteocalcin)
BMD	bone mineral density
BMI	body mass index
BMP-2, -4	bone-morphogenic protein-2, -4
BSP	bone sialoprotein
CAH	congenital adrenal hyperplasia
CaSR	calcium-sensing receptor
CA19-9	carbohydrate antigen 19-9
CEA	carcinoembryonic antigen
COL1A1	type I procollagen alpha 1
COL2A1	type II procollagen alpha 1
COX-2	cyclooxigenase-2
CRC	colorectal cancer
C-Tx	cross-links of C-terminal telopeptide
CV	coefficient of variation
95 % CI	95 % Confidence Intervals
DCN	decorin
DFS	disease free survival
DHEA	dehydroepiandrosterone
DHEAS	dehydroepiandrosterone sulfate
Dpd	deoxypyridinoline
DXA	dual-energy x-ray absorptiometry
ERK	extracellular signal-regulated kinase
EU	European Union

FN-1	fibronectin-1
GnRH	gonadotropin-releasing hormone
12-HETE	12-hydroxyeicosatetraenoic acid
IFN-γ	interferon gamma
IGF-1	insulin-like growth factor-1
IL-1	interleukin-1
IL-6	interleukin-6
IU	international unit
LCT	encoding lactase phlorizin hydrolase enzyme
LH	luteinizing hormone
LHRH	luteinizing hormone releasing hormone
LI	lactose intolerance
LPH	lactase phlorizin hydrolase
LRP-5	low-density lipoprotein receptor related protein 5
MAPK	mitogen-activated protein kinase
MDR-1	multidrug resistence-1 gene
M-MLV	moloney murine leukemia virus
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate type glutamate receptor
NF-κB	nuclear receptor kapppa B
N-Tx	cross-links of N-terminal telopeptide of type-1 collagen
OAT	organic anion transporter family
OATP	organic anion-transporting polypeptide family
OPG	osteoprotegerin
OPN	osteopontin
OP	osteoporosis
OR	odds ratio
OS	overall survival
PKC	protein kinase C
PMS	premenstrual syndrome
PTH	parathyroid hormone
PTHR-1	parathyroid hormone receptor-1
P1NP	serum intact N-terminal propeptide of type-1 procollagen
P21	cyclin-dependent kinase inhibitor-21

P27	cyclin-dependent kinase inhibitor-27
RANKL	receptor for activated nuclear receptor kappa B ligand
RANK	receptor for activated nuclear receptor kappa B
RT-PCR	real-time PCR
SD	standard deviation
SEM	standard error of mean
SERM	selective estrogen receptor modulator
SLE	systemic lupus erythematosus
SMAD-3, -6	mothers against DPP homolog-3, -6
StAR	steroidogenic acute regulatory protein
STS	steroid sulfatase
TAE	tris-acetate-EDTA
TCF-4	T cell factor-4
TGF-β	transforming growing factor beta
TNF-α	tumor necrosis factor-alpha
TNM	tumor, node, metastasis stages
TRAF-6	tumor necrosis factor receptor-associated factor-6
USA	United States of Amercia
χ^2 test	chi-square test

2. Introduction

In the Hungarian population the average calcium intake is low with a range of 400-600 mg per day in adults (Biro et al. 2007). The approximately constant serum calcium level is maintained by regulatory mechanisms in the bones, intestines and kidneys. Almost 99 % of the human body calcium content (1000-1500 g) is located in bones indicating a dynamic calcium store-pool (Heaney 2006). Absorption occurs across the intestinal and renal epithelia. Epidemiological studies suggest a preventive role of calcium intake in osteoporosis (OP), colorectal cancer (CRC) and other chronic diseases (kidney stones, obesity, insulin resistance syndrome (IRS), hypertension, coronary heart disease, stroke, preeclampsia and premenopausal syndrome). Dehydroepiandrosterone sulphate (DHEAS) plays an important role in calcium metabolism, and thus it is also related to the development of OP and perhaps CRC. DHEAS, a precursor molecule for estrogens and androgens, was spread in the USA as the fountain of youth increasing well-being sensation with minor side-effects.

2.1 Role of calcium intake in different diseases

2.1.1 Role of calcium intake in osteoporosis

Calcium seems to have little effect on bone loss if administered within the first five years of the menopause, when mainly estrogen withdrawal has a dominant role (Dawson-Hughes et al. 1990). Among women who were postmenopausal for six years or more, calcium supplementation (400-650 mg/day for two years) reduced bone loss (Dawson-Hughes et al. 1990). Moreover, in a heterogeneous elderly population a meta-analysis showed (n = 52,625) that calcium and vitamin D combined therapy reduce the rate of annual bone loss by 0.54 % at the hip and 1.19 % in the spine and all types of fractures by 12 % over 24-84 months (Tang et al. 2007). These effects were better with calcium doses of 1200 mg or more than with lower doses, and with vitamin D doses of 800 IU (international unit) or more than with lower doses. The relative changes in total hip bone mineral density (BMD) from baseline was -0.5 % and in spine BMD from baseline was -1.1 % in late postmenopausal women (Tanko et al. 2003). However, other studies failed to detect any significant effect of calcium (1000 mg) and

vitamin-D (800 IU) therapy on the rate of bone loss or fracture incidence in an elderly population (age > 70 years; n = 5292) over 24-62 months (Grant et al. 2005).

Lower calcium intake is a key factor in abnormal bone mineralization which acts not only through reducing available calcium for bone mineralization but through the consequently elevated parathyroid hormone level (PTH) (secondary hyperparathyroidism) which results in bone loss via stimulating osteoclast maturation, proliferation and activity (Seeman 2003).

2.1.2 Importance and epidemiology of osteoporosis

Osteoporosis is defined as a "systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fractures."

In Hungary 900,000 people suffer from OP (600,000 women and 300,000 men) (Poór 1997). The number of fractures associated with OP was 30-40,000 in the lumbar spine, 25-28,000 in the wrist, 15,000 in the hip (femoral neck and pertrochanteric fractures) and 8-10,000 in the proximal humerus during one year (Kazár 1987; Poór 1992; Somogyi 2000). Osteoporosis is a major public health threat for more than 200 million people worldwide and 10 million people in the United States of America (USA) (National Osteoporosis Foundation, available at http://www.nof.org/osteoporosis/diseasefacts.htm, accessed on Nov 23, 2007). The incidence of OP fractures in women is dramatically rising above the age of 50 years. Regarding data from Rochester, Minnesota the incidence of OP was about 45 % and also the fracture risk during lifetime was 40 % in women aged 50 years (Melton et al. 1992). In men this lifetime risk of any fracture is almost 13 % (Melton et al. 1992). The most common fracture type alters during aging: between the ages of 40 and 70 years it is the wrist and after this period the hip fracture (Keen 2007). The effect of fractures on survival depends on the type of fracture. Hip fractures are the most serious, as 30 % of women become totally dependent (Bonar et al. 1990) and 15 % of them will die within 6 months (Sexson and Lehner 1987). Vertebral fractures may cause disability via thoracic kyphosis and pain, which also increase mortality (Keen 2007). Another aspect of fractures is the increased risk for secondary fractures at the same or at another sites: fracture (hip, vertebral, forearm) leads to a ten-fold increase in the risk of a subsequent fracture (Sambrook and Cooper 2006). In patients with a history of forearm fracture the cumulative incidence of any subsequent fractures was 55% by ten years (Lauritzen et al. 1993; Mallmin et al. 1993; Cuddihy et al. 1999).

The estimated combined cost of fractures is $20 billion in the USA and about $30

billion in the European Union (EU) per year (Cummings and Melton 2002). Elderly people, a population with high incidence of hip fractures represent the fastest growing age group worldwide. It is predicted that the estimated number of hip fractures worldwide will rise from 1.7 million in 1990 to 6.3 million in 2050 (Sambrook and Cooper 2006; Keen 2007).

2.1.3 Role of calcium intake in colorectal cancer

Even though colorectal tumor genesis is a complex process, epidemiological and experimental data indicate that calcium has a chemopreventive role in the development of CRC (Garland et al. 1991; Lipkin and Newmark 1995; Kallay et al. 2000; Bhagavathula et al. 2005). Low-fat diary products containing up to 1200 mg/day calcium reduce cell proliferation in colonic crypt (Holt et al. 1998). In a heterogeneous population (mean age = 61 years) calcium supplementation (1200 mg/day) for four years was also shown to decreased polyp recurrence by 25 % which is known as the starting point in colorectal carcinogenesis (Baron et al. 1999). Among female subjects (n = 48,115, mean age = 65 years) the calcium intake (500-1000 mg) was associated with reduced risk for large adenoma over twelve years (Oh et al. 2007). In a cohort study with males (n = 85,903, median age = 61 years) and females (n = 105,108, median age = 60) calcium intake (1000 mg) was inversely associated with CRC in both males (risk reduction was 30 %) and females (risk reduction was 36 %) over seven years. Intake of dairy products is also associated with similar risk reduction in both genders (Park et al. 2007). In another study among male CRC patients (n = 45,306, mean age = 60 years), dietary calcium intake (risk reduction was 54 %) was shown to be more beneficial than only total calcium intake (risk reduction was 32 %) with a follow-up of 6 years (Larsson et al. 2006). In a 19-year prospective study the combined therapy of calcium and vitamin D in men (n = 1954) reduced the risk for CRC by three-fold (Garland et al. 1985). However, other studies failed to support the inverse association between calcium intake and CRC pathogenesis (Bergsma-Kadijk et al. 1996; Martinez and Willett 1998; Wactawski-Wende et al. 2006).

Intraluminal calcium precipitates intestinal fatty acids and secondary bile acids (deoxycholic and lithocholic acids), these are known as soluble hydrophobic surfactants with carcinogen effects (Wargovich et al. 1983; Govers et al. 1996). Both factors have show activity in colon epithelial cells and they have strong synergistic cytotoxic effects (Lapre et al. 1992). Moreover, bile acids stimulate cell proliferation directly via an increased generation of 12-hydroxyeicosatetraenoic acid (DeRubertis et al. 1984), a significant lipoxygenase product

in carcinogenesis (Wong et al. 2001). Moreover, proliferative activity of colonic mucosa, reactive oxygen production enhanced by protein kinase C activation (Craven et al. 1987; Govers et al. 1996) and apoptosis resistance induction (Payne et al. 1995) are involved in the effects of bile acids. Consequently, the beneficial effect of calcium on colorectal carcinogenesis may act through the formation of insoluble salts with fatty acids and secondary bile acids which result in altered effects on colon mucosa.

2.1.4 Importance and epidemiology of colorectal cancer

In Hungary, with approximately 8800 newly diagnosed cases annually, CRC is the **second** most common cancer type. The incidence rate is 87 cases (per 100,000) (Szabolcs 2005). Regarding the incidence of rectal cancer in males, the highest rates are found in Hungary with 20 cases (per 100,000) (Wilmink 1997). CRC is the second leading cause of cancer deaths with a death rate of 48 (per 100,000) (Szabolcs 2005).

CRC with 153,000 new cases per year is the third leading cancer type in the USA (Jemal et al. 2007). Its incidence is the highest among african-americans with 70-53 cases (per 100,000 for male-female) and the lowest in the asian-american population with 52-38 cases (per 100,000 for male-female) (Jemal et al. 2007). White people's incidence is between these two with 45-63 cases (per 100 000 for male-female). The lifetime probability of developing CRC is 5.79 % in males and 5.37 % in females. The incidence begins to rise around the age of forty years: it is 0.73-0.93 % for age between 40-59 years and 4.45-4.92 % for age of 70 years or older. CRC is the third leading cause of cancer deaths in the USA with a death rate of 22 (per 100,000) (Jemal et al. 2007). The significance of this disease is emphasized by the poor five-year survival, which is approximately 40 % (Benson 2007).

2.1.5 Role of calcium intake in other diseases

However, only a low portion of oxalate is absorbed from the gut, variations in the quantity appeared to have important role in kidney stones. Surprisingly, calcium intake is inversely associated with kidney stone risk in men, because calcium reduces the absorption of oxalate (Curhan et al. 1993). Higher calcium intake with food decreased stone recurrences by 50 % as compared to calcium-restricted diet (Borghi et al. 2002).

In a cohort of young males and females (n = 3157) CARDIA study showed that in overweight subjects the higher calcium intake from diary products lowers the rate of obesity

by 19 %, the abnormal fasting insulin or glucose level by 13 % and hypertension by 14 % in ten years (Pereira et al. 2002). IRS decreased by more than 50 % as a result of higher calcium intake (Pereira et al. 2002). Adjusting for nutrients in diary products like lactose, proteins and fats as factors which may reduce overweight via enhanced satiety did not lead to significant association which emphasized the role of calcium and not other nutrients (Pereira et al. 2002). As the calcium absorption and conservation have a low efficiency and the calcium is essential for signaling processes, the extracellular calcium level is strictly regulated by an endocrine control system in which PTH plays a main role. PTH alters calcium excretory threshold in the kidneys, osteoclast calcium releasing impact in the bones and calcium absorption in the gut affected by calcitriol synthesis of the kidneys (Heaney 2006). Calcium also can directly act on calcitriol producing cells. Nowadays the calcium intake is decreased as compared to that in the ages of our hunter-gatherer ancestors, which resulted in chronically high PTH and calcitriol levels. The calcium intake stimulates lipolysis and inhibits lipogenesis via altered PTH and calcitriol synthesis as it has been showed both in human (Davies et al. 2000) and animal models (Shi et al. 2001). Calcium also acts on vascular smooth muscle tone through the effect of PTH and renin on intracellular calcium level (Belizan et al. 1988; Hofmeyr et al. 2007) and it directly increases serum magnesium concentrations (Repke et al. 1989). Associated with hypertension, calcium intake decreased the rate of coronary heart disease (Bostick et al. 1999), stroke (Iso et al. 1999) and preeclampsia (Hofmeyr et al. 2007). Observation of an increased risk of OP after menopause in women with premenstrual syndrome (PMS) (Lee and Kanis 1994), similarities between the symptoms of PMS and hypocalcaemia such as depression, anxiety and fatigue (Thys-Jacobs 2000), and the association between calcium supplementation and PMS (Bertone-Johnson et al. 2005) suggested the relationship between calcium and PMS.

2.2 LCT 13910 C/T polymorphism and lactose intolerance

The CC genotype of the 13910 C/T polymorphism of LCT gene (encoding lactase phlorizin hydrolase (LPH) enzyme) has been reported to perfectly match with lactose intolerance (LI) (Enattah et al. 2002). The ability of patients with LI to cleave lactose, the main carbohydrate in milk into glucose and galactose is decreased because of the low activity of lactase phlorizin hydrolase. Lactase phlorizin hydrolase is represented on the apical surface

of enterocytes in the small intestinal brush border with the highest expression in the mid-jejunum (Lomer et al. 2008). Studies on intestinal biopsies demonstrated that the CC genotype is associated with low LPH-specific mRNA expression and low LPH activity because of the lower enhancer activity of the LPH gene (Kuokkanen et al. 2003; Troelsen et al. 2003). Similar results were also shown in a study with indirect lactose tolerance test (Buning et al. 2005).

LI is inherited as an autosomal recessive trait (Sahi et al. 1973). The prevalence of LI is ranging from 2% in Scandinavia to 20-40% in Central Europe and the United States, and increases to almost 100 % in Southeast Asian population (Sahi 1994). In the study country, Hungary the prevalence of LI is 37 % (Vesa et al. 2000).

People with LI (or with LCT 13910 CC genotype) experience discomfort after consumption of milk or diary products, such as abdominal cramps, distension and diarrhea because of the lactose content. Symptoms are explained by the high osmotic load of unabsorbed lactose, which increases the amount of water and electrolytes in the colon resulting in accelerated transit and softened stool. Secondly, lactose is hydrolyzed by bacterial β-galactosidase to galactose and glucose. These monosaccharides are then available for bacterial fermentation by the ileal and colonic flora into short chain fatty acids with byproducts of hydrogen and carbon dioxide causing consequent flatulence (Lomer et al. 2008). Several authors reported decreased milk or diary product consumption due to these symptoms (Carroccio et al. 1998; de Vrese et al. 2001; Obermayer-Pietsch et al. 2004). Moreover, in LI subjects the calcium absorption is lower, too, because it is decreased by the higher remaining lactose content via changing the pH and transit time in the gut (Obermayer-Pietsch et al. 2007). Reduced calcium absorption observed in subjects with positive breathing test without clinical symptoms (11-32% of all lactose intolerant patients) (Carroccio et al. 1998; de Vrese et al. 2001) indicate the additive impact of pathological processes induced by LCT gene polymorphism.

2.2.1 LCT 13910 C/T polymorphism in osteoporosis and colorectal cancer

The influence of LCT 13910 C/T polymorphism on bone loss has been debated controversially. Obermayer et al. (Obermayer-Pietsch et al. 2004) could demonstrate the deteriorating impact on axial BMD in postmenopausal women, other authors could not detect it neither in postmenopausal women nor in men (Enattah et al. 2004; Enattah et al. 2005; Gugatschka et al. 2007).

Colorectal carcinogenesis was also associated with LCT gene: CC genotype is accompanied by increased risk for CRC in a Finnish population, but not in a Spanish and an English sample (Rasinpera et al. 2005). However, the effect of LCT 13910 mutation on CRC progression has not been published yet. In Hungary (where the population is Caucasian and related to the Finns, i.e. both populations belong to the Finn-Ugor tribal family), both lactose intolerance (Vesa et al. 2000) and incidence of CRC (Kasler 2005) is high, suggesting the association between LCT 13910 C/T polymorphism and CRC development.

2.3 Calcium-sensing receptor function, an outlook

Calcium-sensing receptors (CaSR) on cell surface provide a connection between serum calcium level and several different effector mechanisms depending on the cell type. CaSR is a G-protein-coupled receptor that can be activated by calcium, sodium, magnesium, barium, stroncium, gadolium, lantan, aminoglycoside antibiotics and polyamines such as spermine, spermidine and protamin (Garrett et al. 1995; Riccardi and Maldonado-Perez 2005). Activation of CaSR by higher extracellular calcium concentration is associated with decreased parathyroid hormone (PTH) release from parathyroid cells (Brown 1991), decreased synthesis of 1,25-dihydroxyvitamin D_3 in renal proximal cells (Brown 1991; Brown et al. 1998) and increased calcitonin release from thyroid parafollicular cells (McGehee et al. 1997) which results restoring of serum calcium levels.

Furthermore, CaSR plays a role in gastrin and acid secretion in the stomach (Cheng et al. 1999), intestinal motility (Kirchhoff and Geibel 2006), colon fluid transport (Cheng et al. 2002), thirst regulation (Rogers et al. 1997) and growth hormone secretion (Romoli et al. 1999). CaSR alters cell differentiation of keratinocytes (Tu et al. 2004), colon cells (Chakrabarty et al. 2003), osteoblasts (Yamaguchi et al. 1998a), osteoclasts (Kanatani et al. 1999) and cell proliferation in breast ductal epithelium (Cheng et al. 1998), ovarian epithelial cells (McNeil et al. 1998a), monocytes (Yamaguchi et al. 1998b), bone marrow cells (House et al. 1997) and fibroblasts (McNeil et al. 1998b).

2.3.1 Calcium-sensing receptors on osteoblasts and osteoclasts

In MC3T3-E1 osteoblast cells, higher extracellular calcium stimulated chemotaxis,

proliferation, differentiation (Yamaguchi et al. 1998a) and protein expression via CaSR (Yamauchi et al. 2005). Calcium alters alkaline phosphatase (ALP) activity, osteocalcin (OCN) expression and mineralization (Yamauchi et al. 2005). ALP activity is essential for mineralization and production of bone matrix proteins and OCN, which is a predictive marker for osteoblast differentiation appearing at a later stage of osteoblast differentiation. Calcium also stimulates bone-morphogenic protein-2, -4 (BMP-2, -4), known as osteoinductive proteins and type I collagen expression, the main collagen in the bones (Nakade et al. 2001). However, the principle role of CaSR in bone development was debated, because in CaSR deficient mice the elimination of consequent secondary hyperparathyroidism rescued rickets-like skeletal abnormality in these mice (Yamauchi et al. 2005) and also CaSR deficient osteoblasts retained the ability to response higher calcium level (Pi et al. 2000), both suggesting the role of other cation receptors (N-methyl-D-aspartate (NMDA) glutamate receptors) in bone mineralization (Quarles et al. 1997). Glutamate receptors that belong to the same family as the CaSR may be such candidates (Yamauchi et al. 2005). These receptors are present in bones and respond to the changes in calcium (Kubo et al. 1998).

Calcium has an impact on bone resorption as well: it reduces the activity and stimulates apoptosis of osteoclasts (Zaidi et al. 1989; Kanatani et al. 1999; Lorget et al. 2000).

2.3.2 Calcium-sensing receptors on colon cells

CaSR is expressed both in the apical and basolateral membrane of normal colonic epithelial cells (Sheinin et al. 2000; Kirchhoff and Geibel 2006). Furthermore, CaSR expression is the highest in the well-differentiated regions of colon cancer and it is nearly absent in poorly differentiated regions (Chakrabarty et al. 2003). In human colon carcinoma cell lines extracellular calcium regulates the differentiation of colonic epithelial cells via CaSR (Chakrabarty et al. 2003). Higher concentrations of calcium has been shown to inhibit the proliferation of cancer cells (Chakrabarty et al. 2003; Bhagavathula et al. 2005; Chakrabarty et al. 2005).

First event of response to higher calcium may be the activation of c-SRC (McNeil et al. 1998b), then extracellular signal-regulated kinase (ERK) is activated with subsequent E-cadherin induction and β-catenin/T cell factor-4 (TCF-4) complex reduction (Figure 1). Calcium also stimulates the expression of E-cadherin and suppresses the expression of TCF-4. ERK, a member of mitogen-activated protein kinase (MAPK) family consists of a cascade of intracellular signaling elements that respond to numerous extracellular stimuli, i.e. growth

factors and components of extracellular matrix (Bhagavathula et al. 2005). E-cadherin molecule on the cell surface is an important factor in cell differentiation affecting cell adhesion and tumor suppression; the loss of E-cadherin occurs during tumor progression as it advances to a more malignant phenotype (Mareel et al. 1996; Chakrabarty et al. 2005). The cytoplasmatic domain of E-cadherin can bind β-catenin which consequently reduces the intranuclear complexes of β-catenin/TCF-4 and the down-regulation of TCF-4 transcription (Bhagavathula et al. 2005). β-catenin, as a member of the Wnt signal transduction pathway, activates and then combines with TCF4 in the nucleus; the yielded complex is responsible for the expression of several malignant effectors, such as c-myc, gastrin, cyclin D1, cyclooxigenase-2 (COX-2), matrilysin, urokinase-type plasminogen activator, CD44, multidrug resistance-1 gene and its product, P-glycoprotein (Moon et al. 1997; Wong and Pignatelli 2002; Chakrabarty et al. 2003). Furthermore, Wnt2 gene overexpression was observed in CRC (Vider et al. 1996). Another catenin that can bind to E-cadherin is the γ-catenin (Valizadeh A, amp, 1997) which has an inverse effect on TCF-4 compared to β-catenin as inactivating TCF-4 (Winn et al. 2002). Additional event in response to higher calcium levels is the increased expression of cyclin-dependent kinase inhibitor 21 and 27 (P21 and P27) (Giles et al. 2003; Kamei et al. 2003; Bhagavathula et al. 2005). P21 and P27 are cell cycle inhibitors and induce quiescence (Jacks and Weinberg 1998). To sum up, CaSR functions are promoting differentiation (or suppressing malignant properties) in colon carcinoma cells by up-regulating the expression of E-cadherin and down-regulating β-catenin/TCF4 complex (Chakrabarty et al. 2003; Chakrabarty et al. 2005).

CaSR also activates P38 MAPK (Hsiao et al. 2007) and c-Jun NH_2-terminal kinase (Xia et al. 1995; Arthur et al. 2000), all of them mediate growth arrest and apoptosis.

Supporting the essential role of CaSR in these mechanism, silencer RNA for CaSR has been shown to diminish the connection with E-cadherin, β-catenin, c-myc and cyclin D1 expressions in colon carcinoma cell line (Bhagavathula et al. 2007).

2.3.3 CaSR A986S polymorphism

CaSR A986S polymorphism results in higher PTH levels and consequent higher serum calcium and lower phosphate concentration for S allele carriers via altered calcium sensation (Marz et al. 2007). The modified receptor function affects PTH secretion as it has been shown above and we hypothesized that it may decrease the extracellular calcium as a beneficial effect on bone cells developing OP or on colon cells preventing colorectal carcinogenesis.

The association between osteoporosis and this polymorphism is controversial, the S allele was shown to cause decreased BMD in healthy women (Lorentzon et al. 2001; Eckstein et al. 2002); however, others failed to prove this association both in postmenopausal (Takacs et al. 2002; Young et al. 2003; Bollerslev et al. 2004) and premenopausal women (Mo et al. 2004). CaSR A986S polymorphism is associated with cardiovascular disease, S allele cause higher mortality and increased prevalence of coronary artery disease (Marz et al. 2007), underlying the possible relation between osteoporosis and atherosclerosis. The association between CRC development and CaSR A986S has not been published yet.

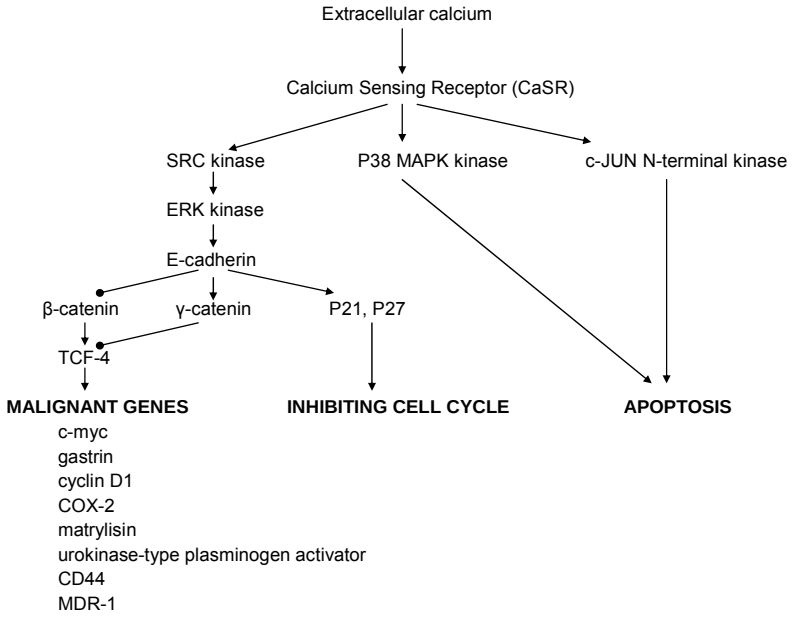


Figure 1. Effects of extracellular calcium on colon cell proliferation via CaSR. ERK: extracellular signal-regulated kinase; TCF-4: T cell factor 4; COX-2: cyclooxigenase-2; MDR-1: multidrug resistance-1 gene; P21: cyclin-dependent kinase inhibitor 21; P27: cyclin-dependent kinase inhibitor 27. → : stimulatory effect; —● : inhibitory effect.

2.4 Dehydroepiandrosterone sulphate and dehydroepiandrosterone

Dehydroepiandrosterone sulfate (DHEAS) and dehydroepiandrosterone (DHEA) are produced by adrenal glands, gonads and the brain. They are synthesized from cholesterol

through pregnenolone and 17α-hydroxypregnenolone (Rainey et al. 2002; Celec and Starka 2003; Miller 2005). DHEA, a prohormone of androgens and estrogens, can be converted into potent androgens such as testosterone, 5-dihydrotestosterone, androstenedione and also into estrogens such as estrogen, estradiol and estrone in the adrenal gland and in peripheral tissues (prostate, external and internal genitals, placenta, adipose tissue, hair follicle) (Labrie 1991; Hoffmann et al. 2001; Miki et al. 2002; Valle et al. 2006). DHEA can be converted into DHEAS catalyzed by sulfotransferase enzyme which is located in the liver, adrenal glands and small intestine. 99 % of DHEA is circulating as DHEAS (Zumoff et al. 1980). DHEAS is a negatively charged molecule at physiological pH and its uptake requires an active transport across the plasma membrane. It is performed by two organic anion carrier protein families: the organic anion-transporting polypeptide (OATP) family and the organic anion transporter (OAT) family, which are expressed in several tissues (Valle et al. 2006). DHEAS is then desulfated by steroid sulfatase (STS), and converts into bioactive androgens and estrogens as DHEA (Martel et al. 1994).

Emphasizing the intracrine formation of DHEA(S) into estogens, DHEA(S) accounts for 75 % of all estrogen production in premenopausal women and it increases to almost 100 % after menopause (Labrie 1991). Also, it provides the half of the amount of androgens both in men and premenopausal women (Labrie 1991; Belanger et al. 2002). Ratios of testosterone/DHEA(S) and estradiol/DHEA(S) levels are approximately 100 and 1000 for men, 500 and 10000 for women, respectively (Labrie et al. 2005).

Circulating DHEAS concentration remains at the same level during the whole day, while DHEA concentration fluctuates together with cortisol, showing a diurnal rhythm (Longcope 1996). There is also a difference between their half-lives: it is seven hours for DHEAS and 15-30 min for DHEA (Belisle et al. 1980; Bird et al. 1984). DHEAS concentrations are given in μmol/l, while DHEA levels are given in nmol/l (as cortisol) (Burger 2002). DHEA(S) levels are higher in men than in women during the whole lifetime; however, only a small amount is produced by the testis (Rainey et al. 2002).

During gestation, large amount of DHEA(S) is secreted by the fetal adrenal glands (Mastorakos and Ilias 2003). At birth the output drops to negligible amounts in both genders and remains unchanged until 5 years of age. At adrenarche the level start to increase and it accelerates through puberty. DHEA(S) output is maximal between the ages of 20 and 30 years, and then it starts to decline by 2 % per year, leaving a residual level of 10-20 % of the highest concentration by the eighth decade of life and 5 % by the age of 90 years (Parker 1991).

Adrenocorticotropin hormone (ACTH) increases DHEA level without a negative feedback; however, the association between ACTH and DHEAS is controversial (Alesci and Bornstein 2001). Prolactin also increases the DHEAS concentration (Vermeulen et al. 1977). Factors that increase DHEA(S) levels include smoking, alcohol and coffee consumption (Bjornerem et al. 2004), physical activity (Ponjee et al. 1994) and posttraumatic stress (Spivak et al. 2000). Medical drugs alter DHEA(S) level, too (Table 1/a, Table 1/b).

DHEAS can be directly excreted to urine or after desulfation it can be metabolized as DHEA: it converts into androstenedione, androsterone or etiocholanolone in the liver and then excretes to the urine as 17-ketosteroides (Longcope 1996) (Figure 2). 30-45 % of DHEA(S) is excreted by fecal elimination. In fetal life the highly expressed CYP3A7 can catalyze the conversion to 16α-hydroxi-DHEAS meaning an important elimination possibility (Kitada et al. 1987b).

Table 1/a. Medications with decreasing effect on DHEA(S) levels

Aminoglutethimide	Phenytoin
Bromocriptine	Pravastatin
Dexamethasone	Dopamine
estrogens	Ampicillin
gestagens	Paroxetine
Carbamazepine	Perazine
Clomipramine	Rifampicin
Cholestyramine	Risperidone
Citalopram	Insulin
Haloperidol	Valproate
Imipramine	Troglitazone
Lithium	Oral contraceptive

Table 1/b. Medications with increasing effect on DHEA(S) levels

Diltiazem	metformin
Benfluorex	alcohol
Amlodipine	retinol
Clomiphene	nicotine
Danazol	testosterone

2.4.1 Role of DHEA(S), estrogens and androgens in osteoporosis

DHEA(S) is the most abundant estrogen and androgen precursor in postmenopausal women (Hammer et al. 2005). Progressive decline in serum DHEA(S) with increasing age is thought to contribute to age-related diseases, such as osteoporosis (Foldes et al. 1997; Haden

et al. 2000; Osmanagaoglu et al. 2004). DHEA supplementation increases spine BMD both in postmenopausal women and andropausal men (Villareal et al. 2000; Hartkamp et al. 2004).

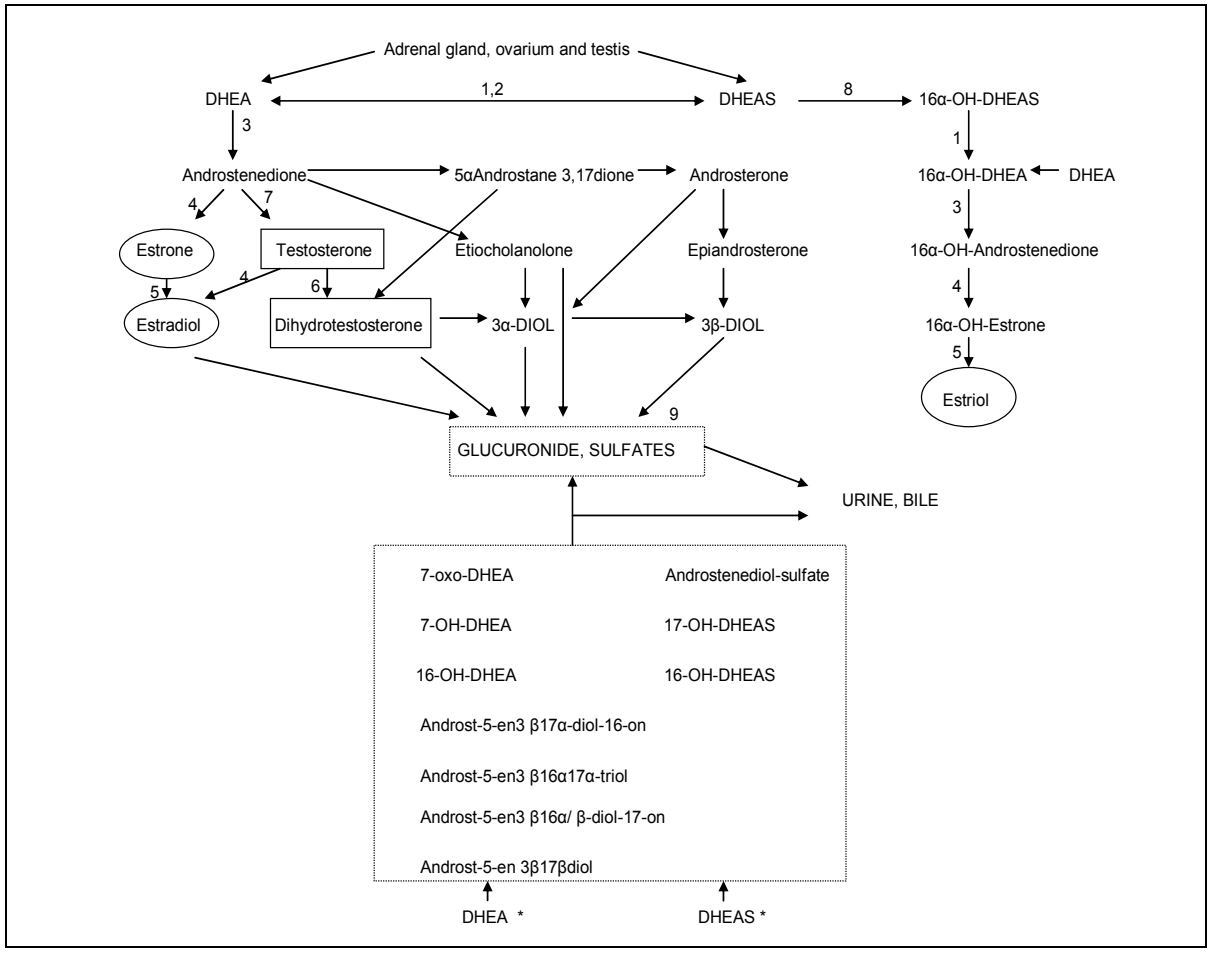


Figure 2. DHEA(S) conversion into androgens and / or estrogens in the periferial intracrine tissues and DHEA(S) metabolism. 1, steroid sulfatase; 2, sulfotransferase; 3, 3β-hydroxysteroid dehydrogenase-1; 4, aromatase; 5, 17β-hydroxysteroid dehydrogenase-1, -7, -12; 6, 5β-reductase-1, -2; 7, 17β-hydroxysteroid dehydrogenase-3, -5, -13; 8, *CYP3A7*; 9, uridine glucuronosyl transferases, sulfotransferase 2B1; * Elimination of steroids via urine or bile

The use of DHEA cream for 12 months results in increasing BMD in the total hip by 2 % in postmenopausal women, and also in a 21 % fall in the level of urinary hydroxyproline/creatinine ratio (a marker of bone resorption), and a 115 % increase compared to control in the serum osteocalcin level (marker of osteoblastic bone formation) (Labrie et al. 1997).

DHEA level positively correlates with IGF-1, the known factor in the pathogenesis of bone loss (Langlois et al. 1998) and is negatively associated with IL-6, an osteoclast

stimulating cytokine. These data suggested that DHEA deficiency may contribute to OP through IFG-1 and IL-6 levels, which are important factors in osteoclastogenesis (Haden et al. 2000). DHEA(S) may act on bone via converting into estrogens or androgens. Deficiency of aromatase (CYP19), the enzyme responsible for estrogen production in osteoblasts leads to OP in mice (Miyaura et al. 2001; Oz et al. 2001), which underlines the role of estrogens in bone homeostasis. Furthermore, correlation of estrone and DHEAS levels with BMD in postmenopausal women suggested the importance of estrogens in the effect of DHEAS (Nawata et al. 1995). Decreased estradiol levels are associated with reduced BMD (Rapuri et al. 2004) and higher hip and vertebral fracture risk in postmenopausal women (Cummings et al. 1998). Besides, estrogen supplementation (conjugated equine estrogen) increased vertebral and total hip BMD by 4.6 % and 3 %, respectively, over three years in postmenopausal women (Reid et al. 2004).

Estrogen deficiency can lead to reduced control over proinflammatory cytokines, like interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), and to decreased production of insulin-like growth factor-1 (IGF-1) and blocked action of NF-κB which is required for activation of IL-6 promoter that results in osteoclastogenesis (Jilka 1998; Lindberg et al. 2006; McCormick 2007). Estrogen binds to transforming growing factor beta (TGF-β) and to receptor for activated nuclear receptor kappa B (RANK)/receptor for activated nuclear receptor kappa B ligand (RANKL)/osteoprotegerin (OPG) systems which both have a significant role in OP (Ohmori et al. 2002; McCormick 2007; Zhang et al. 2007). Estrogens also have beneficial effects through extraosseal mechanisms by increasing calcitonin release and activating calcitriol receptors in the gut (Civitelli et al. 1988).

Androgen (testosterone) levels are inversely correlated with BMD in premenopausal women. However, patients with androgen insensitivity syndrome develop no OP, which emphasizes the importance of androgen-estrogen interconversion in bone development (Steinberg et al. 1989). In a study on cell lines androgens were shown to directly increase type I procollagen alfa 1 transcription (Benz et al. 1991) which is the polypeptide chain of the main collagen in bone (Velleman 2000).

2.4.2 Role of DHEA(S), estrogens and androgens in colorectal cancer

DHEA(S) level is not related to the risk of CRC in humans (Alberg et al. 2000); however, in human adenocarcinoma cell line DHEA treatment resulted in growth inhibition and G1 arrest via depletion of endogenous mevalonate and also in subsequent inhibition of

p21 isoprenylation, a process involved in cell growth and cell cycle progression (Willumsen et al. 1984; Schulz and Nyce 1991; Schulz et al. 1992). Furthermore, DHEA supplementation decreased the number of azoxymethane-induced aberrant crypt foci (ACF) in mice, which is the initial stage in colorectal carcinogenesis (Osawa et al. 2002) and reduced the rate of 1,2-dimethylhydrazine induced colon and anal tumors (Nyce et al. 1984). DHEA also enhanced the effect of azidothymidine chemotherapy (Klann et al. 1992). The possible role of DHEA in carcinogenesis was examined in a mouse model of skin tumor genesis. DHEA inhibited the glucose-6-phosphate dehydrogenize and pentose phosphate pathways, which are important sources of NADPH. NADPH stimulates the generation of oxygen free radicals, which are known to be stimulating factors in hyperplasia (Schwartz and Pashko 1995). Moreover, DHEA(S) can also prevent colorectal carcinogenesis via conversation to estrogens and androgens.

A possible protective effect of estrogens on CRC risk has been suggested by several studies: oral contraceptives used for 96 months or longer lowered the risk for CRC by 40 % (Martinez et al. 1997). Moreover, in postmenopausal women estrogen supplementation reduced the colorectal cancer risk by 19-46 % depending on the duration of therapy (one versus eleven years) and the association disappeared five years after the discontinuation of hormone administration (Calle et al. 1995; Grodstein et al. 1998)! Hormone replacement therapy (HRT) also led to reduced CRC mortality by 30 % (Slattery et al. 1999). In other studies estrogen had beneficial effect on CRC risk only in combination with progestins (Rossouw et al. 2002; Newcomb et al. 2007). Supporting the role of estrogens, women are less likely to develop CRC at all ages than men (Espey et al. 2007).

Estrogen receptor isoforms ER-α and ER-β are expressed in normal colon mucosa in both sexes (Campbell-Thompson et al. 2001). ER-β is the predominant ER-subtype in colon cells and its decreasing levels were associated with colon tumor (Campbell-Thompson et al. 2001). ER gene in CRC is methylated in 90 %; methylation-associated inactivation of ER gene with consequent lower expression is one of the earliest events in CRC development (Issa et al. 1994). Re-expression of ER gene by adding estrogens resulted in growth inhibition of CRC cell lines (Issa et al. 1994). Estrogens may affect microsatellite instability which occurs in approximately 10-15 % of colon tumors (Slattery et al. 2001). Both oral contraceptives and HRT decreased the risk of colon tumors by reducing microsatellite instability (Slattery et al. 2001). In colonic cells estrogens also increased the expression of vitamin D receptors, the known preventive factor of colorectal carcinogenesis (Smirnoff et al. 1999). Moreover,

exogenous estrogens and progestins decreased bile acid production, which resulted in reduced chronic irritation of the colonic mucosa (Gambacciani et al. 2003).

Androgens have been shown to have beneficial effects on CRC development in animal experiments (Stebbings et al. 1989; Izbicki et al. 1990). In human colorectal cell lines 5 - dihydrotestosterone also reduced proliferation (Harrison et al. 1989). Higher level of nuclear androgen receptor (AR) in human normal colonic mucosa as compared to its concentration in colorectal adenoma suggested the protective effect of AR (Marugo et al. 1992). In colorectal cancer samples similar result was failed to be shown, but isoform shifting was seen with higher expression of the functionally inert isoform (Catalano et al. 2000; Ferro et al. 2002). In colon cancer cell lines AR could also repress β-catenin/TCF-4 mediated response, a known important factor in the development of CRC (Chesire and Isaacs 2002).

2.4.3 Role of DHEA(S) in other diseases

DHEA(S) concentration was found to be lower in abdominal obesity (Barrett-Connor and Ferrara 1996), non-insulin dependent diabetes mellitus (Yamauchi et al. 1996), atherosclerosis (Eller et al. 2005), cardiovascular diseases (Barrett-Connor et al. 1986; Haffner et al. 1996), acquired immune deficiency syndrome (Ferrando et al. 1999), systemic lupus erythematosus (SLE) (Straub et al. 2004), rheumatoid arthritis (Imrich et al. 2005), Crohn's disease (Straub et al. 1998), systemic sclerosis (Ramsaransing et al. 2005), pemphigus vulgaris, pemphigoid (de la Torre et al. 1995), Alzheimer's disease (Armanini et al. 2003; Kim et al. 2003), Cushing syndrome with adrenal gland adenoma (Toth et al. 1997), panhypopituitarism (Miller et al. 2001), erectile dysfunction and decreased libido (Reiter et al. 2000; Davis et al. 2005).

50 mg DHEA treatment for six months decreased visceral fat by 7.4 % in aged women and by 10.2 % in aged men. Furthermore, DHEA supplementation lowered blood glucose and total cholesterol level, too (Diamond et al. 1996; Villareal and Holloszy 2004). DHEA(S) acts as an immunomodulator, it can reduce the symptoms of SLE (Casson et al. 1993; Chang et al. 2002). DHEA decreases erectile dysfunction and hot flush (DeFazio et al. 1984; Stomati et al. 2000), improves vaginal epithelial maturation and libido (Labrie et al. 1997; Reiter et al. 1999). It has beneficial effects on the skin: it increases the thickness and hydration of epidermis and decreases skin pigmentation (Johannsson et al. 2002; Spark 2002). Effects of DHEA supplementation with 25-50 mg per day were different between age groups: in young

people DHEA was associated with well-being and in advanced age DHEA mainly increased BMD and libido.

DHEA(S) concentrations are higher in congenital adrenal hyperplasia (CAH), a disease with deficiency of corticosteroid synthesizing enzyme in adrenal glands (21-hydroxylase, 11β-hydroxylase and only for women 3β-hydroxysteroid dehydrogenase) (Falhammar et al. 2007; Willenberg et al. 2007). However, other types of CAH including the congenital lipoid adrenal hyperplasia (steroidogenic acute regulatory protein (StAR) deficiency), cholesterol side change cleavage enzyme (CYP11A1) or aldosterone synthetase deficiency and 3β-hydroxysteroid dehydrogenase deficiency did not lead to altered DHEAS level in men (Miller 2005). Furthermore, DHEAS concentration is elevated in androgen producing tumors (Fernandez-Real et al. 1998; Chang 2004), Cushing disease, glucocorticoid resistance, placental aromatase and sulfatase deficiency, acne vulgaris (Slayden et al. 2001), hirsutism (Cibula et al. 2000) and alopecia (Pitts 1987).

2.4.4 Role of DHEA(S) in intracrinology

Intracrinology is a special field of endocrinology regarding the synthesis of active steroids in peripheral target tissues where the action is exerted in the same cells as where the synthesis takes place without release of the active steroids into the extracellular space and systemic circulation (Labrie et al. 1988; Labrie 1991). This mechanism minimizes the side effects of androgens like masculinization and it is responsible for hirsutism, acne, seborrhea, alopecia and endometriosis without elevated androgen levels in the blood (Cusan et al. 1994; Labrie et al. 1997). In the prostate the 50 % of androgens converted locally from DHEAS (Belanger et al. 1986). This explains the significant level of androgens after castration. Furthermore, in prostate cancer the combined therapy with anti-androgen (flutamide), inhibiting the effects of these androgens resulted in a more effective therapy, than the LHRH (LH releasing hormone) agonist with consequent medical castration alone (Labrie et al. 1982; Bennett et al. 1999). This suggested the significance of controlling androgen level not only in the circulation but in the perifery too. Similarly, in women the locally converted estrogens as much important as androgens in men. The use of aromatase inhibitor (anastrozole) in the treatment of breast cancers inhibiting the DHEAS estrogens conversion, is associated with major benefits over anti-estrogen therapy (tamoxifen) (Nabholtz et al. 2000). Moreover, in postmenopausal women raloxifen, a drug with antiestrogenic effects on breast tissue caused a

76 % reduction in the incidence of breast cancers (Cummings et al. 1999).

CYP3A7*1C polymorphism and DHEA(S)

Cytochrome P450 is a family of hemoproteins that are functioning in the oxidative metabolism of a variety of endogenous and exogenous substrates including steroid hormones (Kitada et al. 1987a; Gonzalez 1988). Among these, CYP3A subfamily has been established as the most abundant P450 enzyme in humans, representing about 30% of the entire hepatic P450 content (Shimada et al. 1994). CYP3A genes are located in a cluster on chromosome 7q21-q22 (Brooks et al. 1988) and consist of isoforms CYP3A4, CYP3A5, CYP3A7 and CYP3A43 (Kitada et al. 1985; Molowa et al. 1986; Aoyama et al. 1989; Westlind et al. 2001).

CYP3A7 is predominantly expressed in fetal liver, and its expression seems to be silenced shortly after birth. In adults only 1.7% of CYP3A7 mRNA can be detected as compared to the fetus (Greuet et al. 1996). In case of a mutant CYP3A7 variant, - CYP3A*1C - the enzyme expression level stands at a higher level (Kuehl et al. 2001).

CYP3A7 catalyzes the 16-α hydroxylation of both dehydroepiandrosterone (DHEA) and its 3-sulphate, dehydroepiandrosterone sulphate (DHEAS) (Kitada et al. 1987a). However, it has also catalytic activity on estrogens, estrone and androgens; the enzyme is responsible for the 2-hydroxylation of 17β-estradiol, for the 16α- and 6β-hydroxylation of estrone (Lee et al. 2003), for 2β-hydroxylation and 6β-hydroxylation of testosterone (Kitada et al. 1987b) and for 6β-hydroxylation of androstenedione (Aoyama et al. 1989).

Smit et al. (Smit et al. 2005) found significant difference in the amount of serum DHEAS level between wild type and mutant allele carriers of CYP3A7*1C genotype in a heterogeneous group of elderly men and women and in another group of elderly men, suggesting the persistence of the enzymatic activity of the CYP3A7 during adult life (corresponding to the CYP3A7*1C allele) resulting in lower DHEAS levels. Furthermore, they found decreased estrone concentration in heterozygous CYP3A7*1C carriers only in men. They could not detect such a relationship with estradiol, testosterone or androstenedione. We hypothesized that CYP3A7*1C polymorphism leads to OP or colorectal carcinogenesis via lower DHEAS levels, which may be the possible factor in the pathogenesis of OP and CRC.

2.5 Relation between serum calcium and DHEAS level

DHEA reduces post-load hypercalcaemia in patients with postmenopausal OP (Hollo et al. 1978).

2.6 Diagnosis of osteoporosis

Besides taking medical history for risk factors, BMD and laboratory measurements are also necessary for the correct diagnosis of OP (Table 2, Table 3). BMD measurement is carried out by dual-energy x-ray absorptiometry (DXA). Results are mostly given in g/cm^2 and are usually converted to T-score or Z-score. Laboratory measurements that describe osteoblast activity include serum osteocalcin, serum bone-specific alkaline phosphatase (ALP) and serum intact N-terminal propeptide of type-1 procollagen (P1NP). ALP and P1NP are early, osteocalcin is a late marker of bone formation. Elevated levels are seen in higher bone turnover and in bone loss.

Table 2. Risks factors for OP and osteoporotic fractures *risk factors that act independently from BMD (Kanis et al. 2005)

Age*	Asian or Caucasian race
Parental history of hip fracture*	Prolonged immobilization
Ever use of steroid*	Low physical activity
Body mass index < 20 kg/m^2*	Loss of height and thoracic kyphosis
Prior fracture after the age of 50 years*	Thyreotoxicosis
Rheumatoid arthritis*	Hypothyroidism
Current smoking*	Hyperparathyroidism
Alcohol intake > 2 units/day*	High cortisol level
High bone turnover*	High follicle stimulating hormone level
Neuromuscular disorder*	Low progesterol level
Poor visual activity*	Low DHEA(S) level
Female gender	Hypercalciuria
Premature menopause	Metabolic acidosis
Primary or secondary amenorrhea	Renal failure
Primary of secondary hypogonadism in men	Vitamin K deficiency
Low dietary calcium intake	Organ transplant
Vitamin D deficiency	
Malabsorption syndromes	
Chronic obstructive pulmonary disease	

Resorption markers including urine cross-links of N-terminal telopeptide of type-1 collagen (N-Tx) or C-terminal telopeptide (C-Tx) and deoxypyridinoline (Dpd) are related to osteoclast activity. Most of these parameters are available to examine the effectiveness of OP therapy and to assess fracture risk (Garnero et al. 1996; Johnell et al. 2002).

Table 3. Medications associated with OP

glucocorticoids	loop diuretics
thiazolidinedione	heparin
aromatase inhibitors	phenytoin
luteinizing hormone releasing hormone agonists	phenobarbiton
	carbamazepine
medroxprogesterone acetate	gonadotropin releasing factor antagonists
proton-pump inhibitors	

2.7 Diagnosis of colorectal cancer

Bleeding, iron deficiency anaemia, change in bowel habits and abdominal pain are the most common symptoms of CRC. Risk factors are shown in Table 4. Diagnosis is established by colonic imaging: radiological (computed tomographic colonography, double contrast barium enema) or endoscopy (standard or flexible sigmoidoscopy) and biopsy. Fecal occult blood tests and measurement of serum tumor markers including carcinoembryonic antigen (CEA), alpha1-fetoprotein (AFP) and carbohydrate antigen 19-9 (CA19-9) are also used. Fecal blood test was shown to be appropriate for asymptomatic people but not for patients with colonic symptoms. Tumor markers may only be useful for follow up of patients because of low sensitivity and specificity. Liver, chest and complete colonic imaging are also necessary to detect metastases (Ballinger and Anggiansah 2007).

Table 4. Risk factors for colorectal cancer

Age	High fat diet
Previous CRC	High red meat diet
Polyps	Physical activity
Family history of CRC or adenomatosus polyps	Obesity
Ulcerative colitis	Smoking
Crohn's disease	Alcohol consumption

3. Objectives

LCT, CaSR and CYP3A7 genes are associated with calcium metabolism, as an important pathogenetical factor in OP and colorectal carcinogenesis. Extracellular calcium also stimulates the activity of osteoblasts. LCT 13910 C/T polymorphism is perfectly mathced with lactose intolerance resulting lower calcium intake. CaSR A986S polymorphism altered calcium sensation. CYP3A7*1C polymorphism leads to decreased serum DHEAS level and consequently lower conversion to estrogens and androgens in peripheral cells. We hypothesized the role of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of OP and CRC.

I. Examining the association between LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms and OP in postmenopausal women

1. Examining allele frequency in postmenopausal osteoporotic and healthy women.
2. Examining association between polymorphisms and risk factors for OP: milk aversion, body mass index (BMI), body height and weight, menopausal age, smoking habits, alcohol consumption, caffeine consumption, history of steroid use and previous fractures.
3. Examining association between polymorphisms and laboratory parameters of calcium metabolism: serum calcium, phosporus, 25-OH-vitamin D_3, beta-crosslaps, serum DHEAS level and alkaline phosphatase activity.
4. Examining association between polymorphisms and BMD.
5. Examining interaction of polymorphisms on risk factors for OP and laboratory parameters.
6. Examining association between serum calcium-, DHEAS level, risk factors for osteoporosis and BMD

II. Examining the importance of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in colorectal carcinogenesis

1. Examining allele frequency in patients with CRC and in healthy subjects.

2. Examining association between polymorphisms and laboratory parameters: serum calcium level and tumor markers (carcinoembryonic antigen (CEA), alpha1-fetoprotein (AFP) and carbohydrate antigen 19-9 (CA19-9) levels).
3. Examining association between polymorphisms and tumor parameters (locoregional or distant metastases, disease-free or overall survival, tumor, node, metastasis stages (TNM) or American Joint Committee on Cancer (AJCC) stages, histological grade, localization).
4. Examining interaction of polymorphisms on laboratory parameters and tumor parameters.
5. Examining association between serum calcium level and tumor parameters (locoregional or distant metastases, disease-free or overall survival, TNM or AJCC stages, histological grade, localization).

III. Examining the effects of calcium supplementation on the activity and protein expression of MC3T3-E1 mouse osteoblast cells

1. Examining association between calcium supplementation and cell proliferation.
2. Examining the role of calcium supplementation in alkaline phosphatase (ALP) activity and gene expression pattern (type I procollagen alpha 1 (COL1A1), type II procollagen alpha 1 (COL2A1), decorin (DCN), bone sialoprotein (BSP), fibronectin-1 (FN-1), bone morphogenetic protein-4 (BMP-4), mothers against DPP homolog-3 (SMAD-3), mothers against DPP homolog-3 (SMAD-6), CaSR, bone gamma carboxyglutamate protein (osteocalcin) (BGLAP)).

4. Patients and Methods

4.1 Patients

Population I/a (OP study): we examined 595 postmenopausal women including 267 subjects with age-related osteoporosis (mean age = 62 ± 10 years), 200 patients with osteopenia (mean age = 62 ± 10 years) and 128 healthy subjects (mean age = 56 ± 10 years) regarding LCT and CaSR genes. Milk consumption, body mass index (BMI), body height and weight, menopausal age, smoking habits, alcohol and caffeine consumption, history of steroid use and previous vertebral or non-vertebral fractures and laboratory parameters (serum calcium, phosporus, 25-OH-vitamin D_3, beta-crosslaps and alkaline phosphatase activity) were also recorded.

Population I/b (OP study): we examined 319 postmenopausal women in the relation to CYP3A7 gene, who were divided into two groups. 217 patients had age-related osteoporosis (mean age = 68 ± 6 years) and there were 102 healthy subjects (mean age = 55 ± 8 years). Body mass index (BMI), body height and weight, menopausal age, smoking habits, alcohol consumption, history of steroid use and previous non-vertebral fractures and laboratory parameters (serum DHEAS, calcium, phosporus, 25-OH-vitamin D_3 and alkaline phosphatase activity) were also recorded. Patients in population I/a and population I/b were recruited through our Metabolic Bone Disease Clinic by selecting successively referred women. Subjects with disorders or medication known to influence bone metabolism were excluded.

Population II (CRC study): we collected 538 participants, 278 subjects (130 female and 148 male) with primary colorectal cancer and 260 healthy blood donors. The age at the time of the diagnosis was 61 ± 11 years. Clinical data, sex, tumor localization, stage, recurrence and time of death were obtained. The tumor, node, metastasis staging system (TNM) and stage groupings of the American Joint Committee on Cancer (AJCC) were used based upon conventional pathological diagnosis. Disease-free survival (DFS) and the duration of time after treatment without cancer-recurrence were calculated. Median DFS was 8 months (range 1-20 months). After staging examination, 64 patients had distant metastasis at the time of diagnosis. All CRCs were histologically adenocarcinoma. Median follow-up period was 17 months (range 1-20 months). Within the follow-up period, 58 patients experienced disease recurrence, the majority (n = 44; 76 %) of them developed distant metastases. Twenty-nine

patients died. Follow-up data were obtained at regular intervals. We analyzed patients separately for the following parameters: sex, presence of distant metastases at the time of diagnosis and localization (rectal and colon tumors). In the CRC group markers of tumor progression (CEA, AFP, CA19-9) as well as serum calcium and albumin levels were measured. The control group consisted of 260 healthy Caucasian blood donors without history of cancer or any chronic diseases. All of our participants were handled anonymously in accordance with previously established ethical protocols. All subjects gave written informed consent. Our studies were approved by ethical committee.

4.2 Methods

4.2.1 Genetic analysis of CaSR A986S G/T and LCT 13910 C/T polymorphisms

Genomic DNA was isolated from EDTA blood using a commercially available kit (Magnesil KF Genomic System, Promega, Madison, WI). Genotyping for CaSR A986S G/T (rs1801725) was performed by a Taqman pre-designed primer/probe set (Applied Biosystems, Foster City, CA) (Livak 1999). This genetic variant causes an amino acid change from alanine (A) to serine (S) in the protein. LCT 13910 C/T (rs4988235) polymorphism involving a thymine (T) - cytosine (C) nucleotide change is located in the initial codon of LCT (in intron 13) (Harvey et al. 1996). It was genotyped with the following sequence of allele specific probes 5'-ATA AGA TAA TGT AGC CCC TGG C; 5'- ATA AGA TAA TGT AGT CCC TGG C and primers (forward) 5'-CTC TGC GCT GGC AAT ACA G and (reverse) 5'-AAA TGC AAC CTA AGG AGG AGA. 5' dyes of probes were VIC and FAM for both polymorphisms. The PCR mixture (20 μL) contained 50 ng of genomic DNA, 900 nM of primers and 200 nM of probes (Sigma-Genosys, Woodlans, Texas), as well as 10 μL of TaqMan Universal PCR Assay Mix (Applied Biosystems, Foster City, CA). Cycling conditions for both genotyping comprised an initial cycle at 60 °C for 1 min and 95 °C for 10 min, followed by 55 cycles at 92 °C for 15 s and 60 °C for 1 min, and a final step at 60 °C for 1 min. The fluorescence intensity was measured by the 7500 RT-PCR System (Applied Biosystems, Foster City, CA).

4.2.2 Genetic analysis of CYP3A7*1C polymorphism

Genomic DNA was isolated from blood samples of the patients using the Magnesil KF Genomic System (Promega, Madison, WI, USA). A restriction fragment length polymorphism of T167G variant (rs11568825) was adapted from Smit et al. (Smit et al. 2005) with the following modifications. Oligonucleotide forward primer 5'-TCTCCTCTTGTCTCTATGG-3' and reverse primer 5'-CTGAGTCTTTTTTTCAGCAGC-3' were used to amplify a 370 bp fragment of the CYP3A7 gene. PCR reaction was carried out in 25 µl final volume using the following materials: 2 µl genomic DNA (50 ng/µl), 12.5 µl 2 x PCR Master Mix (Sigma-Aldrich, St. Louis, MO, USA), 0.5 µl of reverse and 1 µl of forward primer (10 pmol/µl) (Sigma-Genosys, Woodlands, Texas, USA) and 9 µl ultra pure PCR water. Cycling conditions comprised an initial denaturing cycle at 94 °C for 5 min, followed by 40 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s and by a final extension step at 72 °C for 5 min. 17.5 µl PCR product was digested for 2 h at 37 °C in a final volume of 20 µl containing 10 x restriction buffer and 5 U restriction endonuclease SspI (Promega, Madison, WI, USA). Results were quantitated by electrophoresis using standard horizontal Tris-Acetate-EDTA (TAE)-agarose gel-electrophoresis equipment and Fluorchem 8900 imaging system (Alpha Innotech, San Leandro, CA, USA). The wild-type allele fragment has one SspI site, resulting in two fragments of 244 and 126 bp. In contrast, in the CYP3A7*1C allele the splitting site is destroyed, resulting in one fragment size of 370 bp.

4.2.3 Bone densitometry

Bone mineral density (BMD) at the lumbar spine (L1-4), total hip, femoral neck, Ward's triangle and radius were measured by dual energy X-ray absorptiometry (Lunar Prodigy, GE Medical Systems, Diegem, Belgium). For CYP3A7*1C polymorphism we used BMD at lumbar spine (L2-4) and femoral neck. Coefficients of variation were below 1% at all measured sites. Osteoporosis or osteopenia were defined by WHO criteria as a T-score < -2.5 standard deviation (SD) or -1 < T-score < -2.5 SD at any site. The data were given in Z-scores, T-scores and BMD (g/cm^2) for LCT 13910 C/T and CaSR A986S polymorphisms, and Z-scores and T-scores for CYP3A7*1C polymorphism.

4.2.4 Biochemical measurements

Fasting blood samples were drawn in the morning and serums were aliquoted. Serum calcium, serum phosphorus, albumin levels and alkaline phosphatase activity were measured by colorimetric assays (Roche Diagnostics, Indianapolis, IN). Intra- and inter-assay coefficients of variation (CV) for these assays were below 1.9 %, 2.3 % for elevated and normal serum concentrations, respectively. Calcium levels were corrected for serum albumin levels using a standard calculation: corrected serum calcium = serum calcium (mmol/L) – 0.02 x (albumin (g/L) - 40). Serum beta-crosslaps, DHEAS, CEA, AFP and CA19-9 levels were measured by an automated immuno-chemiluminescence assay (Roche Diagnostics, Indianapolis, IN). Beta-crosslaps intra- and inter-assay CVs were below 1.6 %, 4.3 % and 4.7 % for elevated, normal and low serum concentrations, respectively. DHEAS intra- and inter-assay CVs were below 3.2 % and 2.7 % for low, normal and elevated serum concentrations, respectively. The age-related reference range of women for DHEAS are the following: 15-44 year: 1.6-11.0 µmol/l; 45-74 year: 0.3-7.0 µmol/l; >75 year: 0.3-4.2 µmol/l. Intra- and inter-assay CVs for CEA, AFP and CA19-9 were below 4.1 %, 3.8 % and 2.9 %, for elevated, normal and low serum concentrations, respectively. Serum 25-OH-vitamin D_3 measurement was carried out by high performance liquid chromatography (Bio-Rad Laboratories, Hercules, CA). Intra- and inter-assay CVs were below 4.7 %, 4.0 % and 6.5 % for elevated, normal and low serum concentrations, respectively. The reference range for the measured parameters can be seen in Table 6 and Table 12.

4.2.5 Cell cultures

The mouse osteoblast-like MC3T3-E1 cell line deriving from newborn mouse calvaria was cultured in α-MEM containing 1.8 mmol/L of calcium and 25 µg/ml ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10 % fetal calf serum and antibiotics (Sigma-Aldrich, St. Louis, MO, USA).

4.2.6 Determination of viable cells

MC3T3-E1 cells were seeded at a density of 10,400 cells/cm^2. CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA) was used to determine the number of viable cells in culture with non-supplemented α-MEM and medium supplemented

with 25.5 mM $CaCl_2$ after 24 hours. The method based on quantitation of ATP content which signals the presence of metabolically active cells. The cells were centrifuged for 10 min at 1000 rpm at 4 and 28 hour after plating to discard medium and 250 µl CellTiter-Glo reagent (buffer and lyophilized substrate) with 250 µl α-MEM which was added to the cells. Mix contents were shaked for 3 minutes followed by 10 minute incubation at room temperature. Generation of luminescent signal consequent to ATP content is directly proportional to the number of viable cells present in the culture. Luminescence was recorded by Fluoroskan Ascent FL (Thermo Fisher Scientific Inc, MA, USA).

4.2.7 Measurement of alkaline phosphatase activity on culture plates

Medium containing 10 mM β-glycerol-phosphate (Sigma-Aldrich, St. Louis, MO, USA) and 25 ng/mL ascorbic acid (Sigma-Aldrich, St. Louis, MO, USA) was added to the cells. ALP activity was determined by measuring p-nitrophenol generated from p-nitrophenil-phosphate due to enzymatic activity after 15 days of incubation with non-supplemented α-MEM and medium supplemented with 25.5 mM $CaCl_2$ using an Olympus AU2700 analyzer (Olympus Corporation, Tokyo, Japan). ALP levels were normalized against cellular protein levels: cells were digested with CellLytic TM buffer (Sigma-Aldrich, St. Louis, MO, USA) then protein concentrations were measured by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) at 280 nm by using Bradford assay (Sigma-Aldrich, St. Louis, MO, USA).

4.2.8 Gene expression study

To determine the effect of calcium concentration on gene expression (Table 5), MC3T3-E1 cells were cultured with non-supplemented α-MEM and medium supplemented with 25.5 mM $CaCl_2$. Total RNA was extracted from cells after 15 days by using High Pure RNA Isolation Kit (Roche Diagnostics, Indianapolis, IN). RNA quantity and purity was assessed by NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) at 260 nm. 20 µl cDNA was synthesized by the following process: 10 µL of total RNA (50 ng/mL) and 600 ng hexamer primers were incubated for 8 min at 65 °C, then 200 U Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase, 100 nmol dNTP, 20 U RNazin and 4 µL M-MLV 5 x buffer were added and incubated for 60 min at 37 °C (all reagents for reverse transcription were purchased from Promega, Madison, WI, USA). cDNA

amplification was carried out by real-time PCR using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA) (Applied Biosystems, Foster City, CA). 20 μL final reaction volume contained 1 μL cDNA, 10 μL TaqMan 2 x Universal PCR Master Mix NoAmpErase UNG, 1 μL predesigned and validated gene-specific TaqMan Gene Expression Assay 20 x and 8 μL ultra pure PCR water. Three parallel runs of genes were amplified at cycling conditions: initial denaturing cycle at 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 s, and 60 °C for 1 min. GAPDH as housekeeping gene was used to normalize gene expression. Data from amplification were analysed by 7500 SDS software 1.3 package.

Table 5. Examined genes in MC3T3-E1 osteoblasts

GENE ABBREVIATION	GENE NAME
Extracellular structural proteins	
COL1A1	type I procollagen alfa 1
COL2A1	type II procollagen alfa 1
DCN	decorin
BSP	bone sialoprotein
FN-1	fibronectin-1
Members of TGF-β supergene family	
BMP-4	bone morphogenetic protein-4
SMAD-3	mothers against DPP homolog-3
SMAD-6	mothers against DPP homolog-6
Others	
CaSR	calcium-sensing receptor
BGLAP	bone gamma carboxyglutamate protein (osteocalcin)

4.2.9 Statistical analysis

The normal distribution of variables was analyzed by Kolmogorov-Smirnov-Liliefors test. Means of continuous variables were compared between the groups by analysis of variance (ANOVA). The impacts of possible nominal and numerical factors were examined by analysis of covariance (ANCOVA) or multiple regression. We used Mann-Whitney unpaired t-test or Kruskal-Wallis tests to compare numerical variables without normal distribution. Pearson's product moment correlation coefficient was used to measure correlation between the continuous variables. The relation between characteristic variables was examined by Chi-square test or binary logistics regression; we calculated Odds Ratio (OR) and 95 % Confidence Intervals (95% CI). The overall (OS) and disease free survival (DFS) were analyzed by Kaplan-Meier method or Cox regression. Hazard ratio (HR) and 95 % confidence intervals (95% CI) were also calculated. The events were defined as death for OS and disease recurrence for DFS. The survival factor levels were compared with log-rank

test. Furthermore, the survival results were calculated adjusting therapy features. The allele frequency analysis was based upon Hardy-Weinberg probability test. The results were given as mean ± standard deviation (SD) or standard error of mean (SEM) for parameters with normal distribution or median (range, minimum - maximum) for parameters without normal distribution, unless noted otherwise. Significance was defined as $p < 0.05$. The data were analyzed using the SPSS statistical package (SPSS Inc., Chicago, IL; version 15.0 for Windows).

5. Results

5.1 Effects of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of osteoporosis

5.1.1 Basic clinical characteristics and laboratory parameters

Clinical characteristics and laboratory parameters of the whole study population I/a regarding genotypes can be seen in Table 6, Table 7 and Table 8.

5.1.2 LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphism distributions

There was no difference in mutant allele frequencies between subjects with decreased BMD and healthy women (Table 9). Allelic frequencies followed the Hardy-Weinberg equilibrium.

Table 9. The mutant allele frequencies of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in patients with decreased BMD and healthy controls

ALLELE NAME	*ALLELE FREQUENCIES*			*p value*[a]
	OP PATIENTS	*CONTROLS*	*ALL*	
LCT 13910 C	0.65	0.61	0.64	NS
CaSR 986 S	0.17	0.19	0.17	NS
CYP3A7*1C G	0.04	0.02	0.03	NS

[a](patients compared to controls)

5.1.3 LCT 13910 C/T genotype, risk factors of osteoporosis and laboratory parameters

Frequency of aversion to milk consumption was significantly higher for the homozygous mutant genotype (frequency of aversion for CC genotype = 19.66 %, frequency of aversion for TT + TC genotypes = 10.43 %; p = 0.03). The binary logistic regression was also significant in this respect (OR = 2.10, 95 % CI = 1.06 - 4.14; p = 0.032). Moreover, we have seen interaction between LCT 13910 C/T and CaSR A986S polymorphisms on milk consumption; the difference between CCSS (n = 6) and other genotype combinations in this

respect was significant (frequency of aversion for CCSS genotype combination = 66.67 %, for others = 13.53 %; p = 0.0002) (Figure 3).

Albumin-adjusted serum calcium levels were significantly decreased in women with CC as compared to other genotypes (serum calcium for subjects with CC genotype = 2.325 ± 0.09 mmol/L, serum calcium for subjects with TT + TC genotypes 2.360 ± 0.16 mmol/L; p = 0.031) (Figure 4). No other significant difference was observed in other laboratory parameters between genotypes.

Significant association was found between body height and genotypes. Women with CC genotype were shorter by 1.87 cm as compared to other genotypes (p = 0.002) (Figure 5).

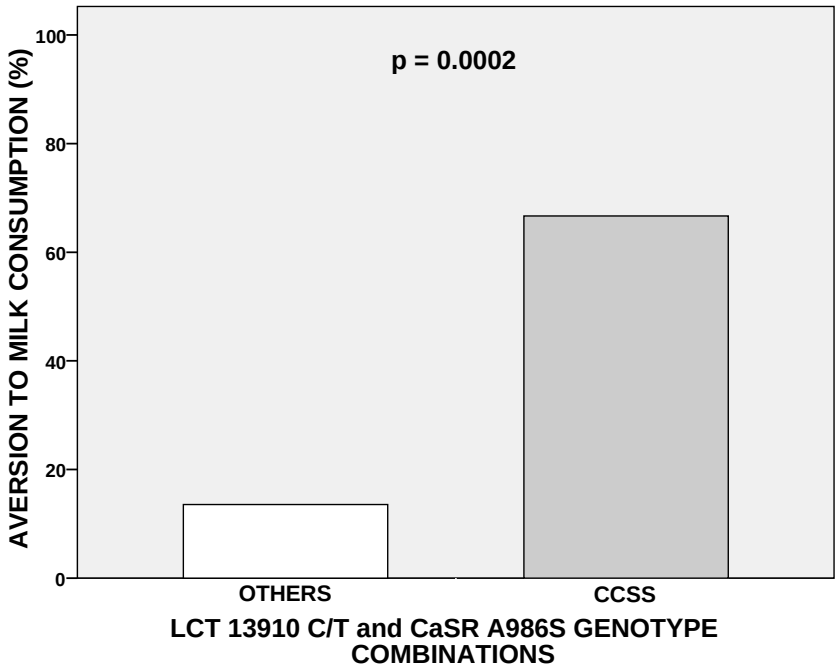


Figure 3. Aversion to milk consumption per 100 subjects in the whole study population (n = 595) according to LCT 13910 C/T and CaSR A986S genotype combinations. Individuals with CCSS genotype combinations have significantly higher frequency of aversion to milk than with other genotype combinations.

5.1.4 CaSR A986S genotype, risk factors for osteoporosis and laboratory parameters

Milk consumption was found to be associated with genotypes (frequency of aversion for SS genotype = 23.52 %, for AS genotype = 7.51 % and for AA genotype = 5.92 %; p = 0.026). There was no association between genotype, other risk factors and laboratory parameters.

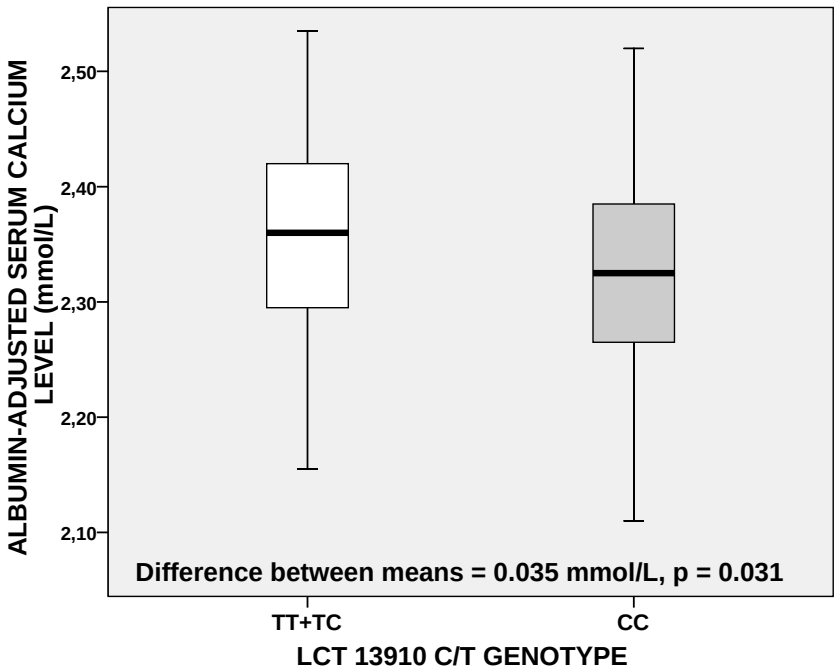


Figure 4. The association between LCT 13910 C/T genotypes and albumin-adjusted serum calcium level in postmenopausal women. The median, the first and the third interquartiles of the distribution are constructing box plots. Whiskers extend 1.5 times the interquartile range above and below the 75th and 25th percentiles. Any values above or below the whiskers are the outliers.

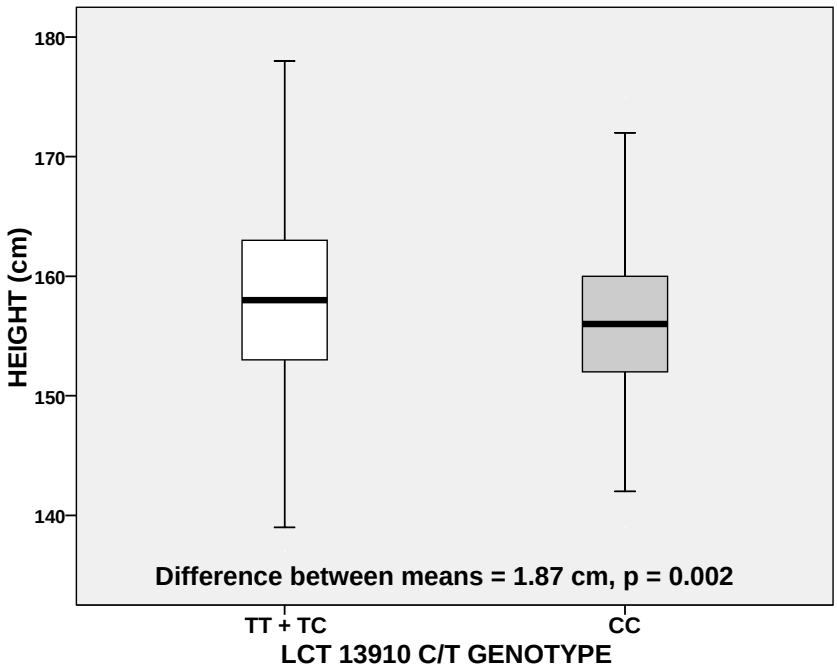


Figure 5. The association between LCT 13910 C/T genotypes and body height in postmenopausal women. The median, the first and the third interquartiles of the distribution are constructing box plots. Whiskers extend 1.5 times the interquartile range above and below the 75th and 25th percentiles. Any values above or below the whiskers are the outliers.

5.1.5 CYP3A7*1C genotype, risk factors for osteoporosis and laboratory parameters

CYP3A7*1C genotype did not related to risk factors for osteoporosis. We did not observe any association between CYP3A7*1C polymorphism and DHEAS concentration in our 319 subjects (Figure 6). Moreover, no correlation was found between genotypes and DHEAS when patients were analyzed separately. Heterozygous carriers of CYP3A7*1C polymorphism had a lower mean DHEAS level by 58.4% compared to that of wild types in the control group that has almost reached significance level (DHEAS level for heterozygous carriers = 1.55 ± 0.56 μmol/l, for wild types = 3.73 ± 2.49 μmol/l; $p = 0.05$). However, after adjustment for age this difference disappeared. Age negatively correlated with serum DHEAS levels in both patient ($r = -0.28$, $p < 0.005$) and control groups ($r = -0.49$, $p < 0.005$), as well as in the whole study population ($r = -0.52$, $p < 0.005$) (Figure 7). We found significant correlation between menopausal age and serum DHEAS levels in controls ($r = -0.43$, $p < 0.005$) and the whole study population ($r = -0.40$, $p < 0.005$). We did not observe other associations between polymorphism and laboratory parameters.

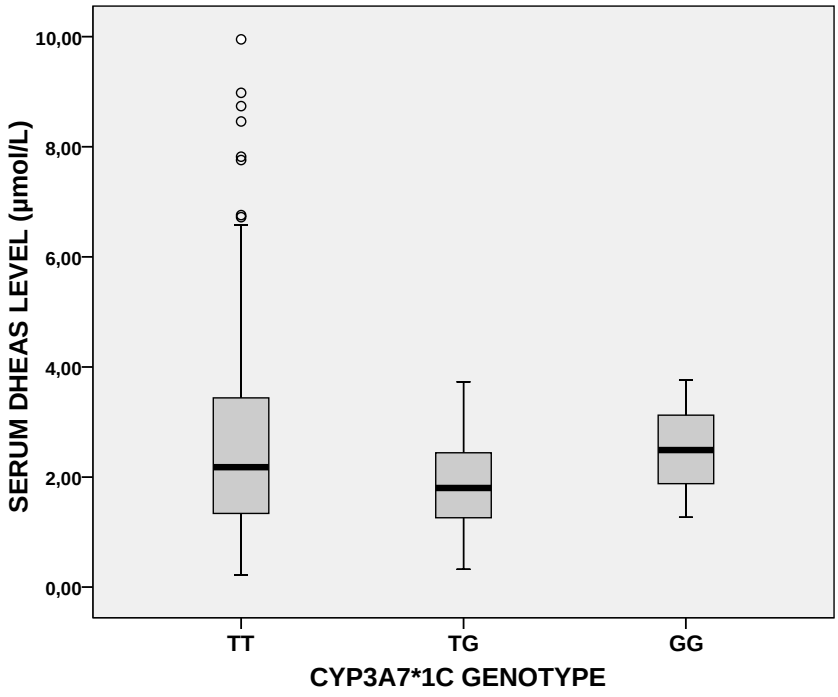


Figure 6. Lack of association between CYP3A7*1C polymorphism and serum DHEAS level in the whole study population. The median, the first and the third interquartiles of the distribution are constructing box plots. Whiskers extend 1.5 times the interquartile range above and below the 75th and 25th percentiles. Any values above or below the whiskers are the outliers.

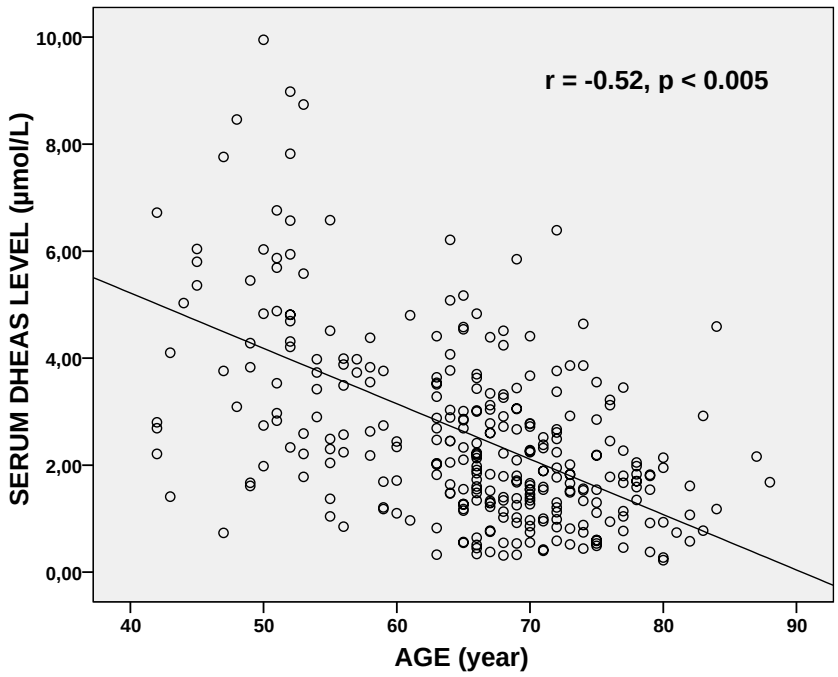


Figure 7. Serum DHEAS levels correlated negatively with age in the whole study population (r = -0.52, p < 0.005).

5.1.6 LCT 13910 C/T genotype and BMD

CC genotypes were associated with significantly reduced density (p = 0.033), T (p = 0.018) and Z-scores (p = 0.038) at the radius as compared to TT + TC genotypes (Figure 8). At the femoral neck, density (p = 0.038), T (p = 0.049) and Z-scores (p = 0.044) of the Ward's triangle were lower for homozygous mutants as compared to those of other genotypes. At the total hip we found a significant difference in Z-scores between CC and TT genotypes (p = 0.041).

By excluding osteopenic subjects and analyzing only a group consisting of osteoporotic and healthy women (n = 395), a more marked influence of CC genotype on BMD could be demonstrated (Table 10). In this group we observed an association between lumbar spine (L1-4) Z-score, T-score and LCT genotypes: all these BMD parameters were significantly decreased in subjects with CC genotype as compared to others. We also observed significantly decreased T- and Z-scores at the Ward's triangle in CC genotype. We have seen a similar reduction at the total hip in CC, however, only difference in Z-scores was found to be significant.

In multiple regression analysis for Z-score using BMI as covariant, the percentage variance of BMD defined by LCT 13910 C/T genotype was 1.1 % at the radius (p = 0.048) and 1.7 % at total hip (p = 0.018). At the lumbar spine no significant result was found for this analysis. BMI in the whole study population accounted for 3.9 % of BMD variation at the radius (p < 0.005) and 13.4 % at the total hip (p < 0.005).

All other variables, such as menopausal age, smoking habits, history of steroid use, previous vertebral or non-vertebral fractures, milk aversion, alcohol and caffeine consumption, daily calcium intake did not influence BMD variance in this model. The vertebral or non-vertebral bone fracture incidence was not different according to genotypes.

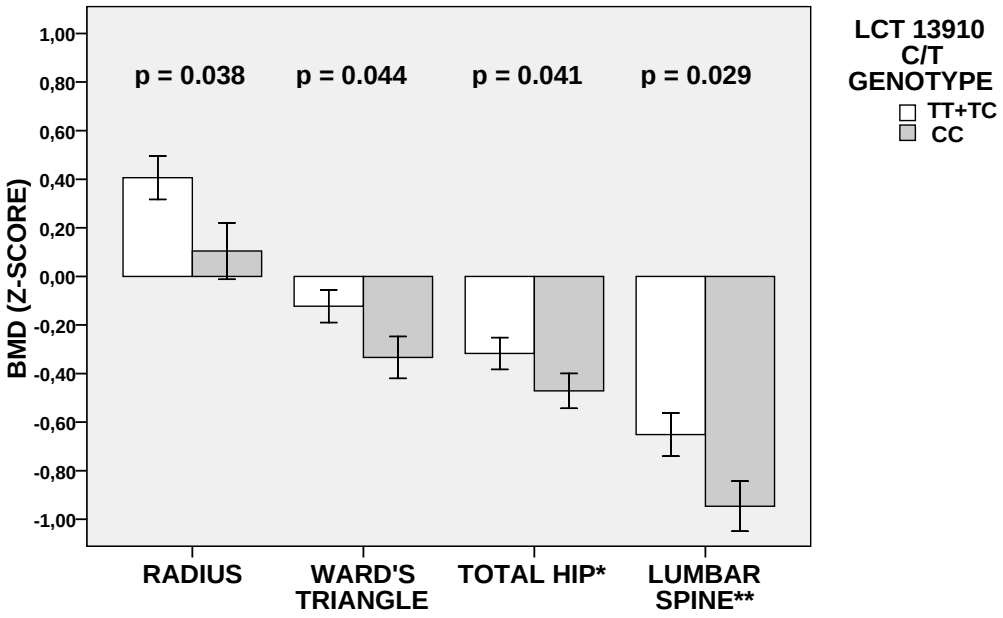


Figure 8. BMD (Z-score) at radius, Ward's triangle and total hip according to LCT 13910 C/T genotypes in the whole study population (n = 595). Individuals with CC genotype have significantly lower BMD compared to other genotypes. The bar chart designates the mean, and the error bars illustrate the standard error of mean.* denotes significant difference only in the CC and TT comparison. ** in group consisting of osteoporotic and healthy women (n=395).

Table 10. Relationship of BMD (Z- and T-score) and genotypes in the subgroup of women with osteoporosis and healthy subjects (n = 395)

Genotypes and p value	Ward's Triangle Z-score	Ward's Triangle T-score	Total hip Z-score	Total hip T-score	Lumbar spine Z-score	Lumbar spine T-score
CC	-0.343 ± 0.91	-1.916 ± 1.24	-0.658 ± 1.16	-1.834 ± 1.61	-0.946 ± 1.29	-2.047 ± 1.53
TT	0.161 ± 0.80	-1.322 ± 1.04	-0.231 ± 1.21	-1.461 ± 1.61	-0.651 ± 1.35	-1.754 ± 1.52
p	0.021	0.027	0.033	NS	0.029[a]	0.043[a]

[a] In the statistical analysis we compared the parameter for CC genotype and for TT + TC genotypes. NS = non significant

5.1.7 CaSR A986S genotype and BMD

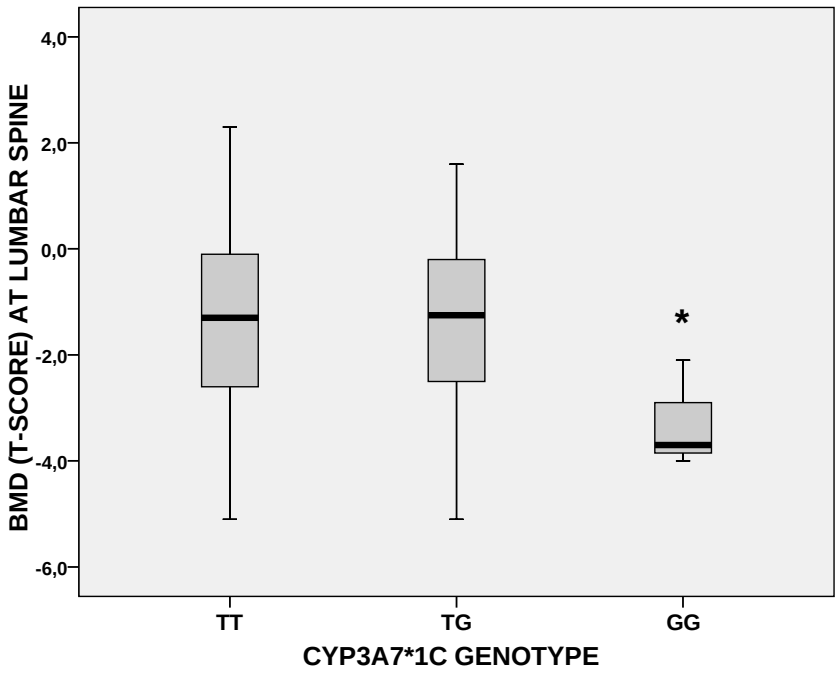
CaSR A986S polymorphism was found to be associated with OP only in the unified group of osteopenic and healthy subjects (n = 328): total hip Z-score was significantly lower for SS than other genotypes (total hip Z-score for SS genotype = -0.372 ± 0.71, Z-score for AA + AS genotype = 0.274 ± 0.92, p = 0.036). In multiple regression analysis for Z-score using BMI as covariant, the percentage variance of BMD defined by CaSR genotype was 1.3 % at total hip (p = 0.04).

5.1.8 CYP3A7*1C genotype and BMD

The homozygous mutant GG genotype associated with significantly reduced T-score (p = 0.041) and Z-score (p = 0.048) at lumbar spine as compared to wild type in the whole study population. This correlation remained significant after adjusting for age and DHEAS level (Figure 9/a., Figure 9/b.). The association between CYP3A7 genotype and BMD remained also significant after adjustment for menopausal age, alcohol consumption, steroid intake, smoking habits and previous fractures (for T-scores p = 0.038, for Z-scores p = 0.047) or BMI and DHEAS levels (for T-scores p = 0.03, for Z-scores p = 0.046). No correlation was found between genotypes and BMD measured at the femoral neck.

5.1.9 Serum calcium, DHEAS level, risk factors for osteoporosis and BMD

Serum calcium level did not correlate with BMD at any sites. Serum DHEAS levels were found to be positively correlated with BMD (T-score) at lumbar spine (L2-4) (r = 0.59, p = 0.042) (Figure 10) after correction for age in the whole study population. Similar correlation could be observed among controls (r = 0.36, p = 0.04). No significant correlation was seen between DHEAS and BMD in the patient group. We found significant regression between serum DHEAS levels and BMD expressed as Z-score at both lumbar spine (r = 0.23, p < 0.005) and femoral neck (r = 0.17, p = 0.003) in the whole study population. Furthermore, there were significant associations between BMD at lumbar spine (Z-score and T-score) and other confounding factors such as age, menopausal age, BMI, alcohol consumption and steroid intake (Table 11). Serum DHEAS also significantly correlated with alcohol consumption (p < 0.005). Daily calcium intake and smoking habits were not associated with BMD.



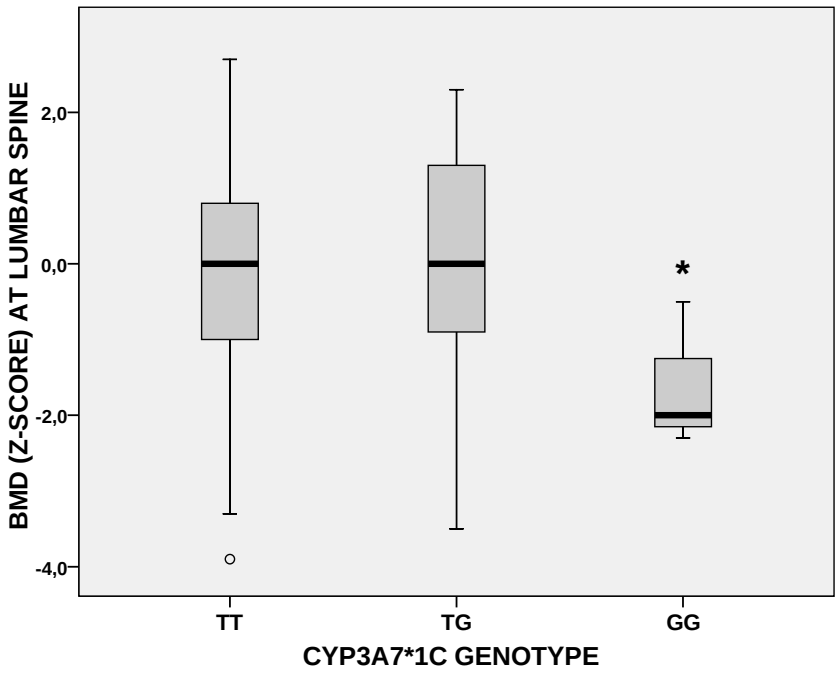


Figure 9/a., 9/b. The association between CYP3A7 genotypes and BMD values at the lumbar spine T-scores (9/a) (* p=0.038) and Z-score (9/b) (* p=0.047) after adjustment for menopausal age, DHEAS level, alcohol consumption, steroid intake, smoking habits, previous fractures in the whole study population. The median, the first and the third interquartiles of the distribution are constructing box plots. Whiskers extend 1.5 times the interquartile range above and below the 75th and 25th percentiles. Any values above or below the whiskers are the outliers.

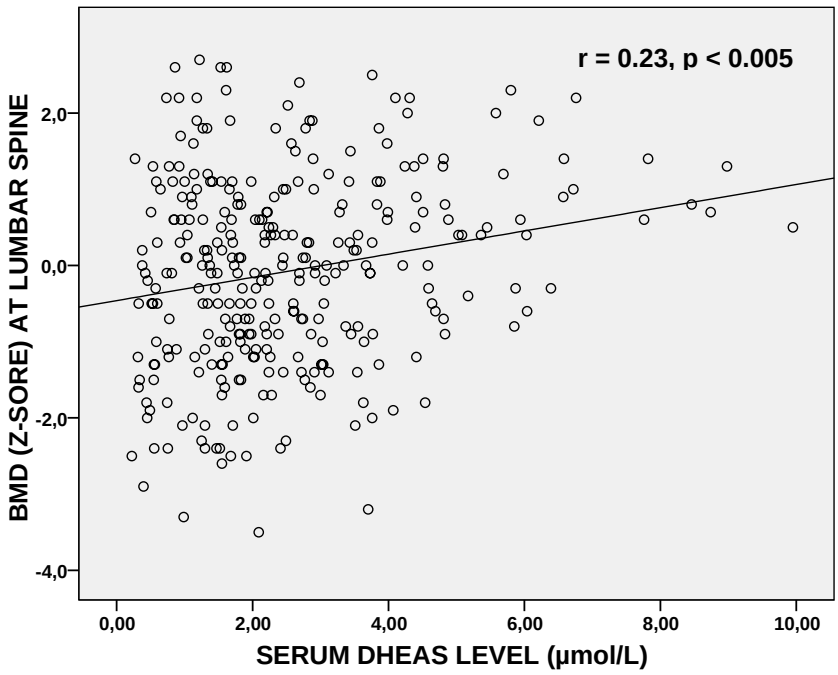


Figure 10. Serum DHEAS levels correlated positively with BMD at the lumbar spine (Z-score) in the whole study population (Z-score: r=0.23, p<0.005).

Table 11. Significant association between risk factors and BMD in the whole study population

RISK FACTORS	*PEARSON'S COEFFICIENTS (p value)* *Lumbar spine*	
	Z score	*T score*
Age (year)	**-0.29 (<0.005)**	**-0.58 (<0.005)**
Menopausal age (year)	**-0.32 (<0.005)**	**-0.55 (<0.005)**
BMI (kg/m^2)	**0.22 (<0.005)**	**0.22 (<0.005)**
DHEAS (µmol/l)	**0.23 (<0.005)**	**0.39 (<0.005)**
Daily consumption of alcohol[a]	**- (0.02)**	**- (0.005)**
History of steroid intake[a]	**- (0.02)**	**- (<0.005)**

[a] In statistical analysis comparing continous varibales of string variables

Table 6. Clinical characteristics, parameters of bone and calcium metabolism and BMD of postmenopausal women according to LCT 13910 C/T genotypes

	LCT 13910 C/T GENOTYPE			
	TT (n = 76)	*TC* (n = 277)	*CC* (n = 242)	*p value*
Clinical charecteristics (mean ± SD or case number [%])				
Age (year)	61.84 ± 10.37	60.54 ± 9.73	61.45 ± 10.05	NS
Menopausal age (year)	13.53 ± 11.16	12.57 ± 11.68	14.27 ± 10.56	NS
Height (cm)	157.68 ± 6.82	157.99 ± 7.05	156.27 ± 6.91	**0.002**[a]
Weight (kg)	67.82 ± 12.35	67.32 ± 11.72	66.58 ± 11.78	NS
Body mass index (kg/m^2)	26.94 ± 4.59	27.79 ± 4.47	27.36 ± 5.51	NS
Serum calcium (mmol/L) Normal range (2.2 – 2.6)	2.47 ± 0.14	2.44 ± 0.18	2.44 ± 0.11	NS
Adjusted serum calcium (mmol/L) Normal range (2.09 – 2.54)	2.37 ± 0.10	2.34 ± 0.18	2.33 ± 0.09	**0.031**[a]
Serum phosphorus (mmol/L) Normal range (0.8 – 1.45)	1.09 ± 0.16	1.16 ± 0.25	1.14 ± 0.15	NS
25-OH-Vitamin D_3 (nmol/L) Normal range (75 – 160)	64.09 ± 18.27	67.86 ± 22.79	67.29 ± 22.86	NS
Serum crosslaps (pg/mL) Normal range (0 – 320)	237.83 ± 192.07	237.88 ± 204.19	240.5 ± 197.10	NS
Serum alkaline phosphatase (U/L) Normal range (35 - 123)	77.39 ± 21.25	85.87 ± 23.97	83.89 ± 22.93	NS
Smoker	13 (17)	43 (16)	44 (18)	NS
Daily consumption of alcohol	44 (58)	164 (59)	140 (58)	NS
Daily consumption of caffeine	71 (94)	258 (93)	227 (94)	NS
History of steroid intake	8 (10)	17 (6)	18 (8)	NS
Frequency of aversion to milk consumption	5 (6)	32 (12)	48 (20)	**0.03**[a]
History of nonvertebral fractures	19 (25)	85 (31)	68 (28)	NS
History of vertebral fractures	14 (18)	47 (17)	46 (19)	NS
BMD				
Radius BMD (g/cm^2)	0.75 ± 0.18	0.73 ± 0.17	0.71 ± 0.17	**0.033**[a]
Radius Z-score	0.43 ± 1.37	0.40 ± 1.31	0.10 ± 1.42	**0.038**[a]
Radius T-score	-1.65 ± 2.06	-1.57 ± 1.94	-2.10 ± 1.99	**0.018**[a]
Ward's triangle BMD (g/cm^2)	0.60 ± 0.18	0.59 ± 0.17	0.55 ± 0.28	**0.038**[a]
Ward's triangle Z-score	0.08 ± 0.79	-0.18 ± 0.83	-0.33 ± 0.87	**0.044**[a]
Ward's triangle T-score	-1.61 ± 1.08	-1.81 ± 1.14	-2.05 ± 1.14	**0.049**[a]
Total hip BMD (g/cm^2)	0.82 ± 0.18	0.82 ± 0.19	0.79 ± 0.16	NS
Total hip Z-score	-0.17 ± 1.09	-0.36 ± 1.22	-0.47 ± 1.08	**0.041**[b]
Total hip T-score	-1.39 ± 1.37	-1.57 ± 1.49	-1.69 ± 1.41	NS
Femur head BMD (g/cm^2)	0.87 ± 0.12	0.87 ± 0.14	0.79 ± 0.51	NS
Femur head Z-score	0.16 ± 0.88	0.18 ± 0.93	-0.03 ± 0.92	NS
Femur head T-score	-1.06 ± 1.09	-1.05 ± 1.16	-1.29 ± 1.16	NS
Lumbar spine BMD (L1-4) (g/cm^2)	0.95 ± 0.19	0.93 ± 0.19	0.90 ± 0.19	NS
Lumbar spine Z-score	-0.45 ± 1.27	-0.56 ± 1.16	-0.71 ± 1.18	NS
Lumbar spine T-score	0.95 ± 0.19	0.93 ± 0.19	0.90 ± 0.19	NS

[a] In the statistical analysis we compared the parameter for CC genotype and for TT + TC genotypes. [b] In the statistical analysis we compared the parameter for CC genotype and for TT genotype. NS = non significant

Table 7. Clinical characteristics, parameters of bone and calcium metabolism and BMD of postmenopausal women according to CaSR A986S genotypes

	CaSR A986S GENOTYPE			
	AA (n = 405)	*AS* (n = 173)	*SS* (n = 17)	*p value*
Clinical charecteristics (mean ± SD or case number [%])				
Age (year)	60.99 ± 9.92	60.88 ± 9.91	61.41 ± 10.51	NS
Menopausal age (year)	13.43 ± 11.36	13.03 ± 10.43	13.36 ± 12.21	NS
Height (cm)	157.61 ± 7.29	157.11 ± 6.16	157.00 ± 5.41	NS
Weight (kg)	67.86 ± 12.05	68.37 ± 10.94	69.22 ± 13.58	NS
Body mass index (kg/m^2)	27.37 ± 5.05	27.75 ± 4.56	28.16 ± 5.73	NS
Serum calcium (mmol/L) Normal range (2.2 – 2.6)	2.44 ± 0.11	2.43 ± 0.22	2.46 ± 0.08	NS
Adjusted serum calcium (mmol/L) Normal range (2.09 – 2.54)	2.35 ± 0.10	2.32 ± 0.21	2.35 ± 0.06	NS
Serum phosphorus (mmol/L) Normal range (0.8 – 1.45)	1.13 ± 0.15	1.16 ± 0.29	1.04 ± 0.22	NS
25-OH-Vitamin D_3 (nmol/L) Normal range (75 – 160)	67.27 ± 21.92	67.50 ± 24.08	62.98 ± 16.91	NS
Serum crosslaps (pg/mL) Normal range (0 – 320)	231.61 ± 206.20	250.57 ± 176.27	167.00 ± 110.37	NS
Serum alkaline phosphatase (U/L) Normal range (35 - 123)	85.17 ± 23.82	81.48 ± 22.34	78.17 ± 17.66	NS
Smoker	63 (16)	29 (17)	1 (6)	NS
Daily consumption of alcohol	223 (55)	94 (54)	11 (65)	NS
Daily consumption of caffeine	267 (66)	107 (62)	10 (59)	NS
History of steroid intake	31 (8)	8 (5)	3 (18)	NS
Frequency of aversion to milk consumption	24 (6)	13 (8)	4 (24)	**0.026**
History of nonvertebral fractures	114 (28)	45 (26)	2 (12)	NS
History of vertebral fractures	34 (8)	14 (8)	1 (6)	NS
BMD				
Radius BMD (g/cm^2)	0.72 ± 0.17	0.73 ± 0.18	0.66 ± 0.14	NS
Radius Z-score	0.27 ± 1.39	0.25 ± 1.34	0.08 ± 1.12	NS
Radius T-score	-1.85 ± 1.98	-1.67 ± 1.94	-2.45 ± 1.89	NS
Ward's triangle BMD (g/cm^2)	0.57 ± 0.25	0.60 ± 0.16	0.54 ± 0.11	NS
Ward's triangle Z-score	-0.24 ± 0.86	-0.12 ± 0.84	-0.41 ± 0.40	NS
Ward's triangle T-score	-1.91 ± 1.14	-1.79 ± 1.18	-2.31 ± 0.44	NS
Total hip BMD (g/cm^2)	0.81 ± 0.17	0.82 ± 0.20	0.77 ± 0.12	NS
Total hip Z-score	-0.41 ± 1.15	-0.29 ± 1.18	-0.66 ± 0.84	NS
Total hip T-score	-1.65 ± 1.42	-1.49 ± 1.43	-1.99 ± 1.05	NS
Femur head BMD (g/cm^2)	0.82 ± 0.40	0.88 ± 0.15	0.83 ± 0.04	NS
Femur head Z-score	0.04 ± 0.91	0.21 ± 0.98	0.09 ± 0.22	NS
Femur head T-score	-1.20 ± 1.13	-1.02 ± 1.25	-1.29 ± 0.29	NS
Lumbar spine BMD (L1-4) (g/cm^2)	0.92 ± 0.04	0.93 ± 0.19	0.91 ± 0.03	NS
Lumbar spine Z-score	-0.63 ± 1.18	-0.58 ± 1.20	-0.56 ± 1.09	NS
Lumbar spine T-score	-1.78 ± 1.29	-1.74 ± 1.33	-1.79 ± 1.15	NS

NS = non significant

Table 8. Clinical characteristics, DHEAS level and BMD of postmenopausal women according to CYP3A7*1C genotypes

	*CYP3A7*1C GENOTYPE*			
	TT (n = 302)	TG (n = 14)	GG (n = 3)	*p value*
Clinical charecteristics (mean ± SD or case number [%])				
Age (year)	64.54 ± 9.77	64.71 ± 7.71	70.33 ± 1.16	NS
Menopausal age (year)	14.75 ± 11.05	14.13 ± 5.87	23.67 ± 3.52	NS
Height (cm)	157.58 ± 7.68	161.58 ± 6.32	153.33 ± 2.52	NS
Weight (kg)	70.44 ± 12.49	69.04 ± 8.68	64.10 ± 7.10	NS
Body mass index (kg/m^2)	32.39 ± 11.58	29.90 ± 7.34	26.96 ± 5.67	NS
Serum calcium (mmol/L) Normal range (2.2 – 2.6)	2.44 ± 0.15	2.48 ± 0.08	2.41 ± 0.07	NS
Adjusted serum calcium (mmol/L) Normal range (2.09 – 2.54)	2.34 ± 0.14	2.37 ± 0.07	2.44 ± 0.06	NS
Serum albumin (g/L) Normal range (35 – 50)	44.04 ± 2.72	44.86 ± 2.04	39.00 ± 2.01	NS
Serum phosphorus (mmol/L) Normal range (0.8 – 1.45)	1.14 ± 0.20	1.15 ± 0.13	0.96 ± 0.11	NS
25-OH-Vitamin D_3 (nmol/L) Normal range (75 – 160)	67.05 ± 22.46	72.91 ± 19.12	70.00 ± 15.92	NS
Serum alkaline phosphatase (U/L) Normal range (35 - 123)	84.30 ± 23.25	67.71 ± 17.56	111.00 ± 13.32	NS
Smoker	48 (16)	3 (38)	0 (0)	NS
Daily consumption of alcohol	40 (13)	1 (13)	1 (33)	NS
History of steroid intake	15 (5)	1 (13)	0 (0)	NS
History of nonvertebral fractures	87 (29)	3 (38)	2 (67)	NS
BMD				
Lumbar spine Z-score (L2-4)	-0.06 ± 1.27	-0.03 ± 1.54	-1.60 ± 0.96	**0.047**[ab]
Lumbar spine T-score	-1.35 ± 1.53	-1.43 ± 1.70	-3.27 ± 1.02	**0.038**[ab]
Femoral neck Z-score	-0.15 ± 0.93	-0.01 ± 0.81	-0.4 ± 0.26	NS
Femoral neck T-score	-1.01 ± 1.24	-1.21 ± 1.02	-1.93 ± 0.21	NS
Hormone				
DHEAS (µmol/l)	2.61 ± 1.92	1.93 ± 0.99	2.51 ± 1.25	NS

[a] after adjustment for menopausal age, DHEAS level, alcohol consumption, steroid intake, smoking habits, previous fractures; [b] TT vs GG . NS= non significant

5.2 Effects of LCT13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of colorectal cancer

5.2.1 Laboratory and tumor parameters

Clinical characteristics of the patient population including TNM and AJCC stages as well as features of therapy are shown in Table 12 and Table 13.

5.2.2 LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphism distributions

The distribution of C allele of the LCT gene appeared to be more frequent among patients with CRC than control subjects, however, the difference did not reach the significance level (0.622 for patients and 0.590 for controls; p = 0.30) (Table 14). LCT genotypes had no association with cancer incidence. However, CCSS (n = 5) genotype distribution significantly differenced between study groups (frequency of CCSS genotype combinations for patients = 1.79 %, for control group = 0 %; p = 0.033).

Frequency of the S allele was found to be significantly higher in CRC patients than in the control population (0.196 for patients and 0.146 for control group, p = 0.026) (Table 14). The same trend was also seen in the subgroups: higher S allele frequency was found in women (0.189, p = 0.024) (n = 95) and in men (0.208, p = 0.028) (n = 120), in patients with metastasis at the time of diagnosis (0.195, p = 0.029) (n = 64) or without metastasis (0.202, p = 0.026) (n = 151) and in subjects with rectum cancer (0.201, p = 0.023) (n = 184). CaSR A986S genotypes were associated with cancer incidence, as well. The homozygous S genotype was found to be more frequent in patients than controls utilizing the recessive (OR = 3.99; 95 % CI = 1.32-12.02; p = 0.014) or interval-scaled model (OR = 1.39; 95 % CI = 1.01-1.91; p = 0.041), or comparing SS to AA as reference (OR = 4.11; 95 % CI = 1.36-12.46; p = 0.012).

There was no difference in CYP3A7*1C allele frequencies among subjects with CRC and controls (Table 14). Allelic frequencies followed the Hardy-Weinberg equilibrium.

Table 14. The mutant allele frequencies of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in patients with CRC and healthy subjects

ALLELE NAME	*ALLELE FREQUENCIES*			*p value*[a]
	CRC PATIENTS	*CONTROLS*	*ALL*	
LCT 13910 C	0.62	0.59	0.61	NS
CaSR 986 S	0.20	0.15	0.17	**0.026**
CYP3A7*1C G	0.03	0.03	0.03	NS

[a](patients compared to controls)

5.2.3 LCT 13910 C/T genotype and laboratory parameters

Serum CA19-9, AFP and CEA levels did not correlate with LCT genotypes. Patients with TCSS genotype combination (n = 7) had higher CA19-9 concentrations as compared to others (CA19-9 level for TCSS = 129.70 (6.88 – 2482.00) U/mL and for other genotype combinations = 8.97 (0.30 – 123429.00) U/mL; p = 0.036). Female patients (n = 95) with LCT 13910 CC genotypes had lower serum calcium concentrations (calcium for CC genotypes = 2.35 ± 0.14 mmol/L and for TT + TC genotypes = 2.41 ± 0.11 mmol/L; p = 0.038). Significant interaction was found between the two polymorphisms on serum calcium concentrations in female patients. Subjects with TCSS genotype combination (n = 3) had significantly elevated serum calcium level as compared to others (calcium for TCSS = 2.45 ± 0.28 mmol/L, calcium for other genotype combinations = 2.29 ± 0.11 mmol/L; p = 0.025). The difference remained significant combining rare genotype combinations as TCSS, TTSS and CCSS (n = 6) (calcium for rare genotype combinations = 2.40 ± 0.19 mmol/L, calcium for others = 2.29 ± 0.10 mmol/L; p = 0.033). No similar relationship could be seen in men.

5.2.4 CaSR A986S genotype and laboratory parameters

An association between AFP levels and CaSR A986S polymorphism in male patients with metastasis at the time of diagnosis (n = 35) was detected: male patients with SS genotype had increased AFP concentrations (AFP level for SS genotypes = 7.29 ± 4.25 ng/mL and for AA + AS genotypes = 3.73 ± 2.08 ng/mL; p = 0.045). No correlations were observed between CaSR A986S genotypes and CA19-9 or CEA levels. In female patients with CaSR 986 SS genotypes, serum calcium levels were higher as compared to other genotypes (calcium for SS genotypes = 2.40 ± 0.19 mmol/L and for AS + AA genotypes = 2.29 ± 0.11 mmol/L; p = 0.033).

5.2.5 CYP3A7*1C genotype and laboratory parameters

CYP3A7*1C polymorphism had no effect on laboratory parameters.

5.2.6 LCT 13910 C/T polymorphism and tumor parameters

In patients with distant metastasis significantly higher CC frequency was seen during the follow-up period, examined by both recessive (OR = 2.52; 95 % CI = 1.23-5.17; p = 0.012) or interval-scaled models (OR = 1.67; 95 % CI = 1.01-2.75; p = 0.045). There was no difference between the mean age of patients with CC genotype (mean age = 60 ± 11 years) and other genotypes (mean age = 61 ± 10 years). In the whole study population we could not see any association between genotypes and DFS. In men (n = 120) an increased CC genotype frequency was observed for locoregional and distant metastasis together compared to TT as the reference genotype (OR = 5.00; 95 % CI = 1.26-19.86; p = 0.022), and the difference remained significant in the recessive (OR = 2.46; 95 % CI = 1.02-5.96; p = 0.046) or interval-scaled models (OR = 2.09; 95 % CI = 1.13-3.85; p = 0.018), as well. The DFS was significantly shorter for CC genotypes in the Kaplan-Meyer method (log-rank test, p = 0.021) (Figure 11) or in the Cox-regression if we used the recessive (HR = 2.69; 95 % CI = 1.12-6.49; p = 0.028) or interval-scaled model (HR = 2.04; 95 % CI = 1.07-3.91; p = 0.031). Results remained significant after adjustment for the type of therapy in both recessive (HR = 2.78; 95 % CI = 1.15-6.72; p = 0.024) or interval-scaled model (HR = 2.09; 95 % CI = 1.09-4.02; p = 0.025). There was no difference between the mean age of patients with CC genotype (mean age = 62 ± 9 years) and other genotypes (mean age = 61 ± 9 years). In women we detected shorter survival by Kaplan-Meyer method only for genotype combination and not for single genotypes: DFS was worse for TCSS (n = 3) (log rank test p < 0.005) (Figure 12). The result remained significant after adjustment for type of therapy (HR = 14.49; 95 % CI = 1.14-184.54; p = 0.039). There was no difference between the mean age of patients with TCSS genotype combinations (mean age = 65 ± 8 years) and other genotype combinations (mean age = 60 ± 11 years).

The T and N stages were related to LCT genotypes in patients with metastasis at the time of diagnosis (n = 67). The presence of C allele was accompanied by higher T stage (p=0.03). Furthermore, in subjects with non-rectal colon cancer (n = 94) higher N stage was found in the CC genotypes (p = 0.037). We could not find any associations between overall

survival (OS), AJCC stages, histological grade or localization and LCT genotypes.

5.2.7 CaSR A986S polymorphism and tumor parameters

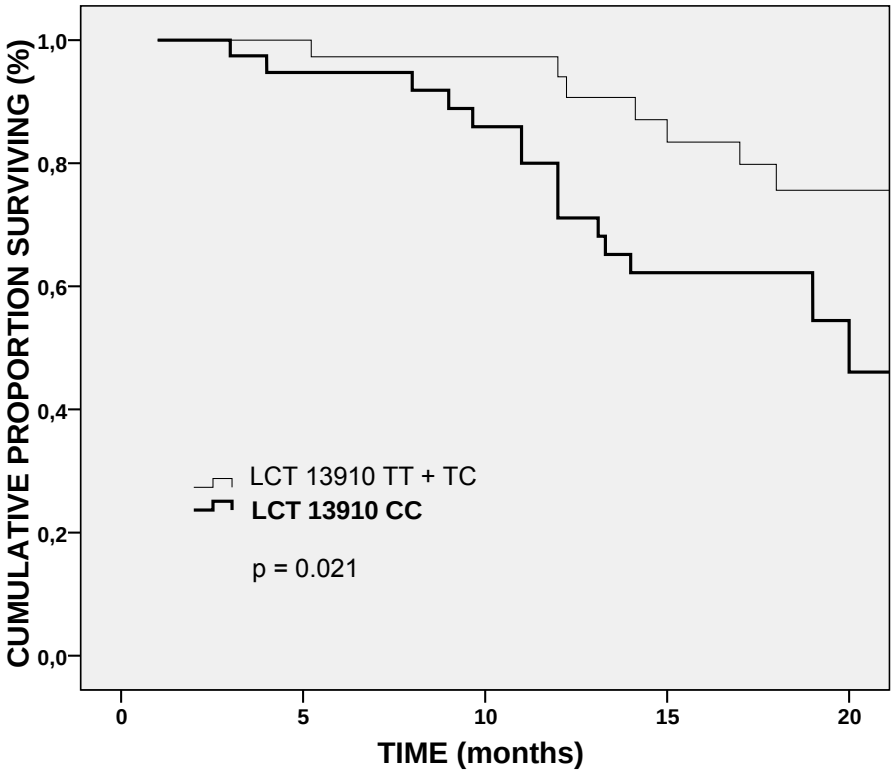
There was no correlation between locoregional or distant metastases, disease-free or overall survival, TNM, AJCC stages, histological grade, localization and CaSR A986S genotypes.

5.2.8 CYP3A7*1C polymorphism and tumor parameters

There was no correlation between locoregional or distant metastases, disease-free or overall survival, TNM, AJCC stages, histological grade, localization and CYP3A7*1C genotypes.

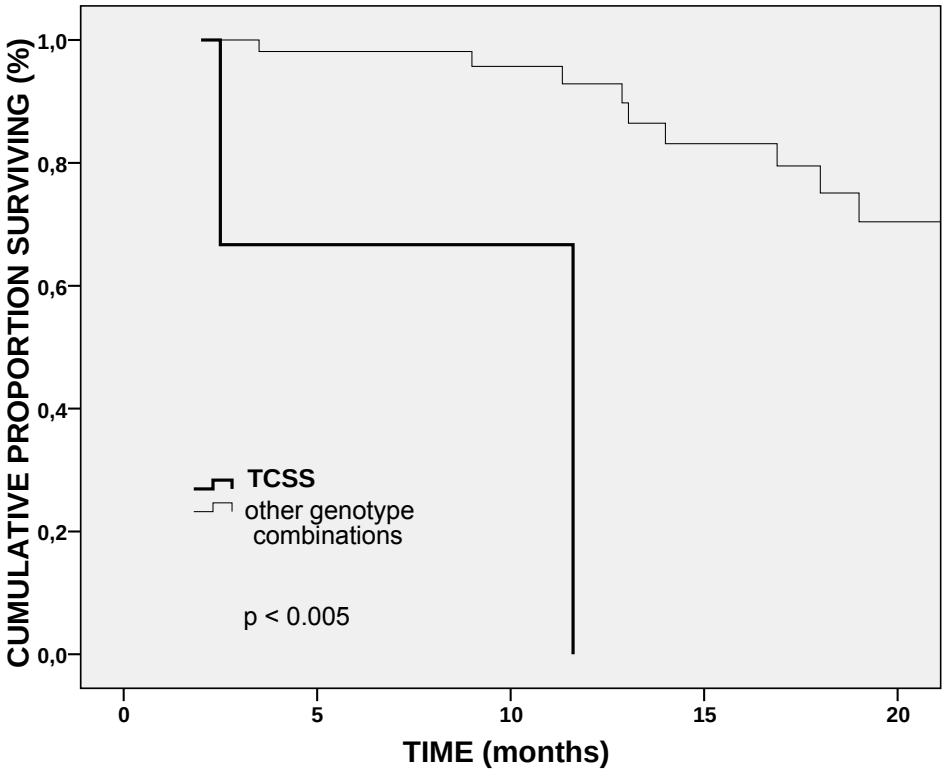
5.2.9 Serum calcium level and tumor parameters

A correlation between serum calcium level and T stages was observed in male CRC patients. In patients with lower serum calcium levels the T stages was higher (serum calcium levels for T1 = 2.52 ± 0.11 mmol/L, for T2 = 2.46 ± 0.09 mmol/L, for T3 = 2.38 ± 0.11 mmol /L, for T4 = 2.34 ± 0.11 mmol/L; p = 0.009). There was no association between serum calcium concentration and other tumor parameters.



LCT 13910 C/T GENOTYPES	NUMBER OF PATIENTS AT RISK				
TT+TC	37	37	31	23	13
CC	39	34	29	21	11

Figure 11. Kaplan-Meyer estimates of LCT 13910 genotypes for disease-free survival (DFS) in male CRC patients. Patients with LCT 13910 CC genotype had worse outcome compared to TC + TT genotypes (log rank test p = 0.021).



LCT 13910, CaSR 986 GENOTYPE COMBINATIONS		NUMBER OF PATIENTS AT RISK			
TCSS	3	1	1	0	0
Other	54	49	37	24	15

Figure 12. Kaplan-Meyer estimates of TCSS genotype combinations for disease-free survival (DFS) in female CRC patients. Patients with TCSS genotype combination had worse outcome compared to others (log rank test $p < 0.005$).

Table 12. Clinical characteristics and tumor parameters of patients according to LCT 13910 C/T and CaSR A986S genotypes

	LCT 13910 GENOTYPE			*CaSR A986S GENOTYPE*		
	TT + TC (n = 164)	CC (n = 114)	p value	AA + AS (n = 261)	SS (n = 17)	p value
Clinical charecteristics (mean ± SD or case number [%])						
Age at time of diagnosis (years)	60 ± 11	61 ± 10	NS	60 ± 10	62 ± 13	NS
Gender			NS			NS
Female	59 (62)	36 (38)		88 (93)	7 (7)	
Male	66 (55)	54 (45)		112 (93)	8 (6)	
Serum calcium (mmol/L)	2.39 ± 0.12	2.38 ± 0.13	NS	2.29 ± 0.11	2.35 ± 0.17	NS
Normal range (2.2 – 2.6)						
Female	2.41 ± 0.11	2.35 ± 0.14	**0.038**	2.29 ± 0.11	2.40 ± 0.19	**0.033**
Male	2.37 ± 0.12	2.39 ± 0.11	NS	2.29 ± 0.12	2.31 ± 0.14	NS
Serum albumin (g/L)	44.3 (27-52)	44.7 (25-51)	NS	44.6 (25-52)	43.1 (35-47)	0.05
Nomal range (35 – 50)						
Tumor parameters						
Tumor localization groups			NS			NS
Colon vs. rectum	0.55	0.46		0.52	0.42	
T stage groups			NS			NS
1, 2,3 vs 4	3.46	4.29		4.00	2.00	
N stage groups			NS			NS
0, 1 vs 2	3.46	2.91		3.16	4.67	
M stages			NS			NS
0	87 (57)	66 (43)		142 (93)	11 (7)	
1	38 (61)	24 (39)		58 (94)	4 (6)	
Histological grade			NS			NS
High	5 (46)	6 (54)		9 (82)	2 (18)	
Middle	98 (57)	75 (43)		162 (94)	11 (6)	
Low	22 (71)	9 (29)		29 (94)	2 (6)	
AJCC stage groups			NS			NS
1-2B	31 (58)	22 (42)		49 (92)	4 (8)	
3A-4	94 (58)	68 (42)		151 (93)	11 (7)	
Recurrence*	28 (48)	30 (52)	NS	54 (93)	4 (7)	NS
Locoregional recurrence*	12 (52)	11 (48)	NS	22 (96)	1 (4)	NS
Distant metastases*	18 (41)	26 (59)	**0.011**	41 (93)	3 (7)	NS
Survival						
DFS (months)	6 (1-20)	9 (2-20)	NS	8 (1-20)	6 (3-20)	NS
OS (months)	16 (1-20)	17 (1-20)	NS	17 (1-20)	17 (3-20)	NS
Death*	17 (58.6)	12 (41.4)	NS	28 (96.6)	1 (3.4)	NS
Tumor markers						
Serum CEA (ng/mL)	3.47 (0.2-4426)	3.42 (0.2-5200)	NS	3.38 (0.2-5200)	3.53 (0.44-2029)	NS
Normal range < 4.3						
Serum CA19-9 (U/mL)	10.25 (0.3-10932)	8.52 (0.3-123429)	NS	9.52 (0.3-123429)	54.43 (0.3-2482)	NS
Normal range < 39						
Serum AFP (ng/mL)	3.72 (1.13-132.1)	3.36 (0.85-14.2)	NS	3.53 (0.85-132.1)	2.85 (2.09-10.3)	NS
Normal range < 13.6						
Therapy						
Neoadjuvant chemotherapy and radiotherapy*	66 (59)	46 (41)	NS	105 (94)	7 (6)	NS
Surgical resection*	103 (57)	79 (43)	NS	171 (94)	11 (6)	NS
Adjuvant chemotherapy*	63 (58)	45 (42)	NS	102 (94)	6 (6)	NS
Palliative therapy*	62 (56)	49 (44)	NS	103 (93)	8 (7)	NS

* χ^2 test was used for the number of patients presenting and lacking the examined parameters.

No tumor parameters and clinical follow-up was available for 63 patients.

Table 13. Clinical characteristics and tumor parameters of patients according to CYP3A7*1C genotypes

	*CYP3A7*1C GENOTYPE*		
	TT n = 259	TG n = 19	p value
Clinical charecteristics (mean ± SD or case number [%])			
Age at time of diagnosis (years)	61 ± 10	57 ± 13	NS
Gender			NS
Female	88 (93)	7 (7)	
Male	112 (93)	8 (7)	
Serum calcium (mmol/L) Normal range (2.25 – 2.61)	2.38 ± 0.12	2.41 ± 0.09	NS
Female	2.38 ± 0.13	2.43 ± 0.10	NS
Male	2.39 ± 0.12	2.39 ± 0.07	NS
Serum albumin (g/L) Nomal range (35 – 50)	43.5 (25-52)	44.9 (35-51)	NS
Tumor parameters			
Tumor localization groups			NS
Colon vs. rectum	0.51	0.58	
T stage groups			NS
1, 2,3 vs 4	3.87	2.75	
N stage groups			
0, 1 vs 2	3.16	4.00	NS
M stadium			NS
0	140 (92)	13 (8)	
1	60 (97)	2 (3)	
Histological grade			NS
High	11 (100)	0 (0)	
Middle	160 (92)	13 (8)	
Low	29 (94)	2 (6)	
AJCC stage groups			NS
1-2B	50 (94)	3 (6)	
3A-4	150 (93)	11 (7)	
Recurrence*	53 (91)	5 (9)	NS
Locoregional recurrence*	22 (96)	1 (4)	NS
Distant metastases*	41 (93)	3 (7)	NS
Survival			
DFS (months)	7 (1-20)	14 (5-20)	NS
OS (months)	17 (1-20)	16 (5-20)	NS
Death*	29 (100)	0 (0)	NS
Tumor markers			
Serum CEA (ng/mL) Normal range < 4.3	3.46 (0.2- 5200)	2.82 (0.2-443.5)	NS
Serum CA19-9 (U/mL) Normal range < 39	9.95 (0.3-123429)	8.17 (0.3-130)	NS
Serum AFP (ng/mL) Normal range < 13.6	3.49 (0.85-132.1)	4.89 (3.02-6.38)	NS
Therapy			
Neoadjuvant chemotherapy and radiotherapy*	105 (94)	7 (6)	NS
Surgical resection *	167 (92)	15 (8)	NS
Adjuvant chemotherapy*	96 (89)	12 (11)	0.027
Palliative therapy*	106 (95)	5 (5)	NS

* χ^2 test was used for the number of patients presenting and lacking the examined parameters.

No tumor parameters and clinical follow-up was available for 63 patients. NS = non significant

5.3 Effects of calcium supplementation on the proliferation, ALP activity and protein expression of MC3T3-E1 osteoblast cells

5.3.1 Proliferation assay

Calcium supplementation (25.5 mmol/L) for 24 hours reduced the proliferation of MC3T3-E1 osteoblast cells (fold increase of cell number with calcium supplementation = 2.07 ± 0.06 (mean ± SEM), without calcium supplementation it was 2.38 ± 0.06 (mean ± SEM); $p < 0.001$).

5.3.2 Alkaline phosphatase activity

ALP activity was found to be significantly higher after a 15 day long calcium supplementation (ALP activity for calcium supplemented cells = 30.07 ± 3.04 (mean ± SEM) U/g protein, without calcium supplementation = 4.44 ± 0.52 (mean ± SEM) U/g protein; $p < 0.001$).

5.3.3 Gene expressions

Calcium supplementation as long as 15 days altered the expression of COL1A1, COL2A1, DCN, BSP, BMP-4, SMAD-3, SMAD-6, CaSR and BGLAP genes (Figure 13). Change in FN-1 expression was not significant.

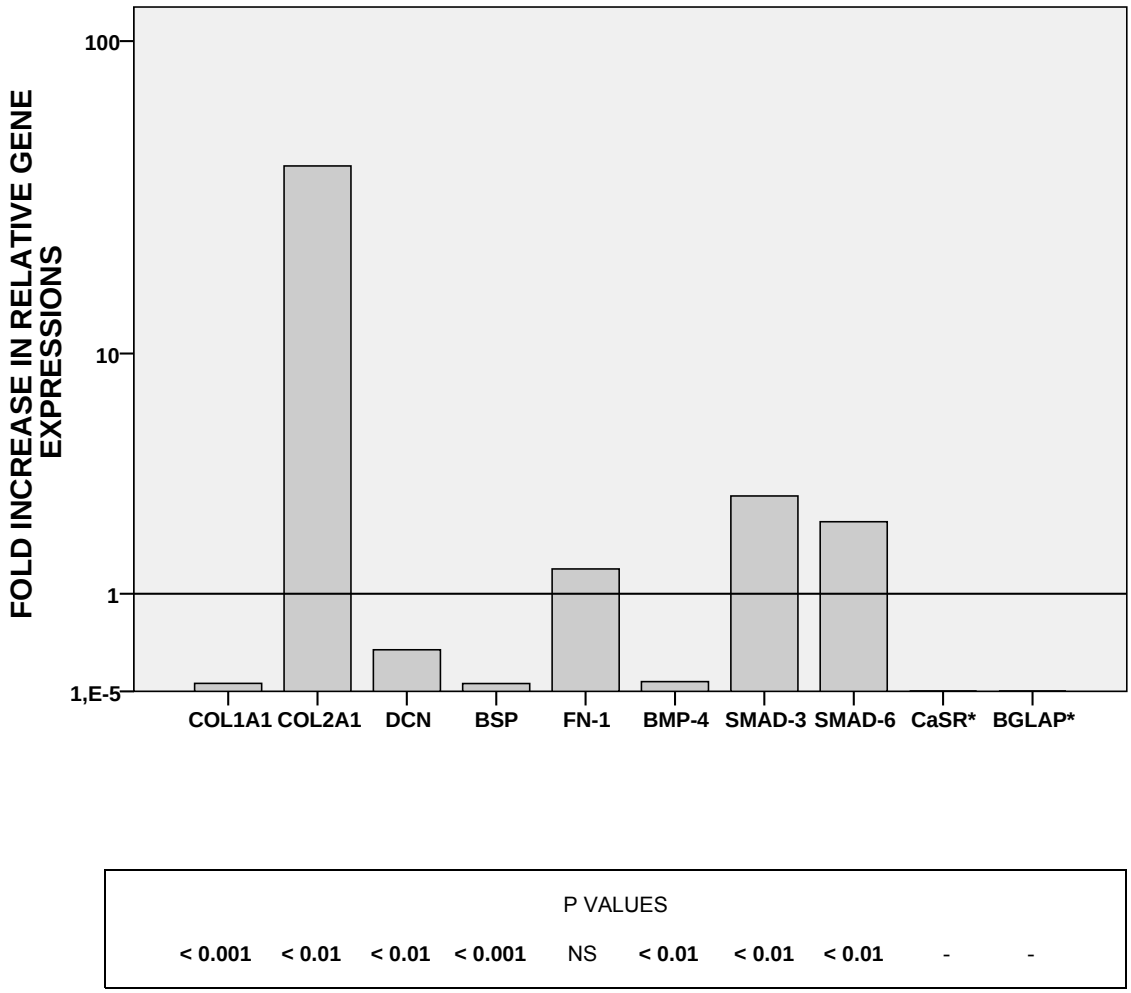


Figure 13. Effect of calium supplementation as long as 15 days on gene expression profile of MC3T3-E1 cells. Bars represent the relation of relative gene expression after and before calcium supplementation. * gene was not expressed in detectable amount after supplementation, NS = non significant

6. Discussion

6.1 Effects of LCT13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of osteoporosis

We focused our investigation on polymorphic variations in LCT, CaSR and CYP3A7 genes to evaluate their potential association with BMD through altered calcium metabolism. We could demonstrate a significant correlation of LCT 13910 C/T polymorphism with milk aversion and altered albumin-adjusted serum calcium levels in postmenopausal women. We have also found a strongly significant correlation between this polymorphism and bone mineral density. CaSR A986S and CYP3A7*1C were also found to be related to BMD.

Decreased serum calcium seen in our subjects with LCT CC genotype could be - at least partly – a result of reduced milk consumption observed in this group. Frequency of aversion to milk consumption was higher in women with CC genotype as it has also been reported by others (Carroccio et al. 1998; de Vrese et al. 2001; Obermayer-Pietsch et al. 2004). Beside reduced milk and diary product consumption, i.e. calcium intake, undigested lactose itself may further reduce calcium absorption (Obermayer-Pietsch et al. 2007). The reduction in calcium absorption can also be seen in subjects with positive breathing test but without gastrointestinal symptoms (11-32 % of all lactose intolerant patients) (Carroccio et al. 1998; de Vrese et al. 2001), indicating the importance of the underlying genetic impairment.

Recently, a meta-analysis of 29 randomized trials (n=63,897) (Tang et al. 2007) reported that calcium supplementation in the general population was associated with a significant 12 % risk reduction for all types of fractures. Also in trials that reported bone-mineral density as an outcome (Tang et al. 2007), calcium treatment was found to be significantly associated with a reduced rate of annual bone loss by 0.54 % at the hip and 1.19 % in the spine. Thus, it is not surprising that we observed decreased BMD in subjects carrying CC genotype. All these subjects were lactose intolerant, consuming less calcium and exhibiting lower serum calcium, as well. Obermayer et al (Obermayer-Pietsch et al. 2004) have also reported reduced BMD at the lumbar spine and hip in CC genotype, however, they did not report decreased serum calcium levels. We could also detect reduced BMD at the radius reflecting the involvement of cortical bone, as well. The contribution of LCT 13910 C/T polymorphism to BMD was between 1.1 - 1.7 % at different skeletal sites.

We have also shown that women with CC genotype had lower body height. This fact could be a consequence of reduced BMD and bone strength leading to accelerated compression of vertebrae or a consequence of population stratification (Campbell et al. 2005). Our population consisted of Caucasian women exclusively providing a homogeneous genetic background. Others could show the association between milk consumption and body height (Gugatschka et al. 2005), however, they did not examine the relationship with LCT polymorphism. Impaired calcium metabolism in subjects with CC genotype appears to influence bone-deleterious effects leading to osteoporosis.

CaSR plays an important role in fluid transport by colonic epithelial cells: calcium reduced fluid secretion which led to absorptive net fluid transportation (Cheng et al. 2004). We hypothesized that this mechanism is involved in milk aversion, as we have seen it in patients with CCSS genotype combination. CaSR was shown to be important but not essential in bone development (Quarles et al. 1997). In accordance with this result, we could demonstrate a weak association between this polymorphism and BMD, however, it was found only in a study subgroup. In studies on postmenopausal women this association could not be proved yet (Takacs et al. 2002).

We did not observe significant association between serum DHEAS levels and CYP3A7*1C genotypes in 319 postmenopausal women after adjusting for age. Smit et al. (Smit et al. 2005) detected a significant association between CYP3A7*1C polymorphism and serum DHEAS levels in a heterogeneous population. They have shown a reduction in serum DHEAS levels by nearly 50 % in the presence of CYP3A7*1C allele. However, they examined two groups of subjects: 208 randomly selected persons (110 women and 98 men) and a group of 345 elderly men. When women were analyzed separately, they failed to detect significant correlation between the CYP3A7 gene polymorphism and DHEAS levels, too.

We could demonstrate significant association between CYP3A7 gene polymorphism and BMD (both in T-scores and Z-scores) at the lumbar spine (L2-L4) with homozygous mutants having lower bone mass. This association remained significant after adjustment for menopausal age, serum DHEAS level, alcohol consumption, steroid intake, smoking habits and previous fractures. These findings suggest that CYP3A7*1C alleles might have an impact on BMD in women, but presumably not via decreased serum DHEAS levels. CYP3A7*1C may act through the accelerated elimination of other substrates including estrone, estradiol, androstenedione and testosterone, all of which are known to protect bone. As a corroboration to this theory, Smit et al (Smit et al. 2005) has found reduced serum estrone in the presence of the mutant allele.

Gender might be an important factor in the relationship between CYP3A7*1C polymorphism and serum DHEAS level. Previous studies have reported differences between CYP3A7 expressions in male and female reproductive tissues. The highest level of CYP3A7 mRNA expression was detected in the prostate, whereas in testis, uterus and ovaries, the expression was very low (Koch et al. 2002; Nishimura et al. 2003; Downie et al. 2005). Similarly, there are gender differences in the circulating levels of DHEAS (and DHEA), as men have higher circulating levels than women. The gender difference between CYP3A7 expressions and DHEAS levels suggests that the impact of CYP3A7 mutation on serum DHEAS level is stronger in male than in female. In line with these results, we and Smit et al (Smit et al. 2005) could not detect a significant correlation between CYP3A7 polymorphism and DHEAS levels in women.

The progressive decline in serum DHEAS with increasing age is believed to contribute to age-related diseases, such as osteoporosis (Haden et al. 2000). Like others, we observed a positive correlation between serum DHEAS level and BMD in women, independently of age. Serum DHEAS levels were significantly decreased in subjects with decreased BMD. Beside the decreased production of estrogens and androgens from DHEAS, reduced serum DHEAS levels may contribute to decreased BMD through subsequently declining levels of IGF-1 and increasing levels of IL-6 (Haden et al. 2000; Koch et al. 2002).

In conclusion, we could demonstrate an association between LCT 13910 C/T polymorphism and BMD that may be mediated through altered calcium consumption and serum calcium level. Lactose-free calcium supplementation could be an important factor in women with CC genotype in order to improve calcium absorption and prevent early reduction in bone mineral content. Also, CaSR A986S polymorphism was found to be related to milk aversion and decreased BMD. Furthermore, CYP3A7*1C polymorphism may be associated with decreased bone mass at the lumbar spine independently of serum DHEAS concentrations. This finding and the lack of association between CYP3A7*1C polymorphism and serum DHEAS level in women support the hypothesis that this genetic variation might influence bone mass via other CYP3A7 hormonal substrates. These theories deserve further investigations.

6.2 Effects of LCT 13910 C/T, CaSR A986S and CYP3A7*1C polymorphisms in the pathogenesis of colorectal cancer

We examined the significance of calcium metabolism in the pathogenesis of colorectal cancer through LCT, CaSR and CYP3A7 genetic variants. We have demonstrated the significance of the LCT 13910 C/T polymorphism in the progression of colorectal cancer. Rasinpera et al (Rasinpera et al. 2005) could also detect elevated C allele frequency in Finnish subjects with colorectal cancer, however, they could not show this association in smaller subpopulations of British and Spanish patients. This relationship could be ethnicity-specific (Finnish-Hungarian tribal relationship) or it might require larger study groups to demonstrate the correlation in other nations, as well. In contrast to the work of Rasinpera et al (Rasinpera et al. 2005), our work had a longitudinal design that facilitated to show the impact of this polymorphism on disease-free survival.

Patients with LCT 13910 CC genotype and consequent lactose intolerance consume less milk products, which is the main source of calcium (Carroccio et al. 1998; de Vrese et al. 2001), and also have decreased calcium absorption due to the impairing effect of lactose on this process (Obermayer-Pietsch et al. 2007). Epidemiological studies suggested the risk reduction effect of calcium intake in colorectal carcinogenesis (Garland et al. 1991; Lipkin and Newmark 1995; Larsson et al. 2006; Park et al. 2007). Calcium could act via intraluminal binding to bile and fatty acids causing their precipitation which is known as a pathogenetic factor in this disease (Lapre et al. 1992; Lapre et al. 1993; Govers et al. 1996). Bile and fatty acids caused lythic effect on colon cells (Wargovich et al. 1983; Govers et al. 1996), bile acids also increased 12-hydroxyeicosatetraenoic acid (DeRubertis et al. 1984) production and cell proliferation enhanced by protein kinase C activation (Govers et al. 1996). Lower milk consumption may act through building of galactose to lectins. Digestion of lactose by LPH enzyme produces galactose. One of the most common glycolysation abnormalities in colon cancer is the increased mucosal expression of the galactose-β-1,3-N-acetilgalactosamine, known as the Thomsen-Friedenreich blood group antigen (Campbell et al. 1995). This compound is able to bind lectins, the known factors of stimulated colon epithelial proliferation. Intestinal galactose produced by LPH has a protective effect against CRC by binding lectins, thus inhibiting mucosal proliferation (Evans et al. 2002). In lactose intolerance, i.e. in the presence of LCT 13910 CC genotype, the amount of intestinal galactose is decreased due to the insufficient LPH concentration.

In addition, calcium itself may inhibit hyperproliferation of colonic epithelial cells (Lipkin and Newmark 1985; Lipkin and Newmark 1995; Kallay et al. 2000). It was also shown that extracellular calcium regulates the differentiation of colonic epithelial cells via the CaSR (Chakrabarty et al. 2003; Bhagavathula et al. 2005; Chakrabarty et al. 2005). CaSR expression is the highest in well-differentiated regions of colon cancer and is nearly absent in the poorly differentiated sites which suggest the role of CaSR in colorectal carcinogenesis (Chakrabarty et al. 2003). The stimulation of the calcium/CaSR system inhibited the process of carcinogenesis by several mechanisms. It was shown to act through c-SRC, ERK activation with subsequent expression of E-cadherin, a transmembran glycoprotein that functions in cell-cell adhesion and epithelial integrity (Chakrabarty et al. 2003). Calcium/CaSR also suppressed the β-catenin - T cell factor (TCF) activation, which is responsible for the expression of many malignant effectors, such as c-myc, gastrin, cyclin D1, cyclo-oxigenase-2 (COX-2), matrilysin, urokinase-type plasminogen activator, CD44, multidrug resistance-1 gene and its product P-glycoprotein (Wong and Pignatelli 2002; Chakrabarty et al. 2003). Furthermore, the stimulation of MAPK (Hsiao et al. 2007) and c-JUN NH2-terminal kinases (Xia et al. 1995; Arthur et al. 2000) by CaSR is responsible for growth arrest and apoptosis, as well. This stimulation is elicited through filamin-A that is bound to the intracellular region of CaSR containing the A986S mutation site (Awata et al. 2001; Hjalm et al. 2001). In case of mutated CaSR, stimulation of MAPK is impaired, thus, cells may proliferate in an uncontrolled manner. As a proof of this putative mechanism, CaSR 986 SS genotype was accompanied by increased risk for CRC in our study. We could also detect similar result for CCSS genotype combination.

CYP3A7*1C polymorphism encoding a steroid metabolizing enzyme though all life has been shown to cause decreased DHEAS concentration in heterogenic population and not to affect other enzyme substrates (Smit et al. 2005). The role of DHEAS in colorectal carcinogenesis is contradictory: no difference in DHEAS level was found between patients with colorectal cancer and healthy controls (Alberg et al. 2000), however in human colon cancer cell line DHEAS supplementation reduced proliferation rate (Willumsen et al. 1984; Schulz and Nyce 1991; Schulz et al. 1992). We could not detect any associations between CYP3A7*1C polymorphism and colorectal cancer development or progression, emphasizing that DHEAS metabolism may have a minor role in colorectal carcinogenesis.

In summary, LCT 13910 C/T and CaSR A986S polymorphisms appear to have an impact on colorectal carcinogenesis via calcium metabolism. LCT 13910 CC genotype mainly affects tumor recurrence and disease-free survival, while CaSR 986 SS genotype could lead to

higher tumor incidence. The importance of these findings requires further investigations. CYP3A7*1C polymorphism did not relate to colorecral carcinogenesis.

6.3 Effects of calcium supplementation on the proliferation, ALP activity and protein expression of MC3T3-E1 osteoblast cells

Our study investigated the effect of higher calcium supplementation on proliferation, ALP activity and gene expressions of MC3T3-E1 osteoblast cells. Calcium has been shown to be essential for bone mineralization, osteoblast chemotaxis and differentiation (Yamaguchi et al. 1998a; Yamauchi et al. 2005). During bone remodeling, substantial amount of calcium (40 mmol/L) was found to be released from mineralized bone matrix, raising the level of calcium in the proximity of osteoclasts (Silver et al. 1988).

Higher calcium level acted on osteoblasts via CaSR (Yamaguchi et al. 1998a) stimulating SMAD-3 gene expression as a critical component of the TGF-β signaling pathways (Massague and Chen 2000) or other cation (NMDA) receptors (Pi et al. 2000). SMAD-3 intracellular effector was found to be associated with higher ALP activity as a marker of osteoblast activity (Sowa et al. 2002). In lining with these results, we found higher SMAD-3 expression and ALP activity in calcium supplementation, emphasizing the role of SMAD-3 in the effect of calcium on osteoblasts. SMAD-6, the inhibitor of osteoblast differentiation (Mukai et al. 2007), was elevated, too. Bone morphogenic proteins (BMPs), other members of TGF-β supergene family, have been used in the treatment of fracture healing, causing formation of a large cartilaginous callus that promotes bone formation (Govender et al. 2002; Einhorn 2003; Okamoto et al. 2006). However, continuous application of BMP-4 has been shown to stimulate osteoclastic bone resorption (Okamoto et al. 2006). In our study, the expression of BMP-4 was reduced paralleling DCN, a small leucine-rich proteoglycan, which can bind growth factors, such as BMP-4 and play a significant role in osteoblast differentiation (Chen et al. 2004). Expression of bone structural extracellular protein COL2A1, which is characteristic for enchondral ossification (Ornitz and Marie 2002) was also found to be stimulated by calcium supplementation. In conclusion, calcium supplementation stimulated the bone protein expression of MC3T3-E1 osteoblastic cells elucidating beneficial effects of higher calcium intake in bone loss regarding epidemiological studies.

7. Conclusion

7.1 Role of LCT 13910 C/T, CaSR A986S, CYP3A7*1C polymorphisms and calcium supplementation in the pathogenesis of osteoporosis

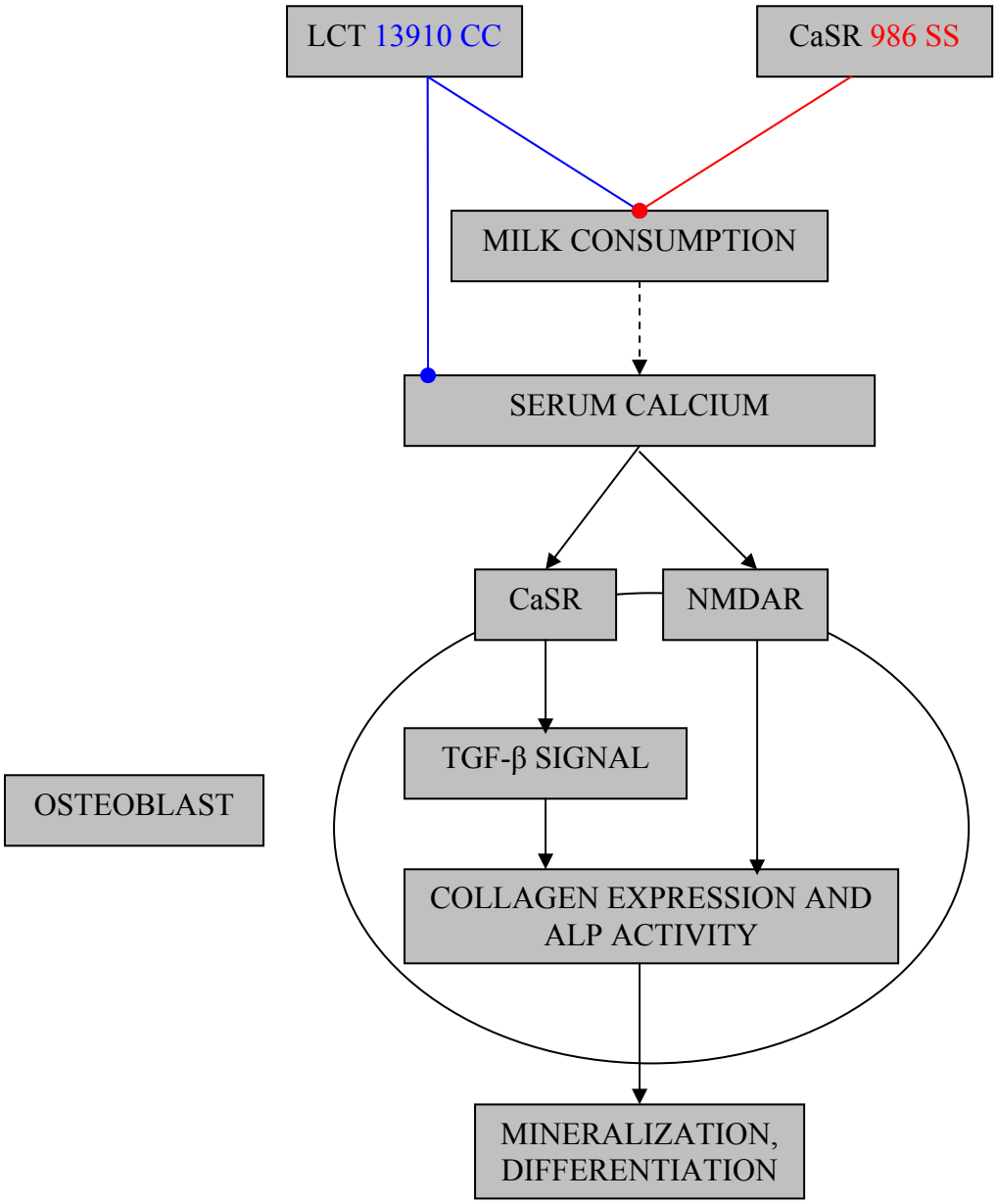


Figure 14. Effect of LCT 13910 C/T and CaSR A986S polymorphism on BMD. Associations of LCT 13910 CC genotype with lactose intolerance, and CaSR 986 SS genotype with a lower sensitivity of calcium were known. We found that in postmenopausal women LCT 13910 C/T and CaSR A986S polymorphisms acts partly via milk consumption resulting in OP. Moreover, LCT 13910 CC genotype altered serum calcium level. Besides, in MC3T3-E1 osteoblasts calcium supplementation led to decreased proliferation, higher expression of COL2A1 and increased ALP activity relating TGF-β signaling pathways. ⟶ : stimulatory effect; ⟶● : inhibitory effect, ⇢: partial effect.

CYP3A7*1C polymorphism with consequent higher steroid (pro)hormone metabolism leads to decreased bone mass in postmenopausal women, independently from DHEAS level suggesting the role of other CYP3A7 substrates: estrogens, androgens, which were thought to be significant pathogenetic factors in OP.

The LCT 13910 C/T, CaSR A986S and CYP3A7 polymorphisms were not associated with bone fracture rate.

7.2 Role of LCT 13910 C/T, CaSR A986S, CYP3A7*1C polymorphisms and calcium supplementation in the pathogenesis of colorectal cancer

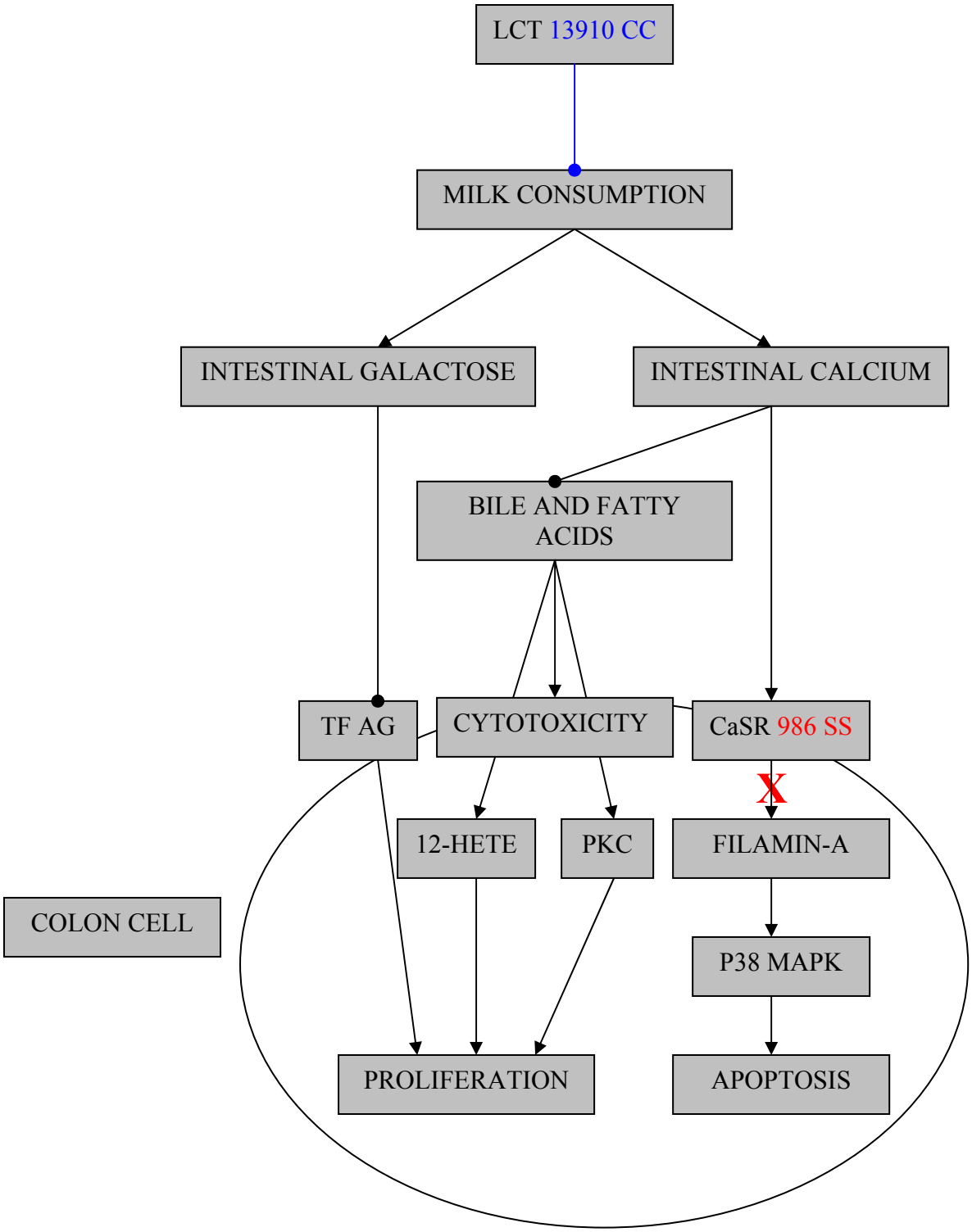


Figure 15. Effect of LCT 13910 C/T, CaSR A986S polymorphisms on colorectal carcinogenesis. Milk aversion and the altered calcium sensing function diminish proliferation control of colon cells. TF AG: Thomsen-Friedenreich blood group antigen, P38 MAPK: P38 mitogen-activated protein kinase, PKC: protein kinase C, 12-HETE: 12-hydroxyeicosatetraenoic acid. ⟶ : stimulatory effect; ⟶●: inhibitory effect, X: diminished effect.

We could not detect any associations between CYP3A7*1C polymorphism and colorectal cancer development or progression, emphasizing that DHEAS metabolism may have a minor role in colorectal carcinogenesis.

8. Summary

Epidemiological studies suggested the preventive role of calcium supplementation in both osteoporosis (OP) and colorectal cancer (CRC). Calcium is necessary to reach normal peak bone mass and to maintain bone, thus it is not surprising that calcium intake decreased bone loss. Calcium also precipitates intestinal secondary bile and fatty acids reducing their carcinogen effect on colon cells.

We genotyped lactase phlorizin hydrolase (LCT) 13910 C/T, calcium-sensing receptor (CaSR) A986S and CYP3A7*1C T/G polymorphisms that are thought to be associated with altered calcium metabolism. Also, we examined the effect of calcium supplementation on MC3T3-E1 osteoblasts.

We showed that in postmenopausal women LCT 13910 CC genotype leads to higher frequency of milk aversion, decreased serum calcium level, body height and bone mineral density (BMD) at both cortical and trabecular bones suggesting the important role of LCT gene in the development of OP. Furthermore, we found that CaSR 986 SS genotype is connected to milk aversion, but it had an effect on BMD only in a smaller study group. We have observed decreased lumbar spine BMD for homozygous CYP3A7*1C GG genotype independently from serum DHEAS level, suggesting that it might influence bone mass via other CYP3A7 hormonal substrates as estrogens and/or androgens.

We found that in CRC patients LCT 13910 CC genotype is related to distant recurrence, moreover, in male subjects it is associated with worse DFS. Also, corroborating our previous findings in postmenopausal women, this genotype was accompanied with reduced serum calcium level in female patients. The CaSR 986 SS genotype was associated with higher CRC incidence probably acted through the altered apoptotic signal transduction, but it was not related to cancer progression. CYP3A7*1C polymorphism was not related to the incidence or progression of colorectal cancer.

In MC3T3-E1 osteoblasts calcium supplementation increased alkaline phosphatase activity, stimulated type II procollagen alpha 1 expression through transforming growing factor beta (TGF-β) pathway.

In conclusion, both LCT and CaSR gene polymorphisms play a role in OP and colorectal carcinogenesis. CYP gene alters BMD possibly via estrogens and/or androgens but it is not related to colorectal carcinogenesis. Based on these results, calcium supplementation,

especially in the lactose intolerant population, might be a cheap and effective measure in the prevention of osteoporosis and colorectal cancer.

9. Összefoglalás

Epidemiológiai vizsgálatok hangsúlyozzák a kalcium pótlás szerepét a csontritkulás és a colorectalis daganat prevenciójában. A kalcium alapvető fontosságú a csúcscsonttömeg elérésében és a csontdenzitás fenntartásában, ezért nem meglepő, hogy a kalcium pótlás csökkenti a csontvesztést. A kalcium oldhatatlan komplexet képez a szekunder epe- és zsírsavakkal a bélben, csökkentve ezzel a vastagbélhám irritációját és proliferációját.

Munkánkban a kalcium metabolizmust befolyásoló laktáz phlorizin hidroláz (LCT) 13910 C/T, kalcium szenzor receptor (CaSR) A986S és CYP3A7*1C T/G polimorfizmusokat és a kalcium szupplementáció MC3T3-E1 osteoblastokra kifejtett hatását vizsgáltuk.

Igazoltuk, hogy posztmenopauzás nőkben az LCT 13910 CC genotípus csökkent tejfogyasztáshoz, alacsonyabb szérum kalcium szinthez, testmagassághoz és csontritkuláshoz vezet mind a kortikális, mind a trabekuláris csontokat érintve, hangsúlyozva az LCT gén fontosságát a csontritkulás kialakulásában. A CaSR 986 SS genotípus is alacsonyabb tejfogyasztással járt, de a csont ásványanyag sűrűgéggel (BMD) való kapcsolatát csak kisebb alcsoportban tudtuk igazolni. Csökkent lumbális gerinc BMD-t találtunk a CYP3A7*1C homozigóta GG genotípus esetén függetlenül a szérum dehidroepiandroszteron szulfát (DHEAS) szinttől, feltételezhetően hatása más CYP3A7 szubsztrát (ösztrogén, androgén) fokozott eliminációján keresztül valósulhat meg.

Colorectalis tumoros betegekben az LCT 13910 CC genotípus gyakoribb távoli recidívát eredményezett, és férfiakban rövidebb betegségmentes túléléssel is járt. Továbbá, hasonlóan a posztmenopauzás nőknél találtakkal, a CC genotípus nőkben csökkentette a szérum kalcium szintet. A CaSR 986 SS genotípus gyakoribb colorectalis daganat incidenciát eredményezett valószínűleg a megváltozott apoptózis szabályozáson keresztül, de nem befolyásolta a daganat progresszióját. Ugyanakkor a CYP3A7*1C polimorfizmus nem kapcsolódott a colorectalis daganat incidenciájához, progressziójához.

MC3T3-E1 osteoblastokon a kalcium pótlás növelte az alkalikus foszfatáz aktivitást, stimulálta a II típusú prokollagén alfa 1 expressziót a transzformáló növekedési faktor béta (TGF-β) rendszeren keresztül.

Összegezve elmondható, hogy igazoltuk az LCT és a CaSR génpolimorfizmusok szerepét a csontvesztés és a colorectalis daganat pathomechanizmusában. A CYP gén befolyásolta a csontdenzitást, feltehetőleg az ösztrogének és/vagy androgének fokozott eliminációján keresztül, de nem volt hatással a colorectalis carcinogenesisre. Eredményeink

alapján felvethető, hogy a kalcium szupplementáció, elsősorban a laktóz intoleráns populációban, feltehetőleg kedvezően hatna az osteoporosis és a colorectalis daganat incidenciájára és progressziójára.

10. References

Alberg, A.J., Gordon, G.B., Hoffman, S.C., Comstock, G.W., and Helzlsouer, K.J. 2000. Serum dehydroepiandrosterone and dehydroepiandrosterone sulfate and the subsequent risk of developing colon cancer. *Cancer Epidemiol Biomarkers Prev* **9**(5): 517-521.

Alesci, S. and Bornstein, S.R. 2001. Intraadrenal mechanisms of DHEA regulation: a hypothesis for adrenopause. *Exp Clin Endocrinol Diabetes* **109**(2): 75-82.

Aoyama, T., Yamano, S., Waxman, D.J., Lapenson, D.P., Meyer, U.A., Fischer, V., Tyndale, R., Inaba, T., Kalow, W., and Gelboin, H.V. 1989. Cytochrome P-450 hPCN3, a novel cytochrome P-450 IIIA gene product that is differentially expressed in adult human liver. cDNA and deduced amino acid sequence and distinct specificities of cDNA-expressed hPCN1 and hPCN3 for the metabolism of steroid hormones and cyclosporine. *J Biol Chem* **264**(18): 10388-10395.

Armanini, D., Vecchio, F., Basso, A., Milone, F.F., Simoncini, M., Fiore, C., Mattarello, M.J., Sartorato, P., and Karbowiak, I. 2003. Alzheimer's disease: pathophysiological implications of measurement of plasma cortisol, plasma dehydroepiandrosterone sulfate, and lymphocytic corticosteroid receptors. *Endocrine* **22**(2): 113-118.

Arthur, J.M., Lawrence, M.S., Payne, C.R., Rane, M.J., and McLeish, K.R. 2000. The calcium-sensing receptor stimulates JNK in MDCK cells. *Biochem Biophys Res Commun* **275**(2): 538-541.

Awata, H., Huang, C., Handlogten, M.E., and Miller, R.T. 2001. Interaction of the calcium-sensing receptor and filamin, a potential scaffolding protein. *J Biol Chem* **276**(37): 34871-34879.

Ballinger, A.B. and Anggiansah, C. 2007. Colorectal cancer. *BMJ* **335**(7622): 715-718.

Baron, J.A., Beach, M., Mandel, J.S., van Stolk, R.U., Haile, R.W., Sandler, R.S., Rothstein, R., Summers, R.W., Snover, D.C., Beck, G.J., Bond, J.H., and Greenberg, E.R. 1999. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N Engl J Med* **340**(2): 101-107.

Barrett-Connor, E. and Ferrara, A. 1996. Dehydroepiandrosterone, dehydroepiandrosterone sulfate, obesity, waist-hip ratio, and noninsulin-dependent diabetes in postmenopausal women: the Rancho Bernardo Study. *J Clin Endocrinol Metab* **81**(1): 59-64.

Barrett-Connor, E., Khaw, K.T., and Yen, S.S. 1986. A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *N Engl J Med* **315**(24): 1519-1524.

Belanger, A., Brochu, M., and Cliche, J. 1986. Levels of plasma steroid glucuronides in intact and castrated men with prostatic cancer. *J Clin Endocrinol Metab* **62**(5): 812-815.

Belanger, C., Luu-The, V., Dupont, P., and Tchernof, A. 2002. Adipose tissue intracrinology: potential importance of local androgen/estrogen metabolism in the regulation of adiposity. *Horm Metab Res* **34**(11-12): 737-745.

Belisle, S., Schiff, I., and Tulchinsky, D. 1980. The use of constant infusion of unlabeled dehydroepiandrosterone for the assessment of its metabolic clearance rate, its half-life, and its conversion into estrogens. *J Clin Endocrinol Metab* **50**(1): 117-121.

Belizan, J.M., Villar, J., and Repke, J. 1988. The relationship between calcium intake and pregnancy-induced hypertension: up-to-date evidence. *Am J Obstet Gynecol* **158**(4): 898-902.

Bennett, C.L., Tosteson, T.D., Schmitt, B., Weinberg, P.D., Ernstoff, M.S., and Ross, S.D. 1999. Maximum androgen-blockade with medical or surgical castration in advanced prostate cancer: A meta-analysis of nine published randomized controlled trials and 4128 patients using flutamide. *Prostate Cancer Prostatic Dis* **2**(1): 4-8.

Benson, A.B., 3rd. 2007. Epidemiology, disease progression, and economic burden of colorectal cancer. *J Manag Care Pharm* **13**(S6): S5-S18.

Benz, D.J., Haussler, M.R., Thomas, M.A., Speelman, B., and Komm, B.S. 1991. High-affinity androgen binding and androgenic regulation of alpha 1(I)-procollagen and transforming growth factor-beta steady state messenger ribonucleic acid levels in human osteoblast-like osteosarcoma cells. *Endocrinology* **128**(6): 2723-2730.

Bergsma-Kadijk, J.A., van 't Veer, P., Kampman, E., and Burema, J. 1996. Calcium does not protect against colorectal neoplasia. *Epidemiology* **7**(6): 590-597.

Bertone-Johnson, E.R., Hankinson, S.E., Bendich, A., Johnson, S.R., Willett, W.C., and Manson, J.E. 2005. Calcium and vitamin D intake and risk of incident premenstrual syndrome. *Arch Intern Med* **165**(11): 1246-1252.

Bhagavathula, N., Hanosh, A.W., Nerusu, K.C., Appelman, H., Chakrabarty, S., and Varani, J. 2007. Regulation of E-cadherin and beta-catenin by Ca2+ in colon carcinoma is dependent on calcium-sensing receptor expression and function. *Int J Cancer* **121**(7): 1455-1462.

Bhagavathula, N., Kelley, E.A., Reddy, M., Nerusu, K.C., Leonard, C., Fay, K., Chakrabarty, S., and Varani, J. 2005. Upregulation of calcium-sensing receptor and mitogen-activated protein kinase signalling in the regulation of growth and differentiation in colon carcinoma. *Br J Cancer* **93**(12): 1364-1371.

Bird, C.E., Masters, V., and Clark, A.F. 1984. Dehydroepiandrosterone sulfate: kinetics of metabolism in normal young men and women. *Clin Invest Med* **7**(2): 119-122.

Biro, L., Zajkas, G., Greiner, E., Szorad, I., Varga, A., Domonkos, A., Agoston, H., Balazs, A., Mozsary, E., Vitrai, J., Hermann, D., Boros, J., Nemeth, R., Keki, Z., and Martos, E. 2007. Hungarian national dietary survey, 2003-2004. Micronutrients: mineral salts. *Orv Hetil* **148**(15): 703-708.

Bjornerem, A., Straume, B., Midtby, M., Fonnebo, V., Sundsfjord, J., Svartberg, J., Acharya, G., Oian, P., and Berntsen, G.K. 2004. Endogenous sex hormones in relation to age, sex, lifestyle factors, and chronic diseases in a general population: the Tromso Study. *J Clin Endocrinol Metab* **89**(12): 6039-6047.

Bollerslev, J., Wilson, S.G., Dick, I.M., Devine, A., Dhaliwal, S.S., and Prince, R.L. 2004. Calcium-sensing receptor gene polymorphism A986S does not predict serum calcium level, bone mineral density, calcaneal ultrasound indices, or fracture rate in a large cohort of elderly women. *Calcif Tissue Int* **74**(1): 12-17.

Bonar, S.K., Tinetti, M.E., Speechley, M., and Cooney, L.M. 1990. Factors associated with short- versus long-term skilled nursing facility placement among community-living hip fracture patients. *J Am Geriatr Soc* **38**(10): 1139-1144.

Borghi, L., Schianchi, T., Meschi, T., Guerra, A., Allegri, F., Maggiore, U., and Novarini, A. 2002. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. *N Engl J Med* **346**(2): 77-84.

Bostick, R.M., Kushi, L.H., Wu, Y., Meyer, K.A., Sellers, T.A., and Folsom, A.R. 1999. Relation of calcium, vitamin D, and dairy food intake to ischemic heart disease mortality among postmenopausal women. *Am J Epidemiol* **149**(2): 151-161.

Brooks, B.A., McBride, O.W., Dolphin, C.T., Farrall, M., Scambler, P.J., Gonzalez, F.J., and Idle, J.R. 1988. The gene CYP3 encoding P450pcn1 (nifedipine oxidase) is tightly linked to the gene COL1A2 encoding collagen type 1 alpha on 7q21-q22.1. *Am J Hum Genet* **43**(3): 280-284.

Brown, E.M. 1991. Extracellular Ca2+ sensing, regulation of parathyroid cell function, and role of Ca2+ and other ions as extracellular (first) messengers. *Physiol Rev* **71**(2): 371-411.

Brown, E.M., Pollak, M., and Hebert, S.C. 1998. The extracellular calcium-sensing receptor: its role in health and disease. *Annu Rev Med* **49**: 15-29.

Buning, C., Genschel, J., Jurga, J., Fiedler, T., Voderholzer, W., Fiedler, E.M., Worm, M., Weltrich, R., Lochs, H., Schmidt, H., and Ockenga, J. 2005. Introducing genetic testing for adult-type hypolactasia. *Digestion* **71**(4): 245-250.

Burger, H.G. 2002. Androgen production in women. *Fertil Steril* **77**(S4): S3-S5.

Calle, E.E., Miracle-McMahill, H.L., Thun, M.J., and Heath, C.W., Jr. 1995. Estrogen replacement therapy and risk of fatal colon cancer in a prospective cohort of postmenopausal women. *J Natl Cancer Inst* **87**(7): 517-523.

Campbell-Thompson, M., Lynch, I.J., and Bhardwaj, B. 2001. Expression of estrogen receptor (ER) subtypes and ERbeta isoforms in colon cancer. *Cancer Res* **61**(2): 632-640.

Campbell, B.J., Finnie, I.A., Hounsell, E.F., and Rhodes, J.M. 1995. Direct demonstration of increased expression of Thomsen-Friedenreich (TF) antigen in colonic adenocarcinoma and ulcerative colitis mucin and its concealment in normal mucin. *J Clin Invest* **95**(2): 571-576.

Campbell, C.D., Ogburn, E.L., Lunetta, K.L., Lyon, H.N., Freedman, M.L., Groop, L.C., Altshuler, D., Ardlie, K.G., and Hirschhorn, J.N. 2005. Demonstrating stratification in a European American population. *Nat Genet* **37**(8): 868-872.

Carroccio, A., Montalto, G., Cavera, G., and Notarbatolo, A. 1998. Lactose intolerance and self-reported milk intolerance: relationship with lactose maldigestion and nutrient intake. Lactase Deficiency Study Group. *J Am Coll Nutr* **17**(6): 631-636.

Casson, P.R., Andersen, Gerrod, H.G., Stentz, F.B., and Buster, J.E. 1993. Oral dehydroepiandrosterone in physiologic doses modulates immune function in postmenopausal women. *Am J Obstet Gynecol* **169**(6): 1536-1539.

Catalano, M.G., Pfeffer, U., Raineri, M., Ferro, P., Curto, A., Capuzzi, P., Corno, F., Berta, L., and Fortunati, N. 2000. Altered expression of androgen-receptor isoforms in human colon-cancer tissues. *Int J Cancer* **86**(3): 325-330.

Celec, P. and Starka, L. 2003. Dehydroepiandrosterone - is the fountain of youth drying out? *Physiol Res* **52**(4): 397-407.

Chakrabarty, S., Radjendirane, V., Appelman, H., and Varani, J. 2003. Extracellular calcium and calcium sensing receptor function in human colon carcinomas: promotion of E-cadherin expression and suppression of beta-catenin/TCF activation. *Cancer Res* **63**(1): 67-71.

Chakrabarty, S., Wang, H., Canaff, L., Hendy, G.N., Appelman, H., and Varani, J. 2005. Calcium sensing receptor in human colon carcinoma: interaction with Ca(2+) and 1,25-dihydroxyvitamin D(3). *Cancer Res* **65**(2): 493-498.

Chang, D.M., Lan, J.L., Lin, H.Y., and Luo, S.F. 2002. Dehydroepiandrosterone treatment of women with mild-to-moderate systemic lupus erythematosus: a multicenter randomized, double-blind, placebo-controlled trial. *Arthritis Rheum* **46**(11): 2924-2927.

Chang, R.J. 2004. A practical approach to the diagnosis of polycystic ovary syndrome. *Am J Obstet Gynecol* **191**(3): 713-717.

Chen, X.D., Fisher, L.W., Robey, P.G., and Young, M.F. 2004. The small leucine-rich proteoglycan biglycan modulates BMP-4-induced osteoblast differentiation. *FASEB J* **18**(9): 948-958.

Cheng, I., Klingensmith, M.E., Chattopadhyay, N., Kifor, O., Butters, R.R., Soybel, D.I., and Brown, E.M. 1998. Identification and localization of the extracellular calcium-sensing receptor in human breast. *J Clin Endocrinol Metab* **83**(2): 703-707.

Cheng, I., Qureshi, I., Chattopadhyay, N., Qureshi, A., Butters, R.R., Hall, A.E., Cima, R.R., Rogers, K.V., Hebert, S.C., Geibel, J.P., Brown, E.M., and Soybel, D.I. 1999. Expression of an extracellular calcium-sensing receptor in rat stomach. *Gastroenterology* **116**(1): 118-126.

Cheng, S.X., Geibel, J.P., and Hebert, S.C. 2004. Extracellular polyamines regulate fluid secretion in rat colonic crypts via the extracellular calcium-sensing receptor. *Gastroenterology* **126**(1): 148-158.

Cheng, S.X., Okuda, M., Hall, A.E., Geibel, J.P., and Hebert, S.C. 2002. Expression of calcium-sensing receptor in rat colonic epithelium: evidence for modulation of fluid secretion. *Am J Physiol Gastrointest Liver Physiol* **283**(1): 240-250.

Chesire, D.R. and Isaacs, W.B. 2002. Ligand-dependent inhibition of beta-catenin/TCF signaling by androgen receptor. *Oncogene* **21**(55): 8453-8469.

Cibula, D., Hill, M., and Starka, L. 2000. The best correlation of the new index of hyperandrogenism with the grade of increased body hair. *Eur J Endocrinol* **143**(3): 405-408.

Civitelli, R., Agnusdei, D., Nardi, P., Zacchei, F., Avioli, L.V., and Gennari, C. 1988. Effects of one-year treatment with estrogens on bone mass, intestinal calcium absorption, and 25-hydroxyvitamin D-1 alpha-hydroxylase reserve in postmenopausal osteoporosis. *Calcif Tissue Int* **42**(2): 77-86.

Craven, P.A., Pfanstiel, J., and DeRubertis, F.R. 1987. Role of activation of protein kinase C in the stimulation of colonic epithelial proliferation and reactive oxygen formation by bile acids. *J Clin Invest* **79**(2): 532-541.

Cuddihy, M.T., Gabriel, S.E., Crowson, C.S., O'Fallon, W.M., and Melton, L.J., 3rd. 1999. Forearm fractures as predictors of subsequent osteoporotic fractures. *Osteoporos Int* **9**(6): 469-475.

Cummings, S.R., Browner, W.S., Bauer, D., Stone, K., Ensrud, K., Jamal, S., and Ettinger, B. 1998. Endogenous hormones and the risk of hip and vertebral fractures among older women. Study of Osteoporotic Fractures Research Group. *N Engl J Med* **339**(11): 733-738.

Cummings, S.R., Eckert, S., Krueger, K.A., Grady, D., and Jordan, V.C. 1999. Effect of raloxifene on risk of breast cancer in postmenopausal women: results from the MORE randomized trial. *JAMA* **281**(16): 2189-2197.

Cummings, S.R. and Melton, L.J. 2002. Epidemiology and outcomes of osteoporotic fractures. *Lancet* **359**(9319): 1761-1767.

Curhan, G.C., Willett, W.C., Rimm, E.B., and Stampfer, M.J. 1993. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. *N Engl J Med* **328**(12): 833-838.

Cusan, L., Dupont, A., Gomez, J.L., Tremblay, R.R., and Labrie, F. 1994. Comparison of flutamide and spironolactone in the treatment of hirsutism: a randomized controlled trial. *Fertil Steril* **61**(2): 281-287.

Davies, K.M., Heaney, R.P., Recker, R.R., Lappe, J.M., Barger-Lux, M.J., Rafferty, K., and Hinders, S. 2000. Calcium intake and body weight. *J Clin Endocrinol Metab* **85**(12): 4635-4638.

Davis, S.R., Davison, S.L., Donath, S., and Bell, R.J. 2005. Circulating androgen levels and self-reported sexual function in women. *JAMA* **294**(1): 91-96.

Dawson-Hughes, B., Dallal, G.E., Krall, E.A., Sadowski, L., Sahyoun, N., and Tannenbaum, S. 1990. A controlled trial of the effect of calcium supplementation on bone density in postmenopausal women. *N Engl J Med* **323**(13): 878-883.

de la Torre, B., Fransson, J., and Scheynius, A. 1995. Blood dehydroepiandrosterone sulphate (DHEAS) levels in pemphigoid/pemphigus and psoriasis. *Clin Exp Rheumatol* **13**(3): 345-348.

de Vrese, M., Stegelmann, A., Richter, B., Fenselau, S., Laue, C., and Schrezenmeir, J. 2001. Probiotics--compensation for lactase insufficiency. *Am J Clin Nutr* **73**(S2): S421-S429.

DeFazio, J., Meldrum, D.R., Winer, J.H., and Judd, H.L. 1984. Direct action of androgen on hot flushes in the human male. *Maturitas* **6**(1): 3-8.

DeRubertis, F.R., Craven, P.A., and Saito, R. 1984. Bile salt stimulation of colonic epithelial proliferation. Evidence for involvement of lipoxygenase products. *J Clin Invest* **74**(5): 1614-1624.

Diamond, P., Cusan, L., Gomez, J.L., Belanger, A., and Labrie, F. 1996. Metabolic effects of 12-month percutaneous dehydroepiandrosterone replacement therapy in postmenopausal women. *J Endocrinol* **150**(S): S43.

Downie, D., McFadyen, M.C., Rooney, P.H., Cruickshank, M.E., Parkin, D.E., Miller, I.D., Telfer, C., Melvin, W.T., and Murray, G.I. 2005. Profiling cytochrome P450 expression in ovarian cancer: identification of prognostic markers. *Clin Cancer Res* **11**(20): 7369-7375.

Eckstein, M., Vered, I., Ish-Shalom, S., Shlomo, A.B., Shtriker, A., Koren-Morag, N., and Friedman, E. 2002. Vitamin D and calcium-sensing receptor genotypes in men and premenopausal women with low bone mineral density. *Isr Med Assoc J* **4**(5): 340-344.

Einhorn, T.A. 2003. Clinical applications of recombinant human BMPs: early experience and future development. *J Bone Joint Surg Am* **85-A**(S3): S82-S88.

Eller, N.H., Netterstrom, B., and Allerup, P. 2005. Progression in intima media thickness-the significance of hormonal biomarkers of chronic stress. *Psychoneuroendocrinology* **30**(8): 715-723.

Enattah, N., Pekkarinen, T., Valimaki, M.J., Loyttyniemi, E., and Jarvela, I. 2005. Genetically defined adult-type hypolactasia and self-reported lactose intolerance as risk factors of osteoporosis in Finnish postmenopausal women. *Eur J Clin Nutr* **59**(10): 1105-1111.

Enattah, N., Valimaki, V.V., Valimaki, M.J., Loyttyniemi, E., Sahi, T., and Jarvela, I. 2004. Molecularly defined lactose malabsorption, peak bone mass and bone turnover rate in young finnish men. *Calcif Tissue Int* **75**(6): 488.

Enattah, N.S., Sahi, T., Savilahti, E., Terwilliger, J.D., Peltonen, L., and Jarvela, I. 2002. Identification of a variant associated with adult-type hypolactasia. *Nat Genet* **30**(2): 233-237.

Espey, D.K., Wu, X.C., Swan, J., Wiggins, C., Jim, M.A., Ward, E., Wingo, P.A., Howe, H.L., Ries, L.A., Miller, B.A., Jemal, A., Ahmed, F., Cobb, N., Kaur, J.S., and

Edwards, B.K. 2007. Annual report to the nation on the status of cancer, 1975-2004, featuring cancer in American Indians and Alaska Natives. *Cancer* **110**(10): 2119-2152.

Evans, R.C., Fear, S., Ashby, D., Hackett, A., Williams, E., Van Der Vliet, M., Dunstan, F.D., and Rhodes, J.M. 2002. Diet and colorectal cancer: an investigation of the lectin/galactose hypothesis. *Gastroenterology* **122**(7): 1784-1792.

Falhammar, H., Filipsson, H., Holmdahl, G., Janson, P.O., Nordenskjold, A., Hagenfeldt, K., and Thoren, M. 2007. Metabolic profile and body composition in adult women with congenital adrenal hyperplasia due to 21-hydroxylase deficiency. *J Clin Endocrinol Metab* **92**(1): 110-116.

Fernandez-Real, J.M., Engel, W.R., Simo, R., Salinas, I., and Webb, S.M. 1998. Study of glucose tolerance in consecutive patients harbouring incidental adrenal tumours. Study Group of Incidental Adrenal Adenoma. *Clin Endocrinol (Oxf)* **49**(1): 53-61.

Ferrando, S.J., Rabkin, J.G., and Poretsky, L. 1999. Dehydroepiandrosterone sulfate (DHEAS) and testosterone: relation to HIV illness stage and progression over one year. *J Acquir Immune Defic Syndr* **22**(2): 146-154.

Ferro, P., Catalano, M.G., Dell'Eva, R., Fortunati, N., and Pfeffer, U. 2002. The androgen receptor CAG repeat: a modifier of carcinogenesis? *Mol Cell Endocrinol* **193**(1-2): 109-120.

Foldes, J., Lakatos, P., Zsadanyi, J., and Horvath, C. 1997. Decreased serum IGF-I and dehydroepiandrosterone sulphate may be risk factors for the development of reduced bone mass in postmenopausal women with endogenous subclinical hyperthyroidism. *Eur J Endocrinol* **136**(3): 277-281.

Gambacciani, M., Monteleone, P., Sacco, A., and Genazzani, A.R. 2003. Hormone replacement therapy and endometrial, ovarian and colorectal cancer. *Best Pract Res Clin Endocrinol Metab* **17**(1): 139-147.

Garland, C., Shekelle, R.B., Barrett-Connor, E., Criqui, M.H., Rossof, A.H., and Paul, O. 1985. Dietary vitamin D and calcium and risk of colorectal cancer: a 19-year prospective study in men. *Lancet* **1**(8424): 307-309.

Garland, C.F., Garland, F.C., and Gorham, E.D. 1991. Can colon cancer incidence and death rates be reduced with calcium and vitamin D? *Am J Clin Nutr* **54**(S1): S193-S201.

Garnero, P., Hausherr, E., Chapuy, M.C., Marcelli, C., Grandjean, H., Muller, C., Cormier, C., Breart, G., Meunier, P.J., and Delmas, P.D. 1996. Markers of bone resorption

predict hip fracture in elderly women: the EPIDOS Prospective Study. *J Bone Miner Res* **11**(10): 1531-1538.

Garrett, J.E., Capuano, I.V., Hammerland, L.G., Hung, B.C., Brown, E.M., Hebert, S.C., Nemeth, E.F., and Fuller, F. 1995. Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. *J Biol Chem* **270**(21): 12919-12925.

Giles, R.H., van Es, J.H., and Clevers, H. 2003. Caught up in a Wnt storm: Wnt signaling in cancer. *Biochim Biophys Acta* **1653**(1): 1-24.

Gonzalez, F.J. 1988. The molecular biology of cytochrome P450s. *Pharmacol Rev* **40**(4): 243-288.

Govender, S., Csimma, C., Genant, H.K., Valentin-Opran, A., Amit, Y., Arbel, R., Aro, H., Atar, D., Bishay, M., Borner, M.G., Chiron, P., Choong, P., Cinats, J., Courtenay, B., Feibel, R., Geulette, B., Gravel, C., Haas, N., Raschke, M., Hammacher, E., van der Velde, D., Hardy, P., Holt, M., Josten, C., Ketterl, R.L., Lindeque, B., Lob, G., Mathevon, H., McCoy, G., Marsh, D., Miller, R., Munting, E., Oevre, S., Nordsletten, L., Patel, A., Pohl, A., Rennie, W., Reynders, P., Rommens, P.M., Rondia, J., Rossouw, W.C., Daneel, P.J., Ruff, S., Ruter, A., Santavirta, S., Schildhauer, T.A., Gekle, C., Schnettler, R., Segal, D., Seiler, H., Snowdowne, R.B., Stapert, J., Taglang, G., Verdonk, R., Vogels, L., Weckbach, A., Wentzensen, A., and Wisniewski, T. 2002. Recombinant human bone morphogenetic protein-2 for treatment of open tibial fractures: a prospective, controlled, randomized study of four hundred and fifty patients. *J Bone Joint Surg Am* **84-A**(12): 2123-2134.

Govers, M.J., Termont, D.S., Lapre, J.A., Kleibeuker, J.H., Vonk, R.J., and Van der Meer, R. 1996. Calcium in milk products precipitates intestinal fatty acids and secondary bile acids and thus inhibits colonic cytotoxicity in humans. *Cancer Res* **56**(14): 3270-3275.

Grant, A.M., Avenell, A., Campbell, M.K., McDonald, A.M., MacLennan, G.S., McPherson, G.C., Anderson, F.H., Cooper, C., Francis, R.M., Donaldson, C., Gillespie, W.J., Robinson, C.M., Torgerson, D.J., and Wallace, W.A. 2005. Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet* **365**(9471): 1621-1628.

Greuet, J., Pichard, L., Bonfils, C., Domergue, J., and Maurel, P. 1996. The fetal specific gene CYP3A7 is inducible by rifampicin in adult human hepatocytes in primary culture. *Biochem Biophys Res Commun* **225**(2): 689-694.

Grodstein, F., Martinez, M.E., Platz, E.A., Giovannucci, E., Colditz, G.A., Kautzky, M., Fuchs, C., and Stampfer, M.J. 1998. Postmenopausal hormone use and risk for colorectal cancer and adenoma. *Ann Intern Med* **128**(9): 705-712.

Gugatschka, M., Dobnig, H., Fahrleitner-Pammer, A., Pietschmann, P., Kudlacek, S., Strele, A., and Obermayer-Pietsch, B. 2005. Molecularly-defined lactose malabsorption, milk consumption and anthropometric differences in adult males. *QJM* **98**(12): 857-863.

Gugatschka, M., Hoeller, A., Fahrleitner-Pammer, A., Dobnig, H., Pietschmann, P., Kudlacek, S., and Obermayer-Pietsch, B. 2007. Calcium supply, bone mineral density and genetically defined lactose maldigestion in a cohort of elderly men. *J Endocrinol Invest* **30**(1): 46-51.

Haden, S.T., Glowacki, J., Hurwitz, S., Rosen, C., and LeBoff, M.S. 2000. Effects of age on serum dehydroepiandrosterone sulfate, IGF-I, and IL-6 levels in women. *Calcif Tissue Int* **66**(6): 414-418.

Haffner, S.M., Moss, S.E., Klein, B.E., and Klein, R. 1996. Sex hormones and DHEA-SO4 in relation to ischemic heart disease mortality in diabetic subjects. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. *Diabetes Care* **19**(10): 1045-1050.

Hammer, F., Subtil, S., Lux, P., Maser-Gluth, C., Stewart, P.M., Allolio, B., and Arlt, W. 2005. No evidence for hepatic conversion of dehydroepiandrosterone (DHEA) sulfate to DHEA: in vivo and in vitro studies. *J Clin Endocrinol Metab* **90**(6): 3600-3605.

Harrison, J.D., Watson, S., and Morris, D.L. 1989. The effect of sex hormones and tamoxifen on the growth of human gastric and colorectal cancer cell lines. *Cancer* **63**(11): 2148-2151.

Hartkamp, A., Geenen, R., Godaert, G.L., Bijl, M., Bijlsma, J.W., and Derksen, R.H. 2004. The effect of dehydroepiandrosterone on lumbar spine bone mineral density in patients with quiescent systemic lupus erythematosus. *Arthritis Rheum* **50**(11): 3591-3595.

Harvey, C.B., Wang, Y., Darmoul, D., Phillips, A., Mantei, N., and Swallow, D.M. 1996. Characterisation of a human homologue of a yeast cell division cycle gene, MCM6, located adjacent to the 5' end of the lactase gene on chromosome 2q21. *FEBS Lett* **398**(2-3): 135-140.

Heaney, R.P. 2006. Calcium intake and disease prevention. *Arq Bras Endocrinol Metabol* **50**(4): 685-693.

Hjalm, G., MacLeod, R.J., Kifor, O., Chattopadhyay, N., and Brown, E.M. 2001. Filamin-A binds to the carboxyl-terminal tail of the calcium-sensing receptor, an interaction that

participates in CaR-mediated activation of mitogen-activated protein kinase. *J Biol Chem* **276**(37): 34880-34887.

Hoffmann, R., Rot, A., Niiyama, S., and Billich, A. 2001. Steroid sulfatase in the human hair follicle concentrates in the dermal papilla. *J Invest Dermatol* **117**(6): 1342-1348.

Hofmeyr, G.J., Duley, L., and Atallah, A. 2007. Dietary calcium supplementation for prevention of pre-eclampsia and related problems: a systematic review and commentary. *BJOG* **114**(8): 933-943.

Hollo, I., Szalay, F., Steczek, K., and Szucs, J. 1978. Effect of intravenous calcium load on the serum calcium level in postmenopausal osteoporosis (a study of the pathogenesis, and diagnostic use of the test). *Acta Med Acad Sci Hung* **35**(1): 53-59.

Holt, P.R., Atillasoy, E.O., Gilman, J., Guss, J., Moss, S.F., Newmark, H., Fan, K., Yang, K., and Lipkin, M. 1998. Modulation of abnormal colonic epithelial cell proliferation and differentiation by low-fat dairy foods: a randomized controlled trial. *Jama* **280**(12): 1074-1079.

House, M.G., Kohlmeier, L., Chattopadhyay, N., Kifor, O., Yamaguchi, T., Leboff, M.S., Glowacki, J., and Brown, E.M. 1997. Expression of an extracellular calcium-sensing receptor in human and mouse bone marrow cells. *J Bone Miner Res* **12**(12): 1959-1970.

Hsiao, P.W., Chang, C.C., Liu, H.F., Tsai, C.M., Chiu, T.H., and Chao, J.I. 2007. Activation of p38 mitogen-activated protein kinase by celecoxib oppositely regulates survivin and gamma-H2AX in human colorectal cancer cells. *Toxicol Appl Pharmacol* **222**(1): 97-104.

Imrich, R., Rovensky, J., Malis, F., Zlnay, M., Killinger, Z., Kvetnansky, R., Huckova, M., Vigas, M., Macho, L., and Koska, J. 2005. Low levels of dehydroepiandrosterone sulphate in plasma, and reduced sympathoadrenal response to hypoglycaemia in premenopausal women with rheumatoid arthritis. *Ann Rheum Dis* **64**(2): 202-206.

Iso, H., Stampfer, M.J., Manson, J.E., Rexrode, K., Hennekens, C.H., Colditz, G.A., Speizer, F.E., and Willett, W.C. 1999. Prospective study of calcium, potassium, and magnesium intake and risk of stroke in women. *Stroke* **30**(9): 1772-1779.

Issa, J.P., Ottaviano, Y.L., Celano, P., Hamilton, S.R., Davidson, N.E., and Baylin, S.B. 1994. Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat Genet* **7**(4): 536-540.

Izbicki, J.R., Hamilton, S.R., Wambach, G., Harnisch, E., Wilker, D.K., Dornschneider, G., Eibl-Eibesfeldt, B., and Schweiberer, L. 1990. Effects of androgen manipulations on

chemically induced colonic tumours and on macroscopically normal colonic mucosa in male Sprague-Dawley rats. *Br J Cancer* **61**(2): 235-240.

Jacks, T. and Weinberg, R.A. 1998. The expanding role of cell cycle regulators. *Science* **280**(5366): 1035-1036.

Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J., and Thun, M.J. 2007. Cancer statistics, 2007. *CA Cancer J Clin* **57**(1): 43-66.

Jilka, R.L. 1998. Cytokines, bone remodeling, and estrogen deficiency: a 1998 update. *Bone* **23**(2): 75-81.

Johannsson, G., Burman, P., Wiren, L., Engstrom, B.E., Nilsson, A.G., Ottosson, M., Jonsson, B., Bengtsson, B.A., and Karlsson, F.A. 2002. Low dose dehydroepiandrosterone affects behavior in hypopituitary androgen-deficient women: a placebo-controlled trial. *J Clin Endocrinol Metab* **87**(5): 2046-2052.

Johnell, O., Oden, A., De Laet, C., Garnero, P., Delmas, P.D., and Kanis, J.A. 2002. Biochemical indices of bone turnover and the assessment of fracture probability. *Osteoporos Int* **13**(7): 523-526.

Kallay, E., Bajna, E., Wrba, F., Kriwanek, S., Peterlik, M., and Cross, H.S. 2000. Dietary calcium and growth modulation of human colon cancer cells: role of the extracellular calcium-sensing receptor. *Cancer Detect Prev* **24**(2): 127-136.

Kamei, J., Toyofuku, T., and Hori, M. 2003. Negative regulation of p21 by beta-catenin/TCF signaling: a novel mechanism by which cell adhesion molecules regulate cell proliferation. *Biochem Biophys Res Commun* **312**(2): 380-387.

Kanatani, M., Sugimoto, T., Kanzawa, M., Yano, S., and Chihara, K. 1999. High extracellular calcium inhibits osteoclast-like cell formation by directly acting on the calcium-sensing receptor existing in osteoclast precursor cells. *Biochem Biophys Res Commun* **261**(1): 144-148.

Kanis, J.A., Borgstrom, F., De Laet, C., Johansson, H., Johnell, O., Jonsson, B., Oden, A., Zethraeus, N., Pfleger, B., and Khaltaev, N. 2005. Assessment of fracture risk. *Osteoporos Int* **16**(6): 581-589.

Kasler, M. 2005. Current state and future perspectives of oncology care in Hungary based on epidemiologic data. *Orv Hetil* **146**(29): 1519-1530.

Kazár, G. 1987. A csípőtáji combtörések epidemiológiája, gyógyítási és rehabilitációs problémái. *OrvHetil* **128**(29): 1505-1508.

Keen, R. 2007. Osteoporosis: strategies for prevention and management. *Best Pract Res Clin Rheumatol* **21**(1): 109-122.

Kim, S.B., Hill, M., Kwak, Y.T., Hampl, R., Jo, D.H., and Morfin, R. 2003. Neurosteroids: Cerebrospinal fluid levels for Alzheimer's disease and vascular dementia diagnostics. *J Clin Endocrinol Metab* **88**(11): 5199-5206.

Kirchhoff, P. and Geibel, J.P. 2006. Role of calcium and other trace elements in the gastrointestinal physiology. *World J Gastroenterol* **12**(20): 3229-3236.

Kitada, M., Kamataki, T., Itahashi, K., Rikihisa, T., and Kanakubo, Y. 1987a. P-450 HFLa, a form of cytochrome P-450 purified from human fetal livers, is the 16 alpha-hydroxylase of dehydroepiandrosterone 3-sulfate. *J Biol Chem* **262**(28): 13534-13537.

-. 1987b. Significance of cytochrome P-450 (P-450 HFLa) of human fetal livers in the steroid and drug oxidations. *Biochem Pharmacol* **36**(4): 453-456.

Kitada, M., Kamataki, T., Itahashi, K., Rikihisa, T., Kato, R., and Kanakubo, Y. 1985. Purification and properties of cytochrome P-450 from homogenates of human fetal livers. *Arch Biochem Biophys* **241**(1): 275-280.

Klann, R.C., Holbrook, C.T., and Nyce, J.W. 1992. Chemotherapy of murine colorectal carcinoma with cisplatin and cisplatin plus 3'-deoxy-3'-azidothymidine. *Anticancer Res* **12**(3): 781-787.

Koch, I., Weil, R., Wolbold, R., Brockmoller, J., Hustert, E., Burk, O., Nuessler, A., Neuhaus, P., Eichelbaum, M., Zanger, U., and Wojnowski, L. 2002. Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. *Drug Metab Dispos* **30**(10): 1108-1114.

Kubo, Y., Miyashita, T., and Murata, Y. 1998. Structural basis for a Ca2+-sensing function of the metabotropic glutamate receptors. *Science* **279**(5357): 1722-1725.

Kuehl, P., Zhang, J., Lin, Y., Lamba, J., Assem, M., Schuetz, J., Watkins, P.B., Daly, A., Wrighton, S.A., Hall, S.D., Maurel, P., Relling, M., Brimer, C., Yasuda, K., Venkataramanan, R., Strom, S., Thummel, K., Boguski, M.S., and Schuetz, E. 2001. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat Genet* **27**(4): 383-391.

Kuokkanen, M., Enattah, N.S., Oksanen, A., Savilahti, E., Orpana, A., and Jarvela, I. 2003. Transcriptional regulation of the lactase-phlorizin hydrolase gene by polymorphisms associated with adult-type hypolactasia. *Gut* **52**(5): 647-652.

Labrie, C., Belanger, A., and Labrie, F. 1988. Androgenic activity of dehydroepiandrosterone and androstenedione in the rat ventral prostate. *Endocrinology* **123**(3): 1412-1417.

Labrie, F. 1991. Intracrinology. *Mol Cell Endocrinol* **78**(3): 113-118.

Labrie, F., Belanger, A., Cusan, L., Gomez, J.L., and Candas, B. 1997. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. *J Clin Endocrinol Metab* **82**(8): 2396-2402.

Labrie, F., Dupont, A., Belanger, A., Cusan, L., Lacourciere, Y., Monfette, G., Laberge, J.G., Emond, J.P., Fazekas, A.T., Raynaud, J.P., and Husson, J.M. 1982. New hormonal therapy in prostatic carcinoma: combined treatment with an LHRH agonist and an antiandrogen. *Clin Invest Med* **5**(4): 267-275.

Labrie, F., Luu-The, V., Belanger, A., Lin, S.X., Simard, J., Pelletier, G., and Labrie, C. 2005. Is dehydroepiandrosterone a hormone? *J Endocrinol* **187**(2): 169-196.

Langlois, J.A., Rosen, C.J., Visser, M., Hannan, M.T., Harris, T., Wilson, P.W., and Kiel, D.P. 1998. Association between insulin-like growth factor I and bone mineral density in older women and men: the Framingham Heart Study. *J Clin Endocrinol Metab* **83**(12): 4257-4262.

Lapre, J.A., De Vries, H.T., Termont, D.S., Kleibeuker, J.H., De Vries, E.G., and Van der Meer, R. 1993. Mechanism of the protective effect of supplemental dietary calcium on cytolytic activity of fecal water. *Cancer Res* **53**(2): 248-253.

Lapre, J.A., Termont, D.S., Groen, A.K., and Van der Meer, R. 1992. Lytic effects of mixed micelles of fatty acids and bile acids. *Am J Physiol* **263**(1): 333-337.

Larsson, S.C., Bergkvist, L., Rutegard, J., Giovannucci, E., and Wolk, A. 2006. Calcium and dairy food intakes are inversely associated with colorectal cancer risk in the Cohort of Swedish Men. *Am J Clin Nutr* **83**(3): 667-673.

Lauritzen, J.B., Schwarz, P., McNair, P., Lund, B., and Transbol, I. 1993. Radial and humeral fractures as predictors of subsequent hip, radial or humeral fractures in women, and their seasonal variation. *Osteoporos Int* **3**(3): 133-137.

Lee, A.J., Cai, M.X., Thomas, P.E., Conney, A.H., and Zhu, B.T. 2003. Characterization of the oxidative metabolites of 17beta-estradiol and estrone formed by 15 selectively expressed human cytochrome p450 isoforms. *Endocrinology* **144**(8): 3382-3298.

Lee, S.J. and Kanis, J.A. 1994. An association between osteoporosis and premenstrual symptoms and postmenopausal symptoms. *Bone Miner* **24**(2): 127-134.

Lindberg, M.K., Svensson, J., Venken, K., Chavoshi, T., Andersson, N., Moverare Skrtic, S., Isaksson, O., Vanderschueren, D., Carlsten, H., and Ohlsson, C. 2006. Liver-derived IGF-I is permissive for ovariectomy-induced trabecular bone loss. *Bone* **38**(1): 85-92.

Lipkin, M. and Newmark, H. 1985. Effect of added dietary calcium on colonic epithelial-cell proliferation in subjects at high risk for familial colonic cancer. *N Engl J Med* **313**(22): 1381-1384.

-. 1995. Calcium and the prevention of colon cancer. *J Cell Biochem Suppl* **22**: S65-S73.

Livak, K.J. 1999. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet Anal* **14**(5-6): 143-149.

Lomer, M.C., Parkes, G.C., and Sanderson, J.D. 2008. Review article: lactose intolerance in clinical practice--myths and realities. *Aliment Pharmacol Ther* **27**(2): 93-103.

Longcope, C. 1996. Dehydroepiandrosterone metabolism. *J Endocrinol* **150**(S): S125-S127.

Lorentzon, M., Lorentzon, R., Lerner, U.H., and Nordstrom, P. 2001. Calcium sensing receptor gene polymorphism, circulating calcium concentrations and bone mineral density in healthy adolescent girls. *Eur J Endocrinol* **144**(3): 257-261.

Lorget, F., Kamel, S., Mentaverri, R., Wattel, A., Naassila, M., Maamer, M., and Brazier, M. 2000. High extracellular calcium concentrations directly stimulate osteoclast apoptosis. *Biochem Biophys Res Commun* **268**(3): 899-903.

Mallmin, H., Ljunghall, S., Persson, I., Naessen, T., Krusemo, U.B., and Bergstrom, R. 1993. Fracture of the distal forearm as a forecaster of subsequent hip fracture: a population-based cohort study with 24 years of follow-up. *Calcif Tissue Int* **52**(4): 269-272.

Mareel, M., Berx, G., Van Roy, F., and Bracke, M. 1996. Cadherin/catenin complex: a target for antiinvasive therapy? *J Cell Biochem* **61**(4): 524-530.

Martel, C., Melner, M.H., Gagne, D., Simard, J., and Labrie, F. 1994. Widespread tissue distribution of steroid sulfatase, 3 beta-hydroxysteroid dehydrogenase/delta 5-delta 4 isomerase (3 beta-HSD), 17 beta-HSD 5 alpha-reductase and aromatase activities in the rhesus monkey. *Mol Cell Endocrinol* **104**(1): 103-111.

Martinez, M.E., Grodstein, F., Giovannucci, E., Colditz, G.A., Speizer, F.E., Hennekens, C., Rosner, B., Willett, W.C., and Stampfer, M.J. 1997. A prospective study of reproductive factors, oral contraceptive use, and risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* **6**(1): 1-5.

Martinez, M.E. and Willett, W.C. 1998. Calcium, vitamin D, and colorectal cancer: a review of the epidemiologic evidence. *Cancer Epidemiol Biomarkers Prev* **7**(2): 163-168.

Marugo, M., Aste, H., Bernasconi, D., Fazzuoli, L., Conio, M., Cuva, A., and Meozzi, M. 1992. Cytosolic and nuclear androgen receptors in colorectal adenomas. *Anticancer Res* **12**(3): 705-708.

Marz, W., Seelhorst, U., Wellnitz, B., Tiran, B., Obermayer-Pietsch, B., Renner, W., Boehm, B.O., Ritz, E., and Hoffmann, M.M. 2007. Alanine to serine polymorphism at position 986 of the calcium-sensing receptor associated with coronary heart disease, myocardial infarction, all-cause, and cardiovascular mortality. *J Clin Endocrinol Metab* **92**(6): 2363-2369.

Massague, J. and Chen, Y.G. 2000. Controlling TGF-beta signaling. *Genes Dev* **14**(6): 627-644.

Mastorakos, G. and Ilias, I. 2003. Maternal and fetal hypothalamic-pituitary-adrenal axes during pregnancy and postpartum. *Ann N Y Acad Sci* **997**: 136-149.

McCormick, R.K. 2007. Osteoporosis: integrating biomarkers and other diagnostic correlates into the management of bone fragility. *Altern Med Rev* **12**(2): 113-145.

McGehee, D.S., Aldersberg, M., Liu, K.P., Hsuing, S., Heath, M.J., and Tamir, H. 1997. Mechanism of extracellular Ca2+ receptor-stimulated hormone release from sheep thyroid parafollicular cells. *J Physiol* **502**(1): 31-44.

McNeil, L., Hobson, S., Nipper, V., and Rodland, K.D. 1998a. Functional calcium-sensing receptor expression in ovarian surface epithelial cells. *Am J Obstet Gynecol* **178**(2): 305-313.

McNeil, S.E., Hobson, S.A., Nipper, V., and Rodland, K.D. 1998b. Functional calcium-sensing receptors in rat fibroblasts are required for activation of SRC kinase and mitogen-activated protein kinase in response to extracellular calcium. *J Biol Chem* **273**(2): 1114-1120.

Melton, L.J., 3rd, Chrischilles, E.A., Cooper, C., Lane, A.W., and Riggs, B.L. 1992. Perspective. How many women have osteoporosis? *J Bone Miner Res* **7**(9): 1005-1010.

Miki, Y., Nakata, T., Suzuki, T., Darnel, A.D., Moriya, T., Kaneko, C., Hidaka, K., Shiotsu, Y., Kusaka, H., and Sasano, H. 2002. Systemic distribution of steroid sulfatase and estrogen sulfotransferase in human adult and fetal tissues. *J Clin Endocrinol Metab* **87**(12): 5760-5768.

Miller, K.K., Sesmilo, G., Schiller, A., Schoenfeld, D., Burton, S., and Klibanski, A. 2001. Androgen deficiency in women with hypopituitarism. *J Clin Endocrinol Metab* **86**(2): 561-567.

Miller, W.L. 2005. Disorders of androgen synthesis--from cholesterol to dehydroepiandrosterone. *Med Princ Pract* **14**(S1): S58-S68.

Miyaura, C., Toda, K., Inada, M., Ohshiba, T., Matsumoto, C., Okada, T., Ito, M., Shizuta, Y., and Ito, A. 2001. Sex- and age-related response to aromatase deficiency in bone. *Biochem Biophys Res Commun* **280**(4): 1062-1068.

Mo, X.Y., Zhang, Y.Y., Lei, S.F., and Deng, H.W. 2004. A986S polymorphism of calcium-sensing receptor gene is not related to bone mineral density or bone size in premenopausal Chinese women. *Di Yi Jun Yi Da Xue Xue Bao* **24**(10): 1097-1101.

Molowa, D.T., Schuetz, E.G., Wrighton, S.A., Watkins, P.B., Kremers, P., Mendez-Picon, G., Parker, G.A., and Guzelian, P.S. 1986. Complete cDNA sequence of a cytochrome P-450 inducible by glucocorticoids in human liver. *Proc Natl Acad Sci USA* **83**(14): 5311-5315.

Moon, R.T., Brown, J.D., and Torres, M. 1997. WNTs modulate cell fate and behavior during vertebrate development. *Trends Genet* **13**(4): 157-162.

Mukai, T., Otsuka, F., Otani, H., Yamashita, M., Takasugi, K., Inagaki, K., Yamamura, M., and Makino, H. 2007. TNF-alpha inhibits BMP-induced osteoblast differentiation through activating SAPK/JNK signaling. *Biochem Biophys Res Commun* **356**(4): 1004-1010.

Nabholtz, J.M., Buzdar, A., Pollak, M., Harwin, W., Burton, G., Mangalik, A., Steinberg, M., Webster, A., and von Euler, M. 2000. Anastrozole is superior to tamoxifen as first-line therapy for advanced breast cancer in postmenopausal women: results of a North American multicenter randomized trial. Arimidex Study Group. *J Clin Oncol* **18**(22): 3758-3767.

Nakade, O., Takahashi, K., Takuma, T., Aoki, T., and Kaku, T. 2001. Effect of extracellular calcium on the gene expression of bone morphogenetic protein-2 and -4 of normal human bone cells. *J Bone Miner Metab* **19**(1): 13-19.

Nawata, H., Tanaka, S., Tanaka, S., Takayanagi, R., Sakai, Y., Yanase, T., Ikuyama, S., and Haji, M. 1995. Aromatase in bone cell: association with osteoporosis in postmenopausal women. *J Steroid Biochem Mol Biol* **53**(1-6): 165-174.

Newcomb, P.A., Zheng, Y., Chia, V.M., Morimoto, L.M., Doria-Rose, V.P., Templeton, A., Thibodeau, S.N., and Potter, J.D. 2007. Estrogen plus progestin use, microsatellite instability, and the risk of colorectal cancer in women. *Cancer Res* **67**(15): 7534-7539.

Nishimura, M., Yaguti, H., Yoshitsugu, H., Naito, S., and Satoh, T. 2003. Tissue distribution of mRNA expression of human cytochrome P450 isoforms assessed by high-sensitivity real-time reverse transcription PCR. *Yakugaku Zasshi* **123**(5): 369-375.

Nyce, J.W., Magee, P.N., Hard, G.C., and Schwartz, A.G. 1984. Inhibition of 1,2-dimethylhydrazine-induced colon tumorigenesis in Balb/c mice by dehydroepiandrosterone. *Carcinogenesis* **5**(1): 57-62.

Obermayer-Pietsch, B.M., Bonelli, C.M., Walter, D.E., Kuhn, R.J., Fahrleitner-Pammer, A., Berghold, A., Goessler, W., Stepan, V., Dobnig, H., Leb, G., and Renner, W. 2004. Genetic predisposition for adult lactose intolerance and relation to diet, bone density, and bone fractures. *J Bone Miner Res* **19**(1): 42-47.

Obermayer-Pietsch, B.M., Gugatschka, M., Reitter, S., Plank, W., Strele, A., Walter, D., Bonelli, C., Goessler, W., Dobnig, H., Hogenauer, C., Renner, W., and Fahrleitner-Pammer, A. 2007. Adult-type hypolactasia and calcium availability: decreased calcium intake or impaired calcium absorption? *OsteoporosInt* **18**(4): 445-451.

Oh, K., Willett, W.C., Wu, K., Fuchs, C.S., and Giovannucci, E.L. 2007. Calcium and vitamin D intakes in relation to risk of distal colorectal adenoma in women. *Am J Epidemiol* **165**(10): 1178-1186.

Ohmori, H., Makita, Y., Funamizu, M., Hirooka, K., Hosoi, T., Orimo, H., Suzuki, T., Ikari, K., Nakajima, T., Inoue, I., and Hata, A. 2002. Linkage and association analyses of the osteoprotegerin gene locus with human osteoporosis. *J Hum Genet* **47**(8): 400-406.

Okamoto, M., Murai, J., Yoshikawa, H., and Tsumaki, N. 2006. Bone morphogenetic proteins in bone stimulate osteoclasts and osteoblasts during bone development. *J Bone Miner Res* **21**(7): 1022-1033.

Ornitz, D.M. and Marie, P.J. 2002. FGF signaling pathways in endochondral and intramembranous bone development and human genetic disease. *Genes Dev* **16**(12): 1446-1465.

Osawa, E., Nakajima, A., Yoshida, S., Omura, M., Nagase, H., Ueno, N., Wada, K., Matsuhashi, N., Ochiai, M., Nakagama, H., and Sekihara, H. 2002. Chemoprevention of precursors to colon cancer by dehydroepiandrosterone (DHEA). *Life Sci* **70**(22): 2623-2630.

Osmanagaoglu, M.A., Okumus, B., Osmanagaoglu, T., and Bozkaya, H. 2004. The relationship between serum dehydroepiandrosterone sulfate concentration and bone mineral density, lipids, and hormone replacement therapy in premenopausal and postmenopausal women. *J Womens Health (Larchmt)* **13**(9): 993-999.

Oz, O.K., Hirasawa, G., Lawson, J., Nanu, L., Constantinescu, A., Antich, P.P., Mason, R.P., Tsyganov, E., Parkey, R.W., Zerwekh, J.E., and Simpson, E.R. 2001. Bone phenotype of the aromatase deficient mouse. *J Steroid Biochem Mol Biol* **79**(1-5): 49-59.

Park, S.Y., Murphy, S.P., Wilkens, L.R., Nomura, A.M., Henderson, B.E., and Kolonel, L.N. 2007. Calcium and vitamin D intake and risk of colorectal cancer: the Multiethnic Cohort Study. *Am J Epidemiol* **165**(7): 784-793.

Parker, L.N. 1991. Control of adrenal androgen secretion. *Endocrinol Metab Clin North Am* **20**(2): 401-421.

Payne, C.M., Bernstein, H., Bernstein, C., and Garewal, H. 1995. Role of apoptosis in biology and pathology: resistance to apoptosis in colon carcinogenesis. *Ultrastruct Pathol* **19**(4): 221-248.

Pereira, M.A., Jacobs, D.R., Jr., Van Horn, L., Slattery, M.L., Kartashov, A.I., and Ludwig, D.S. 2002. Dairy consumption, obesity, and the insulin resistance syndrome in young adults: the CARDIA Study. *JAMA* **287**(16): 2081-2089.

Pi, M., Garner, S.C., Flannery, P., Spurney, R.F., and Quarles, L.D. 2000. Sensing of extracellular cations in CasR-deficient osteoblasts. Evidence for a novel cation-sensing mechanism. *J Biol Chem* **275**(5): 3256-3263.

Pitts, R.L. 1987. Serum elevation of dehydroepiandrosterone sulfate associated with male pattern baldness in young men. *J Am Acad Dermatol* **16**(1): 571.

Ponjee, G.A., De Rooy, H.A., and Vader, H.L. 1994. Androgen turnover during marathon running. *Med Sci Sports Exerc* **26**(10): 1274-1277.

Poór, G. 1992. Az osteoporosis, a csigolyadeformitások és a csípőtáji femurtörések epidemiológiai vonatkozásai. *Orv Hetil* **133**(27): 1695-1701.

-. 1997. A csigolyadeformitás prevalenciája hazánkban: Az Európai Vertebrális Osteoporosis Study. *OrvHetil* **138**(42): 2647-2652.

Quarles, L.D., Hartle, J.E., 2nd, Siddhanti, S.R., Guo, R., and Hinson, T.K. 1997. A distinct cation-sensing mechanism in MC3T3-E1 osteoblasts functionally related to the calcium receptor. *J Bone Miner Res* **12**(3): 393-402.

Rainey, W.E., Carr, B.R., Sasano, H., Suzuki, T., and Mason, J.I. 2002. Dissecting human adrenal androgen production. *Trends Endocrinol Metab* **13**(6): 234-239.

Ramsaransing, G.S., Heersema, D.J., and De Keyser, J. 2005. Serum uric acid, dehydroepiandrosterone sulphate, and apolipoprotein E genotype in benign vs. progressive multiple sclerosis. *Eur J Neurol* **12**(7): 514-518.

Rapuri, P.B., Gallagher, J.C., and Haynatzki, G. 2004. Endogenous levels of serum estradiol and sex hormone binding globulin determine bone mineral density, bone remodeling, the rate of bone loss, and response to treatment with estrogen in elderly women. *J Clin Endocrinol Metab* **89**(10): 4954-4962.

Rasinpera, H., Forsblom, C., Enattah, N.S., Halonen, P., Salo, K., Victorzon, M., Mecklin, J.P., Jarvinen, H., Enholm, S., Sellick, G., Alazzouzi, H., Houlston, R., Robinson, J., Groop, P.H., Tomlinson, I., Schwartz, S., Jr., Aaltonen, L.A., Jarvela, I., and FinnDiane Study, G. 2005. The C/C-13910 genotype of adult-type hypolactasia is associated with an increased risk of colorectal cancer in the Finnish population. *Gut* **54**(5): 643-647.

Reid, I.R., Eastell, R., Fogelman, I., Adachi, J.D., Rosen, A., Netelenbos, C., Watts, N.B., Seeman, E., Ciaccia, A.V., and Draper, M.W. 2004. A comparison of the effects of raloxifene and conjugated equine estrogen on bone and lipids in healthy postmenopausal women. *Arch Intern Med* **164**(8): 871-879.

Reiter, W.J., Pycha, A., Schatzl, G., Klingler, H.C., Mark, I., Auterith, A., and Marberger, M. 2000. Serum dehydroepiandrosterone sulfate concentrations in men with erectile dysfunction. *Urology* **55**(5): 755-758.

Reiter, W.J., Pycha, A., Schatzl, G., Pokorny, A., Gruber, D.M., Huber, J.C., and Marberger, M. 1999. Dehydroepiandrosterone in the treatment of erectile dysfunction: a prospective, double-blind, randomized, placebo-controlled study. *Urology* **53**(3): 590-594.

Repke, J.T., Villar, J., Anderson, C., Pareja, G., Dubin, N., and Belizan, J.M. 1989. Biochemical changes associated with blood pressure reduction induced by calcium supplementation during pregnancy. *Am J Obstet Gynecol* **160**(3): 684-690.

Riccardi, D. and Maldonado-Perez, D. 2005. The calcium-sensing receptor as a nutrient sensor. *Biochem Soc Trans* **33**(1): 316-320.

Rogers, K.V., Dunn, C.K., Hebert, S.C., and Brown, E.M. 1997. Localization of calcium receptor mRNA in the adult rat central nervous system by in situ hybridization. *Brain Res* **744**(1): 47-56.

Romoli, R., Lania, A., Mantovani, G., Corbetta, S., Persani, L., and Spada, A. 1999. Expression of calcium-sensing receptor and characterization of intracellular signaling in human pituitary adenomas. *J Clin Endocrinol Metab* **84**(8): 2848-2853.

Rossouw, J.E., Anderson, G.L., Prentice, R.L., LaCroix, A.Z., Kooperberg, C., Stefanick, M.L., Jackson, R.D., Beresford, S.A., Howard, B.V., Johnson, K.C., Kotchen, J.M., and Ockene, J. 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. *JAMA* **288**(3): 321-333.

Sahi, T. 1994. Genetics and epidemiology of adult-type hypolactasia. *Scand J Gastroenterol Suppl* **202**: S7-S20.

Sahi, T., Isokoski, M., Jussila, J., Launiala, K., and Pyorala, K. 1973. Recessive inheritance of adult-type lactose malabsorption. *Lancet* **2**(7833): 823-826.

Sambrook, P. and Cooper, C. 2006. Osteoporosis. *Lancet* **367**(9527): 2010-2018.

Schulz, S., Klann, R.C., Schonfeld, S., and Nyce, J.W. 1992. Mechanisms of cell growth inhibition and cell cycle arrest in human colonic adenocarcinoma cells by dehydroepiandrosterone: role of isoprenoid biosynthesis. *Cancer Res* **52**(5): 1372-1376.

Schulz, S. and Nyce, J.W. 1991. Inhibition of protein isoprenylation and p21ras membrane association by dehydroepiandrosterone in human colonic adenocarcinoma cells in vitro. *Cancer Res* **51**(24): 6563-6567.

Schwartz, A.G. and Pashko, L.L. 1995. Cancer prevention with dehydroepiandrosterone and non-androgenic structural analogs. *J Cell Biochem Suppl* **22**: S210-S217.

Seeman, E. 2003. Invited Review: Pathogenesis of osteoporosis. *J Appl Physiol* **95**(5): 2142-2151.

Sexson, S.B. and Lehner, J.T. 1987. Factors affecting hip fracture mortality. *J Orthop Trauma* **1**(4): 298-305.

Sheinin, Y., Kallay, E., Wrba, F., Kriwanek, S., Peterlik, M., and Cross, H.S. 2000. Immunocytochemical localization of the extracellular calcium-sensing receptor in normal and malignant human large intestinal mucosa. *J Histochem Cytochem* **48**(5): 595-602.

Shi, H., Norman, A.W., Okamura, W.H., Sen, A., and Zemel, M.B. 2001. 1alpha,25-Dihydroxyvitamin D3 modulates human adipocyte metabolism via nongenomic action. *FASEB J* **15**(14): 2751-2753.

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., and Guengerich, F.P. 1994. Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J Pharmacol Exp Ther* **270**(1): 414-423.

Silver, I.A., Murrills, R.J., and Etherington, D.J. 1988. Microelectrode studies on the acid microenvironment beneath adherent macrophages and osteoclasts. *Exp Cell Res* **175**(2): 266-276.

Slattery, M.L., Anderson, K., Samowitz, W., Edwards, S.L., Curtin, K., Caan, B., and Potter, J.D. 1999. Hormone replacement therapy and improved survival among

postmenopausal women diagnosed with colon cancer (USA). *Cancer Causes Control* **10**(5): 467-473.

Slattery, M.L., Potter, J.D., Curtin, K., Edwards, S., Ma, K.N., Anderson, K., Schaffer, D., and Samowitz, W.S. 2001. Estrogens reduce and withdrawal of estrogens increase risk of microsatellite instability-positive colon cancer. *Cancer Res* **61**(1): 126-130.

Slayden, S.M., Moran, C., Sams, W.M., Jr., Boots, L.R., and Azziz, R. 2001. Hyperandrogenemia in patients presenting with acne. *Fertil Steril* **75**(5): 889-892.

Smirnoff, P., Liel, Y., Gnainsky, J., Shany, S., and Schwartz, B. 1999. The protective effect of estrogen against chemically induced murine colon carcinogenesis is associated with decreased CpG island methylation and increased mRNA and protein expression of the colonic vitamin D receptor. *Oncol Res* **11**(6): 255-264.

Smit, P., van Schaik, R.H., van der Werf, M., van den Beld, A.W., Koper, J.W., Lindemans, J., Pols, H.A., Brinkmann, A.O., de Jong, F.H., and Lamberts, S.W. 2005. A common polymorphism in the CYP3A7 gene is associated with a nearly 50% reduction in serum dehydroepiandrosterone sulfate levels. *J Clin Endocrinol Metab* **90**(9): 5313-5316.

Somogyi, P. 2000. Az osteoporosis eredetű csonttörések számának becslése Magyarországon. *Ca és Csont* **3**(3): 111-117.

Sowa, H., Kaji, H., Yamaguchi, T., Sugimoto, T., and Chihara, K. 2002. Smad3 promotes alkaline phosphatase activity and mineralization of osteoblastic MC3T3-E1 cells. *J Bone Miner Res* **17**(7): 1190-1199.

Spark, R.F. 2002. Dehydroepiandrosterone: a springboard hormone for female sexuality. *Fertil Steril* **77**(S4): S19-S25.

Spivak, B., Maayan, R., Kotler, M., Mester, R., Gil-Ad, I., Shtaif, B., and Weizman, A. 2000. Elevated circulatory level of GABA(A)--antagonistic neurosteroids in patients with combat-related post-traumatic stress disorder. *Psychol Med* **30**(5): 1227-1231.

Stebbings, W.S., Vinson, G.P., Farthing, M.J., Balkwill, F., and Wood, R.F. 1989. Effect of steroid hormones on human colorectal adenocarcinoma xenografts, of known steroid-receptor status, in nude mice. *J Cancer Res Clin Oncol* **115**(5): 439-444.

Steinberg, K.K., Freni-Titulaer, L.W., DePuey, E.G., Miller, D.T., Sgoutas, D.S., Coralli, C.H., Phillips, D.L., Rogers, T.N., and Clark, R.V. 1989. Sex steroids and bone density in premenopausal and perimenopausal women. *J Clin Endocrinol Metab* **69**(3): 533-539.

Stomati, M., Monteleone, P., Casarosa, E., Quirici, B., Puccetti, S., Bernardi, F., Genazzani, A.D., Rovati, L., Luisi, M., and Genazzani, A.R. 2000. Six-month oral dehydroepiandrosterone supplementation in early and late postmenopause. *Gynecol Endocrinol* **14**(5): 342-363.

Straub, R.H., Vogl, D., Gross, V., Lang, B., Scholmerich, J., and Andus, T. 1998. Association of humoral markers of inflammation and dehydroepiandrosterone sulfate or cortisol serum levels in patients with chronic inflammatory bowel disease. *Am J Gastroenterol* **93**(11): 2197-2202.

Straub, R.H., Weidler, C., Demmel, B., Herrmann, M., Kees, F., Schmidt, M., Scholmerich, J., and Schedel, J. 2004. Renal clearance and daily excretion of cortisol and adrenal androgens in patients with rheumatoid arthritis and systemic lupus erythematosus. *Ann Rheum Dis* **63**(8): 961-968.

Szabolcs, O., Kásler, M. 2005. A hazai és nemzetközi daganatos halálozási és megbetegedési mutatók alakulása. *Magy Onkol* **49**(2): 99-107.

Takacs, I., Speer, G., Bajnok, E., Tabak, A., Nagy, Z., Horvath, C., Kovacs, K., and Lakatos, P. 2002. Lack of association between calcium-sensing receptor gene "A986S" polymorphism and bone mineral density in Hungarian postmenopausal women. *Bone* **30**(6): 849-852.

Tang, B.M., Eslick, G.D., Nowson, C., Smith, C., and Bensoussan, A. 2007. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *Lancet* **370**(9588): 657-666.

Tanko, L.B., Felsenberg, D., Czerwinski, E., Burdeska, A., Jonkanski, I., Hughes, C., and Christiansen, C. 2003. Oral weekly ibandronate prevents bone loss in postmenopausal women. *J Intern Med* **254**(2): 159-167.

Thys-Jacobs, S. 2000. Micronutrients and the premenstrual syndrome: the case for calcium. *J Am Coll Nutr* **19**(2): 220-227.

Toth, M., Racz, K., Varga, I., Adleff, V., Jakab, C., Futo, L., Kiss, R., and Glaz, E. 1997. Plasma dehydroepiandrosterone sulfate levels in patients with hyperfunctioning and non-hyperfunctioning adrenal tumors before and after adrenal surgery. *Eur J Endocrinol* **136**(3): 290-295.

Troelsen, J.T., Olsen, J., Moller, J., and Sjostrom, H. 2003. An upstream polymorphism associated with lactase persistence has increased enhancer activity. *Gastroenterology* **125**(6): 1686-1694.

Tu, C.L., Oda, Y., Komuves, L., and Bikle, D.D. 2004. The role of the calcium-sensing receptor in epidermal differentiation. *Cell Calcium* **35**(3): 265-273.

Valle, L.D., Toffolo, V., Nardi, A., Fiore, C., Bernante, P., Di Liddo, R., Parnigotto, P.P., and Colombo, L. 2006. Tissue-specific transcriptional initiation and activity of steroid sulfatase complementing dehydroepiandrosterone sulfate uptake and intracrine steroid activations in human adipose tissue. *J Endocrinol* **190**(1): 129-139.

Velleman, S.G. 2000. The role of the extracellular matrix in skeletal development. *Poult Sci* **79**(7): 985-989.

Vermeulen, A., Suy, E., and Rubens, R. 1977. Effect of prolactin on plasma DHEA (s) levels. *J Clin Endocrinol Metab* **44**(6): 1222-1225.

Vesa, T.H., Marteau, P., and Korpela, R. 2000. Lactose intolerance. *J Am Coll Nutr* **19**(S2): S165-S175.

Vider, B.Z., Zimber, A., Chastre, E., Prevot, S., Gespach, C., Estlein, D., Wolloch, Y., Tronick, S.R., Gazit, A., and Yaniv, A. 1996. Evidence for the involvement of the Wnt 2 gene in human colorectal cancer. *Oncogene* **12**(1): 153-158.

Villareal, D.T. and Holloszy, J.O. 2004. Effect of DHEA on abdominal fat and insulin action in elderly women and men - A randomized controlled trial. *JAMA* **292**(18): 2243-2248.

Villareal, D.T., Holloszy, J.O., and Kohrt, W.M. 2000. Effects of DHEA replacement on bone mineral density and body composition in elderly women and men. *Clin Endocrinol (Oxf)* **53**(5): 561-568.

Wactawski-Wende, J., Kotchen, J.M., Anderson, G.L., Assaf, A.R., Brunner, R.L., O'Sullivan, M.J., Margolis, K.L., Ockene, J.K., Phillips, L., Pottern, L., Prentice, R.L., Robbins, J., Rohan, T.E., Sarto, G.E., Sharma, S., Stefanick, M.L., Van Horn, L., Wallace, R.B., Whitlock, E., Bassford, T., Beresford, S.A., Black, H.R., Bonds, D.E., Brzyski, R.G., Caan, B., Chlebowski, R.T., Cochrane, B., Garland, C., Gass, M., Hays, J., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Jackson, R.D., Johnson, K.C., Judd, H., Kooperberg, C.L., Kuller, L.H., LaCroix, A.Z., Lane, D.S., Langer, R.D., Lasser, N.L., Lewis, C.E., Limacher, M.C., and Manson, J.E. 2006. Calcium plus vitamin D supplementation and the risk of colorectal cancer. *N Engl J Med* **354**(7): 684-696.

Wargovich, M.J., Eng, V.W., Newmark, H.L., and Bruce, W.R. 1983. Calcium ameliorates the toxic effect of deoxycholic acid on colonic epithelium. *Carcinogenesis* **4**(9): 1205-1207.

Westlind, A., Malmebo, S., Johansson, I., Otter, C., Andersson, T.B., Ingelman-Sundberg, M., and Oscarson, M. 2001. Cloning and tissue distribution of a novel human cytochrome p450 of the CYP3A subfamily, CYP3A43. *Biochem Biophys Res Commun* **281**(5): 1349-1355.

Willenberg, H.S., Bahlo, M., Schott, M., Wertenbruch, T., Feldkamp, J., and Scherbaum, W.A. 2008. Helpful diagnostic markers of steroidogenesis for defining hyperandrogenemia in hirsute women. *Steroids* **73**(1): 41-46.

Willumsen, B.M., Christensen, A., Hubbert, N.L., Papageorge, A.G., and Lowy, D.R. 1984. The p21 ras C-terminus is required for transformation and membrane association. *Nature* **310**(5978): 583-586.

Wilmink, A.B. 1997. Overview of the epidemiology of colorectal cancer. *Dis Colon Rectum* **40**(4): 483-493.

Winn, R.A., Bremnes, R.M., Bemis, L., Franklin, W.A., Miller, Y.E., Cool, C., and Heasley, L.E. 2002. gamma-Catenin expression is reduced or absent in a subset of human lung cancers and re-expression inhibits transformed cell growth. *Oncogene* **21**(49): 7497-7506.

Wong, B.C., Wang, W.P., Cho, C.H., Fan, X.M., Lin, M.C., Kung, H.F., and Lam, S.K. 2001. 12-Lipoxygenase inhibition induced apoptosis in human gastric cancer cells. *Carcinogenesis* **22**(9): 1349-1354.

Wong, N.A.C.S. and Pignatelli, M. 2002. Beta-catenin--a linchpin in colorectal carcinogenesis? *Am J Pathol* **160**(2): 389-401.

Xia, Z., Dickens, M., Raingeaud, J., Davis, R.J., and Greenberg, M.E. 1995. Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis. *Science* **270**(5240): 1326-1331.

Yamaguchi, T., Chattopadhyay, N., Kifor, O., Butters, R.R., Jr., Sugimoto, T., and Brown, E.M. 1998a. Mouse osteoblastic cell line (MC3T3-E1) expresses extracellular calcium (Ca2+o)-sensing receptor and its agonists stimulate chemotaxis and proliferation of MC3T3-E1 cells. *J Bone Miner Res* **13**(10): 1530-1538.

Yamaguchi, T., Olozak, I., Chattopadhyay, N., Butters, R.R., Kifor, O., Scadden, D.T., and Brown, E.M. 1998b. Expression of extracellular calcium (Ca2+o)-sensing receptor in human peripheral blood monocytes. *Biochem Biophys Res Commun* **246**(2): 501-506.

Yamauchi, A., Takei, I., Kasuga, A., Kitamura, Y., Ohashi, N., Nakano, S., Takayama, S., Nakamoto, S., Katsukawa, F., and Saruta, T. 1996. Depression of dehydroepiandrosterone in Japanese diabetic men--comparison between non-insulin-

dependent diabetes mellitus and impaired glucose tolerance. *Eur J Endocrinol* **135**(1): 101-104.

Yamauchi, M., Yamaguchi, T., Kaji, H., Sugimoto, T., and Chihara, K. 2005. Involvement of calcium-sensing receptor in osteoblastic differentiation of mouse MC3T3-E1 cells. *Am J Physiol Endocrinol Metab* **288**(3): 608-616.

Young, R., Wu, F., Van de Water, N., Ames, R., Gamble, G., and Reid, I.R. 2003. Calcium sensing receptor gene A986S polymorphism and responsiveness to calcium supplementation in postmenopausal women. *J Clin Endocrinol Metab* **88**(2): 697-700.

Zaidi, M., Datta, H.K., Patchell, A., Moonga, B., and MacIntyre, I. 1989. 'Calcium-activated' intracellular calcium elevation: a novel mechanism of osteoclast regulation. *Biochem Biophys Res Commun* **163**(3): 1461-1465.

Zhang, X., Peng, B., Fan, M., Bian, Z., and Chen, Z. 2007. The effect of estrogen deficiency on receptor activator of nuclear factor kappa B ligand and osteoprotegerin synthesis in periapical lesions induced in rats. *J Endod* **33**(9): 1053-1056.

Zumoff, B., Rosenfeld, R.S., Strain, G.W., Levin, J., and Fukushima, D.K. 1980. Sex differences in the twenty-four-hour mean plasma concentrations of dehydroisoandrosterone (DHA) and dehydroisoandrosterone sulfate (DHAS) and the DHA to DHAS ratio in normal adults. *J Clin Endocrinol Metab* **51**(2): 330-333.

11. Original papers

Publications related to the dissertation

Bácsi, K., Kósa, JP., Borgulya, G., Balla, B., Lazáry, Á., Nagy, Z., Horváth, C., Speer, G., Lakatos, P. 2007. CYP3A7*1C polymorphism, serum dehydroepiandrosterone sulfate level, and bone mineral density in postmenopausal women. *Calcif Tissue Int* **80**(3): 154-9. **IF: 2.483**

Bácsi, K., Kósa, J., Lazáry, Á., Horváth, H., Balla, B., Lakatos, P., Speer, G. 2007. Importance of dehydroepiandrosterone and dehydroepiandrosterone sulfate in different diseases. *Orv Hetil* **148**(14): 651-7.

Bácsi. K., Kósa, J., Lazáry, Á., Balla, B., Horváth, H., Takács, I., Nagy, Z., Speer, G., Lakatos, P. 2007. Impact of CYP3A7*1C polymorphism on serum dehidroepiandrosteron sulphate level and bone mineral density in postmenopausal women. *Orv Hetil* **148**(27): 1273-80.

Lazáry, Á., Balla, B., Kósa, JP., **Bácsi, K.**, Nagy, Z., Takács, I., Varga, PP., Speer, G., Lakatos, P. 2007. Effect of gypsium on proliferation and differentation of MC3T3-E1 mouse osteoblast cells. *Biomaterials* **28**(3): 393-9. **IF: 5.196**

Bácsi, K., Hitre, E., Kósa, JP., Horváth, H., Lazáry, Á., Lakatos, LP., Balla, B., Nagy, Z., Lakatos, P., Speer, G. Effects of the lactose-phlorizin hydrolase 13910 C/T and calcium-sensor receptor A986S G/T polymorphisms on the incidence and recurrence of colorectal cancer (submitted for *Int J Cancer*, Manuscript number: IJC-08-0175)

Bácsi, K., Kósa, JP., Lazáry, Á., Balla, B., Nagy, Z., Lakatos, P., Speer, G. Effect of LCT 13910 C/T polymorphism on serum calcium level and bone mineral density in postmenopausal women (submitted for *Osteoporosis Int*, Manuscript number: OI-2008-01-0049)

Publications not related to the dissertation

Lazáry, Á., Speer, G., Varga, PP., Balla, B., **Bácsi, K.**, Kósa, JP., Nagy, Z., Takács, I., Lakatos, P. Effect of vertebroplasty filler materials on viability and gene expression of human nucleus pulposus cells. *J Orthop Res,* 2008. Jan. 4. (article in press, DOI 10.1002/jor.20532) **IF: 2.784**

Lazáry, Á., Balla, B., Kósa, JP., **Bácsi, K.**, Nagy, Z., Takács, I., Varga, PP., Speer, G., Lakatos, P. 2007. Synthetic bone grafts, the role of the gypsum in bone substitution; molecular biological approach. *Orv Hetil*, **148** (51): 2427-33.

Balla, B., Kósa, JP., Kiss, J., Borsy, A., Podani, J., Takács, I., Lazáry, Á., Nagy, Z., **Bácsi, K.**, Speer, G., Orosz, L., Lakatos, P. 2008. Different gene expression patterns in the bone tissue of aging postmenopausal osteoporotic and non-osteoporotic women. *Calcif Tissue Int*, **82**(1): 12-26 **IF: 2.483**

Kósa, JP., Kis, A., **Bácsi, K.**, Nagy, Z., Balla, B., Lazáry, Á., Takács, I., Speer, G., Lakatos, P. 2007. Identification of novel candidate genes associated with glucocorticoid-induced bone loss in a murine osteoblastic cell line and primary calvarial osteoblasts. *Magyar Belorvosi Archívum,* **60**(5): 443-450.

Balla, B., Kósa, JP., Takács, I., Kiss, J., Podani, J., Borsy, A., Lazáry, Á., **Bácsi, K.**, Nagy, Z., Speer, G., Orosz, L., Lakatos, P. Menopauza hatása a csontszöveti génkifejeződésre posztmenopauzás és premenopauzás korú nem oszteoprotikus nőkben. *Magyar Belorvosi Archívum* (article in press)

Kiss, J., Balla, B., Kósa, JP., Borsy, A., Podani, J., Takács, I., Lazáry, Á., Nagy, Z., **Bácsi, K.**, Szlávy, E., Szendrői, M., Speer, G., Orosz, L., Lakatos, P. Gene expression patterns in the bone tissue of women with fibrous dysplasia (submitted for *J Bone Miner Res*, Manuscript number: J0802072)

Lazáry, Á., Kósa, JP., Tóbiás, B., Balla, B., **Bácsi, K.**, Takács, I., Nagy, Z., Speer, G., Mező, T., Lakatos, P. Single nucleotide polymorphisms in new candidate genes are

associated with bone mineral density in Hungarian postmenopausal women (submitted for *Eur J Endocrinol*, Manuscript number: EJE-08-0021)

Abstracts

Bácsi, K., Kósa, JP., Balla, B., Lazáry, Á., Nagy, Z., Takács, I., Speer, G., Lakatos, P. Decreased activity of lactase phlorizin hydrolase and bone mineral density in postmenopausal women. *American Society for Bone and Mineral Research (ASBMR) 29th Annual Meeting* Honolulu, HI, USA September 16-19, 2007. *J Bone Miner Res* 2007, **22**(S1): S186

Bácsi, K., Kósa, JP., Balla, B., Lazáry, Á., Nagy, Z., Takács, I., Speer, G., Lakatos, P. A laktáz phlorizin hidroláz és a csontdenzitás kapcsolatának vizsgálata posztmenopauzális nőkben. *Magyar Osteoporosis és Osteoarthrologiai Társaság XVI. Kongresszusa* Balatonfüred, 2007. május 23-26. *Ca és Csont* 2007, **10**(2): 50

Lazáry, Á., Varga, P., Speer, G., **Bácsi, K.,** Balla, B., Kósa, JP., Nagy, Z., Takács, I., Lakatos, P. Effect of Vertebroplasty Filler Materials on Viability and Gene Expression of Mouse and Human Nucleus Pulposus Cells. *American Society for Bone and Mineral Research (ASBMR) 29th Annual Meeting* Honolulu, HI, USA September 16-19, 2007. *J Bone Miner Res* 2007, **22**(S1): S160

Balla, B., Kósa, JP., Kiss, J., Borsy, A., Podani, J., Takács, I., Lazáry, Á., Nagy, Z., **Bácsi, K.,** Speer, G., Orosz, L., Lakatos, P. Effect of Estrogen Deficiency on Gene Expression Pattern in the Bone Tissue of Postmenopausal Versus Premenopausal Healthy Women. *American Society for Bone and Mineral Research (ASBMR) 29th Annual Meeting* Honolulu, HI, USA September 16-19, 2007. *J Bone Miner Res* 2007, **22**(S1): S261

Speer, G., Lazáry, Á., Kósa, JP., Tóbiás, B., Mező, T., **Bácsi, K.,** Balla, B., Takács, I., Nagy, Z., Lakatos, P. Multiplex SNP Genotyping and Data Analysis on 360 Hungarian Postmenopausal Women. *American Society for Bone and Mineral Research (ASBMR) 29th Annual Meeting* Honolulu, HI, USA September 16-19, 2007. *J Bone Miner Res* 2007, **22**(S1): S286

Balla, B., Kósa, JP., Takács, I., Kiss, J., Podani, J., Borsy, A., Lazáry, Á., **Bácsi, K.,** Speer, G., Orosz, L., Lakatos, P. Ösztrogén hiányában fellépő génexpressziós változás vizsgálata posztmenopauzás és premenopauzás nem osteoporosisos nők csontszövetében. *Magyar Osteoporosis és Osteoarthrologiai Társaság XVI. Kongresszusa* Balatonfüred, 2007. május 23-26. *Ca és Csont* 2007, **10**(2): 51

Lazáry, Á., Speer, G., Kósa, JP., Tóbiás, B., Balla, B., **Bácsi, K.,** Nagy, Z., Takács, I., Mező, T., Lakatos, P. Multiplex SNP genotipizálás és adatfeldolgozás 360 posztmenopauzás nő vérmintájából. *Magyar Osteoporosis és Osteoarthrologiai Társaság XVI. Kongresszusa* Balatonfüred, 2007. május 23-26. *Ca és Csont* 2007, **10**(2): 59

Kis, A., Balla, B., Lazáry, Á., Takács, I., Nagy, Z., Speer, G., Mező, T., **Bácsi, K.,** Lakatos, P. A tartós glükokortikoid terápia okozta osteoporosis létrejöttében résztvevő gének kimutatása osteoblastsejteken. *Magyar Osteoporosis és Osteoarthrologiai Társaság XV. Kongresszusa* Balatonfüred, 2006. május 24-27. *Ca és Csont* 2006, **9**(1): 19

Balla, B., Borsy, A., Kósa, JP., Kiss, J., Lazáry, Á., Takács, I., Nagy, Z., Speer, G., **Bácsi, K.,** Orosz, L., Lakatos, P. Génexpressziós mintázat vizsgálata posztmenopauzában levő egészséges és osteoporotikus nők csontszövetében. *Magyar Osteoporosis és Osteoarthrologiai Társaság XV. Kongresszusa* Balatonfüred, 2006. május 24-27. *Ca és Csont* 2006, **9**(1): 11

Lazáry, Á., Balla, B., Kósa, JP., Takács, I., Nagy, Z., Speer, G., **Bácsi, K.,** Koppány, V., Lakatos, P. A gipsz in vitro hatása az osteoblastok osztódására és differenciálódására. *Magyar Osteoporosis és Osteoarthrologiai Társaság XV. Kongresszusa* Balatonfüred, 2006. május 24-27. *Ca és Csont* 2006, **9**(1): 22

Balla, B., Kósa, JP., Takács, I., Lazáry, Á., **Bácsi, K.,** Lakatos, P. Génexpressziós mintázat vizsgálata prae- és postmenopausás egészséges nők csontszövetében. *A Magyar Endokrinológiai és Anyagcsere Társaság XXI. Kongresszusa* Debrecen, 2006. május 18-20. Magyar Belorvosi Archívum 2006, **1**: 24

CUMULATIVE IMPACT FACTOR: 12.946

12. Acknowledgements

Firstly, I would like to thank my tutor, Prof. Péter Lakatos for his guidance throughout my studies and for providing physical and theoretical background. His practical thinking and critical ability had a great impact on me. Without his help this work would have never been prepared. I would like to thank Dr. Gábor Speer, my co-tutor for his scrupulous attention and contribution to my work. He also provided me absolute support to progress in scientific research. My sincere thanks to Prof. Ágoston Szél for his important contribution to success in my research. I would like to thank Prof. Éva Szőke for her support. I would also like to acknowledge János Kósa for his methodological experience. I wish to thank Edit Máté for the relaxing conversations. Special thanks to Dr. Zsolt Nagy, Dr. István Takács, Bernadett Balla and Dr. Áron Lazáry for their help as members of our team. I also thank Dr. Erika Hitre for providing optimal research conditions in her institute. Thanks to the staff of our department at Semmelweis University for their generous help.

Thanks to my friends, Gabriella Taba, István Hartung and Dr. Lehel Hagyó for their patience and the conversations always full of fun. Finally but not lastly, special thanks to my parents for their love and moral support. I would also like to say thanks to my brother, András.