The role of plant-food components in pelvic-organ cancer survivors

– From feasibility to effects in randomized controlled dietary interventions

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Cover illustration: By Rebecca Ahlin and Mattias Ahlin.

In the picture on the front cover, the pelvis symbolizes pelvic-organ cancers; the broccoli, rye straw, and soybeans, the dietary interventions of dietary fiber and phytoestrogens; and the mobile phone, the digital application. The picture on the back cover symbolizes prostate cancer by showing stained prostates where the tumors are marked.

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“The fruit of your own hard work is the sweetest.”

Deepika Padukone

To my family – Ni betyder allt för mig

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ABSTRACT

Background: The number of people who are surviving cancer is steadily growing. Dietary components have a potential role in preventing the side effects of treatment for pelvic-organ cancer, as well as tumor recurrence in survivors. However, the evidence for such specific dietary advice is currently lacking. Large-scale randomized controlled trials are needed to strengthen the evidence for the effects of dietary components in pelvic-organ cancer survivors. Since compliance is one of the challenges in dietary interventions, digital tools could provide an effective method of measuring compliance. However, new methods need to be validated against a more established method to assess their precision in correctly measuring dietary intake.

Aim: The aim of this thesis was to test the feasibility of a dietary fiber intervention prior to conducting a large randomized controlled trial in patients with pelvic organ cancer. Secondly, to investigate the effect of a diet high in phytoestrogens on tumor proliferation and if the effect differs between men with different genotypes of estrogen receptor beta (ERβ) in a large randomized controlled trial in patients with prostate cancer. Lastly, to develop and evaluate a new digital dietary assessment method for measuring dietary fiber intake.

Methods: A dietary fiber intervention, using a fiber supplement and a moderate fiber intake from the regular diet, was tested in a feasibility study in patients with gynecological cancer (n = 57, Paper I). A study-specific food frequency questionnaire and a digital application were used, and blood and fecal samples were collected. The effects of a phytoestrogen intervention, using soybeans and flaxseeds, were tested in a randomized trial with patients with prostate cancer (n = 140, Papers II–IV). Tumor proliferation was measured as a Ki-67 index in tumor specimens, and prostate-specific antigen (PSA) and hormone concentrations were measured in the blood. A randomized

crossover trial was used to validate the digital application against a 3-day dietary record to measure dietary fiber intake in a randomly selected female population (n = 26, Paper V).

Results: In the Feasibility study, expected burden of the study or acute side effects of radiotherapy were the most common reasons for declining participation or dropping out (Paper I). The participation rate was highest for blood sampling and lowest for fecal sampling. The phytoestrogen intervention decreased the risk of a higher Ki-67 index, and the effect was most pronounced among men with a specific genotype of ERβ (Paper III). An opposite effect was seen on comparing the groups of ERβ genotypes where the phytoestrogen diet increased or decreased the risk of increased total PSA concentration. The phytoestrogen intervention did not affect blood concentrations of hormones except for decreased risk of increased estradiol concentration in one of the ERβ genotype groups (Paper IV). In the Dietary validation study, a ~2 g difference in measured fiber intake was found between the dietary record and the digital application (Paper V).

Conclusions: Dietary interventions with dietary fiber supplements and phytoestrogen-rich foods are feasible in patients with pelvic-organ cancer and digital tools can be used for the assessment of dietary fiber intake. The design of interventions needs to be carefully adapted to the targeted group to be feasible. The effects of the plant-food components need further investigation. The results of this thesis can be useful for both clinicians and researchers.

Keywords: gynecological cancer, prostate cancer, pelvic radiotherapy, dietary fiber, phytoestrogens, digital application, validation

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SAMMANFATTNING PÅ SVENSKA

Bakgrund: Antalet personer som överlever cancer ökar stadigt. Komponenter i kosten har en potentiell roll att förhindra biverkningar av behandlingen för bäckenorgancancer (ex prostatacancer och gynekologisk cancer) såväl som återfall av cancertumörer hos överlevare. Idag saknas vetenskapligt underlag för sådana specifika kostråd. Storskaliga kontrollerade studier, där deltagarna lottas till vilken kost de ska få, behövs för att stärka bevisen för effekten av olika kostkomponenter hos canceröverlevare. Att deltagarna följer den tilldelade kosten, även kallad kostintervention, är en av utmaningarna i koststudier. Digitala verktyg kan vara en effektiv metod för att mäta hur bra deltagarna följer kosten. Nya metoder behöver dock kontrolleras mot en mer etablerad metod för att undersöka noggrannheten för att korrekt mäta kostintaget.

Syfte: Syftet med denna avhandling var att testa genomförbarheten av en intervention med kostfibrer innan man genomför en stor lottad kontrollerad studie på patienter med bäckenorgancancer. För det andra att undersöka effekten av en kost rik på fytoöstrogener (växtkomponenter som bland annat finns i sojaprodukter och frön) på tumörtillväxt och om effekten skiljer sig åt beroende på vilken genuppsättning männen har av östrogenreceptorn, i en stor lottad kontrollerad studie på patienter med prostatacancer. Slutligen att utveckla och utvärdera en ny digital metod för att mäta kostfiberintag.

Metod: Genomförbarheten av en fiberintervention, med ett fibertillskott och ett måttligt fiberintag från den vanliga kosten, testades i en studie med patienter med gynekologisk cancer (n = 57, delarbete I). Ett studiespecifikt kostfrekvensformulär och en digital applikation användes och blod- och avföringsprover samlades in. Effekterna av en fytoöstrogenrik kost, med sojabönor och linfrön, testades hos patienter med prostatacancer där deltagarna lottades till vilken grupp de skulle tillhöra (n = 140, delarbete II–IV). Tumörtillväxt mättes som ett Ki-67-index i tumörmaterial och koncentrationer av prostataspecifikt antigen (PSA) och hormoner mättes i blodet. En lottad korsdesign användes för att validera den digitala applikationen mot en 3-dagars kostdagbok för att mäta kostfiberintag i en slumpmässig kvinnlig population (n = 26, delarbete V).

Resultat: De vanligaste orsakerna till att man avböjde deltagande eller hoppade av kostfiberinterventionen var förväntad börda eller akuta biverkningar av strålbehandlingen (delarbete I). Deltagandet var högst för blodprovstagningarna och lägst för avföringsinsamlingarna. Fytoöstrogen-

kosten minskade risken för ett högre Ki-67-index, och effekten var mest uttalad hos män med en viss genotyp av östrogenreceptorn (delarbete III). Motsatt effekt sågs vid jämförelse av genotypgrupperna för PSA, där interventionskosten ökade eller minskade risken för höjda koncentrationer i blodet. Fytoöstrogen-kosten påverkade inte blodkoncentrationerna av hormoner förutom en minskad risk för ökad koncentration av östradiol i blodet hos en av genotypgrupperna (delarbete IV). I kostvalideringsstudien var skillnaden i uppmätt fiberintag ~2 g mellan kostdagboken och applikationen (delarbete V).

Slutsats: Kostinterventioner med kostfibertillskott och fytoöstrogenrika livsmedel är möjliga hos patienter med bäckenorgancancer och digitala verktyg kan användas för att mäta intaget av kostfibrer. Designen av interventionerna behöver noggrant anpassas till målgruppen för att vara genomförbar. Effekterna av de växtbaserade komponenterna behöver undersökas ytterligare. Resultaten av denna avhandling kan vara användbara både för kliniker och forskare.

LIST OF PAPERS

This thesis is based on the following papers, referred to in the text by their Roman numerals.

I. **Ahlin R**, Bergmark K, Bull C, Devarakonda S, Landberg R, Sigvardsson I, Sjöberg F, Skokic V, Steineck G, Hedelin M. A Preparatory Study for a Randomized Controlled Trial of Dietary Fiber Intake During Adult Pelvic Radiotherapy. Frontiers in Nutrition; 2021; 8: 756485.

II. **Ahlin R**, Nybacka S, Josefsson A, Stranne J, Steineck G, Hedelin M. The effect of a phytoestrogen intervention and impact of genetic factors on tumor proliferation markers among Swedish patients with prostate cancer: study protocol for the randomized controlled PRODICA trial. Trials 2022; 23(1): 1041.

III. **Ahlin R**, Josefsson A, Nybacka N, Landberg R, Skokic V, Stranne J, Steineck G, Hedelin M. Effects of a phytoestrogen intervention and estrogen receptor β genotype on prostate cancer proliferation and PSA concentrations – a randomized controlled trial. Manuscript.

IV. **Ahlin R**, Nørskov P. N, Nybacka N, Landberg R, Skokic V, Stranne J, Josefsson A, Steineck G, Hedelin M. Effects on Serum Hormone Concentrations after a Dietary Phytoestrogen Intervention in Patients with Prostate Cancer: A Randomized Controlled Trial Nutrients 2023, 15, 1792.

V. **Ahlin R**, Sigvardsson, I, Skokic, V, Landberg, Steineck G, Hedelin M. Development and Validation of a Mobile Phone Application Developed for Measuring Dietary Fiber Intake. Nutrients 2021; 13: 2133.

CONTENT

ABBREVIATIONS

3βAdiol	5α androstane3β,17β-diol
BMI	Body Mass Index
CI	Confidence Interval
cT	Clinical Tumor Category
DHT	Dihydrotestosterone
ERα	Estrogen Receptor Alpha
ERβ	Estrogen Receptor Beta
FFQ	Food Frequency Questionnaire
IGF-1	Insulin-like Growth Factor 1
ISUP	International Society of Urological Pathology
PSA	Prostate-Specific Antigen
SCFA	Short Chain Fatty Acids
SHBG	Sex Hormone-Binding Globulin

DEFINITIONS IN SHORT

Differentiation	How strongly the cancer tissue resembles normal tissue. Poorly differentiated cells are different from normal cells and well-differentiated cancer cells are more similar to normal cells.
Digital application	A program that can be downloaded onto mobile phones or tablets with complete mobile operating systems e.g., Android.
Neoplasia	Presence or formation of abnormal tissue growth.

1 PREFACE

I cannot remember when my interest in nutrition and health started, but it was a long time ago. It inspired me to learn more and to become a registered dietitian. By chance, whilst doing my master's, I saw an advertisement for a research assistant in a cancer research group. Having applied, I was fortunate to be given this position whilst at the same time being able to continue the completion of my master's thesis. For about two years I worked as a research assistant before becoming a PhD student.

I now have six years of experience of meeting patients with prostate cancer and gynecological cancer. I am extremely grateful for the contact I have had with all these patients and for being able to get to know the people behind the data. Most of the patients who accepted participation in our nutritional studies had an interest in nutrition, and several of them have appreciated being able to meet a registered dietitian and receive answers to their previously unanswered questions. Many of the myths around nutrition and cancer, many constantly circulating in the media, have become truths for these patients and I am glad to have been able to give them evidence-based nutritional advice. It is dreadful that cancer patients should feel guilty as a result of the false, and potentially detrimental, information that is spread, for example, that eating sugar will accelerate tumor growth. Some patients have expressed disappointment at the lack of nutritional evidence and that there is nothing more that they themselves can do, because their motivation and dedication are certainly not lacking.

Like me, you have probably been affected by cancer, have relatives who have received a cancer diagnosis or have yourself received this dreaded diagnosis. Over the years, my passion to expand the knowledge of nutritional evidence in cancer has grown to be able to contribute a small step on the way forward. It is this which forms the basis of this thesis.

2 INTRODUCTION

The incidence of cancer is increasing worldwide leading to a greater number of cancer survivors (1, 2). Cancer incidence is projected to continue escalating over the coming years (2). There is, therefore, a great demand for improved treatment options, both during and after cancer treatment. Healthy dietary patterns could have a potential role in preventing tumor recurrence in cancer survivors (3). The dietary advice currently given to patients with all types of cancers is standard, although adapted according to the symptoms the patients are experiencing (4, 5). Dietary components could potentially play a major part in reducing the side effects of cancer treatment and improving the outcome of the cancer disease. Before specific dietary advice can be given to patients, the evidence of its proven effects must be evaluated in large-scale randomized controlled clinical intervention trials, which poses several challenges. Patients might experience side effects from ongoing treatments for the disease, which could increase the risk of them leaving the study. The treatments may already involve multiple hospital visits, which then need to be coordinated with visits for the study. Another challenge in intervention studies is compliance, which could be affected by the side effects of disease treatments. Moreover, obtaining accurate reporting of dietary intake is a major challenge that can have considerable implications for the interpretation of the results from diet-health studies.

2.1 PELVIC-ORGAN CANCER

Pelvic-organ cancers include several malignancies, such as prostate, cervical, vulva, bladder, rectal, and bladder cancer (6). In 2020 there were approximately 4.2 million new cases of, and 1.6 million deaths from, pelvic-organ cancers worldwide (Figure 1) (2). In Sweden, almost 16 000 new cases of pelvic-organ cancers were reported in 2020 (Figure 2) (7). The treatments for pelvic-organ cancers include surgery, radiotherapy, and chemotherapy which can be used either separately or in combination (8).

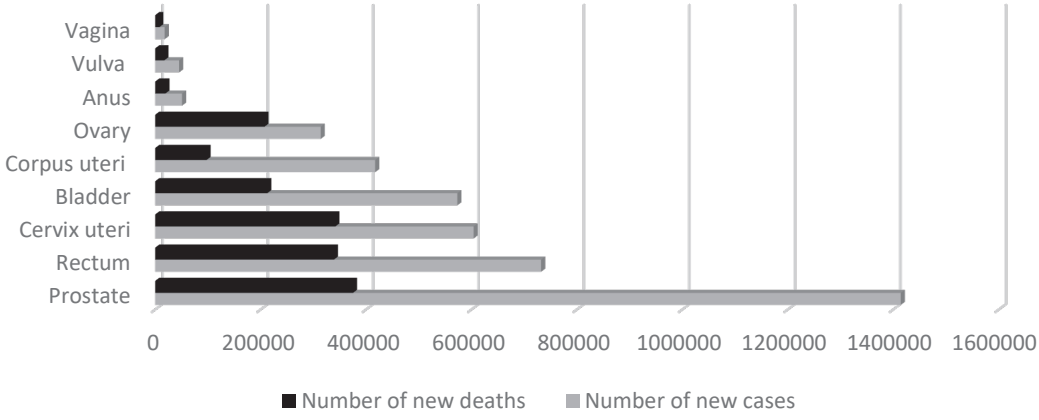

Figure 1. Incidence and mortality of different pelvic-organ cancers worldwide in 2020. Source: GLOBOCAN (2).

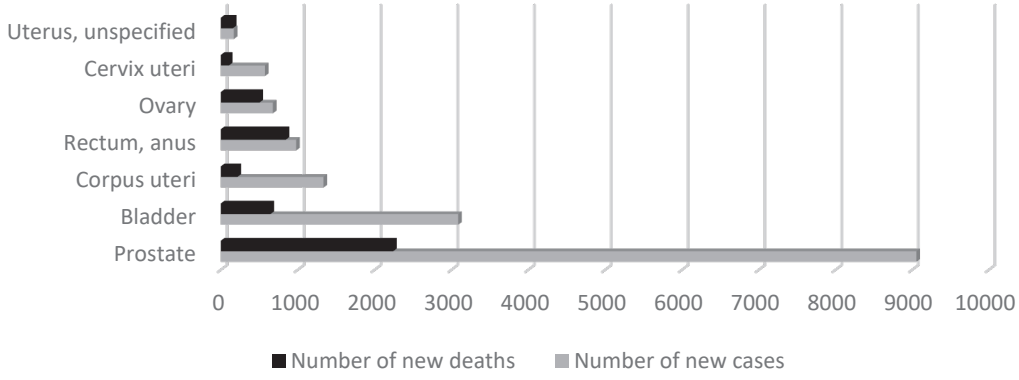

Figure 2. Incidence and mortality of different pelvic-organ cancers in Sweden in 2020. Source: Socialstyrelsen (7, 9).

2.1.1 PELVIC-ORGAN RADIOTHERAPY

In 2007, Andreyev *et al*. estimated that up to 300 000 patients worldwide undergo pelvic-organ radiotherapy annually (10). This number has most likely increased since then and will continue to grow in line with a predicted increase in new cancer cases, which is estimated at 47% over the next 20 years (2). The reported number of patients with pelvic-organ cancers undergoing radiotherapy in Sweden in 2020 was approximately 3600 (11-15). These numbers have been reported to Swedish quality registers but should be interpreted with caution. The coverage rates were high (97–98%) in the registers for anal, prostate, and bladder cancers but only 70% and 80% in the registers for colorectal and gynecological cancers, respectively. The register for anal cancer reported only curative radiotherapy, while the other registers reported radiotherapy that was given as the primary treatment. The register for bladder cancer did not separate radiotherapy from other primary treatments (12).

In external-beam radiotherapy, ionizing radiation is used to damage tumors with the ambition of sparing the surrounding normal tissue (16). Ionizing molecules causes DNA damage in the irradiated tissue. The tumor is more vulnerable to radiation than healthy tissue and, by fractionating the radiation, the healthy tissue can recover between the fractions. Nevertheless, these repetitive injuries come with a price for the healthy tissue with effects being experienced for many years afterwards (17). The radiation starts a very complex process of multiple events whilst, at the same time, the fractions of the radiation continue to be given. These events include activation of the coagulation system, inflammatory processes, formation of granulation tissue, epithelial regeneration, and remodeling, together with molecular signals of chemokines, cytokines, and growth factors (17). Fibrotic tissue may be a result of these processes, but delayed tissue injuries are poorly understood. Both rates of dose accumulation, fraction size, and overall duration of treatment affect the magnitude of structural injury resulting from the radiation (18-21). Chronic inflammatory processes may occur during radiotherapy, but little is currently known (22).

2.1.1.1 ADVERSE EFFECTS OF PELVIC RADIOTHERAPY

In pelvic radiotherapy, the intestines are among the organs exposed to radiation which results in radiation-induced intestinal toxicity. Acute toxicity occurs during or within three months of radiotherapy and chronic toxicity after this time (23). In recent years, the terminology of chronic toxicity has been debated and the term "pelvic radiation disease" has been suggested (24). Steineck *et al*. categorized 30 long-term intestinal symptoms in gynecological-cancer survivors into five syndromes: leakage syndrome, urgency syndrome, blood discharge, excessive mucus discharge, and excessive gas discharge (25). It has been suggested that these syndromes should be called survivorship diseases.

A Swedish study of gynecological cancer survivors and controls found that the cancer survivors reported a higher occurrence of gastrointestinal symptoms compared to controls (26). "Emptying of all stools into clothing without forewarning" and "defecation urgency" were two of the most common symptoms. In a British study, 81% of the patients reported new-onset gastrointestinal problems occurring at least one year after completion of pelvic-organ radiotherapy (27). Quality of life was affected in 52% of these patients. The risk factors for developing gastrointestinal symptoms after pelvic-organ radiotherapy include stage of disease, chemotherapy, radiation dose, dose intensity, age, and acute toxicity (28-30).

2.2 PROSTATE CANCER

In 2020, there were approximately 1.4 million new cases of, and 375 000 new deaths from, prostate cancer worldwide (2). Prostate cancer accounted for 7% of all cancers and was the second most common cancer in men. In Sweden, there were almost 18 000 new cases of, and 4500 deaths from, prostate cancer in 2020 (31). The incidence has steadily increased between 1970 to the beginning of the 2000s while the mortality rate has remained somewhat stable in Sweden (Figure 3). Over the past 15 years, the incidence has fluctuated or decreased. The increasing incidence and fluctuation are likely a result of the use of prostate-specific antigen (PSA) testing for early diagnosis in healthcare and the implementation of several scientific studies on prostate cancer screening (32). The decline in 2020 could be an effect of the Covid-19 pandemic, which led to a decrease in the possibilities and requests for PSA testing, and a reduced tendency for people to seek healthcare for symptoms.

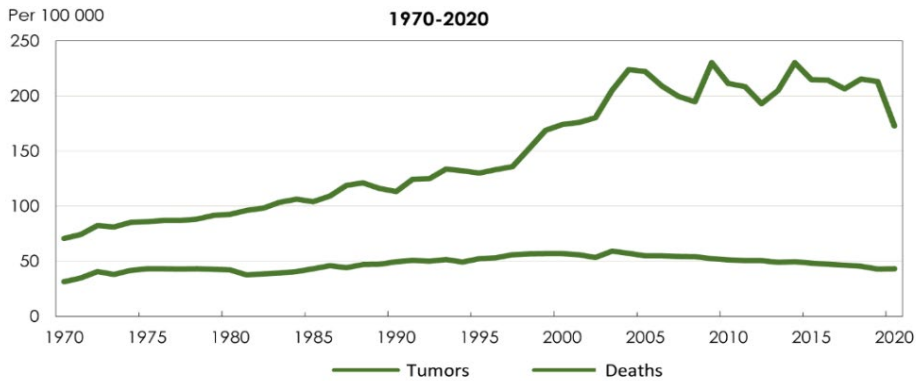

Figure 3. The number of prostate tumors and deaths in Sweden 1970-2020. Source: Socialstyrelsen 2021.

Age is the major risk factor for prostate cancer (33, 34). Other risk factors include a family history of prostate cancer, ethnicity, genetic factors, and environmental factors such as dietary constituents (34). The incidence and mortality of prostate cancer varies enormously worldwide, with the highest incidence in Europe and the lowest in Asia (2). This variation in incidence is probably partly due to diagnostic differences between countries.

2.2.1 PROSTATE CANCER MARKERS

There are several markers in prostate cancer which determine the type of treatment to be used and predict the outcome after treatment (35). All markers have limitations and the accuracy can therefore be improved by using several markers.

2.2.1.1 TUMOR STAGE

The extent of the primary tumor can be categorized into a clinical tumor category (cT) which is based on the results of a physical examination, prostate biopsy, and imaging test (36). Localized prostate cancer is divided into cT1 or cT2, where cT1 includes a non-palpable tumor on digital rectal examination which is not visible on transrectal ultrasound and cT2 includes a palpable tumor on digital rectal examination or is visible on transrectal ultrasound. cT2a tumors involve <50% of one side of the prostate (unilateral), cT2b tumors involve ≥50% of one side of the prostate (unilateral), and cT2c tumors involve both sides of the prostate (bilateral) (36).

2.2.1.2 PROSTATE CANCER GRADING

The Gleason system was created in the 1960s as a unique grading system for prostate cancer and was based on the architectural pattern of the tumor (37). The Gleason score was reported as the sum of the two most common grade patterns 1–5, where the first number was the most common pattern in the tumor. The grading of 1–5 described how cancerous the tissue looked, where 1 was most similar to normal prostate tissue and 5 was most similar to abnormal cells and growth patterns (38). The definitions of the patterns have been modified over the years. In 2014 the International Society of Urological Pathology (ISUP) set up a new grading system to adapt to modern techniques and knowledge (39). The new Grade Groups are graded 1–5 (Table 1).

Table 1. Definitions of ISUP Grade Groups.

ISUP Grade Group	Gleason score	Definition
1	≤6	Separate discrete well-formed glands only.
2	3+4 = 7	Mainly well-formed glands with a minor component of poorly-formed/fused/cribriform glands.
3	4+3 = 7	Mainly poorly formed, fused, or cribriform glands with a minor component of well-formed glands[1].
4	4+4 = 8 3+5 = 8 5+3 = 8	Poorly formed, fused, or cribriform glands only, or mainly well-formed glands and minor component lacking glands[2], or major lacking glands and minor component of well-formed glands[2].
5	9–10	Gland formation is absent (or with necrosis) with or without poorly formed, fused, or cribriform glands[1].

[1] For cases with >95% poorly formed, fused, or cribriform glands or lack of glands on a core or at radical prostatectomy, the component of <5% well-formed glands is not included in the grade.

[2] Poorly formed, fused, or cribriform glands can be a more minor component.

Source: Epstein *et al*. 2014 (39).

2.2.1.3 PROSTATE-SPECIFIC ANTIGEN (PSA)

PSA is predominantly expressed in the prostate (40). Serum concentrations increase during conditions such as neoplasia or inflammation due to the structure of the prostate gland being disrupted causing PSA to leak out into the circulation. For several years, PSA has been the most common measurement used to diagnose prostate cancer and is used routinely in cancer clinics (41). The total serum concentration of PSA includes both the complexed and free forms. Compared with benign prostatic hyperplasia, patients with prostate cancer have a lower ratio of free and total PSA. Rising PSA concentrations are considered an indication of prostate cancer progression. Even if PSA has sometimes been controversial in the role of development and progression of prostate cancer, it is still suggested as an appropriate target in cancer treatment (40). The criticism of using solely PSA concentrations in prostate cancer screening is the detection of low-risk cancers which are given unnecessary cancer treatments causing side effects such as incontinence and impotence resulting in reduced quality of life (42). In addition, previous studies have found significant variability in PSA measurements collected within short intervals (43). To avoid overdiagnosis of prostate cancer, several markers have been tested that are combined with PSA, and diagnosis tools based on gene expression are emerging (44, 45).

2.2.1.4 KI-67

The protein Ki-67 attends the active phases of the cell cycle and is not present in resting cells (46). This makes the expression of Ki-67 strictly associated with cell proliferation and it is frequently used in research as a proliferation marker. The proportion of Ki-67-positive tumor cells is often associated with the clinical outcome of the disease (46). However, Ki-67 is not used as a predictor of prostate cancer outcome in clinical practice.

2.2.2 THE HORMONAL EFFECTS IN PROSTATE CANCER

In the 1970s, a relationship was established between androgens and estrogens and prostate cancer (47). Through the injection of estrogens or reducing androgens by castration, an enzyme in serum that is associated with neoplasm was reduced and was increased with the injection of androgens. These results, together with the studies that followed, led to the hypothesis that a high concentration of testosterone leads to increased growth of prostate cancer. Androgen ablation therapy became, therefore, a treatment for prostate cancer; it is still used in patients with metastatic disease, as an adjunct treatment during radiotherapy for high-risk localized disease, and sometimes in patients with increasing PSA concentrations after local treatment (48). However, over time most patients develop androgen-independent non-curative prostate cancer after androgen ablation therapy (49).

Androgens are primarily formed in the testes and the adrenal gland (50). Testosterone is the main circulating androgen in the body while dihydrotestosterone (DHT) is the predominant androgen circulating in the prostate tissue. In the prostate, testosterone is metabolized to DHT by the enzyme 5α-reductase. DHT binds to the androgen receptor and induces multiple androgenic actions, for example transcriptional activity. Of the total testosterone, about 44% binds to the sex hormone-binding globulin (SHBG) with high affinity, 54% binds to albumin with low affinity, and 1–2% is unbound (50). SHBG is a transport protein for steroid hormones with the highest affinity for testosterone and DHT. However, SHBG is not only a passive carrier but can itself induce responses in different tissues, for example, estradiol and SHBG together have been shown to induce androgen actions in the prostate (51).

Insulin-like growth factors (IGF) are involved in the metabolism of fat, glucose, and protein (52). The part IGF plays in the regulation of apoptosis and cell proliferation is also of importance. Previous research has shown that IGF-1 is upregulated in prostate cancer tissue compared to benign tissue, with higher expression in high-grade tumor areas compared with low-grade areas (53).

Studies have found inclusive results between serum concentrations of testosterone, estradiol, SHBG, and IGF-1 and the risk of prostate cancer (52, 54, 55). Several factors can affect hormone concentrations. Both older age and obesity are associated with lower concentrations of testosterone (56). Furthermore, testosterone plasma concentration has been inversely associated with high-grade prostate cancer (ISUP grade ≥2) and positively associated with low-grade disease (ISUP grade 1) (57). In addition, studies have found that, despite no differences in serum concentrations of androgens, higher concentrations were found in prostate tissue in patients with prostate cancer compared to patients with benign prostatic hyperplasia (58).

2.2.2.1 ESTROGEN AND ESTROGEN RECEPTORS

Endogenous estrogens include estrone, estradiol, and estriol (59). These compounds bind to various types of receptors that induce gene transcription and expression in different organ systems. Of special interest are the estrogen receptors, with estrogen receptor alpha (ERα) and beta (ERβ) being the most known. ERα has been suggested to have proliferative effects and ERβ antiproliferative effects (60). In samples of benign prostatic hyperplasia the expression of ERβ is more pronounced than in malign prostate samples, where the expression of ERβ declines (61).

2.3 THE ROLE OF DIET IN PROSTATE CANCER AND DURING PELVIC-ORGAN RADIOTHERAPY

A diet high in fruit and vegetables and low in energy, fat, meat, dairy products, and calcium may decrease the risk of developing prostate cancer; however, the evidence is limited (62). The current dietary advice is similar for both men diagnosed with prostate cancer and those who want to reduce their risk of the disease. The Asian population has a low incidence of, and mortality from, prostate cancer and besides genetic factors and diagnostic differences, it is thought that their diet is favorable in reducing prostate cancer incidence. Asian citizens immigrating to the USA have shown an increased incidence of prostate cancer, potentially due to changed dietary habits (63). Compared to Western countries, Asian countries have a high intake of soy foods, which contain phytoestrogens (64-66).

Preclinical studies indicate that an increased intake of dietary fiber during pelvic-organ radiotherapy can reduce acute and late radiation-induced inflammation (67-69). The protective effect of dietary fiber can potentially be explained by it contributing to diverse gut microbiota and the protection of the colonic mucus layer (70). However, human studies giving high-quality evidence of the effect of an increased intake of dietary fiber during pelvic-organ radiotherapy are lacking (71-73). Most human studies investigating the effect of dietary fiber intake are small with only 20 or fewer participants per group (Table 2) (74-80). Studies have shown conflicting results concerning effectiveness and have been heterogeneous due to their use of different fiber interventions, such as the supplements and advice given. This makes it difficult to compare studies and draw conclusions meaning that dietary advice cannot be introduced into clinical practice.

In 2017, our research group contacted 15 oncology clinics in Sweden to investigate the types of dietary advice given to patients with prostate and gynecological cancer receiving radiotherapy before, during, and after treatment (81). There was a large variation in the dietary advice given, although this was not unexpected due to the current lack of evidence. However, most clinics recommended decreasing the intake of high-fiber foods, which could

potentially harm the future intestinal health of patients. High-quality studies are, therefore, urgently needed.

Table 2. Examples of human intervention studies assessing the effect of dietary fiber intake during pelvic-organ radiotherapy.

Authors and year	Title	Design	Key findings
Rosli *et al*. (74) 2021	Randomized controlled trial on the effect of partially hydrolyzed guar gum supplementation on diarrhea frequency and gut microbiome count among pelvic radiation patients	Patients receiving pelvic RT were randomized to 2x10 g/d of partially hydrolyzed guar gum (n = 14) or placebo (n = 16) 14 d prior and 14 d during RT. Diarrhea frequency, nutrition status, fecal samples, and quality of life were investigated.	The intervention group had a higher mean diarrhea frequency compared with controls on days 14 and 28, which reduced at day 45. Bifidobacterium count was doubled in the intervention group at 14 d of supplementation, and this was not seen in controls.
Forslund *et al*. (82) 2019	Effects of a nutrition intervention on acute and late bowel symptoms and health-related quality of life up to 24 months post radiotherapy in patients with prostate cancer: a multicentre randomised controlled trial	Patients with prostate cancer referred to RT were randomized to replace insoluble fibers with soluble fibers and reduce lactose intake (intervention; n = 92) or maintain their habitual diet (control; n = 88). Intestinal symptoms, quality of life, and intake of intervention foods were investigated.	The intervention was associated with less blood in stools, flatulence, less appetite in the acute phase, and more bloating in the late phase. However, the findings were small or clinically trivial.
Wedlake *et al*. (83) 2017	Randomized controlled trial of dietary fiber for the prevention of radiation-induced gastrointestinal toxicity during pelvic radiotherapy	Patients receiving pelvic RT were randomized to low-fiber (≤10 g NSP/d; n = 55), habitual-fiber (control; n = 55), or high-fiber (≥18 g NSP/d; n = 56) diets. Intestinal symptoms, stools, macronutrient intake, and concentration of fecal short-chain fatty acid were investigated.	The high-fiber group had a smaller change in intestinal symptoms between the start and end of RT than the habitual-fiber group. The habitual-fiber group also had more intestinal symptoms at 1 y post-RT than the high-fiber group.
Garcia-Peris *et al*. (75) 2016	Effect of inulin and fructo-oligosaccharide on the prevention of acute radiation enteritis in patients with gynecological cancer and impact on quality-of-life: a randomized, double-blind, placebo-controlled trial	Patients with gynecological cancer were randomized to a daily intake of 2x6 g of prebiotics (inulin and fructooligosaccharide; n = 20) or placebo (n = 18) from 1 w before to 3 w after RT. Stool consistency and the number of bowel movements were recorded daily. Quality of life was also evaluated.	The prebiotic group had fewer days with watery stools compared to the placebo group. Insomnia and diarrhea had the greatest impact on quality of life.

Itoh *et al*. (76) 2015	A randomized, double-blind pilot trial of hydrolyzed rice bran versus placebo for radioprotective effect on acute gastroenteritis secondary to chemoradiotherapy in patients with cervical cancer	Patients with cervical cancer undergoing chemoradiotherapy were randomized to hydrolyzed rice bran (n = 10) or placebo (n = 10). Acute gastrointestinal side effects and medications were analyzed.	The intervention group had a lower diarrheal score compared to controls. There were trends in reduced diarrhea symptoms and less need for strong antidiarrheal medications in the intervention group compared to controls.
Nascimento *et al*. (77) 2014	Efficacy of synbiotics to reduce acute radiation proctitis symptoms and improve quality of life: a randomized, double-blind, placebo-controlled pilot trial	Patients with prostate cancer undergoing 3-dimensional conformal RT were randomized to an intake of a synbiotic powder (Lactobacillus reuteri + 4.3 g soluble fiber (inulin + partially hydrolyzed guar gum); n = 10) or placebo (n = 10). Proctitis symptoms and quality of life were measured.	The placebo group had more proctitis symptoms and lower quality of life than the synbiotic group.
Pettersson *et al*. (84) 2014	Effects of a dietary intervention on gastrointestinal symptoms after prostate cancer radiotherapy: long-term results from a randomized controlled trial	Prostate cancer patients referred to RT were randomized to reduce their intake of insoluble dietary fibers and lactose (intervention; n = 64) or continue their habitual diet (control; n = 66). Gastrointestinal side effects, quality of life, and dietary intake were evaluated at 24 m after RT.	The intervention had no distinct effect on the long-term gastrointestinal side effects or quality of life. The symptoms of unintentional stool leakage, mucus discharge, and limited daily activities were pronounced post-RT compared to pre-RT.
Pettersson *et al*. (85) 2012	Effects of a dietary intervention on acute gastrointestinal side effects and other aspects of health-related quality of life: a randomized controlled trial in prostate cancer patients undergoing radiotherapy	Prostate cancer patients referred to RT were randomized to reduce their intake of insoluble dietary fibers and lactose (intervention; n = 64) or continue their habitual diet (control; n = 66). Gastrointestinal side effects and quality of life were evaluated up to 2 m after RT.	The intervention did not affect gastrointestinal side effects or quality of life. During RT, a lower proportion of the intervention group had intestinal symptoms compared to controls but the differences between the groups were not statistically significant.
Murphy *et al*. (86) 2000	Testing control of radiation-induced diarrhea with a psyllium bulking agent: a pilot study	Patients receiving pelvic RT were randomized to take psyllium (Metamucil; intervention; n = 30) or not (control; n = 30). Radiation-induced diarrhea was analyzed.	The intervention decreased the incidence and severity of diarrhea and showed a strong trend in decreased use of anti-diarrheal medication.

Lodge *et al*. (79) 1995	A randomized cross-over study of the efficacy of codeine phosphate versus Ispaghulahusk in patients with gynaecological cancer experiencing diarrhoea during pelvic radiotherapy	Patients with gynecological cancer who had changed bowel habits during RT were randomized to codeine phosphate (n = 5) or Ispaghula husk (n = 5). The interventions were compared concerning control of diarrhea. Patients remained in the study for the duration of their symptoms (max 5 w) or would crossover when the study medication was ineffective (4 d).	The trial was interrupted when all who received codeine phosphate had control of their diarrhea, while the patients allocated to Ispaghula husk were all crossed over to the codeine phosphate group.
Salminen *et al*. (80) 1988	Preservation of intestinal integrity during radiotherapy using live Lactobacillus acidophilus cultures	Patients with gynecological cancer scheduled for RT were randomized to receive 150 ml of a fermented milk product containing Lactobacillus acidophilus and lactulose (intervention; n = 11) or a control group (n = 10). Both groups were given dietary advice regarding a low-fat, low-residue diet during RT. Intestinal side effects and medications were investigated.	The intervention group had a smaller incidence of diarrhea and less use of antidiarrheal medications but experienced more flatulence compared to controls.

Abbreviations: RT; radiotherapy, NSP; non-starch polysaccharides.

2.3.1 DIETARY FIBER

Dietary fiber is a diverse group of compounds with several definitions (87). The definition from the CODEX Alimentarius Commission defined in 2009 is shown in Table 3.

Table 3. Definition of dietary fiber according to the CODEX Alimentarius Commission

Dietary fiber definition
Dietary fiber means carbohydrate (CHO) polymers with ten or more monomeric units[1], which are not hydrolyzed by the endogenous enzymes in the small intestine (SI) of humans and belong to the following categories: • Edible CHO polymers naturally occurring in the food as consumed • CHO polymers, obtained from food raw material by physical, enzymatic, or chemical means[2] • Synthetic CHO polymers[2]

[1] The footnote allows international authorities to decide whether those compounds with DP of 3–9 would be allowed.

[2] For the isolated or synthetic fibers in category '2' or '3', they must show a proven physiological benefit to health as demonstrated by generally accepted scientific evidence to competent authorities Cited from Jones *et al*. 2014 (87).

The main components included in dietary fiber definitions are non-starch polysaccharides (e.g., cellulose, pectins), resistant starch (e.g., retrograded amylose, physically enclosed starch), resistant oligosaccharides (e.g., galacto-oligosaccharides, fructooligosaccharides), and other often minor components associated with dietary fiber polysaccharides (e.g., lignin) (88). Regardless of the fiber definition used, non-starch polysaccharides are the main component. Dietary fiber can be divided into insoluble and soluble fibers, according to their solubility in water (89). Soluble fibers can be further divided according to their viscosity, that is their ability to form gels and thicken. Examples of insoluble fibers are cellulose and wheat bran, while psyllium husk typically contains soluble viscous fibers, and wheat dextrin and inulin are typically composed of soluble non-viscous fibers. However, most fibers are a mixture of insoluble and soluble types (89). Another classification of dietary fibers is according to their fermentation by bacteria in the large intestine and the production of short-chain fatty acids (SCFA), primarily acetate, propionate, and butyrate. SCFA, in particular butyrate, play an important role in colonic health by being the

main energy source for the colonocytes (90). Soluble non-viscous fibers, such as inulin and wheat dextrin, are highly and rapidly fermentable and could potentially produce a large amount of gas, whereas soluble viscous fibers, such as psyllium husk, are intermediately fermentable, and insoluble fibers are relatively nonfermentable, which results in less gas production (89, 91). Large amounts of, and rapidly produced, gas from the fermentation of dietary fiber may cause side effects such as bloating, cramps, and abdominal pain (89, 92).

The intake of dietary fiber provides several health benefits, for example reducing the risk of diseases, such as diabetes and coronary heart disease (93, 94), and improving physical parameters, for example lowering blood pressure and low-density lipoprotein cholesterol (95, 96). Citizens in most countries worldwide do not achieve the recommended fiber intake (97). An intake of 25–35 g/d (approximately 3 g/MJ) of dietary fiber is recommended in Nordic countries (88). The major dietary sources recommended are wholegrain cereals, vegetables, whole fruit, legumes, and nuts. According to *Riksmaten,* the Swedish national survey of dietary habits in adults conducted in 2010-2011, the mean dietary fiber intake in Sweden was 19.9 g/d (women: 18.8 g; men 21.3 g) (98). The largest sources were bread (28%), vegetables (12%), fruit and berries (11%), potatoes and potato dishes (11%), and breakfast cereals (5%). These fiber values refer to official analytical methods accepted by the Association of Official Analytical Chemists and include non-starch polysaccharides, lignin, and analytically resistant starch. Other minor components in Nordic diets, such as inulin and resistant oligosaccharides, are not included (88).

2.3.2 PHYTOESTROGENS

Phytoestrogens are plant compounds that have similar structures to natural and synthetic estrogens and antiestrogens (e.g., estradiol, diethylstilbesterol, tamoxifen) (99). Phytoestrogens are often divided into three subclasses: isoflavones, coumestans, and lignans (Table 4). The primary sources of isoflavones are soybeans and soy foods (Table 5), and secondary sources include other legumes and clover sprouts (100). The processing of soy foods affects both the type and amounts of isoflavones found in the foods (101). Flaxseeds are the main source of lignans but legumes, cereals, and some vegetables also contain lignans, although in much lower concentrations (Table 5) (100). The main sources of coumestans are sprouts of alfalfa and clover, while other sources are seeds and legumes (99).

Table 4. The subclasses of phytoestrogens and examples of compounds in the groups.

Isoflavones	Coumestans	Lignans
Daidzein	Coumesterol	Secoisolariciresinol
Genistein	4´-methoxycoumesterol	Lariciresinol
Glycitein		Matairesinol
Formononetin		Medioresinol
Biochanin A		Pinoresinol
Equol		Syringaresinol
		Enterodiol
		Enterolactone

Source: Kurzer *et al*. (99) and Landete *et al*. (102).

Table 5. Sources and content of isoflavones and lignans.

Isoflavone sources		Lignan sources
(total content of isoflavones, mg/kg)		(total content of lignan, mg/kg)
Non-fermented	Fermented	
Roasted soybeans (170–2020)	Miso (260–890)	Flaxseed meal (675)
Tofu (80–670)	Tempeh (69–625)	Flaxseed flour (527)
Dried soybeans, boiled (470)	Natto (460–870)	Lentil (179)
Green soybeans, boiled (550)	Fermented bean curd (390)	Soybean (9)
Sprouted soybeans (250–530)	Soy sauce (13–75)	Oat bran (7)
Soy milk (13–211)		Wheat bran (6)

Source: Fletcher *et al*. (99).

The metabolism of phytoestrogens is dependent on the intestinal microbiota (103, 104). The isoflavones daidzein and genistein, both found in soybeans, can also be converted from formononetin and biochanin A, respectively (Figure 4) (99, 103). Daidzein can be further metabolized to equol in some people. The plant lignans matairesinol and secoisolariciresinol, mainly found in flaxseeds, are converted to the mammalian lignans enterolactone and enterodiol (Figure 4) (99). Enterodiol can be further converted to enterolactone. There is a major individual variation in the metabolism and excretion of phytoestrogens (105, 106). In addition, only approximately 20–35% of individuals excrete significant amounts of equol after consumption of isoflavones (107-111).

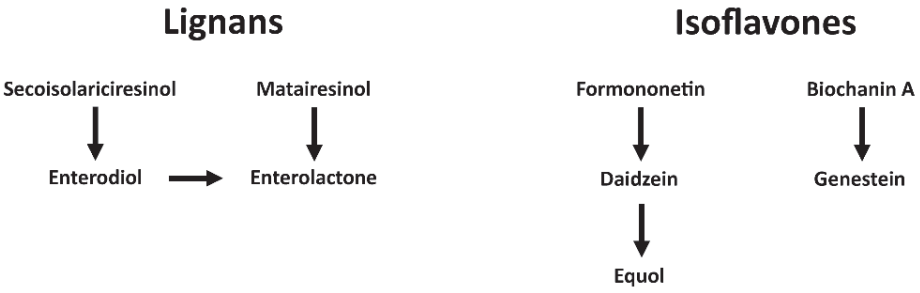

Figure 4. Metabolic pathways induced by the intestinal microbiota of the major lignans in flaxseeds and major isoflavones in soybeans. Modified from Setchell et al. (103) and Borriello et al. (112).

The average intake of isoflavones in Asian populations has been estimated to 20–40 mg per day (64, 65). These amounts of isoflavones correspond to about 40–100 g of soy products per day. The average intake of isoflavones in European populations is less than 1 mg per day (66). In Asian populations, the main source of isoflavones is tofu (64, 65). The intake of soy foods is overall low in European populations and the main source of isoflavones is bread (66).

The lower intakes of isoflavones in European populations compared to Asian populations are reflected in the excretion of phytoestrogens. The concentration of phytoestrogens in the blood is generally higher in Asian populations compared to European populations (Table 6) (113, 114). However, there is still substantial variation between different countries, both in Europe and in Asia

(115, 116). Furthermore, the differences between the populations are specifically regarding concentrations of isoflavones rather than lignans, likely reflecting different intakes of soy foods (Table 6) (113).

Table 6. Blood concentrations of phytoestrogens in Asian and European populations.

	Blood concentration (nmol/L)		
Population	Daidzein mean (95% CI)	Genistein mean (95% CI)	Enterolactone mean (95% CI)
Japanese men	283 (210, 355)	493 (376, 609)	33 (21, 44)
European men	3[1] (3, 4)	7[1] (6, 7)	8[1] (8, 9)
Swedish adults	2[1] (1, 3)	3[1] (2, 6)	13[1] (8, 20)

[1] Adjusted geometric mean.

Abbreviation: CI, Confidence Interval.

Source: Morton *et al*. (112), Peeters *et al*. (114).

2.3.2.1 THE RELATIONSHIP BETWEEN PHYTOESTROGENS AND ENDOGENOUS HORMONES

Testosterone and DHT may cause proliferation by binding to the androgen receptor (Figure 5) (50). In contrast, 5α androstane3β,17β-diol (3ßAdiol), a metabolite of DHT, can suppress the expression of the androgen receptor and androgen-driven proliferation, as well as increase cell differentiation (117, 118). Since the structure of phytoestrogens is similar to estrogens and they therefore bind to ERβ with high affinity, they could act as a substitute for 3ßAdiol thereby increasing differentiation in prostate tissue and inhibiting cancer growth (Figure 5) (119). These pathways of phytoestrogens have been confirmed in in-vitro cell studies and preclinical studies (120-122).

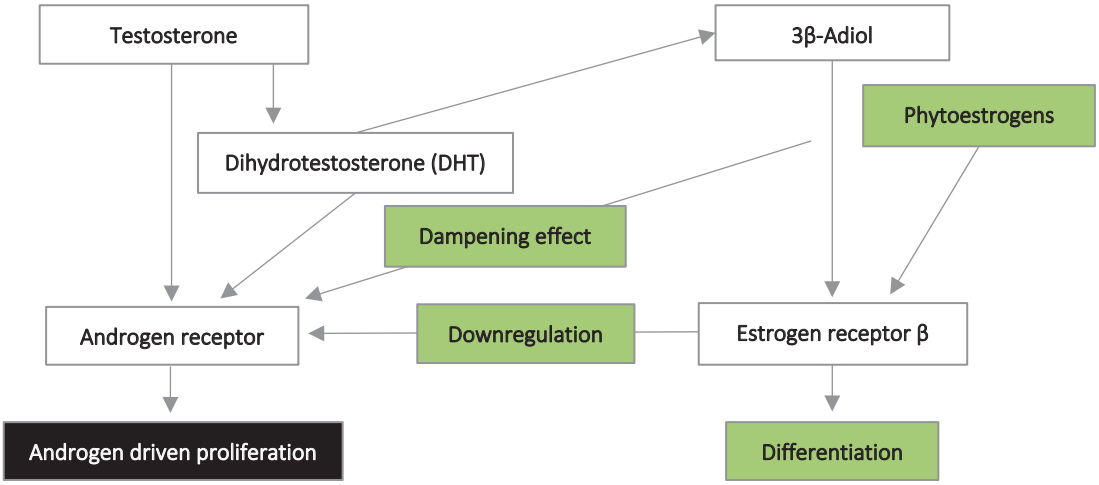

Figure 5. The potential pathways of phytoestrogens in their protective role in prostate cancer. Abbreviation: 3βAdiol, 5α androstane3β,17β-diol.

2.3.2.2 PHYTOESTROGEN INTERVENTIONS IN PROSTATE CANCER PATIENTS

Most previous research exploring the effects of phytoestrogen interventions on hormones and proliferation markers in patients with prostate cancer have used phytoestrogen supplements (Table 7) (123-130). Landberg *et al*. and Bylund *et al*. used rye bread as a source of phytoestrogens, Demark-Wahnefried *et al*. flaxseeds, and Dalais *et al*. soy grits (131-134). All the studies in Table 7 involved groups of fewer than 50 participants. Varying results have been found regarding the effect of phytoestrogen intervention on hormones related to prostate cancer and proliferation markers (Table 7).

Table 7. Examples of studies using phytoestrogen interventions in patients with prostate cancer where the outcomes were proliferation markers and hormones related to prostate cancer.

Authors and year	Title	Design	Key findings
Kumar *et al.* (123) 2020	A phase II randomized clinical trial using aglycone isoflavones to treat patients with localized prostate cancer in the pre-surgical period prior to radical prostatectomy	Patients with localized prostate cancer were randomized to 40 mg (n = 36) of aglycone isoflavones daily or placebo (n = 34) prior to radical prostatectomy. Toxicity, concentrations of PSA and steroid hormones, and tissue markers of proliferation were investigated.	The intervention did not reduce tissue markers of proliferation or serum steroid hormones. The concentration of PSA decreased with isoflavone intervention compared to placebo in Caucasian men but not in African American men. The concentration of IGF-1 decreased in African American men with isoflavone supplementation.
Hamilton-Reeves *et al.* (124) 2013	Short-term soy isoflavone intervention in patients with localized prostate cancer: a randomized, double-blind, placebo-controlled trial	Patients with localized prostate cancer were randomized to soy isoflavones (80 mg/d) (n = 42) or placebo (n = 44) capsules for 6 w before scheduled prostatectomy. Changes in concentrations of e.g., free and total testosterone, total estrogen, estradiol, and PSA were analyzed.	Twelve genes involved in cell cycle control and nine genes involved in apoptosis were downregulated in the intervention tumor tissues compared to the placebo group. Changes in concentrations of serum free and total testosterone, total estrogen, estradiol, and PSA in the intervention groups were not statistically significant compared to the placebo group.
Lazarevic *et al.* (125) 2012	The effects of short-term genistein intervention on prostate biomarker expression in patients with localised prostate cancer before radical prostatectomy	Prostate cancer patients (n = 47) were randomized to 30 mg/d genistein or placebo capsules for 3-6 w prior to prostatectomy. Androgen-related biomarkers were analyzed in luminal cells from isolated malignant and benign glands. Androgen-, proliferative- (Ki-67 nuclear antigen), cell cycle-, and apoptotic biomarkers were analyzed in normal and prostate tissue.	There were no effects of the intervention on proliferative-, apoptotic, or cell cycle-biomarkers. However, the intervention reduced the mRNA level of KLK4 in tumor cells.
Lazarevic *et al.* (126) 2011	Efficacy and safety of short-term genistein intervention in patients with localized prostate cancer prior to radical prostatectomy: a randomized, placebo-controlled, double-blind Phase 2 clinical trial	Patients with localized prostate cancer were randomized to 30 mg/d synthetic genistein (n = 23) or placebo (n = 24) for 3–6 w before prostatectomy. Concentrations of biomarkers in serum and tissue were investigated.	The concentration of PSA decreased by 7.8% in the intervention group and increased by 4.4% in the placebo group. In the intervention group, concentrations of PSA were comparable in normal and tumor tissue. In the placebo group, concentrations of PSA were reduced in tumor tissue compared to normal tissue. No significant effects were found on sex hormones.

deVere White *et al.* (127) 2010	Effects of a High Dose, Aglycone-Rich Soy Extract on Prostate-Specific Antigen and Serum Isoflavone Concentrations in Men With Localized Prostate Cancer	Patients with prostate cancer (n = 53) in an active surveillance program were randomized to a daily isoflavone supplement (450 mg genistein, 300 mg daidzein, and other isoflavones) or placebo for 12 months. Concentrations of PSA, genistein, daidzein, and equol were investigated.	There was no change in PSA concentrations in any of the groups after 6 or 12 months. The concentrations of genistein and daidzein at 6 months (39.85 and 45.59 μmol/l) were significantly higher than at baseline and considerably higher compared to reported concentrations in other studies. Concentrations of equol did not change.
Landberg *et al.* (134) 2010	Rye Whole Grain and Bran Intake Compared with Refined Wheat Decreases Urinary C-Peptide, Plasma Insulin, and Prostate Specific Antigen in Men with Prostate Cancer	In a randomized controlled, crossover trial, patients with prostate cancer (n = 17) were provided with 485 g rye whole grain and bran products or refined wheat products. Concentrations of e.g., PSA were analyzed.	The concentrations of plasma total PSA were lower after the rye products compared with the wheat products. The mean treatment effect was 214% (P = 0.04).
Kumar *et al.* (128) 2010	Results of a randomized phase I dose-finding trial of several doses of isoflavones in men with localized prostate cancer: administration prior to radical prostatectomy	Patients with prostate cancer (n = 45) were randomized to 40, 60, and 80 mg of purified isoflavones or no supplement prior to prostatectomy (30 ±3 d). Compliance with the supplement, toxicity, and changes in concentrations of plasma isoflavones, serum steroid hormones and PSA, and tissue Ki-67 were investigated.	Concentrations of plasma isoflavones significantly increased in all doses compared with controls. For the doses of 40 and 60 mg isoflavones, concentrations of serum total estradiol were significantly increased. For the dose of 60 mg isoflavones, an increased concentration of serum-free testosterone was also observed. The changes in concentrations of SHBG and PSA, and the percentage of tissue Ki-67, were not statistically significant.
Demark-Wahnefried *et al.* (133) 2008	Flaxseed supplementation (not dietary fat restriction) reduces prostate cancer proliferation rates in men presurgery	Patients with prostate cancer (n = 161) with ≥21 days before scheduled prostatectomy were randomized to 1. control/usual diet, 2. flaxseed-supplemented diet (30 g/d), 3. low-fat diet, or 4. flaxseed-supplemented, low-fat diet. Concentrations of e.g., PSA, SHBG, IGF-1, and testosterone were investigated in blood, and e.g., Ki-67 was analyzed in tumors.	The flaxseed interventions had lower proliferation rates than the other diets. The median Ki-67-positive cells/total nuclei ratios were 1.66 (flaxseed-supplemented diet), 1.50 (flaxseed-supplemented, low-fat diet), 3.23 (control diet), and 2.56 (low-fat diet). No statistically significant differences were observed for measured hormones between the groups.

Kumar *et al.* (129) 2007	A Phase II randomized, placebo-controlled clinical trial of purified isoflavones in modulating steroid hormones in men diagnosed with localized prostate cancer	Patients with early-stage prostate cancer (Gleason score ≤6) (n = 53) were randomized to 80 mg purified isoflavones or placebo for 12 w. Changes in e.g., concentrations of plasma isoflavones and serum steroid hormones were analyzed.	Plasma concentrations of isoflavones increased without any clinical toxicity. The difference between the groups was nonsignificant for changes in concentrations of serum SHBG, total estradiol, and testosterone.
Dalais *et al.* (132) 2004	Effects of a diet rich in phytoestrogens on prostate-specific antigen and sex hormones in men diagnosed with prostate cancer	Patients with prostate cancer (n = 29) scheduled for radical prostatectomy were randomized to a study bread containing 50 g of heat-treated soy grits (high phytoestrogen), 50 g of heat-treated soy grits and 20 g of linseed (high phytoestrogen), or wheat (low phytoestrogen). Concentrations of e.g., total and free PSA, testosterone, SHBG, and dihydrotestosterone were investigated.	Statistically significant differences were found between the soy group and the wheat group for the percentage of change in concentrations of total PSA and free/total PSA ratio, and between the soy group and the soy/linseed group for the percentage of change in free/total PSA ratio.
Kumar *et al.* (130) 2004	The Specific Role of Isoflavones in Reducing Prostate Cancer Risk	Patients with early-stage prostate cancer (Gleason score ≤6; age 50–80) (n = 76) were randomized to 60 mg of soy isoflavones or a placebo for 12 w. Changes in concentrations of serum PSA and steroid hormones were investigated.	The concentration of free testosterone was reduced or unchanged in 61% of participants in the intervention group compared to 33% in the placebo group. The concentration of total PSA was reduced or unchanged in 69% of the participants in the intervention group compared to 55% in the placebo group. No increase was observed in the concentration of SHBG.
Bylund *et al.* (131) 2003	Randomised controlled short-term intervention pilot study on rye bran bread in prostate cancer	Patients with prostate cancer were randomized to 295 g of rye bran bread (n = 10) or 275 g of wheat bread (control, n = 10) with similar fiber content for 3 w. Concentrations of e.g., plasma lignans, PSA, sex hormones, IGF-1, and apoptotic index were analyzed.	In the intervention group, the concentration of plasma enterolactone increased significantly. The apoptotic index increased significantly from 2.1% to 5.9% in 4 participants in the intervention group and 7 participants in the control group. Only small changes were found in concentrations of plasma PSA, sex hormones, excreted estrogens, and IGF-1.

Abbreviations: IGF-1, insulin-like growth factor-I; PSA, prostate-specific antigen; SHBG, sex hormone-binding globulin.

2.4 DIETARY ASSESSMENT

Measuring dietary intake is challenging. A person's daily diet varies regarding the type of foods and amounts eaten. Measurements of dietary intake are often only feasible over a limited period of time (135). Underreporting of dietary intake is common, especially in some subgroups, for example, women and individuals with overweight or obesity (136). When new methods to measure dietary intake are developed it is important to validate their capacity for validity and precision. However, this also entails a requirement that the reference method chosen to validate the new method is accurate.

2.4.1 DIETARY ASSESSMENT METHODS

2.4.1.1 DIETARY RECORDS

In dietary records, all foods consumed are recorded over a specified period and the amounts can be estimated or weighed (135). Weighed dietary records provide more precise measurements than estimated but could, due to their high burden on the participant, affect habitual dietary intake. A weighed record is therefore often only collected for 1–4 days, while an estimated record can be collected for up to 7 days. Dietary records, especially weighed, are time-consuming and place high demands on the respondent (135).

2.4.1.2 24-H DIETARY RECALLS

In 24-h dietary recalls, the respondent is interviewed about their dietary intake over the previous 24 hours (135). The recall may be over a longer period than 24 hours but this can make it more difficult for the responder to remember their dietary intake. The interview is often performed in several steps to help the respondent remember all the foods they consumed. Photographs or pictures of portion sizes can help the respondent to quantify the amounts. The advantages of the 24-h dietary recall include the collection of detailed information about dietary intake, they require less effort from the respondent, and they can be carried out by telephone, computer, or face-to-face. Disadvantages include the reliance on memory, which makes it inappropriate for some people, and that they must be repeated on multiple occasions to capture habitual dietary intake (135).

2.4.1.3 FOOD FREQUENCY QUESTIONNAIRE (FFQ)

Food Frequency Questionnaires (FFQs) are often structured as a list of foods with response options indicating how often each food is consumed (e.g., daily, 3–4 times/w, 1–2 times/m, <1 times/m, never) (135). The number of foods included in the FFQ depends on the type of dietary intake that the investigator wants to capture. FFQs are primarily self-completed by the respondent which makes it possible to collect long-term dietary intake in a cost-effective and standardized way in a large number of respondents. Portion sizes can be added to the FFQ to improve precision. Disadvantages of the FFQ include lack of detailed information about the foods and the complexity of estimating dietary intake over time, which introduce large random errors (135).

2.4.1.4 BIOMARKERS

Biomarkers can be used as a marker of specific foods, nutrient intake, or status and can be measured in, for example, samples of urine, blood, and feces (137). The advantages of using biomarkers are that they do not have the same errors as traditional dietary assessment methods since they are objective and do not rely on the respondents' ability to estimate dietary intake or dietary cultural norms. In addition, biomarkers are not dependent on nutrient data from food composition databases, which are based on chemical analyses or estimations of other adequate data (138). However, cooking methods influence nutrient content and absorption, and there are interactions when combinations of foods are eaten (139, 140). Individual variations in physiology and nutrient metabolism, variations in different tissues, and at what time point the biomarker is measured also affect nutritional status (137). Depending on the purpose, these factors could constitute both advantages and disadvantages of biomarkers. Another disadvantage is that some biomarkers can be affected during storage, which can lead to laboratory analytical errors.

2.4.1.5 DIGITAL APPLICATIONS

In 2017, 85% of the Swedish population had a smartphone and 69% had access to a tablet in their household (141). This brings opportunities for the use of technology to measure dietary intake. The advantages of using digital applications in measuring dietary intake are that additional automatic functions can be added for potentially forgotten items or items incorrectly reported, the researcher can follow the respondent's registration in real time, and data can be easily exported for analysis (142). The quality of data depends on the underlying food composition database, search functions, estimations of portion sizes, and the user's technological skills. The use of digital applications may be more challenging in older age groups of which a smaller proportion have access to a smartphone and because older individuals use the internet less, both overall and on smartphones, compared to younger people (141).

3 AIM

3.1 GENERAL AIM

The overall aim of the thesis was to develop and evaluate the feasibility and effects of dietary fiber and phytoestrogen interventions in patients with pelvic-organ cancer for the alleviation of side effects during radiotherapy and for secondary prevention, respectively. This was addressed by testing the feasibility of a dietary fiber intervention before conducting a large randomized controlled trial in patients with pelvic organ cancer. Furthermore, the effects of a diet high in phytoestrogens were investigated in a large randomized controlled trial in patients with prostate cancer. Finally, a new digital dietary assessment method was developed and evaluated for convenient dietary fiber intake assessment among patients with cancer enrolled in dietary interventions. The thesis is based on the intervention trials FIDURA (dietary Fiber Intake DUring pelvic RAdiotherapy and PRODICA (impact of DIet and individual genetic factors on tumor proliferation rate in men with PROstate CAncer).

3.2 SPECIFIC AIMS

In patients with gynecological cancer, the specific aims were to:

- Improve logistics and investigate participation rate and compliance, as well as their determinants of a dietary fiber intervention during radiotherapy.

In patients diagnosed with low and intermediate-risk prostate cancer scheduled for radical prostatectomy, the specific aims were to:

- Design and evaluate the effect of a dietary phytoestrogen intervention and if there is a synergetic effect with a specific genotype of ERβ on tumor proliferation and concentrations of PSA, and hormones related to prostate cancer.
- Investigate the influence of a phytoestrogen intervention on plasma concentrations of phytoestrogens.
- Assess the agreements between reported intakes of phytoestrogens and blood concentrations of phytoestrogens.

In a randomly selected population of women, the specific aims were to:

- Validate the ability of a digital application to measure dietary fiber intake compared to a dietary record.
- Investigate food group sources of total, insoluble, and soluble dietary fiber intake.

4 PARTICIPANTS AND METHODS

The papers included in this thesis are based on three different data collections and are summarized in Table 8. An overview of the different study designs is shown in Figures 6–8 and the periods of the three data collections are found in Figure 9.

Table 8. Overview of the study designs of the papers included in the thesis.

Paper	Design	Participants	Measurements of exposure	Outcomes	Statistical analyses
I	Observational feasibility trial	57 women with gynecological cancer scheduled for radiotherapy	FFQ Digital application Blood samples Fecal samples	Reasons for non-participation and dropout Participation rates Fiber intake	Mann-Whitney U test Kruskal-Wallis test Post-hoc test Linear mixed-effects model
II	Protocol	25 men with prostate cancer, cT1-cT2	FFQ 24-h dietary recall	Compliance	-
III	Randomized controlled trial	140 men with prostate cancer, cT1-cT2	FFQ 24-h dietary recall Reported remaining intervention foods	PSA concentrations (blood samples) Ki-67 index (tumor material)	Shapiro-Wilk test Mann-Whitney U test Generalized linear model
IV	Randomized controlled trial	135 men with prostate cancer, cT1-cT2	Blood samples	Hormone concentrations	Shapiro-Wilk test. Mann-Whitney U test / Independent samples T-test Linear regression Generalized linear model
V	Randomized cross-over trial	26 randomly selected women, 35–85 y	Digital application 3-d dietary record	Difference between methods Correlation between methods Fiber sources	Subsequent data-driven analysis Shapiro-Wilk test Mann-Whitney U test Bland-Altman plot Pearson correlation Linear regression analyses

Abbreviation: FFQ, Food Frequency Questionnaire; PSA, prostate-specific antigen.

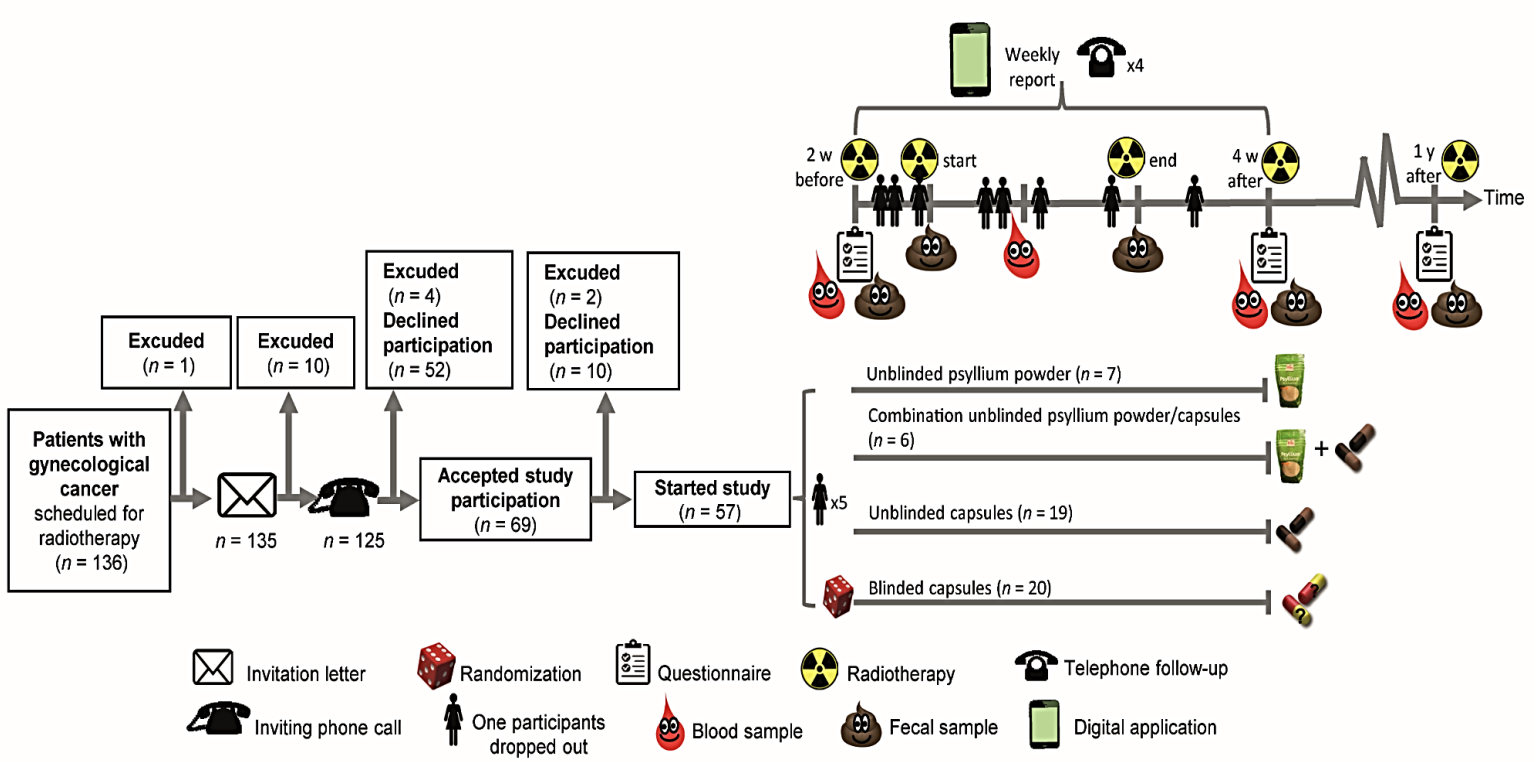

Figure 6. The study design and number of participants included in the Feasibility study (Paper I).

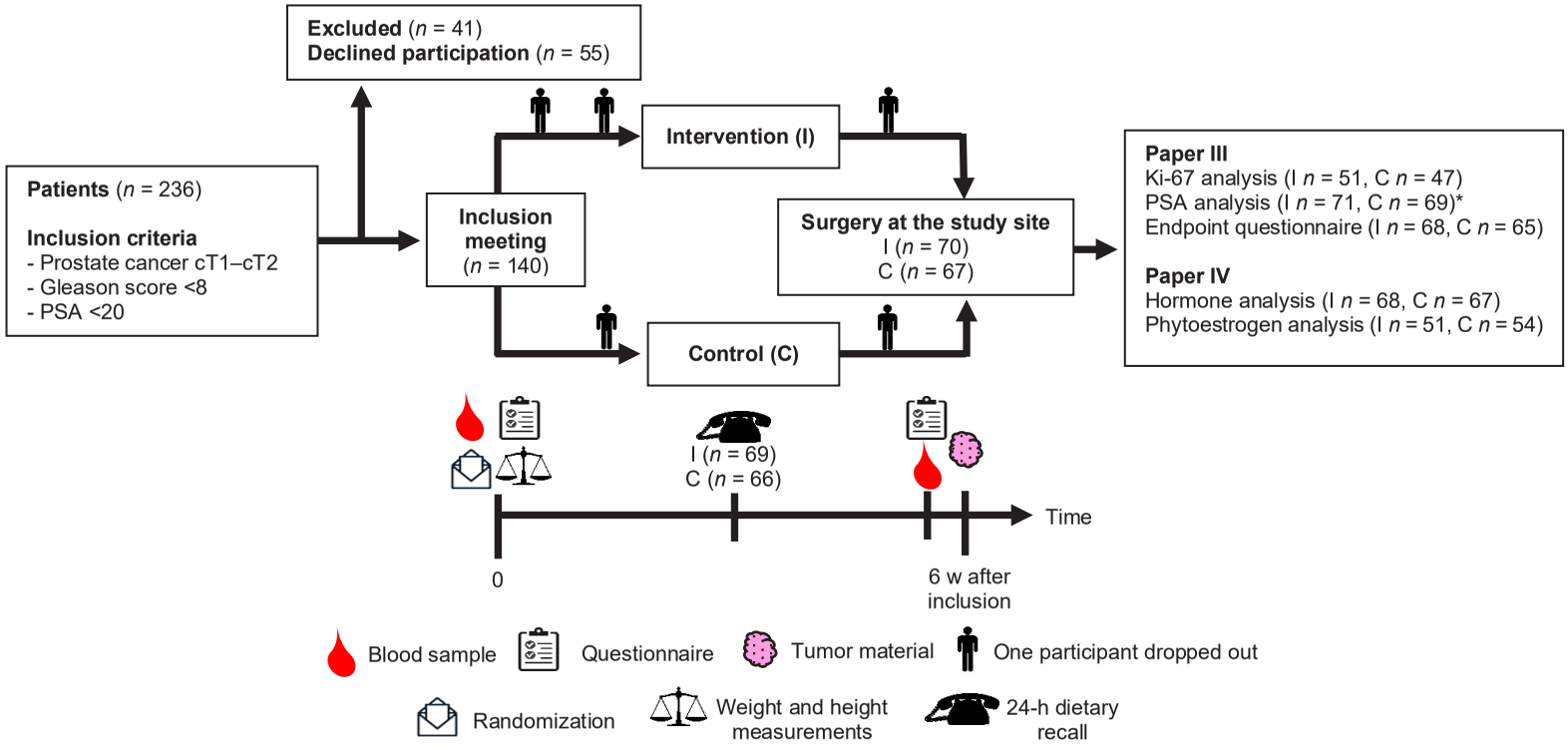

*Figure 7. The study design and number of participants included in the PRODICA study (Papers II–IV). * The prostate-specific antigen (PSA) concentrations from three participants in the intervention group and two participants in the control group were collected from the National Prostate Cancer Register of Sweden.*

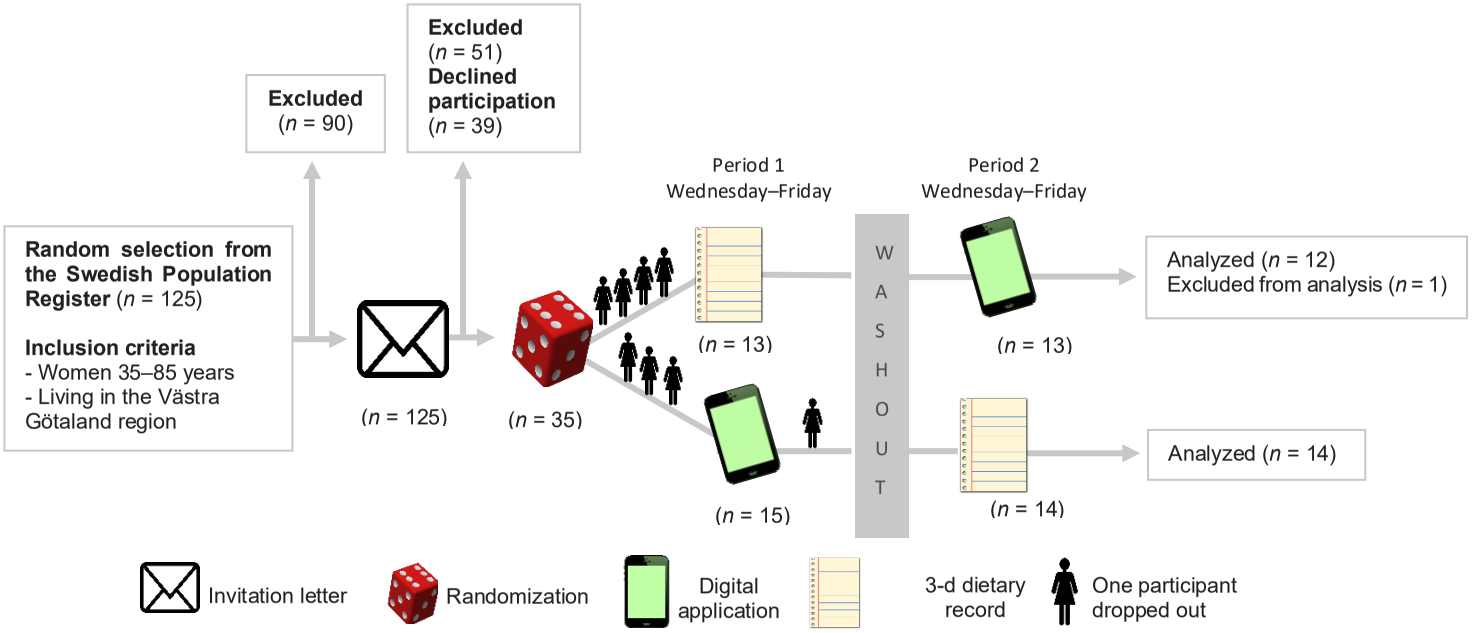

Figure 8. The study design and the number of participants included in the Validation study (Paper V).

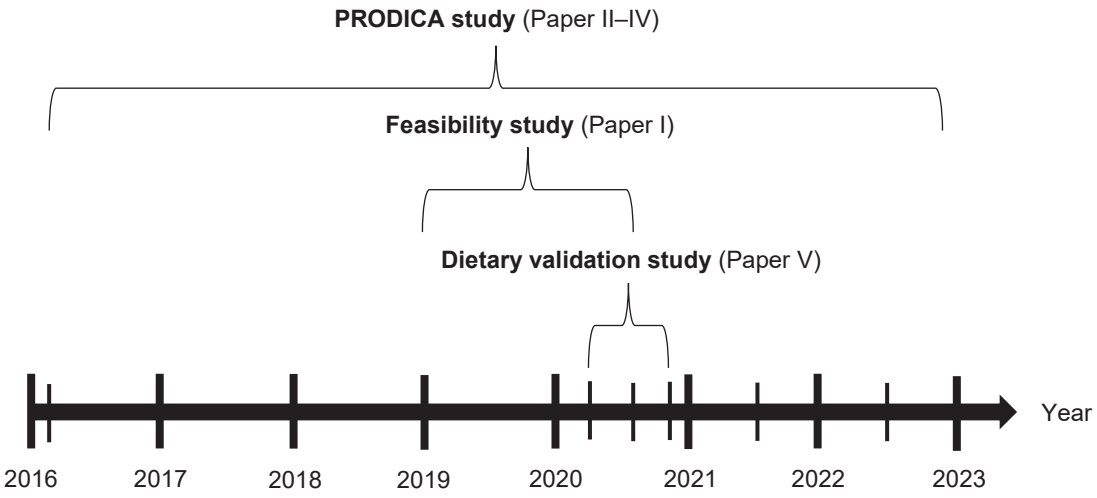

Figure 9. Overview of the time periods for data collection in the studies.

4.1 STUDY PARTICIPANTS

Details of the participants in the different studies are summarized in Table 8. The Feasibility study (Paper I) included 57 patients with gynecological cancer scheduled for curative radiotherapy at Sahlgrenska University Hospital in Gothenburg, Sweden. Patients with an intestinal stoma or reservoir, and those who had difficulty reading or understanding Swedish, were not included. Participants in the PRODICA study (Papers II–IV) were patients diagnosed with low to intermediate-risk prostate cancer and scheduled for radical prostatectomy at Sahlgrenska University Hospital in Gothenburg, Sweden. Patients with ongoing hormone therapy, psychiatric or mental disorders, or cognitive dysfunction, and those who were allergic to the intervention diet, were not included in the study. Paper III included 140 patients and Paper IV 135 patients. The Dietary validation study (Paper V) included 26 randomly selected women aged 35–85 years who were living in the Västra Götaland Region in Sweden. They understood written Swedish, had access to a mobile phone or tablet, and were able to download digital applications.

4.2 STUDY INTERVENTIONS AND RANDOMIZATION

4.2.1 THE FEASIBILITY STUDY

The interventions in the Feasibility study (Paper I) were the use of unblinded psyllium husk powder and capsules with a known content of psyllium husk, and blinded capsules with an unknown content of psyllium husk or placebo (Figure 6). Only the participants with blinded capsules were randomized (allocation 1:1) and this was automatized by a digital study-specific database. The participants also received dietary advice and were instructed to daily eat ≥16 g of fiber from their regular diet.

Pectin was initially intended as the fiber intervention due to its promising results in preclinical studies as a radioprotective agent (67-69). A pectin powder was tested but the solubility in foods such as porridge and yogurt was poor. Psyllium husk was discovered to have better solubility in these foods and had been used with promising results in an intervention study with patients during pelvic-organ radiotherapy (86). When the decision was subsequently made to use psyllium capsules instead of powder, there was no time to test pectin capsules as the study was due to start.

4.2.2 THE PRODICA STUDY

The intervention in the PRODICA study (Papers II–IV) was a daily addition of phytoestrogen-rich foods, which were provided to the participants. Controls were not provided with any food items, but all participants received a brochure with general healthy dietary recommendations. Participants were randomized to the groups by drawing a folded note from an envelope containing 26 notes (allocation 1:1). The intervention foods consisted of frozen green soybeans (47 g), flaxseeds (28 g), and roasted yellow soybeans (28 g), which corresponded to approximately 200 mg of phytoestrogens per day (Figure 10).

The chosen intervention foods were excellent sources of phytoestrogens, both isoflavones and lignans, and would hopefully, for different reasons, result in

good compliance. First, unlike other beans, soybeans have a less floury texture and the variants chosen needed no soaking or boiling. The flaxseeds also needed no preparation compared with other phytoestrogens sources, such as rye flour. Second, choosing frozen green and roasted yellow soybeans increased the variance, and the servings of both flaxseeds and soybeans could be varied with different recipes to satisfy different food preferences. Third, using prepared sources of rye, such as bread, would also exclude people with celiac disease or those following a trend of decreasing their intake of major carbohydrate sources.

Figure 10. Food items provided to participants in the intervention group in the PRODICA study (Papers II–IV). From the left: frozen green soybeans, flaxseeds, and roasted yellow soybeans.

4.2.3 THE DIETARY VALIDATION STUDY

The women in the Dietary validation study (Paper V) were randomized to start registering their dietary intake in either a digital application or a 3-day dietary record for three consecutive days. The women were randomized by the study personnel using a closed envelope with four folded notes (allocation 2:2). After the first registration, a washout period of ≥2 weeks was scheduled and the participants then registered their intake using the other method.

Block randomization was chosen to keep the groups comparable in size. The study personnel had to pick the note for randomization to enable the study to be performed remotely. Randomization to start with either the digital application or the dietary record, and the washout period, prevented the methods from systematically affecting the registration of the other.

4.3 ASSESSMENT OF EXPOSURES AND OUTCOMES

4.3.1 QUESTIONNAIRES

The questionnaires used in the PRODICA study (Papers II–IV) were study-specific and included a FFQ covering 12 food categories (Table 9). The same FFQ was used when the questionnaires for the FIDURA study were designed (Paper I). In this study, more food items and supplements containing dietary fiber were added to the FFQ. Questions covering gastrointestinal symptoms and potential confounders were also included, and the questions were adapted to suit all patients receiving pelvic-organ radiotherapy rather than just patients with prostate cancer. Examples of questions in the questionnaires used in the FIDURA study (Paper I) are shown in Appendix I and in the PRODICA study (Papers II–IV) in Appendix II. The FFQs used in the studies were chosen to capture participants' habitual dietary intake.

Table 9. Food categories in the food frequency questionnaires in the FIDURA study (Paper I) and the PRODICA study (Papers II–IV).

Food categories
Sandwiches
Porridge[1], breakfast cereals, and muesli
Nuts and seeds
Drinks
Vegetables and fruit
Meat and meat products
Fish and shellfish
Egg dishes and vegetarian alternatives
Potato, pasta, rice, and grains
Fast food
Sauce and dressing
Sweets, snacks, and dessert cheese

[1] In Sweden porridge can traditionally be made using various types of cereals, often either oats, wheat, or rice (rice pudding).

Most of the questions in the FFQ were designed according to the format below:

8. How often do you eat porridge? Fill in only one alternative (day, week, month). If you never eat porridge tick in the box "never".

Some kind of porridge	____ times	☐ per day	☐ per week	☐ per month	☐ never

9. Imagine eating porridge <u>ten times</u> in a row. How many times is the porridge usually:

___ Oatmeal

___ Porridge made of wheat or rice

___ Porridge made of rye

Please note! The total sum should always be 10, enter only integers

4.3.2 24-H DIETARY RECALL

The 24-h dietary recall was used to measure compliance in the PRODICA study (Papers II–III). It was performed over the telephone by a dietitian who followed a standard template. The participants' intake of foods over the previous 24 hours was documented. A list was then used to check for potentially forgotten food items. The timing of meals and other details were also recorded. Finally, the list of food items was read out to the participant to check if the record of their food intake was complete.

The 24-h dietary recall aimed to capture a more detailed dietary intake, especially the intake of phytoestrogens, compared to the FFQ. In contrast to the FFQ, the 24-h dietary recall is a better tool to measure portion sizes. Compliance could therefore be measured more accurately in both groups. However, a limitation is that only one recall was performed, which is not enough to capture habitual dietary intake.

4.3.3 THE DIGITAL APPLICATION

We built a database of common Swedish food items high in dietary fiber. Food groups that did not contain fiber or contained only a small amount were not included (e.g., animal protein sources, drinks, sweets). The database was connected to the digital application developed for the FIDURA study (Paper I). The application was validated in Paper V.

The digital application included a main menu of the different options "daily dietary intake", "daily stools", "weekly events" and "my intake" (Figure 11A). "Daily dietary intake" included the different food categories "vegetables/legumes/roots/potatoes", "fruits/berries", "bread/crackers", "plant-based substitutes" (Quorn and soy protein products), "pasta/grains/porridge/cereals/flour/muesli", "nuts/seeds", "snacks", "recipes", and "saved meals" (Figure 11B). The food items were registered in specified quantities to one decimal place, i.e., slices, numbers, deciliters, or tablespoons (Figure 11C). "My intake" showed all registered dietary items on the specific date, the items could be edited or deleted, or new items could be added on the selected date (Figure 11F). In "daily stools" the consistency of all stools was registered according to the Bristol Stool Form Scale (Figure 11D). "Weekly events" include questions about intake of gas- or bowel regulators (e.g., anti-motility, laxatives), abdominal bloating or pain, flatulence, and intake of the study capsules (Figure 11E).

The creation of a digital application was to be able to follow patients' choice of fiber sources during the entire radiotherapy period. The patients were already enduring a challenging time due to radiotherapy treatment, which often takes 5–6 weeks, so it was important that registration of dietary intake should be as easy as possible. It is well known that performing a dietary record implies a substantial commitment and, since food items contributing to fiber intake were the primary interest, food items with a small amount or with no dietary fiber content were therefore excluded. A disadvantage of not registering the entire dietary intake is that it was not possible to adjust for energy intake.

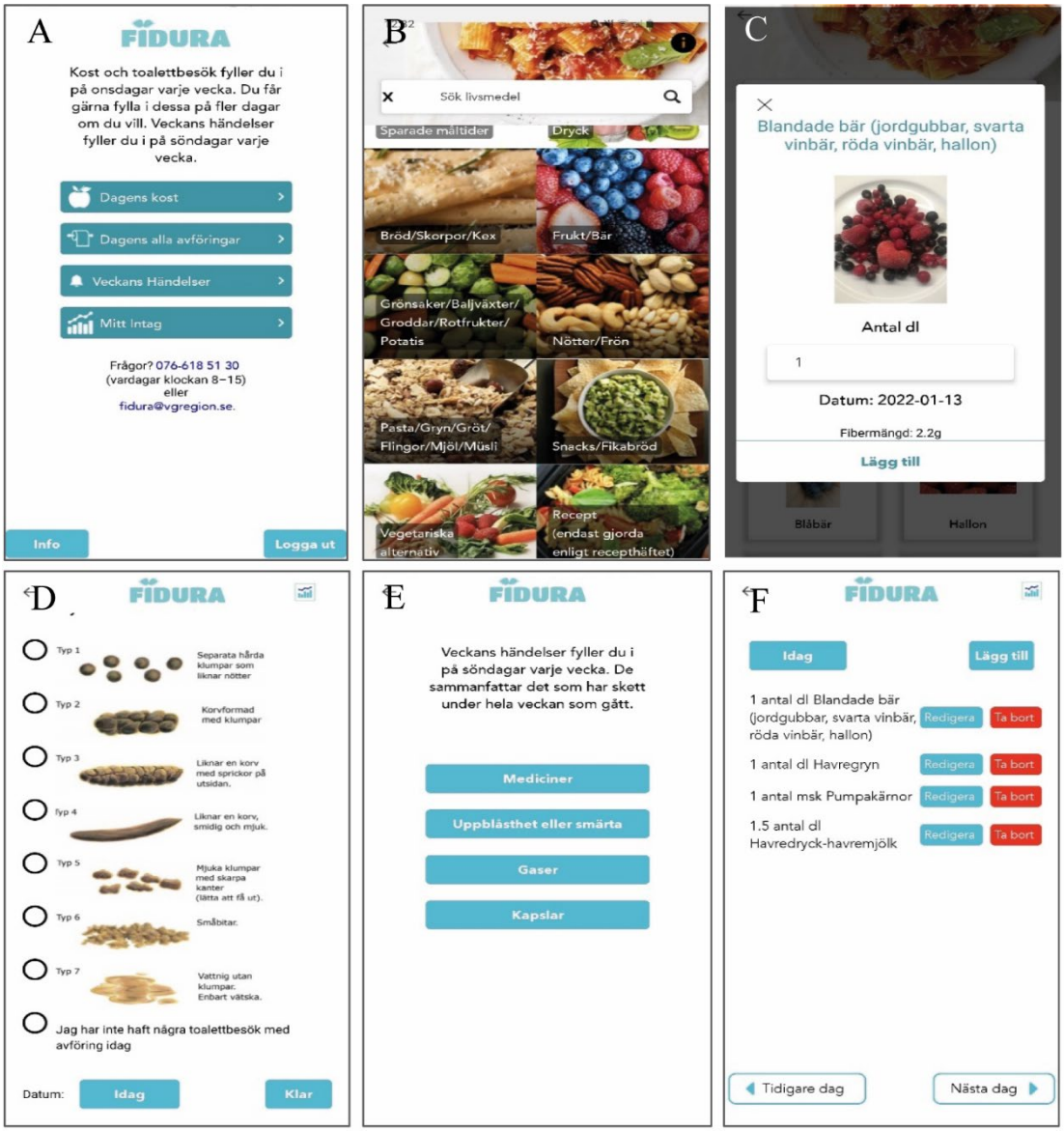

Figure 11. The different layouts of the digital application. (A) The main menu consisted of "daily dietary intake", "daily stools", "weekly events" and "my intake". (B) Foods could be searched or selected under different food categories in "daily dietary intake". (C) Selected foods were registered in specific quantities e.g., dl. (D) In "daily stools", stool consistency was registered according to the Bristol Stool Form Scale. (E) "Weekly events" included questions about bowel-related medicines, abdominal bloating/pain, flatulence, and the study capsules. (F) The registered dietary items were shown under "my intake".

4.3.4 DIETARY RECORD

A 3-day dietary record was used as the reference method in the Dietary validation study (Paper V). Participants were instructed to write down all consumed foods and drinks. They were also instructed to note the quantities of the consumed items using, if possible, a household scale, or otherwise household measures or a portion guide that they were provided with. Other details, such as brand, fat percentage, and cooking methods, were also to be recorded.

The weighed dietary record is currently the best way to capture dietary intake in detail. However, the effort required to register a dietary record may influence the participant's choice of foods and therefore not capture their habitual dietary intake (141). The registration was eased by the use of household measures and the portion guide if participants had no household scale available or ate out at a restaurant or other venue. The choice of a 3-day registration was made to avoid study dropouts and tiring the participants during the registration.

4.3.5 BLOOD AND TISSUE SAMPLES

Blood samples were collected in the Feasibility study and the PRODICA study (Papers I–IV). All blood samples were collected by healthcare professionals, either in the clinic or at a sampling center. The samples were then handled according to clinical routines and frozen before being analyzed. In the PRODICA study (Paper III–IV) concentrations of free and total PSA and hormones (estradiol, testosterone, IGF-1, and SHBG) were analyzed in serum, different phytoestrogens (lariciresinol, secoisolariciresinol, enterodiol, enterolactone, daidzein, genistein, glycitein, and equol) in plasma, and the genotype of ERβ (TT or TC/CC) in whole blood.

In the PRODICA study (Paper III), after radical prostatectomy, the prostate tissue was embedded with formalin-fixed paraffin according to the pathological clinical routine. The prostate tissue was then pretreated before immunohistochemical staining. A pathologist evaluated Ki-67 by assessing a total of at least 500 cells in 5 different areas, which were randomly selected in the prostate slice containing the dominant tumor content of the primary tumor.

If the intended prostate slice was missing, a similar slice was evaluated instead. The Ki-67 indexes were calculated as follows:

$$\frac{the\ number\ of\ immunohistochemical\ positive\ prostate\ cancer\ nuclei}{the\ total\ number\ of\ evaluated\ tumor\ cells} \times 100$$

The individual median, mean, and maximum values of the five Ki-67 indexes were calculated and the median index was used as the primary index. The Ki-67 index was chosen as the primary outcome in the PRODICA study, and PSA and hormone concentrations were chosen as secondary outcomes.

Initially, Ki-67 was also intended to be evaluated in patients' diagnostic biopsies. Unfortunately, however, the number of diagnostic biopsies available to analyze was more limited than expected, which led to the Ki-67 only being evaluated at endpoint. At endpoint, Ki-67 was evaluated in prostatectomy specimens instead of biopsies to avoid missing data from participants who dropped out of the study or if biopsy collection was missed at surgery. The evaluation was then not dependent on where the biopsies were taken from, for example avoiding only including benign prostate tissue.

4.4 STATISTICAL ANALYSES

In all the studies, there were different null hypotheses (no difference between the two groups), which were accepted or rejected with different statistical methods. In the Feasibility study (Paper I), the null hypotheses were that there is no difference in the lengths of the intake periods between the different psyllium or placebo interventions, no change in dietary fiber intake before and after radiotherapy, and no difference in age between those who chose to use the web or paper version of the questionnaires. In the PRODICA study (Papers III–IV), the null hypothesis was that there was no difference in the Ki-67 index or concentrations of PSA and hormones between the intervention and the control groups. In the Dietary validation study (Paper V), the null hypothesis was that there was no difference in fiber intake when registering dietary intake in the digital application and the 3-day dietary record.

To test the differences between the two groups, an independent samples T-test was used if the data were normally distributed and the Mann-Whitney U test if the data were non-normally distributed. The Shapiro-Wilk test was primarily used for testing for normality. The Shapiro-Wilk test is used to test the probability that the data belongs to a normally distributed population and *P* values below 0.05 are considered non-normally distributed (142).

4.4.1 THE FEASIBILITY STUDY

The participants were divided into five groups depending on the time point when they had been included in the Feasibility study, to investigate if the participation rates for the third blood sample and the first questionnaire had changed during the study (Paper I). The Kruskal-Wallis test and a post hoc test were used to examine if the length of the intake periods differed between the different interventions of psyllium or placebo. For the analysis of total fiber intake registered using the digital application, a linear mixed-effect model was used, and sensitivity analyses were performed where outliers and participants with ≤2 days of registration were excluded.

4.4.2 THE PRODICA STUDY

Most analyses were stratified according to the genotype of ERβ. In Paper III, a generalized linear model was used to calculate risk differences. This was used to explore the risk of having a Ki-67 index over the median value of the study population and increasing concentrations of PSA during the intervention, comparing the intervention and control groups (adjusted for age, BMI, tumor stage, the most recent biopsy, and intake of polyunsaturated fatty acids). A sensitivity analysis of the risk differences was performed, stratified by intakes of antibiotics. Median values of reported dietary intakes, the Ki-67 index, and concentrations of PSA were also compared between the intervention and the control groups. For outcomes of Ki-67 and PSA, a per-protocol analysis was performed with compliant participants, calculated from the report of the remaining intervention foods (intervention group) and the 24-h dietary recall (control group).

In Paper IV, median values of hormone and phytoestrogen concentrations were compared between the intervention and the control groups. Risk differences were calculated to explore the risk of increasing different hormone concentrations during the intervention, comparing the intervention and the control groups (adjusted for BMI, age, and smoking). A linear regression was used to explore the relationship between blood concentrations of phytoestrogens and hormones (adjusted for BMI, age, and smoking). For the phytoestrogen concentrations, sensitivity analyses were performed with users and non-users of antibiotics during the past five years and different intervention lengths. The Kruskal-Wallis test was used to compare results between different intervention lengths. For lignan concentrations, a sensitivity analysis was performed with those receiving crushed and whole flaxseeds.

4.4.2.1 EFFECT MODIFICATION

The genotype of ERβ was tested as an additive effect-modifying factor in the PRODICA study (Papers II–IV). Effect modification or interaction, sometimes used as synonyms and other times as two different terms, can be confusing (143, 144). The terms are often used differently in epidemiology and statistics, where they are measured on additive and multiplicative effect scales, respectively. Hereafter, the focus is on the effect modification measured on an additive effect scale. The definition of an effect-modifying factor is that the effect of the exposure on an outcome varies depending on the presence of a third factor (144). Hence, some treatments could be more favorable in some subgroups. The hypothesis was that the genotype of ERβ would be an effect-modifying factor on the effects of high intake of phytoestrogens on prostate tumor proliferation. It was hypothesized that the phytoestrogen intervention would produce a favorable effect in patients with the TC/CC genotype of ERβ but not in the TT genotype.

4.4.2.2 AGREEMENT BETWEEN REPORTED INTAKE OF PHYTOESTROGENS AND BLOOD CONCENTRATIONS OF PHYTOESTROGENS

A linear regression and Kappa statistics were used to compare the reported intake of phytoestrogens with blood concentrations of phytoestrogens. Reported intakes of phytoestrogens were calculated in three ways: firstly, the intake of phytoestrogens, based on the report of the remaining intervention foods at the end of the intervention, divided by the number of days from the inclusion meeting to surgery (intervention group only); secondly, the intake of phytoestrogens reported at the 24-h dietary recall; and thirdly, the intake of phytoestrogens reported in the endpoint FFQ.

In the linear regression, estimated intakes of phytoestrogens were explanatory variables and plasma concentrations of phytoestrogens were outcomes. These variables were skew and were therefore logarithmized using the natural logarithm. The regression model was adjusted for intakes of energy (tertiles), saturated fat (tertiles), alcohol (tertiles), and antibiotics (users, non-users or not knowing their intake during the past five years). A separate analysis was carried out with participants with different intervention lengths (<28, 28–56, >56 days). Kappa statistics were used to compare the agreement of tertile classifications. Kappa values <0 were considered poor, 0.00–0.20 slight, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 substantial, and 0.81–1.00 almost perfect (143). A sensitivity analysis was performed with users and non-users of antibiotics over the past 5 years.

4.4.3 THE DIETARY VALIDATION STUDY

A Bland-Altman plot and Pearson correlation coefficient were used to compare the registered fiber intakes using the digital application and the dietary record (Paper V). Linear regression analyses were used to investigate if there was a trend between the measurement deviations and the mean measurements of the two methods or the participants' age, BMI, or weight. The calculated fiber intakes were also divided into low, moderate, and high intakes, and the two methods were compared. The most common food groups were calculated as a percentage of the total, insoluble, and soluble fiber intakes of the group. The sources of total fiber intake were compared between the two methods. Sensitivity analyses were carried out without potential under-reporters and outliers.

5 RESULTS

5.1 DEMOGRAPHICS

The median age of the patients with gynecological cancer who were included in the Feasibility study (Paper I) was 69 years. The sites of the participants' gynecological cancers are shown in Figure 12. The number of radiotherapy fractions they received ranged from 11 to 35 (median 28) and the external radiation dose ranged from 33 to 77 Gy (median 56Gy). Most patients had received chemotherapy before their radiotherapy (Figure 13).

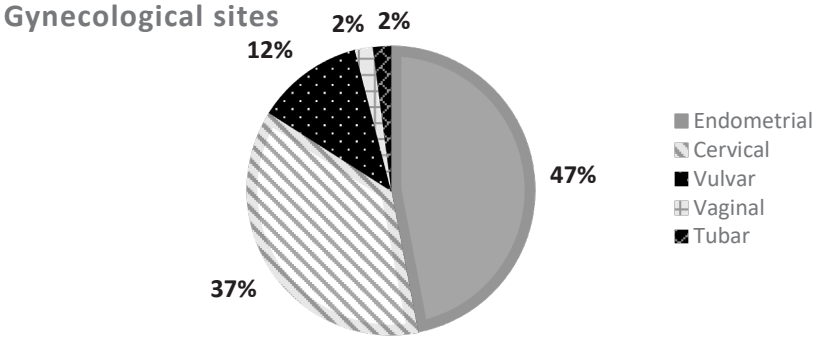

Figure 12. Distribution of gynecological sites of the gynecological cancers in the 57 patients included in the Feasibility study (Paper I).

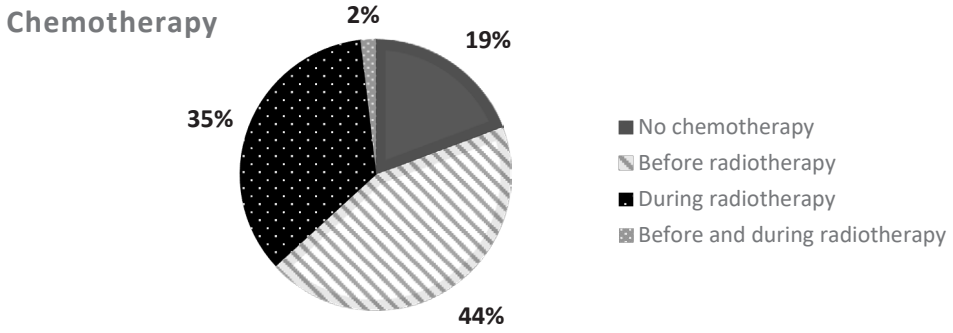

Figure 13. Timepoint of chemotherapy in the 57 patients included in the Feasibility study (Paper I).

The patients with prostate cancer included in the PRODICA study had a median age of 66 years (Papers III–IV). The participants' tumor stage and ISUP grade are shown in Figure 14 and Figure 15, respectively. Most of the men had a moderate physical activity level, an education to upper secondary school level, were married or had a partner, and were retired (Table 10). The randomly selected women included in the Dietary validation study (Paper V) had a median age of 57.5 years. Their median BMI corresponded to a normal weight (24.1 kg/m^2) and the median energy intake was 1871 kcal.

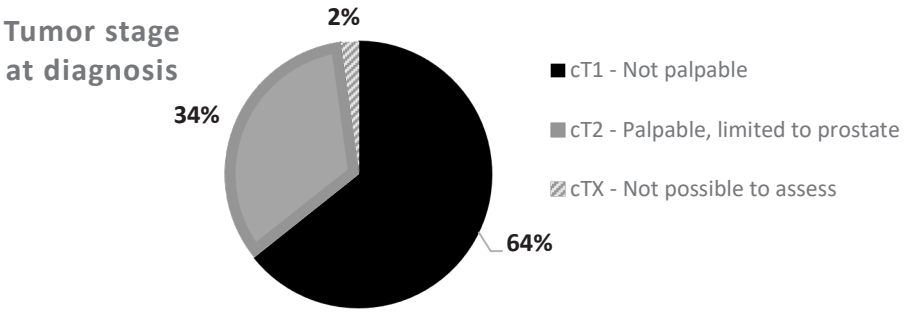

Figure 14. Distribution of tumor stage at diagnosis of the 140 patients included in the PRODICA study (Papers III–IV).

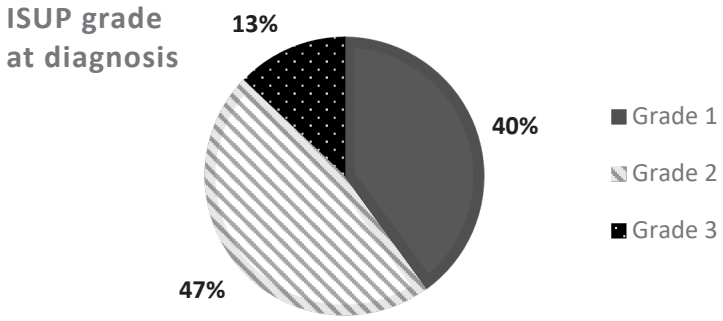

Figure 15. Distribution of International Society of Urological Pathology (ISUP) grade at diagnosis of the 140 patients included in the PRODICA study (Papers III–IV).

Table 10. Characteristics of the patients included in the PRODICA study (Papers III–IV).

	Intervention (*n* = 71)		**Control (*n* = 69)**	
	Genotype TT[1] (*n* = 37)	Genotype TC/CC[1] (*n* = 34)	Genotype TT[1] (*n* = 28)	Genotype TC/CC[1] (*n* = 41)
	n (%)	*n* (%)	*n* (%)	*n* (%)
Education[2]				
Primary school	2 (5)	6 (18)	5 (18)	5 (12)
Upper secondary school	21 (57)	15 (44)	15 (54)	19 (46)
University degree or equivalent	14 (38)	13 (38)	8 (29)	17 (41)
Civil state				
Married/partner	31 (84)	28 (82)	23 (82)	33 (80)
Unmarried/widower/ divorced/living apart	6 (16)	6 (18)	5 (18)	8 (20)
Employment				
Full-time work	15 (41)	8 (24)	12 (43)	21 (51)
Full-time pensioner/disability pensioner	16 (43)	21 (62)	13 (46)	17 (41)
Part-time work/part-time pensioner	5 (14)	4 (12)	3 (11)	3 (7)
Unemployed	0 (0)	1 (3)	0 (0)	0 (0)
Other	1 (3)	0 (0)	0 (0)	0 (0)

[1] Participants were allocated to the estrogen receptor beta genotype TT, TC, or CC.

[2] Elementary school and secondary school were included in "primary school". High school, vocational school, and other education were included in "upper secondary school". College and university were included in "university degree or equivalent".

The data were collected from the baseline questionnaire.

5.2 MAIN FINDINGS

5.2.1 FEASIBILITY OF THE FIBER INTERVENTION

It was essential to provide patients with concise and relevant information at the right moment in order for patients to agree to participate and to not leave the study (Paper I). It was important to customize the ambition level of individuals' participation in other parts of the study and instead prioritize the primary endpoints in order to keep the dropout rate acceptable. Expected burden of radiotherapy or the acute side effects of radiotherapy were the most common reasons for declining participation or dropping out of the study. The time points for dropout were in general spread over the radiotherapy period. If participants completed the follow-up four weeks after radiotherapy ended, they also completed the follow-up one year after radiotherapy ended (Figure 6). The participation rates in the four blood samplings were 84%, 75%, 63%, and 67% and in the five fecal samplings 51%, 49%, 44%, 42%, and 40% (Figure 16). The participation rates in the three questionnaires were 82%, 70%, and 67% (Figure 17). Younger patients used web versions of the questionnaires and the application to a higher extent, and older patients used the paper versions to a higher extent (Figure 18).

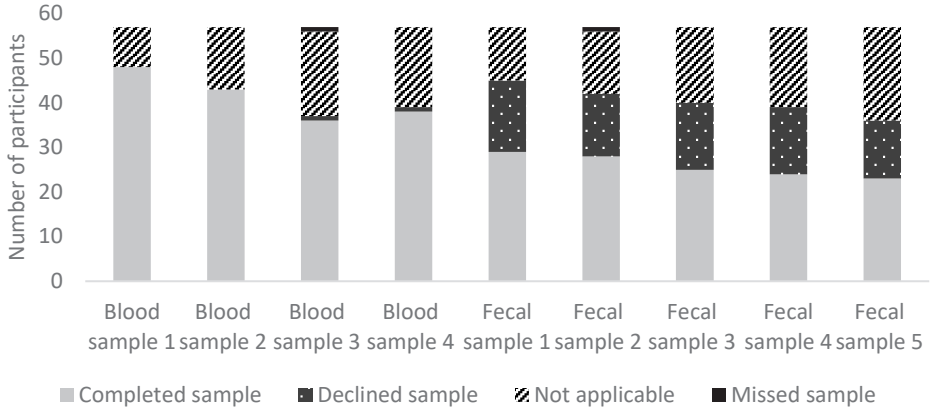

Figure 16. Participation in blood and fecal samplings for the 57 participants in the Feasibility study (Paper I). Not applicable was defined as missed sampling due to study dropout, deceased patient, late inclusion in the study, sampling procedures not completed, or canceled clinic visit. The figure has been updated after the paper was published.

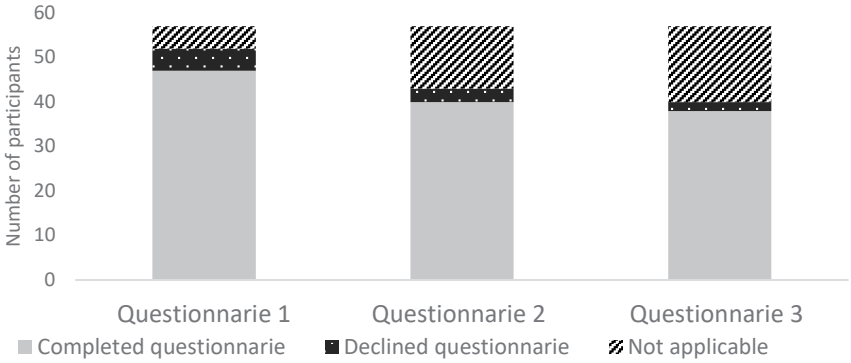

Figure 17. Participation in the questionnaires for the 57 participants in the Feasibility study (Paper I). Not applicable was defined as study dropout or deceased patient.

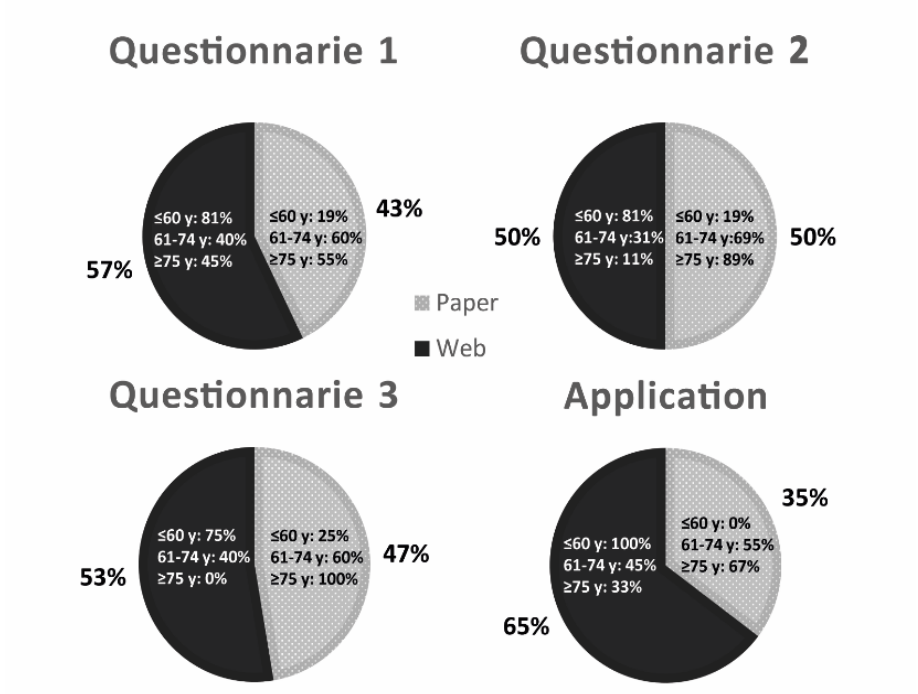

Figure 18. Formats used by participants in different age groups for the different questionnaires and the application in the Feasibility study (Paper I). There were statistically significant differences in the use of web and paper formats between the different age groups for all questionnaires and the application (P <0.05; Fisher's exact test). Questionnaire 1 (n = 42); five participants were not included because they were recruited before the web version was finished. Questionnaire 2 and questionnaire 3 (n = 57) and application (n = 34).

5.2.2 EFFECTS OF THE PHYTOESTROGEN INTERVENTION ON KI-67 AND CONCENTRATIONS OF PSA AND HORMONES

The study protocol was evaluated after ten participants had been included and the collaborations and arrangements were found to work practicably (Paper II). Compliance was tested in 25 participants and the intervention group was found to have increased their intake of phytoestrogens and the control group had not.

During the intervention period, the intervention group increased their intake of lignans, isoflavones, coumestrol, and total phytoestrogens compared to controls (Paper III). The intervention group also increased plasma concentrations of secoisolariciresinol, daidzein, genistein, glycitein, enterolactone, enterodiol, and equol during the intervention, compared to participants in the control group who maintained or decreased their concentrations (Paper IV).

Participants in the intervention group had a decreased risk of a higher Ki-67 index compared to controls, and the effects were more pronounced in participants with the TT genotype of ERβ (Figure 19, Paper III). There were also different effects on the risk of increasing the concentration of total PSA in the different groups of genotypes (Figure 20). When comparing the intervention group and the control group, participants with genotype TC/CC had a reduced risk of increasing total PSA concentration and participants with genotype TT had an increased risk. In addition, participants with the TT genotype had higher mean and maximum values of the Ki-67 index in the control group compared to participants with the TC/CC genotype.

No effect from the intervention diet on hormone concentrations other than estradiol was found (Paper IV). There was a trend of a decreased risk of increasing estradiol concentrations in participants with the TC/CC genotype, comparing the intervention and control groups. Within the intervention group, participants with the TC/CC genotype decreased their concentration of SHBG between baseline and endpoint compared to participants with the TT genotype who increased their concentration.

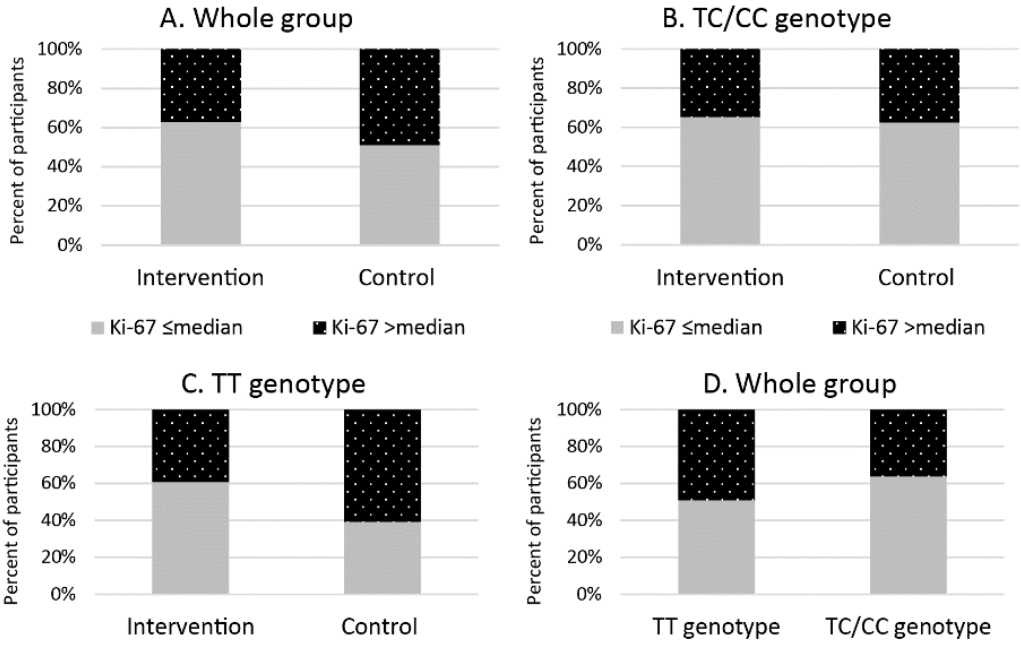

Figure 19. The percentage of participants with a Ki-67 index ≤ median or >median in the whole group (A, D) and in the different genotypes of estrogen receptor beta (B, C) in the PRODICA study (Paper III).

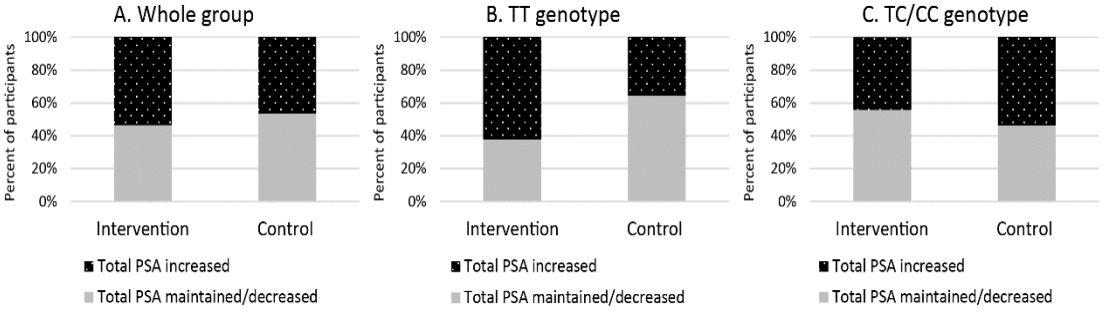

Figure 20. The percentage of participants with increased or maintained/decreased total prostate-specific antigen (PSA) concentration in the whole group (A) and in the different genotypes of estrogen receptor beta (B, C) in the PRODICA study (Paper III).

5.2.3 AGREEMENT BETWEEN REPORTED INTAKE OF PHYTOESTROGENS AND BLOOD CONCENTRATIONS OF PHYTOESTROGENS

A 10% increase in reported intake of total phytoestrogens was associated with an 8.2% increase in total plasma concentrations of phytoestrogens in the intervention group (FFQ, adjusted $P = 0.053$, Table 11). In the control group, a 10% increase in reported intake of lignans was associated with a 3.7% increase in plasma concentration of lignans (adjusted $P = 0.001$). The stratified analysis according to intervention length showed associations between intake of reported intervention foods and plasma concentrations of total phytoestrogens in the intervention group in 28–56 days (β 2.09; 95% confidence interval (CI) 0.096, 4.17), and between reported intake of lignans and plasma concentration of lignans in the control group in <28 days (β 0.67; 95% CI 0.11, 1.23) (data not shown).

According to the tertile classifications, 45%, 47%, and 50% of participants were classified in the correct tertile for intake and plasma concentrations of lignans, isoflavones, and total phytoestrogens, respectively (Table 12). In contrast, the proportion of participants misclassified into the opposite tertile was 9%, 8%, and 5% for lignans, isoflavones, and total phytoestrogens, respectively. Kappa statistics showed a slight agreement for lignans and isoflavones, and a fair agreement for total phytoestrogens. Kappa statistics including only non-users of antibiotics during the past five years were higher compared with analyses including users of antibiotics ($Kappa_{non\text{-}users} = 0.36$, $P < 0.001$; $Kappa_{users} = 0.17$, $P = 0.0305$).

Table 11. Linear regression analyses between intake of phytoestrogens reported with different dietary assessment methods (explanatory variables) and plasma concentrations of phytoestrogens at endpoint (outcomes) in patients with prostate cancer.

		Control (*n* = 54)		Intervention (*n* = 51)	
Models		β	95% CI	β	95% CI
Total phytoestrogens[1]					
Intake reported in 24-h dietary recall (mg) and plasma concentrations (nmol/L)	Unadjusted	0.17[2]	-0.093, 0.43[2]	0.80	0.036, 1.57
	Adjusted[3]	0.18[2]	-0.096, 0.45[2]	0.75	-0.063, 1.57
Intake of reported intervention foods[4] (mg) and plasma concentrations (nmol/L)	Unadjusted	-	-	0.68[5]	-0.18, 1.54[5]
	Adjusted[3]	-	-	0.68[5]	-0.25, 1.60[5]
Intake reported in FFQ at endpoint (µg) and plasma concentrations (nmol/L)	Unadjusted	0.12	-0.089, 0.32	0.83	0.064, 1.60
	Adjusted[3]	0.15	-0.075, 0.37	0.82	-0.013, 1.66
Isoflavones[6,7]					
Intake reported in FFQ at endpoint (µg) and plasma concentrations (nmol/L)	Unadjusted	-0.016[8]	-0.25, 0.21[8]	0.041	-0.46, 0.54
	Adjusted[3]	-0.045[8]	-0.27, 0.18[8]	0.021	-0.51, 0.55
Lignans[7,9]					
Intake reported in FFQ at endpoint (µg) and plasma concentrations (nmol/L)	Unadjusted	0.31	0.11, 0.51	0.15	-0.47, 0.78
	Adjusted[3]	0.37	0.16, 0.58	0.14	-0.52, 0.79

[1] Includes isoflavones, lignans, and coumestrol in the reported intake and isoflavones and lignans in the plasma concentrations.

[2] Two participants are missing.

[3] The analyses were adjusted for intake of energy (kcal) (tertiles), saturated fat (g) (tertiles), alcohol (g) (tertiles), and antibiotics (0 = reported "do not know" or "no intake of antibiotics" during the study and the past five years on the questionnaires; 1 = reported ≥1 intake of antibiotics during the study and the past five years on the questionnaires).
[4] Calculated from the amount of intervention foods consumed during the study divided by the number of days from the inclusion meeting to surgery. Only the intervention group is included.
[5] Six participants are missing.
[6] Daidzein, genistein, biochanin, formononetin, and equol were included in the reported intake. Daidzein, genistein, glycitein, and equol were included in the plasma concentrations.
[7] Reported intakes of enterolactone, enterodiol, and equol were only available for milk products in the FFQ.
[8] One participant is missing.
[9] Lariciresinol, matairesinol, medioresinol, pinoresinol, secoisolariciresinol, syringaresinol, enterolactone, and enterodiol were included in the reported intake. Lariciresinol, secoisolariciresinol enterolactone, and enterodiol were included in the plasma concentrations.
The intake of phytoestrogens and the concentrations of phytoestrogens were logarithmized using the natural logarithm.
Abbreviations: β, beta-coefficient; FFQ, Food Frequency Questionnaire.

Table 12. Agreement of classifications in tertiles from estimated intake of phytoestrogens and plasma concentrations of phytoestrogens at endpoint.

	Plasma concentrations of lignans[1]			
Estimated intake of lignans[2] from the FFQ	Low, *n* (%)	Moderate, *n* (%)	High, *n* (%)	Total, *n* (%)
Low, *n* (%)	20 (19)	11 (10)	5 (5)	36 (34)
Moderate, *n* (%)	11 (10)	12 (11)	15 (14)	38 (36)
High, *n* (%)	4 (4)	12 (11)	15 (14)	31 (30)
Total, *n* (%)	35 (33)	34 (33)	35 (33)	105 (100)
	Plasma concentrations of isoflavones[3]			
Estimated intake of isoflavones[4] from the FFQ	Low, *n* (%)	Moderate, *n* (%)	High, *n* (%)	Total, *n* (%)
Low, *n* (%)	20 (19)	10 (10)	4 (4)	34 (32)
Moderate, *n* (%)	11 (10)	12 (11)	14 (13)	37 (35)
High, *n* (%)	4 (4)	13 (12)	17 (16)	34 (32)
Total, *n* (%)	35 (33)	35 (33)	35 (33)	105 (100)
	Plasma concentrations of total phytoestrogens[5]			
Estimated intake of total phytoestrogens[6] from the FFQ	Low, *n* (%)	Moderate, *n* (%)	High, *n* (%)	Total, *n* (%)
Low, *n* (%)	22 (21)	12 (11)	2 (2)	36 (34)
Moderate, *n* (%)	10 (10)	13 (12)	15 (14)	38 (36)
High, *n* (%)	3 (3)	10 (10)	18 (17)	31 (30)
Total, *n* (%)	35 (33)	34 (33)	35 (33)	105 (100)

[1] Includes lariciresinol, secoisolariciresinol enterolactone, and enterodiol.

[2] Includes lariciresinol, matairesinol, medioresinol, pinoresinol, secoisolariciresinol, syringaresinol, enterolactone, and enterodiol.

[3] Includes daidzein, genistein, glycitein, and equol.

[4] Includes daidzein, genistein, biochanin, formononetin, and equol.

[5] Includes isoflavones and lignans.

[6] Includes isoflavones, lignans, and coumestrol.

Abbreviation: FFQ, Food Frequency Questionnaire.

Kappa statistics for lignans = 0.17 ($P = 0.0064$); isoflavones = 0.20 ($P = 0.0019$); total phytoestrogens = 0.26 ($P < 0.001$).

5.2.4 EVALUATION OF THE DIGITAL APPLICATION TO ESTIMATE DIETARY FIBER INTAKE

The measured mean fiber intake differed between the dietary record and the digital application by 2 g per day (Paper V). The absolute measured difference between the methods was independent of the total fiber intake and the relative difference between the methods decreased in older participants. The correlation between the methods was rho = 0.65 (P <0.001). The most common sources of total and soluble fiber intake were vegetables and roots (Figure 21, Figure 22). Bread and crackers were the most common sources of insoluble fiber intake (Figure 22).

Of the participants, 38% preferred using the digital application, 27% preferred using the dietary record, and 35% had no opinion regarding the two methods. There was no difference in age between the participants who preferred using the application and the dietary record. The features of the application were considered both simple and difficult to use by the participants in the Dietary validation study. Most participants thought it was an advantage that the features of the application were simple, but a few participants found the application to be lacking with regard to more advanced features, such as being able to scan food products and connect to a Bluetooth scale.

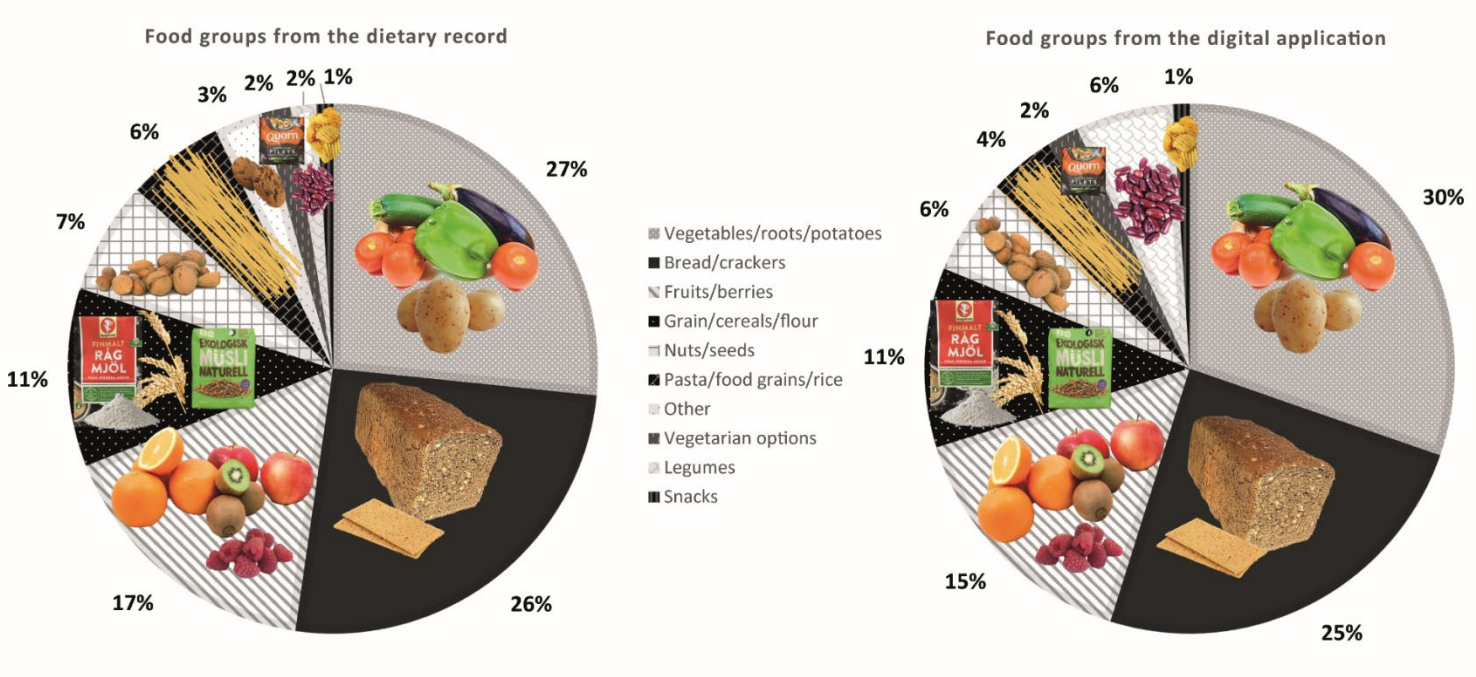

Figure 21. Food groups contributing to total dietary fiber intake from registrations in the dietary records and the digital application in the Dietary validation study (Paper V, n = 26).

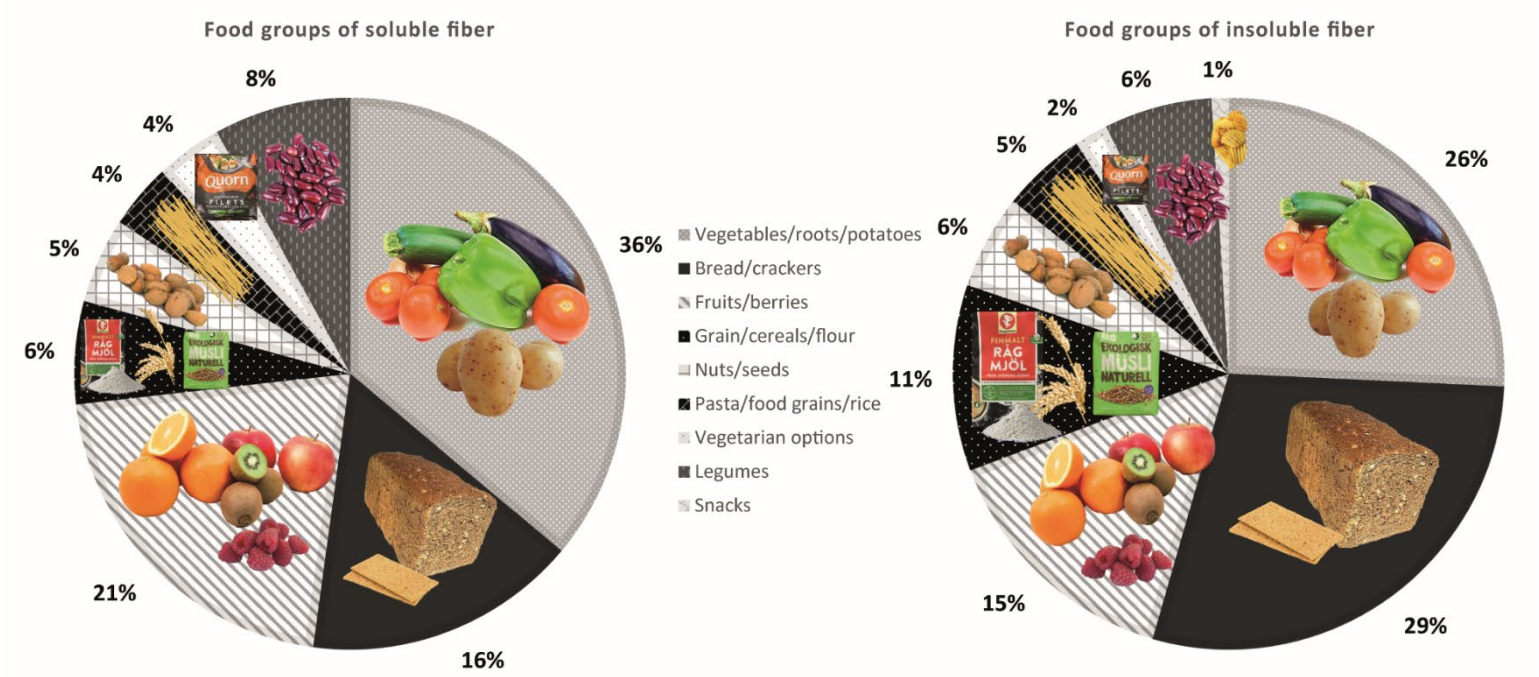

Figure 22. Food groups contributing to soluble and insoluble dietary fiber intake from registrations in the digital application in the Dietary validation study (Paper V, n = 26).

6 ETHICAL CONSIDERATIONS

The benefits of research must outweigh the risks or suffering. Responding to dietary questions may be sensitive for some people. In the Feasibility study, patients may have experienced embarrassment from collecting fecal samples and answering questions about gastrointestinal symptoms and bowel habits. The overall aim of the PRODICA trial and the FIDURA trial is to gain increased evidence regarding dietary advice given to patients with pelvic-organ cancers. In clinical practice, it would not be ethical to give patients dietary advice to increase their intake of phytoestrogens in order to decrease prostate tumor proliferation or increase dietary fiber intake in order to reduce gastrointestinal inflammation without scientifically proven effects. In addition, increasing the intake of dietary fiber may increase intestinal symptoms, such as bloating or flatulence, causing patients discomfort or abdominal pain (144). The participants received dietary advice, based on evidence-based information or clinical experience, and personal contact with a dietitian in consideration of the time that they had spent on participation in the studies. This is of great value since cancer survivors often wish for dietary advice and, in the absence of such advice, search the media and online sources with the risk of finding misinformation (145).

Participation in all the studies included in this thesis was voluntary, and all participants signed informed consent. All statistical analyses were encoded, and the participants were not affected by them. The main outcomes of the FIDURA trial are as yet unknown; however, if the fiber intervention gives the hypothesized result, the control groups will have missed out on this effect, or if the opposite result is shown, the intervention group may experience adverse effects without any positive outcomes.

All studies have been approved by the Ethical Review Board in Gothenburg, Sweden, or the Swedish Ethical Review Authority. The FIDURA study (Paper I) has registration number 803-18 and amendments numbers T1085-18 and 2020-01974, the PRODICA study (Papers II–IV) has registration number 410-14 and amendments numbers T124-15, 2020-02471, 2021-03320, and 2021-05878-02, and the Dietary validation study (Paper V) has registration number 2019-06252.

7 DISCUSSION

7.1 METHODOLOGICAL CONSIDERATION

This thesis is based on three randomized controlled intervention studies. Compared to observational studies, randomized controlled intervention studies minimize the risk of confounding, which improves the chance of proving causal evidence. The methodological strengths and limitations of the thesis will be discussed according to a hierarchical step model (Figure 23) (146) and each error is discussed in detail in separate paragraphs later in the discussion. The hierarchical step model of potential biases starts from a study hypothesis (Figure 23). In an optimal research study, there would be two identical worlds where the exposure categories could be tested in one world with the other world acting as a control during the same period of time. This enables the researcher to observe the differences in the outcome categories and thereby see the effect of the intervention (perfect person-time). Unfortunately, there are no perfect research studies and there are no identical worlds. The errors between the perfect person-time and the targeted person-time are called confounding factors. All participants are not able to, or do not want to, complete the study (dropout), which will lead to the studied person-time not being equal to the targeted person-time. Misclassification is the step between the studied person-time and the data set, and analytical errors can then cause bias before the calculated effect measure is received. Incorrect interpretation can error the statement of causality. Finally, depending on the targeted person-time, the results could lack generalizability.

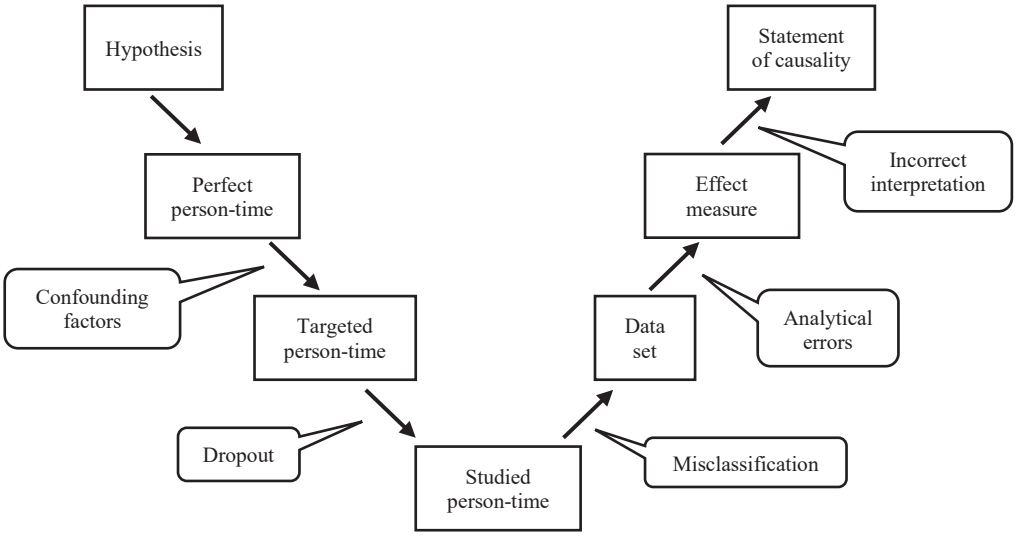

Figure 23. A hierarchical step model of the potential biases during the research process (146). Courtesy of Prof. Gunnar Steineck and Ass. Prof. Maria Hedelin.

7.1.1 CONFOUNDING

All the papers in the thesis are based on randomized trials. If the size of the groups is big enough, randomization will distribute both known and unknown confounding factors equally between the groups that are compared. Data on known confounding factors were collected in the studies to allow adjustment for them in statistical models.

Only a proportion of the participants in the Feasibility study (Paper I) were randomized and outcomes that were not affected by the randomization were investigated, for example participation rate during different periods. The highest participation rate in the last recruited participants is probably partly due to improvements in the logistics, but may also have been affected by random variation over time, for example the Covid-19 pandemic. In the FIDURA trial, possible confounding factors are those affecting inflammation and intestinal syndromes, for example anti-inflammatory drugs, overweight, and radiation dose. The questionnaires are one of the methods by which this information is collected, which makes it important to have a high participation

rate in the questionnaires. The placebo effect is a possible confounding factor that cannot be adjusted for in statistical analyses. The decision to use blinded capsules in the Feasibility study (Paper I) was therefore an important factor in increasing the validity of the FIDURA trial.

In the PRODICA study (Papers III–IV), participants' characteristics were studied to see if the groups were comparable. Unfortunately, there were some differences in baseline characteristics between the intervention and the control groups. There were differences between the unadjusted and the adjusted analyses of the risk differences, indicating confounding effects. The Dietary validation study (Paper V) had a crossover design, which means all the participants were their own control and reported dietary intakes were therefore only estimated within subjects which is more powerful since no between-subject-variation, independent of the reported differences, needs to be overcome (147).

7.1.2 DROPOUT

Errors caused by dropout occur when the dropout is related to the outcome or, in the worst case, both the exposure and the outcome. This is a problem since it is not known how the missing data would have affected the results of the study. Dropout errors are therefore a major threat to the validity of the data in all studies. To minimize the problem, the data collection prioritized the primary outcomes and attempts to collect information about those lost to follow up and reasons for dropout were made.

The dropout rates of those who started the interventions in the PRODICA study (4%, Papers III–IV) and in the Dietary validation study (4%, Paper V) were low compared to the Feasibility study (23%, Paper I). In addition to the low dropout rate in the PRODICA study, the collection of tumor material was not dependent on whether the participants completed the study (Paper III). Missing information on total PSA concentration at endpoint could also be collected from the National Prostate Cancer Register of Sweden. The small dropout rate in the Dietary validation study (Paper V) would affect the results minimally.

The participants in the Feasibility study (Paper I) were undergoing a challenging period of ongoing radiotherapy treatment and a lot of information was collected. To reduce the number of dropouts, the study design was adjusted to ease the burden for the participants. Some of the dropouts in the Feasibility study was related to gastrointestinal symptoms, which are also likely to occur in the FIDURA trial, and this is therefore a potential source of bias.

7.1.3 MISCLASSIFICATION

Decreased sensitivity or specificity in measuring an outcome that does not vary between treatment groups (non-differential misclassification) will either dilute or not affect the average effect measure, although the random error of the effect measure may increase (146, 148). However, if the error varies between the treatment groups (differential misclassification), the effect measure may mislead us regarding the actual effect.

The FFQ, 24-h dietary recall, and dietary record all include misclassification. The validity of self-reporting dietary intake is also generally low (149). However, poor reporting probably did not vary between the intervention and the control groups, which means non-differential misclassification potentially diluted the results concerning compliance and the agreement between reported intake of phytoestrogens and blood concentrations of phytoestrogens. Compliance was estimated by calculating the reported intake of phytoestrogens in different ways (Paper III). However, there were differences in the estimated compliance in the intervention group. This was potentially caused by misclassification from the 24-h dietary recall not capturing the participants' habitual intake of phytoestrogens on the recalled day. Measurements of outcomes, such as the Ki-67 index and concentrations of PSA and hormones, will likewise include non-differential misclassification.

In the Dietary validation study (Paper V), a 3-day dietary record was used as the reference method to measure participants' intake of dietary fiber. Due to misclassification, it is not known if the dietary record reflects the participants' true fiber intake. The difference in fiber intake between the digital application and the dietary record can be a result of a true individual variation between the two registered periods (150). The most optimal approach would have been to compare the two methods over the same three days, but two different periods

were chosen to avoid the registrations majorly affecting each other. Using 24-h dietary recalls would have affected the registration in the digital application less. However, the disadvantages of using 24-h dietary recalls would have been that the portion sizes would not have been as accurately recorded as in a weighed dietary record, greater resources would have been required from the study secretariat, and the risk of missing data if the participants did not answer the telephone when the 24-h dietary recalls were planned.

7.1.4 ANALYTICAL ERRORS

In all the papers, *P* values below 0.05 have been considered statistically significant and the null hypothesis has been rejected for values below that number. "Goodness of fit" was practiced, ensuring correct statistical models adapted to the characteristics of the data in the studies. Parametric tests were used for normally distributed data and non-parametric tests for non-normally distributed data, and the normal distribution was primarily tested with the Shapiro-Wilk test.

In the cases of Shapiro-Wilk test values near 0.05, both parametric and non-parametric tests were used for the relevant data. In most cases, the interpretation of the results did not differ regardless of whether a parametric or a non-parametric test was used. The use of parametric tests in non-normally distributed data can cause errors due to the assumption that the population is normally distributed, while the use of non-parametric tests in normally distributed data could lack the power to prove a true effect. We therefore used a non-parametric test in case of uncertainties about whether the data were normally distributed.

In the PRODICA study (Papers III–IV), the outcomes of Ki-67, PSA, and hormones were dichotomized in higher/lower Ki-67 index and increased or maintained/decreased PSA and hormone concentrations. The limitation when dichotomizing an outcome is that one loses information about the size of how high or low the index is or the size of the increased or decreased concentrations. The advantage is that the noise from the individual variation is decreased.

The limitation of the correlation analysis in the Dietary validation study (Paper V) is that it only shows the covariation between the digital application and the dietary record. In other words, the two methods can include the same systematic errors and show a high correlation coefficient. A third method with uncorrelated measurement errors could have therefore been used to estimate the correlation with the true underlying exposure (i.e., estimated validity coefficients).

7.1.5 INTERPRETATION OF DATA

An identified association in a data set can be a result of 1) bias, 2) chance, or 3) a true causal effect. To interpret the results correctly, one must be aware of the limitations of the study and the study design and understand statistical effect measurements and pathological mechanisms. Type 1 or type 2 errors may occur because of different errors in the hierarchical step model (Figure 23) and could therefore cause incorrect interpretations. In type 1 errors, the null hypothesis is erroneously rejected – the intervention is concluded to have an effect but there is actually no effect. In type 2 errors, the null hypothesis is erroneously accepted – the intervention is concluded to not have an effect but there is actually an effect. Type 2 errors can be avoided by using bigger sample sizes, but type 1 errors cannot. Not finding an effect of the phytoestrogen intervention on the continuous Ki-67 index could, potentially, have been caused by a type 2 error due to an underpowered sample size (Paper III).

In the Feasibility study (Paper I), the conclusions are primarily based on the authors' own experiences, which is a potential bias. The different periods during the Covid-19 pandemic and individual variation also challenge the conclusions about the increasing participation rate caused by the modifications made to the study logistics. In the Dietary validation study (Paper V), the registration during the two different periods is the main limitation and could have contributed to the underestimation of the application. Repeated registrations of the methods would have strengthened the results in the Dietary validation study. The results regarding the decreased difference between the methods and increasing age could be a result of chance, since it was not statistically significant.

7.1.6 GENERALIZABILITY

The generalizability of a study is the degree to which the study results can be applied to other populations. Several factors may decrease the generalizability of the results in this thesis.

The participants in the Feasibility study (Paper I) and the PRODICA study Paper III–IV) had an education level that was higher than the general Swedish population (151). The education level of the participants in the Dietary validation study (Paper V) is not known, but it may be reasonable to assume that most participants had a higher education than the general population. Higher socioeconomic status is associated with higher dietary quality (152), and one might suspect that people enrolling in nutritional studies have a higher interest in, and knowledge of, nutrition than the general population. However, it is also not uncommon that people who receive a cancer diagnosis develop an increased interest in nutrition and become more motivated to adopt a healthier diet (153). This means that in a population with lower nutritional interest, the participation rates found in the Feasibility study could be even lower and the difference in fiber intake between the digital application and a 3-day dietary record could be greater than 2 g at group level. The generalizability of the results in the Dietary validation study is also limited concerning its application to men and to other age groups since dietary habits vary between the sexes and between different age groups (98, 150).

The results of the PRODICA study (Papers III–IV) cannot be generalized to patients with high-grade cancer. The expression of ERβ is reduced in prostate tissue in high-grade prostate cancer compared to low and intermediate-risk cancers (154), which could decrease the effects of the ERβ genotype and the eventual effects of phytoestrogens.

7.2 DISCUSSION OF MAIN FINDINGS

7.2.1 FEASIBILITY OF A FIBER INTERVENTION DURING PELVIC-ORGAN RADIOTHERAPY

When planning a large-scale intervention study, it is of great importance to identify the major obstacles before starting the main data collection. In the Feasibility study (Paper I), several challenges that patients experienced during the study were identified, and a number of adjustments were therefore made to increase the participation rate and decrease the dropout rate. The fiber intervention was feasible, but it was more challenging than the other studies in this thesis and this was reflected in the high dropout rate. Aspects related to radiotherapy treatment were the major reasons given for declining participation in and dropout from the study. Radiotherapy treatment involves many healthcare visits and debilitating side effects, which probably affected study participation and may have sometimes become overwhelming. In addition, before starting treatment, patients are unaware of the side effects that may affect them, making it even harder for them to imagine the total burden of radiotherapy.

The participation rates reflected the prioritized outcomes in the Feasibility study. It can often be necessary to compromise on what is most important in research. The decision had been made initially to collect several outcomes in the study, but it was later necessary to make a choice between receiving all the information concerning the outcomes with the consequence of risking the participants dropping out of the study or only receiving information about the primary outcomes. The number of tasks needs to be carefully considered when planning interventions in groups experiencing high psychological and physiological burdens. It is beneficial to choose primary endpoints which are independent of whether the participants complete the study or not, such as in the PRODICA study (Paper III).

In hindsight, it would have been interesting to try to make capsules containing pectin. If it had been easier to fill the capsules with pectin, the number of capsules recommended to the participants could have been decreased. Using psyllium rather than pectin could be a disadvantage because pectin is highly

fermentable in the colon compared to psyllium, which is intermediately fermentable (91). The use of pectin could therefore have led to higher production of SCFA. However, in the FIDURA study, the psyllium capsules are combined with a moderate intake of dietary fiber from the regular diet. Psyllium has been shown to delay fermentation and shift the fermentation site leading to a higher butyrate concentration in the distal colon (155), which is potentially advantageous for patients receiving pelvic-organ radiotherapy. In addition, the lower fermentation grade of psyllium compared to pectin could be beneficial in patients undergoing pelvic-organ radiotherapy due to less gas being formed, which decreases the risk of discomfort and pain (144).

Since the Feasibility study (Paper I) was published, only one intervention study has been reported studying the effect of a fiber intervention in patients undergoing pelvic-organ radiotherapy. This was a small study investigating the effect of a supplement of partially hydrolyzed guar gum on diarrhea frequency and gut microbiota among patients undergoing pelvic-organ radiotherapy (74). The study reported that the supplement had an effect on the gut microbiota (increased count of the bifidobacterial) and reduced the frequency of diarrhea after completion of radiotherapy. This agrees with the hypothesis in the FIDURA study that dietary fiber will positively affect the gut microbiota and reduce gastrointestinal effects after radiotherapy.

Our Swedish national investigation of the dietary advice given to these patients in the clinical situation revealed that several oncology clinics give non-evidence-based advice for patients to decrease their intake of dietary fiber (81). Söderström *et al*. (156) studied associations between a modified dietary fiber intake (switching from insoluble to soluble fiber) and nutritional intake in patients with prostate cancer during radiotherapy. Their intervention seemed successful in that patients did not decrease their fiber intake but it also highlighted the role of the dietitian in giving advice regarding dietary modifications. In another study, the impact of an anti-fermentative diet in elderly patients with prostate cancer receiving radiotherapy was investigated since this diet is sometimes used as standard care during radiotherapy for patients with prostate cancer (157). They found that the diet reduced the patients' intakes of energy, dietary fiber, and some other nutrients, and that their body weight decreased. It is of particular concern when patients are given dietary advice without the involvement of a dietitian. This can result in the nutritional intake of patients with cancer becoming inadequate. Malnutrition is

a predictor for poor clinical outcomes in cancer patients (158). These patients are vulnerable, which is why the dietitian has a crucially important role to play in their care.

7.2.2 IMPACT OF AGE ON THE CHOICE OF FORMAT OF DIETARY ASSESSMENT METHODS

We found that age affected the choice of using paper or web versions of the questionnaires and the application in the Feasibility study (Paper I). This result has been confirmed by others. In a study where 3406 patients with colorectal cancer aged 18–85 years were invited to fill out a questionnaire on the web or on paper, patients ≥70 years were found to more often choose to fill out the paper questionnaire compared to the web-based questionnaire (159).

In the Dietary validation study (Paper V), however, age did not affect whether participants preferred the digital application or the dietary record. A systematic review found lower acceptance of nutrition applications in older populations compared to younger (160). This was reflected in more negative attitudes toward, and lower usage rates of, these applications. The participants' median age in the Feasibility study was higher than the participants in the Dietary validation study and could have affected the results. The study populations were also different. Kelfve *et al*. found that paper respondents in their study were associated with being retired, female, single, having lower education, lower self-reported health, and a higher level of depression compared to web respondents (161). They concluded that a web-based survey is feasible after retirement age but also offering a paper questionnaire is important to avoid missing data in a small but important group, which could potentially bias the results. I believe that age is an important factor when using digital assessments and that it is necessary to offer participants a paper complement, particularly in older populations.

7.2.3 EFFECTS OF A PHYTOESTROGEN-RICH DIET IN PATIENTS WITH PROSTATE CANCER

A modest or no effect of the phytoestrogen intervention was found on the Ki-67 index and total PSA concentration in the whole study population. However, when the analysis was stratified by the genotype of ERβ, different effects of the phytoestrogen intervention were found. In the TT genotype group the strongest effect on the decreased risk of a higher Ki-67 index was identified, while in the TC/CC genotype group a decreased risk of increasing total PSA concentration was found. Ki-67 is a well-known proliferation marker (46), however, it is not known whether total PSA concentration reflects a response from the benign prostate cells or the cancer prostate cells. The effects of phytoestrogens in benign and cancerous prostate cancer tissue may be different – lower concentrations of genistein have been found in prostate cancer tissue compared to benign hyperplasia prostate tissue (162). Nevertheless, prostate cancer is a heterogeneous disease and Ki-67 was only evaluated in the dominating tumor. In prostate cancer, it is common for patients to have more than one tumor (163), and smaller tumors can be more aggressive than larger ones (164). The intended aim was to calculate the change in the Ki-67 index in prostate biopsies, as was done for the PSA concentrations. However, Ki-67 was not evaluated at baseline and Ki-67 was evaluated in prostatectomy specimens instead of in biopsies at endpoint. Nevertheless, it is a limitation that baseline values of the Ki-67 index could not be compared.

The only effect of the intervention diet on the risk of increased PSA concentrations found was when the analyzes were stratified by the genotype of ERβ. A systematic review published in 2021 concluded that isoflavones do not seem to influence PSA concentrations in patients with localized prostate cancer (165). This systematic review included published articles up until 2019. The publication of phytoestrogen interventions in patients with prostate cancer has since been scarce. Kumar *et al*. published a study in 2020 where they did not find any effect of an isoflavone supplement on PSA concentration or serum steroid hormones in the whole study population (123). However, they found different effects of the supplement in concentrations of PSA and IGF-1, where PSA concentration decreased in Caucasian men, and IGF-1 concentration decreased in African American men. The PRODICA study also consisted predominantly of a Caucasian population, but no effect on total PSA concentration was found in the whole group. Zhang *et al*. (2019) found that their genistein intervention altered the expression of four genes, of which three had established connections to cancer cell motility and metastasis (166). Gene

expression may be a better outcome of tumor proliferation compared to the Ki-67 index.

When the intervention and control groups were compared, a trend of decreased risk of increased estradiol concentration was found in participants with the TC/CC genotype. The group of the TC/CC genotype also had a decreased risk of increasing total PSA concentration. The effect of estradiol could be a mechanism of the effect on PSA. Even though absolute serum concentrations of estradiol have not been proven to be a risk factor for prostate cancer, some studies have shown a higher incidence of prostate cancer in some populations with higher circulating estrogen (59). This could indicate a positive effect of lower concentrations of estrogen.

No effect of the phytoestrogen intervention was found in the other investigated blood concentrations of hormones. Reed *et al*. (2021) investigated the effect of soy and isoflavone intake on reproductive hormones in men in a meta-analysis of clinical studies (167). They found no significant effects on concentrations of testosterone, estradiol, and SHBG, even regardless of study duration and dose. It is possible that other hormones can be involved in the effect of phytoestrogens in prostate cancer, which were not investigated in this thesis, for example 3ßAdiol and DHT. To my knowledge, no one else has investigated the effects of a phytoestrogen intervention on blood concentrations of 3ßAdiol. The effect of phytoestrogens on blood concentrations of DHT has been less studied than testosterone, but like testosterone, the results have varied (167). However, the blood concentrations of DHT badly reflect the concentrations in the prostate tissue (168). Another possible mechanism of phytoestrogens in prostate cancer is through the expression of ERβ and the androgen receptor in the tumor tissue (169).

The findings in the PRODICA study suggest that genotype TC/CC could be favorable in prostate cancer compared to the TT genotype. In participants with the TC/CC genotype, a decreased concentration of SHBG was also found. It is possible that this finding is connected. SHBG is a transport protein for androgens and could be involved in the androgenic response in prostate cancer (50, 51).

The clinical relevance of the results of the PRODICA study can be difficult to interpret. If there is a true effect of phytoestrogens in reduced prostate tumor proliferation, this would result in increased survival or decreased cancer recurrence in patients. The association between blood concentrations of phytoestrogens and prostate cancer survival and mortality has been studied but no associations have been found (170). However, different genotypes of ERβ were not studied. The varying results in research on the effects of phytoestrogens in patients with prostate cancer may partly be explained by not considering different genotypes. Further research is needed to investigate the impact of the ERβ genotype on the effect of phytoestrogen intake in patients with prostate cancer. To my knowledge, no other group has studied this, which makes it a promising topic for future research.

If there is a truly different effect of phytoestrogens on tumor proliferation, by groups of ERβ genotypes, the implication of this for clinical practice could be challenging. Genotyping leads to increased costs, which could be justified if it results in savings due to decreased cancer recurrence. However, if there are no adverse effects in one of the genotypes, advice regarding increased intake of phytoestrogens could be given to all patients and genotyping would not be necessary.

7.2.4 AGREEMENT BETWEEN REPORTED INTAKE OF PHYTOESTROGENS AND PLASMA CONCENTRATIONS OF PHYTOESTROGENS

The agreement classification between estimated intake and plasma concentrations of total phytoestrogens was fair and consistent with previous studies (171). The metabolism of phytoestrogens is dependent on the intestinal microbiota (103) and previous studies have revealed a substantial individual variation in metabolism and excretion of phytoestrogen after consumption of phytoestrogen-rich foods (105), indicating that these factors have a major impact on estimated intake and plasma concentrations of phytoestrogens. As expected, the agreement classification of total phytoestrogens was stronger in non-users of antibiotics. This was probably caused by the intake of antibiotics negatively impacting the intestinal microbiota and thereby affecting the metabolism of phytoestrogens (172). The overall stronger agreement between estimated intake and plasma concentrations of phytoestrogens in the intervention group compared with the control group could be explained by the

intervention group having a more regular intake of phytoestrogen-rich foods compared to the control group.

The reason for the poor associations between estimated intake from the FFQ and plasma concentrations of isoflavones and lignans is probably in part because only a limited number of isoflavones and lignans in plasma could be analyzed and the estimated intake from the FFQ included more of the different isoflavones and lignans. Measurement errors in the FFQ could also be a contributing factor (173), which is in line with the findings of Grace *et al.* (174) but in contrast to those of French *et al.* (171). Soybeans were included under the category "Beans, lentils or chickpeas (e.g., kidney beans, soybeans, white beans)" in the vegetable category in the FFQ, which could have made it more difficult for participants to estimate actual intake, thereby leading to a false low registered intake for participants in the intervention group at endpoint, but which was still higher than the controls. Another plausible explanation is decreased absorption of lignans due to decreased bioavailability owing to mainly using whole rather than crushed flaxseeds in this study (175). In addition, the plasma concentrations of the phytoestrogens only reflect the concentration at the sampling occasion and are, therefore, affected by dietary intake recently prior to sampling (110).

7.2.5 EVALUATION OF THE DIGITAL APPLICATION

The 2 g difference in recorded fiber intake between the digital application and the dietary record could be smaller for the digital application that is now being used in the FIDURA study since more food items have been added. However, it is highly likely that the application still underestimates the true fiber intake due to people omitting to register relevant food items in the digital application. This will not be a problem when analyzing data in the FIDURA trial due to the intervention and control groups using the same application. However, there could be implications if the results are converted to dietary advice concerning fiber intake during pelvic-organ radiotherapy. The underestimation of the dietary fiber intake must then be considered and an interval for the fiber recommendation presented might be preferable.

Since the Dietary validation study (Paper V) was published, some new studies in this research area have been published. However, the applications used in

these studies have been designed to cover the person's whole dietary intake and not just dietary fiber intake; the digital applications have also not been developed for cancer patients. Moyer *et al*. validated their image-assisted food-tracking mobile application in a healthy population and a population with either type 1 or type 2 diabetes (176). Their study design was similar to ours and their application was also found to underestimate dietary fiber intake by a mean of 2 g compared to an automated self-administered 24-h dietary recall. They also examined the usability of both methods and found that the application had a higher usability score compared to the 24-h dietary recall, due, for example, to participants being able to copy meals and to shorter tracking times (176). There is also a function to save meals and reuse them in the digital application used in this thesis. The limited number of food categories in the digital application is also something that hopefully leads to shorter tracking times and improved usability.

The advantage of validating the digital application in the general population is that the application could then be used for various research purposes and also within healthcare. It could be used both to measure the fiber intake or as a guide to how to achieve different fiber intakes. Even though the digital application was found to be most useful for ranking individuals' fiber intakes rather than assessing their absolute intakes, this is of minor relevance in the above-mentioned areas. However, its areas of use are limited given that it is only designed to measure dietary fiber intake. Another disadvantage of the application is that it has not been validated for use by men.

The main source of total dietary fiber in the Dietary validation study (Paper V) was found to be vegetables and roots. This finding is in contrast to those of previous studies where bread and cereals have been the largest sources of fiber (98, 177). However, in Hedelin *et al*. this was probably affected by the choices made regarding the division of food groups, i.e., all cereals were grouped together (177). In the Dietary validation study, on the other hand, bread and other cereals were grouped separately as is also the case in Amcoff *et al*. (98). Studies can, therefore, be difficult to compare depending on the division of food groups. Nevertheless, in Europe, Sweden is one of the few countries where bread contributes a higher proportion of fiber than vegetables, including potatoes (178). However, grain products have been the largest source of fiber in most European countries, even when vegetables and potatoes are grouped together. Knowledge about fiber sources can be useful in regulating fiber

intake. For example, if the food group vegetables and roots is the major fiber source, the food group bread and crackers could be targeted to increase fiber intake, for example by changing the type of bread consumed. If allergies or intolerances are an issue, the focus can be on the relevant food groups that are tolerated. Nevertheless, it will be interesting to see if the fiber sources differ in a population of patients undergoing pelvic-organ radiotherapy compared to a randomly selected population of women.

The participants' opinions regarding the digital application varied in the Dietary validation study (Paper V), probably reflecting their previous experiences of using digital applications and their technical skills. A few participants found the digital application to be lacking in more advanced features. However, this may not be relevant for patients undergoing pelvic-organ radiotherapy who can be otherwise mentally preoccupied during their treatment. A systematic review investigating barriers and facilitators for using nutrition applications concluded that there is no "one-size-fits-all" approach for their use (179). Examples of the highlighted barriers and facilitators regarding the use of applications were technical issues, the features, and the usability of applications.

7.3 THE OVERALL STRENGTHS AND LIMITATIONS OF THE THESIS

The strengths of this thesis include it being based on a variety of outcomes from randomized trials in nutrition, the majority of which are clinical studies. In addition, the PRODICA study (Papers II–IV) is built on a large clinical sample. Clinical studies are both time-consuming and challenging, with the need for coordination of recruiting, baseline and endpoint visits, and sampling. This can explain why several clinical studies are small in size with short duration. I was part of the planning of the study design and data collection of the Feasibility study (Paper I) and the Dietary validation study (Paper V); I also collected most of the data included in the thesis, built the database for the digital application, and designed the features of the digital application. Previous publications in this area have been rare which further strengthens this thesis because the evidence available in these research areas is still limited. The limitations of this thesis include the main outcomes of the FIDURA study not being included and the small study sample in the Dietary validation study (Paper V). The knowledge obtained in this thesis could be utilized in further research, as well as by physicians, nurses, and dietitians in clinical practice.

8 CONCLUSION

Dietary interventions using dietary fiber supplements and phytoestrogen-rich foods are feasible in patients with pelvic-organ cancer and digital tools can be used for the assessment of dietary fiber intake. For interventions to be feasible, their design needs to be carefully adapted to the target group. The effects of the plant-food components need further investigation. The specific conclusions are highlighted below.

- A dietary fiber intervention, including psyllium and placebo capsules in combination with a moderate intake of dietary fiber from the regular diet, is feasible in patients with pelvic-organ cancer undergoing radiotherapy. However, the number of tasks and measurements that patients should carry out needs to be individually adapted to their ability and their status during radiotherapy.
- People's age affects their choice to use digital applications and web questionnaires. This should be considered when such formats are planned to be used in research studies.
- We designed a phytoestrogen intervention in patients with prostate cancer and the effects on the Ki-67 index and total PSA concentration were modest in the whole group. However, there may be stronger effects depending on the genotype of ERβ.
- A phytoestrogen-rich diet did not affect blood concentrations of testosterone, SHBG, and IGF-1 in patients with prostate cancer. However, there may be a modest effect on estradiol concentration depending on the ERβ genotype.
- The genotype of ERβ appeared to have a greater effect than the phytoestrogen intervention on both tumor proliferation and some blood concentrations of hormones in patients with prostate cancer.
- A phytoestrogen-rich diet increased the plasma concentration of phytoestrogens in patients with prostate cancer.
- The agreement between reported intakes of total phytoestrogens and blood concentrations of total phytoestrogens was acceptable when categorized in tertiles of patients with prostate cancer.
- A digital application was developed and found to be technically feasible to use but underestimated the intake of dietary fiber by ~2 g compared to a 3-d dietary record.

- Vegetables and roots were the primary sources of total and soluble fiber intake, and bread and crackers were the primary sources of insoluble fiber intake in a randomly selected female population.

9 FUTURE PERSPECTIVES

- In order to maintain a high participation rate in studies with patients receiving pelvic-organ radiotherapy, the information needs to be condensed and the number of tasks minimized or individualized to ensure high compliance and a low dropout rate.
- Formulations with large volumes and coarse textures may be problematic for patients with gynecological cancer to take during radiotherapy due to nausea. Supplements of capsules with a small size and limited number are preferred.
- Access to digital and paper versions of dietary assessment methods is recommended to enable adaptation according to individual preferences.
- In patients receiving pelvic-organ radiotherapy, investigations could be conducted regarding different dose-effect responses with different levels and solubility of dietary fiber intake, the associations between different fiber intakes and stool forms and stool frequencies, and if the effects differ between cancers in different pelvic organs and age groups. In addition, investigations regarding major fiber sources and if patients prefer taking fiber in the diet, as supplements, or as medicines.
- The compilation of national guidelines for dietary fiber intake during pelvic-organ radiotherapy.
- If psyllium turns out to be an effective addition for patients undergoing pelvic radiotherapy, tests could be conducted to investigate if pectin could be even better.
- It would be interesting to investigate how a phytoestrogen intervention such as ours affects the concentrations of phytoestrogens and hormones in prostate tissue.
- Exploration of the effect of a phytoestrogen intervention on the expression of ERβ, androgen receptor, and genes involved in tumorigenesis in tumor material.
- Studying the effect of a high intake of phytoestrogens and ERβ genotype in the survival and recurrence of prostate cancer.
- Digital applications could be connected to existing expanded nutritional databases using suitable portion sizes.
- Establishing biomarkers for total and specific fiber intakes.

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