



Mammary Adipose Tissue Control of Breast Cancer Progression: Impact of Obesity and Diabetes

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Mammary adipose tissue (AT) is necessary for breast epithelium. However, in breast cancer (BC), cell-cell interactions are deregulated as the tumor chronically modifies AT microenvironment. In turn, breast AT evolves to accommodate the tumor, and to participate to its dissemination. Among AT cells, adipocytes and their precursor mesenchymal stem cells (MSCs) play a major role in supporting tumor growth and dissemination. They provide energy supplies and release a plethora of factors involved in cancer aggressiveness. Here, we discuss the main molecular mechanisms underlining the interplay between adipose (adipocytes and MSCs) and BC cells. Following close interactions with BC cells, adipocytes lose lipids and change morphology and secretory patterns. MSCs also play a major role in cancer progression. While bone marrow MSCs are recruited by BC cells and participate in metastatic process, mammary AT-MSCs exert a local action by increasing the release of cytokines, growth factors and extracellular matrix components and become principal actors in cancer progression. Common systemic metabolic diseases, including obesity and diabetes, further modify the interplay between AT and BC. Indeed, metabolic perturbations are accompanied by well-known alterations of AT functions, which might contribute to worsen cancer phenotype. Here, we highlight how metabolic alterations locally affect mammary AT and interfere with the molecular mechanisms of bidirectional communication between adipose and cancer cells.

Keywords: mammary adipose tissue, breast cancer, obesity, diabetes, molecular signals, adipocytes, mesenchymal stem cells

INTRODUCTION

Breast cancer (BC) is the most common tumor in women and represents the second cause of cancer-caused death after lung cancer (1). In 2018, over 2 million new BC cases were estimated worldwide (2). In the past 3 decades, patient survival rate has increased, thanks to improvements in treatment and detection (3). However, patients' quality of life is still negatively affected by chemotherapy side effects. Targeted and hormone therapies in most cases do not have long lasting effects; and a number of patients display or acquire resistance to treatments, with a significant reduction of therapy efficacy (3, 4).

The increased incidence and the worse prognosis for BC are parallel to the alarming increase of metabolic disturbances. BC risk is about twofold higher in obese and 16% higher in women with type 2 diabetes (T2D), independently of obesity (5). Patients with obesity and T2D have larger tumors at diagnosis, and worse outcome, with increased risk of distant metastases and mortality (6–8). Moreover, obesity and T2D affect chemotherapy toxicity and surgical complications (9–11).

Breast tissue is composed by 90% of adipose tissue (AT) with permanent interactions between epithelial cells and adipose cells (12). Adipocytes and their precursor mesenchymal stem cells (MSCs) may sustain tumor phenotypes by either acting as energy reservoirs for neighboring cancer cells or through secretion of signaling molecules and vesicles containing proteins, lipids and nucleic acids (13, 14). The dysfunction of AT is now considered a central mechanism for the development of obesity and T2D metabolic complications (15).

In this manuscript, we overview the role of mammary AT as a support for BC cell growth and progression, and describe the known molecular mechanisms underlying the AT/tumor bidirectional crosstalk, especially in the presence of metabolic disorders.

MAMMARY ADIPOSE TISSUE AND BREAST CANCER

AT is a loose connective tissue characterized by marked cellular heterogeneity. It is made up of about one-third of adipocytes and two-thirds of stromal-vascular fraction cells, a combination of MSCs, endothelial precursor cells, fibroblasts, smooth muscle cells, pericytes, macrophages and preadipocytes in various stages of development (16). MSCs are located in perivascular niches and participate to cell turnover and to the vascular network for AT tropism (17). For a long time, AT has been considered as an energy depot. Since 90s, the role of AT has been revised and broadened to an active endocrine organ, able to control systemic energy and metabolic homeostasis through a complex network of signals (16, 18).

In the mammary gland, adipose cells are characterized by high plasticity and support the growth and function of the mammary epithelium (19). Mammary AT surrounds the epithelial ducts, which are the milk-producing structures of the breast. *In vitro* and *in vivo* studies have shown the importance of mammary AT for the growth, the branching and the preservation of the ducts and for the functional differentiation of the epithelium ahead of pregnancy (20). For instance, *A-Zip* mice, which have mammary gland lacking mature adipocytes, display rudimental epithelial ducts with reduced branching and severe distention (21).

The role of AT, and more specifically of adipocytes and their precursors MSCs, in BC progression and metastasis, is a quite new area of research. However, adipose cells communicate with cancer cells within the breast, and this may contribute to cancer progression, through different mechanisms.

- *Release of signaling molecules*: Either locally released molecules, either those coming from distal sites converge

in the interstitial fluids of the mammary AT (19). Some AT-released factors contribute to AT remodeling, adipogenesis, innervation and angiogenesis by acting through autocrine and paracrine ways. Other AT-factors act in an endocrine manner and influence the functions of many tissues, thus controlling appetite, food intake, glucose disposal and energy expenditure (16, 18, 22). Over 350 proteins have been identified in mammary AT by using proteomic approaches. These factors are called “adipokines” and include leptin, adiponectin, resistin, growth factors (IGF1, insulin-like growth factor 1; VEGF, vascular endothelial growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; NGF, nerve growth factor; TGF β , transforming growth factor), enzymes (autotaxin) and cytokines (interleukin [IL]-1, IL-6, IL-8, CCL5, tumor necrosis factor-TNF- α) (16, 22, 23). These molecules are crucial for the physiology and development of AT and breast epithelium and for the entire organism. However, the same factors may as well contribute to proliferation, motility, invasiveness, epithelial to mesenchymal transition (EMT) and stemness of BC cells, as well as to tumor angiogenesis by activating different molecular mechanisms (12, 24–26).

- *Mechanical support*: Among AT-secreted proteins, there is a wide variety of extracellular matrix (ECM) proteins needed for the tissue structure, but also involved in cell-cell communication systems and in the sequestering of growth factors for a time- and context-dependent release (27). Some of these factors may sustain cancer progression. For instance, adipocyte-derived collagen VI promotes BC progression via bNG2/chondroitin sulfate proteoglycan receptors, while endotrophin, a cleavage product of collagen VI, contributes to tissue fibrosis and EMT of BC cells through enhanced TGF- β signaling (28, 29).
- *Energy supply*: Lipids and metabolites are largely released by AT in mammary glands. Mammary epithelium is able to utilize and metabolize fatty acids to a variety of derivatives (19). However, lipids are taken up also by cancer cells which display a characteristic “lipid metabolic reprogramming.” BC cells take advantage of fatty acids and glycerol for the biosynthesis of membranes, needed for their proliferation, and for the generation of lipid-derived biomolecules such as steroid hormones, diacylglycerol, eicosanoids, phospholipids and sphingolipids which sustain all the functions of cancer cells (12, 30). Recently, it has been shown that the inhibition of fatty acid receptor CD36 impairs metastasis in human BC-derived tumors (31).

Therefore, adipose cells may accommodate BC cells with stimulatory, supportive and nutritive functions.

BC-ASSOCIATED ADIPOSE CELLS

Emerging evidence indicates that relevant phenotypic changes occur in AT surrounding BC. Indeed, the invasion of AT by BC cells located at the margin of tumor mass is associated with tumor aggressiveness and poor prognosis (32).

Overall, AT adjacent to malignant breast tumors displays down-regulation of the expression of adipogenesis-related genes Homeobox C Cluster (HOXC) 8, HOXC9, fatty acid binding protein 4 (FABP4) and hormone sensitive lipase (HSL) and up-regulation of inflammatory cytokines, like TNF- α and monocyte chemoattractant protein 1 (MCP-1) and of leptin, with a decrease of adiponectin levels (33).

The impact of BC cells specifically on adipocytes has been documented by analyzing adipocytes isolated from human and mouse tumor samples and by using *in vitro* systems of BC cells co-cultured with different types of human and murine adipocytes. Compared to normal adipocytes, the so called "cancer-associated adipocytes" (CAA) are smaller cells, with a reduction in the number and size of lipid droplets and modification of basement membranes and ECM (34). Adipocytes adjacent to breast tumors display increased levels of matrix metalloproteinase-11 (MMP11), which inhibits pre-adipocyte differentiation and reverses mature adipocyte differentiation to maintain the invasive property of cancer cells (34, 35). Impaired adipogenic differentiation program of CAAs is accompanied by the downregulation of Peroxisome Proliferator Activated Receptor Gamma (PPAR γ), CCAAT-enhancer-binding protein α (C/EBP α), FABP4, and resistin mRNA levels (36, 37). Moreover, BC cell-released TNF- α and IL-11 drive a desmoplastic reaction in pre-adipocytes, leading to downregulation of adipogenic master genes (37). PPAR γ reduction is also related to the increase of miRNA-155 in exosomes of adipocyte-BC co-cultures (38). Notably, the reduction of lipid droplets takes place with the metabolic reprogramming that adipocytes undergo in contact with BC cells and with the acquisition of a brown-like phenotype. Indeed, cancer cells induce the lipolysis in CAAs *via* HSL and adipose triglyceride lipase (ATGL). Free fatty acids enter into cancer cells, are transported through FABP4 and degraded to provide ATP and bioactive lipids needed for cell invasion, angiogenesis and immunosuppression (11, 30). Consistently, during BC progression, in cancer cells there is an increase of FABP4 and of lipogenic enzymes, like fatty acid synthase (FASN). FASN controls the response of BC cells to E₂-stimulated ER α signaling and its levels are associated to a poor clinical outcome in patients with BC (39, 40). Gene expression profiling of breast adipocytes shows greater brown adipocyte-related activity next to breast tumors than in benign breast lesions (33). This is consistent with the finding that in co-cultures of adipocytes- BC cells there is an increase of the exosomal miRNA-144 that promotes beige/brown adipocyte differentiation by downregulating MAP3K8/ERK1/2/PPAR γ and of exosomal miRNA-126 that plays a crucial role in metabolic reprogramming of adipocytes, targeting IRS1 (Insulin receptor substrate 1) and AMPK (5' AMP-activated protein kinase) (41). In addition to MMP11, CAAs express MMP1, MMP7, MMP10, MMP14 and PAI, thus damaging ECM integrity in BC environment (34). Furthermore, cancer cells induce adipocytes to secrete fibronectin, which, in turn activates STAT3 signaling pathway in BC cells, thus promoting EMT (42). Fibronectin activates also AKT2 in BC cells, interfering with p38 pathway and docetaxel-induced apoptosis (43). Beside ECM proteins, CAAs display an imbalanced secretion of adipokines, cytokines and growth

factors or associated proteins. For instance, compared to normal adipocytes, CAAs secrete a higher amount of leptin, IL-1b, IL-6, CCL5, MCP-1, TNF- α , VEGF and insulin-like growth factor binding protein-2 (IGFBP-2) which in turn promote invasion and metastasis of BC (24, 44, 45). Indeed, CCL5 immuno-detection in peritumoral AT of women with triple negative BC (TNBC) correlates with lymph node and distant metastases and shows a negative correlation with the overall survival of patients (46). In addition, adipocytes co-cultured with BC cells induce the expression of IL-6 in cancer cells, resulting in the phosphorylation of effector kinase CHK1 and the acquisition of a radio-resistant phenotype in BC cells (47). Mature adipocytes also contribute to HER2 + BC cell resistance to trastuzumab-mediated antibody-dependent cellular cytotoxicity and impair immunotherapy efficacy by the hyperexpression of programmed death- ligand 1 (PD-L1), that prevents the antineoplastic functions of CD8 + T cells (24, 48).

Similar to adipocytes, MSCs are largely modified by cancer cells. *Per se* involved in tissue repair, angiogenesis and immunomodulation, MSCs, in contact with cancer cells become cancer supportive cells, so called carcinoma-associated MSCs (CA-MSCs). Nowadays, the contribution of MSCs in BC progression has been investigated mainly by using bone marrow-derived MSCs (BM-MSCs). BM-MSC homing in BC is mediated by tumor (and CAA) -derived chemokines (MCP-1, CCL5, CXCL 16- chemokine [C-X-C motif] ligand 16, SDF1-stromal cell-derived factor 1), growth factors (VEGF, IGF1, TGF β , FGF) and miRNAs (i.e., miRNA-126/miRNA-126*) (49, 50). Once educated by cancer cells, BM-MSCs secrete CXCL1, CXCL2, SDF1, IL-6, IL-8, TGF β and microvesicles containing miRNA, such as miRNA-21 and miRNA-34a, all factors implicated in BC survival, progression and chemo-resistance (51, 52). For instance, BC cell-released TNF- α stimulates BM-MSCs to secrete CXCR2 (C-X-C Motif Chemokine Receptor 2) ligands which, in turn, recruit CXCR2 + neutrophils into the tumor, thus promoting metastases (53). Following MSC co-culture, BC cells upregulate IL-6 and CXCL7 pathways with enhanced mammosphere formation efficiency (54). BM-MSCs enhance angiogenesis by releasing soluble factors (VEGF, Leukemia inhibitory factor- LIF, Macrophage Inflammatory Protein 2-MIP2) and exosomes that induce VEGF expression in cancer cells by activating extracellular signal-regulated kinase1/2 (ERK1/2) pathway (55). Moreover, BC cell migration is fostered by BM-MSCs through ER (estrogen receptor)-SDF-1/CXCR4 crosstalk and CXCR2 activation (56, 57). BC cells prompt BM-MSCs to secrete large amount of CCL5, which, in turn, were shown to increase BC metastatic potential of about 5 fold (58). Occurrence of BC metastases in lungs and bones is also supported by the induction of TWIST transcription by BM-MSC production of lysyl oxidase enzyme (59) and by the production of exosomes containing miRNA-222/223 (60). Finally, it has been shown that, in contact with BC cells, BM-MSCs are able to transdifferentiate into cancer associated fibroblasts (CAFs), the best companions for BC cells (50, 51, 61).

The crosstalk between BM-MSCs and BC cells is highly relevant since BC typically metastasizes to bone, and bone marrow could represent an ideal environment for the

development of BC micro-metastatic niches. However, the interaction of BC cells with mammary AT resident MSCs should be taken into account for its potential role in BC progression.

Recently, it has been shown that MSCs isolated from mammary AT of patients with BC, express high levels of brain-derived neurotrophic factor (BDNF), neurogenic locus notch homolog protein 1 (NOTCH1) and cytoskeletal Vimentin, and reduction of growth differentiation factor 15 (GDF15), IGF1, MMP2, platelet-derived growth factor receptor b (PDGFRB) and TGF β . Moreover, when co-injected with BC cells in immune-compromised SCID/beige mice, MSCs generate tumors with an increased volume and innervation (52). MSCs isolated from mice bearing BC xenograft tumors can be incorporated in tumor vessels and display up-regulation of SDF1 and α -smooth muscle actin (α -SMA - marker of CAF) (62). Mammary MSCs significantly promote ER-negative BC cell migration and invasion *in vitro* and tumor invasion in a co-transplant xenograft mouse model by producing IL-6 upon activation of cofilin-1, a well-known regulator of actin dynamics (63). Noteworthy, in another study, using co-culture models, AT-derived MSCs promote BC cell migration and invasion through P2X-mediated purinergic signaling and ATP-loaded microvesicles (64). AT-derived MSC exosomes lead to an up-regulation of WNT target genes Axin2 and Dkk1, and β -catenin in BC cells, thus enhancing cell migration (65). Moreover, mammary MSCs promote mammosphere formation via cytokines, EGF/EGFR/Akt and adiponectin pathways (26, 44, 66). In addition, it has been shown that AT-derived MSCs fuse with BC cells spontaneously and this fused population is enriched in BC stem cells (CSC) CD44⁺CD24^{-/low}EpCAM⁺ (67). Finally, MSCs isolated from BC patients with pathological stage III disease, induce up-regulation of mRNA expression levels of *IL-4*, *TGF- β 1*, *IL-10*, *CCR4* and *CD25* in peripheral blood leukocytes and an increase of the percentage of CD4⁺CD25^(high)Foxp3⁽⁺⁾ T regulatory cells *in vitro*, thus sustaining an anti-inflammatory response within the tumor microenvironment (68).

Taken together, these data indicate that both adipocytes and MSCs are largely modified by cancer cells and, once educated by BC cells, become principal actors in metastatic process.

ADIPOSE CELLS AS LINK BETWEEN METABOLIC DERANGEMENTS AND BC PROGRESSION

Several studies have highlighted that hyperlipidemia, hyperglycemia, hyperinsulinemia and anti-diabetic drugs may be determinant in the association between metabolic imbalance (i.e., obesity and T2D) and BC (7, 8). Changes in body weight and genetic polymorphisms may significantly interact to increase pre- and post-menopausal BC risk (69). However, it is still poorly understood how mammary AT changes related to obesity and T2D conditions might influence BC progression.

In general, in presence of chronic overnutrition, AT expands beyond its capacity in order to maintain a sufficient angiogenesis, leading to persistent hypoxia, fibrosis, cellular senescence, necrotic adipocyte death and a large secretion of

pro-inflammatory cytokines, such as TNF- α , IL-6, IL-8 and MCP-1. Compromised AT cells recruit immune cells, particularly monocytes that amplify the local inflammation, determining the “low grade chronic inflammation.” The unhealthy AT expansion largely contributes to the systemic metabolic derangements associated to obesity and T2D (15, 27, 70).

It has been reported that adipocytes and MSCs from human lipoaspirate of obese donors, compared to adipocytes/MSCs from lean subjects, enhance BC cell growth at higher extent and promote tumor metastasis at least in part by IGF1 and leptin pathways (71, 72). Leptin from obese-derived MSCs increases expression of *SERPINE 1*, *SNAI2*, *IL-6*, *TWIST1*, and cyclooxygenase-2 -COX-2, which are crucial in EMT and CSC programs in BC cell lines and in TNBC PDX-derived cells (72). Moreover, leptin modulates exosome biogenesis in BC cells and promotes invasive ductal and lobular carcinoma *in vivo* (12, 73). Leptin increase and adiponectin reduction are hallmarks of obesity. While leptin involvement in BC progression is widely recognized (12, 74), the role of adiponectin is still controversial and depends on ER α expression in BC (75). In ER α + cells, low adiponectin levels, like those observed in obesity, stimulate cell proliferation. In contrast, in ER α - cells, adiponectin is able to inhibit cell growth and progression *in vitro* and *in vivo* (76). In AT of obese subjects and mouse models an increase of survivin has also been observed. Survivin is an antiapoptotic protein strongly linked to cancer cell growth. Consistently, its increase in obesity protects MSCs from apoptosis and controls adipocyte lipolysis and lipid storage and may contribute to cancer progression (77, 78). Increased lipid content and external lipid stimuli largely modify adipocyte-BC cell communication. Indeed,

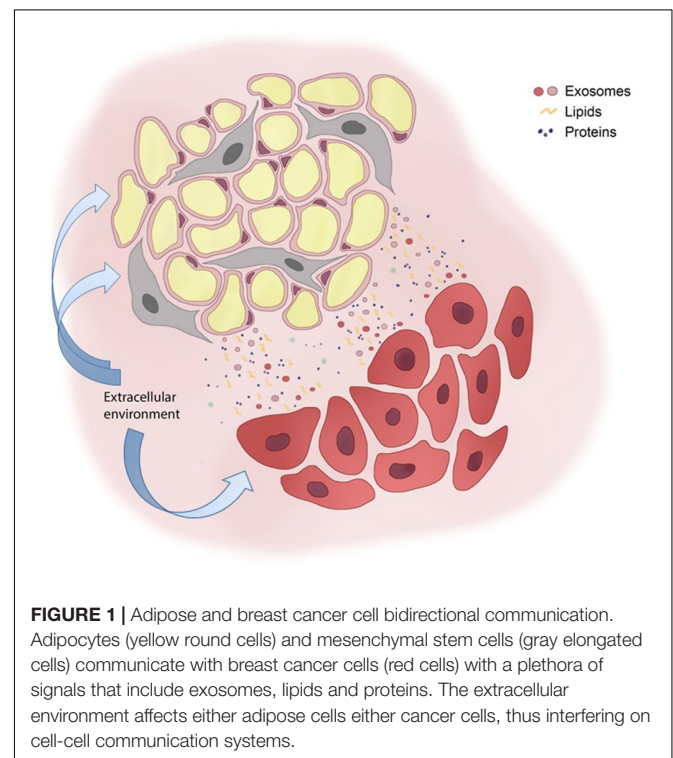


FIGURE 1 | Adipose and breast cancer cell bidirectional communication. Adipocytes (yellow round cells) and mesenchymal stem cells (gray elongated cells) communicate with breast cancer cells (red cells) with a plethora of signals that include exosomes, lipids and proteins. The extracellular environment affects either adipose cells either cancer cells, thus interfering on cell-cell communication systems.

lipid-overloaded 3T3-L1 adipocytes transfer about twofold more fatty acids to BC cells, compared to “normal” adipocytes. BC cells in turn up-regulate Carnitine palmitoyltransferase 1A (CPT1A) and electron transport chain complex protein levels and display an increase of proliferation and migration that is parallel to adipocyte lipolysis (79). Mammary adipocytes, incubated with palmitate or oleate or cultured in high glucose medium release higher amounts of IGF1 and CCL5 and promote BC cell proliferation and invasiveness (46, 71). High glucose concentrations also stimulate mammary adipocytes to secrete IL-8, which contributes to tamoxifen resistance in BC cells possibly through up-regulation of Connective Tissue Growth Factor (CTGF) (80). CTGF levels in tumoral tissues of patients with ER + BC correlates with hormone therapy resistance, distant metastases, reduced overall and disease-free survival (80). In addition, tumor-surrounding adipocytes induce multi-drug resistance in BC cells through up-regulation of a transport-associated major vault protein (MVP) which mediates the efflux of the chemotherapeutic agent doxorubicin. This effect is amplified by obesity (81). Recently, it has been shown that in mice bearing triple negative BC, diet-induced obesity (DIO) inhibits fatty acids storage and amplify local inflammation in mammary AT. In parallel, cancer cells increase fatty acid synthesis and change fatty acid composition. Lipid saturated cell membranes protect cancer cells from the cytotoxic effects of doxorubicin (82). Moreover, lipid peroxidases secreted by visceral AT of DIO rats are able to modify carcinogenesis-related genes in non-tumoral breast epithelium cells, thus indicating that in obesity, AT-secreted factors are also involved in early stages of tumor development (83). AT hypoxia and BC progression seem to be strongly linked. A high amount of adipocytes enhances cancer progression inducing the expression of Hypoxia-inducible factor-1 α (HIF-1 α) and its target genes, which causes the loss of ER α protein in BC cells (84). A positive correlation between breast adipocyte size and the presence of crown-like structures (CLS; spread inflammatory loci characterized by M1 macrophages around necrotic adipocytes) in peritumoral AT has been observed, likely reflecting local inflammation. In patients with both obesity and BC, the presence of CLS accumulation in mammary AT is concomitant with aggressive, high-grade tumors and positive lymph node involvement (85). In the inflamed breast tissue of obese BC patients, higher levels of both NF- κ B binding activity and aromatase expression have been reported (85). Mammary AT inflammation, characterized by CLS, inflammatory cytokines and recruitment of a number of immune cells, is largely considered the link between metabolic derangements and worse BC prognosis (70, 86). For this reason, great efforts are being made for disrupting the obesity-cancer link by targeting inflammation with omega-3 fatty acids, non-steroidal anti-inflammatory drugs, monoclonal

antibodies against specific cytokines, selective COX-2 inhibitors or polyphenolic compounds. Resveratrol, for example, appears to protect against the protumorigenic effects of obesity in a murine model of BC, at least in part, by inhibiting adipocyte hypertrophy, CLS formation, pro-inflammatory cytokines and COX-2 expression (87).

CONCLUSION AND PERSPECTIVES

As genetic and epi-genetic mutations accumulate in cancer cells (88, 89), functional alterations appear in AT and support BC progression. AT and BC cells communicate through a network of exosomes, lipids and proteins; extracellular environment may further interfere with these signals (**Figure 1**). However, most of the studies are based on MSCs/adipocytes isolated from different AT reservoirs (i.e., abdominal, dermal, umbilical), both of murine and human origin. Since the functional diversity of AT depots is well established (20), it is necessary to strengthen the research activity on AT-BC communication in the context of the breast. In addition, the expanded crosstalk of adipocytes and MSCs between each other and with other components of breast microenvironment, such as endothelial cells, pericytes and immune cells should also be exploited concerning BC progression.

AUTHOR CONTRIBUTIONS

VD'E, PF, CM, and FB conceived the idea and edited the manuscript. VD'E, MA, and MG wrote the manuscript. SC contributed to literature search and prepared the figure. PF supervised the final version of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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