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Review

The link between mammary cancer, excessive adipose tissue and cholesterol

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Abstract: Mammary cancer remains the most frequent worldwide type of cancer in females. From a health point of view, it is a huge challenge. As a definition, we can say that a group of biologically and molecularly heterogeneous diseases is represented by mammary cancer. An important causal factor for this disease is genetic predisposition, especially mutations in the BRCA1 or BRCA2 gene. The mammary gland is stimulated by hormones both morphologically and physiologically. The most significant of these are estrogens.

Estrogen is the main female hormone, but it is present in both females and males. Elevated levels of this hormone may increase the risk of developing mammary cancer. In post-climacteric excessive adipose tissue, estrogens biosynthesis is catalyzed by aromatase, converting adrenal androgens into estrogen. Risk factors for developing mammary cancer, such as excessive adipose tissue, age at menarche and the use of exogenous hormones may increase the risk of developing it.

The aim of this paper is to show the link between cholesterol, excessive adipose tissue and the increased risk of developing mammary cancer.

Keywords: cancer, estrogen, hormones, cholesterol, obesity

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Introduction

Obesity is a risk factor for both mammary cancer as well as a strong prognosis, which predicts side effects of the disease. Clinical evidence shows that obese patients with mammary cancer who are being treated with chemotherapy or aromatase inhibitors are more likely to have a recurrence of the disease compare to females with normal body mass index [1]. Information about adipose tissue has increased significantly in recent years. Although adipose tissue has always been described for lipid storage, it is now identified as a true organ that possesses both metabolic and endocrine functions. It releases a variety of substances into the bloodstream to communicate with other organs and tissues [2].

Adipokines, adipose tissue-specific substances, are essential in determining a group of physiological responses, namely glucose and lipid metabolism, homeostasis, angiogenesis, inflammation and satiety [3]. On one hand, disorder of the hormonal role and uncontrolled expression of adipokines in adipose tissue, causes overweight or obese, eventually connecting obesity with mammary cancer risk [4]. On the other hand, there are studies that show that increased adiposity induces the growth and advancement of mammary cancer in post-climacteric females through the secretion of estrogen [4].

2. The role and effects of estrogens

The main roles of estrogen are to induce cell proliferation and the development of genitals and other tissues involved in reproduction [5]. Estrogens help with:

1. Development of the stromal tissue of the breasts;
2. Formation of a well-represented pipeline system;
3. Deposition of adipose tissue in the breasts.

The mammary lobes and alveoli develop only to a small extent under the influence of estrogen. Progesterone and prolactin are the hormones that have a decisive effect on the growth and functioning of these structures [6].

2.1 Effects of estrogen on metabolism and adipose tissue deposition - estrogen synthesis in obese adipose tissue.

Estrogen biosynthesis occurs primarily in the adipose tissue in post-climacteric females, through the conversion of adrenal androgens to estrogen by aromatase [7]. Several cellular and molecular changes in obese adipose tissue alter the biosynthesis and metabolism of estrogen.

Activation of the NF-κB pathway leads to an increase in aromatase expression in breast adipocytes and, therefore, to a higher estrogen synthesis (Figure 1) (Simpson et al., 2013). Similarly, several cytokines that are regulated in obese adipose tissue, such as TNF-α and IL-6, stimulate aromatase activity [8]. Excess breast fat, microscopic outbreaks of adipocytes surrounded by macrophages, present increased aromatase activity [9].

Moreover, in post-climacteric females, BMI is directly proportional to serial concentrations of estrone and estradiol and inversely proportional to hormone-binding globulin levels, leading to an increase in total bioavailable estrogen [7]. Compared to females with a BMI <22.5 kg / m2, obese females have an 86% increase in circulating estradiol, a 60% increase in estrone, and a 20% increase in testosterone [10].

3. Mammary cancer and excessive adipose tissue-mechanism

Chronic activation of NF-κB in adipose tissue not only causes obesity-mediated inflammation, but also stimulates anti-apoptotic genes and the proliferation of mammary cancer, invasion, angiogenesis and metastasis [11].

Concentrations of IL-6 in peritumoral fat are higher than in all other regions and grow with increasing tumor size and lymph node involvement. Some studies have shown that the interaction between cancer cells and adipocytes induces both cell types to increase the secretion of cytokines IL-6, IL-8, CCL2 and CCL5. In addition, this phenomenon promotes tumor invasion and metastasis [11, 12].

Adipose tissue normally helps stimulate up to 35% of circulating IL-6. It is also responsible for the increase in serum IL-6 after climacterium. In this way it can help increase the risk of mammary cancer and the progression of the tumor. IL-8 resulting from cancer cells, surrounding adipocytes, endothelial cells, infiltrating neutrophils, and tumor-associated macrophages (TAM) develops angiogenesis, tumor growth, metastasis, and resistance to chemotherapy [13].

4. The link between overweight and tumorigenesis

Along with obesity, the secreted cytokines go from an anti-inflammatory profile to a pro-inflammatory and proangiogenic profile. The secretion of pro-inflammatory and proangiogenic cytokines also increases in adipocytes, and ultimately results in the multiplication of cancer cells. In this way, the stimulation of angiogenesis, the expansion of cancer stem cells, invasion and metastasis takes place [14].

Excessive adipose tissue accumulates mediators of antitumor immunity, such as CD8-positive (CD8 +) T cells, natural killer (NK) cells, and dendritic cells, myeloid-derived suppressor cells (MDSC), and tumor-associated macrophages (TAM) which suppresses antitumor immunity (fig.1). In addition, stimulation of adipocyte aromatase results in higher estrogen synthesis and thus potentiates the development of estrogen receptor-positive (ER +) mammary cancer [13].

Adipocyte hypertrophy induces the secretion of inflammatory cytokines, chemokines, and leptin as a result of an increase in the number and size of adipocytes. These adipokines then induce macrophage recruitment and polarization. Macrophages secrete inflammatory cytokines, which can act directly to stimulate mammary cancer. In the end, all this leads to an increase in the production of aromatase and estrogen. In addition, they induce the expression of pro-angiogenic factors [13].

Inflammation of adipose tissue also promotes the development of insulin resistance, leading to the release of insulin and insulin-like growth factor (IGF). Insulin resistance and IGF can directly promote mammary cancer. Similarly, adipocyte leptin acts directly on cancer cells. Moreover, the decrease in adiponectin caused by excess adipose tissue has the same effect [13].

5. Cholesterol and the risk of mammary cancer

A number of measures have been implemented to detect and treat elevated cholesterol, largely through the use of statins (Figure 2) and more recently by PCSK-9 inhibitors [15]. PCSK9 inhibitors are monoclonal antibodies that inhibit proprotein convertase *subtilisin/kexin type 9* (PCSK9). PCSK9 is a protein that binds to LDL receptors for degradation and thus reduces the liver's ability to remove LDL-cholesterol from the blood, also called "bad" cholesterol [16].

The PCSK9 inhibitor is synthesized to bind to PCSK9 and prevent PCSK9 from binding to LDL (low density lipoprotein) receptors on the surface of liver cells. In the absence of PCSK9, there will be more LDL receptors on the surface of liver cells to remove LDL-cholesterol from the blood, resulting in a lower concentration of LDL cholesterol in the blood. In the last decade, there have been more and more results associating cholesterol with other modifiable risk factors, such as obesity and diabetes [17, 18]. Unfortunately, the combined action of these factors is reflected in a number of cancers, including mammary cancer [19].

High blood cholesterol is often associated with obesity [20]. Its impact as a risk factor in the occurrence of mammary cancer is not very clear and it has not been established which of the 3 parameters: total cholesterol, LDL or HDL contributes to the appearance of the disease [21]. The systematic analysis and meta-analysis of prospective studies that investigated the association between total cholesterol (CT), HDL-C, LDL-C, ApoA1 and ApoB and the risk of mammary cancer suggested an inversely proportional, statistically significant link [22].

At the end of the twentieth century, some epidemiological research (small sample size) studied the impact of serum cholesterol on the incidence of malignancy, but the results were inconclusive [23]. Others have suggested that red and processed meat, which contain a higher amount of LDL cholesterol, are risk factors for colorectal, mammary and endometrial cancer [24]. A larger study published in Science shows the role of cholesterol in the development of mammary cancer in mice. Moreover, the authors also showed that knockout mice in which cholesterol levels increased and were treated with statins had a lower predisposition to developing mammary cancer [25].

At the cellular level, it has been shown that there are many physiological mechanisms. Low serum cholesterol can increase the fluidity of the cell membrane which may result in the spread of cancer cells [26]. Conversely, the loss of membrane cholesterol can decrease the antigenicity of tumor cells, which results in the avoidance of the action of the immune system [27].

5.1 Cholesterol, HFD (high-fat diet), 27HC and cancer biology

Cholesterol represents a major factor mammary cancer risk. Unfortunately, the mechanism by which it takes place is not fully known. Most likely the increase in cholesterol content in cell membranes and after the affecting of the membrane fluidity caused by dyslipidemia may represent a possible explanation. Also, there are researches that show the functionality of the metabolite, 27-hydroxycholesterol (27HC) as an estrogen **[**21, 28]. The intensive proliferation of estrogen receptor (ER)-positive mammary cancer cells is a result of its action. In this way, the treatments used in mammary cancer in order to lower the concentration of cholesterol are justified [21].

In animals, the specific contribution of cholesterol, a comorbidity of obesity, in the pathogenesis of cancer was underestimated. This may be due to the fact that no increase in cholesterol was observed after a hyperlipidemic diet (HFD high-fat diet). To address this, a high-fat diet was used in humanized APOE3 mice. Circulating cholesterol levels were subsequently determined [25].

The observed increase in circulating cholesterol in HFD-fed mice is the result of intensified of novo synthesis. However, the effect of HFD on tumor growth was attenuated by statin treatment or inhibition of CYP27A1 [25]. These findings confirm the importance of cholesterol and dyslipidemia in mammary cancer and highlight the importance of 27HC and ER as mediators of these effects.

On the other hand, there are studies showing that the effect of statins in mammary cancer is not beneficial [29].

6. Conclusions

The effects of excessive adipose tissue on the risk of mammary cancer differ depending on the status of the ER. Obesity is associated with a significantly higher risk of ER-positive mammary cancer. The effect is insignificant for ER-negative mammary cancer after climacterium [30].

High concentrations of cytokines and leptins secreted by adipose tissue increase the number of preadipocytes, that release free fatty acids (FFA) and activate the NF-κB pathway. This pathway controls DNA transcription, cytokine production, and apoptosis in both adipocytes and immune cells in order to produce chronic inflammation [31]. The high number of preadipocytes causes high concentrations of IL-6, IL-8, CCL2, CCL5 and VEGF with a positive effect on the production of NF-κB and cytokines [32]. In addition, contact between adipocytes and invasive cancer cells synergistically regulates cytokine secretion. 9 TNF-α also called tumor necrosis factor alpha and IL-6 affects insulin receptor activation [33]. Finally, there is insulin resistance that has a positive impact on the development and growth of mammary cancer.

The NF-κB, TNF-α and IL-6 pathway also stimulates the expression of aromatase in stromal and adipocyte fibroblasts [8] breast. The effect is an increased estrogen production in both cancer and stromal cells. In addition to increasing insulin resistance, IGFs, adipokines, and local estrogen production, there is clear evidence to support the independent role of cholesterol as a mediator of the effects of dyslipidemia and/or obesity on the pathogenesis of mammary cancer [21].

A major mechanism by which cholesterol triggers mammary cancer includes its metabolite, 27HC, a molecule with SERM activity (which is a selective modulator of estrogen receptors). Studies highlight the immediate therapeutic potential for modulating cholesterol levels, either through diet or medication, such as statins, PCSK9 inhibitors, or niacin [34].

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, M.C. and P.B.; methodology,

P.B.; software, M.C.; validation, M.C., and P.B.; formal analysis, P.B.; investigation, P.B.; resources, P.B.; data curation, P.B.; writing—original draft preparation, P.B.; writing—review and editing, C.M.; visualization, C.M.; supervision, C.M. All authors have read and agreed to the published version of the manuscript".

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References

1. Lee, K., Kruper, L., Dieli-Conwright, C. M., Mortimer, J. E. The Impact of Obesity on Breast Cancer Diagnosis and Treatment. *Current oncology report* **2019**, 21(5), 41. https://doi.org/10.1007/s11912-019-0787-1
2. Coelho M, Oliveira T, Fernandes R. Biochemistry of adipose tissue: an endocrine organ. *Arch Med Sci.* **2013**;9(2):191-200. doi:10.5114/aoms.2013.33181
3. Balistreri, C. R., Caruso, C., Candore, G. The role of adipose tissue and adipokines in obesity-related inflammatory diseases. *Mediators of inflammation* **2010**, 802078. https://doi.org/10.1155/2010/802078
4. Mohanty, S. S., Mohanty, P. K. Obesity as potential breast cancer risk factor for postmenopausal women. *Genes & Disease* **2021**, 8(2), 117-123.
5. Hamilton, K. J., Hewitt, S. C., Arao, Y., Korach, K. S. Estrogen Hormone Biology. *Current topics in developmental biology* **2017** 125, 109–146. https://doi.org/10.1016/bs.ctdb.2016.12.005
6. Brisken, C., O'Malley, B. Hormone action in the mammary gland. *Cold Spring Harbor perspectives in biology* **2010**, 2(12), a003178. https://doi.org/10.1101/cshperspect.a003178
7. Liedtke, S., Schmidt, M. E., Vrieling, A., Lukanova, A., Becker, S., Kaaks, R., Zainedin, A.K., Buck, K., Benner, A., Chang-Claude, J., Steindorf, K. Postmenopausal sex hormones in relation to body fat distribution. *Obesity* **2012**, 20(5), 1088-1095.
8. Purohit, A., Reed, M. J. Regulation of estrogen synthesis in postmenopausal women. *Steroids* **2002**, 67(12), 979-983.
9. Mullooly, M., Yang, H. P., Falk, R. T., Nyante, S. J., Cora, R., Pfeiffer, R. M., Radisky, D.C., Visscher, D.W., Hartmann, L.C., Carter, J.M., Degnim, A.C., Stanczyk, F.Z., Figueroa, J.D., Garcia-Closas, M., Lissowska, J., Troester, M.A., Hewitt, S.M., Brinton L.A., Sherman M.E., Gierach, G. L. Relationship between crown-like structures and sex-steroid hormones in breast adipose tissue and serum among postmenopausal breast cancer patients. *Breast Cancer Research* **2017**, 19(1), 1-10.
10. Key, T. J., Appleby, P. N., Reeves, G. K., Travis, R. C., Brinton, L. A., Helzlsouer, K. J., Dorgan, J.F., Gapstur, S.M., Gaudet, M.M., Kaaks, R., Riboli, E., Rinaldi, S., Manjer, J., Hallmans, G., Giles, G.G., Marchand, L.LE, Kolonel, L.N., Henderson, B.E., Tworoger, S.S., Hankinson, S.E., Zeleniuch-Jacquotte A., Koenig, K., Krogh, V., Sieri, S., Muti, P., Ziegler, R.G., Schairer, C., Furhman, B.J., Barrett-Conner, E., Laughlin, G.A., Grant, E.J., Cologne, J., Ohishi, W., Hida, A., Cauley, J.A., Fourkala, E.O., Menon, U., Rohan, T.E., Strickler, H.D., Gunter, M. J. Endogenous Hormones and Breast Cancer Collaborative Group. Steroid hormone measurements from different types of assays in relation to body mass index and breast cancer risk in postmenopausal women: reanalysis of eighteen prospective studies. *Steroids* **2015**, 99(Pt A), 49-55.
11. Dirat, B., Bochet, L., Dabek, M., Daviaud, D., Dauvillier, S., Majed, B., Wang, Y.Y., Meulle, A., Salles, B., Gonidec, L.S., Garrido, I., Escourrou, G., Valet, P., Muller, C. Cancer-associated adipocytes exhibit an activated phenotype and contribute to breast cancer invasion. *Cancer research* **2011**, 71(7), 2455-2465.
12. Mueller MM, Fusenig NE. Friends or foes—bipolar effects of the tumour stroma in cancer. *Nat Rev Cancer* **2004**; 4:839–49.

13. Kim, J. H., Bachmann, R. A., Chen, J. Interleukin-6 and insulin resistance. *Vitamins & Hormones* **2009**, 80, 613-633.
14. Makki, K., Froguel, P., Wolowczuk, I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. *ISRN inflammation* **2013**, 139239. https://doi.org/10.1155/2013/139239
15. Chaudhary, R., Garg, J., Shah, N., Sumner, A. PCSK9 inhibitors: A new era of lipid lowering therapy. *World journal of cardiology* **2017**, 9(2), 76–91. https://doi.org/10.4330/wjc.v9.i2.**76**
16. Fattori, E., Cappelletti, M., Lo Surdo, P., Calzetta, A., Bendtsen, C., Ni, Y. G., Pandit, S., Sitlani, A., Mesiti, G., Carfí, A., Monaci, P. Immunization against proprotein convertase subtilisin-like/kexin type 9 lowers plasma LDL-cholesterol levels in mice. *Journal of lipid research* **2012**, 53(8), 1654–1661. https://doi.org/10.1194/jlr.M028340
17. Klop, B., Elte, J. W., Cabezas, M. C. Dyslipidemia in obesity: mechanisms and potential targets. *Nutrients* **2013**, 5(4), 1218–1240. https://doi.org/10.3390/nu5041218
18. Onat, S., Ates, G., Avcı, A., Yıldız, T., Birak, A., Ozmen, C. A., Ulku, R. The role of mediastinoscopy in the diagnosis of non-lung cancer diseases. *Therapeutics and clinical risk management* **2017**, 13, 939.
19. La Vecchia C, Giordano SH, Hortobagyi GN, Chabner B. Overweight, obesity, diabetes, and risk of breast cancer: interlocking pieces of the puzzle. Oncologist. 2011;16(6):726-729. doi:10.1634/theoncologist.2011-0050
20. Must, A., Spadano, J., Coakley, E. H., Field, A. E., Colditz, G., Dietz, W. H. The disease burden associated with overweight and obesity. *Jama* **1999**, 282(16), 1523-1529.
21. Nelson, E. R., Chang, C. Y., McDonnell, D. P. Cholesterol and breast cancer pathophysiology. *Trends in Endocrinology & Metabolism* **2014**, 25(12), 649-655.
22. Touvier M, Fassier P, His M, Norat T, Chan DS, Blacher J, Hercberg S, Galan P, Druesne-Pecollo N, Latino-Martel P. Cholesterol and breast cancer risk: a systematic review and meta-analysis of prospective studies. *Br J Nutr.* **2015**, 14;114(3):347-57. doi: 10.1017/S000711451500183X. Epub 2015 Jul 15. PMID: 26173770.
23. Salonen, J. T. Risk of cancer and death in relation to serum cholesterol: a longitudinal study in an eastern Finnish population with high overall cholesterol level. *American journal of epidemiology* **1982**, 116(4), 622-630.
24. Guo, H., Callaway, J. B., Ting, J. P. Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nature medicine* **2015**, 21(7), 677-687.
25. Nelson, E. R., Wardell, S. E., Jasper, J. S., Park, S., Suchindran, S., Howe, M. K., Carver, N.J., Pillai, R.V., Sullivan, P.M., Sondhi, V., Umetani, M., Geradts, J., McDonnell, D. P. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. *Science* **2013**, 342(6162), 1094-1098.
26. Oliver, M. F. (1981). Serum cholesterol-the knave of hearts and the joker. The Lancet, 318(8255), 1090-1095.
27. Shinitzky, M. Membrane fluidity and receptor function. In Membrane fluidity. *Springer* **1984**, pp. 585-601. Boston, MA.
28. Nelson, E. R. The significance of cholesterol and its metabolite, 27-hydroxycholesterol in breast cancer. *Molecular and cellular endocrinology* **2018**, 466, 73-80.
29. Wu, Q., Ishikawa, T., Sirianni, R., Tang, H., McDonald, J. G., Yuhanna, I. S., Thompson, B., Girard, L., Mineo, C., Brekken, R.A., Umetani, M., Euhus, D.M., Xie, Y., Shaul, P. W. 27-Hydroxycholesterol promotes cell-autonomous, ER-positive breast cancer growth. *Cell reports* **2013**, 5(3), 637-645.
30. Picon-Ruiz, M., Morata-Tarifa, C., Valle-Goffin, J. J., Friedman, E. R., Slingerland, J. M. Obesity and adverse breast cancer risk and outcome: Mechanistic insights and strategies for intervention. *CA: a cancer journal for clinicians* **2017** 67(5), 378–397. https://doi.org/10.3322/caac.21405

31. Zatterale, F., Longo, M., Naderi, J., Raciti, G. A., Desiderio, A., Miele, C., Beguinot, F. Chronic Adipose Tissue Inflammation Linking Obesity to Insulin Resistance and Type 2 Diabetes. *Frontiers in physiology* **2020**, 10, 1607. https://doi.org/10.3389/fphys.2019.01607
32. Simons, P. J., van den Pangaart, P. S., van Roomen, C. P., Aerts, J. M., Boon, L. Cytokine-mediated modulation of leptin and adiponectin secretion during in vitro adipogenesis: evidence that tumor necrosis factor-α- and interleukin-1β-treated human preadipocytes are potent leptin producers. *Cytokine* **2005**, 32(2), 94-103.
33. Kern, P. A., Ranganathan, S., Li, C., Wood, L., Ranganathan, G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. *American Journal of Physiology-Endocrinology And Metabolism* **2001**, 280(5), E745-E751.
34. Thelen, K. M., Lütjohann, D., Vesalainen, R., Janatuinen, T., Knuuti, J., von Bergmann, K., Lehtimäki, T., Laaksonen, R. Effect of pravastatin on plasma sterols and oxysterols in men. *European journal of clinical pharmacology* **2006**, 62(1), 9-14.