The use of cholesterol lowering medicine for postmenopausal women with estrogen receptor positive breast cancer

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Abstract

Cancer is a world-wide problem. One third of cancer cases are breast cancer. Most of the breast cancer cases are in postmenopausal women due to increased estrogen production. Postmenopausal women have an increased risk of developing breast cancer because the estrogen production takes place in the adipose tissue. Furthermore postmenopausal with increased BMI are at greater risk of developing breast cancer due to increased estrogen production in the adipose tissue. Statins are used as treatment of hypercholesterolemia, which more often occurs in postmenopausal women. It is suggested that statins have other effects than preventing hypercholesterolemia. One of these effects is an anticancer effect. This is suggested due to statins influence on different pathways and intermediates. In this report, the purpose was to investigate whether it could be estimated if statin use reduces the recurrence risk of breast cancer. Through analyzing different meta-analyses and studies it could be estimated that statins did have a positive effect on breast cancer recurrence but further research should be made.

Indhold

Preface

This report is written by a 4th semester group on the subject module of Medicinal Biology at Roskilde University in the spring semester 2015.

We would like to thank our supervisor, Peter Michael Vestlev, for his supervision.

Target group

The target group of the following report is 4th semester students studying medicinal- and molecular biology with basic education on and knowledge of human physiology and cell biology. The project could also be of interest for those who have knowledge of and interest in the topic.

Project scope

The focus in the project report will be on postmenopausal women with estrogen-receptor positive (ER +) breast cancer treated with a third generation nonsteroidal aromatase inhibitor, preferably letrozole.

The meta-analyses and studies processed in this report do not necessarily encompass all these criteria but at least one of them.

The specific statistics and methods utilized in the processed meta-analyses and studies are not accounted for in this report due to none of us have had a statistics course yet.

Method

To answer the problem of interest the report examined literature studies and data extraction from patients' medical history and wished to use recent information based on criteria from studies, books, reviews, meta-analyses and results from studies. The report selected two articles from the meta-analysis based on the criteria to examine the results in the articles compared to the meta-analyses.

Introduction

Cancer is a world-wide disease with estimated 1,658,370 new cases in 2015 (American Cancer Society.com). Up to one third of these cases are breast cancers (Russo et al., 2000). In Denmark 37,000 new cases of cancers were registered in 2013 (Cancerregistret.dk). In regard to breast cancer in women in Denmark, approximately 4,700 new cases were registered in 2013 (Cancerregistret.dk). The risk of breast cancer increases in proportion to increasing age. Thus postmenopausal women have a higher risk of developing breast cancer. Furthermore, breast cancer and breast cancer

risk are to a great extent associated with estrogen which initiates and promotes breast cancer development (Yager & Davidson, 2006). The primary production of estrogen in postmenopausal women is not in the ovaries in premenopausal women, but in the peripheral tissue, especially the adipose tissue. Thus postmenopausal women with a high BMI are particularly at risk of developing breast cancer. Increased BMI also needs to be taken into consideration in regard to recurrence risk. 90 % of all women with breast cancer in Denmark are at risk of recurrence (cancer.dk). In Denmark postmenopausal women with (HER+) breast cancer receives an anti-hormonal treatment called aromatase inhibitors, typically letrozole (cancer.dk). Treatment with letrozole has been shown to lead to a five-year disease-free survival of 84 % (BIG1-98 Collaborative Group).
Furthermore approximately 30 % of postmenopausal women in Denmark are prescribed statins to treat hypercholesterolemia (SSI.dk) resulting from elevated cholesterol levels (Lehninger, 2013). Statins function by inhibiting HMG-CoA reductase and thus preventing the synthesis of cholesterol (Ahern et al., 2014). Besides statins' use as treatment of hypercholesterolemia, statins have some pleiotropic effects including anticancer effects (Ahern et al., 2014) and in some cases they have been shown to reduce the recurrence risk of breast cancer among Danish women (Ahern et al., 2011a).
Statins are very inexpensive and tolerable, and thus they would be ideal in the treatment of breast cancer patients (Ahern et al., 2014) if they are shown to have a considerable positive effect, but this needs to be further investigated. The aim of this report was to estimate such an association based on the findings in epidemiological studies.

Problem of interest

With basis in epidemiologic studies, is it possible to estimate whether statins reduce the risk of recurrence of ER+ breast cancer in postmenopausal women receiving letrozole treatment, or not?

Theory

1.1 Breast cancer

Breast cancer is the uncontrolled growth of abnormal cells in the breast tissue and the most common cancer type among women(James et al., 2015). It has been estimated that 1,200,000 new cases occur every year worldwide (Lumachi et al., 2013) and represents 11 % among all cancer types globally (Majeed et al., 2014). Breast cancer is more common in postmenopausal women (Smith & Dowsett, 2003) and one third of all new cases of women's cancer in North (Russo, Hu, Yang, & Russo, 2000). The breast cancer mortality is declining in certain industrialized countries but the opposite is observed in nations with lower breast cancer incidence (Russo et al., 2000). In the last 5 decades the death from breast cancer has remained almost the same, due to the lack of knowledge about cancer progression and initiation (Russo et al., 2000). There are several factors causing the occurrence of breast cancer such as smoking, drinking alcohol, hormone therapies, physical inactivity, postmenopausal obesity, early menarche, delayed first childbirth and family history of cancer in breast, endometrium and ovary (Russo et al., 2000). The major cause is inherited mutations in the genes BRCA1 and BRCA2. Gene mutation contribute to approximately 10-15 % of all cases (Majeed et al., 2014). After normal cells have completed their life cycle, they undergo apoptosis. Before initiation of apoptosis, several pathways (PI3K/AKT and RAS/MEK/ERK) and proteins (PTEN proteins) protect the cells. Mutation in genes that are associated with those pathways result in continuous cell division and proliferation and prevent apoptosis (Majeed et al., 2014). PTEN proteins turn off the PI3K/AKT pathway, so a mutation in these proteins causes uncontrolled proliferation of tumor cells (Majeed et al., 2014).

1.2 Breast cancer subtypes

There are several types of breast cancers. Among these, 70 % is hormone-dependent (HD) (Lumachi et al., 2013) meaning that these cancer cells and tumors have hormone receptors (HR). There are two kinds of these receptors in the nucleus, the estrogen receptor (ER) and progesterone receptors (PR) (Lumachi et al., 2013). ER is the primary transcription factor driving oncogenesis in HR+ breast cancer (Lumachi et al., 2013). There are two kinds of ER in the ER tumor cells, those that found in the cell membrane and those in the cell nuclei (Edwards & Boonyaratanakornkit, 2003). HD breast cancers depend on the presence of estrogen and/or progesterone for cell growth and proliferation (Lumachi et al., 2013). Another receptor is the human epidermal growth factor receptor 2 (HER2), a protein (Lumachi et al., 2013) that is located on the cell surface. The activa-

tion of HER2 leads to activation of downstream pathways that promotes cell growth, survival and differentiation (Iqbal & Iqbal, 2014).

There are two hypotheses explaining the association between estrogen and breast cancer:
1) The binding between estrogen and ER enhances cell proliferation of mammary cells, and increase in cell division and DNA synthesis elevates the risk for replication errors that may result in mutations (Deroo & Korach, 2006).
2) Estrogen metabolism leads to the production of genotoxic byproducts that could damage DNA, leading to point mutations (Deroo & Korach, 2006). The link between estrogen and breast cancer may explain why breast cancer occurs 100 fold more frequently in women than men because of their higher estrogen levels (Santen, Yue, & Wang, 2014). The breast cancer incidence increases with age but slow down at age 50 which is the average age of menopause, which indicates that female reproductive hormones are involved in the breast cancer risk.
Breast carcinomas are classified into four subtypes (figure 1.2) according to which receptors they express and whether they are associated with low or high amounts of the Ki-67 antigen. Ki-67 is a protein associated with cell proliferation (Lumachi et al., 2013).
The four subtypes:

1) Luminal A breast carcinomas represents 50-60 % of all breast cancers (Lumachi et al., 2013). Patients with this type of cancer have a good prognosis (Eroles et al., 2012).
2) Luminal B have a more aggressive phenotype than luminal A and makes up 10-20 % of all breast cancers. The difference between luminal A and B is that luminal B has an increased expression of proliferation genes (Lumachi et al., 2013).
3) Non-luminal, with HER2 overexpression (HER2+): this type of breast cancer has an overexpression of HER2 and genes associated with the HER2 pathway. Non-luminal with HER2 expression represent 15-20 % of all breast cancers (Eroles et al., 2012).
4) Basal-like tumors represent 10-20 % of all breast cancers. They do not express the HR and HER2. They are similar to triple-negative breast cancer but the concordance between these two subtypes is not 100 %. This type of breast cancer have a poor prognosis and are very aggressive (Eroles et al., 2012).

The knowledge of these subtypes can tell which patients that will benefit from hormonal or cytoxic therapy (Eroles et al., 2012).

Parameter	Luminal A	Luminal B HER2-	Luminal B HER2+	Non Luminal	Basal-Like
ER and/or PR	Positive	Positive	Positive	Negative	Negative
Ki-67	Low	High	Low or high	Low or high	Low or high
HER2	Negative	Negative	Amplified or overexpressed	Amplified or overexpressed	Negative

Figur 1.2 (Lumachi et al., 2013)

2.1 Cholesterol

Cholesterol is a sterol; a structural lipid with a steroid nucleus, and it is the major sterol in animal tissue (Nelson & Bulun, 2001). It is a component in the lipid membranes of the cells and the precursor for steroid hormones, bile salts and various specialized molecules (Widmeier et.al. 2014) figure 2.1

2.2 Structure

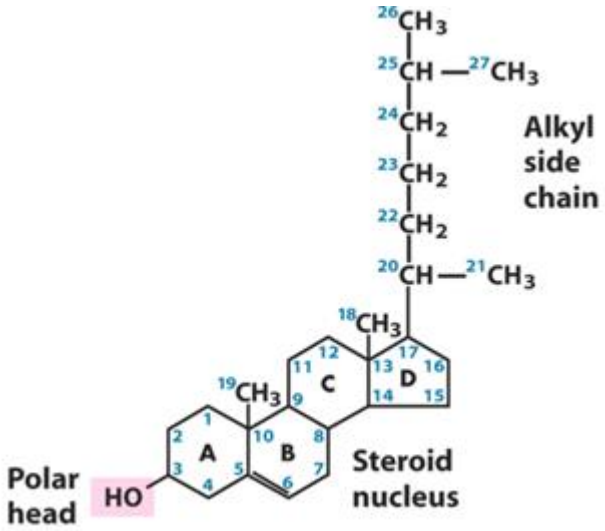


Figure 2.1 shows molecular structure of cholesterol (Nelson & Cox, 2013).

The hydrophobic steroid nucleus and alkyl sidechain with the polar head makes cholesterol amphipathic, which is a common trade for the membrane lipids. The steroids derived from cholesterol are missing the alkyl side chain that binds to the steroid nucleus.

2.3 Synthesis

All mammalian cells can synthesize cholesterol, but the synthesis is regulated by dietary cholesterol so that the plasma cholesterol stays somewhat stable. The homeostasis of plasma cholesterol is mainly controlled by the liver, which can both deliver cholesterol to the blood stream and remove it

(note that only liver cells and the cells lining in the gastrointestinal tract secrete cholesterol into the blood (Widmeier et al., 2014)). The regulation happens via a negative feedback on the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase which is inhibited when the plasma cholesterol increases and is released from inhibition when the plasma cholesterol decreases (Nelson & Cox, 2013; Widmeier et al., 2014). Excess cholesterol extracted from the blood is also secreted to the bile to maintain homeostasis (Widmeier et al., 2014).

Cholesterol's precursor is acetate and the synthesis happens in four stages (Nelson & Cox, 2013):
I: *Acetate → mevalonate*
This step consists of two condensations; from 2 acetyl-CoA to acetoacetyl-CoA, catalyzed by acetyl-CoA acetyl transferase (CoA-SH), which condenses with a third acetyl-CoA to HMG-CoA, catalyzed by HMG-CoA synthase (Nelson & Cox, 2013).
A third reaction reduces HMG-CoA to mevalonate, this reaction is catalyzed by HMG-CoA reductase.

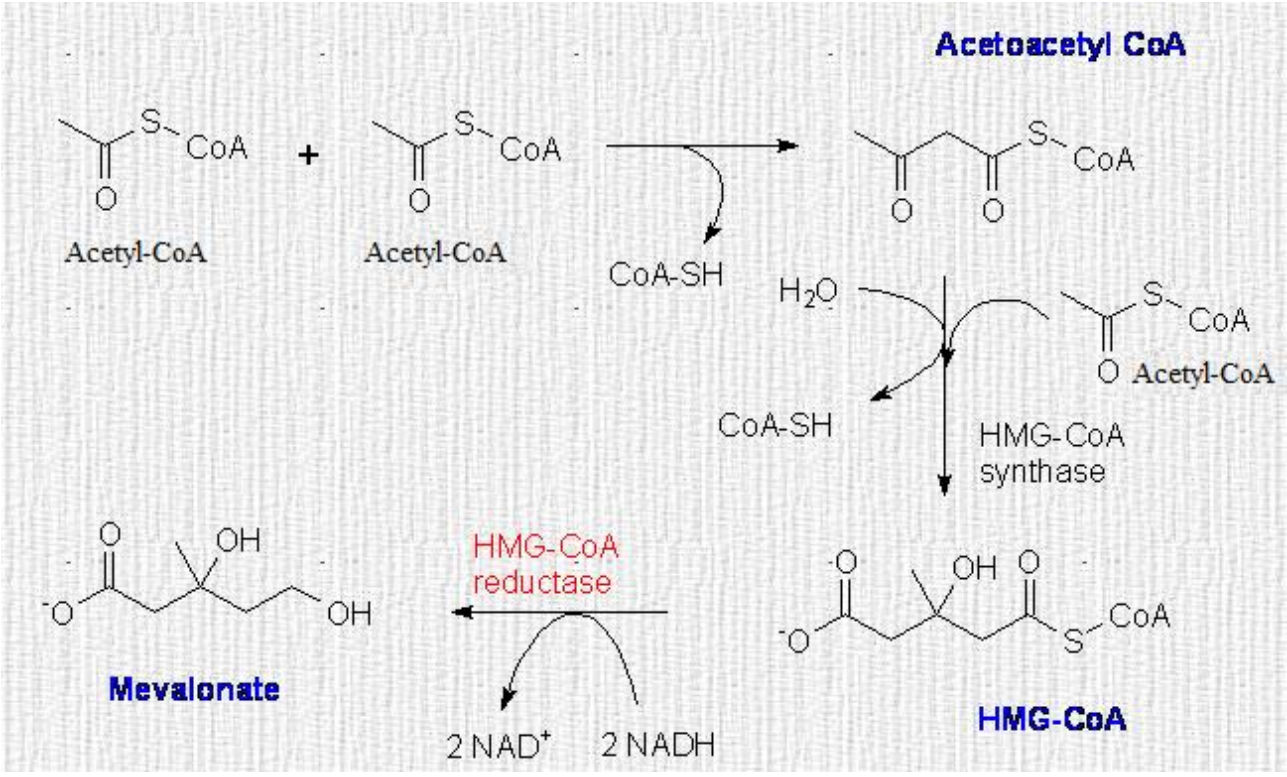
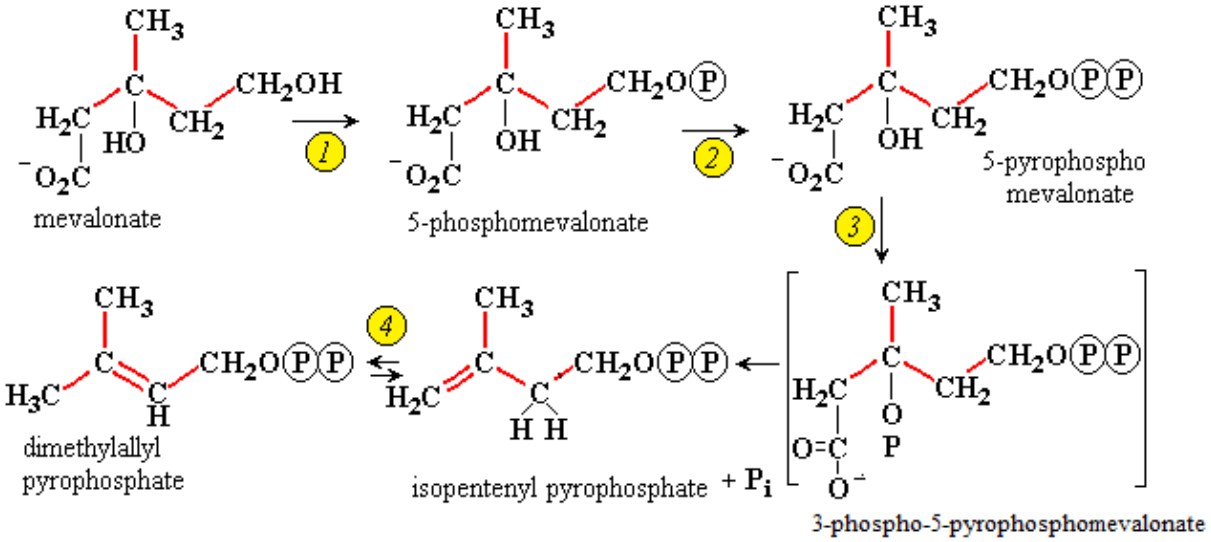


Figure 2.3.1 shows the conversion of acetate to melanovate.
(http://chemistry.umeche.maine.edu/CHY431/Cholest2.html)

II: *Mevalonate → 2 activated isoprene*

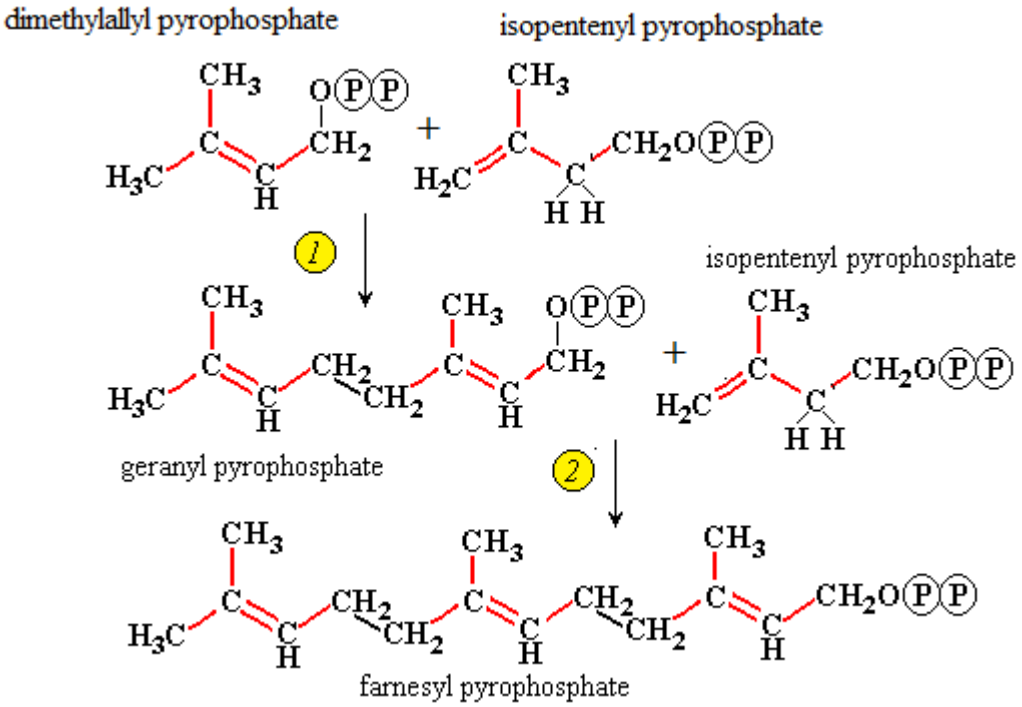
First mevalonate is phosphorylated in two steps catalyzed by three different enzymes: mevalonate 5-phosphotransferase, phosphomevalonate kinase and pyrophosphomevalonate decarboxylase; the intermediates are 5-phosphomevalonate, 5-pyrophosphomevalonate and 3-phospho-5-pyrophosphomevalonate. The next step is a decarboxylation of 3-phospho-5-pyrophosphomevalonate to 3-isopentenyl pyrophosphate, catalyzed by pyrophosphomevalonate decarboxylase. An isomerization of 3-isopentenyl pyrophosphate, catalyzed by isopentenyl pyrophosphate (IPP) isomerase, then creates an equilibrium between 3-isopentenyl pyrophosphate and dimethyallyl pyrophosphate.



Figur 2.3.2"*Mevalonate → 2 activated isoprene* " (modified from http://www.chembio.uoguelph.ca/educmat/chm452/lectur16.htm)

III: *6 activated isoprene units → squaline*

In this step isopentenyl pyrophosphate and dimethylallyl pyrophosphate condense head-to-tail where one pyrophosphate is displaced, and forms the intermediate geranyl pyrophosphate (GPP), which in the same way condenses with another isopentenyl pyrophosphate to form another intermediate; farnesyl pyrophosphate (FPP). Squaline is formed when two FPPs condense head-to-tail and in the same process, FPP loses both pyrophosphate groups.



Figur 2.3.3 shows the conversion of activated isoprene units to farnesyl pyrophosphate (http://www.chembio.uoguelph.ca/educmat/chm452/lectur16.htm)

IV: *Squaline → four-ring steroid nucleus*

In this step squaline monooxegynase adds one oxygen atom to the end of the squaline chain to form squaline2,3-epoxide, which in turn undergoes a cyclization that results in lanosterol. Lanosterol is then, by multiple steps that are not going to be explained in this report, finally converted into cholesterol.

2.4 Functions

Cholesterol has various, different functions in the human body. The main function relevant for this report is that cholesterol is a precursor for steroids, as androgens are converted to estrogens (Nelson & Cox, 2013).

2.5 Transport within the body

Many cells use cholesterol, but they only synthesize little themselves. Due to this, cholesterol is transported through the blood by various different transport proteins; chylomicrons, very-low-density lipoproteins (VDL), low-density lipoproteins (LDL) and high-density lipoproteins (HDL). LDL is the main cholesterol carrier and is responsible for delivering cholesterol to cells throughout the body. LDL binds to membrane receptors and is then absorbed into the cell via endocytosis. Cho-

lesterol, bound to LDL, is simply called LDL-cholesterol (LDL-C) (Widmeier et al., 2014). At the target cells LDL-C binds to receptors and is absorbed through the cell membrane by receptor-mediated endocytosis where it is stored in non-membrane bound lipid droplets. It is released as free cholesterol by cholesterol esterase when the cell is stimulated in its use of cholesterol (Widmeier et al., 2014).

HDL does the opposite and transports excess cholesterol in the blood to the liver, that metabolizes the excess cholesterol into bile salts. HDL also transports cholesterol to steroid producing endocrine cells, but. The main transport is to the liver (Widmeier et al., 2014).

3.1 Estrogen Structure

Estrogen is a naturally occurring steroid hormone, which functions as the primary female sex hormone. There are three classes of estrogen: estrone (E1), 17β-estradiol (E2), and estriol (E3)

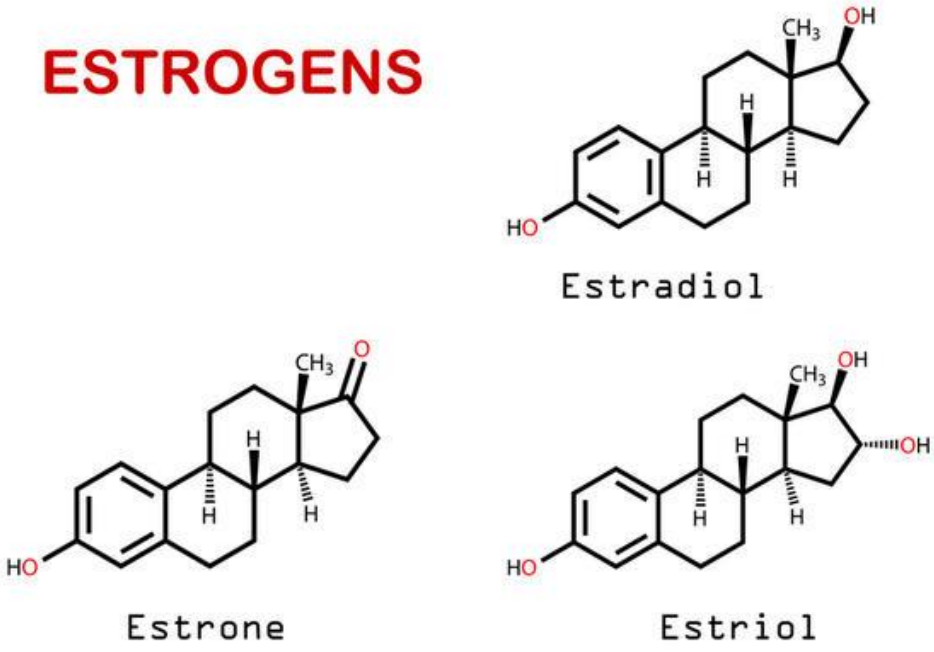


Figure 3.1.1 “Estrogen types”, all derived from cholesterol (Nilsson et al., 2001).
(http://www.blurrent.com/article/26-intriguing-facts-that-you-didn-t-know-about-estrogen-).

Estrogen is secreted in large amounts from the ovaries (Nilsson et al., 2001). Estradiol is the predominant estrogen in women in the plasma. Estradiol is primarily produced in the ovaries and is called estrogen, even though estrogen counts all three types (Nilsson et al., 2001). Throughout the rest of the report it will be referred to as estrogen, when it is not specified. Estrone is also produced

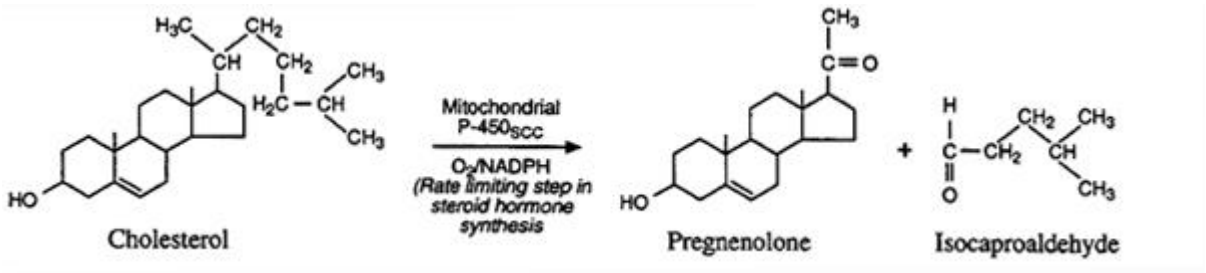
in the ovaries, and estriol is mostly produced in pregnant women in the placenta (Nilsson et al., 2001). In postmenopausal the estrogen production primarily takes place in the peripheral tissue (adipose tissue and skin). All three types of estrogen are produced from androgens (androstenedione or testosterone) by aromatase (Nilsson et al., 2001).

3.2 Synthesis from cholesterol

Estrogen is synthesized from cholesterol in several steps. In this chapter is a summary in five steps:

I: *Cholesterol pregnolone*

Within the inner mitochondrial membrane in steroid synthesizing tissue, cholesterol is converted to pregnenolone. This happens via, a hydroxylation sequence catalyzed by the enzyme cytochrome P450scc (CYP11A1) via the mechanism shown in fig 3.2.1 where the side chain of cholesterol is cleaved (Tuckey, 2005).



$$\text{cholesterol} \xrightarrow{2e^-} \text{22R-hydroxycholesterol} \xrightarrow{2e^-} 20\alpha,\text{22R-dihydroxycholesterol} \xrightarrow{2e^-} \text{pregnenolone}$$

fig. 3.2.1 “Cholesterol side chain cleavege”

(http://chemistry.gravitywaves.com/CHE452/21_Adrenal%20Steroid17.htm - 7/4-2015; Tyckey, 2004)

II: *Pregnolone progesterone*

Pregnenolone is then converted into progesterone by type 1 3-hydroxysteroid dehydrogenase (3-HSD) (Tyckey, 2004).

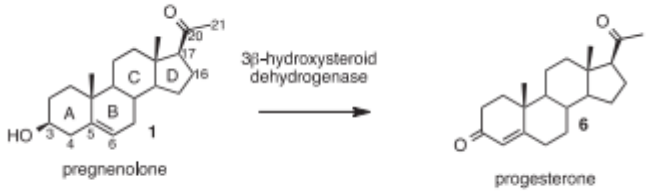


Figure 3.1.2 “conversion of pregnenolone to progesterone” (Yoshimoto & Auchus, 2014).

III: *progesterone androstenedione*

Androstenedione is derived from progesterone via hydroxylation catalyzed by the enzyme CYP17A1.

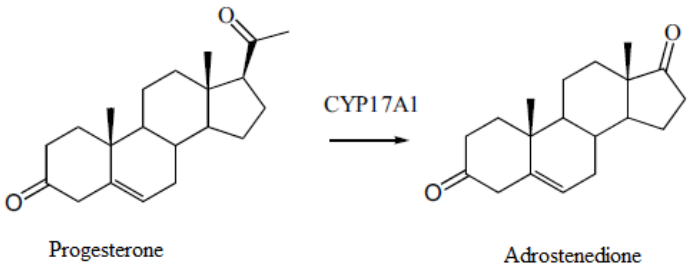
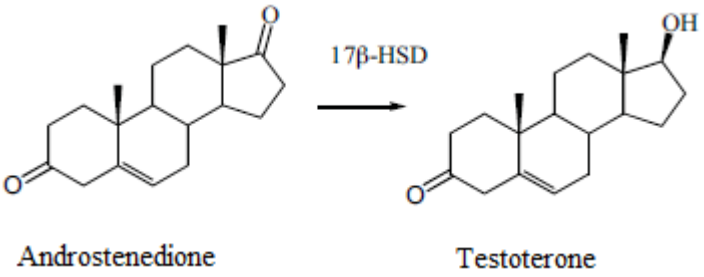


Figure xx “progesterone to adrostenedione” (Porubek, 2013)

IV: *Androstenedione to testosterone*

Adrostenedione is then reduced to testosterone by 17-hydroxysteroid dehydrogenase (17-HSD) (Nelson & Bulun, 2001) :



V: *Androstenedione estrone & testosterone estradiol*

Androstenedione and testosterone are secreted in small amounts by the ovaries and in larger amounts by the adrenal cortex (Nelson & Bulun, 2001), and some of these are converted to estrogen in other organs than the ovaries, such as adipose tissue (Nelson & Bulun, 2001).

Aromatization of androgens is the primary mechanism of estrogen production in postmenopausal women (Nelson et al, 2001).

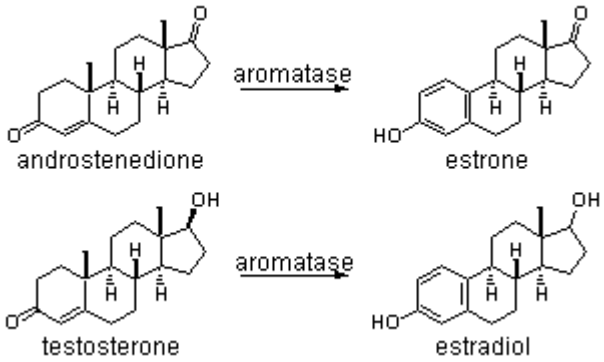


Figure x.x "aromatization of androstedeione and testoterone"
https://www.staff.ncl.ac.uk/i.r.hardcastle/antiendocrine.html

The androgens used in this mechanism are primarily circulating androstenedione from the adrenal cortex, which is converted into estrone. Estrone is weakly estrogenic and is locally reduced to estradiol for more estrogen activity. This reduction can cause cancer in postmenopausal women (Nelson et al, 2001).

In premenopausal women the estrogen production is fluctuating according to the menstruational cyclus (Widmeier et al.2014), but in postmenopausal women the estrogen production is constant. As a consequence of this, the breast tissue in these women is continuously exposed to estrogen (Nelson et at, 2001). However, the estrogen level is reduced in postmenopausal women whom's mean serum estrogen level is 25 pmol/l which is synthesized mainly in other tissues than the glandular, whereas in premenopausal women the mean serum estrogen level is 400 pmol/l which is synthesized primarily in the ovarians (Zidan, el al., 2010).

4.1 Estrogen receptors

There are two types of estrogen receptors, estrogen receptor (ER) and estrogen receptor (ER), which belongs to the steroid superfamily of nuclear receptors (Nilsson et al, 2001). They have three independent but interacting functional domains: the NH_2-domain, the DNA-binding domain (DBD), and the COOH-terminal, also called the ligand-binding domain (LBD). The NH_2-domain functions independently of ligands. The DBD plays an important role in receptor dimerization and binding of receptors to specific DNA sequences. The COOH-terminal is responsible for the change in conformation when ligands bind to an estrogen receptor and mediates receptor dimerization, nuclear translocation and transactivation of target gene expression. It is in the NH_2-domain the difference between ER and ER is found. In ER the NH_2-domain is very active in the stimulation of reporter-gene

expression from a variety of estrogen response element (ERE)-constructs in different cell lines but in ER the NH_2-domain is almost not active in this stimulation (Nilsson et al, 2001).
Both ERs have two transcriptional activation domains AF1, located in the NH_2-domain and AF2 located in the COOH-terminal. (Edwards & Boonyaratanakornkit, 2003). The core of the COOH-terminal is inactive as a transcription factor, but sufficient for mediating many rapid estrogen response pathways (Edwards & Boonyaratanakornkit, 2003).
It is commonly known that it is the ER α that is responsible in the development of breast cancer (Wang & Yin, 2015), and thus only this receptor will be discussed further.

4.2 Function

ER are located in the nucleus and estrogens diffuse freely over the cell walls (Deroo & Korach, 2006). The major actions of estrogens is the slow (within hours) direct transcriptional regulation of specific gene networks (Edwards & Boonyaratanakornkit, 2003). The estrogen-ER complex binds directly or indirectly to ERE-sequences, through protein-protein interactions with activator protein 1 (AP1) or specificity protein 1 (SP1) sites in the promotor regions of estrogen-responsive genes (Deroo & Korach, 2006). These interactions result in recruitment of coregulatory proteins (coactivators and -repressors) to the promoter; increased or decreased mRNA levels and associated increased or decreased protein production and other physiological responses, depending on the cell (Deroo & Korach, 2006). These are slow mechanisms that happen over the course of hours. Estrogens can also act more swiftly (within seconds or minutes) by not travelling all the way to the nucleus. It can bind either to ERs located in or adjacent to the plasma membrane or to non-ER estrogen-binding proteins associated with the plasma membrane (Figure 4.2.1) (Deroo & Korach, 2006). These interactions result in cellular responses such as increased levels of Ca^{++} or NO and activation of kinases. The rapid non-transcriptional activation of various signaling molecules and signal transduction pathways are independent of the synthesis of new mRNA and associated protein synthesis (Edwards & Boonyaratanakornkit, 2003).[5] [6] ERs are, as other steroid receptors, latent activators that require ligand binding for activation (Edwards & Boonyaratanakornkit, 2003). Binding of a ligand to ER changes the rate of transcription of estrogen-regulated genes. (Nilsson et al, 2001).
In the nucleus, ER mediates estrogen effects by different interactions with DNA, dimerization with other receptors, recruitment and interaction with transcription factors such as coactivators, and formation of preinitiation complex (Nilsson et al, 2001; Deroo & Korach, 2006). These DNA interactions happen through AF1 and AF2 (Edwards & Boonyaratanakornkit, 2003). It is unclear if the non-ER estrogen-binding proteins are unrelated to the ERs or whether they are a subpopulation to

the different associates of ERs, which associates with the plasma membrane to mediate rapid non-transcriptional actions of estrogen (Edwards & Boonyaratanakornkit, 2003).
The response to estrogen is dependent on the specific tissues and cells (Edwards & Boonyaratanakornkit, 2003).

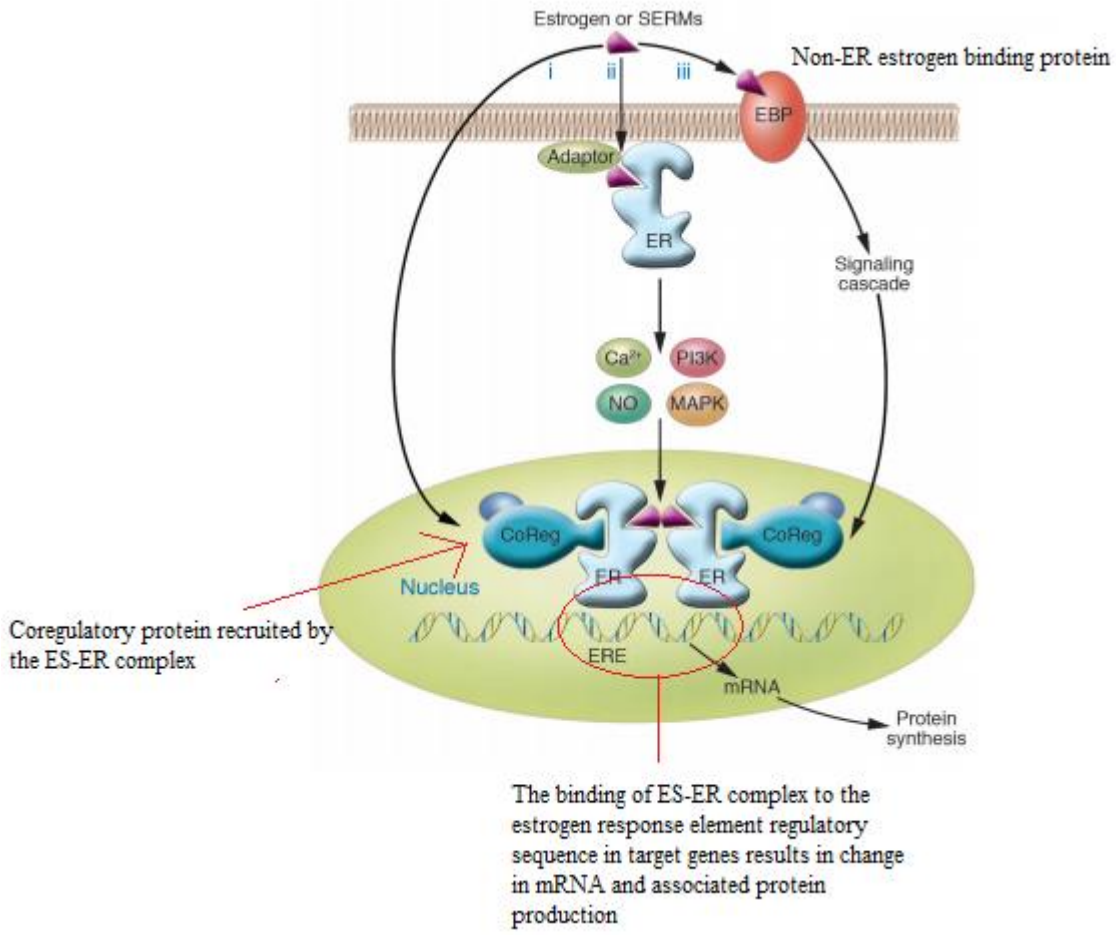
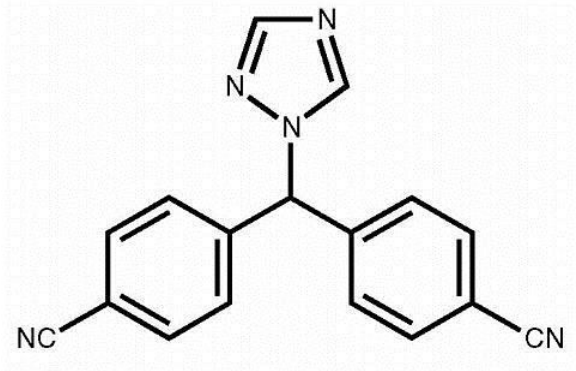


Figure 4.2.1 “Estrogen’s binding with ER and non-ER” (modified from Deroo & Korach, 2006)

5.1 Letrozole and its structure

Letrozole is a nonsteroidal, third generation, type II aromatase inhibitor (AI), where the type of the AI refers to the biological functions (Smith & Dowsett, 2003; (Scott & Keam, 2006)). Letrozole function as a competitive inhibitor and is highly selective in its inhibition (Scott & Keam, 2006).



5.2 How does letrozole function – its role in breast cancer?

AIs function by inhibiting or inactivating aromatase, the enzyme synthesizing estrogen from cholesterol, or more specifically estradiol and estrone from the androgens testosterone and androstenedione, respectively, and thus decreasing plasma estrogen levels (Smith & Dowsett, 2003). Aromatase belongs to the superfamily of the cytochrome P450 enzymes and is the rate-limiting step in the synthesis of estrogen (Smith & Dowsett, 2003). The nitrogen-containing parts of letrozole (Bhatnagar, 2007)bind reversibly to the heme group of the cytochrome P450 subunit of the aromatase enzyme (Smith & Dowsett, 2003). By inhibiting aromatase, letrozole inhibits estrogen biosynthesis throughout the entire body (Scott & Keam, 2006), but it has no effect on the synthesis of certain other hormones, such as thyroid hormones (Haynes et al., 2003)

Estrogen is believed to initiate and promote ER+ breast cancer (Yager & Davidson, 2006). Reduction of estrogens in the breast, by inhibiting aromatase (Smith & Dowsett, 2003), functions as prevention and treatment of breast cancer (Jeon et al., 2009).

In postmenopausal women with large primary breast cancer, treated neoadjuvant with AIs, it has been shown that letrozole profoundly inhibits in situ aromatase activity and thus it is reducing endogenous estrogen within the breast (Miller, 1999).

In a review, the use of AIs for treatment of postmenopausal endocrine-responsive breast cancer in the metastatic, adjuvant, and preoperative settings is processed (Briest & Davidson, 2007). AIs are widely accepted as treatment in postmenopausal women and are standard-care in first-line therapy of breast cancer (Briest & Davidson, 2007).

5.3 The use and potency of letrozole

Letrozole is used as first-line therapy and extended adjuvant therapy in postmenopausal women with hormone-responsive, early breast cancer (Scott & Keam, 2006). Breast cancer is the most common type of cancer in women in Western countries, especially in postmenopausal women

(Tobias, 2004). Estrogen is well-established as an important agent in development of breast cancer, as almost 70 % of postmenopausal having hormone-receptor positive (HR+) tumors (Tobias, 2004). Thus components reducing estrogen synthesis can be used in the treatment of HR+ breast cancer (Baum, 2004) because the stimulation of the estrogen-dependent tumors is reduced (Michaud, 2005). Letrozole significantly inhibits proliferation in estrogen-dependent tumors independent of human epidermal growth factor receptor (HER) status (Ellis et al., 2003).

5.4 Compared to other AIs and tamoxifen

Letrozole and other AIs have shown to be more effective than tamoxifen in the endocrine treatment of breast cancer (Smith & Dowsett, 2003). But tamoxifen has other beneficial effects. Tamoxifen functions as an agonist and thus it reduces the lipid levels (Bell et al., 2012).

The antitumor effects of letrozole were demonstrated and a prediction of letrozole's superiority to tamoxifen was made as well as predictions of letrozole's possibly superiority to other AIs. It was shown that letrozole is highly potent and selective of intracellular aromatase with an almost complete suppression of all body aromatization of estrogen (Bhatnagar, 2007). Furthermore letrozole was shown to be more potent that tamoxifen and to be the most potent AI of the third generation AIs in regards to both early breast cancer (EBC) and advanced breast cancer (ABC) (118, 123) (Bhatnagar, 2007).

A recapitulation of the current role of AIs and their potential for clinical use was made (Smith & Dowsett, 2003). It seems that letrozole is convincingly more potent of the AIs (Smith & Dowsett, 2003).

Successful translational research has shown that of the AIs the third generation is more efficient than both first and second generation AIs (Lønning, 2004).

It is unclear if one third generation AI is better than the other (Briest & Davidson, 2007). All third generation AIs are effective in regard to reducing breast cancer recurrence but in some cases no overall superiority to tamoxifen has been shown (Briest & Davidson, 2007). In other cases, letrozole have been shown to cause a greater reduction in breast cancer recurrence (disease-free survival) in women with breast cancer compared to tamoxifen Figure x.x (BIG 1-98 Collaborative Group, 2005). Furthermore it has been shown in the follow-up study (Regan, 2012) on BIG 98-1 Collaborative Group that letrozole use can lead to hypercholesterolemia (Keating, 2009).

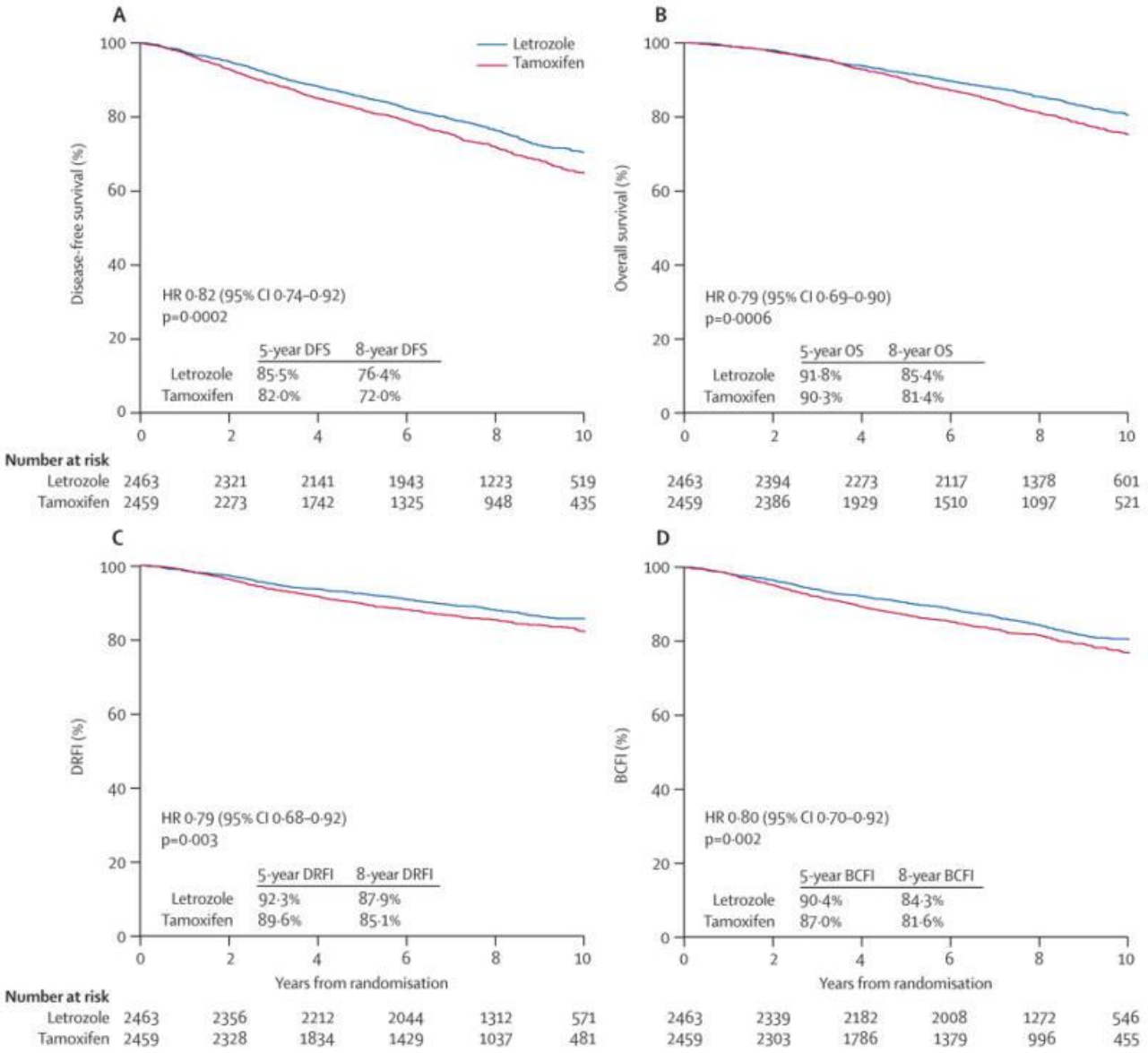


Figure x.x shows the disease-free survival among breast cancer patients treated either with tamoxifen or letrozole (Regan et al., 2011).

5.5 Effect on estrogen and lipid levels

In a prospective study the effect of letrozole on plasma lipids, triglyceride lipase, and estradiol levels in women with metastatic breast cancer was evaluated (Zidan et al., 2010). Total cholesterol and HDL-C was insignificantly increased after three months but returned to baseline values after six months (Zidan et al., 2010). Also LDL-C increased insignificantly but after six months, this value returned to baseline value after 12 months (Zidan et al., 2010). The estradiol value decreased from 44 pmol/l to less than 18 pmol/l before six months, thus indicating that letrozole has a safe effect on lipids and triglyceride lipase (Zidan et al., 2010).

Letrozole has shown no significant alteration but small increases of total serum cholesterol, LDL-cholesterol, and triglyceride levels after up to 36 months treatment among 347 participants (Wasan et al., 2005). But the more comprehensive study (BIG 1-98 Collaborative Group, 2005) which encompasses 8010 participants, showed the opposite in approximately 30 % of the patients (BIG 1-98 Collaborative Group, 2005).

5.6 Short- and long-term effects

Long-term effects of profound suppression are unknown and careful monitoring for bone demineralization should be made (Smith & Dowsett, 2003).

Furthermore AIs are believed to play a key role in future adjuvant breast cancer therapy but lack of cross-resistance between steroidal and nonsteroidal AIs still needs to be investigated (Lønning, 2004). The importance of postmenopausal estrogen levels to subsequent breast cancer development, body mass index, and plasma estrogen correlation has been substantiated by the plasma estrogen levels, bone demineralization, and breast density (Lønning, 2004). (Meta-analysis of the above: body mass index, serum sex hormones, and breast cancer risk in postmenopausal women).

Furthermore the long-term effects as bone demineralization, cardiovascular disease, and cognition still need to be evaluated (Briest & Davidson, 2007). Co-administration/drug-drug interaction of AIs has partly shown to affect some growth factors and their downstream products that interact with ER signaling (E-hypersensitivity, increase of signal transduction pathways, cross-talk between other signal transduction and ER pathways) (Briest & Davidson, 2007).

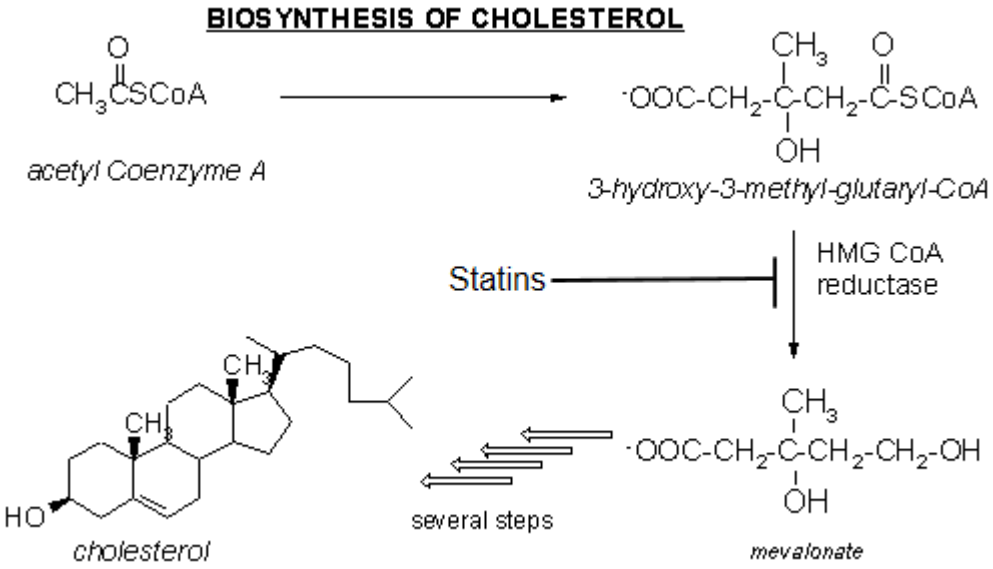
Due to AIs' inhibition of aromatase in every type of tissue, use of AIs could lead to a fear of bone loss and cognitive function, and thus a tissue-specific inhibition of aromatase expression is desirable and, as it turns out, possible because of decrease of estrogen synthesis in the ovaries in postmenopausal women (Simpson & Dowsett, 2002). In postmenopausal women estrogen is primarily local paracrine and intracrine, and tissue-specific promoters regulate each tissue (Simpson & Dowsett, 2002).

5.7 Pharmacokinetics of letrozole

Letrozole is rapidly absorbed after ingestion (Simpson et al., 2004). At steady state letrozole has nonlinear pharmacokinetics (Femara, prescription) and is highly distributed in tissues (Sioufi et al., 1997). Furthermore it is converted to its inactive metabolite by CYP3A4 and CYP2A6 isoenzymes and with cimetidine and warfarin co-administrations, no drug-drug interaction was found (Femara, prescription). But when letrozole is co-administered with tamoxifen the effect of letrozole is reduced (Femara, prescription).

6.1 Statins

Statins are cholesterol-lowering drugs used to reduce high cholesterol levels in the blood. Statins act as a strong competitive inhibitor with a 1.000-fold greater affinity to HMG-CoA reductase than HMG-CoA. Statins inhibit HMG-CoA reductase in the liver by binding to its active site (Manuscript, 2014), and thereby inhibit the conversion of HMG-CoA to mevalonate, used in the synthesis of cholesterol (figure 6.1.1) (Ahern et al., 2014).



Figur x http://www.people.vcu.edu/~urdesai/intro.htm

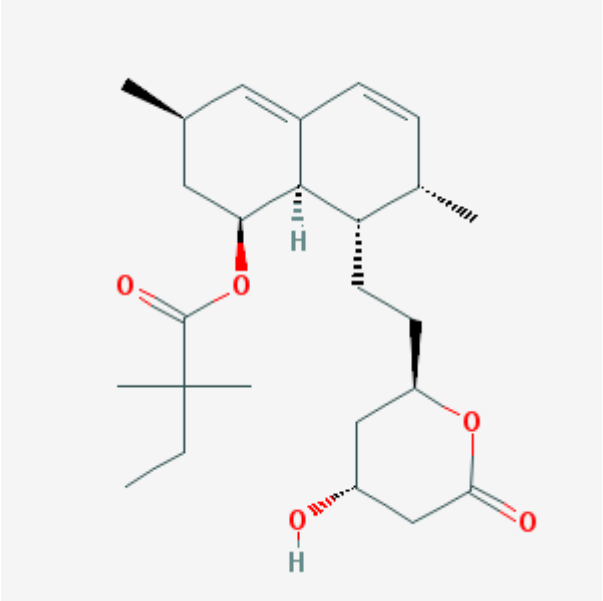
A resume of cholesterol biosynthesis and where statins inhibits it. Statins have a 1.000 greater affinity for HMG CoA reductase than HMG CoA and inhibits the reduction of HMG CoA to molanovate by binding to HMG CoA redutase's active site.

Statins are used to treat familial hypercholesterolemia, atherosclerosis and other diseases resulting from elevated cholesterol levels (Nelson & Cox, 2013). The lowering of LDL-C leads to the upregulation of LDL-receptors (Wang et al., 2008) and is the primary pathway of clearing circulating LDL-C from the blood (Lagor & Millar, 2010). Beside statins' cholesterol-lowering effects, (dos Santos et al., 2014) showed some association between LDL-C and cancer cell proliferation and migration. Statins may therefore contribute to the treatment of cancer.

Observational studies and clinical trials of statin use and breast cancer risk, have shown diverse results; some show no association, while others show an increase in breast cancer risk while report a protective effect by the use of statins compared to those patients not using statins (Desai et al., 2013). The most serious side effects by using statins are myopathy and rhabdomyolysis. It is assumed that myopathy is caused by the inhibition of FPP, which is an intermediate for ubiquinone or

coenzyme Q10 (CoQ10) and is important in mitochondrial energy production in the electron transport chain (Marcoff & Thompson, 2007). The FPP inhibition leads to CoQ10 deficiency and may lead to mitochondrial dysfunctional (Marcoff et al., 2007). The minor side effects are nausea, gastrointestinal pain, constipation and dyspepsia (Wong, Dimitroulakos, (Wong, Dimitroulakos, Minden, & Penn, 2002)).

Statins can be categorized as lipophilic or hydrophilic, they differ in their potency and solubility. Hydrophilic statins are more restricted to the liver where lipophilic statins easily can spread through extra hepatic tissues and therefor have greater potential to exert pleotropic effects (Ahern et al., 2014). There are several different statins categorized as lipophilic: simvastatin, cerivastatin, pitavastatin lovastatin, atorvastatin, fluvastatin and a few categorized as hydrophilic: pravastatin and rosuvastatin (Wong et al., 2002). The first natural statin discovered was mevastatin in 1976, but clinical trials in rats showed that mevastatin caused hepatocellular toxicity (Liao & Laufs, 2009). In 1979 lovastatin, a more potent inhibitor and not causing hepatocellular toxicity in rats, was isolated from *Aspergillus terreus* (Liao & Laufs, 2009). Therefore lovastatin became the first natural statin drug for human use (Liao & Laufs, 2009). Other natural statins are simvastatin and pravastatin. Statins are administered as active hydroxy acids except for simvastatin and lovastatin, which are consumed as lactones (inactive form) (Wong et al., 2002) pro-drugs (Gazzerro et al., 2012) and metabolized in the liver by CYP enzymes to active and inactive metabolites. Simvastatin (figure 6.1.2) the most prescribed statin is metabolized by CYP3A4 to beta-hydroxyacid, which is the active form of simvastatin. All statins share HMG-like moiety and can therefore inhibit HMG-CoA reductase by similar mechanisms (Liao & Laufs, 2009). The doses range between 5-80 mg/day for all statins and are consumed orally (Gazzerro et al., 2012).



Figur 6.1.2 http://pubchem.ncbi.nlm.nih.gov/compound/simvastatin

The molecular structure of simvastin.

6.2 Pleiotropic effects

The inhibition of HMG-CoA reductase leads not only to inhibition of cholesterol synthesis but also to the inhibition of downstream intermediates of the mevalonate (MVA) pathway, such as isoprenoids, squalene, steroids and others (Liao & Laufs, 2009). The inhibition of these other metabolites, leads to cholesterol-independent effects (pleiotropic effects), such as improvement of endothelial function, decreasing oxidative stress and enhancing the stability of atherosclerotic plaques (Liao & Laufs, 2009). These effects will be explained more detailed later in this chapter

6.3 Statins as a possible chemotherapeutic agent

The MVA pathway is responsible for synthesizing all mammalian isoprenoids. Isoprenoids in the MVA pathway such as FPP and geranylgeranyl pyrophosphate (GGPP) are metabolites important for post-translational prenylation of GTP-binding proteins (GTPase). There are many different/similar GTPases; Rac, Rho, Ras are some of them. GTPases acts as molecular switches controlling multiple pathways, cell functions, proliferation and apoptosis (Liao & Laufs, 2009) (Gazzerro et al., 2012). GTPases cycle between their inactive, GDP bound state and active, GTP-bound state and is depended on prenylation before they can exists in their active form (Wang et al., 2008). Statin use therefore leads to the accumulation of inactive GTPases (Liao & Laufs, 2009). The Ras proteins (H-Ras, K-Ras, N-Ras) also known as the Ras oncogene superfamily, depends on farnesylation to become active where Rho proteins (RhoA/B/C. Rac-1 and Cdc42) depends on geranyl-

geranylation. Activation of Ras proteins, in an inappropriate way, has a key role in proliferation, malignant transformation and signal transduction (Wang et al., 2008). The inhibition of HMG-CoA reductase and depletion of FPP and GGPP may therefore result in chemoprevention (Ahern et al., 2014) which leads to the possibility of using statins as a chemotherapeutic agent (Wong et al., 2002).

6.4 Endothelial dysfunction

Endothelial dysfunction is a result of hypercholesterolemia (Liao & Laufs, 2009). Some of the side effects of endothelial dysfunction are impaired synthesis, activity and release of endothelial-derived nitric oxide (NO) (Liao & Laufs, 2009). Endothelial-derived NO is important for vasodilation, the decreasing of vascular smooth-muscle proliferation, and inhibition of platelet aggregation (Liao & Laufs, 2009). Endothelial dysfunction is one of the earliest events that leads to atherosclerosis and studies suggest that endothelial function could be restored by statins (Liao & Laufs, 2009). Statin increase the NO production, by inhibition of GGPP or RhoA, resulting in upregulation of eNOS expression by prolonging eNOS mRNA half-life (Liao & Laufs, 2009).

6.5 Atherosclerotic plaques

Atherosclerosis is the formation of plaques in the blood vessels and is a type of cardiovascular disease (Reddy & Seshaiyer, 2015). Atherosclerosis starts when LDL-C accumulates in the intima of artery walls. In the arterial intima LDL-C undergo oxidation and becomes ox-LDL-C, which promote inflammatory processes (Wang et al., 2008), that causes endothelial cells to release monocyte chemoattractant protein (MCP-1). MCP-1 then attracts monocytes to the arterial intima where it matures to macrophages, which in turn engulfs the ox-LDL-C. This makes them lose motility and turns the macrophages into foam cells. Foam cells sends positive feedback that result in the releasing chemokines, which leads to recruitment of more macrophages. The macrophages release growth factors that stimulates the smooth muscles cells in the media, causing them to migrate into the arterial intima. When foam cells and smooth muscle cells die in the intima, they release their lipid contents, which forms a lipid core. A healing process against lipid core is the formation of a fibrous cap which creates plaques named thin-capped fibroatheromas. Macrophages release matrix metalloproteinases that will degrade the plaques and eventually lead to rupture (Reddy & Seshaiyer, 2015). This results in the contact between lipid core and blood, which in turn results in thrombosis. Statins contribute to plaque stability by lowering lipid concentration (Liao & Laufs, 2009).

6.6 Inhibition of ROS generation

NADPH oxidase complex is the collection of proteins that donate an electron from NADPH to oxygen and produce superoxide. The complex needs activation by GTPases Rac1 and rap1a. As mentioned before, statins inhibit isoprenoids, and Rac and rap1a therefore does not get activated (Wang et al., 2008).

Analysis

BMI, estrogen and breast cancer risk

In this part of the analysis the meta-analysis "Body Mass Index, Serum Sex Hormones, and Breast Cancer Risk in Postmenopausal Women" is analyzed to assess some possible factors in the relationship of BMI, estrogen and breast cancer. The different studies in the analysis are mainly commented on, on the basis of their abstracts and the information avaiable in the meta analysis. Two of the studies are analyzed further to see which factors may have contributed to the conclusion.

In a meta-analysis (Key et al., 2003), which was conducted by the Endogenous Hormones and Breast Cancer Collaborative Group (EHBCCG), it was investigated whether the increased risk of breast cancer caused by increased body mass index (BMI) could be associated with increased estrogen levels. The meta-analysis consists of eight prospective studies selected on specific terms, including:

- The data encompassing endogenous hormones and breast cancer risk using prospectively gathered blood samples from postmenopausal women (Key et al., 2003).
- Data on reproductive and anthropometric factors
- Date of diagnosis of case patients, date of birth, date of blood collection
- Cohort study where blood samples were collected from healthy women who were followed afterwards to identify breast cancer subjects
- None of the women used hormone replacement therapy or other exogenous sex hormones at time of blood collection
- Data on concentrations of the hormones were retrieved from blood samples
- All women were postmenopausal
- The studies were nested which means control subjects were paired with case subjects after diagnosis

The studies included in the meta-analysis have been examined before in another paper by EHBCCG to investigate the overall associations of sex-hormones with breast cancer risk in postmenopausal women. Common criteria for all the women in the studies included in this meta-analysis were data on BMI and prediagnostic estradiol levels which was available for 624 case subjects and 1669 control subjects. Additionally, the studies provided data on free estradiol, non-sex hormone-binding

globulin-bound (SHBG) estradiol (free plus albumin-bound estradiol), estradiol (total), estrone, estrone sulfate, androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone but these were available for fewer participants (Key et al., 2003). Furthermore height, weight, date of diagnosis of case patients, date of birth, and date of blood collection was part of the inclusion criteria. Women fulfilling these five criteria were eligible. The studies included women with both normal weight, overweight and obese women, according to WHO's definition of BMI[3] which make it representative for a larger population of women, and makes it easy to compare to for other studies.

The characteristics of the eight studies can be seen in table x.x

Table 1. Characteristics and body mass index (BMI) by case–control status and study*

Study†	Subjects	No.	Mean age, y	Mean y to diagnosis	BMI, kg/m^2 <22.5	22.5–24.9	25.0–27.4	27.5–29.9	⩾30.0	Mean (SD)
Columbia, MO, USA *(7,8)*	Case	71	61.4	3.3	6 (8.5)	23 (32.4)	16 (22.5)	16 (22.5)	10 (14.1)	26.5 (3.8)
	Control	133	61.8	—	23 (17.3)	39 (29.3)	29 (21.8)	13 (9.8)	29 (21.8)	26.6 (5.3)
Guernsey, UK *(9)*	Case	61	58.6	7.7	9 (14.8)	15 (24.6)	18 (29.5)	11 (18.0)	8 (13.1)	26.0 (3.2)
	Control	177	58.5	—	33 (18.6)	49 (27.7)	48 (27.1)	26 (14.7)	21 (11.9)	25.6 (3.8)
Nurses' Health Study, USA *(10)*	Case	155	61.8	2.4	32 (20.6)	28 (18.1)	36 (23.2)	20 (12.9)	39 (25.2)	26.9 (5.5)
	Control	310	61.8	—	68 (21.9)	83 (26.8)	59 (19.0)	37 (11.9)	63 (20.3)	26.2 (4.7)
NYU WHS, USA *(11,12)*	Case	127	58.7	2.0	21 (16.5)	33 (26.0)	31 (24.4)	23 (18.1)	19 (15.0)	26.1 (4.2)
	Control	246	58.5	—	73 (29.7)	71 (28.9)	48 (19.5)	20 (8.1)	34 (13.8)	25.1 (4.5)
ORDET, Italy *(13)*	Case	65	58.6	2.6	8 (12.3)	17 (26.2)	14 (21.5)	14 (21.5)	12 (18.5)	26.5 (4.0)
	Control	264	58.1	—	41 (15.5)	60 (22.7)	58 (22.0)	56 (21.2)	49 (18.6)	26.7 (4.1)
Rancho Bernardo, USA *(14,15)*	Case	31	64.3	10.4	9 (29.0)	8 (25.8)	7 (22.6)	4 (12.9)	3 (9.7)	24.8 (3.3)
	Control	286	64.9	—	86 (30.1)	100 (35.0)	50 (17.5)	26 (9.1)	24 (8.4)	24.5 (3.7)
RERF, Japan *(16)*	Case	23	62.6	7.5	9 (39.1)	4 (17.4)	8 (34.8)	2 (8.7)	0 (0.0)	23.5 (3.3)
	Control	45	62.3	—	22 (48.9)	11 (24.4)	7 (15.6)	3 (6.7)	2 (4.4)	22.3 (4.3)
SOF, USA *(17)*	Case	97	70.9	3.2	16 (16.5)	24 (24.7)	17 (17.5)	14 (14.4)	26 (26.8)	27.6 (5.4)
	Control	243	71.8	—	35 (14.4)	63 (25.9)	53 (21.8)	45 (18.5)	47 (19.3)	26.5 (4.3)
Total	Case	630	62.0	3.6	110 (17.5)	152 (24.1)	147 (23.3)	104 (16.5)	117 (18.6)	26.5 (4.6)
	Control	1704	62.4	—	381 (22.4)	476 (27.9)	352 (20.7)	226 (13.3)	269 (15.8)	25.8 (4.4)

*SD = standard deviation; NYU WHS = New York University Women's Health Study; ORDET = Study of Hormones and Diet in the Etiology of Breast Tumors; RERF = Radiation Effects Research Foundation; SOF = Study of Osteoporotic Fractures.

†For each study and in total, the numbers of case and control subjects with a BMI measurement, their mean age at blood collection, and years from blood collection to diagnosis (case subjects only), the numbers (percentage) in each BMI category, and the mean (SD) BMI are shown.

Table x.x "Characteristisc and body mass index by case-control status and study" (Key et al, 2003)

For the studies that weren't nested, this was done subsequently by EHBCCG within the cohort and control groups to make all eight studies comparable (Key et al, 2003). This means that the matching of control and case subjects were not as precise as the nested ones, as good matches were not always possible within the cohort. In one of the studies included in the meta-analysis (Berrino et al, 1996) it was proved that matching case and control subjects was important for controlling undesired variability. Only 4 out of 8 studies were nested. This means that the studies that were not nested contributes to an uncertainty when adjusting for age, menopausal status and time for the collection of blood samples. As half of the studies contributes to this, there might be some some undesired variabilities that are not accounted for.

Even though all studies adjusted for age when comparing hormone levels, the age did not seem to affect the amount of years from registration to diagnosis. This tendency can be seen in table x.x as the two groups with the lowest mean age has the next highest amount of years to diagnosis (Guemsey, UK) and the lowest amount of years to diagnosis (NYU WHS, USA).
The association with BMI was not assessed in the abstract of the studies involved in the meta-analysis, but anthropometric data was available in them, as that was one of the inclusion criteria for the meta analysis. In the two studies that is analyzed later in this chapter, BMI was not found to have a significant effect on the relative risk for breast cancer, but it is also mentioned that this might be due to lack of statistical power (Berrino F., 1996). Information about BMI was gathered both by self report and measured by trained staff at the institutions were the blood samples were taken (see appendix 1). The BMI from the SOF study is the least precise data, as the subjects (both case and control) who were at least 62 years old, had to report their height from when they were 25, which is even less precise than relying on women who had to report their current height. This was done to asses the problem that some of the women could have experienced osteoporotic height loss after vertebral fractures. A similar situation asserted itself in the Nurses's health study, as weight and height was reported as of the age of 18. Self-reported information about BMI is not as precise as if it was measured by the staff at the same time as the blood sample, because subjects might report it just by memory or might not have proper equipment and might report numbers that are rounded up or down. In the case of the SOF and Nurses's health study, the BMI from when subjects were younger is not representative for a woman in her 60'es or 70'es (mean age as seen in table x.x.), not to mention that at that time, they were most likely premenopausal.
That the effect of BMI on BC risk was not found significant in Berrino et al. (1996), possibly due to lack of statistical power, do not need to affect that the Key et al. (2003) found an association, as they had access to all the data from the included studies, which could contribute to the statistical power that Berrino et al (1996) was lacking.
The association with BMI and breast cancer was made as in "indirect link" as it was compared to the hormone levels and a positive association was found. This can be seen in figure x.x where BMI is compared to the mean concentration of estradiol (to the left) and the relative risk for developing breast cancer (on the right).

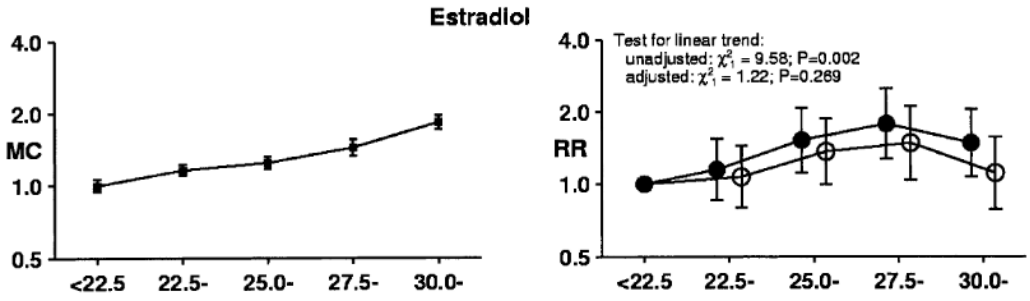


Figure AN.1.

Overall the case subjects had higher BMI than the control subjects (26,5 relative to 25,8). The two studies with the lowest mean BMI (Ranco Bernado, USA & RERF, Japan) have the largest amount of years from registration to diagnosis. This fits with the hypothesis that high BMI is a risk factor, but is not commented in the meta-analysis.

The relative risk according to BMI was calculated relative to category 1 (<22,5kg/m^2)

In this report only results for estradiol is shown, as that is the only one all the studies reported. Also it is the only hormone of interest to the group as, out of this selection of hormones, only estrogens are of relevance to the estrogen receptors and through them are of relevance to ER+BC.[9] Also estradiol is the most interesting one as the production of it increases with the adipose tissue in postmenopausal women and therefore is assumed to increase with BMI.

Conclusion summary for the meta-analysis regarding BMI, estrogen, and breast cancer risk

After adjusting for free estradiol, other estrogens, and risk factors the results still suggested that the association between increased breast cancer risk with increased BMI was the result of increased estrogen (Key et al., 2003).

Studies have shown positive association between estrogen levels and breast cancer risk and high BMI is considered a general risk factor for postmenopausal women. On top of that increase in BMI is positively associated with increased estrogen levels (see figure AN.1) .

The group has further more analyzed two of the studies included in the meta-analysis (Hankinson et al., 1998); Berrino et al, 1996) to see which factors may have contributed to the conclusion and especially how the studies assessed the connection with BMI.

The two studies were selected because they represented self-reported BMI and measured BMI thus could contribute to understand how this was different and were the largest studies in these two categories. A table of the characteristics for the two studies can be seen in appendix 2.
The cohort in Hankinson et al (1998) was almost 3 times as large as the cohort in Beronni et al. (1996). This was adjusted for in Key et al. (2003) by working with mean values and pooled values as all the cohorts were different size. All the different studies were large enough to asses an actual effect, but the studies with a case group of less than 50 subjects could be argued to only being hypothesis generating due to lack of cases to show an effect.

As mentioned before, the matching of case and control was important to avoid undesired variabilities. Beronni et al. (1996) had better matches over all, except for date of blood sample, which was the same day in Hankinson et al (1998). Both have good matches and opposite to the four studies in the meta-analysis which are not nested, possible contributes to better comparability between hormone levels, BMI and relative risk.

The subject information for all the women in the cohort in the Barroni et al. (1996) study was retrieved by trained nurses, including check for diagnosis. In the other study informations were retrieved, along with blood samples, from self-report. The interesting thing is that in this study the cohort consists of nurses, who assumably is more qualified to give precise information due to their profession, than the cohort from Italy, whom's professions were not taken into account. This makes the information more comparable than if it had been a cohort not consisting of nurses that had to self-report all the information.

Both studies had blood samples traveling: samples in Hankinson et al (1998) traveled from wherever the nurses got their blood samples taken, and samples in Barroni et al. (1996) from the other institute, so this shouldn't contribute to an uncertainty, but as it is not recorded how far the Hankinson et al (1998)'s samples had been moved other than it was within 26 hours, it is not sure.

The blood samples were stored on in a freezer for a different amount of time in the two studies.That it is not recorded for how long the individual blood samples had been stored in the freezer has no effect on the hormone levels. This was tested by Borrino et al (2006) and the levels were stable even after 3 years of storage. On the contrary that it made it possible to asses the blood samples more or less at the same time, with the same equipment, by the same staff makes the samples more comparable. The samples from Hankinson et al. (1998) was analyzed in three batches with 2 years

in between, this could make a difference if the same equipment was not used, but according to the stability of the hormones in the blood samples, it should not make a difference in comparability.

Metabolic syndrome and breast cancer risk

In a meta-analysis (Laezza et al., 2010) the potential association between metabolic syndrome (MS) and postmenopausal breast cancer risk was investigated by reviewing and analyzing nine epidemiological studies published between 2009 and 2012. Four of these were prospective cohort studies, two were nested case-control, two were case-control, and one was a retrospective Japanese cohort study (Table). In total the articles encompassed 6,417 cancer cases and for the cohort studies the sizes ranged from 5,450 to 287,320 participants while the case-control studies' control groups consisted of between 261 and 4,082 participants. All included studies were human studies, and should either provide adequate data to estimate the risks or report the risk estimates themselves besides definition of the syndrome. The components of the syndrome considered in the meta-analysis were BMI/waist circumference, hyperglycemia, higher blood pressure, higher triglycerides, and LDL-C. Studies were excluded if not published as full reports. Inclusion criteria comprised quality of election, comparability, exposure, and outcome of study participants (Esposito et al., 2013). Several data were extracted from each study, concerning name of the first author, year of publication, country where the study was performed, follow-up time, total number of individuals, number of cases, and risk estimates and 95 % confidence interval. Furthermore risk estimates for every single component were collected and compared to risk estimates of the full syndrome within the same study. For all studies the heterogeneity was quantified to describe the total variation across studies for subgroups according to populations and definition of MS, and the quantification showed that the overall heterogeneity among the studies was high. Publication bias was examined and indicated by analysis and was made insignificant by exclusion of biased studies after sensitivity analysis. Lastly, tests to assess true statistical significance were performed (Esposito et al., 2013).
In general, MS was associated with a 52 % increased risk for postmenopausal breast cancer, which changed a little depending on the populations and the definition of the syndrome but was significant irrespective of country. Separate and combined analyses were made in addition to sensitivity analyses. Meta-regression, a permutation test, and regression asymmetry test were performed. On the basis of this, BMI, triglycerides, and HDL-C were analyzed and discussed in this meta-analysis among others. Furthermore these factors were examined and mentioned as risk estimates and it was concluded that increased BMI and decreased HDL-C were associated with increased postmenopau-

sal breast cancer risk and that triglycerides were not associated with postmenopausal breast cancer risk, and that decreased HDL-C was associated with increased postmenopausal breast cancer risk. Overall the risk of postmenopausal breast cancer was moderately increased. Furthermore neither did increased BMI explain the risk that is related to the full syndrome nor did high levels of triglycerides or decreased HDL-C individually. It is unknown if the risk conveyed by the full syndrome is greater than the components combined, which means that maybe some of the components of the syndrome combined equals the risk of the full syndrome. This meta-analysis is the first to evaluate the role of hypertension, high triglycerides, and low HDL-C in postmenopausal breast cancer risk.

Letrozole and breast cancer risk

A prospective study conducted by Zidan et al. (2010) investigated the association between letrozole and lipid profiles, trigylceride lipase, and estradiol plasma levels in women with metastatic breast cancer (MBC), each receiving letrozole 2.5 mg/day. Blood samples were collected at baseline, after three, six, and 12 months. The characteristics of the study are gathered in table x.x where it is compared to another study by Bell et al. (2012).

The other study (Bell et al., 2012) on the AIs' (exemestane and letrozole) effect on the plasma lipid profile in postmenopausal women with breast cancer measured total cholesterol, HDL-C, LDL-C, and triglycerides before and after three months of treatment with either exemestane or letrozole. This was investigated due to AIs' deprivation of estrogen which is known to affect lipid concentrations and therefore cardiovascular disease. Furthermore postmenopausal women already have increased risk of altered lipid profiles and cardiovascular disease and thus this population is important to examine (see Appendix 3).

The duration of prior tamoxifen use was not taken into consideration during the analysis of the results, it was only considered if the patients had taken tamoxifen prior to AI treatment or not.

In the study conducted by Bell et al. (2012) the blood samples were collected after 3 months of AI treatment. Compared to the other study (Zidan et al., 2010) and the "normal" AI treatment duration (which is 2-5 or more years), this is a rather short time of investigation. Furthermore the study of Bell et al. (2012) made a comparison of an entire cohort, an exemestane group, and a letrozole group on the basis of the baseline values and the values after 3 months treatment whereas Zidan et al. (2010) compared letrozole with letrozole on the basis of the baseline values, the values after 3, 6, and 12 months of letrozole treatment. Neither of the studies compare to a placebo or control group but to the baseline values. In the study by Zidan et al. (2010) the percentages for description of par-

ticipants are not calculated as it is in the study by Bell et al. (2012). In both studies a patient characterization is made but it is not mentioned whether all these variables are taken into consideration or not. In Bell et al. (2012) a multivariate analysis was made, which showed that only baseline HDL-C was a significant predictor of the reduction in HDL-C following AI treatment. Also for exemestane, baseline HDL-C was an independent predictor. Both baseline LDL-C and prior use of tamoxifen were statistically significant predictors of change in LDL-C. This was also the case for letrozole (Bell et al., 2012). Furthermore the patients were stratified by prior use of tamoxifen but this did not affect the exemestane-induced reduction in HDL-C, but was partially responsible for observed elevations in LDL for letrozole patients because only women that had used tamoxifen prior to the letrozole treatment experienced a significant increase in LDL-C (Bell et al., 2012). The change in LDL-C in the entire lipid analysis cohort was significantly associated with baseline TC, LDL-C, LDL/HDL-ratio, and prior use of tamoxifen (Bell et al., 2012).

In patients receiving letrozole treatment no significant altering in HDL was shown (Bell et al., 2012). The explanation could be that letrozole does not produce an androgenic metabolite that binds to the androgen receptor, which exemestane does. Additionally the exemestane group had a higher baseline value for HDL-C.

The characterization is not completely similar in the two studies. The methods and statistics used by Zidan et al. (2010) are not very comprehensively described, and thus it is difficult to know what has been taken into account and what has not. The duration of the study of Bell et al. (2012) is 4 years in which the duration of the AI treatment is 2 years and for Zidan et al. (2010) it is 3 years but the duration of the treatment is "until disease progression", which is not defined. Thus, due to the duration of the entire study of Zidan et al. (2010) being shorter that the duration of the entire study of Bell et al. (2012), the progression of disease maybe can be considered shorter than the 2 years of treatment in Bell et al. (2012). In Zidan et al. (2010) the criteria for termination of previous anticancer treatment was determined to be at least 1 month before initiation of letrozole treatment and the median time for the termination was 12 months. In Bell et al. (2012) no such termination time is set (or at least described) and thus the effects of prior treatment can possibly influence the measured values at both baseline and after 3 months of AI treatment. In the exclusion from lipid analysis by Bell et al. (2012) the sum of the excluded participants (n=254) subtracted from all the enrolled participants (n=503-254) gives another number (n=249) that the number of the included participants (n=246). This is not mentioned in the study but perhaps it is due to deaths during the trial. Furthermore some of the exclusion criteria are made to adjust for confounders. Because of Bell et al.

(2012) only measuring values at baseline and after 3 months of AI treatment it is impossible to say, if the changed lipid values would have returned to baseline values after 6 or 12 months or treatment. Table x.x is a comparison of the measured values in each of the studies after 3 months of treatment with letrozole (modified from Zidan et al., 2010; Bell et al., 2012).

Alteration/measured	Bell et al., 2012	Zidan et al., 2010
TC	Significantly increased	Insignificantly increased
HDL-C	Insignificantly decreased	Insignificantly increased
LDL-C	Significantly increased	Significantly increased
LDL/HDL ratio	Significantly increased	Not registered
TG	Insignificantly increased	Baseline-like value

Table shows the results after 3, 6, and 12 months of treatment with letrozole (modified from Zidan et al., 2010).

Alteration/measured	After 3 months	After 6 months	After 12 months
TC	Insignificantly increase	Returned to baseline value	Returned to baseline value
HDL-C	Insignificantly increased	Returned to baseline-like value	Returned to baseline-like value
LDL-C	Significantly increased	Significantly increased	Returned to baseline value
TG	Baseline-like value	Insignificantly increased	Insignificantly increased
Estradiol	Significantly decreased	Significantly decreased	Significantly decreased

In the substudy conducted by Bell et al. (2012) the participants besides AI treatment also used lipid-altering medication. The population was generally older and had an increased BMI but was otherwise similar. The results of the substudy showed that all the measured values significantly were lower at baseline compared to the patients only treated with AI except for triglycerides. Overall AI therapy induced a significant decrease in TC and HDL-C. Also these patients were stratified in regard to prior tamoxifen use (Bell et al., 2012).

Adverse effects on lipids are undesirable due to cholesterol and triglycerides indicating long-term cardiovascular disease risk (Bell et al., 2012). HDL-C was not significantly changed in letrozole patients but for women both taking letrozole and lipid-lowering medication a significant reduction was observed. The study by Bell et al. (2012) is the first comparison of steroidal and nonsteroidal AI in postmenopausal women with breast cancer and the first to independently analyze patients treated with both an AI and lipid-lowering medication.

Prior use of tamoxifen was an independent predictor of increased LDL both in the entire cohort and for letrozole users which makes sense because tamoxifen reduces LDL-C. Prior tamoxifen use in the exemestane group did not increase LDL-C as pronounced as in letrozole, especially in the lipid-lowering group which means that letrozole possibly increases LDL.

Conclusively, long-term observations of effects of AI are important (Bell et al., 2012).

In the study conducted by Zidan et al. (2010) it was concluded that the letrozole does not affect the lipid profiles or triglycerides of women with breast cancer but suppresses estradiol to a high degree. Whereas the conclusion of the other study (Bell et al., 2012) was that significant and adverse changes of the lipid profiles of breast cancer patients was observed but it was not possible to determine whether the observations were due to tamoxifen washout or AI treatment.

Other studies have proposed that nonsteroidal and steroidal AIs are equally effective and safe due to indirect comparisons but yet others found different results in regard to lipid profiles for exemestane and letrozole (Bell et al., 2012). This is suggested by Bell et al. (2012) to possible be due to differences in study design and genetic and/or population differences.

Statins and breast cancer risk

Intro: The articles this report used for its analysis of the association between statin use and cancer-related mortality and breast cancer mortality were selected on the basis of the meta-analysis by Zhong et al. (2015) that used 41 articles; “thirty-nine cohort studies and two case-control studies”(Zhong et al., 2015).

The articles used for this analysis were based on criteria; the size of the study population, ethnicity, statins' impact on cancer mortality and breast cancer mortality, and that they fulfilled the search criteria.

Zhong et al. (2015) examined the therapeutic value of statins and their association with survival in cancer patients for both cancer-specific mortality and all-cause mortality in relation to pre-diagnosis and post-diagnosis. The study (Zhong et al., 2015) involved 990,649 participants. The articles used for the meta-analysis were only included if they met the inclusion criteria;

a) The exposure of interest was statin use assessed before or after diagnosis
b) The study design was case control or cohort
c) The outcomes of interest were all-cause mortality or cancer-specific mortality
d) The follow-up period was longer than 1 year
e) Risk estimates of mortality and 95% confidence intervals (CIs) were reported (or information to calculate them (Zhong et al., 2015).

The results from the 41 articles were all gathered in a table (appendix 4) after "name of the author, year, country, follow-up period, study design, patient characteristics, statin use, cancer-specific mortality, all-cause mortality, and covariate adjustment" (Zhong et al., 2015). One of the largest cancer groups in (Zhong et al., 2015) was breast cancer, which showed a benefit from statin use. It was observed that post-diagnosis statin use, reduced the "risk of death from cancer in breast patients"(Zhong et al., 2015). Statin use prior to diagnosis reduced the risk of any cause of death in breast cancer patients and a decrease in death from cancer was also observed (Zhong et al., 2015).

Conclusion regarding statins and breast cancer risk

The average effect of statin use compared with non-users had a beneficial effect (Zhong et al., 2015) for overall survival and cancer-specific survival (Zhong et al., 2015) in both pre-diagnosis and post-diagnosis (figure A and B).

Table ST.1 The association with post-diagnosis statin use with mortality (Zhong et al., 2015)**.**

Group	Pre-diagnosis statin use in relation to all-cause mortality	Post-diagnosis statin use in relation to all-cause mortality
Stratified by	Reduced risk of death from any cause was	Statin use was associated with decreased

cancer type	found in both breast and colorectal cancer patients	risk of death from prostate and ovarian cancer
Subgroup by gender	Significant benefit for overall survival in both male and females	Significant benefits for overall survival in both male and females

Table ST.2 The association with pre-diagnosis statin use and mortality (Zhong et al., 2015).

Group	Pre-diagnosis statin use in relation to cancer-specific mortality	Post-diagnosis statin use in relation to cancer-specific mortality
Stratified by cancer type	Decreased risk of death from cancer in breast, colorectal and prostate cancer patients	Decreased risk of death from cancer found in breast and prostate cancer patients
Subgroup by gender	Significant beneficial for survival in both male and females	No association in women, but in males a decrease in mortality was observed

The first article selected from the meta-analysis Zhong et al., (2015) conducted by (Nielsen, Nordestgaard, & Bojesen, 2012)), that examined the use of statins and cancer mortality in patients from the Danish population who had received a diagnosis between 1995 and 2007 (Nielsen et al., 2012) and followed them until December 31, 2009. The group consisted of 295,925 patients out of which 18,721 had used statins regularly before diagnosis and 277,204 reported to never had used statins (Nielsen et al., 2012). The cancer patients included were age 40 or older, those under age 40 were excluded. Statin users that have statin prescriptions filled 2 years before diagnosis and down to at least 6 months before diagnosis were defined as regular users (Nielsen et al., 2012).
Statin use was only recorded before the date of cancer diagnosis from patients prescription and was used to indicate statin use before and after cancer diagnosis. Information about diagnosis of cardio-vascular diseases and diabetes was gathered, but did not have any effect on the outcome of the study. Information about covariates such as race, ethnic descent, levels of education, size of residen-tial area was gathered for all patients (Nielsen et al., 2012).

Results regarding statins and breast cancer

During the follow-up which ended december 31, 2009, 195,564 patients died, 162,067 deaths were cancer related, 14,489 deaths was caused by cardiovascular diseases and 19,038 deaths were due to other causes (Nielsen et al., 2012). There was a difference between statin-users and non-users. Both any cause of death and deaths caused by cancer were lower among statin-users (figure A). thatmay be caused by statins cholesterol-independent effects. Because of the cardiovascular mortality among statin users the curve converge after 5 years (Nielsen et al., 2012).

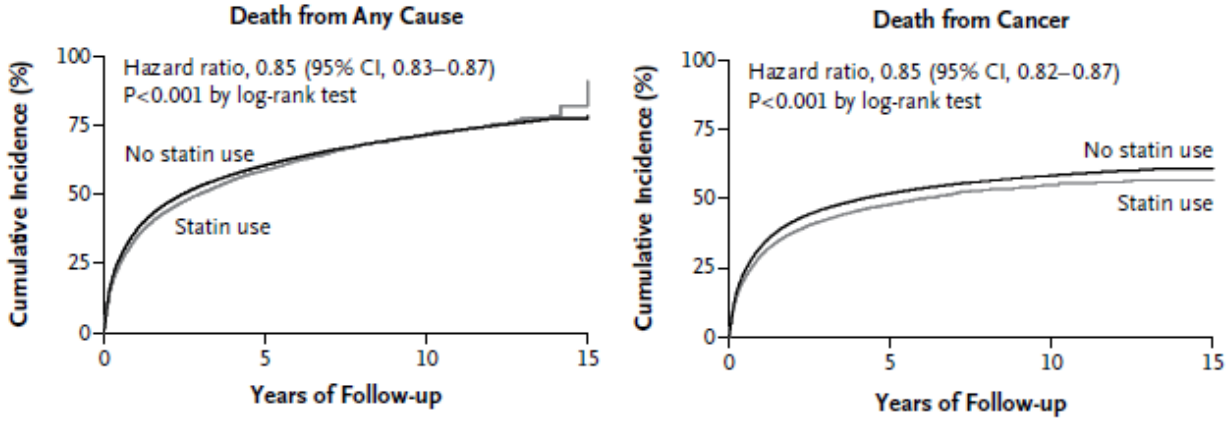


Figure A (Nielsen et al., 2012).

The second article that fulfilled the search criteria was a study conducted by Desai et al. (2015) used the Women's Health Initiative to collect data and investigate prior statin use and breast cancer stage at diagnosis and mortality. The results showed that breast cancer mortality was slightly lower in statin users compared with non-users and that statins were associated with lower breast cancer stage at diagnosis. The study was composed of an observational study (n= 93,676) and clinical trials (n = 68,132). Desai et al. (2015) excluded women "who did not report a mammogram within 5 years of study entry, women with no health insurance, no reported medical care provider, prior history of breast cancer, no information on follow-up, missing information on stage in incident breast cancer, and missing information on baseline statin use" (Desai et al., 2015). This resulted in a study population including 128,675 postmenopausal women aged 50 – 79 years (Desai et al., 2015). At the beginning of the study, there were diagnosed 7,883 newly cases of in situ, local, regional and distant stage breast cancer (Desai et al., 2015). The cases were stratified into early (in situ and local) versus late (regional and distant) stage breast cancer (Desai et al., 2015). The study was terminated in 2010. "Information on statin intake was collected at years 1, 3, 6 and 9 in the clinical trials, and year

3 in the observational study" (Desai et al., 2015). The participants were listed in table 1 (appendix x) that included socio-demographic and medical history, variables that could have an impact on the outcome of the study (Desai et al., 2015).

Results regarding statins and breast cancer

Lipophilic statins were associated with a reduction in diagnosis of late-stage breast cancer compared to non-users and also a significantly lower risk among women with late-stage ER-positive breast cancer in the time-dependent analysis was found (Desai et al., 2015). The mortality was slightly lower in statin users compared with non-users in the time dependent analysis but not in the multivariable model (Desai et al., 2015). The result fit to the theory that statins may act as chemotherapeutic agents through cholesterol-independent pathway. In the late-stage breast cancer models, women were censored if they had early-stage breast or died during follow-up. Censoring the women who died under follow-up shows a misleading association between statin use and late-stage breast cancer. Censoring women that died under the follow-up, they are not accounted for in the effect of statins and their association with late-stage breast cancer, which shows a wrong result of statins chemotherapeutic effects.

Model	Multivariable model	Multivariable time-dependent analysis
Late-stage breast cancer (LSBC)	No significant association between statin use at baseline and LSBC	Modest but non-significant reduction in LSBC
LSBC, statin use, and ER+	A nonsignificant trend toward a lower risk of LSBC ER+ among statin users	Significantly lower risk of LSBC and ER+
Statin lipophilicity	Statin lipophilicity did not have a significant development of LSBC	Significantly lower hazard of LSBC among women that used lipophilic statins
BREAST CANCER mortality	No significant relationship between statin use and BREAST CANCER-specific mortality	Statin use associated with decreased risk of BREAST CANCER- mortality over time
BREAST CANCER,	No information given	No significant association between

statin use, and ER+		statin use and BREAST CANCER mortality and ER+
Statin lipophilicity	No significant relationship between type of statin and BREAST CANCER-mortality	No information given

As the table shows there was a non-significant association between statin use, late-stage breast cancer and cancer-specific mortality in the multivariable models also when stratified by ER status. In the multivariable time-dependent model, there was a reduction in LSBC with statin use but non-significant. There was a significantly lower risk of LSBC when stratified by ER status. Statin use was also associated with decreased risk of BC mortality in time-dependent analysis but no association was seen when stratified by ER status.

Discussion

The discussion will be based on the meta-analyses and the associated studies used in the analysis. Most of the analysis and studies focus on the risk and the mortality of cancer and breast cancer. This report tries to estimate whether statins reduce recurrence or not. Thus it will be assumed that the risk of breast cancer can be compared to recurrence in the following discussion.

BMI, estrogen, and breast cancer risk

In this part of the discussion it is mainly the first theory mentioned in chapter 1 about the association between estrogen and breast cancer: *The binding between estrogen and ER enhances cell proliferation of mammary cells, and increase in cell division and DNA synthesis elevates the risk for replication errors that may result in mutations (Deroo & Korach, 2006)* that will be focused on (see chapter 1.1). The reason for this is that no studies were found to assess the second thesis point mutations

In the meta-analysis "Body Mass Index, Serum Sex Hormones, and Breast Cancer Risk in Postmenopausal Women" (Key et al., 2003) an association between an increase in BMI and increased serum estradiol is not surprising. This is however not entirely surprising. As obesity is established as a risk factor for breast cancer in postmenopausal women (Cleary & Grossmann, 2009) and the main estrogen source for postmenopausal women is production in the adipose tissue (Yaghjyan & Colditz, 2011), it can be assumed that an increase in BMI also would cause an increase in estrogen producing cells and thereby an increase in the serum estrogen. The studies involved in the meta-analysis by Key et al (2003) all contributed to the hypothesis that high serum sex hormone levels and more specific estrogens preceded the development of breast cancer. This association between serum estrogens, BMI and the risk of developing breast cancer, might be applicable to the the risk factors of recurrence. Especially the association between risk of recurrence of breast cancer and high estrogen levels is supported by the fact that patients are given letrozole as a prevention for recurrence. The estrogen levels are reduced and so is the risk of recurrence. (Fedele et al., 2014) contribute to this possible association between high estrogen levels and breast cancer with the association they found that patients with increased BMI after treatment have increased risk of recurrence. The risk of recurrence was a significantly increased if the patient gained 2 kg/m^2 or more after treatment. This means that a postmenopausal woman with a normal BMI at 23kg/m^2 who were 1,65m tall would have an increased risk of recurrence if she gained 5.45 kg. An argument against this association with increase in BMI for increase in risk of breast cancer, is that

serum levels of hormones measured in the blood is not a complete representative for the estrogen production in the adipose tissue, but merely a representative of travelling estrogen in the blood. But as the proliferation of cells is stimulated by the ER which are activated by estrogen, it is not really important where the estrogen in the blood comes from, if the focus is only on the association of risk for related to estrogen. But as mentioned before, the adipose tissue is the main location for estrogen production in postmenopausal women, thus making the association with increased BMI possible.
The connection between breast cancer risk and BMI could also be a connection to hypercholesterolemia as obesity and hypercholesterolemia is often connected and BMI is recognized as a risk factor for breast cancer. If BMI increases with hypercholesterolemia, then the connection that is shown in Zohng which is that statins reduce the risk of breast cancer could be possible. The argument for this is that if hypercholesterolemia contributes to the increase in BMI, then lowering of cholesterol will make it easier to decrease BMI and through that maybe the amount of adipose tissue and thereby the estrogen production and the risk for recurrence.

MS, letrozole, and breast cancer risk

It is known that women with breast cancer have higher estrogen levels than women without breast cancer (Key et al., 2003). Also women with increased BMI have higher estrogen levels due to increased adipose tissue and aromatization. Furthermore postmenopausal women's estrogen production in the ovaries is highly reduced and most of the production happens in the adipose tissue (Yaghjan & Colditz, 2011). Thus postmenopausal women have increased risk of ER+ breast cancer, especially postmenopausal women with increased BMI. In the study concerning BMI and breast cancer risk (Key et al., 2003) it was found that the higher breast cancer risk related to higher BMI is due to an increased estrogen production.
Women with metabolic disease generally have increased BMI, decreased HDL-C, and increased levels of TG. In the study concerning MS (Esposito et al., 2013) both increased BMI and decreased HDL-C were associated with increased breast cancer risk but increased TG showed no association (Esposito et al., 2013). It could not be identified whether the individual components combined or the full syndrome, were associated with a greater risk of postmenopausal breast cancer or not (Esposito et al., 2013). Furthermore it is not possible to determine if it is all of the components or only some or if it is the association of the components of the syndrome that induces the increased risk of postmenopausal breast cancer (Esposito et al., 2013).

Letrozole is suspected to induce hypercholesterolemia but suppresses estrogen and thus reduces breast cancer recurrence risk to a very high degree. It is therefore important to investigate letrozole's influence on lipid profiles to determine its possible adverse effects compared to its desired and effective suppression of estrogen. In one (Bell et al., 2012) of the two studies (Zidan et al., 2010; Bell et al., 2012) concerning letrozole processed in the analysis of this report, an adverse effect on the lipid profile of women with breast cancer after 3 months of letrozole treatment was observed. But it must be taken into consideration that prior to letrozole treatment, 43 % of the letrozole group had been treated with tamoxifen so the alteration of the lipid profile could partly be due to tamoxifen washout because tamoxifen has a reducing effect on the lipids. The possible effect of tamoxifen washout depends on the termination of tamoxifen treatment and tamoxifen's "washout-time". The study does not mention how long before letrozole treatment the prior treatment was terminated. Additionally, the altered lipid profiles could possibly change further after the 3 months measurement and this is not accounted for in the study. Conclusively, the study demonstrated adverse short-term effects of treatment with letrozole that could possibly be due to tamoxifen washout. In regard to the results accomplished in the study (Bell et al., 2012) letrozole treatment could possibly induce hypercholesterolemia due to the significantly increased TC, LDL-C, and LDL/HDL ratio after the 3 months treatment. Furthermore HDL-C was insignificantly decreased which also can be a component in inducing hypercholesterolemia.

The other study (Zidan et al., 2010) concerning letrozole also showed an adverse effect of letrozole after 3 months treatment but the adverse changes in the lipid profiles were all returned to baseline after 12 months of treatment. Only TG was insignificantly increased after 12 months treatment but this do not affect the risk of breast cancer due to the meta-analysis regarding MS (Esposito et al., 2013) which showed that high levels of TG were not associated with increased breast cancer risk. However increased levels of TG may be associated with hypercholesterolemia.

The differences between the results after three months treatment of letrozole in the two studies (Zidan et al., 2010; Bell et al., 2012) may be due to several factors. Some of the values are different according to increase or decrease, whereas the others differ by the significance in alteration or no alteration at all. First of all, both the TC and the LDL-C values are increased. In both studies LDL-C is increased significantly. This could either mean that letrozole influences the LDL-C directly or that the potential washout of tamoxifen does. The TC value is significantly increased and the TG value is insignificantly increased in the study by Bell et al. (2012) whereas the TC value is insignificantly increased and the TG value is baseline-like (Zidan et al., 2010). This shows that all these

three values (TC, LDL-C, and TG) from the study conducted by Bell et al. (2012) are more distinctively increased than those in the other study. However it should be noted that these three values (Bell et al., 2012) all were lower at baseline than those in the study by Zidan et al. (2010). The HDL-C on the other hand was respectively decreased (Bell et al. 2012) and increased (Zidan et al., 2010) but insignificantly for both studies. None of the studies included patients using lipid-altering medication, the mean age did not differ much. It was 57 years for patients receiving letrozole (Bell et al., 2012) and 56 years (Zidan et al., 2010). But the number of participants varied greatly by 50 compared to 246. Furthermore the ethnicity varied between the two studies and among the participants within the study. Additionally the termination of prior anticancer treatment was not accounted for in one of the studies (Bell et al., 2012) while it should be at least 1 month before initiation of letrozole treatment in the other (Zidan et al., 2010). This can possibly influence the differences in the results. Also the type of prior treatment may affect the results together with lack of adjustment for this. Another condition to take into consideration is that Bell et al. (2012) investigated both exemestane and letrozole and thus the overall conclusion is marked by this, e.g. due to exemestane's significant decrease of HDL-C which could be caused by exemestane producing an androgenic metabolite that binds to the androgen receptor and androgens having an adverse effect on HDL-C.
In the study by Bell et al. (2012) a substudy was conducted. Approximately 24 % of the enrolled participants in the primary study used lipid-altering medication. The substudy consisted of participants (n=95) receiving either letrozole (n=54) or exemestane (n=41) combined with lipid-altering medication. 86 of the 95 participants used statins.
The results showed that the group using lipid-altering medication was not protected against the adverse effect of letrozole on the lipid profile (Bell et al., 2012) even though it was only the significant decrease in HDL-C and thus an increase in the LDL/HDL ratio which was adverse overall. This is not necessarily unexpected because statins and letrozole (possibly) affect cholesterol in two different ways. But it has not been investigated whether these two different effects are related – directly or indirectly – through pathways or reversible mechanisms. The alteration of the lipid profiles may be especially adverse in the substudy group to whom an alteration is undesirable due to their increased risk of cardiovascular disease besides the general increased risk of breast cancer recurrence. This indicates that in regard to letrozole's effect on altered lipid profiles which is related to increased breast cancer risk and thus recurrence, patients using lipid-lowering medication (statins) do not have a decreased risk of recurrence of breast cancer and furthermore they are at risk of getting cardiovascular disease.

In regard to this report the alteration in lipid profiles are interesting because patients with hypercholesterolemia are prescribed statins and statins may have a beneficial effect on breast cancer. Thus, if letrozole alters the lipid profiles negatively that could maybe lead to hypercholesterolemia which when diagnosed can be treated with statins.

Statins and breast cancer risk

Several studies have examined the association between statins and cancer, but show inconsistent results. It is assumed that statins have anti-cancer effects by inhibiting HMG-CoA reductase and inhibit the downstream products of the MVA pathway that are important for proper cellular functions (see chaper 6.1).

Zhong et al., (2015) observed that statin use, both post-diagnosis and pre-diagnosis, was beneficial for overall and cancer-specific survival. This did not differ from the results in Desai et al., (2015) and Nielsen et al., (2012), which observed beneficial effects of statin use and cancer-related mortality compared to statin non-users. Desai et al., (2015) observed that statins were associated with lower breast cancer-stage at diagnosis and a reduced cancer-related mortality was observed in cancer patients (Nielsen et al., 2012). The association between statins and cancer showed beneficial effects compared to non-users in all three studies (Desai et al., 2015; Nilsson et al., 2001; Zhong et al., 2015). There were a lot of similarities in the methods in studies which may explain some of the similarities. The results from Desai et al., (2015) differed a bit from Nielsen et al., (2012) and Zhong et al., (2015) because the beneficial effects of statins were found in the multivariable time-dependent analysis and not it the multivariable model. It may be assumed on the basis of the results in (Desai et al., 2015) that statins' beneficial effects only works in a time-frame. May due to the explanation that the patients became resistant to statins over time. Desai et al., (2015) & Nielsen et al., (2012) are some of the studies with a greater population included in the meta-analysis Zhong et al., (2015) and may therefore have a more reliable result compared to a smaller study as (Lipworth et al., 2013) used in the meta-analysis (Zhong et al., 2015). This study observed no association between statin use and cancer-mortality (Lipworth et al., 2013). The explanation for a different result may be due to the ethnicity of the population, that contained black and white people in the Southeastern United States. Little is known about statin use in black people, they may not have the same beneficial effect from statins as the study population in Desai et al., (2015) and Nielsen et al., (2012), which involved patients from Denmark (WHI and Danish Cancer registry). The majority of the study population in Desai et al., (2015) and Lipworth et al., (2013) used lipophilic statins and

the same is applied in (Nielsen et al., 2012) although it is not mention, it is assumed out from the data from Roskilde hospital that showed that the majority of the breast cancer patients were prescribed lipophilic statins. The use of lipophilic statins may be the reason for that the cancer-mortality in (Lipworth et al., 2013) did not decrease. If the majority of the patients in (Lipworth et al., 2013) had been prescribed hydrophilic statins instead, the decrease in cancer-mortality may had been decreased. Statins anticancer and anti-invasive effetcts may be caused by cholesterol independent effects such as the inhibition of the isoprenoids FPP and GGPP (see chap 6.1) are involved in posttranslational modification of many proteins, that are important for cellular functions such as cell proliferation (Desai et al., 2015), gene expression and more (Nielsen et al., 2012). Disruption of these proteins malignant cells results in inhibition of cancer growth and metastasis (Nielsen et al., 2012).

To investigate the connection between statins and reduction in recurrence risk further might be beneficial to make an intervention study where some postmenopausal women without hypercholesterolemia is given statins and then compared to a control group with the same criteria. The group have investigated the statin use in postmenopausal women in Region Sjaelland (appendix 6) which showed that approximately 30% of the population were given statins. It is possible that if, as a part of the intervention study, all these woman were screened for hypercholesterolemia, even more woman would prove to benefit from the statin use. This would leave a small population, that then had to be split in two to have a control group and a group who were given statins. The group given statins would be too small to be more than hypothesis generating.

Conclusion

The studies showed a lot of different results which perhaps was due to different populations and genetic variation. BMI was associated with increased risk of breast cancer due to increased estrogen levels. Metabolic disease showed that some of the components were associated with increased breast cancer risk but these components were not necessarily affected by letrozole treatment, which was suspected. The results of the letrozole treatment differed both due to duration-time, population, and prior treatment, especially tamoxifen. Thus it could not be concluded whether letrozole affected the lipid profiles of postmenopausal women with breast cancer negatively. In the studies concerning statin the results also differed. This was due to time-dependency, ethnicities, and type of statin. Furthermore our own data showed that approximately 30 % of the postmenopausal breast cancer patients were treated with statins. Thus it was not possible for us to answer the problem of interest. But a way to investigate this could be done through an intervention study to investigate statins influence on postmenopausal women with breast cancer. However this cannot be done because 30 % of the patients already receive statins and in an investigated population more of the patients would probably have hypercholesterolemia and would have to be censored. After all, statins do seem to have a positive association with breast cancer risk and thus breast cancer recurrence but this needs further investigation.

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