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A role for 5-lipoxygenase products in obesity-associated inflammation and insulin resistance

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There is a growing amount of evidence that obesity-induced low-grade inflammation is an important causative link between obesity and many of its associated pathologies such as type 2 diabetes and atherosclerosis. In the quest to identify the triggers of obesity-associated inflammation of adipose tissue, our laboratory recently demonstrated that adipocytes can secrete leukotrienes, and that these pro-inflammatory lipid mediators contribute to obesity-associated inflammation and insulin resistance in mice. Together with other recent studies, our recent findings identify an important role for the enzyme 5-lipoxygenase and its products in the induction and resolution of adipose tissue inflammation. Therefore, pharmaceutical approaches that target this enzyme or its products should be considered as novel treatments aimed at preventing or resolving obesity-induced inflammation and its associated pathologies.

Keywords: obesity, inflammation, 5-lipoxygenase, insulin resistance, adipose tissue

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Obesity has become a worldwide public health crisis. Globally, obesity is now a bigger threat than malnutrition in every region aside from sub-Saharan Africa, according to the most recent Global Burden of Disease Study.[1] Deaths due to obesity now occur at three times the rate of deaths due to malnutrition. Obesity itself would be of little concern if it were not associated with the "metabolic syndrome", a constellation of metabolic abnormalities that portend the development of type 2 diabetes, atherosclerosis, and non-alcoholic fatty liver disease.[1] The list of diseases associated with obesity is ever growing. This inspires the question of how obesity triggers these complications and what can be done to intervene in the progression from obesity to disease. Low-grade, chronic inflammation is now considered a common link between obesity and the majority of its associated pathologies. In both obese mice and humans, macrophage accumulation within insulin-sensitive tissues (i.e., white adipose tissue [WAT], liver, and skeletal muscle [SKM]) has been demonstrated, together with the recently described increase in WAT T lymphocytes.[2] Recent studies have established that obesity leads in WAT to a shift in balance of anti-inflammatory M2 macrophages and Th2 and regulatory T cells (Tregs) toward pro-inflammatory Th1 cells and influx of $CD8^+$ effector T cells, subsequently resulting in the recruitment and differentiation of pro-inflammatory M1 macrophages.[2] The resulting increase in production/secretion of pro-inflammatory factors leads to insulin resistance and type 2 diabetes.[2] This interplay between immunological and metabolic processes has recently been defined as immunometabolism; an emerging field of investigation at the interface between the historically distinct disciplines of immunology and metabolism.[3] Over the past years, many research efforts have focused on identifying the trigger(s) driving the recruitment of inflammatory cells to obese AT. Several recent studies have demonstrated a prominent role for the 5-lipoxygenase/leukotriene pathway in AT inflammation and the insulin resistance that is associated with it.

Leukotrienes (LTs) are potent proinflammatory mediators and many important aspects of both innate and adaptive immune responses, such as leukocyte and lymphocyte cytokine production and cell proliferation, differentiation, and migration, are regulated by LTs.[4] Their biosynthesis starts with cytosolic phospholipase A2 (cPLA2) that catalyzes the release of arachidonic acid (AA) from cell-membrane phospholipids. AA is subsequently converted to leukotriene A4 (LTA4) by the enzyme 5-lipoxygenase (5-LO) in concert with 5-lipoxygenase activating protein (FLAP). Although FLAP does not have enzymatic activity, it enhances the ability of 5-LO to interact with its substrate. LTA4 is converted by LTA4 hydrolase (LTA4-H) to leukotriene B4 (LTB4), or it can be conjugated with reduced glutathione by leukotriene C4 (LTC4) synthase (LTC4-S) to yield LTC4. LTB4 and LTC4 are exported from the cell by specific transporter proteins; the released LTC4 is converted to LTD4 which undergoes conversion to LTE4 by sequential amino acid hydrolysis. LTB4 is a potent leukocyte chemoattractant and activator.[4] This leukotriene promotes the generation of M1 macrophages.[5] On the other hand, cysteinyl-containing LTs (CysLTs; LTC4, D4, and E4) contract smooth muscles, particularly in the peripheral airways, and are regarded as pivotal mediators of bronchial asthma.[4] In addition, both LTB_4 and CysLTs are potent chemoattractants for T cells.[6-8] Furthermore, LTB_4 inhibits Treg differentiation and stimulates T_H17 differentiation, a T cell subset recently shown to be increased in obesity.[9,10] LTs exert their biological functions through activation of G-protein-coupled receptors. LTB_4 binds to BLT1 and BLT2 receptors with high and low affinities, respectively, and the CysLTs bind to the CysLT1 and CysLT2 receptors with varying affinities.[4]

Several studies have suggested a potential link between the LT pathway and AT inflammation.[11] Both 5-LO and FLAP expression were shown to be increased in obese AT.[12,13] These major players in LT biosynthesis were found to be expressed both in adipocytes and adipose tissue macrophages (ATMs) in obese AT.[12,13] Furthermore, a recent study demonstrated that adipose tissue from obese mice, compared with that from lean mice, exhibits increased LTB4 levels. In the same study, FLAP inhibition resulted in decreased ATM infiltration and improvement of insulin sensitivity.[14] Moreover, mice lacking the LTB_4 receptor BLT1 exhibit a similar decrease in ATM infiltration and improvement of insulin sensitivity in a model of diet-induced obesity.[15]

Recently, we reported that mouse and human adipocytes produce LTs.[16] This adipocyte LT production increases with obesity, positively correlates with adipocyte size, and is stimulated by inflammatory stimuli. Furthermore, preadipocytes do not produce LTs and this is in agreement with low mRNA levels of LT synthesis pathway enzymes in preadipocytes compared with mature adipocytes. Adipocytes account for 45% of the total LTB_4 production and 24% of the total CysLT production in AT from normal chow fed mice. This contribution decreases to 33% and 12%, respectively in mice fed a high fat diet (HFD). This decrease is presumably due to a reduction of adipocytes per gram of AT (consequence of increased adipocyte size), and perhaps an increase of stromal vascular cell content (consequence of infiltration of inflammatory cells). Using in vitro macrophage and T cell chemotaxis assays, we showed that primary adipocytes isolated from obese AT secrete more chemotactic activity for these cells than those isolated from normal AT.[16] This obesity-induced increase in chemotactic activity is lacking in primary adipocytes isolated from obese mice that lack the enzyme 5-LO and are therefore incapable of producing LTs, suggesting an important role for 5-LO products in the chemotactic activity secreted by adipocytes. This was further confirmed by the observation that inhibition of adipocyte 5-LO activity with zileuton led to a dose-dependent decrease in secreted chemotactic potential by these adipocytes. From these in vitro chemotaxis experiments, we concluded that inhibiting 5-LO activity in adipocytes can fully block their chemotactic potential for macrophages and T cells. We showed that these effects on chemotaxis could be partially due to an indirect effect of blocking 5-LO activity on the production of other chemokines such as monocyte chemoattractant protein 1 (MCP-1), since our data suggests that 5-LO products produced by adipocytes can stimulate MCP-1 production by those cells in an autocrine fashion. 5-LO-deficient mice exhibit a decrease in HFD-induced AT macrophage and T cell infiltration and are partially protected from HFD-induced insulin resistance.[16] Similarly, 2 weeks of daily treatment with zileuton could counteract the insulin resistance and AT inflammatory state already established in HFD-fed wild-type mice. The decrease in AT macrophages was largely due to a reduction in proinflammatory M1 macrophages with, as a consequence, a proportional increase in M2 macrophages. These findings suggest that lack or inhibition of 5-LO leads to a shift in proportion of M1 vs. M2 macrophages in AT. Lastly, we showed that lack or inhibition of 5-LO resulted in an improved inflammatory state in HFD-fed mice, both systemically and locally in AT.[16]

Our finding that only 2 weeks of zileuton treatment already leads to such a significant reduction of AT macrophages and T cells suggests that there is a fast turn-over of these cells. However, it was shown before that ATMs are retained longer in HFD-fed compared with NC-fed mice and it was suggested that ATMs in NC-fed mice have a faster turnover rate than HFD-fed mice.[17] Perhaps the increased levels of LTs found in obese AT are partially responsible for retaining the ATMs and therefore blocking LT production accelerates their departure and decreases their attraction.

One question that we were not able to answer in our recent study is whether the phenotypes observed in the 5-LO knockout mice and the zileuton-treated mice (i.e., decreased inflammation/insulin resistance) are due to the absence or decrease, respectively, in LT production in the adipocytes, stromal vascular cells (SVCs; e.g., macrophages), or both. We have given much thought to how we could determine the relative roles of LT production from both cell compartments to the local and systemic inflammation and development of insulin resistance. We intended to investigate this by performing bone marrow transplant studies. The idea was that by transplanting bone marrow from the 5-LO KO into WT mice we would create mice that would

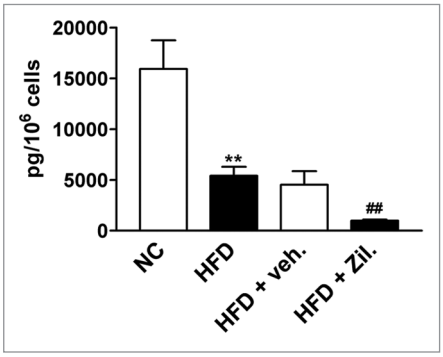

Figure 1. Adipose tissue lipoxin A4 levels. Lipoxin A4 levels in epi-WAT derived from NC- and HFD-fed wild-type mice and HFD-fed wild-type mice treated p.o. daily for a 2-week period with either vehicle (10% DMSO) or zileuton (100 mg/kg). Data are expressed as mean ± SEM ($n \geq 6$). $^{**}p < 0.01$ compared with NC; $^{\#\#}p < 0.01$ compared with HFD + veh.

not be able to produce LTs by the bone marrow-derived stromal vascular cells (e.g., macrophages) but would still produce LTs by adipocytes. Similarly, bone marrow transplants from WT mice to 5-LO KO mice would result in mice that can produce LTs by bone marrow-derived stromal cells (e.g., macrophages) but not by adipocytes. However, we realized that these bone marrow studies would be inconclusive as a consequence of LT production resulting from transcellular biosynthesis. This phenomenon occurs when a donor cell, that has the capabilities to produce LTA_4 (i.e., expresses 5-LO and FLAP), will transfer the LTA_4 to a neighboring acceptor cell that expresses LTA_4-H and/or LTC_4-S, which will then lead to LT synthesis by the acceptor cell.[18] Direct cell-to-cell contact, or at least close proximity between the cells, seems to be necessary for the LTA_4 transfer to occur. This would mean that when, for example, we would transplant 5-LO KO bone marrow to WT mice, adipocytes could transfer LTA4 to neighboring 5-LO-deficient macrophages that could subsequently still produce LTs. Making a cell type-specific 5-LO KO using a Cre-lox system would suffer from the same dilemma. To overcome this problem, instead of transplanting WT BM into 5-LO-deficient recipients we could transfer WT BM into LTA_4-$H^{-/-}$ and/or LTC_4-$S^{-/-}$ mice (and vice versa), thereby preventing any possible LTA_4 conversion to LTB_4 and/or cys-LTs by non-BM derived cells. A similar approach can also be used to investigate whether transcellular biosynthesis does indeed take place in adipose tissue; trancellular biosynthesis does occur if we can still measure BLT_4 or CysLT production in adipose tissue from 5-LO-deficient mice that received BM from LTA_4-H or LTC4-S mice, respectively. Since in that case, LTA_4 from BM-derived cells must have been transferred to 5-LO-deficient surrounding cells that can still convert LTA_4 to BLT_4 or CysLT by the action of LTA_4-H or LTC_4-S, respectively. Following the same line of thought, cell type-specific knockouts of LTA_4H or LTC_4 (or both) using the Cre-lox system would also allow us to circumvent the above described problem of transcellular biosynthesis and study the relative roles of LT production from a specific cell type (e.g., adipocyte or macrophage) in the local and systemic inflammation and development of insulin resistance observed in obese mice. Furthermore, these kinds of approaches would also allow us to delineate the specific roles of LTB_4 vs. CysLTs. In this regard, an important role for LTB_4 has already been demonstrated by the finding that BLT1 knockout mice exhibit a decrease in ATM infiltration and improvement of insulin sensitivity in a model of diet-induced obesity.[15]

In our study, we focused on leukotrienes as products of 5-LO but this enzyme also plays a crucial role in the synthesis of lipoxins, resolvins, and maresins, three families of specialized pro-resolving mediators (SPM).[19] These SPM play an important role in the resolution of inflammation, in an actively coordinated program that permits restoration of tissue homeostasis and involves the effective removal of inflammatory stimuli and the spatio-temporal control of leukocyte trafficking as well as chemical mediator generation.[19] These anti-inflammatory lipid mediators are synthesized by transcellular pathways involving both 12/15-LO and 5-LO. There seems to be an inverse relationship between LT and SPM biosynthesis.[20] Based on this, one could expect to see a decrease of SPM in obese AT. Indeed, it was recently demonstrated that the production of SPM is deficient in inflamed obese AT.[21] We confirm that AT lipoxin A4 levels decrease with HFD (**Fig. 1**). Furthermore, we observed a further decrease when we blocked 5-LO activity with zileuton, confirming that lipoxin A4 production depends on 5-LO activity (**Fig. 1**).

The role for 5-LO in the synthesis of SPM suggests that resolving inflammation in obese AT by stimulating a switch from LT to SPM production by 5-LO could be an alternative way of treating insulin resistance and other pathological conditions that have been shown to be associated with the obesity-induced low-grade inflammation. In line with this, it was shown that stimulation of SPM formation (accompanied by a decrease in LTB_4) in leptin-deficient obese mice, by feeding them an omega-3-fatty acid-enriched diet, led to reduced insulin resistance and hepatic steatosis.[22] The same study showed that directly administering SPM intraperitoneally mimicked these effects. Similarly, two other studies administering either resolvin D1 to leptin receptor-deficient diabetic mice or lipoxin A4 to an age-associated adipose inflammation model, respectively, both showed reduced AT inflammation and improvement of insulin sensitivity.[23,24] Furthermore, resolvin D1 treatment was shown to induce M2 macrophage polarization in obese AT.[23,25]

In summary, further research is needed to elucidate the specific roles of LTB_4 vs. CysLTs in adipose tissue inflammation and the relative role of adipocyte- vs. SVC-derived LTs. However, based on our recent findings and those of others, we can already conclude that 5-LO and its inflammation-inducing or -resolving products play an important role in obesity-induced AT inflammation and subsequent development of insulin resistance. New or already existing therapeutic approaches that target 5-LO or the action of its products might therefore be promising tools for treating obesity-induced inflammation and associated pathologies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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