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ARTICLE

Evidence of depot-specific regulation of *all-trans*-retinoic acid biosynthesis in human adipose tissue

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Abstract

The prevalence of obesity continues to rise, underscoring the need to better understand the pathways mediating adipose tissue (AT) expansion. *All-trans*-retinoic acid (*at*RA), a bioactive vitamin A metabolite, regulates adipogenesis and energy metabolism, and, in rodent studies, aberrant vitamin A metabolism appears a key facet of metabolic dysregulation. The relevance of these findings to human disease is unknown, as are the specific enzymes implicated in vitamin A metabolism within human AT. We hypothesized that in human AT, family 1A aldehyde dehydrogenase (ALDH1A) enzymes contribute to *at*RA biosynthesis in a depot-specific manner. To test this hypothesis, parallel samples of subcutaneous and omental AT from participants ($n = 15$) were collected during elective abdominal surgeries to quantify *at*RA biosynthesis and key *at*RA synthesizing enzymes. ALDH1A1 was the most abundant ALDH1A isoform in both AT depots with expression approximately twofold higher in omental than subcutaneous AT. ALDH1A2 was detected only in omental AT. Formation velocity of *at*RA was approximately threefold higher ($p = 0.0001$) in omental AT (9.8 [7.6, 11.2]) pmol/min/mg) than subcutaneous AT (3.2 [2.1, 4.0] pmol/min/mg) and correlated with ALDH1A2 expression in omental AT (β-coefficient = 3.07, $p = 0.0007$) and with ALDH1A1 expression in subcutaneous AT (β-coefficient = 0.13, $p = 0.003$). Despite a positive correlation between body mass index (BMI) and omental ALDH1A1 protein expression (Spearman $r = 0.65$, $p = 0.01$), BMI did not correlate with *at*RA formation. Our findings suggest that ALDH1A2 is the primary mediator of *at*RA formation in omental AT, whereas ALDH1A1 is the principal *at*RA-synthesizing enzyme in subcutaneous AT. These data highlight AT depot as a critical variable for defining the roles of retinoids in human AT biology.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Rodent data suggest that dysregulated production of *all-trans*-retinoic acid (*at*RA), the primary bioactive metabolite of vitamin A, may contribute to body weight gain and its complications. However, the key enzymes responsible for *at*RA biosynthesis in human adipose tissue have not been identified, nor has the relationship between body weight and adipose tissue *at*RA biosynthesis been evaluated in humans.

WHAT QUESTION DID THIS STUDY ADDRESS?

This study sought to identify the key enzymes involved in *at*RA biosynthesis in human omental and subcutaneous adipose tissue. This study also quantified *at*RA formation velocity and explored the potential relationship between body mass index (BMI) and *at*RA biosynthesis in both adipose tissue depots.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

This study establishes that among the aldehyde dehydrogenase (ALDH) isoforms, ALDH1A1 and ALDH1A2 both contribute to *at*RA biosynthesis in human omental adipose tissue, whereas only ALDH1A1 contributes to *at*RA biosynthesis in subcutaneous adipose tissue. Both ALDH1A1 expression and *at*RA formation velocity are substantially higher in omental than subcutaneous adipose tissue. Omental ALDH1A1 protein expression exhibits a positive correlation with BMI, but *at*RA formation velocity in both omental and subcutaneous adipose tissue shows no correlation with BMI. Thus, these findings highlight discrepancies between human and rodent adipose tissue biology and, moreover, reveal depot-specific regulation of vitamin A metabolism in human adipose tissue.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

This line of research ultimately is intended to define the roles of vitamin A metabolites in the regulation of tissue remodeling and energy partitioning in human adipose tissue. This knowledge could contribute to the delineation of mechanisms underlying progressive obesity and its complications.

INTRODUCTION

The prevalence of obesity continues to rise, and an estimated 42% of US adults now live with obesity,[1] underscoring the need to gain better understanding of the mechanisms that underlie adipose tissue expansion and body weight gain. Vitamin A (retinol) is a fat-soluble vitamin that plays critical roles in vision, immune and barrier function, reproduction, and energy metabolism. Hallmark signs of systemic vitamin A deficiency include impaired immunity, delayed growth, and infertility. In contrast to this systemic, nutritional deficiency, mounting preclinical data suggest that a state of relative, tissue-specific vitamin A deficiency may arise in metabolic disorders including obesity and hepatic steatosis.[2,3] Further, retinoid administration has been shown to ameliorate these metabolic derangements in rodent models,[4,5] suggesting that delineation of vitamin A homeostasis within human metabolic tissues could lend novel, mechanistic insight into obesity and its complications.

All-trans-retinoic acid (*at*RA), the bioactive metabolite of retinol, is a versatile transcriptional regulator with high affinity for its canonical nuclear receptors, retinoic acid receptors (RARs).[6] Numerous roles for *at*RA signaling in adipose tissue biology have been identified, including regulation of adipocyte differentiation, adipose tissue browning, lipogenesis, and fatty acid oxidation.[7–10] In rodent studies, both genetic and dietary models of obesity resulted in diffuse tissue retinol deficiency accompanied by reduced retinoid signaling.[3] Inversely, moderate systemic *at*RA depletion through genetic manipulation led to increased adiposity and body weight gain in both male and female mice, and this metabolic phenotype was reversed with *at*RA administration.[4] Further, treatment with RA or an RARβ agonist has been shown to confer metabolically protective effects in rodent models of obesity.[11,12] However, comparable *at*RA doses used pharmacologically in clinical settings have been shown to confer risk of hyperlipidemia, increased truncal adiposity, and

metabolic syndrome.[13] Moreover, ALDH1A1-specific inhibitors have been shown to protect mice on a high-fat diet from progressive body weight gain and adiposity and accordingly have been proposed as a novel treatment strategy for obesity.[14,15] These collective findings underscore the importance of delineating *at*RA-synthesizing pathways in metabolic tissues specifically in humans, both to better understand apparent discord between preclinical and clinical data and to inform the design of targeted therapeutics for obesity.

Fundamental questions regarding vitamin A metabolism within human adipose tissue remain unanswered, including which enzymes are responsible for *at*RA biosynthesis. The aldehyde dehydrogenase 1A (ALDH1A) family of enzymes,[16,17] also known as retinaldehyde dehydrogenases (RALDHs), are the predominant enzymes contributing to *at*RA biosynthesis in many tissues, but their expression varies among tissues, cell types, and with developmental stage.[18–20] In addition, aldehyde oxidase (AOX) may contribute to *at*RA biosynthesis.[21] At present, the respective, depot-specific roles of these enzymes in modulating human adipose tissue *at*RA concentrations and vitamin A homeostasis are unknown. Further, the potential influence of body weight on *at*RA formation requires additional investigation. We hypothesized that *at*RA biosynthesis would differ between subcutaneous and omental adipose tissue depots, and, based on rodent models, we predicted that diminished *at*RA biosynthesis would be evident in omental adipose tissue with increasing body mass. To test these hypotheses, we characterized *at*RA biosynthesis in human omental and subcutaneous adipose tissue and identified the respective enzymes responsible for *at*RA biosynthesis in these depots. We also explored the potential impact of body mass on adipose tissue depot-specific *at*RA formation.

METHODS

Chemicals and reagents

Retinaldehyde (RAL), *all-trans*-retinoic acid (*at*RA) and nicotinamide adenine dinucleotide (NAD^+) were purchased from Millipore Sigma (Burlington, MA) and *at*RA-d_5 from Cambridge Isotope Laboratories (Tewksbury, MA). All mass spectrometry grade solvents were purchased from Thermo Fisher Scientific (Waltham, MA). Stable isotope labeled (SIL) peptides labeled with [$^{13}C_6{}^{15}N_2$] arginine and [$^{13}C_6{}^{15}N_2$] lysine were from Thermo Fisher Scientific (Waltham, MA) and used as internal standards (IS) for AOX, aldehyde dehydrogenase 1 family member A1 (ALDH1A1) and family member A2 (ALDH1A2) protein quantification.[21,22] Human recombinant ALDH1A1, ALDH1A2, and AOX were expressed and purified as described previously and used as quantification standards.[21]

Participants

Men and women ages 18–65 years old who were planning to undergo elective abdominal surgical procedures were recruited through University of Washington (UW) Medical Center surgery clinics. The study protocol was approved by the UW Institutional Review Board and conducted in accord with the Declaration of Helsinki principles. Participants provided written informed consent prior to performance of any study procedures. Exclusion criteria included a history of endocrine or other secondary causes of obesity, diabetes, current use (within 3 months) of any weight loss or glucose-lowering medication, major systemic illness or infection, daily vitamin A supplementation ≥10,000 international units, malabsorptive gastroenterological disease, anemia, or bleeding disorders.

Adipose tissue sample acquisition and processing

Biopsies of omental and subcutaneous adipose tissues were performed by surgeons during the elective surgical procedures, with tissue yields of 2–23 g from each depot. Acquisition of the adipose tissue samples by research staff was within 5 min of excision, and tissue was immediately rinsed with ice-cold phosphate-buffered saline, flash-frozen on dry ice, and stored at −80°C until preparation of S9 fractions.

Preparation of S9 fractions from adipose tissue

Human adipose tissue (150–260 mg) was homogenized in homogenizing buffer (10 mM potassium phosphate [KPi], pH 7.4, 250 mM sucrose) supplemented with cOmplete Protease Inhibitor Cocktail (Millipore Sigma, Burlington, MA) in a ratio of 3:1 of buffer to tissue weight. The homogenization was performed using an Omni Bead Ruptor 24 Bead Mill homogenizer (Omni International, Inc., Kennesaw, GA) with ceramic beads at 4°C. The adipose tissue homogenate was transferred to a 1.7 ml Eppendorf tube and centrifuged at 9000 *g* at 4°C for 30 min. After the top lipid layer was gently removed, the supernatant (S9 fraction) was aliquoted and stored at −80°C until use. A bicinchoninic acid (BCA) assay was performed to determine protein concentrations in all S9 fractions and S9

protein yield per gram of tissue was calculated for each tissue sample.

Measurement of *at*RA formation velocity and ALDH1A, AOX, and CRBP-I protein expression in adipose tissue S9 fractions

Formation of *at*RA and the expression of ALDH1A and AOX protein in adipose tissue were quantified in tissue S9 fractions using liquid-chromatography tandem mass spectrometry (LC-MS/MS) following published procedures, and CRBP-I expression was quantified by enzyme-linked immunosorbent assay (ELISA; Supplementary Methods, Sections S1–3).[21,22] All quantification was done blinded to the individual participant and adipose tissue depot analyzed.

Predictions of *at*RA formation velocity in adipose tissue S9 fractions

The *at*RA formation velocity in individual adipose tissue S9 samples was predicted using Equation 1.

$$v_{\text{predicted}} = \frac{[\text{ALDH1A1}] * k_{cat,1A1} * [\text{S}]}{K_{m,\,\text{ALDH1A1}} + [\text{S}]} + \frac{[\text{ALDH1A2}] * k_{cat,1A2} * [\text{S}]}{K_{m,\,\text{ALDH1A2}} + [\text{S}]} + \frac{[\text{AOX}] * k_{cat,\,\text{AOX}} * [\text{S}]}{K_{m,\,\text{AOX}} + [\text{S}]} \quad (1)$$

In Equation 1, $v_{\text{predicted}}$ is the predicted *at*RA formation velocity at the given retinaldehyde concentration [S]. [ALDH1A1], [ALDH1A2], and [AOX] are the measured expression levels (mean of the replicate analyses of each sample) in units of pmol/mg S9 protein for each enzyme in the individual donor S9 fractions from a given depot based on the LC-MS/MS quantification. The K_m and k_{cat} values are as previously measured for recombinant ALDH1A1 (K_m = 285 nM, k_{cat} = 1.1 min^{-1}),[20] ALDH1A2 (K_m = 56 nM, k_{cat} = 3.7 min^{-1}),[20] and AOX (K_m = 1500 nM, k_{cat} = 3.6 min^{-1}).[21] The average fold error (afe) of predictions of *at*RA formation velocity in adipose tissue S9 fractions was calculated using Equation 2.

$$\text{afe} = 10^{\frac{1}{n}\sum \log \frac{v_{\text{predicted}}}{v_{\text{observed}}}} \quad (2)$$

In Equation 2, $v_{\text{predicted}}$ and v_{observed} are the predicted (based on Equation 1) and observed *at*RA formation velocities, respectively, in individual donor S9 samples. Afe values ranging from 0.5 to 1.5 were considered acceptable for assuming enzymes forming *at*RA in adipose tissue are adequately characterized. The mean predicted fraction of *at*RA formation by a particular enzyme (f_m) in adipose tissue S9 fractions was calculated using Equation 3:

$$f_{\text{m,predicted}} = \frac{v_{\text{enzyme}}}{v_{\text{total}}} \quad (3)$$

In Equation 3, v_{total} is the average *at*RA formation velocity ($v_{\text{total}} = v_{\text{ALDH1A1}} + v_{\text{ALDH1A2}} + v_{\text{AOX}}$) calculated from Equation 1 with retinaldehyde [S] concentrations ranging from 0.001 to 2 μM, [ALDH1A1], [ALDH1A2], and [AOX] are the average values of the enzyme expression calculated from individual adipose tissue S9 samples for a given depot, and v_{enzyme} is the predicted *at*RA formation velocity by an individual enzyme (ALDH1A1, ALDH1A2, and AOX) in a given depot calculated from the average enzyme expression in S9 samples and the kinetic values measured with recombinant enzyme. The expression levels of ALDH1A2 and AOX in subcutaneous adipose tissue were assigned as 0 for the prediction calculation as the expression was below lower limit of detection (LLOD) in all samples for ALDH1A2 and in all but one sample for AOX.

Statistical analyses

Protein expression and *at*RA formation data are reported as normalized to both adipose tissue S9 protein and adipose tissue mass. All data are presented as median (interquartile range [IQR]). The comparisons of ALDH1A1 protein expression and *at*RA formation velocity between omental and subcutaneous whole adipose tissue S9 samples were conducted with a Wilcoxon matched-pairs signed rank test. For all statistical analyses, a value of 0 was assigned for samples with enzyme expression levels below the LLOD. To evaluate the relationships between protein expression and *at*RA formation with body mass index (BMI), nonparametric correlation analyses were performed, and Spearman r values are presented. Multiple linear regression analyses were used to test correlations between *at*RA formation and ALDH1A and AOX expression. For all regression models, enzyme expression levels and BMI were included as independent variables. Both statistically significant variables ($p < 0.05$) and those evident as statistical trends ($p < 0.1$) were included in the final models, and standardized β-coefficients are shown. All statistical analyses were performed using GraphPad Prism 8.0 (GraphPad Software; San Diego, CA). A p value threshold less than 0.05 was considered statistically significant.

RESULTS

Study participants

Participants were overall healthy, without history of diabetes or significant cardiac, pulmonary, kidney, or liver disease. Two participants had hypertension that was well controlled on a single antihypertensive agent, and two participants were taking antidepressant medications. The other 11 participants had no other major comorbid conditions. Basic demographic and clinical information for the 15 participants are listed in Table 1, and the study design is depicted in Figure 1. The median BMI for study participants was 39.9 (26.0, 43.2) kg/m^2, and the median age was 39 (34, 42) years. Omental adipose tissue was not collected from one participant, as the indicated surgical procedure did not provide access to this depot for biopsy. Race and ethnicity were self-reported. One study participant experienced a hematoma at the port site used for subcutaneous adipose tissue biopsy. This was self-resolving, and no other study-related adverse events occurred.

ALDH1A1 is the most highly expressed *at*RA-synthesizing enzyme in human omental and subcutaneous adipose tissue

In order to identify the key *at*RA-synthesizing enzymes in omental and subcutaneous adipose tissue, protein expression of ALDH1A1, ALDH1A2, ALDH1A3, and AOX was quantified (Figure 2a–d). In both depots, ALDH1A1 was the most abundant of these proteins, and its expression was higher in omental than subcutaneous adipose tissue (35.5 [29.3, 42.5]) pmol/mg S9 protein in omental adipose tissue vs. 12.8 [10.0, 19.7] pmol/mg S9 protein in subcutaneous adipose tissue, $p = 0.0001$). In omental adipose tissue, ALDH1A2 and AOX also were detected, albeit at expression levels substantially lower than ALDH1A1 (1.2 [0.8, 1.6] pmol/mg S9 protein for ALDH1A2 and 1.1 [0.8, 1.3] pmol/mg S9 protein for AOX; Figure 2e). ALDH1A2 and AOX were detectable in 13 of the 14 omental adipose samples but below the LLOQ in one of these samples; the two samples with low or undetectable expression for ALDH1A2 and AOX were from the same two study participants. In contrast, ALDH1A2 was not detected in any subcutaneous adipose tissue samples, whereas AOX was quantifiable in one study participant. ALDH1A3 protein was not detected in either omental or subcutaneous adipose tissue.

When protein expression was normalized to adipose tissue mass rather than S9 protein, ALDH1A1 expression was over twofold higher in omental than in subcutaneous adipose tissue (238.0 [203.2, 324.6] pmol/gm omental adipose tissue vs. 99.2 [84.3, 127.7] pmol/gm subcutaneous adipose tissue, $p = 0.0006$; Figure 2f).

CRBP-I was expressed both in subcutaneous and omental adipose tissue (1.5 [1.2, 1.7] ng/mg S9 protein in subcutaneous adipose tissue versus 1.3 [1.9, 1.4] ng/mg S9 protein in omental adipose tissue) with the expression in subcutaneous adipose tissue modestly but significantly higher ($p = 0.003$).

TABLE 1 Demographic and clinical information for study participants

Age, years	Sex, M/F	Body mass index, kg/m^2	Surgical procedure	Race/ethnicity	Comment
62	M	26.0	Hernia repair	White/not Hispanic	
27	M	20.7	Hernia repair	White/not Hispanic	
38	F	26.5	Hernia repair	Black or African American	Subcutaneous adipose tissue only
51	M	23.0	Hernia repair	White/not Hispanic	
41	M	25.7	Cholecystectomy	White/Hispanic or Latino	
42	F	42.4	Gastric bypass	White/not Hispanic	
38	F	43.0	Gastric bypass	White/not Hispanic	
24	F	40.0	Sleeve gastrectomy	White/not Hispanic	
63	F	39.9	Gastric bypass	White/Hispanic or Latino	
40	M	36.0	Cholecystectomy	White/not Hispanic	
35	F	51.9	Gastric bypass	White/not Hispanic	
38	F	45.8	Gastric bypass	White/not Hispanic	
39	M	39.8	Sleeve gastrectomy	White/not Hispanic	
31	M	43.2	Gastric bypass	White/not Hispanic	
34	F	52.9	Gastric bypass	White/Hispanic or Latino	

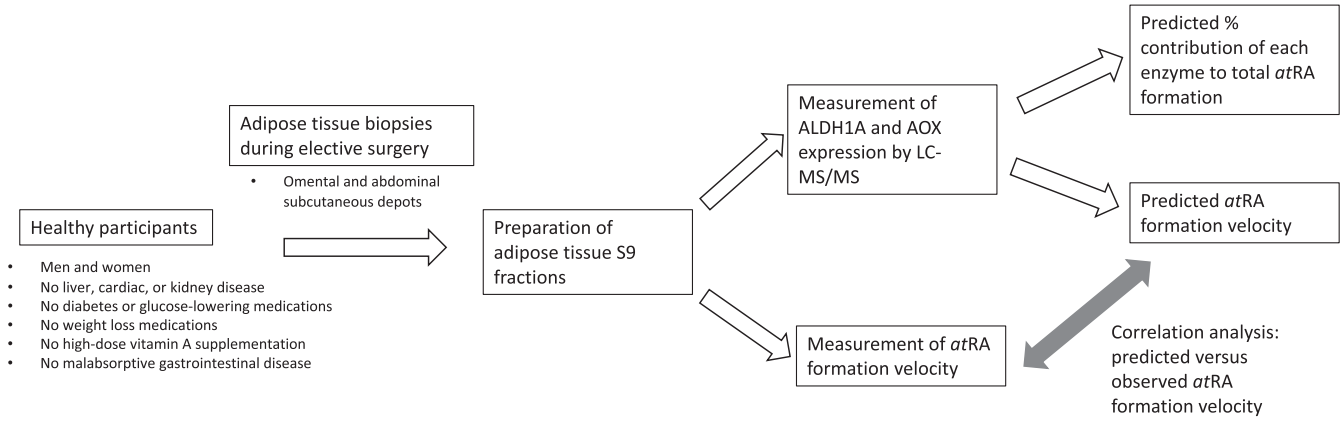

FIGURE 1 Schematic of the study design. Subcutaneous and omental adipose tissue were collected through surgical biopsy from healthy participants across a broad spectrum of body mass index. Tissue S9 fractions were isolated and used to measure expression of *at*RA synthesizing enzymes and *at*RA formation velocity in both adipose tissue depots. Protein expression data were used to predict the relative contribution of each enzyme to total *at*RA formation and *at*RA formation velocity for each adipose tissue depot. The correlation between the predicted and observed *at*RA formation velocities was determined to further test the validity of the observed findings. ALDH, aldehyde dehydrogenase; AOX, aldehyde oxidase; *at*RA, *all-trans*-retinoic acid; LC-MS/MS, liquid-chromatography tandem mass spectrometry

Human omental and subcutaneous adipose tissue exhibit distinct patterns of enzymatic regulation of *at*RA formation

The formation velocity of *at*RA was quantified for both omental and subcutaneous adipose tissue depots. Velocity was measured both in the presence of NAD^+, an essential cofactor for ALDH1A but not AOX activity, and in its absence to provide an estimate of AOX activity alone. Formation of *at*RA was observed in omental and subcutaneous adipose tissue in the absence of NAD^+, indicating the presence of AOX activity, but formation velocity was much higher in the presence of NAD^+ in all samples (Figure 3).

In parallel with ALDH1A1 protein expression, *at*RA formation was higher in omental than subcutaneous adipose tissue in the presence of NAD^+ (9.8 [7.6, 11.2] pmol/min/mg S9 protein in omental adipose tissue versus 3.2 [2.1, 4.0] pmol/min/mg S9 protein in subcutaneous adipose tissue, $p = 0.0001$; Figure 3B). Formation velocity of *at*RA in the absence of NAD^+ was markedly (~90%) lower than in the presence of NAD^+ in both omental (0.8 [0.3, 1.1] pmol/min/mg S9 protein) and subcutaneous (0.3 [0.2, 0.5] pmol/min/mg S9 protein] adipose tissue, supporting only a minor role for AOX in *at*RA biosynthesis (~10% of total formation) in both adipose tissue depots. The formation velocity of *at*RA in the absence of NAD^+ was higher in omental than subcutaneous adipose tissue ($p = 0.002$).

When *at*RA formation velocity was normalized to adipose tissue mass, *at*RA formation remained significantly higher in omental than subcutaneous adipose tissue (70.9 [48.9, 96.2] pmol/min/gm omental adipose tissue versus 24.3 [11.2, 26.7] pmol/min/gm subcutaneous adipose tissue, $p = 0.0006$; Figure 3c). Formation velocity of *at*RA in the absence of NAD^+ also exhibited a similar pattern when normalized to adipose tissue mass (5.2 [3.5, 7.5] pmol/min/gm omental adipose tissue versus 2.2 [0.8, 3.9] pmol/min/gm subcutaneous adipose tissue, $p = 0.06$).

Based on the measured protein expression of ALDH1A1, ALDH1A2, and AOX and the known kinetics of *at*RA formation by the individual enzymes, *at*RA formation velocity was predicted for each omental and subcutaneous adipose tissue S9 fraction, and the predicted values were compared to the observed *at*RA formation velocities (Figure 4a,d). The afe values were 1.27 for omental adipose tissue and 1.37 for subcutaneous adipose tissue, demonstrating acceptable characterization of *at*RA formation in the samples. The fraction of retinaldehyde metabolized to *at*RA by ALDH1A1, ALDH1A2, and AOX (the flux of *at*RA biosynthesis via each of these enzymes) in adipose tissue S9 fractions was predicted at various concentrations of retinaldehyde (0.001–2 μM), with values spanning far below and above the expected physiologic concentration in adipose tissue (~0.1 μM). Due to the differences in the K_m values of retinaldehyde with ALDH1A1 and ALDH1A2, the predicted fraction metabolized (f_m) by ALDH1A1 in omental adipose tissue increased from 66% to 84% with increasing retinaldehyde concentrations, and the ALDH1A2 f_m decreased from 33% to 11%. The predicted f_m by AOX was generally low (~1%) and consistent with the observed *at*RA formation in adipose tissue S9 fractions in the absence of NAD^+. At a physiologically relevant concentration of retinaldehyde (0.1 μM) in omental adipose tissue,[23] ALDH1A1, ALDH1A2, and AOX were predicted to contribute 65%, 35%, and 1% of total *at*RA formation, respectively. In contrast to omental adipose tissue,

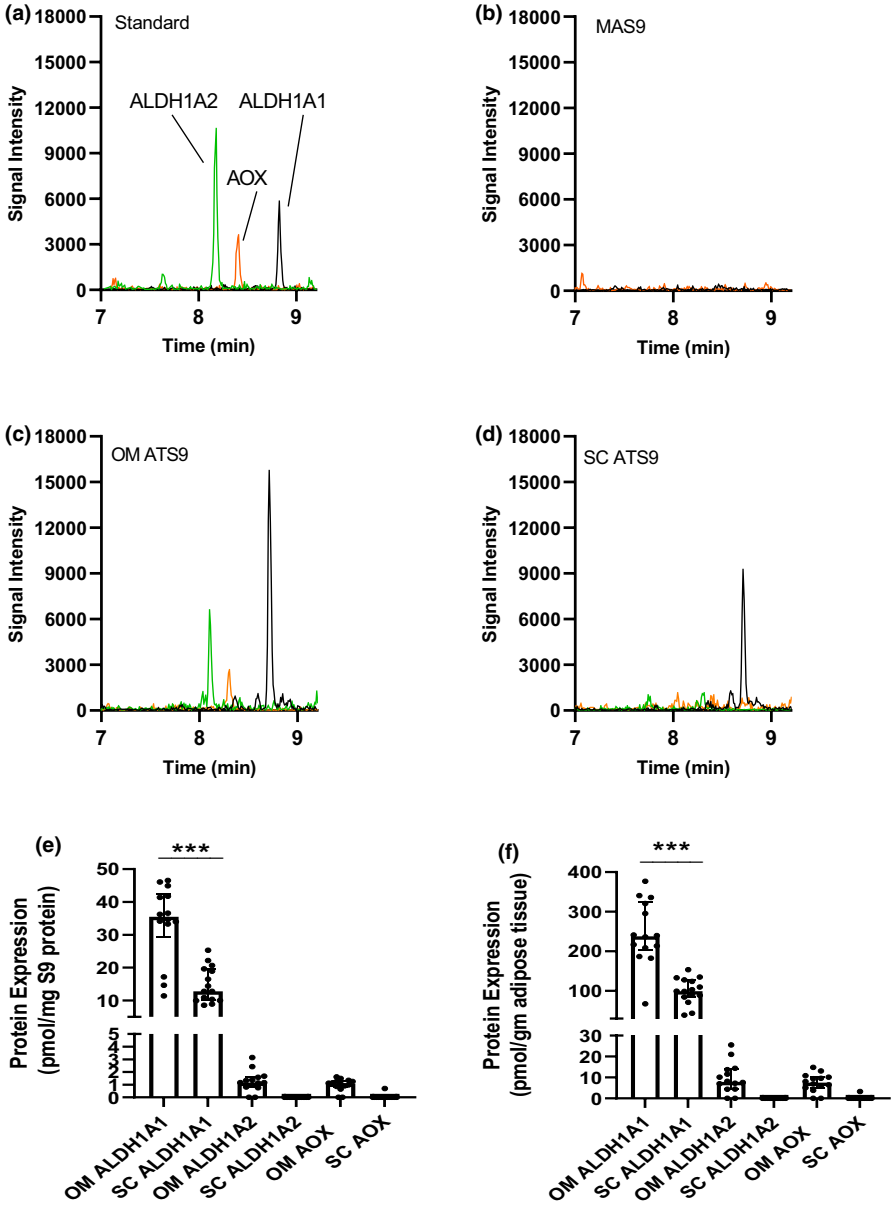

FIGURE 2 Chromatograms and expression data for *at*RA-synthesizing enzymes in human adipose tissue. LC-MS/MS chromatograms of target peptides of ALDH1A1 (black trace, *m/z* 795.4>879.5; ANNTFYGLSAGVFTK), AOX (orange trace, *m/z* 605.3>963.5; VFFGEGDGIIR) and ALDH1A2 (green trace, *m/z* 714.7>846.4; ILELIQSGVAEGAK) are shown. A sample of mouse adipose tissue S9 fraction spiked with 0.2 pmol purified recombinant human ALDH1A1, 0.02 pmol AOX, and 0.08 pmol ALDH1A2 (standard) is shown in (a). No target peptides of human ALDH1A1, AOX, or ALDH1A2 were detected in blank mouse adipose tissue S9 fractions (b). Representative chromatograms from a study participant showing detection of all three enzymes in the omental S9 fraction (c) and detection of ALDH1A1 in the subcutaneous (d) S9 fraction. ALDH1A1 is the most highly expressed *at*RA-synthesizing enzyme in both omental and subcutaneous human adipose tissue, irrespective of whether data are normalized to S9 protein (e) or gram of tissue (f). For values below the lower limit of quantification, data points are shown as 0. Bars and lines depict median and interquartile values in panels (e) and (f). ALDH, aldehyde dehydrogenase; AOX, aldehyde oxidase; *at*RA, *all-trans*-retinoic acid; LC-MS/MS, liquid-chromatography tandem mass spectrometry; MAS9, mouse adipose tissue S9 fraction; OM, omental; SC, subcutaneous; ATS9, adipose tissue S9 fraction; ***, $p < 0.001$; **, $p < 0.01$

essentially all *at*RA formation was predicted to be mediated by ALDH1A1 in subcutaneous adipose tissue, with the remaining formation attributed to AOX, although its contribution could not be quantitatively predicted. This largely was because ALDH1A2 was not detected in subcutaneous adipose tissue.

Multiple linear regression analyses were performed to support the predicted importance of each *at*RA-synthesizing enzyme to total *at*RA formation in both omental and subcutaneous adipose tissue. In omental adipose tissue, the correlation between ALDH1A1 expression and *at*RA formation was not significant (β-coefficient = 0.14,

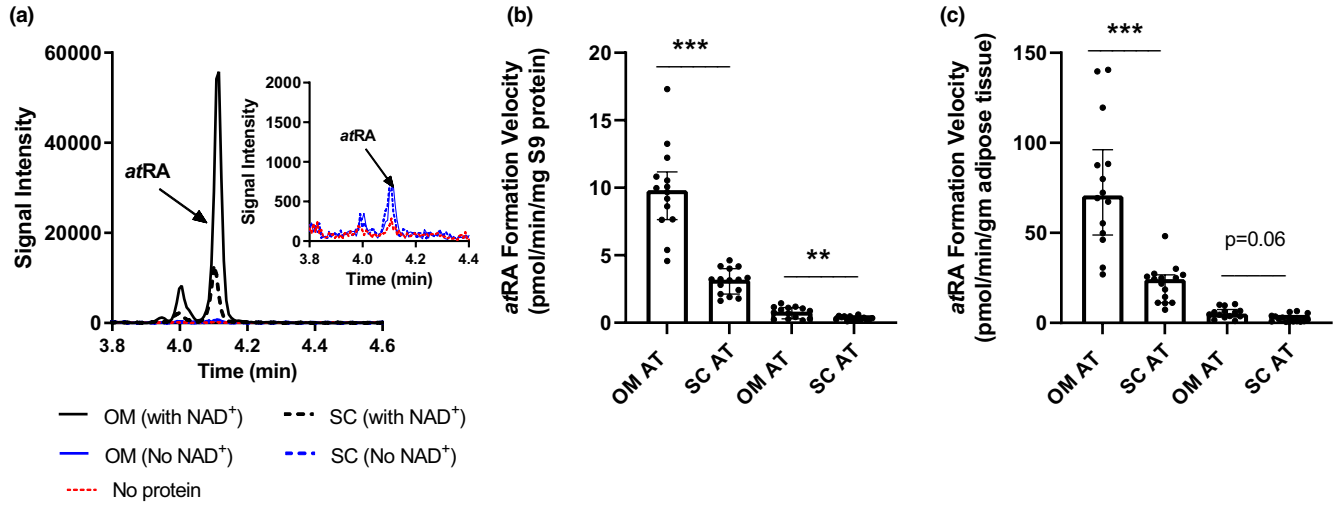

FIGURE 3 Formation of *at*RA in human omental and subcutaneous adipose tissue. Representative chromatograms of *at*RA formation in incubations with adipose tissue S9 fractions in the presence and absence (inset) of NAD^+ (a). Omental and subcutaneous S9 fractions shown are from a single participant, and the MRM transition for *at*RA is m/z 301>205. In both the presence and absence of NAD^+, *at*RA formation velocity is higher in omental than subcutaneous human adipose tissue irrespective of whether data are normalized to S9 protein (b) or gram of adipose tissue (c). All incubations were done in duplicate and repeated on three separate days at retinaldehyde (substrate) concentration of 100 nM. Each data point shown is the mean value of the measurements over 3 days. Experimental details can be found in supplemental methods. NAD^+, nicotinamide adenine dinucleotide; SC AT, subcutaneous adipose tissue; OM AT, omental adipose tissue; *at*RA, *all-trans*-retinoic acid; ***, $p < 0.001$; *, $p < 0.05$

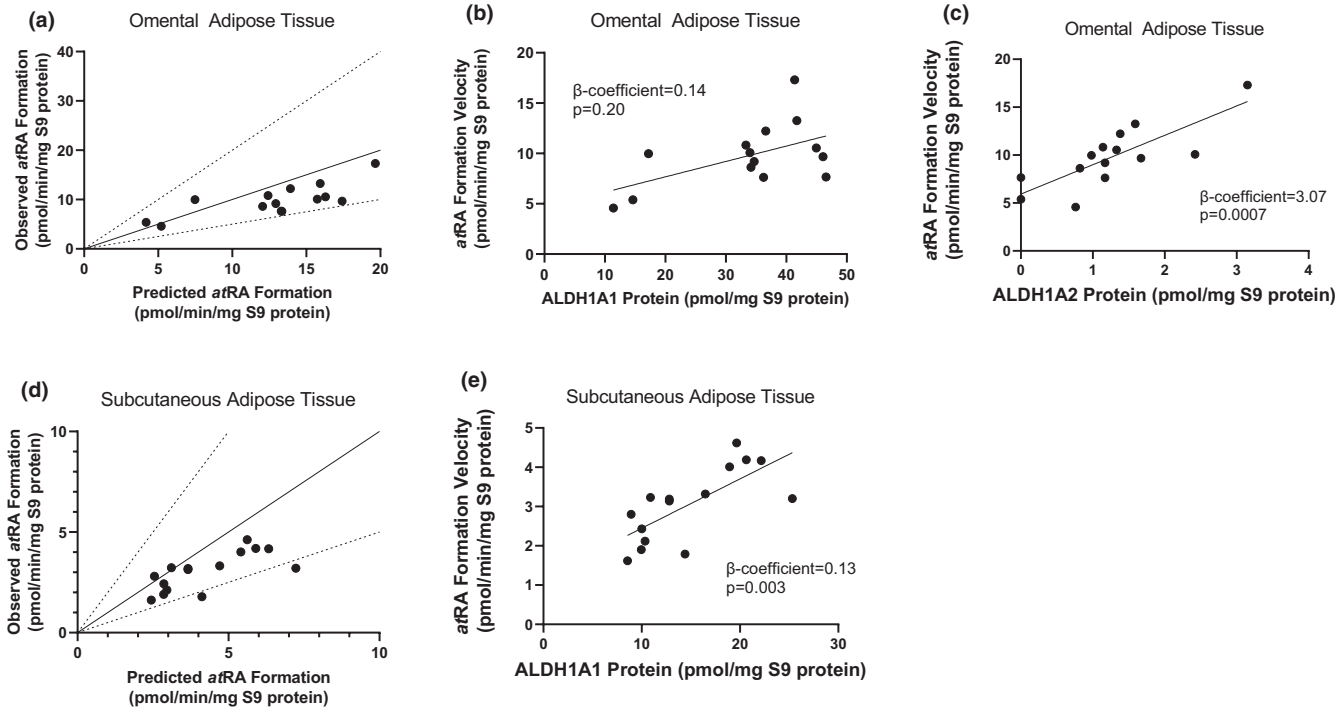

FIGURE 4 Predicted and observed *at*RA formation velocities in human adipose tissue. Predictions of *at*RA formation velocity in individual human omental (a) and subcutaneous (d) adipose tissue were within acceptable range (2-fold) of observed values. *at*RA formation was measured at 100 nM retinaldehyde concentrations as described in supplemental methods, and *at*RA formation velocity was predicted in each participant according to Equation 1 using the retinaldehyde concentration of 100 nM and the measured enzyme expression levels in each participant. In omental adipose tissue, the correlation between ALDH1A1 and *at*RA formation did not achieve significance (b), and ALDH1A2 was the strongest correlate of *at*RA formation (c). ALDH1A1 was the sole correlate of *at*RA formation in subcutaneous adipose tissue (e). Enzyme expression and *at*RA formation were measured as described in supplemental methods. OM, omental adipose tissue; SC, subcutaneous adipose tissue; ALDH, aldehyde dehydrogenase; AOX, aldehyde oxidase; *at*RA, *all-trans*-retinoic acid

$p = 0.20$; Figure 4b), whereas a strong, positive correlation was found between ALDH1A2 expression and total *at*RA formation (β-coefficient = 3.07, $p = 0.0007$; Figure 4c). In contrast, ALDH1A1 expression was the only significant correlate of *at*RA formation in subcutaneous adipose tissue (β-coefficient = 0.13, $p = 0.003$; Figure 4e).

BMI exhibits a positive correlation with ALDH1A1 protein expression but not *at*RA formation velocity in omental adipose tissue

To explore the relationship between body mass and adipose tissue-specific retinoid metabolism, we first examined the correlation between BMI and ALDH1A1 expression in omental adipose tissue. Consistent with a prior study that assessed ALDH1A1 mRNA,[24] a positive correlation was evident between BMI and ALDH1A1 protein in omental adipose tissue (Spearman $r = 0.65$, $p = 0.01$; Figure 5a). This positive correlation was not statistically significant when ALDH1A1 expression was normalized to gram of adipose tissue rather than mg of S9 protein (Spearman $r = 0.42$, $p = 0.14$; Figure 5b). However, no correlation was found between BMI and *at*RA formation velocity, irrespective of whether *at*RA formation was normalized to S9 protein (Figure 5c) or gram of omental adipose tissue (Figure 5d). Consistent with this absence of a correlation between *at*RA formation and BMI, no correlation was found between ALDH1A2 protein expression and BMI; this was again irrespective of normalization strategy (Figure 5e,f).

In subcutaneous adipose tissue, no correlation was found between BMI and either ALDH1A1 protein (Figure 6a,b) or *at*RA formation velocity (Figure 6c,d), irrespective of normalization strategy.

DISCUSSION

Our study was designed to define which enzymes contribute to *at*RA biosynthesis in human adipose tissue and to test the hypothesis that *at*RA biosynthesis rates differ in omental and subcutaneous adipose tissue. In order to test whether *at*RA biosynthesis is diminished in omental adipose tissue with increasing body mass as suggested by rodent models, we also explored the relationship between body mass and *at*RA biosynthesis in both omental and subcutaneous adipose tissue depots in

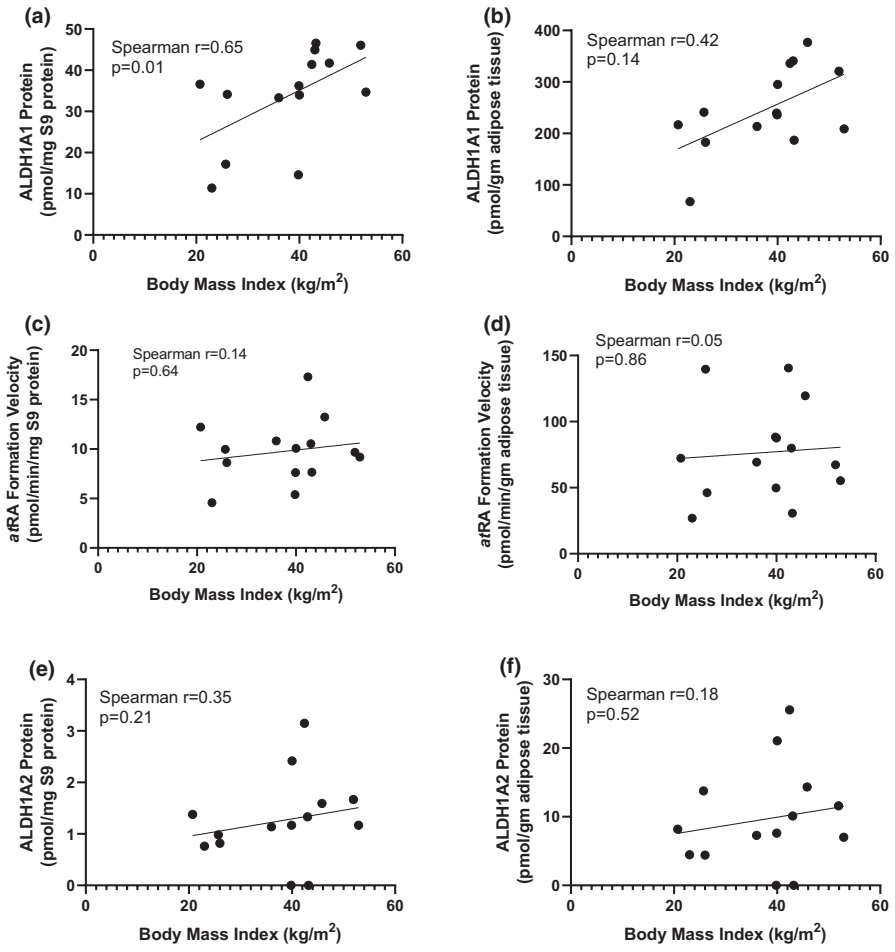

FIGURE 5 Correlations between body mass and both ALDH1A enzyme expression and *at*RA formation in human omental adipose tissue. A positive correlation was evident between ALDH1A1 protein expression and body mass index (BMI) (a) in omental adipose tissue, with a weaker correlation seen when data were normalized to gram of adipose tissue (b). However, no association was found between *at*RA formation velocity and BMI regardless of whether data were normalized to S9 protein (c) or adipose tissue mass (d). No correlation was found between ALDH1A2 protein expression and BMI, also irrespective of whether data were normalized to S9 protein (e) or adipose tissue mass (f). ALDH, aldehyde dehydrogenase; *at*RA, *all-trans*-retinoic acid

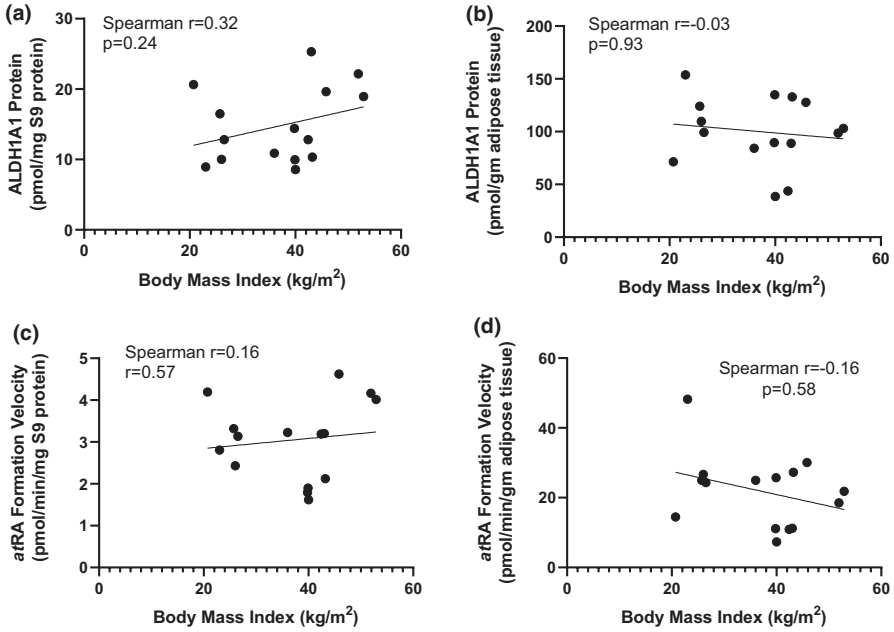

FIGURE 6 Correlations between body mass index and both ALDH1A1 expression and *at*RA formation in human subcutaneous adipose tissue. In human subcutaneous adipose tissue, body mass index did not correlate with ALDH1A1 protein expression whether normalized to S9 protein (a) or gram of adipose tissue (b). Formation of *at*RA similarly did not correlate with body mass index across the entire cohort, regardless of whether data were normalized to S9 protein (c) or gram of tissue (d). ALDH, aldehyde dehydrogenase; *at*RA, *all-trans*-retinoic acid

healthy men and women across a broad range of body mass. Our data unequivocally show that ALDH1A1 and ALDH1A2 are the main enzymes contributing to *at*RA biosynthesis in human adipose tissue and exhibit depot-specific expression. The formation velocity of *at*RA is higher in omental than subcutaneous adipose tissue whether normalized to S9 protein or tissue mass. This is likely due to expression of ALDH1A2 solely in omental adipose tissue and the higher expression of ALDH1A1 in omental relative to subcutaneous adipose tissue. Although AOX, which may also synthesize *at*RA, was detected in omental and subcutaneous adipose tissue, its contribution to *at*RA biosynthesis in human adipose tissue was minimal. Further, our data show novel evidence that ALDH1A2 together with ALDH1A1 determine *at*RA biosynthesis within human omental adipose tissue and that variability in ALDH1A2 expression is a primary determinant of variability in *at*RA formation in this depot. In subcutaneous adipose tissue, only ALDH1A1 contributed to *at*RA formation. In contrast with our original hypothesis, we find no evidence of diminished *at*RA biosynthesis in adipose tissue with increasing body mass.

Our finding of ALDH1A2 protein expression in human omental adipose tissue and its predicted contribution to *at*RA biosynthesis in this depot are significant, as previous studies have focused primarily on ALDH1A1 in human adipose tissue based on rodent data.[24–26] Our measurement of ALDH1A2 protein expression is consistent with the prior detection of ALDH1A2 mRNA in human omental adipose tissue from four female donors[26] and significantly higher ALDH1A2 mRNA expression in visceral than subcutaneous adipose tissue-derived stem cells.[27] Our data show that ALDH1A2 is predicted to contribute ~35% of *at*RA biosynthesis in human omental adipose tissue, highlighting its discord with mesenteric or perigonadal depots in rodents. The correlation analyses further suggest that the contribution of ALDH1A2 to omental adipose tissue *at*RA synthesis is greater than 35%. This is likely due to the impact of CRBP-I expression in adipose tissue on *at*RA biosynthesis, as CRBP-I has been shown to increase ALDH1A2- but decrease ALDH1A1-mediated *at*RA biosynthesis.[20] In subcutaneous adipose tissue, ALDH1A1 appears to mediate greater than 90% of *at*RA biosynthesis, consistent with rodent studies.

Across all participants, *at*RA formation was roughly threefold higher in omental than in subcutaneous adipose tissue. The higher *at*RA formation in omental adipose tissue is likely driven by ALDH1A2 expression, as ALDH1A1 protein expression was only twofold higher in omental than subcutaneous adipose tissue. Although we did not measure tissue *at*RA concentrations, *at*RA biosynthesis velocity as assayed in this study has been shown to correlate with tissue *at*RA concentrations,[20] suggesting that *at*RA concentrations also would be higher in omental than subcutaneous adipose tissue.

The finding of higher ALDH1A1 protein expression in omental than subcutaneous adipose tissue is consistent with a previous study that showed higher ALDH1A1 mRNA expression in omental than subcutaneous adipose tissue.[24] However, two other studies, one in mice[28] and another that analyzed human adipose tissue-derived stem cells,[27] have suggested similar ALDH1A1 mRNA expression in visceral and subcutaneous adipose tissue. These discrepancies may be due to differences between mRNA and protein expression, differences in the specific visceral

depot collected in mice (perigonadal and mesenteric), and differences between adipose tissue stem cells and whole adipose tissue, underscoring the need to determine depot specificity and the discrete cell types involved in adipose tissue vitamin A metabolism. Our lack of detection of ALDH1A3 protein in either adipose tissue depot is in contrast to a previous finding of ALDH1A3 mRNA expression in adipose tissue in both mice and women.[26] This discrepancy is likely due to discord between protein and mRNA expression, as the prior study found ALDH1A3 mRNA transcript levels that were comparable to those of ALDH1A2 in omental adipose tissue; a similar degree of protein expression would unequivocally be captured by our highly sensitive and specific mass spectrometric method for protein quantitation.

We found a positive correlation between ALDH1A1 protein expression and BMI in omental adipose tissue. Previously, a correlation between omental adipose tissue ALDH1A1 mRNA expression and BMI was observed,[24] but this study did not report quantitative data for protein expression. Another prior study observed higher ALDH1A1 mRNA expression in stromovascular cells from omental adipose tissue in women with obesity than in women without obesity.[29] Thus, our findings generally corroborate these prior data from mRNA expression and verify a similar relationship between ALDH1A1 protein and BMI in omental adipose tissue. However, no relationship was evident between *at*RA formation velocity and BMI, a finding likely due to the importance of ALDH1A2 in *at*RA biosynthesis in omental adipose tissue and the lack of correlation between ALDH1A2 protein expression and BMI. The relationship between BMI and ALDH1A1 in the absence of a correlation between *at*RA formation and BMI may reflect increased oxidation of an alternative ALDH1A1 substrate that increases with progressive body weight gain. One potential, alternative substrate is reactive lipid aldehydes. Indeed, the primary role of ALDH1A1 is the detoxification of reactive lipid aldehydes in the human lens and mouse liver.[30,31] Our group previously reported that whereas ALDH1A1 expression is high in human hepatocytes, retinoid homeostasis localizes to the stellate cells,[18] again indicating an alternative function for ALDH1A1 in tissues with high exposure to reactive lipids. These data are consistent with a prior mouse study demonstrating that high fat feeding led to increased ALDH1A1 expression but not tissue *at*RA concentrations in white adipose tissue[25] and provide a potential model to reconcile these seemingly discordant findings.

In subcutaneous adipose tissue, we found no correlation between BMI and either *at*RA formation velocity or ALDH1A1 expression. A previous study identified higher ALDH1A1 mRNA in subcutaneous adipose tissue in men with obesity.[25] However, our study benefitted from protein quantification using mass spectrometry, whereas prior measurements of ALDH1A in human adipose tissue have reported only relative quantitation of transcript expression.[24,25,29]

Although our sample size is small, this study unequivocally demonstrated depot-specific differences in *at*RA biosynthesis and ALDH1A2 expression, consistent with the goal of this study to elucidate basic facets of retinoid biology within human adipose tissue. A potential limitation of the study is that only a single location of subcutaneous and visceral tissue was obtained for analysis. It is possible that adipose tissue from other locations differs from the abdominal subcutaneous and omental depots sampled in this study; thus, further studies are needed to assess ALDH1A expression in different adipose tissue locations. The analyses regarding the influence of BMI on *at*RA biosynthesis were exploratory and limited to a small cohort of healthy participants with and without obesity and therefore require validation. Additional metabolic phenotyping, including assessments of total and regional adiposity and glucose homeostasis, also will be essential in future studies to provide additional context for our findings. Data from our current study will enable statistical power calculations for further assessment of these relationships in future studies. Future work also will include individuals with type 2 diabetes and other obesity-related comorbidities, enabling enhanced understanding of changes in systemic vitamin A metabolism that may occur across a spectrum of metabolic disorders.

Our study provides novel insights into vitamin A metabolism within human adipose tissue and demonstrates distinct vitamin A enzymology within omental and subcutaneous adipose tissue depots. Our data show several key inconsistences with rodent models, highlighting the importance of verifying preclinical data in clinical studies. Collectively, our findings constitute an essential biological basis for elucidating the functions of retinoids in human adipose tissue remodeling, obesity, and metabolic disease.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

K.B.R., G.Z., and N.I. wrote the manuscript. K.B.R. and N.I. designed the research. K.B.R., G.Z., L.C.C., J.Y.C., E.W., Z.P., S.K., D.K., J.L., and N.I. performed the research. K.B.R., G.Z., and N.I. analyzed the data.

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SUPPORTING INFORMATION

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