Adipose Tissue 12/15 Lipoxygenase Pathway in Human Obesity and Diabetes


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Context: Visceral adipose tissue (VAT) is a key contributor to chronic inflammation in obesity. The 12/15-lipoxygenase pathway (ALOX) is present in adipose tissue (AT) and leads to inflammatory cascades that are causal for the onset of insulin resistance in rodent models of obesity.

Objective: The pathophysiology of the ALOX 12/15 pathway in human AT is unknown. We characterized the ALOX pathway in different AT depots in obese humans with or without type 2 diabetes (T2D).

Design: This study includes a cross-sectional cohort of 46 morbidly obese (body mass index > 39 kg/m²) nondiabetic (n = 25) and T2D (n = 21) subjects.

Setting: This study was conducted at Eastern Virginia Medical School (Norfolk, Virginia) in collaboration with Sentara Metabolic and Weight Loss Surgery Center (Sentara Medical Group, Norfolk, Virginia).

Patients: Twenty-five obese (body mass index 44.8 ± 4.4 kg/m²) nondiabetic (hemoglobin A1c 5.83% ± 0.27%) and 21 obese (43.4 ± 4.1 kg/m²) and T2D (hemoglobin A1c 7.66% ± 1.22%) subjects were included in the study. The subjects were age matched and both groups had a bias toward female gender.

Main Outcomes and Measures: Expression of ALOX isoforms along with fatty acid substrates and downstream lipid metabolites were measured. Correlations with depot-specific inflammatory markers were also established.

Results: ALOX 12 expression and its metabolite 12(S)-hydroxyeicosatetraenoic acid were significantly increased in the VAT of T2D subjects. ALOX 15A was exclusively expressed in VAT in both groups. ALOX 12 expression positively correlated with expression of inflammatory genes IL-6, IL-12a, CXCL10, and lipocalin-2.

Conclusions: ALOX 12 may have a critical role in regulation of inflammation in VAT in obesity and T2D. Selective ALOX 12 inhibitors may constitute a new approach to limit AT inflammation in human obesity. (J Clin Endocrinol Metab 99: E1713–E1720, 2014)
tabolites hydroperoxyeicosatetraenoic acid and hydroxyeicosatetraenoic acids (HETEs) are involved in insulin resistance (3). Sequential action of the 12-LO or 15-LO and 5-LO on docosahexanoic acid (DHA) leads to the formation of antiinflammatory lipid compounds such as maresins and resolvins (4). A recent paper described the presence and diversity of both the pro- and antiinflammatory lipid mediators derived from the lipoxigenase pathway in sc and perivascular fat in humans (5). We have previously demonstrated that in obese humans, ALOX 12, ALOX 15a, and ALOX 15b are more highly expressed in omental (OM) than in sc adipose tissue (AT) (6). Little is known about the upstream activators of the 12/15 ALOX enzymes in human AT. A potential candidate is lipocalin-2 (LCN2), a recently characterized proinflammatory molecule with positive correlation with obesity and insulin resistance (IR) (7) that was shown to stimulate activity of 12-LO in AT (8).

Here we characterize the ALOX12/15 family of enzymes and their pro- and antiinflammatory metabolites in the sc and OM depots of obese humans with and without type 2 diabetes (T2D). Results suggest a proinflammatory role for ALOX 12 and its metabolites and significant correlations between expression of LCN2, proinflammatory cytokines and ALOX 12 in OM fat in T2D.

Selective targeting of ALOX 12 may provide a novel therapeutic approach to reduce inflammation in visceral fat and resulting complications.

Materials and Methods

Research subjects

The study included a cross-sectional cohort of morbidly obese T2D (n = 21) and nondiabetic subjects (n = 25), aged 18–65 years, undergoing bariatric surgery at Sentara Metabolic and Weight Loss Surgery Center (Sentara Medical Group, Norfolk, Virginia). Exclusion criteria included autoimmune disease including type 1 diabetes mellitus, conditions requiring chronic immunosuppressive therapy, antiinflammatory medications, thiazolidinediones, active tobacco use, chronic or acute infections, or a history of malignancy treated within the last 12 months. T2D was defined as a fasting plasma glucose of 126 mg/dL or greater, a glucose of 200 mg/dL or greater after a 2-hour glucose tolerance test, or use of antidiabetic medications. Informed consent was obtained, and the research project was approved by the Eastern Virginia Medical School Institutional Review Board.

Quantitative real-time PCR and gene arrays

These procedures were described previously (6). Results were normalized to 18S RNA and data were expressed as 1/Δ cycle threshold. The multiplex gene array PCR for AT was performed using the Cytokine and Chemokine RT² Profiler PCR array from SA Bioscience.

Immunohistochemistry

Formalin-fixed, paraffin-embedded tissue biopsies were incubated with antibodies for human leukocyte antigen (HLA-D-related [DR]; Dako; 1:100 dilution), CD68 (Dako; 1:100 dilution), or CD3 (Dako; 1:100 dilution) and detected using the avidin-biotin-peroxidase method. Quantification of the data was done using a MetaMorph software version 6.3 (Molecular Devices).

Lipid extraction and analysis by liquid chromatography and tandem mass spectrometry and gas chromatography

Lipids were extracted from liquid N₂-pulverized AT samples using methanol/NaOH followed by the addition of the deuterated internal standards and hydrolysis. Fatty acids were extracted using boron trifluoride/methanol and pentadecanoic acid and further transesterified to corresponding methyl esters that were analyzed using a gas chromatograph equipped with a flame ionization detector. For lipid metabolites, a solid-phase extraction procedure using Varian Bond-Elut Certify II was performed as described by Rivera et al (9). The liquid chromatography, electrospray ionization, and tandem mass spectrometry method for the determination of metabolites was described previously (10, 11).

Statistical analysis

The SAS MIXED analysis (repeated measures ANOVA) was used to evaluate the main and interactive effects of type of adipose tissue depot (omental vs sc) and diabetes status (diabetic vs nondiabetic), taking into account the clustering of data. Correlations were established using the linear regression analysis and Pearson’s coefficient.

Results

Subjects

Twenty-five nondiabetic (three males, 22 females), aged 41.4 ± 9.2 years and 21 T2D subjects (six males, 15 females), aged 48.7 ± 6.8 years were included. The body mass index was 44.8 ± 4.4 kg/m² for nondiabetic and 48.7 ± 6.8 kg/m² for T2D subjects (P < .05). Hemoglobin A1c was significantly higher in the T2D vs nondiabetic subjects (7.66% ± 1.22% vs 5.83% ± 0.27%), whereas fasted insulin was not significantly different (6.21 ± 3.24 vs 7.05 ± 2.48 mU/L).

Expression and activity of ALOX 12/15 lipoxygenases

We examined the expression of ALOX 12, ALOX 15a, and ALOX 15b in sc and OM depots of T2D or nondiabetic subjects and found significantly higher expression of ALOX 12 in the OM vs sc depot independent of the presence of T2D. In addition, the effect of T2D on ALOX 12 expression is depot specific, with a significant increase in the OM depot only (Figure 1A). ALOX 15a expression...
was absent in the sc depot from both T2D and nondiabetic subjects, and we did find an increase in ALOX15B in the OM depot that was independent of T2D presence (Figure 1A).

Next, we profiled the 12/15 ALOX fatty acid substrates and metabolites in T2D and nondiabetic sc and OM (Figure 1, B and C). Linoleic acid (LA) showed 10-fold higher values compared with AA and 100-fold higher than DHA. The amount of AA, LA, and DHA did not differ between T2D and nondiabetic subjects in either of the depots (Figure 1B). Proportionally similar abundances were found for the major lipid metabolites originating from the three substrates (Figure 1C). Interestingly, the ALOX 12-derived metabolites followed the pattern of ALOX 12 expression. The expression of 12-HETE was independently increased in the OM depot and in T2D. In addition, the effect of diabetic status on 12-HETE expression is depot dependent, as suggested by the significant interaction effect (Figure 1C). This suggests that 12-HETE is a key inflammatory metabolite associated with T2D in the OM fat. Expression of 14(S)-HDHA [(S)-hydroxy-docosahexaenoic acid], the DHA-derived metabolite of ALOX 12 was also increased in the OM depot independent of the presence of T2D (Figure 1C). The levels of 13S-hydroxy-9Z,11E-octadecadienoic acid, the most abundant metabolite, were influenced only by the T2D presence and were depot independent.

The absence of ALOX 15A expression in the sc depot from both T2D and nondiabetic subjects, and we did find an increase in ALOX15B in the OM depot that was independent of T2D presence (Figure 1A).

![Figure 1. ALOX isoform gene expression, fatty acid substrates, and lipid metabolites in human adipose tissue. Gene expression (A) for ALOX 12, ALOX 15A, and ALOX 15B was measured using specific Taqman probes, and data were expressed as 1/Δ cycle threshold after normalization to 18S expression. Results represent mean ± SEM, and the null hypothesis was rejected for a value of P < .05. Fatty acid substrates (B) and metabolites (C) of the ALOX 12/15 pathway were measured in a subgroup of six diabetic and six nondiabetic subjects; the ALOX isoforms involved in formation of the various metabolites are illustrated based on the reported data. Data were analyzed using the SAS MIXED model and comparisons between depots (OM vs sc) and disease groups [nondiabetic (ND) vs diabetic (T2D)] as well as the interaction term (depot × disease) are illustrated below each of the graphs; the null hypothesis was rejected for a value of P < .05. Graphs show the median (solid line) and mean (dotted line) values for each group including the confidence interval and the outliers (dots).](https://academic.oup.com/jcem/article-abstract/99/9/E1713/2537538/doi-10-1210-jcem-2013-4461)
The increased levels of this antiinflammatory metabolite in the OM and T2D subjects suggest a protective compensatory mechanism in the setting of increased inflammation. 5-HETE was comparable with the other HETE levels; however, no differences in the abundance were found between depots or with T2D (not shown). This suggests that the formation of the antiinflammatory DHA-derived metabolites is primarily accounted

![Figure 2](https://academic.oup.com/jcem/article-abstract/99/9/E1713/2537538)
for by differences in ALOX 12 and ALOX 15 expression.

**Correlation between ALOX expression and AT inflammatory markers**

The relationship of other AT inflammatory markers to changes in ALOX isoform expression was tested. We found an increased expression of *IL-6*, *IL-12a*, and chemokine ligand 10 (*CXCL10*) in the OM of T2D subjects compared with nondiabetic subjects as well as significant correlations between the expression of the latter and ALOX 12 expression (Figure 2A). Gene array analysis of 72 cytokines and chemokines indicated several additional proinflammatory molecules significantly up-regulated in the OM and sc of T2D subjects, notably the proinflammatory genes *TNFRS11b* and *SPP1* (osteopontin) (Figure 2B).

We next examined the immune cell composition in AT and found an increase in the number and activation of the macrophages in the OM fat, reflected by an increase in CD68 and HLA-DR markers (Figure 2D). T2D had a significant effect on the number of macrophages and CD3+ cells in both of the OM and sc depots (Figure 2D).

LCN2, a proinflammatory neutrophil elastase, up-regulates the expression of 12-LO in murine models of obesity and IR. We found a significantly higher mRNA expression...
of LCN2 in OM vs sc that was influenced by T2D in a depot-independent manner. Also, LCN2 protein expression was elevated in the OM of T2D vs nondiabetic subjects (Figure 2C). Importantly, LCN2 and ALOX12 expressions were significantly correlated in the OM depot of T2D subjects (Figure 2C). These results support LCN2 as a candidate molecule responsible for the up-regulation of ALOX 12 expression in T2D.

**Discussion**

Visceral AT inflammation is a major contributor to IR, T2D, and cardiovascular complications of obesity. The ALOX pathway was extensively described in visceral AT and vasculature in animal models and was causally related to the development of IR in obesity via generation of pro-inflammatory metabolites (2). Mice with a targeted deletion of 12-/15-LO in adipose tissue are protected from IR and pancreatic islet inflammation, suggesting a key role of AT 12-/15-LO for the onset of IR in obesity (12). We previously reported that ALOX 12, ALOX 15A and ALOX 15B have a depot- and cell-specific expression in human AT that differs from that of animal models of obesity (6), which emphasizes the importance of findings validation in humans (1).

One novelty of the current study is identifying ALOX 12 as the major isoform up-regulated in OM and increased by T2D in a depot-independent manner. A key feature of this study was that the fatty acid substrates for ALOXs 12/15 and the major classes of lipid metabolites were measured in parallel with ALOX expression in both sc and OM fat in T2D and nondiabetics. Despite the large difference in abundance between the AA, LA, and DHA, there were no differences between the depots or with T2D. This suggests equal substrate exposure but does not rule out possible differences in substrate preference as was previously seen for human ALOX 12, ALOX 15a, and ALOX 15b (13, 14).

In accordance with an earlier report (15), we found 13S-hydroxy-9Z,11E-octadecadienoic acid as the most abundant metabolite in AT. This LA metabolite was significantly increased by the presence of T2D in a depot-independent manner, suggesting a substantial contribution of the ALOX 12 isoform. The AA-derived metabolites, 12-HETE and 15-HETE, were more abundant in the OM vs sc depot. Also, 12-HETE was the only metabolite that was up-regulated in T2D in a depot-specific manner. The antiinflammatory resolvin D1 precursor 17(S)-HDHA, generated by ALOX 15a, was significantly increased by T2D in a depot-independent manner, possibly as an attempt to resolve persistent inflammation.
These results suggest that in T2D subjects, ALOX 12 is producing predominantly proinflammatory metabolites, whereas the ALOX 15a produces more of the antiinflammatory metabolites. Future therapeutic development should focus on highly selective ALOX 12/15 inhibitors that target primarily the proinflammatory isoform.

Another key finding is a positive correlation between ALOX 12 expression and proinflammatory cytokines in the OM fat of T2D subjects. Macrophages abundantly infiltrate adipose tissue in obesity (16). We previously showed that ALOX 12 is mainly expressed in the CD34+ cellular component of the stromal vascular compartment including macrophages (6). Indeed, we find that the macrophage marker CD68 and the myeloid activation marker HLA-DR are both significantly increased in the OM fat of T2D subjects, suggesting that the increased macrophage infiltration and activation may contribute to the increased ALOX 12 expression. Also, IL-12 production by lipopolysaccharide-stimulated macrophages is dependent on 12-/15-LO (17), and therefore, increases in ALOX 12 in the OM depot may account for the increased IL-12 expression. We also found in OM of T2D subjects significant elevation of additional inflammatory molecules reportedly increased in obesity and vascular disease (osteopontin and TNFRSF11b). Similar increases in cytokines and inflammatory cell markers were reported recently in the visceral fat of obese subjects with a high correlation of IL6 expression and T2D (18). Importantly, ALOX variants in humans are associated with induced expression of IL-6, TNFα, and IL-1β, indicating a broad role for the enzyme in systemic inflammation (19). Therefore, the correlations found between ALOX 12 and proinflammatory cytokines support a role for ALOX 12 in the initiation and/or propagation of inflammation in visceral AT in T2D.

Reports show that LCN2, a neutrophil elastase produced by adipocytes, is elevated with aging and obesity in rodents and can stimulate the expression and activity of 12/15-lipoxygenase isoenzymes in adipocytes, is elevated with aging and obesity in rodents and can stimulate the expression and activity of lipoprophage-activated protein (20). However, a role for insulin is less likely because no differences were found in fasting insulin between the T2D and nondiabetic groups.

In summary, data establish the depot-specific pattern of expression and activity of the 12/15 ALOX enzymes and identifies ALOX 12 as the main proinflammatory isoform increased by T2D in a depot-dependent fashion.

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