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Reduced adiponectin expression after high-fat diet is associated with selective up-regulation of ALDH1A1 and further retinoic acid receptor signaling in adipose tissue

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ABSTRACT: Adiponectin is an adipocyte-derived adipokine with potent antidiabetic, anti-inflammatory, and anti-atherogenic activity. Long-term, high-fat diet results in gain of body weight, adiposity, further inflammatory-based cardiovascular diseases, and reduced adiponectin secretion. Vitamin A derivatives/retinoids are involved in several of these processes, which mainly take place in white adipose tissue (WAT). In this study, we examined adiponectin expression as a function of high dietary fat and high vitamin A conditions in mice. A decrease of adiponectin expression in addition to an up-regulation of aldehyde dehydrogenase A1 (ALDH1A1), retinoid signaling, and retinoic acid response element signaling was selectively observed in WAT of normal vitamin A– and high-fat diet–fed mice. Reduced adiponectin expression in WAT was also observed in high vitamin A diet–fed mice. Adipocyte cell culture revealed that endogenous and synthetic retinoic acid receptor (RAR)α- and RARγ-selective agonists, as well as a synthetic retinoid X receptor agonist, efficiently reduced adiponectin expression, whereas ALDH1A1 expression only increased with RAR agonists. We conclude that reduced adiponectin expression under high-fat dietary conditions is dependent on *i*) increased ALDH1A1 expression in adipocytes, which does not increase all-*trans*-retinoic acid levels; *ii*) further RAR ligand–induced, WAT-selective, increased retinoic acid response element–mediated signaling; and *iii*) RAR ligand–dependent reduction of adiponectin expression.—Landrier, J.-F., Kasiri, E., Karkeni, E., Mihály, J., Béke, G., Weiss, K., Lucas, R., Aydemir, G., Salles, J., Walrand, S., de Lera, A. R., Rühl, R. Reduced adiponectin expression after high-fat diet is associated with selective up-regulation of ALDH1A1 and further retinoic acid receptor signaling in adipose tissue. FASEB J. 31, 000–000 (2017). www.fasebj.org

KEY WORDS: vitamin A · nuclear hormone receptor · obesity · diabetes · retinaldehyde dehydrogenase

Obesity is considered to be one of the most common nutritional disorders of Western society and is characterized by a disproportionate expansion of body fat mass [reviewed in Gasbarrini and Piscaglia (1)]. In addition to being an energy storage site, white adipose tissue (WAT) also functions as a highly active metabolic regulator and

ABBREVIATIONS: ALDH1A1, aldehyde dehydrogenase 1A1; ATRA, all-*trans*-retinoic acid; CTRL, control; FABP4, fatty acid binding protein 4; HF, high fat; HODE, hydroxyoctadecadienoic acid; LF, low fat; LXR, liver X receptor; NF, normal fat; PPAR, peroxisome proliferator-activated receptor; RALDH, retinaldehyde dehydrogenase; RAR, retinoic acid receptor; RARE, retinoic acid response element; RE, retinol equivalents; RETSAT, all-trans-retinol 13,14-reductase; RXR, retinoid X receptor; TG2, transglutaminase 2; VDR, vitamin D receptor; WAT, white adipose tissue

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major endocrine organ that secretes various adipokines (2–4). Adiponectin is a major adipokine with strong antidiabetic, anti-inflammatory, and antiatherogenic activity, and its expression is decreased in WAT under high dietary fat conditions [reviewed in Ouchi *et al.* (5)]. With the exception of the putative role of inflammation (6–8), the precise mechanisms that mediate this down-regulation remain to be elucidated.

Retinoids are important regulators of adipogenesis. Diets that are high in vitamin A (9), excess of retinoic acid (10, 11), and diet high in β-carotene (12–14), result in increased adipocyte apoptosis and inhibition of adipogenesis, while low concentrations of retinoic acid were described to be proadipogenic (reviewed in refs. 9, 11, 15, 16). Retinoids [*i.e.*, naturally occurring and synthetic retinol analogues (reviewed in refs. 17, 18)], are responsible for activation of specific nuclear receptors: the retinoic acid receptor (RAR) and the retinoid X receptor (RXR). Bioactive retinoic acids are formed from precursor retinaldehydes by the action of retinaldehyde dehydrogenase enzymes (RALDHs/ALDH1A) (19).

RALDH1/ALDH1A–null adult mice have been shown to be resistant to high-fat diet–induced weight gain (20, 21), which suggested that ALDH1A1 and its metabolic products are necessary for high-fat diet–induced obesity (22–24). ALDH1A1 can synthesize retinoic acids (25), such as all-*trans*-retinoic acid (ATRA), 9-*cis*-retinoic acid, and, presumably, the newly found endogenous RXR ligand, 9-*cis*-13,14-dihydroretinoic acid (26). The last 2 are ligands for both RXRs and RARs, whereas ATRA only binds RAR. Unfortunately, only a few studies have detected retinoic acids in low concentrations in adipose tissue (27, 28), but no study has addressed the presence of retinoic acids in WAT when comparing ALDH1A1$^{+/+}$ or ALDH1A1$^{-/-}$ mice. Whether retinoic acids, and which retinoic acids, are the major metabolites of ALDH1A1 in WAT is yet unknown. Moreover, ALDH1A1 expression has been shown to be regulated by liver X receptor (LXR) (29) as well as estrogen receptor–mediated pathways (30, 31).

Various nuclear hormone receptor pathways are involved in adipokine secretion and adipocyte differentiation, proliferation, and lipid accumulation (reviewed in refs. 7, 32). In particular, RXRs, the central heterodimer-forming partners, play important roles during obesity (33–35). RXRα-KO as well as RXRγ-KO mice and RXR-antagonist treatment induce resistance to weight gain after high-fat diet and also promote a higher metabolic rate (36–38). RXRs can also interact with several nuclear receptors, such as RAR, LXR, peroxisome proliferator-activated receptor (PPAR), vitamin D receptor (VDR), or NR4A-orphan nuclear receptors (39, 40), and the activation of various so-called permissive heterodimers (RXR-PPAR, -LXR, -VDR, and -NR4A1/2) by an RXR ligand can initiate heterodimer-mediated signaling (39–41).

The aim of our study was to find out how high-fat diet reduces adiponectin expression in WAT, focusing primarily on vitamin A–mediated RAR- and RXR-dependent pathways.

MATERIALS AND METHODS

Experimental diets

Manually prepared diets were made with wheat starch (Weizenstärke, Foodstar, Germany; provided by Kröner-Stärke, Ibbenbüren, Germany), saccharose (purchased from a local supermarket in Hungary), casein (Sigma-Aldrich, Budapest, Hungary), cellulose (Vivapur; JRS Pharma GmbH; Rosenberg, Germany), vitamin mix (Vitamin-Vormischung C1000; Altromin GmbH, Lage, Germany), mineral mixture (Mineral-Spurenelemente-Vormischung C100; Altromin GmbH), and sunflower oil (Henry Lamotte, Bremen, Germany).

Animal experiments

Animal experiments were performed in the Laboratory Animal Core Facility of the University of Debrecen. Experiments were performed according to Hungarian ethical guidelines.

Experiment with low, normal, or high-fat supplementation diets

After the acclimatization period, animals received a vitamin A–deficient [0 retinol equivalents (RE)/kg diet] diet for 10 wk that contained 5% sunflower oil as a dietary lipid, which represented a diet with normal fat (NF) content (42). Animals were divided into different feeding groups (n = 6 per group) and were fed for 4 wk with specific diets that contained different amounts of dietary fat and equal amounts of vitamin A (2500 RE/kg diet, normal vitamin A). Sunflower oil was added as dietary fat, which contained either 2% [as weight %; low-fat (LF) diet], 5% (NF diet), or 25% [high-fat (HF) diet]. The source of the fat was always sunflower oil in different proportions added to feed. On the basis of the analyzed feed of the NF diet, it contained 11.6% saturated fats, 20% monounsaturated fatty acids, and 68.4% polyunsaturated fatty acids (Weiss *et al.*, in preparation). Furthermore, dietary composition was 180 g/kg casein, 10 g/kg vitamin mix, 45 g/kg mineral mix, and 20 g/kg cellulose for all applied diets (42). As a result of the increased amount of fat in the diet, carbohydrate proportion was lower; the low LF contained 29.5% sucrose and 43% starch, the NF diet 28% sucrose and 41.5% starch, and the HF diet contained 17% sucrose and 32.5% starch (42).

Experiment with normal or high vitamin A supplementation diets

For vitamin content, diets were supplemented with vitamin mix (Vitamin-Vormischung C1000) that contained either 2500 RE/kg as normal vitamin A diet, or for high vitamin A diets, an additional retinyl-palmitate (RetPal) supplement (final 326,500 RE/kg; Sigma-Aldrich) was added to the normal vitamin A diet (42, 43).

After euthanizing mice, blood collection was carried out by cardiac puncture. Blood was centrifuged for 20 min and plasma was stored at −80°C. Mice were anatomized and WAT samples were immediately frozen in liquid nitrogen after dissection and later stored at −80°C until RNA extraction.

Bioimaging

Retinoic acid response element (RARE)-Luc female mice (n = 6) were obtained from Cgene (Oslo, Norway) and received LF, NF, or HF diets for 4 wk or the oral retinoid treatments as described before (43, 44).

We conducted *ex vivo* organ analysis by bioluminescence imaging. All animals were treated with 120 mg/kg D-luciferin

(Bioscience, Budapest, Hungary) *via* intraperitoneal injections 15 min before euthanasia and further organ screening. Mice were euthanized by cervical dislocation. After sacrifice, mouse liver, WAT, intestine, and brain were collected for bioluminescence imaging. Organs were analyzed for bioluminescence signal by using an Andor-Ixon CCD camera (Belfast, United Kingdom), and analysis was performed by Andor-IQ software. After imaging, integrated intensity/area was calculated for liver, WAT, intestine, and brain of each treated animal.

Cell culture

3T3-L1 preadipocytes (American Type Culture Collection, Manassas, VA, USA) were seeded in 3.5-cm-diameter dishes at a density of 15×10^4 cells/well. Cells were grown in DMEM that was supplemented with 10% FBS at 37°C in a 5% CO_2 humidified atmosphere, as previously reported (45, 46). To induce differentiation, 2-d postconfluent 3T3-L1 preadipocytes (day 0) were stimulated for 48 h with 0.5 mM isobutylmethylxanthine, 0.25 μM dexamethasone, and 1 μg/ml insulin in DMEM that was supplemented with 10% FBS. Cells were then maintained in DMEM that was supplemented with 10% FBS and 1 μg/ml insulin (47). To examine the effects on gene expression of ATRA (a gift from BASF AG, Ludwigshafen, Germany), an RARα agonist (BMS753), an RARγ agonist (BMS189961; both were prepared in our laboratories as described in the original patents (48, 49)], and an RXR agonist (LG268; gift from Ligand Pharmaceuticals, San Diego, CA, USA), 3T3-L1 adipocytes were incubated with 1 μM of these molecules for 24 h, as previously reported (47). Data presented are the mean of 3 independent experiments each performed in triplicate.

Human adipose biopsies

Eleven lean (body mass index: 22.5 ± 0.5 kg/m^2) and 14 obese (body mass index: 31.7 ± 0.9 kg/m^2) male participants were recruited, as previously reported (50). Lean and obese volunteers were age 44 ± 7 y and 44 ± 5 y, respectively. Subcutaneous adipose tissue biopsies were performed between 6:30 AM and 7:30 AM after an overnight fast. Biopsies were obtained by needle aspiration in the periumbilical area under local anesthesia. Adipose tissue samples were rinsed in physiologic serum, immediately frozen in liquid nitrogen, and stored at −80°C until RNA extraction. The experimental protocol was performed in accordance with the guidelines in the Declaration of Helsinki and was approved by the Ethical Committee of the Auvergne Region (agreement No. AU 800, March 2010). Participants gave their written informed consent to participate in the study.

Analysis of mRNA expression

Analysis of total cellular RNA extracted from 3T3-L1 cells was performed in France by using Trizol reagent according to manufacturer instructions. Human adipose tissue sample extraction was also performed in France, whereas WAT and liver sample analysis from mice was done in Hungary.

For the cell culture material in the French laboratory, cDNA was synthesized from 1 μg of total RNA in 20 μl by using random primers and Moloney murine leukemia virus reverse transcriptase. Real-time quantitative RT-PCR analyses for genes were performed by using the Mx3005P Real-Time PCR System (Stratagene, La Jolla, CA, USA) as previously described (51). For each sample, expression was quantified in duplicate and 18S rRNA was used as the endogenous control in the comparative cycle threshold (C_T) method.

For WAT and liver tissue analysis of human and murine origin, tissues were homogenized in Tri reagent solution (Thermo Fisher Scientific, Waltham, MA, USA) and total RNA was isolated from tissue according to manufacturer guidelines and as previously described (52). Concentration and purity of RNA was measured by using the NanoDrop spectrophotometer (Thermo Fisher Scientific).

For real-time quantitative PCR, total RNA was reverse transcribed into cDNA by using the Super Script II First-Strand Synthesis System (Thermo Fisher Scientific). Quantitative real-time PCR was carried out in triplicate using predesigned MGB assays (Thermo Fisher Scientific) on an ABI Prism 7900 (Applied Biosystems, Villebon-sur-Yvette, France). Relative mRNA levels were calculated by using the C_t method and were normalized to cyclophilin A mRNA. Sequence Detector Software (v. 2.1; Applied Biosystems) was used for data analysis.

Analytical procedures

WAT samples were collected and stored in dark vials at −80°C until analysis. Sample preparation was performed as previously described for retinoid (53) and eicosanoid/docosanoid (54) analysis. HPLC–tandem mass spectrometry analyses for retinoids as well as eicosanoids and docosanoids, which focused on eicosanoids with known PPAR activation potential, were also performed as previously explained (53, 54).

ELISA assays

To examine the effect of retinoids on adiponectin secretion, 3T3-L1 adipocytes were incubated with 1 μM of the retinoids (ATRA, RARα, RARγ, or RXR ligand) for 48 h. Adiponectin quantification was realized on the culture supernatant by using adiponectin ELISA assay according to manufacturer protocol (Quantikine ELISA; R&D Systems, Lille, France).

Statistics

Data are expressed as means ± SEM. Significant differences between control and treated cells/groups were determined by Student's *t* test using Statview software (SAS Institute, Cary, NC, USA). Values of $P < 0.05$ were considered significant.

RESULTS

Effects of HF diet on body weight gain

Body weight gain was observed in animals after 4 wk of HF diet compared with LF or NF diet supplementation (LF: 1.07 ± 0.08 g; NF: 0.92 ± 0.11 g; HF: 3.24 ± 0.37 g; LF-HF $P = 0.03$ and NF-HF $P = 0.04$). Food intake slightly decreased in the HF diet group (LF: 2.93 g/d/animal; NF: 2.70 g/d/animal; HF: 2.25 g/d/animal).

Supplementation of HF diet results in up-regulation of ALDH1A1 and down-regulation of adiponectin expression

ALDH1A1 was significantly increased only in WAT (LF: 1 ± 0.80; NF: 1.83 ± 0.36; HF: 5.26 ± 0.23) of HF diet–supplemented mice compared with LF diet– and NF diet–fed mice and tissue selective for WAT (**Table 1**, WAT) compared with unchanged expression in the liver (Table 1, liver). ALDH1A2 expression remained unchanged in liver and WAT (Table 1). ALDH1A3 was also significantly

TABLE 1. *Relative adiponectin and ALDH1A1 mRNA expression*

Gene	Fold activation			Significance		
	LF	NF	HF	LF:NF	NF:HF	LF:HF
WAT						
ALDH1A1	1 ± 0.80	1.83 ± 0.36	*5.26 ± 0.23*	0.46	0.05	*0.01*
ALDH1A2	1 ± 0.11	1.05 ± 0.14	1.09 ± 0.19	0.77	0.87	0.69
ALDH1A3	1 ± 0.17	*1.65 ± 0.08*	*2.59 ± 0.24*	*0.03*	0.12	*0.02*
Adiponectin	1 ± 0.31	0.86 ± 0.12	*0.15 ± 0.37*	0.72	*<0.01*	*0.04*
Liver						
ALDH1A1	1 ± 0.10	1.10 ± 0.11	1.39 ± 0.13	0.53	0.64	0.09
ALDH1A2	1 ± 0.12	0.79 ± 0.09	0.74 ± 0.10	0.17	0.64	0.09

Expression shown in WAT and liver of LF (set as 1), NF, and HF diet–fed mice with a normal content of vitamin A in the diet. Gene expression (all $n = 6$) of adiponectin and retinoic acid synthesizing enzymes (ALDH1A1, ALDH1A2, ALDH1A3). Significant values *vs.* LF are in italics.

increased in adipose tissue of HF diet– and NF diet–fed animals compared with LF (Table 1, WAT). In addition, expression of RAR pathway target genes, such as CYP26A1 and CYP26B1, remained unchanged (**Table 2**), whereas expression of the highly sensitive common RAR/RXR pathway target gene transglutaminase 2 (TG2) was strongly increased (Table 1; LF: 1 ± 0.36; NF: 9.56 ± 0.13; HF: 15.36 ± 0.17) in HF diet–supplemented mice.

In addition to increased retinoid signaling, expression of adiponectin was reduced in HF diet–fed mice (LF: 1 ± 0.31; NF: 0.86 ± 0.12; HF: 0.15 ± 0.37).

Increased ALDH1A1 and reduced adiponectin expression in obese volunteers

Experiments using adipose tissue biopsies from normal weight and obese human volunteers confirmed increased ALDH1A1 (healthy volunteers were set as 1; 1.20 ± 0.07) and reduced adiponectin (0.85 ± 0.04) expression in the obese volunteers (**Table 3**).

High vitamin A–supplemented diet results in increased expression of ALDH1A1 and reduced adiponectin expression

Expression of ALDH1A1 increased (NF, normal vitamin A was set as 1: 1 ± 0.19; NF, high vitamin A: 2.32 ± 0.47) in the WAT of high vitamin A– and NF diet–supplemented mice with NF content, whereas adiponectin expression (NF, normal vitamin A was set as 1: 1 ± 0.47; NF, high vitamin A: 0.37 ± 0.21) was decreased in WAT (**Table 4**).

Decreased retinoic acid concentrations present in WAT of HF diet–supplemented animals do not correspond to increased RARE-mediated signaling in RARE-Luc mice, and PPARγ ligands remain mainly unchanged

Retinol levels remained stable in the WAT of LF-, NF-, and HF-supplemented animals, whereas ATRA (LF: 2.2 ± 0.1 ng/g; NF: 1.7 ± 0.2 ng/g; HF: 0.6 ± 0.1 ng/g) levels were lower in the WAT of HF diet–supplemented animals (**Table 5**).

Increased retinoid signaling was confirmed in RARE-Luc mice, with increased RARE-mediated signaling detected specifically in adipose tissue of HF diet– compared with LF and NF diet–supplemented animals, whereas in liver, intestine, and brain, no increased RARE-mediated signaling was observed (**Fig. 1**).

Endogenous PPAR ligands [9-hydroxyoctadecadienoic acid (HODE), 13-HODE, 13-keto-octadecadienoic acid, 12-keto-eicosatetraenoic acid, PgJ2 and d15d12PgJ2] were mainly unchanged, except the adipose tissue–specific PPARγ ligand, hepoxilin B3, which is increased in adipose tissue of HF diet–supplemented animals (Table 5).

TABLE 2. *Relative gene expression of genes involved in RAR and PPAR signaling in mouse WAT*

Gene	Fold activation			Significance		
	LF	NF	HF	LF:NF	NF:HF	LF:HF
RAR pathway						
CYP26A1	1 ± 0.50	0.16 ± 0.41	0.43 ± 0.49	0.13	0.26	0.33
CYP26B1	1 ± 0.63	0.58 ± 0.52	0.93 ± 0.66	0.60	0.65	0.94
TG2	1 ± 0.36	*9.56 ± 0.13*	*15.36 ± 0.17*	*<0.01*	0.08	*<0.01*
PPAR pathway						
PPARγ	1 ± 0.12	1.23 ± 0.09	0.93 ± 0.09	0.18	0.07	0.67
RETSAT	1 ± 0.21	1.96 ± 0.23	1.38 ± 0.26	0.09	0.37	0.37
FABP4	1 ± 0.03	1.00 ± 0.10	1.08 ± 0.10	0.99	0.57	0.47
FADS2	1 ± 0.43	1.31 ± 0.41	1.46 ± 0.48	0.71	0.88	0.64

Expression in WAT of LF (set as 1), NF, and HF diet–fed mice with a normal content of vitamin A in diet (all $n = 6$). Significant values *vs.* LF are in italics.

TABLE 3. *Relative expression of human adiponectin and ALDH1A1 in WAT in humans*

Gene	Fold activation NV (n = 20)	OB (n = 26)	Significance
ALDH1A1	1.00 ± 0.08	*1.20 ± 0.07*	*0.03*
Adiponectin	1.00 ± 0.04	*0.85 ± 0.04*	*0.01*

Expression in WAT of obese (OB) and normal volunteers (NV). Significant values *vs.* NV are in italics. NV was calculated to be set as 1.

NF and HF diet supplementation does not result in altered PPARγ-mediated signaling

Expression of PPARγ and PPARγ target genes retinol saturase (RETSAT)/fatty acid binding protein 4 (FABP4)/FADS2 remained unaffected by NF- and HF-supplemented diet compared with LF-supplemented diet in mouse WAT (Table 2).

Adiponectin expression is reduced by RAR and RXR agonists using 3T3-L1 adipocytes cell culture

Treatment of cultured adipocytes with synthetic RARα-selective ligands [control (CTRL) set as 1; adiponectin: 0.22 ± 0.01 and ALDH1A1 2.37 ± 0.04], RARγ-selective ligands (CTRL set as 1; adiponectin: 0.22 ± 0.01 and ALDH1A1 2.64 ± 0.01), and the natural RAR ligand ATRA (CTRL set as 1; adiponectin: 0.25 ± 0.03 and ALDH1A1 3.19 ± 0.07), in addition to a synthetic RXR agonist (LG268; CTRL set as 1; adiponectin: 0.51 ± 0.04 and ALDH1A1 1.04 ± 0.04), resulted in increased ALDH1A1 expression for RAR agonists, whereas adiponectin expression was reduced for all RAR and RXR ligands. In addition, these results were confirmed at the protein level in cell culture supernatants where adiponectin secretion was reduced for all administered RAR and RXR ligands, except for the RARγ-selective ligand, which displayed a nonsignificant decrease (Table 6).

DISCUSSION

Obesity is classically associated with a decrease of adiponectin plasma level in humans and rodents, as well as a decreased expression in adipose tissue (5). This relationship between obesity and decreased adiponectin expression is suspected to be linked to the increased inflammatory status of adipose tissue, as TNF-α, one of the main inflammatory markers produced by adipose tissue (55), is known to reduce adiponectin expression (56). However, this mechanism is probably not exclusive, and other pathways—RAR signaling among them—could be involved in this regulation.

In this study, we reported that, in mice, reduced adiponectin expression in WAT after HF diet supplementation was associated with an increase of ALDH1A1 expression. Similar results were also obtained by comparing lean *vs.* obese WAT biopsies. Surprisingly, increased ALDH1A1 expression in mice does not result in increased ATRA levels in WAT.

ALDH1A1, the major enzyme for retinoic acid synthesis using retinaldehyde as a substrate, is highly likely to play an important role in the relationship between retinoid signaling and obesity (20, 21, 57). Indeed, its expression is increased in WAT during HF-induced obesity (58). In ALDH1A1$^{-/-}$-deficient adipocytes as well as in ALDH1A1$^{-/-}$ mice, adipogenesis is impaired and mice are resistant to HF diet–induced obesity (20), which is suggested to be related to altered retinoid signaling [(25) and reviewed in refs. 9, 11, 15]. Retinoic acids, the products from ALDH1A1 metabolism, are the endogenous activators of RARs and RXRs. Reduced retinaldehyde and retinol levels were measured in adipose tissue of HF diet–supplemented animals, and ATRA levels were speculated to be increased upon ALDH1A1 activity (20). However, the detection and quantification of retinoic acid levels in adipose tissue have been scarcely examined (27, 28) and, unfortunately, the connection of retinoic acids in response to ALDH1A1 expression in adipose tissue has not been studied before. In the present study, we report that increased ALDH1A1 expression in mice does not result in increased ATRA levels in WAT. On the contrary, ATRA levels were even lower in WAT of HF *vs.* LF or NF diet–supplemented animals. Similar reduced levels of ATRA were confirmed in serum and adipose tissue of obese volunteers compared with obese volunteers after a weight loss diet (unpublished data), which indicates that obesity is related to reduced local and systemic retinoid levels in humans. These findings of reduced local retinoid levels in adipose tissue of obese animals fit well with previous studies on vitamin A–deficient diet–fed animals, which were found to become obese upon reduced ATRA synthesis, levels, and ATRA-mediated signaling [reviewed in Bonet *et al.* (11)]. In addition, it is well established that retinoids, and especially ATRA, as signaling ligands, have the ability to inhibit proliferation of adipocytes; enhance up-regulation of genes involved in lipid oxidation, energy dissipation, and insulin response; and thereby prevent obesity and insulin resistance [reviewed in Bonet *et al.* (11)], probably by targeting adipocyte oxidative phosphorylation and mitochondriobiogenesis (59).

As a result of this unclear evidence and inconclusive determination of retinoic acids levels in WAT, we opted, like others [(21, 57) plus follow-up reviews (15, 22)], for an indirect method of detection of retinoid signaling by using a RARE-reporter mouse model (44) and we confirmed increased WAT-selective, RARE-mediated signaling in the WAT of HF diet– *vs.* LF diet–fed mice (57). Previous experimental studies claimed, without any analytical proof,

TABLE 4. *Relative adiponectin and ALDH1A1 mRNA expression levels in WAT depending on vitamin A*

Gene	Fold activation Normal vitamin A	High vitamin A	Significance
ALDH1A1	1 ± 0.19	*2.32 ± 0.47*	*0.05*
Adiponectin	1 ± 0.47	*0.37 ± 0.21*	*0.02*

Expression in WAT of normal vitamin A and high vitamin A diet–fed mice with a NF diet (set as 1; n = 6). Significant values *vs.* NF, normal vitamin A are in italics.

TABLE 5. *HPLC–tandem mass spectrometry analysis of retinoids and eicosanoids in WAT*

Compound	Levels (ng/g)			Significance		
	LF	NF	HF	LF:NF	NF:HF	LF:HF
Retinoid						
ATRA	2.2 ± 0.1	1.7 ± 0.2	*0.6 ± 0.1*	0.19	*0.01*	*<0.01*
ROL	1461 ± 97	1591 ± 94	1520 ± 50	0.35	0.38	0.41
Eicosanoid						
13-HODE	557 ± 46	605 ± 79	803 ± 83	0.40	0.21	0.11
9-HODE	186 ± 17	157 ± 18	211 ± 27	0.29	0.22	0.35
13-KODE	228 ± 18	674 ± 266	433 ± 95	0.22	0.34	0.16
12-KETE	10.3 ± 2.4	7.6 ± 1.1	19.6 ± 4.6	0.31	0.12	0.20
PgJ2	0.2 ± 0.0	0.4 ± 0.0	0.2 ± 0.2	0.08	0.09	0.44
d15d12PgJ2	UQL	UQL	UQL			
HXB3	0.5 ± 0.2	2.2 ± 0.5	*5.3 ± 1.1*	0.08	0.13	*0.03*

Analysis of retinoids, ATRA and retinol, as well as the endogenous relevant PPAR ligands, 13-HODE, 9-HODE, 13-keto-octadecadienoic acid (KODE), 12-keto-eicosatetraenoic acid (KETE), PgJ2, d15d12PgJ2, and hepoxilin B3 (HXB3); all in ng/g ± SEM of WAT samples from LF, NF, and HF diet–fed mice with a normal content of vitamin A in diet (all $n = 4$). Significant values *vs.* LF are in italics. ROL, retinol; UQL, under the quantification limit.

the involvement of ALDH1A1-synthesized ATRA in adipose tissue and, only on the basis of increased RARE signaling (21, 57), that ATRA is the metabolite of ALDH1A1 in adipose tissue and that the described effects of ALDH1A1, by consequence, are mediated by ATRA-RAR signaling. Furthermore, they claimed that the ALDH1A1 product ATRA must be involved in the ALDH1A1-mediated increase of adipose tissue expansion and diet-induced obesity. Our solid data, generated by using HPLC–tandem mass spectrometry quantification of ATRA in adipose tissue, contradicts these claims and warns about the common obtainment of false-positive data from RARE-Luc activation models (60).

We concluded that either a still-uncharacterized endogenous RAR ligand must be synthesized in the WAT of HF diet–supplemented mice to induce WAT-selective, RARE-mediated signaling or alternative mechanisms that possibly involve transporter protein–mediated signaling [reviewed in (61) and speculated by (62)] or post-translational modifications in adipocytes [(21) and reviewed in ref. 63] must be taken into consideration. With regard to ligands other than ATRA, it is still unknown which RAR- and/or RXR-activating ligands could be synthesized upon ALDH1A1 expression in WAT, and we could not conclusively suggest a possible structure using our current analytical expertise (26, 53). However, several known and unknown candidates, including 9-*cis*- and all-*trans*-13,14-dihydroretinoic acid, retinal, apo-lycopenoic acids, apo-13′-carotenone, apo-10′-carotenoic acid, and apo-14′-carotenoic acid (20, 26, 44, 47, 64–72) were recently identified and could constitute potential endogenous retinoids.

To further exclude the involvement of PPARγ, the key regulator of adipogenesis (7, 73), as a major nuclear receptor responsible for adiponectin reduction after a HF-supplemented diet, endogenous PPAR ligands were determined. Levels in adipose tissue were mainly unaltered after LF, NF, or HF diet supplementation (74–77). Only the levels of the endogenous and adipose tissue–specific PPARγ ligand, hepoxilin B3, (78, 79) were significantly increased in HF diet– *vs.* LF diet–supplemented animals. In addition, our data show no increased expression of PPARγ and known PPARγ target genes, RETSAT, FABP4, and FADS2, in the WAT of HF diet–supplemented mice, which, in part, contrasts with previous studies. In general, increased PPARγ expression in adipose tissue after HF diet is mainly related to omental and not subcutaneous fat in humans, as reviewed in (80). In mice, increased PPARγ expression is observable just after diets with extreme

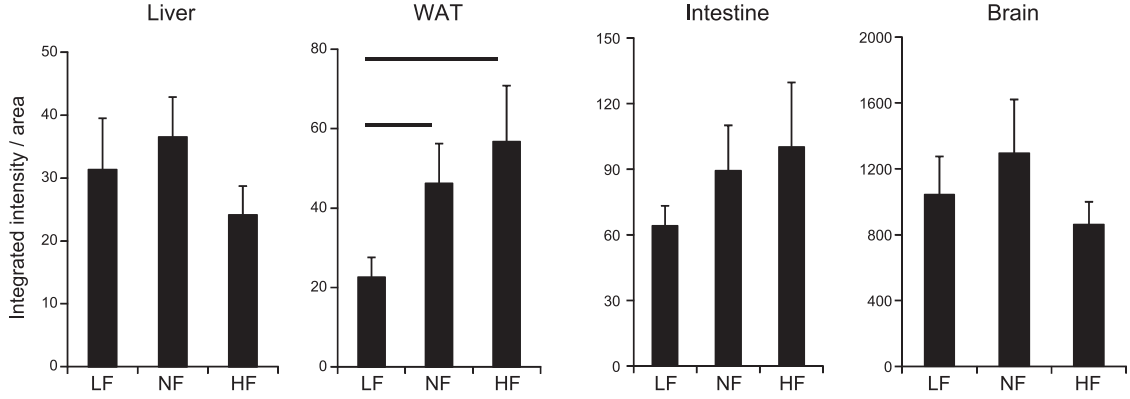

Figure 1. Integrated intensity areas of bioluminescence imaging of various organs of RARE-LUC mice ($n = 6$) that were fed with LF, NF, and HF diets, with normal vitamin A content in the diet. The line over the bars indicates statistical significance.

TABLE 6. *Relative adiponectin concentrations and relative adiponectin and ALDH1A1 mRNA expression in 3T3-adipocytes*

Retinoid	Adiponectin (ELISA)	Adiponectin (relative expression)	ALDH1A1 (relative expression)	Adiponectin (ELISA)	Adiponectin (relative expression)	ALDH1A1 (relative expression)
ATRA	*0.57 ± 0.14*	*0.25 ± 0.03*	*3.19 ± 0.07*	*0.02*	*<0.01*	*<0.01*
RARα-LIG	*0.67 ± 0.04*	*0.22 ± 0.01*	*2.37 ± 0.04*	*0.05*	*<0.01*	*0.01*
RARγ-LIG	0.81 ± 0.04	*0.22 ± 0.01*	*2.64 ± 0.01*	0.20	*<0.01*	*<0.01*
RXR-LIG	*0.64 ± 0.04*	*0.51 ± 0.04*	1.04 ± 0.04	*0.05*	*0.03*	0.14

Expression after 24 h in cultured 3T3-L1 adipocytes with ATRA (1 μM), an RARα-specific agonist BMS753/RARα-LIG (1 μM), an RARγ-specific agonist BMS189961/RARγ-LIG (1 μM), and an RXR ligand RXR-LIG/LG268 (1 μM) calculated with CTRL treatments set as 1. Significance and SEM are based on $n = 6$ parallel treatments. Significant values *vs.* CTRL are in italics. LIG, ligand.

HF conditions, strong weight gain, and after a long time of HF diet supplementation (<8 wk) (81–84). Finally, it is well established that PPARγ signaling activation increases secretion of adiponectin rather than decreases it (85). In summary, all these data strongly imply that PPARγ-mediated signaling in adipose tissue of HF diet–supplemented animals is unlikely to be of major importance for further reduced adiponectin expression.

To evaluate RAR- and RXR-mediated signaling pathways in adipocytes directly, 3T3-L1 adipocyte cell culture models were used, and we determined that ALDH1A1 was increased after administration of ATRA and synthetic RARα- and RARγ-selective RAR ligands, and not by a synthetic RXR ligand, whereas adiponectin expression and secretion in the cell supernatant were decreased after administration of RAR or RXR agonists. We conclude, therefore, that this direct down-regulation of adiponectin is an RAR- or RXR-mediated pathway and that ALDH1A1 expression is regulated by an RAR ligand.

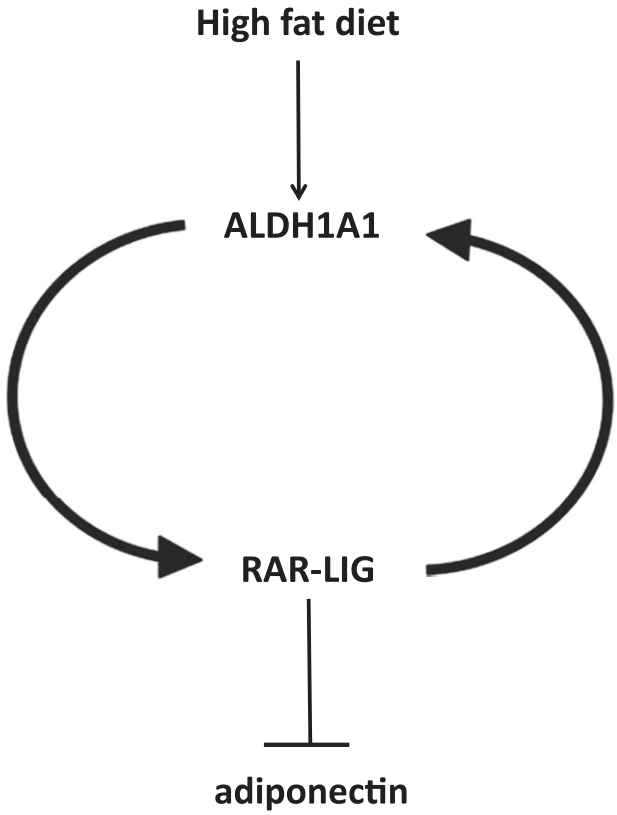

Figure 2. Simplified scheme showing how HF diet induces ALDH1A1 expression, increased RAR ligand (RAR-LIG), and reduced adiponectin expression selectively in WAT.

In summary (**Fig. 2**), we found that reduced adiponectin expression in the WAT of mice is under the control of retinoid-mediated signaling, mainly *via* RAR-mediated signaling pathways. We suggest that altered retinoid signaling in adipose tissue is an important mechanism of HF diet–induced obesity. In particular, ALDH1A1 seems to be the key enzyme that is responsible for the synthesis of alternative endogenous RAR ligands selectively in WAT. This increased ALDH1A1 and reduced adiponectin expression was also confirmed to occur in adipose tissue from obese human volunteers. Endogenous as well as synthetic RAR ligands were shown to further directly inhibit adiponectin expression in cultured adipocytes. The nature of the endogenous RAR/RXR agonists or antagonists synthesized by ALDH1A1 in WAT remains elusive and is the topic of future studies. Characterization of these novel endogenous retinoids with mainly RAR, as well as potential RXR, ligand activation potential and their metabolic pathways can help clarify the controversy of the altered retinoid signaling in adipose tissue. On the basis of these data, novel strategies can be developed to selectively inhibit distinct retinoid signaling, especially that which involves ALDH1A1 products under HF diet, focused on adipose tissue to enable sufficient beneficial adiponectin expression. FJ

AUTHOR CONTRIBUTIONS

R. Rühl and J.-F. Landrier designed the experiments; E. Kasiri, E. Karkeni, J. Mihály, G. Béke, K. Weiss, R. Lucas, G. Aydemir, J. Salles, and S. Walrand performed the experiments; E. Karkeni, J. Mihály, G. Béke, and G. Aydemir analysed the data; and A. R. de Lera provided reagents.

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