Liver Research 7 (2023) 189–195

Contents lists available at ScienceDirect

Liver Research

journal homepage: http://www.keaipublishing.com/en/journals/liver-research

Review Article

Retinoic acid signaling in fatty liver disease☆

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ARTICLE INFO

Article history:
Received 23 February 2023
Received in revised form
17 June 2023
Accepted 10 July 2023

Keywords:
Retinoic acid (RA)
All-trans-RA (AtRA)
Fatty liver disease
Fatty acid oxidation
Lipogenesis
Obesity

ABSTRACT

Retinoic acid (RA) is a metabolite of vitamin A and is essential for development and growth as well as cellular metabolism. Through genomic and nongenomic actions, RA regulates a variety of physiological functions. Dysregulation of RA signaling is associated with many diseases. Targeting RA signaling has been proven valuable to human health. All-trans-RA (AtRA) and anthracycline-based chemotherapy are the standard treatment of acute promyelocytic leukemia (APL). Both human and animal studies have shown a significant relationship between RA signaling and the development and progression of non-alcoholic fatty liver disease (NAFLD). In this review article, we will first summarize vitamin A metabolism and then focus on the role of RA signaling in NAFLD. AtRA inhibits the development and progression of NAFLD by regulating lipid metabolism, inflammation, thermogenesis, etc.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) is a spectrum of liver diseases ranging from simple steatosis, also known as non-alcoholic fatty liver (NAFL), to non-alcoholic steatohepatitis (NASH). The global NAFLD prevalence of NAFLD has reached 32.4%, and the prevalence rate is increasing due to obesity.[1] Many people with NASH develop liver cirrhosis and hepatocellular carcinoma (HCC) as the disease progresses.[2,3] The pathogenic mechanisms of NAFLD are complex and may involve interactions among over-nutrition, genetics, gut microbiota, etc, leading to insulin resistance, lipotoxicity, apoptosis, mitochondrial dysfunction, oxidative stress, inflammation, and fibrogenesis.[2–4] So far, no drugs have been approved to treat NASH by the U.S. Food and Drug Administration (FDA).

Retinoids are metabolites of vitamin A (retinol) that include retinaldehyde/retinal, retinyl esters, oxidized retinol, retinoic acid (RA), and conjugates of these compounds, which are essential for cell growth and differentiation.[5,6] Abnormal retinoid levels have been linked to a wide variety of clinical issues, including cardiovascular disease, diabetes, obesity, fatty liver disease, osteoporosis, skin illnesses, and cancer.[7–10] Mammals cannot synthesize vitamin A. Vitamin A is absorbed by intestinal epithelial cells, stored in the liver, and metabolized in target cells to more biologically active metabolites, RA and 4-oxo-RA.[11]

2. Vitamin A metabolism

Vitamin A is found in meat, dairy products, and beta (β)-carotene. In enterocytes, β-carotene is converted to retinal by β-carotene 15,15' oxygenase-1 (BCO1) and then reduced to retinol by a retinal reductase. Retinyl esters are hydrolyzed to form retinol by retinyl ester hydrolase (REH) prior to absorption. Retinol is then re-esterified with long-chain FAs by lecithin-retinol acyltransferase (LRAT) to regenerate retinyl esters, which are secreted with chylomicrons (CM) from the intestine and up-taken by hepatocytes in the form of CM remnants (Fig. 1). In hepatocytes, retinyl esters are hydrolyzed by REH to produce retinol. Retinol binds to a retinol-binding protein (RBP) for release into the circulation and is up-taken by other cell types via one of the membrane receptors, such as the signaling receptor and transporter of retinol STRA6. Within cells, cellular RBPs (CRBPs) participate in the transport and metabolism of retinol. Retinol is converted to retinal by retinol dehydrogenase (RDH) or retinyl esters by LRAT (Fig. 1).

More than 80% of the vitamin A in the liver is stored in hepatic stellate cells (HSCs).[12] Retinal is converted by retinal dehydrogenase, also known as retinaldehyde dehydrogenase (RALDH), to all-trans-RA (AtRA), 9-cis-RA, 13-cis-RA, 9,13-di-cis-RA, and 11-cis-RA. AtRA and 9-cis-RA are the major biologically active forms of

☆ Edited by Peiling Zhu.

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https://doi.org/10.1016/j.livres.2023.07.002

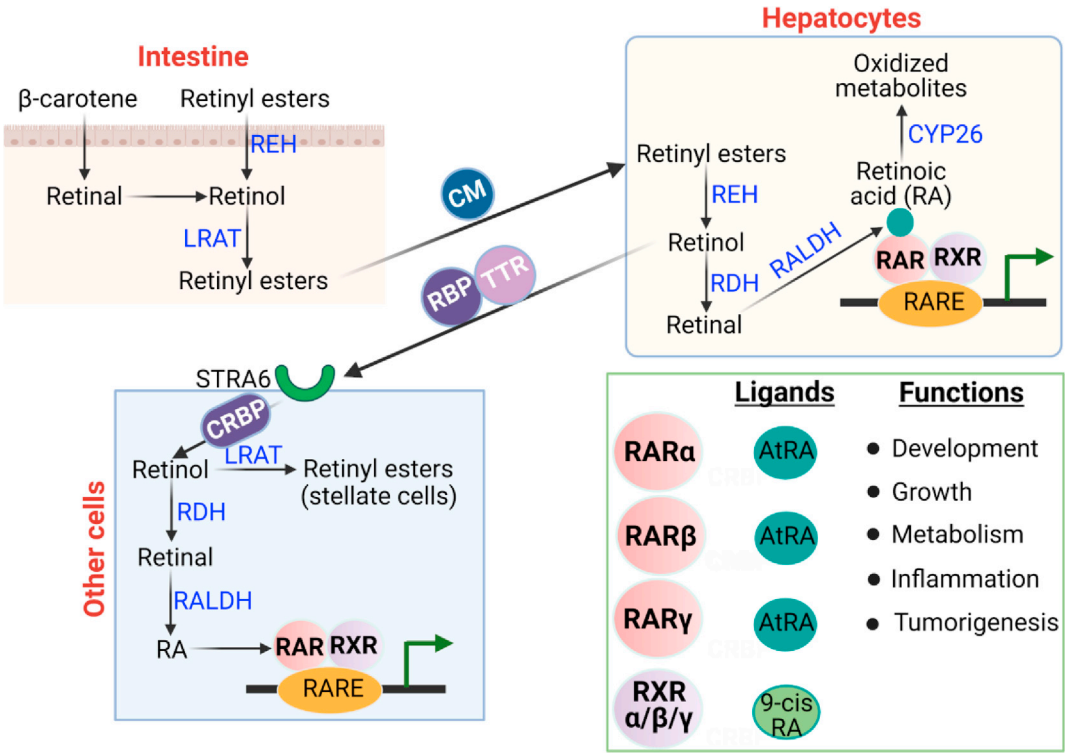

Fig. 1. Overview of vitamin A metabolism and RA signaling pathway. In the intestine, retinyl esters (REs) are hydrolyzed by RE hydrolase (REH) to form retinol. Retinal derived from β-carotene is reduced to retinol by retinaldehyde reductase. Retinol is esterified by lecithin-retinol acyltransferase (LRAT) to form REs, which are assembled into chylomicrons (CM) and secreted to the circulation. REs are uptaken by hepatocytes and are hydrolyzed to form retinol by hepatic REH. Retinol is secreted into the circulation and binds to the retinol-binding protein (RBP)/transthyretin (TTR) complex. The membrane protein STRA6 (signaling receptor and transporter of retinol STRA6) recognizes RBP and transports retinol into cells. In the cells, retinol is converted to retinal by retinol dehydrogenase (RDH), which is further converted to retinoic acid (RA) by retinaldehyde dehydrogenase (RALDH). All-trans-RA (AtRA) activates retinoic acid receptors (RARs), whereas 9-cis-RA activates retinoid X receptors (RXR). RAR and RXR form heterodimers and bind to retinoic acid elements (RAREs) to regulate gene transcription and a variety of pathways, *e.g.,* development, growth, metabolism, inflammation, and tumorigenesis. RAR or RXR has three isoforms, α, β, and γ. Retinol may also be esterified to form REs by LRAT. About 80% of REs are stored in hepatic stellate cells (HSCs). Loss of REs from HSC may result in HSC activation. RA may be metabolized by CYP26A1, CYP26B1, or CYP26C1 to form 4-hydroxy-RA, 4-oxo-RA, etc.

RAs. Cellular RA-binding proteins (CRABPs) transport RA into the nucleus, where AtRA and 9-cis-RA bind to the RA receptor (RAR) and retinoid X receptor (RXR), respectively, to regulate gene transcription (Fig. 1). Excess RA is metabolized by P450 family enzymes (CYP26A1, CYP26B1, and CYP26C1) into polar chemicals, including 4-hydroxy-RA and 4-oxo-RA, which are glucuronidated and then removed from the body via the kidneys or liver into bile.[13]

3. RAR/RXR

RAR has three isoforms, RAR alpha (RARα, NR1B1), RARβ (NR1B2), and RAR gamma (RARγ, NR1B3). RXR also has three isoforms, RXRα (NR2B1), RXRβ (NR2B2), and RXRγ (NR2B3). RAR heterodimerizes with RXR and the dimers bind to the RA response element (RARE) in the target genes. Ligand binding to the RAR/RXR heterodimers results in the change in associated cofactors and activation or repression of gene transcription. More than 532 genes may be regulated by RA through the traditional genomic route.[14] In addition to the traditional genomic functions, RARs may also be engaged in nongenomic biological functions, such as the initiation of translation and kinase cascades, *e.g.,* the p38 or mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK).[15–17] The discovery of RA has proven valuable to human health. For instance, AtRA and anthracycline-based chemotherapy are the standard treatment for acute promyelocytic leukemia (APL), a highly curable disease.[18,19]

4. Altered retinoid metabolism in NAFLD

HSC activation is associated with reduced hepatic retinyl esters and retinol concentrations and vitamin A metabolism.[20] In rats, vitamin A deficiency causes HSC activation to produce the extracellular matrix (ECM) and potentiates CCl_4-induced liver fibrosis.[21,22] In contrast, supplementation with vitamin A inhibits CCl_4-induced liver fibrosis in pigs.[23] Vitamin A is also shown to reduce mortality of animals with induced liver fibrosis by copper sulfate.[24] Clearly, vitamin A and its metabolites may play a key role in liver fibrogenesis.[25]

Multiple studies on patients have shown an inverse relationship between serum retinol levels and the severity of NAFLD.[26–28] Chaves *et al.*[29] showed that serum and hepatic retinol levels decrease in NAFLD by 35.9% and 67.9%, respectively, and that a significant association exists between hepatic retinol concentrations and the severity of NAFLD. Similarly, serum RA levels are also shown to be inversely correlated with the severity of NAFLD.[30] Zhong *et al.*[31] showed that in NAFLD patients, hepatic vitamin A metabolites, including retinyl-palmitate esters, AtRA, 13-cis-RA, and 4-oxo-AtRA, are reduced while the levels of retinol (the inactive form of vitamin A) do not change. They suggested the levels of metabolites of vitamin A, rather than retinol, are more reliable for predicting the disease progression of NAFLD.[31]

In animal models of NAFLD, Trasino *et al.*[32] showed that hepatic retinol levels are decreased in high-fat diet (HFD)-induced obese mice or genetically obese mice (*db/db* or *ob/ob* mice) accompanied

by reduced RAR and RNA-binding protein (RBP1) messenger RNA (mRNA) levels in HSC and elevated serum retinol levels. Saeed *et al.*[33] reported that hepatic retinyl palmitate levels are significantly increased along with upregulated hepatic mRNA levels of genes related to retinol storage and metabolism in hepatocytes of high fat/high cholesterol diet-fed mice and *ob/ob* mice. In rats fed a methionine-choline deficient (MCD) diet, hepatic and serum retinol levels are decreased.[34]

The changes in hepatic vitamin A and their metabolite levels are likely due to the change in genes involved in retinol metabolism. Another gene associated with NAFLD is aldo-keto reductase family 1 member B10 (AKR1B10), which is a key enzyme of retinol metabolism with a very efficient and high all-trans-retinaldehyde reductase activity in converting all-trans-retinaldehyde to retinol, is significantly overexpressed in human NASH liver and HCC tumors.[35] Pettinelli *et al.*[36] showed that NASH patients have highly induced AKR1B10 expression and reduced aldehyde dehydrogenase 1, family member A2 (ALDH1A2), and ALDH1A3 expression in the liver as well as elevated plasma retinol levels. Seventeen-beta hydroxysteroid dehydrogenase 13 (HSD17B13) has RDH activity and a loss-of-function mutation in HSD17B13 reduces the progression of NAFLD.[37] Patatin-like phospholipase domain-containing 3 (PNPLA3), is reported to have retinyl-palmitate lipase activity, releasing retinol from lipid droplets in HSCs.[38] The genetic association studies showed that the genetic variant in I148 M (rs738409), is a risk factor for NAFLD as it reduces the lipase activity and decreases circulating serum retinol levels in NAFLD patients.[38–40] Thus, it is evident that disruption in the retinoid metabolism is often associated with NAFLD.

5. Mechanisms underlying the regulation of NAFLD by RA signaling

Hepatic lipid accumulation occurs from an imbalance between lipid absorption/uptake, synthesis, and secretion/disposal, which are regulated by several pathways, including uptake of circulating free fatty acids (FFAs), *de novo* lipogenesis (DNL), lipolysis, FA oxidation (FAO), and secretion of lipids in very low-density lipoproteins (VLDL) or cholesterol to bile. Obesity is also associated with the development of NAFLD. Next, we will discuss how RA signaling affects hepatic lipid metabolism, inflammation, fibrogenesis, and obesity.

5.1. RA signaling in hepatic lipid metabolism

Accumulation of FFAs may cause lipotoxicity. Amengual *et al.*[41] showed that AtRA treatment induces hepatic expression of peroxisome proliferator-activated receptor α (PPARα), RXRα, uncoupling protein 2 (UCP2), liver-type carnitine palmitoyltransferase 1 (CPT1), and carnitine/acylcarnitine carrier (CAC), and a reduction in the mRNA expression levels of sterol regulatory element binding protein 1c (SREBP1c) and FA synthase (FASN), and reduces hepatic triglyceride (TG) levels and VLDL secretion and increases circulating 3-hydroxybutyrate levels.[41] AtRA is also shown to induce FAO in HepG2 cells and mouse primary hepatocytes.[42,43] We found that AtRA induces FAO independent of the activation of RARa.[43] PPARα is a key regulator of FAO; activating PPARα protects from trans-FA-induced steatohepatitis while PPARα inhibition increases the susceptibility to steatohepatitis.[44] PPARα binds to deoxyribonucleic acid (DNA) as a heterodimer with RXR. Both AtRA and 9-cis-RA can induce the expression of RXR which in turn activates PPAR: RXR heterodimers leading to the transcription of PPARα target genes.[45] In addition to reducing hepatic TG accumulation, activation of PPARα: RXR also decreases the production of TG-rich VLDL and plasma TG levels.[46] PPARβ/delta (PPARβ/δ) is another transcription factor that is known to stimulate FAO. PPARβ/δ may prevent dyslipidemia, insulin resistance, obesity, and NAFLD by regulating hepatic glucose catabolism and FAO and by inhibiting DNL via AMP-activated protein kinase (AMPK) signaling.[44] Apart from its canonical RARs, AtRA binds to PPARβ/δ with high affinity depending on the expression levels of cellular RA-binding protein II (CRABPII) and FA binding protein 5 (FABP5) which delivers AtRA to RAR and PPARβ/δ respectively.[47]

Circulating FFA uptake is a major source of the FA pool in the liver. During fasting and insulin resistance, hepatocytes extract FFAs which increase lipogenesis and lipotoxicity.

FA translocase (CD36/FAT) is a transmembrane glycoprotein that acts as a scavenger receptor capable of binding several ligands, including long-chain FAs, lipoproteins, and oxidized lipids. Even though CD36 expression is considered low in the normal liver, its expression is increased in the liver of NAFLD patients.[48] It is well known that CD36 increases FFA uptake and drives hepatosteatosis onset and its progression to NASH.[49] CD36 is a well-characterized PPARγ target.[50,51] It is reported recently that Alisol B, a natural compound isolated from a plant called *Alisma orientalis*, attenuates HFD and CCl_4-induced liver steatosis by inhibiting CD36 by regulating the RARα-hepatocyte nuclear factor 4α (HNF4α)-PPARγ transcriptional cascade.[52] Tang *et al.*[53] indicated that activating RARβ2 inhibits PPARγ and CD36 levels in HFD-fed mice. We revealed that AtRA inhibits hepatocyte FA uptake and CD36 expression and that the inhibition of CD36 expression is dependent on the activation of RARα.[43]

DNL is the process of the synthesis of endogenous FAs from acetyl-CoA produced by other metabolic pathways such as glycolysis. About 26% of TG in the livers of NAFLD patients come from DNL suggesting that impairment in DNL contributes to the pathogenesis of NAFLD.[54] Two major pathways downstream of the insulin receptor activate SREBP1c, both involving the phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB, or Akt) pathway, one resulting in the phosphorylation of the nascent SREBP1c itself and the other in the activation of the liver X receptor (LXR).[55] Insulin resistance leads to hypertriglyceridemia and hepatic steatosis which is associated with increased SREBP1c activity. Therefore, inhibiting SREBP1c activation has the potential for the treatment of hypertriglyceridemia and NAFLD.[56] Treatment of HFD-fed mice by RA reduces hepatosteatosis and this effect is suggested through sirtuin 1 (SIRT1)-mediated inhibition of SREBP1c.[57] Although AtRA inhibits lipogenic genes in the liver, DNL is not affected when mice are injected with heavy water followed by the analysis of newly synthesized FAs or TGs, suggesting that AtRA lowers hepatic TG levels likely independent of DNL.[43]

Hepatic TG and cholesterol esters are secreted to the circulation in the form of VLDL. AtRA is shown to lower lipid contents in VLDL.[41] Nonetheless, controversial data have been reported on the role of AtRA in hepatic lipogenesis and VLDL secretion.[45] Retinoids have been reported to induce hypertriglyceridemia due to enhanced hepatic lipogenesis and VLDL production and secretion as well as VLDL clearance.[45]

5.2. RA signaling in hepatic inflammation

Retinoids have been known to possess anti-inflammatory effects for 40 years, which may be mediated through downregulation of Th1 cytokines, such as tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), and interleukin-12 (IL-12).[58–63] In the THP-1 monocyte/macrophage cell line, AtRA reduces liposaccharide (LPS)-induced production of the proinflammatory cytokines TNF-α and IL-12 and enhances IL-10 production.[64] Mechanistically, retinoids inhibit the phosphorylation of IkB kinase a/b (IKKa/b), the nuclear factor kappa B (NF-kB)-DNA interaction or its translocation to the nucleus.

Consistent with these observations, deletion of RARα in macrophages or hepatocytes aggravates inflammatory response, whereas RARb2 activation inhibits inflammatory cytokine secretion, suggesting a critical role of RARa and RARb2 in mediating retinoid's effects on inflammation.[43,63,65–68]

5.3. RA in hepatic fibrosis and HSC activation

Fibrosis is a wound-healing process characterized by ECM accumulation that causes scarring and impaired liver function. The effects of RA on ECM accumulation and fibrosis are controversial. It is shown that the loss of retinyl esters in HSCs is often a characteristic of HSC activation during liver injury.[69] Earlier studies showed that 9-cis-RA enhanced plasminogen activator (PA)/plasmin levels and thereby induced proteolytic activation of transforming growth factor-β1 (TGF-β1), a fibrogenic master cytokine, resulting in enhanced ECM production.[70] However, 9-cis-RA activates RXR, which can form heterodimers with a variety of nuclear receptors to exert its functions. Later reports showed a protective effect of RA signaling on liver fibrosis. Hisamori *et al.*[71] showed that AtRA is shown to attenuate CCl_4-induced liver fibrosis by reducing the production of TGF-β, IL-6, and collagen from HSCs in mice. They further showed that AtRA inhibits TGF-β-dependent transdifferentiation of the cells and the activities of NFkB p65 and p38 mitogen-activated protein kinase.[71] Wang *et al.*[72] also showed that AtRA reduces liver fibrosis induced by common bile duct ligation via inhibition of TGF-β and connective tissue growth factor (CTGF) in rats.[72] *In vitro* studies showed that AtRA inhibits HSC proliferation and collagen production by suppressing active protein-1, c-Jun N-terminal kinase signal, and expression of profibrogenic genes (TGF-β1, CTGF, MMP-2, TIMP-1, TIMP-2, PAI-1), and inducing MMP-3 and MMP-13.[73] RA may also synergize with PPARγ to reverse fibrosis by modulating senescence of HSC.[74] In terms of specific RARs, expression of the dominant negative form RARα is shown to induce fibrosis.[75] However, genetic ablation of RARα in the liver does not affect fibrogenesis.[43]

5.4. RA signaling in obesity and insulin resistance

Obesity and insulin resistance are among the most common risk factors for NAFLD as the majority of obese and diabetic patients have NAFLD.[76,77] Thus, treating insulin resistance may help to fight NAFLD. Circulating RA concentrations are lower in subjects with NAFLD and are associated with hepatic lipid metabolism and insulin resistance.[30] AtRA treatment is known to attenuate obesity and insulin resistance.[78] The effect of AtRA on obesity is likely through inhibition of adipogenesis and induction of energy expenditure.[79,80] At molecular levels, AtRA is suggested to inhibit obesity by activation of both PPARb/d and RAR.[81] We demonstrated that hepatic RARa plays an important role in mediating AtRA's effect on diet-induced obesity.[43] Tsuchiya *et al.*[82] showed that AtRA improves insulin sensitivity likely by induction of leptin receptor-mediated phosphorylation of signal transducer and activator of transcription 3 (STAT3) and insulin receptor substrate 1 (IRS1) and RARa activation is significant for these effects. Thus, the inhibition of obesity may play a role in AtRA-mediated amelioration of NAFLD.

6. Therapeutic potential of RA in NAFLD

Some studies have aimed to identify the therapeutic potential of vitamin A metabolites in the treatment of NAFLD. Matsumoto *et al.*[83] showed that feeding obese Zucker (*fa/fa*) rats with brown rice, an animal model of NAFLD, increases RA synthesis which in turn, protects against NAFLD by increasing FAO and VLDL secretion. Zarei *et al.*[84] reported that AtRA significantly reduces liver steatosis in HFD-fed rabbits. We showed that AtRA prevents and reverses Western diet-induced liver steatosis in mice.[43] Zhu *et al.*[85] and Kim *et al.*[86] also showed that AtRA prevents HFD-induced liver steatosis in mice. Liu *et al.*[30] reported that RA levels are significantly reduced in NAFLD patients and correlated with hepatic TG levels. It is also reported that the intake of β-carotene is inversely associated with liver steatosis in humans.[87] However, it remains to be investigated whether AtRA or other retinoids attenuate liver steatosis in humans.

7. Conclusions and prospects

In this review, we primarily discuss the role of RA signaling in the liver and to some extent in adipose tissue. However, RA signaling in other cells and tissues affects the progression of NAFLD. The gut-liver axis plays a key role in the pathogenesis of liver diseases, including NAFLD.[88,89] Dysregulation of gut microbiota, barrier, and permeability contributes to the development of NAFLD.[90–92] There is a rich array of literature describing the role of RA signaling in reshaping gut microbiomes, inflammation, immunity, and barrier functions.[93,94] Serum retinol levels and gut permeability display an inverse relationship.[95,96] RA protects against a leaky gut likely through direct modulation of intestinal permeability and autoimmunity as well as regulating gut microbiota (*e.g., Lactobacillus* spp.).[93] RA inhibits gut microbiota dysbiosis, which may in turn regulate nutrient absorption, gut permeability, and hepatic metabolism thus protecting against NAFLD.[94]

Over the past decades, many studies from different groups investigated the role of retinoids, particularly AtRA, in metabolic homeostasis and cancer development. In addition to APL, AtRA is also being used to treat a range of human cancers in clinical trials.[97,98] In rodents, AtRA may attenuate Western diet-induced liver steatosis, inflammation, and fibrosis by inducing FAO and energy expenditure and inhibiting FA uptake, NF-kB, and TGF-β (Fig. 2).

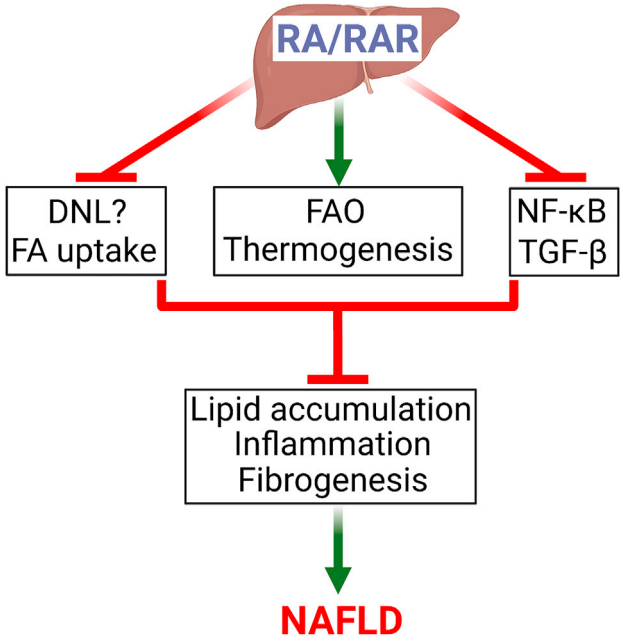

Fig. 2. Regulation of the development of NAFLD by the RA/RAR signaling. Activation of the retinoic acid receptor (RAR) by RA inhibits the development of non-alcoholic fatty liver disease (NAFLD) likely via several pathways. Activation of RAR induces FA oxidation (FAO) and thermogenesis and inhibits fatty acid (FA) uptake, nuclear factor kappaB (NF-κB), and transforming growth factor beta (TGF-β), leading to a reduction in hepatic lipid accumulation, inflammation, and fibrogenesis. The role of RAR activation in the inhibition of *de novo* lipogenesis (DNL) remains to be further clarified.

AtRA activates RARa, RARb, and RARg. The relative role of these RARs in the liver and other cell types/organs (*e.g.,* adipocytes, intestine) in the development of NAFLD remains to be elucidated. Understanding the cell-specific effects of RA signaling and the functions of other less-studied retinoids may offer new therapeutic approaches to the treatment of NAFLD.

Authors' contributions

Fathima N. Cassim Bawa and Yanqiao Zhang wrote the manuscript. Both authors approved the final version for publication.

Declaration of competing interest

The authors declare that there is no conflicts of interest.

Acknowledgements

This work was supported in part by the U.S. NIH grants DK102619 and DK118805 to Y.Z. Both figures were created with BioRender.com and are original.

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