



RĪGAS STRADIŅA
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ALKYLRESORCINOL,
LIGNAN METABOLITES
AND VITAMIN D
IN PROSTATE CANCER
PATIENTS

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CONTENTS

Abbreviations	5
Introduction	6
Actuality of scientific work	6
Hypothesis of the study	8
Aims of the study	8
Objectives	8
Novelty of the study	10
Personal contribution	10
Structure of the work.....	11
1. Materials and methods	12
1.1. Study subjects and design	12
1.2. Assessment of risk factors	19
1.3. Assessment of dietary intake	19
1.4. Determination of lignans and alkylresorcinols in different types of bread	20
1.5. Laboratory examination.....	21
1.6. Morphological examination	22
1.7. Statistical analysis	23
2. Results	24
2.1. Alkylresorcinols and lignans in Latvian bread	24
2.2. Diet: general characteristic, alkylresorcinols and lignans in diet.....	26
2.3. Correlation between dietary parameters and concentration of alkylresorcinol and lignan metabolites in plasma and urine	31
2.4. Intervention study „ Influence of whole-grain rye bread on prostate cancer progression”	43
2.5. Vitamin D	45

3. Discussion	48
3.1. Alkylresorcinols and lignans in Latvian bread	48
3.2. Diet, alkylresorcinol and lignan intake	49
3.3. Alkylresorcinol metabolites and enterolactone	53
3.4. Intervention with rye bread in PC patients	58
3.5. Vitamin D	61
4. Conclusions	64
5. Practical recommendations	65
6. Acknowledgements	66
References	68
List of publications	75

ABBREVIATIONS

25(OH)D	–	25-hydroxyvitamin D
AIAT	–	Alanine Aminotransaminase
AMP	–	Adenosine monofosfate
AR	–	Alkylresocinol
CV	–	Coefficient of variation
DHBA	–	3,5-dihydroxybenzoic acid
DHPPA	–	3-(3,5-dihydroxyphenyl)-1-propanoic acid
DRI	–	Digital rectal examination
ENL	–	Enterolactone
FAI	–	Free androgen index
fPSA	–	Free Prostate Specific Antigen
FSH	–	Follicle-stimulating hormone
GC-MS	–	Gas chromatography-mass spectrometry
HPLC-CAD	–	High performance liquid chromatography with Coulometric Electrode Array Detection
IGF	–	Insulin-like growth factor
IU	–	International Unit
LAR	–	Lariciresinol
LH	–	Luteinizing Hormone
MAT	–	Matairesinol
MED	–	Medioresinol
nd	–	Not detectable
NMS	–	National medicine service
PC	–	Prostate cancer
PIN	–	Pinoresinol
PSA	–	Prostate Specific Antigen
SD	–	Standard deviation
SECO	–	Secoisolariciresinol
SHBG	–	Sex hormone-binding globulin
SYR	–	Syringaresinol
TRUS	–	Transrectal Ultrasound of the Prostate

INTRODUCTION

Actuality of scientific work

The evidence data of last decades point to the fact, that the diet and nutrition have become essential in the risk of oncological diseases. The development of cancer in 30-40% cases is considered to be related to nutrition and some lifestyle factors. In its recent report World Research Fund/American Institute for Cancer Research has postulated, that one third of oncological diseases can be prevented by diet [World Cancer Research Fund / American Institute for Cancer Research, 2007].

Prostate cancer (PC) is the second most common oncological disease in men round the world, as well as the sixth commonest cause of death among all cancer types worldwide, and the commonest one in Latvia [International Agency for Research on Cancer, 2008; Slimību profilakses un kontroles centrs, 2012]. PC is considered to be the key candidate for prevention because of its relatively slow progression, it can take years or decades before the development of dysplastic changes in prostate tissues [Schmid, 2011]. There are the data of studies on the influence of environmental factors, including the diet, that give evidence as to the regional differences in PC incidence and prevalence, as well as studies of migrants: in Asian men there is a remarkable increase of PC incidence when changing the residence from Asia to the United States [World Cancer Research Fund/ American Institute for Cancer Research, 2007]. Traditionally the basis of Asian diet is soy and soy products which are rich in phytoestrogens – isoflavones whose protective role in PC development has been widely studied [Virk-Baker, 2010]. In Western European countries the most important sources of phytoestrogens are lignans [Zamora-Ross, 2012]. In Northern European countries the main source of lignans is rye bread [Hedelin, 2006; Nurmi, 2010]. Extra to it, rye bread contains a lot of fiber which are bound to bioactive compounds, such as alkylresorcinols (AR) and lignans.

Alkylresorcinols, which are mainly localized in the outer part of grain, are considered as whole-grain markers. Alkylresorcinol metabolite concentration in plasma and urine are correlated to the whole-grain product intake [Adlerceutz, 2010; Ross, 2012].

In Latvia the whole-grain diet is part of traditional cuisine, although the intake of refined flour products are lately taking much greater part in the diet [Pudule, 2011]. In order to investigate the possible protective role of rye bread and fiber, it is important to know the concentration of alkylresorcinols and lignans in Latvian rye bread, to investigate the rye bread and rye bread fiber, as well as the amount of some alkylresorcinols and lignans in the diet. In order to judge about the biological effects of alkylresorcinols and lignans, one should evaluate their metabolism by analyzing and comparing kinetics of alkylresorcinol and lignan metabolites in biological fluids (plasma and urine) in PC patients and control group. Results might give evidence, that in PC patients the protective effect of biologically active components in the diet is changed. In our study we tried to focus our attention on these issues.

Another possible factor which could affect PC development, is the vitamin D level in serum. Studies show, that vitamin D possesses anticancer effect, perhaps vitamin D not only prevents prostate cancer, but disease progression as well. It is possible, that protective activity of vitamin D depends on its effect on the prostate's nuclear vitamin D receptors, preventing cancer cell proliferation, to having an effect on cell cycle, cancer cell invasion, angiogenesis, promoting apoptosis, as well as preventing the inflammatory effect of prostaglandins. [Karlsson, 2010; Krishnan, 2010]. Our intention to study the deficiency of vitamin D in relation to PC was initiated by the fact that in Latvia the exposition of the sun is low as a result majority of men may have insufficiency of vitamin D, which might affect cancerogenesis. In our study we determined the vitamin D level in serum, as well as its concentration in food in PC patients and a control group, and assessed its possible protective activity.

Hypothesis of the study

1. AR metabolite DHPPA concentration and kinetics in blood and AR metabolites DHPPA and DHBA concentration and kinetics 12 h day and 12 h overnight in urine being different in PC and control group patients;
2. Lignan metabolite enterolactone (ENL) concentration in plasma, 12 h day and 12 h overnight in urine being different in PC and control group patients;
3. Whole-grain rye bread in the diet delays PC progression.
4. In men over 45 years of age the concentration of vitamin D in the diet is insufficient. The vitamin D level in serum is affected by vitamin D concentration in the diet; vitamin D insufficiency or deficiency is widespread in Latvia in men over 45 years of age; vitamin D deficiency is associated with a higher PC risk.

Aims of the study

1. To study the correlation of AR and lignan metabolites with PC risk and possible effect on PC progression.
2. To assess the possible protective effect of vitamin D in relation to PC risk.

Objectives

1. To analyze Latvian breads.
To determine the content of lignans and alkylresorcinols in the most popular Latvian and Finish breads, compare with North and East European region breads and to supplement Latvian food data base with our determined lignan and alkylresorcinol values of different Latvian breads.

2. To analyze diet in PC and control group:
 - to develop the food frequency questionnaire which could be suitable for analysis of Latvian dietary habits, in which there would be included Latvian foodstuffs, emphasizing whole-grain products and lignan-containing products.
 - to determine the amount of AR and lignans in the diet and the main dietary sources of lignans.
3. To study AR and lignan metabolites in biological fluids in PC and control groups:
 - to measure AR metabolites (DHPPA in plasma; DHPPA and DHBA 12 h day and 12 h overnight in urine) and to evaluate which AR metabolite is the most appropriate to evaluate the intake of rye bread and rye fiber;
 - to estimate whether there exists twenty-four hour kinetics differences of AR metabolite (DHPPA in plasma; DHPPA and DHBA 12 h day and 12 h overnight in urine) in PC and control group;
 - to measure ENL in plasma, in 12 h day and 12 h overnight urine in PC and control group and to assess their correlation with the lignan intake.
4. To study the possible protective effect of whole-grain rye bread, delaying the prostate cancer progression, analyzing PSA, sex hormone concentration and apoptosis activity in the prostate cancer and risk group patients.
5. To determine the vitamin D concentration, its intake and dietary sources in both study groups and to assess the possible correlation of vitamin D with PC risk.

Novelty of the study

There has been done the assessment of the food intake in PC patients and the men in PC risk age group, using two methods: annual food frequency questionnaire and 3-day food diary. Up to now there have not been published any data on the elderly people's diet in Latvia.

According to the food intake data there is analyzed AR and lignan metabolite concentration in plasma, 12 h day and 12 h overnight urine separately for PC and control group patients, and we have estimated which is the most appropriate examination in plasma and in urine, which could be used as a biomarker for whole-grain bread and fiber concentration in the diet. Taking into account the information available to us, no data have been published on AR and lignan metabolites concentration in 12 h day urine, as well as AR metabolites have not previously studied in PC patients.

There is studied the influence of Latvian rye bread on PC progression.

Vitamin D concentration in plasma and its relation to D vitamin intake in PC patients and control group participants in Latvia have been studied. Up to now no such data are published.

Personal contribution

The literature analysis, developing a study design and its coordination at P.Stradiņš Clinical University Hospital Development Fund, Medicinal and Pharmaceutical Research Clinical Research Ethical committee, carrying out the survey of study subjects, using the lifestyle, annual food frequency questionnaire, explanatory work with study participants as to writing an accurate food diary and collection of urine, referral of test material to laboratories, participation in laboratory work, analysis of acquired results, writing of articles, thesis and promotional work.

Structure of the work

Promotional work is written in the Latvian language. It contains 10 chapters: introduction, literature survey, material and methods, results, discussion, conclusions, practical recommendations, acknowledgments, references, list of publications, supplements.

Promotional work has 161 pages (not including appendices 28 pages).

It contains 33 tables and 37 figures. 281 literature sources have been used as references.

1. MATERIALS AND METHODS

1.1. Study subjects and design

Two studies were carried out: case control study and intervention study. Both studies (case control study and intervention study) were coordinated at the P.Stradiņš Clinical University Hospital Development Fund, Medicinal and Pharmaceutical Clinical Research Ethics Committee (conclusions of Ethics Committee on 25.11.2008 and 17.03.2011). The studies were registered also at the Science Department of Rīga Eastern Clinical University Hospital. Each study participant had to sign a written consent.

Design of case control study

In the study were included a PC group and a control group. The study participants were recruited within the period from October 2009 till February 2012. The total study scheme is shown in Figure 1.1.

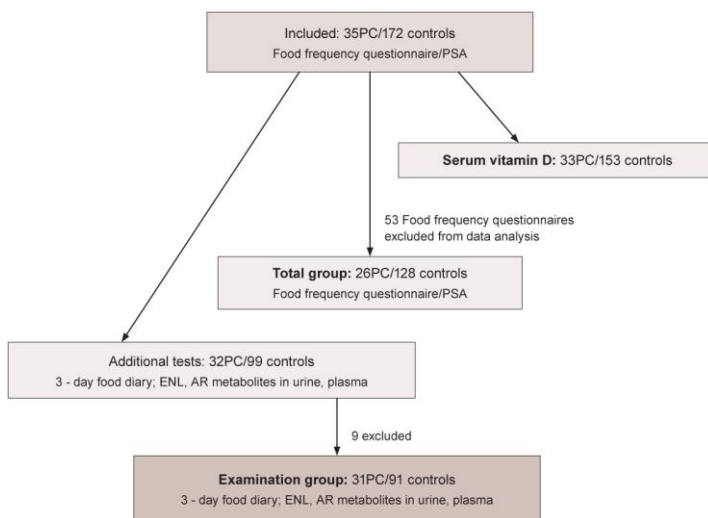


Figure 1.1. Total scheme of case control study

Inclusion criteria for both study groups: men, aged from 45 to 80 years, residing at home, eating habitual food. Exclusion criteria for both study groups: PC in family history, chronic renal disease, chronic liver disease, type I diabetes, a different localization tumour, eating habits being changed within the last 5 years, symptoms of acute lower urinary pathways, vegetarian diet.

Inclusion criteria for PC group: PC – firstly diagnosed, diagnosis affirmed histologically, corresponds to T1/T2/T3 N0 M0, PSA (prostate specific antigen) within the limits from 2,5 $\mu\text{g/L}$ to 20 $\mu\text{g/L}$, *Gleason* grade <8), PC treatment not undertaken. Inclusion criteria for control group: no data of PC by digitally rectal examination (DRE), no data on PC by transrectal ultrasonoscopy (TRUS), PSA – up to 20 $\mu\text{g/L}$.

The examinations for the included study participants: annual food frequency questionnaire, lifestyle questionnaire, PSA, 25(OH)D, urine test, a urologist’s examination performing DRE (digitally rectal examination), TRUS with prostate gland biopsy in patients whose PSA > 4,0 $\mu\text{g/L}$ or PSA \leq 4 $\mu\text{g/L}$, but TRUS with biopsy is indicated by the urologist after DRE. After obtaining the biopsy results the study participants were divided into a PC or a control group (Figure 1.2.).

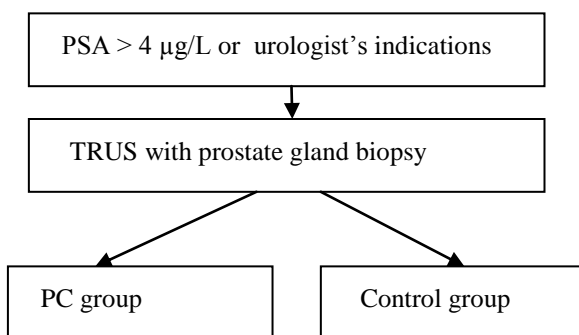


Figure 1.2. Study scheme – distribution into study groups

For the further data analysis, three groups were identified: the general group, examination group and the group to detect vitamin D level in serum.

General group: the group with annual food frequency questionnaire corresponding to the data analysis. When doing statistical data analysis, according to *Willett* method [Willett, 2013], from the further dietary data analysis were excluded those annual food frequency questionnaires (n=53), which were found to have an inadequately high or low consumed energy intake (< 700 kcal/d un > 4000 kcal/d) in relation to the person's age, body mass, height and degree of physical activity. As a result, from the General group were analyzed 26 patients in PC group and 128 participants in the control group.

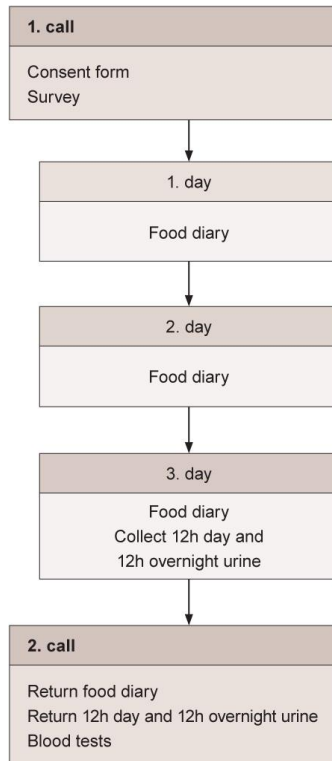


Figure 1.3. Study scheme for Examination group

Examination group: a group with additional tests. The number of study subjects, included in the Examination group: 31 patients in PC group and 91 participants in the control group. Extra exclusion criteria for the 3rd group: use of antibiotics within the last 3 months, bowel resection, inflammatory bowel disease, diarrhea (in such conditions gut microflora is affected and therefore lignan metabolism as well), use of paracetamol within the last 3 days (interfere with results of DHBA using gas chromatography-mass spectrometry (GC-MS)). Examination group participants were writing 3-day food diary, on the 3rd day they were collecting 12 h day and 12 h overnight urine, and in the morning of the 4th day they had to have a blood test (see Fig. 1.3.) to identify also ENL and AR metabolite concentration in blood plasma and in urine. Blood samples were taken prior to the prostate gland biopsy (before receiving the antibiotics) (Fig. 1.3.). Obtained blood samples and urine were referred to two laboratories (Fig. 1.3.).

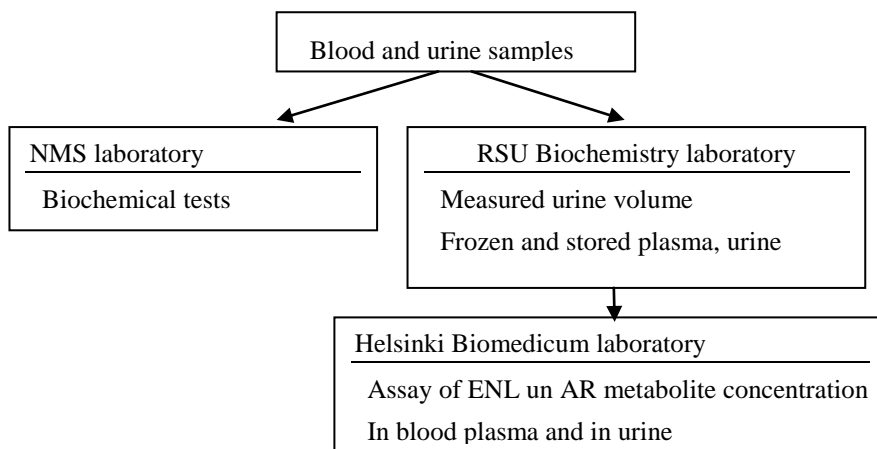


Figure 1.4. Scheme of performance of examination for Examination group

NMS – National medicine service

Helsinki Biomedicum – Institute for Preventive Medicine, Nutrition, and Cancer
Folkhälsan Research Center, Biomedicum Helsinki, University of Helsinki

Additional tests for Examination group: 3-day food diary, Helsinki University Institute of Preventive Medicine, Nutrition and Cancer *Biomedicum* laboratory (further in the text Helsinki *Biomedicum* laboratory): DHPPA detection in plasma, DHPPA 12 h day and 12 h overnight urine, ENL in plasma, ENL 12 h day and 12 h overnight urine, in NMS laboratory: testosterone, SHBG, LH, FSH, ALAT, creatinine. Initially in Examination group were included 32 PC patients and 99 control group participants. When doing the data statistical analysis for the study group, that was writing the food diary and had to have additional tests done, those study participants were excluded from the further data statistical analysis whose diary data contained inadequately high or low consumed energy intake (< 700 kcal/d and > 4000 kcal/d), test results contained extremely high values, or urine was collected not corresponding to the study requirements (n=9). As a result, the data of 31 patients were analyzed in PC group and 91 – in control group.

A group to state vitamin D concentration in serum was studied separately (33 in PC group and 153 in control group). Correlation of vitamin D concentration with dietary data was estimated only for that part of participants whose annual food frequency questionnaires corresponded to the data analysis (22 in PC group and 128 in control group).

Design of intervention study

The intervention study was carried out by involving into the European Regional Development Fund co-financed Project 2.1.1.1.activity „Assessment of potential of local origin whole-grain species and obtaining of species to be used for acquisition of specific diet” (Project Nr.2010/0237/2DP/2.1.1.0/10/APIA/VIAA/083).

Another selection group was included into the intervention study. The study participants were recruited from November 2011 till January 2013.

Inclusion of study participants into the intervention study. In the intervention study with rye whole-grain bread there were included 38 subjects, but the study was completed having 37 patients with histologically confirmed PC in the period before the operative therapy, or the patients who had been planned to have a different therapeutic method.

Inclusion criteria: age – 45–80 years, residing at home, eating habitual food, PC – firstly identified diagnosis was approved histologically, it corresponded to T1/T2/T3 N0 M0 classification, PC therapy had not been started. Exclusion criteria: vegetarian diet, prostate cancer in the family history, use of antibiotics within the last 3 months(antibiotics change the intestinal microflora and along with it the lignan metabolism as well), the use of paracetamol within the last 3 days (it interferes with assay in GC-MS), bowel resection, inflammatory bowel disease, diarrhea, chronic renal disease, chronic liver disease, type I diabetes, tumor of other localization, eating habits had been changed in the last 5 years, symptoms of acute lower urinary tract symptoms.

The study scheme with 3 study stages is shown in Figure 1.5. In the first study stage, the study participants were using habitual diet. In the second stage all participants were indicated the use of 350 g refined wheat bread („wash out” period) for 2 weeks, and then, in the third stage – 6-week-long intervention with rye bread (350 g). The study was carried out with a specially prepared rye bread. The result was estimated after 2 and 6 weeks. Blood tests (in the morning, on an empty stomach) were done at the beginning of the study, after 2 and 6 weeks. Within this period the study participants did not use any other bread, except the one received for the study. The rest of the daily diet was not changed. A nutritionist, once a week, contacted the study participants by telephone to supervise their compliance.

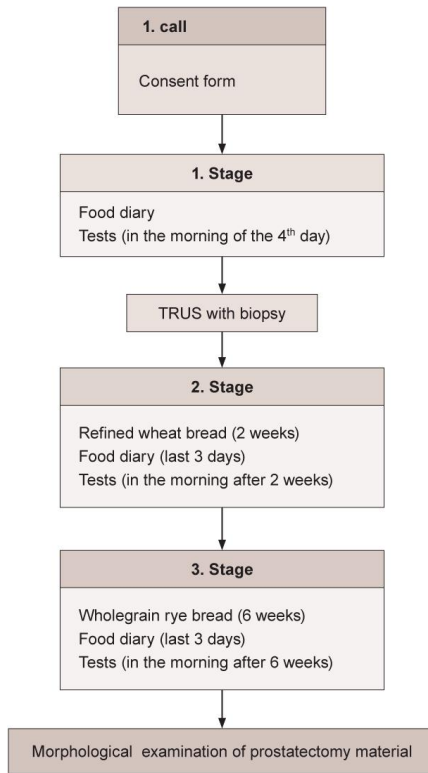


Figure 1.5. Scheme of intervention study

The examination consists of – an annual food frequency questionnaire, lifestyle questionnaire, 3-day food dairies, each time prior to making tests, urologist’s examination, doing DRE and TRU with the prostate gland biopsy, in Helsinki *Biomedicum* laboratory: DHPPA, DHBA, ENL in plasma; in NMS laboratory: PSA; fPSA (free PSA), testosterone, SHBG, LH, FSH, FAI (free androgen index). The prostate gland biopsy material was examined morphologically and the operation (prostatectomy) material was identified. In both prostate tissue materials (in prostate gland cancer cells) the apoptosis was investigated.

1.2. Assessment of risk factors

To assess various possible PC risk factors, the study participants were interviewed, using the lifestyle questionnaire. The questionnaire was made, adopting the lifestyle questionnaire of Swedish mammography cohort study (SMC87). The questionnaire included the questions about the stature, body mass, family status, sexual activity, education, smoking, diseases, the use of medicines and food supplements, physical activity during the lifetime and eating habits. The data about the stature and the body mass is based on self-reported information by the study participants themselves.

1.3. Assessment of dietary intake

To assess the dietary habits of the study subjects, intake of food and nutrients within the last year, there was used a specially designed annual food frequency questionnaire. The questionnaire was developed, adapting European Prospective Investigation of Cancer (EPIC) food frequency questionnaire [University of Cambridge, 2008a]. The questionnaire included 159 questions on the food intake from all food groups. The study participants indicated how often, within a day/week/month, they were using or not using the respective product/foodstuff at all. In order to estimate the amount of dietary intake, the food frequency questionnaire was used together with a special food product atlas of photographs of serving size [Lietuvas Republikas Veselības Aizsardzības ministrija, 2007].

Part of the study participants were filling in a specially designed food diary for extra 3 days, adapted from the European Prospective Investigation of Cancer (*EPIC: European Prospective Investigation of Cancer*) food diary [University of Cambridge, 2008]. The study participants filled in the food diary, estimating the size of the serving from the booklet. In the intervention

study, in order to assess the dietary intake, recorded in the food diary, each study participant was given the food scales (*Soehnle*, precision 1 g).

Dietary data were processed at the Scientific Institute of Food Safety, Animal Health and Environment (further in the text BIOR) by a BIOR developed programme on the basis of *Microsoft Dynamics Ax 2009* programme. BIOR data base of food composition was made, using German BLS *Max Rubner* institute-developed data base of food composition, which was supplemented with products and recipes characteristic for Latvia. Especially for this study, BIOR data base was supplemented by lignan values in food products. There was used Canadian lignan data base [Thompson, 2006], which contains values of four lignan types (MAT, SECO, LAR, PIN). The data base was supplemented also by AR values set by Helsinki *Biomedicum* laboratory [Meija, 2013a] and values of 6 lignan types (MAT, SECO, LAR, PIN, SYR, MED) in Latvian bread [Meija, 2013b].

There was separately analyzed the food frequency questionnaire, mean data from 3-day food diary and the data of the 3rd food diary-writing day.

1.4. Determination of lignans and alkylresorcinols in different types of bread

For the study, the most frequently sold brands of bread in 2008 were chosen, taking into account unpublished data of the Latvian Bakers' Association (private communication with Daiga Kunkulberga). Bread samples were chosen from each type of bread: rye bread, fine rye bread, seed-bran bread and wheat bread. In total there were analyzed 9 types of bread.

Alkylresorcinol detection. Total AR and its homologous content in bread was analyzed with gas chromatography-mass spectrometry (GC-MS) method, using AR C20:0 homologue as an internal standard [Ross, 2001]. All

results were obtained, calculating AR concentration in dry matter. Each sample was analyzed twice and the results were reported as mean values.

Lignan detection. We used the gas chromatography-mass spectrometry instrument. Bread samples were freeze-dried, milled and analyzed according to modified Penalvo [Penalvo JL, 2005a] and Milder [Milder, 2004] protocols. All results were obtained by calculating lignant concentration in dry sample. Each sample was analyzed twice, and the results were reported as mean values.

1.5. Laboratory examination

PSA was analyzed in serum by chemiluminescence microparticle immunochemical method (*Architect i2000SR*, Abbott). Testosterone, SHBG, LH, FSH was analyzed in serum by an immunochemiluminescence method (*Immulite 2000*, Siemens). Vitamin D (25 hydroxyvitamin D: 25(OH)D) in serum was determined by immunochemiluminescence method (*Liaison*). 25(OH)D serum concentration ≤ 20 ng/mL was considered to be vitamin D deficiency, 25(OH)D serum concentration 21–29 ng/mL – as vitamin D insufficiency [Holick, 2011; Latvijas Osteoporozes un kaulu metabolo slimību asociācija, 2011; Płudowski, 2013]. ALAT was analyzed by the IFCC method without pyridoxal-5 phosphate (*Architect c8000*), creatinine was analyzed by Jaffes reaction (*Architect c8000*).

Blood samples, envisaged for further transfer to Helsinki, were taken in vacuum heparin tubes (*BD Vacutainer®*, BD, Plymouth, UK), which were immediately centrifuged (3000 speed/min) for 10 min at 4 °C temperature. Plasma samples were stored in 4,5 ml cryotubes (*CryoPure Tube*, Sarstedt Ag & Co, Numbrecht, Germany) at -20°C temperature.

12h day and 12h overnight urine was collected in 2l plastic containers (*Sarstedt Ag & Co, Numbrecht, Germany*), which were containing 1 g ascorbic acid, to protect from oxidation. Urine was stored in the refrigerator at +4°C, in

the morning it was taken to RSU Biochemistry laboratory, where the urine volume was measured. Urine was stored in 4,5 ml cryotubes (*CryoPure Tube, Sarstedt Ag & Co, Numbrecht, Germany*) at -20°C temperature. Frozen urine and plasma samples were transported in containers with dry ice to Helsinki *Biomedicum* laboratory.

In Helsinki *Biomedicum* laboratory, using the high performance liquid chromatography with Coulometric Electrode Array Detection (HPLC-CAD), DHBA, DHPPA were analyzed in 12h day urine, 12h overnight urine and DHPPA in plasma, using Koskela methods [Koskela, 2007; Koskela, 2008]. ENL in plasma was detected in the same laboratory with time-resolved fluorescence immunoassay method (TR-FIA), based on fluorescence measuring [Adlercreutz, 1998; Stumpf, 2000].

1.6. Morphological examination

The prostate gland biopsy material was estimated using histological preparation routine (hematoxiline and eosine) staining method. Preparations were examined under the light microscope 40 x, 100 x, 200 x amplification. Malignization degree of the prostate carcinoma was assessed, using the Gleason method.

In the intervention study the apoptosis activity was determined in the prostate cancer cells. The prostate biopsy material was compare with the operation material in 11 patients, who were operated on in RAKUS hospitals. Only the reaction in cell nuclei was estimated. Immunohistochemically positive cancer cells were counted for 100 total cell count in 5 visual fields in 400x amplification. The average percentage of positive cell count was marked. Along with it there was marked the Gleason grade, tumor T stage, as well as relative volume of the tumor against the prostate volume.

1.7. Statistical analysis

In order to calculate the nutrient intake, including lignans, concentration, the data acquired from the food frequency questionnaire and food diaries were processed in BIOR programme envisaged for food intake data collection.

The data obtained from food frequency questionnaires – amount of food intake, amount of nutrient intake – they were correlated to the age, total energy intake received, using residual method [Willet, 2013], transforming them (to log). For the comparison of the study data, the non-parametric methods were used: between the groups - *Mann-Whitney U*, *Wilcoxon* and *chi-square* tests, and between the intervention stages – *Friedman* and *Wilcoxon* tests. Correlation between metabolites, laboratory tests and assessment of food products *Spearman* correlation were used. P value, less than $p < 0,05$, is accepted as statistically significant. Data statistical analysis was done in statistical programme SPSS (20.0 version).

2. RESULTS

2.1. Alkylresorcinols and lignans in Latvian bread

The concentration of alkylresorcinol and some of their homologues, as well as lignan concentration in 9 Latvian and 11 Finnish breads with a various fiber concentration – wheat, rye, mixed flour bread and wheat flour bread with added seed were determined. It was done for more accurate evaluation of the amount of whole-grain, especially rye bread, in the diet and its possible protective effects [Meija, 2013a].

The highest AR concentrations were in rye breads (~560–840 µg/dry matter) and in wheat flour breads (white bread made of refined wheat flour) the concentrations of AR was the lowest (~25–31 µg/dry matter) (Table 2.1.). AR concentration in rye and wheat bread samples did not essentially differ in Latvian and Finnish bread types. A considerable difference of AR concentration was seen in breads which were made of mixed flour. In Latvian fine rye-bread (made of fine wheat-flour and refined wheat flour) AR concentration was considerably lower (~200–300 µg/dry matter) than in Finnish bread types from mixed rye and wheat flour (~500–700 µg/dry matter).

In different bread types the AR homologue composition varied. In rye bread dominated the homologues C19:0; C17:0 and C21:0, while in wheat bread – the homologues C21:0 and C19:0. AR ratio C17:0/C21:0 were similar in Latvian and Finnish bread: in rye bread 1,1–1,3, in wheat bread 0,1–0,2 and 0,3–1,3 in bread from mixed rye and wheat flour (Table 2.1.).

Table 2.1.

Alkylresorcinol content in selected Latvian breads [Meija, 2013a]

Sample breads	Fiber	AR homologue composition (%)						Total ARs	Ratio of ARs
		g/100 g	15:0	17:0	19:0	21:0	23:0	25:0	15:0–25:0 μg/g DM
WB1	*	nd	7,3	30,6	42,2	16,3	3,6	24,7	0,2
WB 2	*	nd	5,8	31,7	42,8	16,8	2,9	26,8	0,1
WB WS									
1	3,4	nd	5,7	26,1	42,1	18,6	7,4	27,7	0,1
WB WS									
2	3,9	nd	5,0	26,2	43,3	18,3	7,3	26,4	0,1
MFB 1	5,2	0,9	22,5	28,0	23,9	14,4	10,3	217,4	0,9
MFB 2	*	0,9	23,9	25,5	22,4	16,0	11,4	298,6	1,1
RB 1	7,4	1,0	26,2	27,8	21,7	14,0	9,3	825,8	1,2
RB 2	*	1,0	25,6	27,2	22,0	13,5	10,7	569,6	1,2
RB3	*	1,0	24,9	26,0	21,8	15,4	11,0	651,7	1,1

Fiber – concentration marked on the package

* fiber content not indicated on the labeling

WB – wheat bread (from refined flour)

WB WS – wheat bread with seeds

KSM – wheat bread with seeds

MFB – fine rye-bread (from mixed rye and wheat flour)

RB – rye bread

DM – dry matter

nd– not detected

The highest total lignan concentration was found in bread with added seeds (~3800–10 000 μg/100 g). Lignan concentration in rye bread was also high (~800–1400 μg/100 g). Lignan concentration in mixed rye and wheat flour bread was lower (~500–900 μg/100 g), and the lowest one was found in refined wheat flour bread (~80–100 μg/100 g). Analyzing separate lignans, the highest SECO concentration was in bread with seeds and SYR was dominating in rye bread (Table 2.2. tab.).

Table 2.2.

Lignan content in selected Latvian breads ($\mu\text{g}/100\text{ g wet weight}$)
[Meija, 2013b]

Lignans	SECO	MAT	LAR	PIN	SYR	MED	Total
Latvian bread							
White bread 1	3,0	nd	8,4	nd	70,7	3,4	85,4
White bread 2	11,4	nd	9,8	nd	80,5	4,0	105,8
Wheat bread with seeds 1	7332,1	29,2	197,4	1230,6	109,7	27,3	8926,0
Wheat bread with seeds 2	9442,4	12,8	196,8	291,2	95,2	5,9	10044,0
Fine rye-bread (rye, wheat flour) 1	19,1	2,6	43,1	45,8	324,5	18,6	453,6
Fine rye-bread (rye, wheat flour) 2	31,6	4,7	56,4	62,9	405,7	23,6	584,7
Rye bread 1	15,2	9,0	137,1	111,9	749,9	45,9	1069,0
Rye bread 2	10,3	6,1	101,4	80,7	588,6	33,0	820,1
Rye bread 3	13,9	8,1	141,1	117,8	682,2	45,7	1009,0
Mean CV% n=18	10,8	6,6	2,7	6,0	3,7	4,5	34,3

MAT – matairesinol; SECO – secoisolariciresinol; LAR – lariciresinol; PIN – pinoresinol;

SYR – syringaresinol; MED – medioresinol

nd = not detected

CV (coefficient of variation) % was calculated per wet weight

Defined AR and lignan values in different types of Latvian bread were added to BIOR food database

2.2. Diet: general characteristics, alkylresorcins and lignans in diet

General characteristics of study subjects of General group

Mean body mass index of 154 study subjects was 28,4 (SD 3,8) kg/m^2 , overweight or obesity was frequent (81,2% (n=125) of the man). No statistically significant differences were found in control and cancer groups in such parameters as education, physical activity, smoking, height, incidence of

oncological diseases in the family, use of antibiotics within the last year and sexual activity during one's lifetime ($p>0,05$). The mean age in PC group was 64,9 (SD 7,8) and 58,3 (SD 8,5) years in control group.

General characteristics of diet in General group

Energy intake was statistically significantly higher in control group – 3304,1 (2712,1; 3891,1) kcal/d than 2627,9 (2399,6; 3540,3) kcal/d in PC group ($p=0,006$). After adjusting to the age, no statistically significant differences were found in control and PC group ($p>0,05$) in respect to energy intake, nutrients (proteins; fat; carbohydrates; total, insoluble, soluble fiber) and foodstuffs (cereals, bread, fish, dairy products, tomatoes and products made from tomatoes, fruits and berries, nuts, coffee and tea) used in the diet.

The main source of fiber, both in General group and in PC, as well in control group was cereals – 41% of the total consumption of fiber. Rye bread took the central place, making up to 28% of the total fiber intake and 61% of cereal fiber intake. Fiber content in the diet, when analyzing the annual food frequency questionnaires, was negatively correlating with the total fat concentration in the diet, both in PC group ($r=-0,642$; $p=0,001$), and control group ($r=-0,575$; $p<0,001$), as well as with the saturated fat content in both study groups (PC group $r=-0,605$; $p=0,001$; control group $r=-0,62$; $p<0,001$).

Alkylresorcinols in diet in General group

Median AR intake in General group was 55,8 (25,3; 98,2) mg/d. Mean AR amount in the diet in General group was 72,1 (SD 62,2) mg/d; in PC group 72,8 (SD 59,0) mg/d and in control group 71,9 (63,1) mg/d. No statistically significant differences were found between PC and control group neither in concentration of AR intake, nor separate AR homologues ($p>0,05$).

Analyzing the questionnaires of food frequency, in both groups AR concentration in the diet correlated with the total fiber concentration in the diet (in PC group $r=0,852$; $p<0,001$; in control group $r=0,716$; $p<0,001$), insoluble fiber concentration (on PC group $r=0,814$; $p<0,001$; in control group $r=0,639$; $p<0,001$) and soluble fiber concentration (in PC group $r=0,598$; $p=0,002$; in control group $r=0,498$; $p<0,001$). In a similar way, AR concentration in the diet within the last year in both groups correlated with the intake of bread (in PC group $r=0,826$; $p<0,001$; in control group $r=0,773$; $p<0,001$) and also separately with rye bread intake (in PC group $r=0,998$; $p<0,001$; in control group $r=0,986$; $p<0,001$).

Lignans in diet in General group

Analyzing food frequency questionnaires in General group, there were no statistically significant differences between PC and control groups neither in the total concentration of lignans, nor in specific lignan concentration in the diet. Median lignan amount in the diet for both groups was 2782 (1266–6815) $\mu\text{g}/\text{d}$, mean amount 5151 (SD 6364) $\mu\text{g}/\text{d}$. In both study groups the main source of lignan in the diet was bread with seeds and rye bread (in total 86%), linseed made up 7%, however, only 2% of men used linseed, while 99% of men consumed bread. The other lignan sources were insignificant (see Table 2.3.).

Table 2.3.

Sources of lignan intake in men [Meijja, 2013b]

	Users		MAT		SECO		LAR		PIN		Total	
	%	µg/d *	%**	µg/d	%**	µg/d	%	µg/d	%	µg/d	%	µg/d
<u>In total</u>		27,9 (23,0)		2885,2 (5359,0)		311,8 (213,0)		652,2 (833,4)		5151,3 (6563,7)		
<i>Cereals</i>	100,0	22,8 (21,2)	82,1	2465,2 (4691,2)	85,4	271,6 (206,9)	87,1	575,8 (790,0)	88,3	4609,5 (5765,3)	89,5	
Rye bread	86,0	12,4 (11,6)	44,6	21,0 (19,6)	0,7	189,0 (176,9)	60,6	154,2 (144,4)	23,6	1473,5 (1379,1)	28,6	
Seed bread	45,3	9,7 (18,7)	34,9	2437,4 (4692,5)	84,5	65,6 (126,3)	21,0	409,1 (787,6)	62,7	2967,3 (5712,6)	57,6	
<i>Nuts, seeds</i>	71,5	0,3 (1,4)	1,1	365,2 (2858,7)	12,7	9,73 (27,94)	3,1	6,8 (45,5)	1,0	382,7 (2886,3)	7,4	
Flaxseeds	2,3	0,2 (1,2)	0,5	358,8 (2858,4)	12,4	2,68 (21,38)	0,9	0,70 (5,56)	0,1	362,3 (2886,5)	7,0	
Sunflower seeds	30,8	0,0 (0,1)	0,1	1,0 (3,1)	<0,1	5,73 (17,54)	1,8	1,30 (3,97)	0,2	8,06 (24,64)	0,2	
Sesame seeds	2,3	0,1 (0,8)	0,3	0,0 (0,1)	<0,1	0,10 (0,99)	<0,1	4,60 (45,06)	0,7	5,40 (52,88)	0,1	
<i>Drinks</i>	96,5	0,6 (0,5)	2,2	36,39 (29,88)	1,3	4,42 (3,51)	1,4	9,04 (9,22)	1,4	50,51 (37,64)	1,0	
Coffee	84,3	0,3 (0,3)	1,2	15,42 (13,50)	0,5	2,95 (2,59)	0,9	0,66 (0,57)	0,1	19,35 (16,95)	0,4	
Tea	78,5	0,3 (0,3)	1,0	16,22 (22,94)	0,6	0,44 (0,48)	0,1	8,21 (9,20)	1,3	25,13 (29,67)	0,5	
Beer	25,6	0,0 (0,0)	0,0	0,08 (0,24)	<0,1	0,23 (0,71)	0,1	0,12 (0,36)	<0,1	0,43 (1,31)	<0,1	
<i>Vegetables</i>	100,0	2,7 (3,0)	9,8	11,33 (9,89)	0,4	18,01 (13,75)	5,8	40,99 (38,53)	6,3	73,07 (60,46)	1,4	
Cabbage	93,0	0,0 (0,0)	0,1	0,69 (0,76)	<0,1	8,54 (9,46)	2,7	11,68 (12,94)	1,8	20,91 (23,16)	0,4	
Garlic	95,9	0,3 (0,3)	1,0	2,41 (2,96)	0,1	3,13 (3,83)	1,0	27,70 (33,96)	4,2	33,52 (41,09)	0,7	
Onion	99,4	2,3 (2,7)	8,2	5,52 (6,56)	0,2	0,08 (0,09)	<0,1	0,20 (0,24)	<0,1	8,09 (9,61)	0,2	
<i>Fruits and berries</i>	99,4	1,2 (1,1)	4,4	6,40 (6,68)	0,2	7,44 (6,47)	2,4	4,88 (6,08)	0,7	19,88 (17,60)	0,4	

Continued Table 2.3.

	Users		MAT		SECO		LAR		PIN		Total	
	%	µg/d *	%***	µg/d	%**	µg/d	%	µg/d	%	µg/d	%	µg/d
Berries	91,9	0,0 (0,0)	0,1	2,65 (4,73)	0,1	0,92 (1,64)	0,3	0,02 (0,04)	<0,1	3,60 (6,44)	0,1	
Fruits	99,4	1,2 (1,1)	4,3	3,75 (3,84)	0,1	6,53 (5,99)	2,1	4,86 (6,08)	0,7	16,28 (15,06)	0,3	
<i>Olive oil</i>	58,7	0,0 (0,1)	0,1	0,10 (0,20)	<0,1	0,18 (0,33)	0,1	14,51 (27,36)	2,2	14,81 (27,93)	0,3	

MAT, matairesinol; SECO, secoisolariciresinol; LAR, lariciresinol; PIN, pinoresinol.

Presented mean values (SD); SD – Standard deviation; n = 172 men

* mean intake per day µg/d

%** Percent lignan contribution of food item A = sum of lignan content (µg) from food item A divided by sum of lignan content from all food items

Analyzing specific lignan amount in the diet, the dominating one was SECO where main intake sources were bread with seeds and linseed. PIN main intake sources were bread with seeds, rye bread, root-crops and other vegetables, among which garlic was prevalent. LAR and MAT intake sources were rye bread and bread with seeds, while SYR and MED originated from rye bread. Lignan density in the diet ($\mu\text{g}/\text{kcal}$) did not differ in relation to BMI, age and education level. In non-smokers diet the lignan concentration was statistically significantly higher than in the smokers' diet ($p=0,041$). In control group the lignan concentration in the diet (analyzing the food frequency questionnaire) negatively correlated with the fat concentration in the diet ($r=-0,406$; $p<0,001$). In the PC group such a correlation was not found ($p=0,171$).

2.3. Correlation between dietary parameters and concentration of alkylresorcinol and lignan metabolites in plasma and urine

Alkylresorcinol metabolites

Diet parameter correlation with plasma and urine tests, as well as AR metabolism in biological fluids were analyzed in Examination group in 31 PC patients and 92 control group subjects. Analyzing 3-day food diary data (mean values of 3 days) , no statically significant differences were found between PC and control group neither in energy intake, nor basic nutrient intake or AR, nor separate bread type intake ($p>0,05$).

DHPPA and DHBA concentration 12h day, 12h overnight and 24h night urine did not differ in PC patients and control group men, while DHPPA concentration in plasma was statistically significantly higher in PC group ($p=0,005$) (Table 2.4). .

Table 2.4.

DHPPA, DHBA urine concentration and DHPPA plasma concentration in PC and control group subjects in Examination group

Median (IQR), ng/mL	PC patients (n=31)	Controls (n=91)	P
DHPPA in plasma nmol/L	82,54 (55,53; 122,66)	62,93 (35,45; 85,47)	0,005
DHPPA μ mol/24h urine	20,08 (13,2; 25,87)	19,03 (11,82; 27,86)	0,535
DHPPA μ mol/12h day urine	8,45 (5,37; 12,46)	8,46 (5,10; 13,01)	0,704
DHPPA μ mol/12h overnight urine	11,2 (6,64; 14,65)	9,49 (5,51; 13,74)	0,270
DHBA μ mol/24h urine	11,74 (5,42; 17,91)	9,56 (5,26; 17,49)	0,475
DHBA μ mol/12h day urine	5,51 (2,05; 8,37)	4,62 (2,60; 8,61)	0,800
DHBA μ mol/12h overnight urine	5,77 (3,37; 11,49)	5,72 (3,15; 8,86)	0,374
24h urine, ml	1570 (1140; 2130)	1648 (1360; 2420)	0,209
12h day urine, ml	760 (600; 1114)	900 (664; 1230)	0,155
12h overnight urine, ml	800 (520; 1050)	800 (580; 1264)	0,493

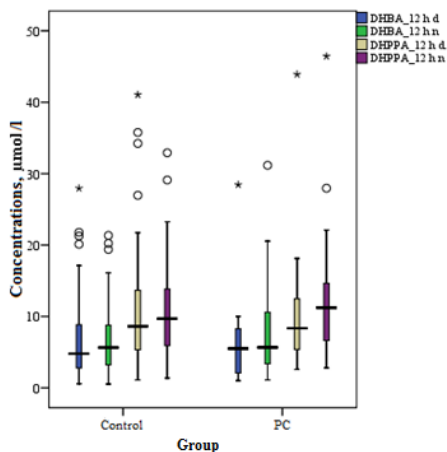
IQR - Interquartile range

DHPPA – 3-(3,5-dihydroxyphenyl)-1-propanoic acid

DHBA – 3,5-dihydroxybenzoic acid

In PC group DHBA and DHPPA median concentration in 12h overnight urine did differ statistically significantly from 12h day urine ($p=0,010$ and $p=0,007$ respectively), while in the control group no difference was found either between DHBA ($p=0,511$), or between DHPPA ($p=0,417$) day and overnight concentration (Figure 2.1.).

In both group DHPPA and DHBA concentration in 12h day urine correlated with DHPPA and DHBA concentration in 12h overnight and 24h urine, also DHPPA concentration in plasma correlated with DHPPA concentration in 12h day, 12h night and 24h urine (for all mentioned AR metabolite group $r=0,420 - 933$; $p<0,010$).



DHPPA – 3-(3,5-dihydroxyphenyl)-1-propanoic acid
 DHBA – 3,5-dihydroxybenzoic acid
 d – 12h day urine, n – 12h overnight urine

Figure 2.1. DHBA and DHPPA excretion in 12 h day and 12 h overnight urine in PC and control group subjects in Examination group

Intake data were analyzed separately in two ways: mean data from 3-day food diary and separately food diary 3rd-day data.

Mean 3-day food diary data. In PC group were found statistically significant correlations between DHBA and DHPPA amounts in 12h overnight and 24h urine and amount of bread fiber, rye bread, rye bread fiber and AR in the diet, but there was not found any correlation between DHPPA concentration in 12 h day urine and food intake data. In the control group the rye bread and rye fiber concentration in the diet statistically significantly correlated with DHPPA concentration in 12 h day, 12 h overnight and 24 h urine, as well as with DHBA concentration in 24 h urine. DHPPA plasma concentration in PC group correlated with bread, bread fiber, rye bread and rye bread fiber concentration in the diet. In the control group such correlations were not found. Correlation coefficients and significance level can be seen in Table 2.5.

Table 2.5.

Correlation coefficients data of AR metabolite and dietary intake in PC and control group subjects in Examination group

	DHPPA plasma	DHBA d	DHBA n	DHBA 24 h	DHPPA d	DHPPA n	DHPPA 24 h
PC group							
Dietary data: mean values of 3-day food diary							
Bread ¹	0,656**	0,426*	0,297	0,350	0,292	0,356*	0,317
Bread fiber ¹	0,607**	0,466**	0,403*	0,443*	0,336	0,490**	0,411*
Rye bread ¹	0,528**	0,417*	0,463*	0,474*	0,375	0,556**	0,476*
Rye bread fiber ¹	0,528**	0,417*	0,463*	0,474*	0,375	0,556**	0,476*
Alkylresorcinols	0,471**	0,375*	0,502**	0,494**	0,333	0,558**	0,450*
Dietary data: 3rd-day of food diary							
Bread ¹	0,468*	0,313	0,122	0,206	0,253	0,317	0,276
Bread fiber ¹	0,361	0,305	0,Table 2.5.135	0,212	0,267	0,333	0,29
Rye bread ¹	0,334	0,221	0,152	0,135	0,335	0,293	0,26
Rye bread fiber ¹	0,382	0,101	0,099	0,062	0,238	0,268	0,203
Alkylresorcinols	0,237	0,253	0,246	0,290	0,317	0,423*	0,376*

Continued Table 2.5.

	Control group							
	DHPPA plasma	DHBA d	DHBA n	DHBA 24 h	DHPPA d	DHPPA n	DHPPA 24 h	
	Dietary data: mean values of 3-day food diary							
Bread ¹	0,115	0,283**	0,253*	0,287**	0,278**	0,323**	0,336**	
Bread fiber ¹	0,199	0,350**	0,336**	0,374**	0,302**	0,350**	0,360**	
Rye bread ¹	0,151	0,231	0,224	0,282*	0,247*	0,270*	0,285*	
Rye bread fiber ¹	0,153	0,230	0,225	0,282*	0,247*	0,271*	0,285*	
Alkylresorcinols	0,397**	0,458**	0,534**	0,552**	0,424**	0,506**	0,526**	
	Dietary data: 3rd -day food diary							
Bread ¹	0,087	0,187	0,246*	0,209	0,18	0,266*	0,253*	
Bread fiber ¹	0,170	0,330**	0,430**	0,385**	0,207	0,343**	0,312**	
Rye bread ¹	0,000	0,260	0,187	0,236	0,213	0,203	0,241	
Rye bread fiber ¹	0,031	0,246	0,199	0,234	0,158	0,183	0,199	
Alkylresorcinols	0,304**	0,391**	0,519**	0,483**	0,368**	0,460**	0,471**	

* Correlation is significant at the 00.05 level

¹ Adjusted for energy intake

DHPPA – 3-(3,5-dihydroxyphenyl)-1-propanoic acid

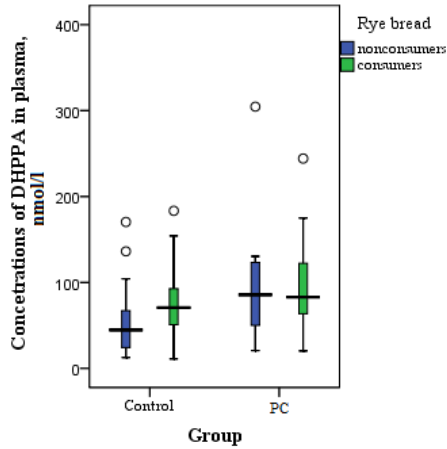
DHBA – 3,5-dihydroxybenzoic acid

d – 12 h day urine; n – 12 h overnight urine; 24 h – 24 h urine

Data from the 3rd-day food diary. In PC group no correlation between the amount of bread, bread fiber, rye bread and rye bread fiber was found and DHBA and DHPPA concentration in 12h day, 12h overnight and 24h urine. In the control group, however, the concentration of bread fiber correlated with DHBA concentration in 12h day, 12h overnight and 24h urine, as well as with DHPPA concentration in overnight and 24h urine (Table 2.5.). Analyzing DHPPA concentration in plasma with the 3rd-day food intake data, in PC group there was found a correlation between DHPPA concentration in plasma and bread amount in the diet. In the control group, however, no correlation between DHPPA concentration in plasma and bread, bread fiber, rye bread and rye bread fiber was found in the diet (correlation coefficients and significance level in Table 2.5.).

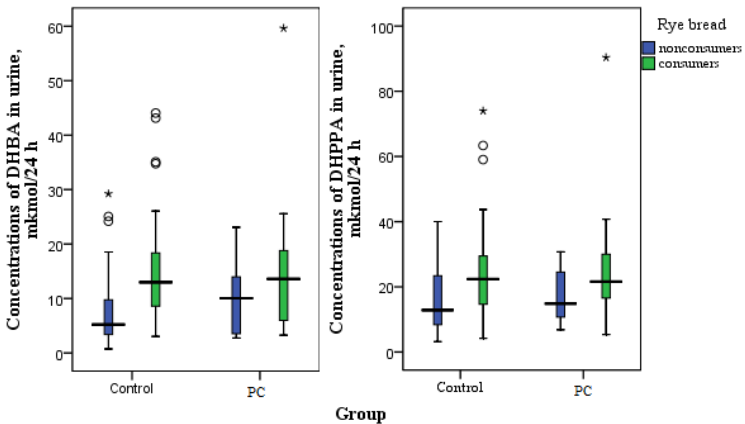
In PC group no statistically significant difference was found between DHPPA concentration in plasma in those subjects, who in their diet had used, and those who had not used rye bread on the 3rd-day of food diary writing ($p=0,680$), while in the control group the differences were statistically significant ($p<0,001$) (Fig. 2.2.).

Quite similarly also DHBA and DHPPA concentration in 12 h day, 12 h overnight and 24 h urine in PC group did not differ between those subjects who had used rye bread, and those who had not used it ($p>0,05$), but in the control group the differences were statistically significant ($p<0,05$) (Figure 2.3.).



DHPPA – 3-(3,5-dihydroxyphenyl)-1-propanoic acid; PC – prostate cancer group

Figure 2.2. DHPPA concentration in plasma in rye bread users and not-users in PC patient group and control group in studied Examination group



DHPPA – 3-(3,5-dihydroxyphenyl)-1-propanoic acid in 24 h urine

DHBA – 3,5-dihydroxybenzoic acid in 24 h urine

PC – prostate cancer group

Figure 2.3. DHPPA and DHBA concentration in 24 h urine in rye bread users and not-users in PC patient and control group in studied Examination group

No correlation was found between glomerular filtration velocity (GFV) and DHPPA concentration in plasma, as well as DHPPA and DHBA concentration in 12h day, 12h overnight and 24h urine.

Enterolactone

ENL concentration in 12 h day, 12 h overnight urine, 24 h urine and in plasma was determined in 31 patients in PC group and 91 control group subjects (the same group who was analyzed for AR metabolites). No statistically significant differences were found between both groups either in the intake of bread with seeds, or rye bread, or lignan content ($p>0,05$). Analyzing 3-day food diary data, lignan median concentration in the diet was 2133,6 (587,0–2441,7) $\mu\text{g/d}$ in PC group and 1213,5 (393,4–21422) $\mu\text{g/d}$ in the control group. Evaluating ENL excretion in 12h day, 12h overnight, 24h urine and in plasma, no statistically significant differences were found between both groups (Table 2.6.).

Table 2.6.

Enterolactone median concentration in urine and in plasma in PC and control group subjects in Examination group

Metabolite	PC patients (n=31)	Control group (n=91)	p
ENL $\mu\text{mol/in}$ 12 h day urine	1,84 (1,17; 2,89)	1,39 (0,83; 2,82)	0,381
ENL $\mu\text{mol/}$ in 12 h overnight urine	1,59 (1,06; 2,90)	1,49 (0,55; 3,16)	0,424
ENL $\mu\text{mol/}$ in 24 h urine	3,49 (2,02; 5,51)	2,79 (1,40; 5,73)	0,417
ENL in plasma, nmol/L	29,17 (14,33; 48,49)	26,41 (14,87; 43,97)	0,646

ENL – enterolactone

In the brackets – interquartile range

In both study groups all ENL concentration indices (ENL in 12h day, 12h overnight, 24h urine and plasma) mutually correlated ($r=0,579-0,953$; $p<0,001$). No statistically significant difference in ENL concentration between day and overnight urine was found neither in PC group ($p=0,131$), nor in the control group ($p=0,773$). Neither of ENL examinations (ENL in 12h day, 12h overnight, 24h urine and in plasma) in neither group correlated with Gleason grade ($p>0,05$) in PC group. In PC group was not found correlation of these parameters with PSA ($p>0,05$). In the control group there was found a positive correlation between PSA and ENL 12h day urine ($r=0,301$; $p=0,005$), ENL 12h overnight urine ($r=0,406$; $p<0,001$), ENL 24h urine ($r=0,376$; $p<0,001$) and ENL concentration in plasma ($r=0,255$; $p=0,015$). In neither group there was found the correlation between ENL concentration in 12h day, 12h overnight, 24h urine and in plasma and SHBG, LH, FSH, testosterone, 25(OH)D concentration, as well as fat, alcohol amount in the diet and smoking (in all cases $p>0,05$).

Both examination groups were divided into subgroups – one subgroup, those who had used antibiotics during the last year, and the second subgroup – those who had not used antibiotics during the last year. The differences between study groups in the frequency of antibiotic use were not statistically significant ($p=0,202$).

In PC group there were no statistically significant differences between both groups, evaluating ENL concentration in 12h day, 12h overnight, 24h urine and in plasma. In the control group, however, ENL concentration in 12h day, 12h overnight, 24h urine and in plasma was statistically significantly lower in those subjects who had used than who had not used antibiotics during the last year (Table 2.7.).

Table 2.7.

Enterolactone median concentration in urine and in plasma in PC and control group subjects in Examination group depending on the antibiotic use during the last year

Metabolite	Had used antibiotics during the last year	Had not used antibiotics during the last year	P
PC group			
	n=8	n=14	
ENL $\mu\text{mol/}$ in 12 h day urine	1,41 (0,76; 7,23)	1,94 (1,50; 2,53)	0,733
ENL $\mu\text{mol/}$ in 12 h overnight urine	1,43 (0,83; 4,37)	1,59 (1,06; 3,00)	0,950
ENL $\mu\text{mol/}$ in 24 h urine	3,00 (2,00; 10,70)	3,65 (2,84; 4,85)	0,614
ENL in plasma (nmol/L)	29,23 (9,53; 56,47)	34,10 (14,26; 50,80)	0,705
Control group			
	n=21	n=14	
ENL $\mu\text{mol/}$ in 12 h day urine	0,95 (0,56; 1,49)	1,83 (0,97; 3,38)	0,020
ENL $\mu\text{mol/}$ in 12 h overnight urine	0,90 (0,39; 1,50)	1,76 (0,79; 3,45)	0,032
ENL $\mu\text{mol/}$ 24 h in urine	2,11 (0,86; 2,65)	3,74 (1,52; 7,10)	0,023
ENL in plasma (nmol/L)	18,59 (9,33; 26,41)	29,29 (16,25; 49,17)	0,032

ENL – enterolactone

In the brackets – interquartile range

Correlation of dietary data with ENL examination results in this case was also analyzed in two ways: there were separately analyzed 3-day mean data and separately – the 3rd-day food diary writing data.

Mean 3-day food diary data. In PC group the concentration of bread in the diet correlated with ENL concentration in plasma ($r=0,445$; $p=0,0212$), in 12h day urine ($r=0,521$, $p=0,003$), 12h overnight urine ($r=0,487$, $p=0,005$) and 24h urine ($r=0,550$, $p=0,001$). With bread fiber there correlated ENL in 12h day ($r=0,419$; $p=0,021$), ENL 12h overnight ($r=0,424$; $p=0,017$) urine and ENL in

plasma ($r=0,348$; $p=0,055$). ENL concentration in plasma, in 12h day, overnight and 24h urine did not correlated with the amount in of rye bread, bread lignan and total lignan intake in the diet. In the control group no correlation was found between ENL concentration in 12 h day, 12 h overnight, 24 h urine and in plasma, and bread, rye bread, bread fiber concentration in the diet ($p>0,05$). ENL concentration in 12 h day urine correlated with the total lignan concentration in the diet ($r=0,215$, $p=0,046$) and bread lignan amount ($r=0,238$, $p=0,027$). ENL in 24h urine correlated with bread lignan amount ($r=0,226$, $p=0,034$).

Data from the 3rd-day food diary In PC group the correlations were found in a subgroup, whose subjects had not used antibiotics during the last year– between ENL concentration in day urine and bread ($r=0,587$, $p=0,027$), rye bread ($r=0,736$, $p=0,0100$), bread fiber ($r=0,538$, $p=0,047$), rye bread fiber ($r=0,700$, $p=0,016$), total lignan ($r=0,622$, $p=0,018$) and bread lignan amount in the diet ($r=0,613$, $p=0,020$). ENL concentration in plasma correlated with rye bread amount in the diet ($r=0,673$, $p=0,023$). In the control group no correlation was found between ENL concentration in 12 h overnight, day, 24 h urine and in plasma with bread, rye bread, bread with seeds, fiber, bread fiber and lignan amount in the diet ($p>0,05$).

2.4. Intervention study „Influence of whole-grain rye bread on prostate cancer progression”

37 PC patients’ data were analyzed. Study subjects’ age was from 46 till 79 years. Mean indices: age 64,5 (SD 7,9) year, BMI 27,9 (SD 4,2) kg/m².

In the first stage AR metabolite concentration in plasma did not statistically differ between those subjects who had used rye bread on the previous day preceding analysis and those who had not used it ($p=0,353\div0,775$).

Figure 2.4. shows PSA changes in three study stages (with habitual diet, after the use of refined wheat and the use of rye bread). PSA has statistically significantly lower concentration in the 3rd study stage in comparison to the 2nd study stage, median value is respectively 6,84 (4,49; 9,80) and 8,05 (5,21;12,15) $\mu\text{g/L}$ ($p=0,016$). One can also observe reduction of PSA concentration amplitude (differences between the largest and smallest value).

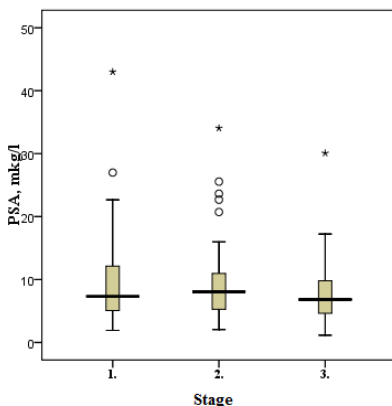


Figure 2.4. PSA changes in PC patients in the three study stages

Analyzing fPSA, testosterone, BAI, LH, FSH changes in different study stages, no statistically significant changes were found between the study stages ($p>0,05$). Evaluating SHBG changes in the study stages, one can observe the increase of median value in the 3rd stage compared to the 2nd stage from 9,5 till 44,9 nmol/L ($p=0,001$).

There is observed a statistically significant decrease of median enterolactone concentration in blood plasma from 9,99 (5,12; 28,08) nmol/L till 7,78 (3,50; 13,91) nmol/L in the 2nd stage ($p= 0,002$) and the increase in the 3rd stage to 16,92 (5,26; 16,92) nmol/L ($p<0,001$). There was found a statistically significant difference for DHBA plasma concentration between the 1st and the 2nd, between the 1st and the 3rd stage, as well as between the 2nd

and the 3rd stage ($p < 0,001$). DHBA median concentration: in the 1st stage 36,50 (20,10; 85,63) nmol/L; in the 2nd stage 16,75 (11,13; 22,55) nmol/L and in the 3rd stage 160,80 (83,70; 259,85) nmol/L. Also significantly differs DHPPA concentration between the 1st and the 2nd, between the 1st and the 3rd stage, as well as between the 2nd and the 3rd stage ($p < 0,001$). DHBA median concentration: in the 1st stage 33,25 (21,78; 48,70) nmol/L; in the 2nd stage 13,40 (9,50; 18,60) and in the 3rd stage 110,90 (70,25; 184,10) nmol/L.

Prostate tissue of 11 patients from preintervention biopsy and surgery (after rye bread intervention) was analyzed for apoptosis activity. Apoptosis activity was increased in 10 patients and unchanged in one. Apoptotic activity in tumour cells showed great variations: 1-35%, and even in one tumor various regions apoptosis was expressed to different degrees. Evaluation of rye bread influence to apoptosis is more difficult because apoptotic activity is influenced by degree of tumor differentiation – the higher the Gleason grade is the higher apoptotic activity. Even in same degree of tumor differentiation there was quite a great variation of apoptosis parameters. Gleason grade in 6 cases differed from one found in surgery: in 5 cases it was higher, in one case –lower than in biopsy found.

Although due to great variations and low number of study subjects statistics could not be used but it is still possible to conclude that rye bread seems to delay PC progression. This was suggested by the apoptosis increase in 10 of 11 apoptosis materials. Thus as a result of the consumption of rye bread to be found a tendency to increased apoptosis activity.

2.5. Vitamin D

Vitamin D concentration in serum was determined in 33 PC patients and 153 control group males in the case control study. The mean age of study subjects of vitamin D group was 59,6 (SD 8,6) years. Mean BMI was 28,1 (SD 3,8) kg/m². No statistically significant differences were found in the control and

cancer group in respect to such parameters as BMI, smoking, height and place of living ($p>0,01$).

Vitamin D amount in the diet was analyzed in 22 PC and 128 control group subjects. Vitamin D intake with diet in PC group was on average 286 (SD 347) IU – from 27 till 1846 IU, in the control group 244 (SD 171) IU – from 31 till 935 IU. Vitamin D concentration in the diet in both groups did not differ ($p=0,096$). Only 5% ($n=8$) of 150 study subjects consumed the necessary vitamin D amount. The main vitamin D intake source in both study groups was fish (82% in PC group and 77% in control group). Other sources of vitamin D intake were dairy products (7% in PC group and 8% in control group), eggs (6% in PC group and 8% control group) and liver (2% in PC group and 3% control group). Dietary intake of fish, dairy products and eggs between both groups did not differ ($0,578 < p > 0,141$). Vitamin D concentration in the diet did not correlate either with the total, or saturated fat intake ($p > 0,05$).

Vitamin D deficiency was 55% ($n=18$) in PC group and 70% ($n=107$) in the control group. Vitamin D insufficiency was 30% ($n=10$) in PC group and 23% ($n=40$) in the control group. Adequate vitamin D level was 15% ($n=5$) in PC group and 7% ($n=11$) in the control group. In PC group the median serum 25(OH)D concentration 18,3 (13,5; 25,5) ng/mL was statistically significantly higher than the value 15,2 (15,2; 21,8) ng/mL in the control group males ($p=0,017$). A tendency was noticed (without statistical significance), that vitamin D concentration in serum in PC group was higher in summer and autumn than in winter and spring, while in a control group vitamin D concentration in serum was lower only in spring. Regarding vitamin D concentration in serum and vitamin D intake there was a positive correlation in the control group ($r=0,363$; $p<0,001$), that was not found in the PC group ($r=0,103$; $p=0,630$). Also the correlation between vitamin D serum concentration and the age was found in the control group ($r=0,209$; $p=0,023$), but not in PC group ($r=0,176$; $p=0,411$).

In neither group we found statistically significant correlation between vitamin D level in serum and BMI (in control group $r=0,042$; $p=0,650$; in PC group $r=-0,288$; $p=0,173$) and in PC group – with the Gleason grade ($r=-0,092$; $p=0,677$). Vitamin D concentration in serum correlated with PSA in the control group ($r=0,363$; $p<0,001$), but not in PC group ($r=-0,103$; $p=0,630$).

3. DISCUSSION

3.1. Alkylresorcinols and lignans in Latvian bread

Taking into account the big importance of bread in the Latvian populations' diet, it is important to know the amounts of biologically active substances in different types of Latvian bread. Knowing AR content in Latvian bread types, one can much better judge about the amounts of whole-grain products in the diet, which is hard to evaluate either for bread users, and scientists.

The greatest AR concentration was found in rye bread (~560–840 µg/dry matter), lesser in fine rye-bread (mixed rye and wheat flour bread) (~200–330 µg/dry matter), and the lowest AR concentration was in refined wheat flour bread (~25–31 µg/dry matter). Since AR are localized in the grain outer coat, and they are considered to be whole-grain markers [Chen, 2004; Ross, 2004], the food guidelines in the whole world, including the Latvian Ministry of Health [Veselības ministrija 2008], recommends to use whole-grain products. In Latvia the industrially produced bread, thus bread, which is used by the majority of the population, does not contain whole-grain flour – flour does not contain the grain's germ part, and bran is added to wheat bread separately. However, AR concentration in Latvian rye bread [Meija, 2013a] is similar to that of Finnish [Mattila, 2005], Swedish [Ross, 2003] and Polish [Kulawinek, 2008] whole-grain rye bread. It shows that in the rye grain production process in all countries there is removed a small part of the grain coat. But AR concentration in fine rye-bread (mixed rye and wheat flour bread) was considerably less. It can be explained by the fact, that rye flour, which is used for the production of fine rye-bread, is finely ground, quite often there is used fine wheat flour. In wheat bread AR concentration depends on the amount of bran added.

In conclusion – AR concentration in different countries is similar in rye and wheat bread, but great differences are seen in mixed wheat and rye flour bread. There are no compulsory requirements to indicate the percentage content of each flour type, therefore for a consumer it is problematic to evaluate how much grain bran particles contained in the respective bread. AR concentration in bread can be used as bran or bread fiber amount markers, but not the whole-grain content marker, because not all countries in bread baking use whole-grain flour.

Analyzing the lignan concentration, the highest was in wheat bread with seeds (~9000–10 000 µg/100 g). The high lignan concentration was determined by the added seeds, most of all – linseed amount. In Finnish bread with seeds there was a comparatively lower lignan concentration (~3800 µg/100 g). In rye bread, as well, there was a high lignan content (800–1100 µg/100 g). In Finnish rye bread the lignan concentration was higher (1000–1400 µg/100 g), because in Finnish rye bread production there are used whole-grain rye flour in which there is a higher lignan content. The lowest lignan concentration was in white bread (90–100 µg/100 g). Results show, that lignan concentration in Latvian bread with seeds and in rye bread is equivalent, or even higher than in Finnish bread [Meija, 2013b], as a result, bread can be a significant lignan source in the diet.

3.2. Diet, alkylresorcinol and lignan intake

Study groups. Such parameters as the mean BMI, smoking, physical activity, oncological diseases in the family history, antibiotic use during the last year, sexual activity within the lifetime in PC and control group subjects did not differ. Both study groups had almost identical intake of nutrients. Our study results are in agreement of some of previous studies but not with all [Kristal, 2010; Schmid, 2011]. In both study groups also amount of bread and bread type did not differ. Also in the large Danish cohort study, which lasted for

12 years, there was not found any connection between the use of whole-grain products and PC risk [Egeberg, 2011]. But the study in Iceland shows, that just the use of rye bread at teenage (at 14–19 years of age) is connected with a smaller PC risk [Torfadottir, 2012].

The main fiber sources in both study groups in our study were cereals (41%), followed by vegetables (20%) and fruits (18%). Also in a majority of European countries for the men , over 50 years, the main fiber source in their diet is grain (40%). In EPIC study, the highest fiber intake was in men in Denmark and Spain (Finland was not included in this study), the least – in Sweden [Suzuki, 2009]. In our study the main fiber source was grain products, similarly like in Finland [Lang, 2003, Nordic Council of Ministers 2002]. Fiber amount in the diet can be considered to a certain extent also as one of the „healthy diet indicators”, because it negatively correlated with the total and saturated fat amount in the diet in both groups, though there were no differences as to fiber concentration in PC and control groups. Also in EPIC study there was not found any correlation between the total fiber and separate grain fiber amount in the diet and PC risk [Suzuki, 2009], though previously in the Italian case control study there was described a negative correlation between PC risk and fiber amount in the diet [Pelucchi, 2004].

Although it has been described, that products, containing a lot of fiber and simultaneously lignans as well, reducing PC risk, increasing SHBG and reducing PSA level [Adlercreutz, 2002], we did not find any correlation between fiber concentration in the diet either with SHBG, or PSA in neither of the study groups.

Alkylresorcinols in diet

Also AR median concentration in the diet during the last year was 55,8 mg/d, and no differences between the groups were found. This concentration is similar to that, which was found in Finland, Denmark, while in Great Britain it

is considerably lower (11,9 mg/d). [Ross, 2005]. Similarly as in other studies, AR concentration in the diet correlated with the concentration of bread, rye bread in the diet [Ross, 2012].

Evaluating AR concentration in the diet, the strong side of the present study was, that during the study we found AR concentration in various Latvian bread types [Meija, 2013a]. But the accuracy of the obtained study data limited the study subjects' possibility to precisely define the bread type, they have used, especially in respect to bread with added seeds and bran. Different rye bread types as well, most commonly contain wheat flour in different proportions to which the consumer quite often does not pay attention.

Lignans in diet

The lignan concentration in the diet during last year was 2782 µg/d, there were no differences between both study groups as to the concentration of the total lignan intake and intake of separate lignans. We analyzed the concentration of four (SECO, MAT, LAR, PIN) lignan types in the diet, we evaluated additionally also SYR and MED, however, we evaluated these two lignan types only in bread. It is known, that SYR in great concentrations is found in rye [Adlercreutz, 2010], therefore it is important to estimate it in the populations where bread is used in large quantities, the one typically being the Latvian population. The lignan concentration in the diet is compared only with those studies, where at least four lignan type concentrations are estimated.

Median lignan concentrations in the diet are greater than the concentration of four lignan type concentration in the diet in European population in the recent published EPIC study, which included 10 European country cohort [Zamora-Ros, 2012]. Compared with concentration of six lignan types, median concentration in Latvian men's diet was less than in Finnish men [Hedelin, 2006]. The main lignan source in men was bread with seeds and rye bread (86%), another significant lignan source was linseed (7%). These results

coincide with the data on lignan sources in Swedish and Finnish men, although in Sweden and Finland the most significant source was rye bread, while in Latvia – bread with seeds [Hedelin, 2006; Nurmi, 2010]. EPIC study data showed, that the main lignan sources of the Mediterranean region countries were fruits (31%), vegetables (26%) and wine (8%), while in the rest of countries there dominated vegetables (23%) and grain products (20%) [Zamora-Ros, 2012]. Similarly to the previously reported [Milder, 2005; Suzuki, 2008], in our study the lignan concentration correlated with the fiber concentration in the diet as well, because the main lignan sources were bread rich in fiber and linseed.

Our study results indicate, that men in Latvia at the age of 45–80 years comparatively more than in other countries with the diet consume such protective substances like lignans and fiber [Touillaud, 2007; Cotterchio, 2008; Nurmi, 2010; Pellegrini, 2010; Zamora-Ros R, 2012]. However, hormonally-dependant tumor prevalence is high [Slimību profilakses un kontroles centrs, 2012]. The explanation might be the high fat concentration in the diet. Fat delays ENL formation in the bowels [Adlercreutz, 2010].

Limitations of our study are due to food frequency questionnaire, which inevitably deals with the possibility of error. We used Canadian lignan basis [Thompson, 2006], but it does not depict the lignan concentration in Latvian foodstuffs, as well as does not cover completely all lignan sources of Latvian population. However, our study is the first in which there is analyzed the lignan concentration and the sources in the diet of older men in Latvia. Strong sides of the study are those, where the four lignan types and bread – six lignan types concentration were evaluated. It is essential, that lignan values in most significant lignan diet sources in Latvia – in different Latvian bread types were determined. Of course, it should be noted, that study subjects used different bread types in their diet, and not in all cases lignan value in these

bread types corresponded to those values, which had been determined in most popular Latvian bread types and had been used for data processing.

In conclusion lignan concentration in older men's diet in Latvia is high. The greatest lignan intake was that of SECO. Between PC and control group men there were no differences as to the consumed lignan amount. The main lignan source in the diet is bread. Potentially protective effect of lignans is, possibly, hindered by the abundant concentration of fat in the diet. In order to more precisely evaluate the lignan concentration in Latvian population diet, one should perform studies with a larger number of study subjects, to increase the lignan data base and to state lignan concentration for a wider range of local foodstuffs.

3.3. Alkylresorcinol metabolites and enterolactone

Alkylresorcinol metabolites

DHPPA concentration in 12 h overnight urine and in plasma did not essentially differ from the results obtained in other studies [Guyman, 2008; Aubertin-Leheudre, 2010].

In both study groups correlation was found between AR metabolites in urine (DHBA 12 h day, 12 h overnight and 24 h urine; DHPPA 12 h day, 12 h overnight and 24 h urine) and bread fiber, rye bread and rye bread fiber concentration during the last 3- day diet. Summarizing the data obtained, we conclude, that DHPPA in 12 h overnight urine was the most appropriate AR metabolite, which can be used as biomarker for bread fiber, rye bread and rye bread fiber concentration in the diet which coincides with the data of other studies, in which DHPPA in 12 h overnight urine is recognized as the indicator, depicting whole-grain wheat and rye bread amount in the diet [Guyman, 2008].

Reliability of food survey data has always been a topical problem in epidemiological studies. The use of appropriate biomarkers, assessing the

intake of whole-grain wheat and rye concentration would ensure a greater reliability of the data acquired and the possibility to get information in situations when it is impossible to get precise data on food. Since AR is found mainly in outer coat of wheat and rye grains, they can be found only in such foodstuffs in which wheat or rye bran have been used. In many countries there is used whole-grain flour and in such a case AR metabolites can be considered as markers for whole-grain wheat or rye bread concentration in the diet. Since in Latvia in bakery almost no whole-grain wheat flour is used, then DHPPA in 12 h overnight urine should be considered as a marker for rye bread, rye fiber and wheat bran concentration in the diet used the last three days.

We found statistically significant differences between both study groups:

1) in PC group DHPPA concentration in plasma was statistically significantly higher than in the control group;

2) in PC group DHPPA in plasma correlate with total bread, bread fiber, rye bread and rye bread fiber concentration in 3-day mean food data, which was not found in the control group;

3) in PC group AR metabolites (except DHPPA in 12 h day urine) in urine correlated with the mean 3-day food data, but did not correlated with the 3rd day food data.. In the control group the correlations mentioned were either for 3-day mean, or separate 3-day food data;

4) in PC group DHPPA in 12 h day urine did not correlated with bread fiber, rye bread concentration and rye bread fiber concentration in 3-day diet, while in the control group such a correlation was not found;

5) in PC group DHBA and DHPPA concentration in the overnight urine was essentially higher than in day urine. In the control group such a difference was not seen.

6) in PC group in plasma there was preserved high DHPPA concentration despite the fact, whether rye bread in the previous day was eaten

or not. In the control group, however, a statistically significant difference of DHPPA plasma concentration was found between those subjects who had eaten rye bread and those who had not.

These acquired data make us think, that PC patients, perhaps, have a delayed metabolism in comparison to the control group men. We do not have any indications in our study on renal dysfunctions. Perhaps, AR metabolism in liver is changed, the place where AR breakdown occurs into DHBA and DHPPA. In healthy, young people DHPPA concentration first of all reaches maximum in urine and only then in blood [Söderholm, 2009, 2011]. Perhaps, PC patients have delayed glucuronidation, therefore a higher DHPPA concentration is preserved in the blood. Previous studies with healthy individuals showed, that at night there may be delayed AR metabolite excretion [Söderholm 2011]. In our study PC patients' DHPPA and DHBA concentration in overnight urine was higher than in day urine, which can indicate, that in PC patients AR metabolites remain for a longer time in the blood and get later into urine. That is may be why PC patients had higher concentration of DHPPA in plasma independent they had consumed rye bread on the previous day or not. It is essential, that these results coincided with our intervention study results. Besides, metabolites in urine do not depict the dietary content of the previous day as found in the control group, but indicates to the food eaten one or two days ago. Another possibility is, that PC patients after metabolic processes in liver the AR elimination type has got changed. We know, that AR, with the increase of AR portion in the diet, the elimination changes from excretion via urine to biliary excretion [Landberg, 2009a], but in PC group biliary excretion, perhaps, is not efficiently used and a high metabolite concentration is preserved in plasma.

Possible explanation why PC patients have a higher potentially protective AR metabolite DHPPA concentration in plasma is, that the metabolite mentioned does not reach the target organs and its protective activity

is hindered. One should think in which way more precisely to assess AR metabolite kinetics in biological fluids, they should be analyzed in relation to the previous 5-day food diary data, at the same time taking into account exact time for bread intake.

Strong sides of the study are: acquired correlation within collection of food data (filling in of food diary) and time of urine collection and blood plasma tests, as well as recording of day-night time. We, in difference to other studies, were analyzing separately 3-day food data and the 3rd day separately, which allows us in more detail to judge about AR kinetics in biological systems. As to the limitation of the current study is – a comparatively small number of study subjects, which represents only part of relatively homogenous population, therefore the results cannot be generalized to the whole population. Food diaries were filled in different seasons, thus the intake of food content differed. However bread eating habits are less dependent on season.

Summary: DHPPA in 12 h overnight urine best of all correlates with rye bread and rye bread fiber concentration in the diet, which makes DHPPA the most appropriate biomarker for the estimation of rye bread and rye bread fiber intake in the population. Perhaps, PC patients have a delayed AR metabolism.

Enterolactone

In both study groups ENL concentration in plasma, in comparison to EPIC study data [Peeters, 2007], was similar to that of the population of Denmark, while for Italian population ENL concentration in plasma was considerably lower. ENL concentration in urine was similar to that which was found in Finnish men [Nurmi, 2010]. Differences in ENL concentration between the study groups were not found in neither ENL examination, which agrees with previous studies [Kilkinen, 2003b; Stattin, 2004; Ward, 2008]. In the Scottish study, in the control group ENL serum concentration was higher

than in PC group [Heald, 2007]. But one of the Swedish studies indicated, that ENL serum concentration in relation to PC risk is not linear and just the mean ENL serum concentrations (15–24 nmol/L) are associated with a decreased PC risk [Hedelin, 2006].

In PC group no correlation of ENL and PSA was found, which agrees with results of other studies [Hedelin, 2006; Venkitaraman, 2008]. Although in the control group there was found a positive correlation between ENL and PSA, it cannot be interpreted as ENL negative effect, because PSA for the majority of study subjects was within the norm.

In the groups we found the ENL concentration had no relation to sex hormone concentration. Up to now, this correlation has been more investigated in women in postmenopausal age, when large lignan doses have increased SHBG concentration [Wu, 2006]. In EPIC Norfolk study, however, was found, that ENL has a positive relation to androgen level in plasma, but no correlation with SHBG was found [Low, 2005]. There is still not clear the role of different sex hormones in PC cancerogenesis.

We also did not find any correlation between ENL concentration in plasma or urine and vitamin D concentration in plasma. Although the hypothesis is set, whether phytoestrogens (genistein), together with vitamin D can act synergically protectively, hindering PC development [Swami, 2007], there are still no data on lignans interaction with the vitamin D. At present our study data results do not confirm ENL protective effects.

In PC group no differences were found between ENL concentration in 12 h day, 12 h overnight and 24 h urine and in plasma in those subjects who had used antibiotics within the last year, and those who had not, while in the control group there was a statistically significant difference found. These results identify, that in PC group, evidently, after the use of antibiotic, the intestinal changes in the microflora were preserved much longer than in the control group, or also PC patients already initially had a specific microflora.

Possible effect of intestinal microflora is seen by the fact, that, by analyzing the 3rd –day dietary data, correlation was found only in that PC group, who had not used antibiotics within the last year. Perhaps, this group had a faster ENL formation and lignan metabolism.

Analyzing ENL correlation with dietary data (bread, bread fiber, rye bread, bread with seeds, bread lignan, total lignan concentration), the results do not show a clear correlation. There was found correlation with a part of food data, with another part – no. One should add, that precision of results were also affected by receiving not always quite precise information on bread type in the diet – study subjects said, that they had used „seed-bran bread”, but concentration of seeds in such breads has a wide range. All in all, our results do not contradict with other study results, of which a part shows the correlation between ENL concentration in serum or in urine and lignan concentration in the diet [Kilkinen, 2003a; Milder, 2007], while in other studies they do not find either in PC, or in the control group [Hedelin, 2006].

Biological activity of lignans is affected by many factors. It is still not clarified, whether after absorption and metabolism the conjugated lignan forms bind with estrogen receptors like non-conjugated forms. Besides, lignan absorption is hindered by fat in the diet, therefore a lot of fat can be one of the reasons why ENL correlation with lignan concentration in the diet was only partial. And still, the main factor which determines ENL formation, is intestinal microflora, which was not possible to assess in our study.

3.4. Intervention with rye bread in PC patients

In our intervention study the use of rye bread significantly lowered PSA in comparison to white bread (refined wheat bread) use ($p=0,016$), as well as reduced PSA concentration amplitude. Changes in fPSA (free PSA) concentration were not found. Previously only two small studies with

individuals on the effect of whole-grain rye bread with high fiber concentration on PSA prostate cancer patients had been done. Our study results agree with Landberg data [Landberg, 2010], who in a 6-months long intervention study with rye bread products found PSA reduction in comparison with the use of wheat bread use, but he did not find any fPSA changes. In a different study [Bylund, 2003] such a result was not found.

In the baseline stage (using habitual diet) DHBA and DHBA concentration did not differ among those study subjects, who had used rye bread on the previous day before doing analysis, and those who had not used bread, which agrees with our case control study results. Analyzing AR metabolite changes in various study stages, DHBA and DHPPA concentration in plasma differed between the baseline stage and after the use of wheat bread, as well as between the baseline stage and after rye bread use, or between the stages after wheat and rye bread use. AR metabolite changes in PC patients in intervention conditions with rye bread have not been studied up to present, but there are studied AR concentration changes in PC patients after rye and wheat bread intervention, and the concentration increase has been found after the use of rye bread, and the concentration decrease after the use of wheat bread [Landberg, 2009b], which agrees with results of our study.

Analyzing sex hormone concentration changes after the use of rye bread, we found SHBG concentration increase after the use of rye bread, in comparison with the situation after the use of wheat bread. But changes in testosterone, BAI, LH and FSH concentration were not found. In Bylund study there were not either SHBG, or FSH, or LH concentration changes [Bylund, 2003].

ENL concentration in our study decreased after the use of wheat bread and increased after the use of rye bread, which agrees with Landberg's study data [Landberg, 2010].

Studying the effect of rye bread on cell apoptosis, we could not get statistically significant results due to a small number of study subjects and great variation, though our results indicate that apoptosis activity has a tendency to grow, and they agree with Bylund's results, which showed, that after the use of rye bread the apoptosis index significantly increased, while in the control group, those who had used wheat bread, such changes had not been found [Bylund, 2003]. Apoptosis activity increase is evidently determined by lignan effect. Lignans can influence PC progression, acting on SHBG either directly hindering the tumor growth, or indirectly acting on IGF and affecting PSA [Adlercreutz, 2002; 2007]. In favour of this hypothesis serves our study results, showing that after the use of rye bread there increased both lignan metabolite ENL concentration, as well as increase of SHBG and decrease of PSA concentration.

We still have to think, that ENL is not the only active substance and there act also other whole-grain rye bread biological mechanisms. Rye bread fiber provide lesser energy utilization because the absorption in small intestines decreases, while the overweight, as well exceedingly great kcal concentration in the diet are PC risk factors. [Hsieh, 2003; Ma, 2008]. Another possible rye fiber protective activity mechanism is fermentation process in the bowels which activates liver AMP-dependant proteinkinase, which, in turn, regulates the energy homeostasis in the body, hinders glyconeogenesis, as a result there is reduced glucose production and insulin secretion [Hu, 2010]. Insulin, in turn, can promote PC progression [Hammarsten, 2005].

The study had its strong sides and limitations. The strong sides was a rather great number of included subjects in the intervention study (n=37) and high compliance. Limitations: it was not a blind study and there was no comparison to the control group. Although the results of our study coincide with other study results, in order to interpret results we still need to continue

research with a larger number of study subjects, it has to be done for a longer period of time and using rye bread with a much greater fiber concentration (enriched with rye fiber).

Summary. The evidence found in the study states that rye bread, perhaps, hinders PC progression. Potential mechanisms of activity: SHBG, ENL, DHBA and DHPPA concentration increase, as a possible result of which PSA decreased and there was observed the tendency for apoptosis indices to increase.

Summary of case control and intervention study on AR and lignan metabolite use

DHPPA concentration in plasma depict rye bread and rye bread fiber concentration in the diet under intervention conditions, when in a longer period of time there is consumed a constant amount of bread. But in the conditions when a man uses habitual diet and the amount of bread in the diet varies, the most appropriate examination to describe rye bread and rye bread fiber in the diet is detection of DHPPA concentration in 12 h overnight urine. Similarly, also ENL in intervention situations depict the lignan intake and rye bread concentration, while when using habitual diet, such a connection is not found.

3.5. Vitamin D

Our study results show, that in both study groups was widespread vitamin D deficiency and/or insufficiency. Only a slight part of men had the recommended vitamin D level in serum. The obtained study results are similar to the Latvian women in postmenopausal age in winter, but vitamin D level is considerably lower than average vitamin D serum concentration of Latvian women [Lejnieks, 2013]. Our results correspond also to study results in other countries and, perhaps, point to the fact, that in Latvia, men over 45 years, vitamin D deficiency or insufficiency are more expressed than in other world

regions [Travis, 2009; Holick, 2011] and may influence PC development but it is against our results because in PC group vitamin D serum concentration was higher than in the control group. In this situation it is hard to judge as to vitamin D possible protective effect, including its influence on PSA and sex hormones, because in both groups its concentration was low, besides PC patient number was numerically small. Perhaps, serum vitamin D level does not depict vitamin D concentration in local tissues [Travis, 2009].

Vitamin D concentration in the diet was insufficient in both groups. Our results coincide with the data on vitamin D insufficient amount in the diet in such countries as Austria, Germany, Great Britain, Italy, the Netherlands, Norway, Ireland [European Food Safety Authority, 2006] and with the previous studies in Latvia [Nacionālais diagnostikas centrs, 2009]. The main vitamin D source of the diet in both study groups was fish, which in both groups is used insufficiently..As both study groups consumed the most important foodstuff equally, one can think about specificities of vitamin D metabolism in PC patients. The data in favour of it show that vitamin D in the diet in the control group correlated with vitamin D serum concentration, but did not correlate in PC group. As a result, the low vitamin D serum concentration might be explained by insufficient exposition of the sun, or insufficient concentration of vitamin D in the diet.

Part of studies show, that higher age is connected with a lower vitamin D concentration [Trump, 2009]. In our study in the control group subjects, growing with age, there increased vitamin D serum concentration, which, perhaps is due to the fact that control group men have been more outside, though physical activity for them was lesser than in PC patients. The references in the literature mention, that by the rise of BMI, vitamin D concentration decreases [Ahn, 2008], in the study with Latvian women such a correlation was found in summer, but not in winter [Lejnieks, 2013]. We did not find any correlation between vitamin serum concentration and BMI, which can be

explained because majority of our study subjects were overweight or obesity I degree.

Investigating vitamin D, strong sides of our study were the strict patient selection criteria, homogenous study subject group, detailed information as to the food intake, lifestyle factors and demographic indices. The limitations of the study were the small number of PC patients.

Summary: vitamin D deficiency or insufficiency is widespread in PC group and control group men..Food consumed does not sufficiently ensure the necessary vitamin D concentration.. To evaluate the possible protective effect of vitamin D in PC prevention, it is advisable to use vitamin D non-active form preparations to achieve the desirable vitamin D serum concentration and to do prospective studies.

4. CONCLUSIONS

1. Lignan concentration in bread with seeds and rye bread in Latvian and Finnish bread is equivalent. Alkylresorcinol concentration in Latvian rye bread is as high as in Scandinavian and Polish rye bread. Alkylresorcinol concentration in bread can be used as a marker for bran and fiber concentration in bread.
2. The developed food frequency questionnaire, together with supplemented BIOR food data base is suitable in order to carry out studies on whole-grain in the diet.
3. The main lignan sources for both groups are bread with seeds and rye bread. No differences were found between PC and control group in the intake of nutrients, including alkylresorcinols and lignans.
4. DHPPA in 12 h overnight urine possibly is the most suitable AR metabolite to estimate intake of rye bread and rye bread fiber.
5. Alkylresorcinol metabolites concentrations in plasma and urine indicate possibly delayed AR metabolism in PC patients.
6. ENL concentration in biological fluids reflects lignan concentration in the diet only under intervention conditions. Effects of antibiotics on ENL concentration in biological fluids could be different in PC and control group, which indicates possible differences in gut microflora in PC and control groups. This assumption requires further research.
7. Whole-grain rye bread possibly hinders PC progression.
8. Vitamin D deficiency and/or insufficiency is widespread in PC and control group in men over 45 years. The necessary vitamin D concentration with habitual diet is not ensured.

5. PRACTICAL RECOMMENDATIONS

1. DHPPA in 12 h overnight urine can be used as biomarker to estimate rye bread and rye bread fiber in epidemiological studies.
2. In intervention studies for evaluation the possible rye bread effect on PC progression it is advisable to use rye bread with a higher rye fiber concentration - with added rye bran.
3. It is recommended to choose whole-grain rye and seed-bran bread which is rich in biologically active substances in daily diet. In bread production it is advisable to use whole-grain flour. Consumers' demand for whole-grain bread has to be promoted. In public education has to be cooperation between nutritionists, food technologists, grain breeders, educators and mass media.
4. To use inactive vitamin D supplements 1000 IU regularly, as well as to include in daily diet vitamin D food sources, from which the most important is the fish.

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LIST OF PUBLICATIONS

Publications in refereed international journals

1. Meija L., Samaletdin A., Koskela A., Lejnieks A., Lietuvietis V., Adlercreutz H. Alkylresorcinols in Latvian and Finnish breads // International Journal of Food Sciences and Nutrition, 2013a; 64(1): 117-121.
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