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# Vitamin A as a Transcriptional Regulator of Cardiovascular Disease

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**Abstract:** Vitamin A is a micronutrient and signaling molecule that regulates transcription, cellular differentiation, and organ homeostasis. Additionally, metabolites of Vitamin A are utilized as differentiation agents in the treatment of hematological cancers and skin disorders, necessitating further study into the effects of both nutrient deficiency and the exogenous delivery of Vitamin A and its metabolites on cardiovascular phenotypes. Though vitamin A/retinoids are well-known regulators of cardiac formation, recent evidence has emerged that supports their role as regulators of cardiac regeneration, postnatal cardiac function, and cardiovascular disease progression. We here review findings from genetic and pharmacological studies describing the regulation of both myocyte- and vascular-driven cardiac phenotypes by vitamin A signaling. We identify the relationship between retinoids and maladaptive processes during the pathological hypertrophy of the heart, with a focus on the activation of neurohormonal signaling and fetal transcription factors (Gata4, Tbx5). Finally, we assess how this information might be leveraged to develop novel therapeutic avenues.

**Keywords:** micronutrient; heart failure; vitamin A; retinoid; nuclear receptor; cardiomyocyte; cardiac regeneration

## 1. Introduction

Vitamin A and its retinoid metabolites are known differentiation inducers and antioxidants used in the treatment of blood cancers and skin disorders [1–3]. Additionally, Vitamin A/retinoids are essential for mammalian embryogenesis [4,5], and excessive vitamin A/retinoids are known to cause congenital heart diseases (CHDs, full list of abbreviations in Table 1) in rodents and humans [6–9]. Intriguingly, population studies have identified a relationship between serum levels of vitamin A/retinoids and adult cardiovascular diseases (CVDs) [10], the global leading cause of death and one for which regenerative therapies are lacking. Thus, an improved understanding of teratogenic and postnatal mechanisms-of-action of vitamin A/retinoid signaling is of importance to both fetal and adult health.

**Table 1.** Abbreviations used in the current study.

| Abbreviation | Full Name | Abbreviation | Full Name |
|---|---|---|---|
| 9CRA | 9-cis retinoic acid | PSC | pluripotent stem cell |
| ATRA | all-trans retinoic acid | RA | retinoic acid |
| atrRFP | atrial RFP reporter | RAR | retinoic acid receptor |
| CaMKII | $Ca^{2+}$/calmodulin-dependent protein kinase II | RARE | retinoic acid response element |
| CHD | congenital heart disease | RBP | retinol binding protein |
| CPC | cardiac progenitor cell | RXR | retinoid x receptor |
| CRBP | cellular retinol binding protein | SMC | smooth muscle cell |
| CVD | cardiovascular disease | venGFP | ventricular GFP reporter |
| MI | myocardial infarction | VD3 | vitamin D3 |

We here review the molecular mechanisms of vitamin A/retinoid signaling in mammals and describe how these contribute to both cardiogenesis and postnatal cardiovascular function. Furthermore, we review evidence from population, genetic, and pharmacological studies on the role of vitamin A/retinoids in cardiac regeneration, myocardial/arterial homeostasis, and CVDs. Finally, we highlight the interaction of vitamin A and prototypical disease signaling pathways and examine how this evidence contributes to an understanding of the pathophysiology of CVDs, micronutrient deficiency, and therapeutic cardiac regeneration.

## 2. Molecular Mechanisms of Vitamin A/Retinoid Signaling and Homeostasis

Cell- and tissue-level effects of vitamin A/retinoid signaling are dependent on a complex series of steps, encompassing dietary intake, storage, mobilization, transport, metabolism to active forms, and activation of retinoic acid receptors (Figure 1). Dietary vitamin A/retinoids are ingested in the form of β-carotene, retinol, or retinyl esters [1], and a lack of dietary vitamin A is one of the most common micronutrient deficiencies [11]. After ingestion, β-carotene and retinol are converted into retinyl esters, and these are stored in the liver until mobilized, at which point they are usually re-converted into retinol [1].

After mobilization, retinol is transported from the liver to tissues bound to retinol binding proteins (RBPs) [12], though retinyl esters may also be transported to distant tissues within chylomicrons [13]. Intracellularly, retinol is bound to cellular retinol binding proteins (CRBPs) [12], and retinol and retinal dehyrodgenases oxidize retinol into active retinoid isoforms, the most predominant of which are all-trans retinoic acid (ATRA) and its stereoisomers, 9-cis retinoic acid (9CRA) and 13-cis retinoic acid [1]. In order to exert its effects on gene expression, ATRA/9CRA bind to homo- or heterodimers of retinoid x receptors (RXRs) and retinoic acid receptors (RARs), which themselves include various subtypes (α, β, γ) [1]. Interestingly, both ATRA and 9CRA activate RARs, whereas only 9CRA is a natural activator of RXRs [14–16]. Moreover, a diverse set of RAR/RXR receptor homo- and heterodimers can be formed in response to ligand binding, and these bind to DNA to regulate transcriptional activity [14]. RARs/RXRs are expressed in numerous tissues/organ systems, and gene expression and genetic loss-of-function analyses have revealed tissue-specific roles of specific combinations of RARs/RXRs, expertly reviewed elsewhere [17,18]. Importantly, synthetic retinoids have been developed which are selective for RARα, RARβ-γ, RARγ and RXRs [19–23], and these serve as tool compounds for probing specific receptor activity.

The biological activity of retinoids is also regulated by their degradation. Cell and tissue levels of active retinoid forms are controlled by Cyp26a1, Cyp26b1, and Cyp26c1, enzymes that degrade both ATRA and 9CRA [24]. Interestingly, Cyp26 enzymes are themselves under the transcriptional control of RAR/RXR, thus forming a negative feedback loop that regulates levels of active retinoids at the cellular and tissue level [24]. Though in vitro studies allow precise perturbation of retinoic acid (RA) signaling, in vivo effects are regulated by the mobilization of vitamin A in the liver [25]. Interestingly, vitamin A levels were reported to increase in the hearts of rats following myocardial infarction (MI), in conjunction with increased hydrolysis of vitamin A stores in the liver and the transcription of RA-dependent reporter genes [26,27]. Furthermore, there were increased levels of retinol in the hearts of mice lacking β-carotene-15,15′-dioxygenase, the enzyme responsible for converting β-carotene into active retinoids [28]. Thus, RA activity is a function of dietary intake, the synthesis of active ATRA/9CRA forms, degradation, binding of retinoids to nuclear RAR/RXR receptors, and modulation of transcriptional outcomes.

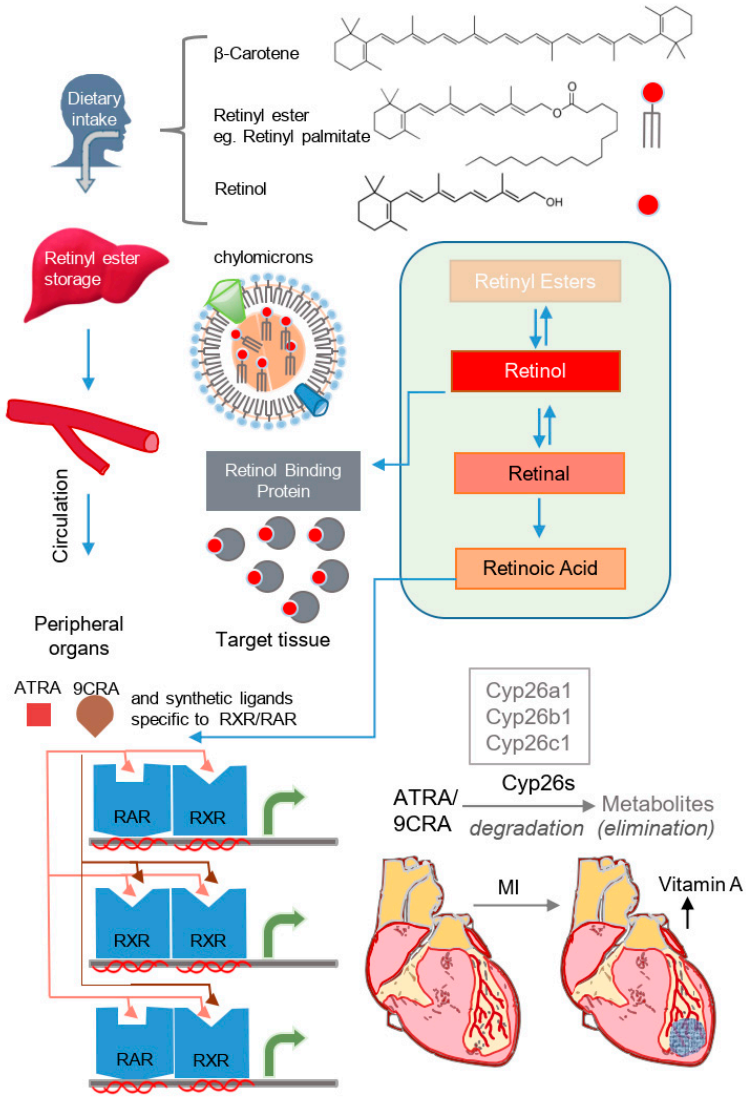



**Figure 1.** Overview of Vitamin A/retinoid signaling. Dietary intake of retinoids is commonly in the form of β-carotene, Retinyl esters, or retinol. These are stored in the liver and transported to target tissues via retinol binding proteins. Active retinoid forms of all-trans retinoic acid (ATRA) and 9-cis retinoic acid (9CRA) bind to homo and heterodimers of retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Levels of ATRA and 9CRA are controlled via Cyp26-mediated degradation of active metabolites. Following myocardial infarction, vitamin A levels have been shown to increase in the heart, as evidenced by direct measurement of vitamin A levels and RA-induced transcription. All-trans retinoic acid (ATRA), 9-cis retinoic acid (9CRA), Retinoic acid receptor (RAR), Retinoid X receptor (RXR).

## 3. Effects of Vitamin A/Retinoids on the Formation of the Heart and Differentiation of Multipotent Cardiovascular Progenitor Cells

Maternal vitamin A deficiency was long ago identified as a cause of congenital heart abnormalities in rodents [6,7], and the exposure of the fetus to exogenous ATRA is highly teratogenic to the embryonic human heart [8]. In addition to providing insights into the onset of CHDs, a more thorough understanding of the molecular mechanisms by which retinoids exert effects on the formation of the heart could provide novel strategies for regeneration of the adult heart following MI. Furthermore, knowledge of these mechanisms could guide efforts to impede the re-activation of developmental gene regulatory networks in the failing heart [29].

The effects of retinoids on the formation of the heart have been well-defined by embryological studies. During early cardiogenesis, RA signaling is integral to the following developmental processes: (1) negative regulation of multipotent cardiac progenitor cells (CPCs)/promotion of cardiomyocyte differentiation [30–33]; (2) patterning of multipotent progenitors into anterior (atrial) and posterior (ventricular) cell fates [34–36]; (3) rightward looping of the heart [32,37]; and (4) Formation of mature structures in the heart, such as septa, atrioventricular cushions, epicardium, ductus arteriosus, and mature ventricular muscle [38–42].

Interestingly, RA receptors appear to have specific functions during cardiogenesis. For instance, RXRα is necessary for embryonic viability, and the deletion of RXRα led to ventricular dysfunction, persistent atrial gene expression in the ventricles, thin muscular walls more resembling atria, and defects in the formation of the ventricular septum [43,44]. However, both single and co-deletion of RARα1 and RARβ do not result in cardiac phenotypes in embryos and adults [45], suggesting that RXRα is a predominant mediator of RA signaling in the developing heart. Interestingly, embryonic RA signaling is subject to complex feedback mechanisms defined by the presence or absence of specific RA receptors, evidenced by zebrafish experiments in which the deletion of RARα led to subsequent overall increases in RA signaling [46].

Developmental effects of RA signaling are also determined by the degradation of RA by Cyp26 enzymes. Deletion of both Cyp26a1 and Cyp26c1 in zebrafish led to an increase in atrial cells and a decrease in vascular cells, in addition to a loss of ventricular cardiomyocytes derived from first heart field progenitor cells [47,48]. Interestingly, overexpression studies of Cyp26a1 revealed that this enzyme regulates RA levels at a tissue, rather than a cellular level, and this further helped to shape developmental gradients [49]. Thus, Cyp26 enzymes regulate the availability of retinoids during development, thereby affecting tissue formation.

The effects of RA during development appear to occur partially through the activation of HOX genes, key drivers of developmental gene activation in the formation of diverse tissues, including the heart [50–52]. RA target genes within second-heart field progenitor cells include Hoxa1, Hoxa3, and Hoxb1 (mouse), as well as Hoxb5b (zebrafish) [52,53]. Interestingly, the overexpression of Hoxb5b exerted similar phenotypes to that of excessive RA in zebrafish embryos, suggesting that HOX genes are major target genes of RA signaling during early embryogenesis [54]. In addition to HOX genes, RA signaling is upstream of several transcription factors key to cardiogenesis, such as Gata4, Tbx5, Pitx2, and Nr2f2 [55–58]. Thus, vitamin A/retinoids exhibit effects on key developmental processes during the formation of the heart, and this occurs via the activation of cardiac transcription factors.

## 4. Vitamin A Signaling and Regeneration of the Myocardium

The regeneration of lost myocardium has recently emerged as a potential therapeutic strategy for the treatment of MI, and vitamin A/retinoids have been implicated in cardiac regeneration, driven by both endogenous mechanisms and cell transplantation (Figure 2a). Expression of the retinoic acid synthesis gene Raldh2 was shown to be induced in the epicardium of the ventricle, atrium, and outflow tract of the regenerating zebrafish heart following myocardial injury [59]. A follow-up study showed that Raldh2 is activated in proliferating cells of the endocardium of fish following cardiac injury, and the inhibition of RARα led to decreased cardiomyocyte proliferation and impeded regeneration of the myocardium [60]. Indeed, the secretion of RA by the epicardium was demonstrated to be required for the proliferation of embryonic cardiomyocytes in the chick model [61]. Furthermore, a population of Raldh2-expressing epicardial cells were shown to migrate into the ventricular myocardium in the chick model, and the authors suggested that these cells may serve as a source of retinoic acid that promotes the thickening of the embryonic myocardium. Moreover, the authors observed that Raldh2+ epicardial cells differentiate into smooth muscle and endothelial cells of the coronary vessels [62]. Additionally, RA signaling regulated secretion of erythropoietin by the liver during embryogenesis, and this in turn stimulated expression of Igf2 in the epicardium, resulting in cardiomyocyte proliferation [63]. Thus, RA signaling

has been implicated in endogenous cardiomyocyte proliferation and regenerative processes native to the hearts of lower vertebrates.

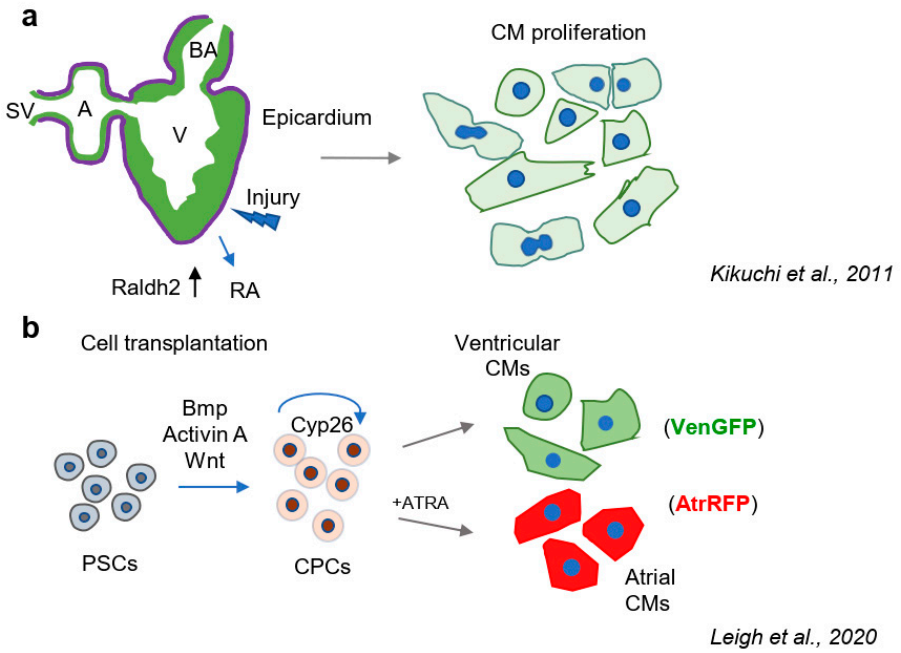



**Figure 2.** Vitamin A signaling and cardiac regeneration. (**a**) During zebrafish heart regeneration, Raldh2 increases in the epicardium of injured hearts, promoting cardiomyocyte proliferation and regeneration of lost myocardial tissue. Secreted retinoic acid has also been shown to promote proliferation of the embryonic chick heart, indicating the possibility to manipulate this pathway to induce therapeutic cardiac regeneration via cardiomyocyte proliferation. (**b**) Effects of Vitamin A/retinoids on the differentiation of pluripotent-stem cell-derived cardiomyocytes observed in Leigh et al. 2020. Vitamin A/retinoids promote the differentiation of pluripotent stem cells to atrial cardiomyocytes in a process involving the expression of retinoic acid-degrading Cyp26 enzymes in multipotent cardiac progenitor cells. Reporter cell lines in Leigh et al. allow assessment of differentiation to atrial (atrRFP) and ventricular (venGFP) cell fates and demonstrate the stage-specific effects of retinoids. Atria (A), Ventricle (V), Sinus Venosus (SV), Bulbus arteriosus (BA), Retinoic Acid (RA), Cardiomyocyte (CM), Pluripotent Stem Cells (PSCs), Cardiac Progenitor Cells (CPCs), Retinoic acid (RA), All-trans retinoic acid (ATRA), Ventricular GFP reporter (venGFP), atrial RFP reporter (atrRFP).

In addition to the induction of endogenous proliferation, the injection of stem cell-derived cardiomyocytes into the infarcted heart has recently emerged as a therapeutic strategy to replace the cardiomyocytes lost following MI [64]. Importantly, RA was identified as a general inducer of differentiation of pluripotent stem cells (PSCs) [65–67]. Moreover, RA has been shown in numerous studies to promote the directed differentiation of PSC- and CPC-derived cardiomyocytes. For instance, ATRA promoted the differentiation of H9c2 cardiomyoblast cells, leading to the expression of cardiomyocyte gene expression programs and providing evidence that ATRA is a differentiation inducer of CPCs [68,69]. Additionally, several in vitro studies in PSCs have reported retinoids as specific inducers of the atrial cardiomyocyte cell fate [58,70,71] (Figure 2b). In our own study, dose- and stage-specific effects of retinoids were observed on the differentiation of atrial and ventricular PSC-derived cardiomyocytes using markers of early cardiogenesis to detect differentiation status, mirroring embryonic phenotypes [70]. Studies by a separate group showed that the titration of BMP and Activin A were shown to regulate the levels of Cyp26 RA degrading enzymes, thereby potentiating RA response in CPCs and determining whether cells assumed an atrial or ventricular fate [72]. A phenotypic screen of 10,000 compounds for the induction of proliferation of human multipotent CPCs led to the identification of RAR, but not RXR agonists as potent inducers of CPC proliferation.

Importantly, the authors found that treating CPCs with retinoids on day 4 of differentiation promoted the atrial cardiomyocyte fate, whereas day 5 treatment led to the induction of cardiac progenitor proliferation [73]. Therefore, the effect of manipulation of RA signaling in PSCs and CPCs is determined by the stage of differentiation, and this pathway can be manipulated chemically to generate specific cardiac cell subtypes. Moreover, in vitro platforms provide tractable systems for the examination of temporal effects on vitamin A/retinoid signaling and serve as a potential source of cell subtypes to regenerate the heart following myocardial injury.

## 5. Population-Based Studies Implicating Vitamin A Deficiency in the Onset of CVDs

In addition to the evidence for the role of RA signaling in cardiogenesis, population-based studies have implicated RA signaling in the development of adult CVDs. A Finnish study of men aged 46–65 reported that low serum β-carotene levels were associated with an increased risk of acute MI [74], and low serum β-carotene levels were associated with an increased risk of cardiovascular mortality, especially among smokers [75]. In a Chinese study, low serum RA levels (median 21 nM vs. 39 nM) were associated with an increased risk of mortality in patients with coronary artery disease, and high RA levels were associated with a decreased risk of overall mortality [10]. A separate study in a US population of over-50-year-olds showed that both low (<30 μg/dL) and high (>80 μg/dL) serum RA levels were associated with increased mortality due to CVDs [76]. Indeed, patients with elevated serum vitamin A (>3.10 μM) were also observed to have elevated apolipoprotein B and to be at a greater risk for CVDs [77]. However, not all population studies display a relationship between vitamin A and coronary artery disease, as evidenced by a Singaporean study in which no significant differences in vitamin A levels were observed in the livers of patients who died of coronary artery disease [78]. Collectively, these studies indicate the potential relevance of RA signaling to CVDs in human patients.

## 6. Effects of Vitamin A/Retinoids in the Healthy and Diseased Postnatal Myocardium

In addition to the relationship between low serum RA and CVDs identified in population studies, there is ample evidence from animal and in vitro studies demonstrating that RA signaling affects myocardial function and CVDs. A recent study showed that RARα decreases in wild type mice during aging and when subjected to metabolic stress, and the genetic deletion of RARα in adult mouse myocardium resulted in diastolic dysfunction, impaired $Ca^{2+}$ handling, and increased oxidative stress [79]. Similarly, Cellular retinoic acid binding protein 1 (Crabp1) knockout mice were more susceptible to heart failure and remodeling induced by isoproterenol, an agonist of adrenergic signaling, and this was attenuated by treatment with ATRA (5 mg/kg/day delivered intraperitoneally) [80].

Animal models have also implicated RA signaling in the pathogenesis of MI. A retinoic acid response element (RARE)-luciferase reporter was activated in the heart following MI in mice, in addition to the RA synthesis gene Aldh1a2 and the RA degradation gene Cyp26b1 [27]. RA receptors RARα and RARγ were more prominently upregulated in cardiac fibroblasts, whereas RARβ was specifically upregulated in cardiomyocytes [27]. Importantly, separate studies showed that exogenously administered ATRA (1 μM; 0.1 μM−10 μM) decreased the proliferation of primary cardiac fibroblasts in vitro [27,81], suggesting that ATRA might have anti-fibrotic properties in the infarcted heart. A separate study demonstrated that dietary vitamin A supplementation (0.3 mg/kg given daily) in rats subjected to MI led to decreased myocyte hypertrophy and an increased maximum rate of rise of left ventricular pressure six months after injury [82].

Evidence exists that RA-signaling is an important component of cardiomyopathies. Dilated cardiopathy was observed in a transgenic mouse line containing a cardiac-specific overexpression cassette of RARα [83], suggesting deleterious effects of prolonged activation of RA signaling. In mouse models of obesity, daily delivery of ATRA by stomach intubation (2 μg/g/day) restored natriuretic peptide levels and prevented adverse cardiac remodeling [84]. Similarly, dietary supplementation with RA decreased cardiac injury in mouse models of gestational diabetes mellitus (3 mg/kg/day), tobacco smoke, and methylglyoxal (1 mg/kg/day) [85–87]. Beneficial effects of RA

in the MI model might be attributed to its anti-hypertrophic effects in cardiomyocytes (Figure 3a). Exogenous ATRA (0.1 μM−1 μM) attenuated phenylephrine-induced hypertrophy in cultured neonatal cardiomyocytes, leading to reductions in cell size and activation of atrial natriuretic peptide, suggesting that ATRA prevents adverse effects due to excessive activation of adrenergic receptors [88]. Similarly, ATRA (10 μM) attenuated endothelin-induced hypertrophy and the activation of natriuretic peptide transcription in neonatal cardiomyocytes in vitro, and this effect was more pronounced in combination with 1,25(OH)2 vitamin D3 (VD3) [89]. Thus, RA signaling interferes with pro-hypertrophic effects of neurohormonal signaling characteristically activated in the context of heart failure.

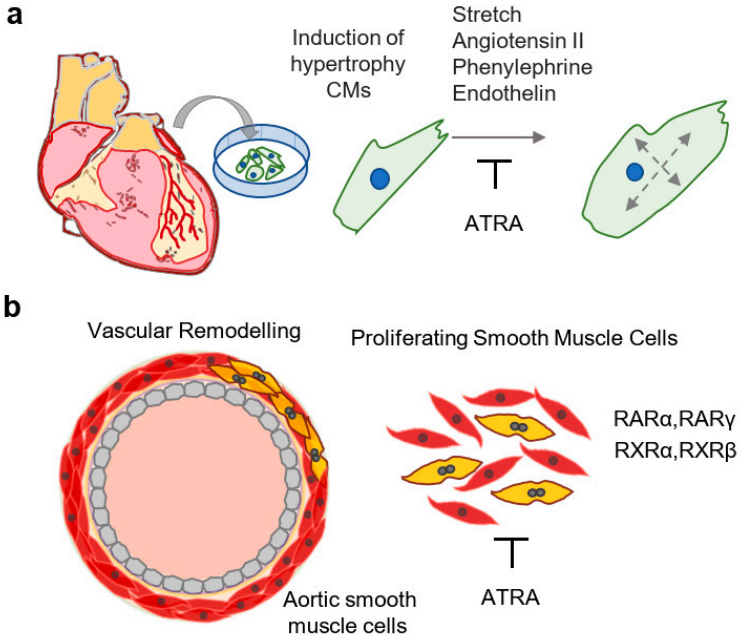



**Figure 3.** Anti-hypertrophic and anti-proliferative effects of Vitamin A/retinoid signaling in the postnatal mammalian heart. (**a**) Anti-hypertrophic effects of retinoids on neonatal cardiomyocytes. Retinoids interfere with the induction of cellular hypertrophy induced by physical stretch and neurohormonal signals, such as Angiotensin II, Phenylephrine, and Endothelin. This suggests a mechanism through which retinoids could promote favorable changes in the diseased heart; (**b**) Anti-proliferative effects of retinoids on vascular smooth muscle cells. ATRA inhibits the proliferation of smooth muscle cells in vitro and in vivo. Inhibition of smooth muscle cell proliferation impedes thickening of the arteries and pathological vascular remodeling. Cardiomyocytes (CMs), All-trans retinoic acid (ATRA), Retinoic acid receptor (RAR), Retinoid x receptor (RXR).

Beneficial effects of modulation of RA signaling have also been observed in other CVD models in vivo. In rats subjected to aortic banding, ATRA (30 mg/kg/day by gavage) prevented deterioration in systolic and diastolic dysfunction, in addition to inhibiting hypertrophic gene expression [90]. ATRA (5 mg or 10 mg/kg/day by intraperitoneal injection) impeded cardiac damage caused by isoproterenol treatment in mice and rats [91,92] and regulated calcium signaling by inhibiting phosphorylation of $Ca^{2+}$/calmodulin-dependent protein kinase II (CaMKII) and phospholamban [91]. Furthermore, ATRA (10 μM–20 μM) attenuated pro-arrhythmic effects of isoproterenol and lysophosphatidylcholine in cultured neonatal cardiomyocytes, and reperfusion-induced ventricular tachycardia and fibrillation were attenuated by the intravenous infusion of ATRA (1 mM delivered by perfusion at 8 mL/hour) in vivo [93]. Thus, ATRA has been reported in various studies to provide beneficial effects in diverse CVD models in vivo.

In addition to the study of exogenous delivery of ATRA in disease models, several studies have investigated the effects of Vitamin A deficiency and/or dietary supplementation. Separate studies found adverse remodeling was induced, rather than impeded by depleted vitamin A stores, and Vitamin-A

depleted hearts underwent more severe adverse remodeling following MI [94,95], though another study reported beneficial effects of vitamin A deficiency [96]. Additionally, in contrast to other studies, ATRA dietary supplementation (24 μg/kg/day for 90 days or 0.3 mg–10 mg/kg/day for two months) in normal rats induced adverse remodeling of the ventricle [97,98]. These studies indicate context-dependent effects of manipulation of retinoids/Vitamin A signalling in the healthy vs diseased heart. Additionally, these findings broadly support the complex relationship between RA levels and CV function, mirrored by human studies in which both low (<30μg/dL) and high (>80μg/dL) serum RA levels led to adverse effects [76]. Further experimentation might be necessary to fully optimize therapeutic concentrations of serum RA upon exogenous treatment. Regardless, animal studies broadly indicate a role for retinoids in maintaining myocardial homeostasis, and potentially indicate RA signaling as a therapeutic target for CVDs.

## 7. Effects of Vitamin A on Arterial Homeostasis

Thickening of the intima via the proliferation of vascular smooth muscle cells is an important component of the pathophysiology of atherosclerosis, the underlying cause of a majority of CVDs [99]. Importantly, ATRA has been shown to inhibit proliferative and adverse phenotypical changes of vascular smooth muscle cells (SMCs), in vitro and in vivo (Figure 3b). For instance, rat aortic SMCs were shown to express RARα, RARγ, RXRα, and RXRβ, and treatment with ATRA (2 μM–20 μM) inhibited the proliferation of rat aortic SMCs [100]. Though RARα was suggested as the major mediator of antiproliferative effects of ATRA (0.125 μM–2 μM) on SMCs [101], a separate study demonstrated that RARγ agonists also inhibited the proliferation of SMCs [102]. Importantly, several studies confirmed anti-proliferative as well as anti-apoptotic effects of ATRA (1 μM; 5 μM–10 μM; 2 μM) on cultured rat aortic SMCs [103–105]. Anti-proliferative effects were also observed in human cells, as ATRA (0.022 μM, 0.01 μM–1 μM, 0.12 μM) inhibited the proliferation and migration of human arterial smooth muscle cells in vitro [106–108]. Interestingly, the proliferation of human arterial endothelial cells was not affected by ATRA, suggesting differential responses of stromal cells to retinoids [106]. This was supported by the observation that retinol (1.25 μM–20 μM) inhibited the proliferation of intimal SMCs but increased the proliferation of medial SMCs, reportedly due to different abilities of these cell types to metabolize retinol to ATRA [109]. In a separate study, ATRA (1 μM) inhibited the proliferation of human aortic SMCs and adult murine cardiofibroblasts, while increasing the expression of RA target genes (Rarβ, Cyp26b1) in mouse adult cardiomyocytes [110]. However, in human umbilical vein endothelial cells, exogenous ATRA (1 μM) led to increased vascular endothelial growth factor (VEGF) signaling, thereby potentiating angiogenesis [111]. Thus, in vitro studies are largely supportive of an anti-proliferative effect of retinoids in aortic SMCs, in addition to showing cell-type specific phenotypic changes.

Exogenous retinoids have also shown beneficial effects on diseases of the arteries in vivo. Dietary ATRA (1.5 mg/day, 30 mg/kg/day) reduced neointima area in both the carotid artery and aorta of rats subjected to balloon injury [112,113]. Similarly, following balloon angioplasty or jugular vein bypass grafts, the oral administration of ATRA (0.6 mg/kg/day, 10 mg/kg/day by gavage) and the local delivery of ATRA (10 mL of 10 μM infusion) decreased intimal thickening in rabbits by decreasing proliferative activity [114–116]. Oral administration of Am80, a RARα agonist, impeded formation of the neointima following vascular injury and modulated SMC phenotypes in a rabbit model of stent placement [117]. In a separate study, daily ATRA (5 mg–10 mg/kg/day by gavage for three months) attenuated the thickening of intramyocardial and intrarenal arteries, as well as ventricular fibrosis in spontaneously hypertensive rats, though cardiac function was not improved [118].

Interestingly, some studies support the beneficial effects of retinoids in arterial pathologies which may not be accounted for by their anti-proliferative activities. For instance, ATRA (6 mg/kg/day) was reported to achieve favorable vascular remodeling without inhibiting proliferation [119]. Additionally, a separate study reported the upregulation of vascular integrins in SMCs by ATRA (1 mg/kg every other day for 3 weeks) in adult rats, suggesting an alternative mechanism by which

ATRA can induce vascular remodeling [120]. ATRA (1 μM–10 μM) was also reported to modulate contractility of the aorta in organ culture, suggesting another mechanism by which ATRA could regulate blood pressure [121]. Indeed, vitamin A-deficient rats appeared to have SMCs with reduced differentiation capacity and decreased contractility [122]. ATRA stimulation (0.1 μM–1 μM) was also reported to impede osteogenic differentiation of cultured human coronary artery SMCs, leading to the suppression of calcification [123]. However, this may be a delicate balance, as excess dietary vitamin A caused aortic valve calcification in mice fed retinyl palmitate (200 IU/g for 12 months) [124].

Despite the abundance of preclinical studies in rodent and animal models, only a limited number of human studies have examined the involvement of RA signaling in atherosclerosis. Measurement of vitamin A metabolites in human samples revealed that ATRA was increased in the left ventricles of patients with end-stage heart failure caused by coronary artery disease, in addition to describing the upregulation of RA genes (ALDH1A2, CRABP2, etc.) in atherosclerotic plaques [110]. Furthermore, a polymorphism in Cyp26b1, which leads to increased RA degradation, was implicated in the size of atherosclerotic lesions [125]. Further studies with human biopsies or human induced pluripotent stem cell models might help to uncover the specific roles of retinoids in regulating the proliferative capacity and phenotype of human smooth muscle cells.

## 8. Effects of Vitamin A/Retinoids on Prototypical Disease Signaling Pathways

In line with the beneficial effects observed in CVD cell and animal models, RA signaling interacts with signaling pathways known to regulate CVD progression. For instance, neurohormonal signaling is activated during maladaptive pathological hypertrophy [126], and several studies indicate that these pathways can be modulated by retinoids. In separate studies, ATRA (0.1 μM–1 μM; 10 μM) inhibited the deleterious effects of adrenergic and endothelin signaling on cardiomyocytes [88,89]. Similarly, ATRA (0.01 μM–2 μM) attenuated the endothelin-induced proliferation of rat ASMCs [127]. Additionally, an analysis of the hearts of LRAT (−/−) mice lacking hepatic retinol stores and fed a vitamin A-depleted diet identified vitamin A as a potential regulator of natriuretic peptides Nppa and Nppb [96]. In a separate study, exogenous ATRA (0.5 μM) was shown to regulate the transcription of natriuretic peptide receptor A by direct binding to the promoter and the modulation of histone acetylation [128]. Thus, RA signaling modulates the neurohormonal signaling characteristic of maladaptive remodeling in the post-MI heart.

Antagonists of the renin-angiotensin-aldosterone system are commonly used as heart failure treatments in order to support vasodilation and a favorable hemodynamic state [126]. Furthermore, angiotensin II is a key mediator of pathological ventricular remodeling during heart failure [126]. Exogenous ATRA (0.1 μM–10 μM) was reported to inhibit effects of angiotensin II on both cardiac fibroblasts and neonatal cardiomyocytes [129], and exogenous ATRA (1 μM) also inhibited the proliferation of vascular SMCs induced by angiotensin II [130]. In primary neonatal cardiomyocytes subjected to stretch, ATRA (0.1 μM–5 μM) inhibited renin-angiotensin signaling by the transcriptional repression of renin, angiotensinogen, angiotensin-converting enzyme, and the angiotensin type 1 receptor [90]. Thus, RA signaling is also an inhibitor of the angiotensin pathway, an important mediator of ventricular remodeling during heart failure.

A hallmark of maladaptive cardiac remodeling is the re-activation of fetal transcription factors [29,131], and RA signaling has been proven to modulate the activity of fetal transcription factors. For instance, ATRA (0.2 μM, 0.01 μM–0.1 μM) induced the expression and DNA binding of Gata4 [132,133], a key regulator of cardiomyocyte hypertrophy [134]. Additionally, vitamin A-deficient embryos exhibited the downregulation of Gata4 [55], suggesting that Gata4 is downstream of RA in both the embryonic and adult contexts. Importantly, the overexpression of Gata4 following MI induced angiogenesis and prevented adverse cardiac remodeling [135], suggesting that the upregulation of Gata4 by RA signaling could engage therapeutic transcriptional pathways. Similarly, Tbx5, a core cardiac transcription factor and the causative factor in Holt–Oram syndrome, cardiomyocyte differentiation, and formation of the interventricular septum was also downstream of RA signaling [56,136–138].

RA signaling was also reported to activate the transcription of Pitx2 in the embryo, another important developmental cardiac transcription factor [57]. Importantly, Pitx2 and Tbx5 were recently shown to regulate atrial rhythm [139], providing another potential mechanism by which activated RA signaling could modulate cardiovascular function.

The effects of RA signaling might also be via the modulation of downstream kinases. The anti-hypertrophic effects of ATRA (5 μM) in neonatal cardiomyocytes were reported to occur in conjunction with upregulation of expression of MAP kinase phosphatase-1 and 2, thereby inhibiting MAP kinase signaling [140]. ATRA (1 μM–4 μM) was also reported to activate AMP-activated protein kinases during the impediment of proliferation in mouse vascular SMCs [141]. Finally, ATRA (0.1 μM–1 μM) was reported to modulate plasminogen activator inhibitor 1 gene expression, and this was impeded by tyrosine kinase inhibitors [142]. Thus, beneficial effects of the activation of vitamin A/retinoid signaling may be accounted for by the regulation of neurohormonal signaling, developmental transcription factors, and downstream kinases.

## 9. Antioxidant and Metabolic Properties of Vitamin A/Retinoids

Some beneficial effects of vitamin A/retinoids may be due to their antioxidant properties or other changes to metabolism rather than interference with disease signaling pathways. Vitamin A/retinoids are well known antioxidants [143–146], and antioxidant properties of vitamin A given to rats (25 IU/kg/day) were reported to underlie its ability to protect the heart from doxorubicin-induced toxicity [147]. Similarly, antioxidant activity of endogenous vitamin A was increased in response to oxidative stress induced by doxorubicin, a common cancer treatment with dose-limiting cardiotoxicity [148]. Importantly, ATRA (10 mg/kg/day) attenuated the oxidative stress-induced apoptosis associated with myocardial ischemia/reperfusion injury. However, anti-apoptotic effects were more clearly linked to RA-driven upregulation of pro-survival signals at the transcriptional level, rather than direct antioxidant activity of vitamin A [149]. In another study, vitamin A deficiency led to an increased proportion of saturated fatty acids and phospholipids in the heart, in conjunction with an upregulation of peroxisome proliferator-activated receptor [150]. Furthermore, in conjunction with a cardiac-specific binding partner CERIP, RXR regulated transcription of the human MCAD gene, an important component of fatty acid oxidation in the mature heart [151]. Thus, vitamin A/retinoids might also exert beneficial effects, due to their antioxidant effects and the modulation of metabolic pathways.

## 10. Exogenous Retinoids or Retinoid Inhibition as Therapeutic Agents in Cardiovascular Diseases

Despite ample evidence for the role of RA signaling in diverse CVD pathologies, clinical trials examining the effects of β-carotene supplementation on CVDs have not shown any benefits [152–155]. However, such results are not necessarily in contrast to preclinical findings (especially in vitro), as β-carotene supplementation may not result in elevated RA signaling at a cellular or organ level, due to the multiple levels of control of active retinoid concentrations. Indeed, differences might exist between the systemic and local effects of retinoids in CVDs, and RA-containing formulations that are directly injected into the heart might represent a feasible therapeutic avenue. For instance, hyaluronan conjugated to RA and butyric acid enhanced the differentiation of PSCs and mesenchymal stem cells to spontaneously differentiating cardiomyocytes, and this led to myocardial repair in vivo [156–158]. Additionally, intramyocardial delivery of a hyaluronan mixed ester of butyric acid and RA was beneficial following MI, and led to the transcriptional upregulation of VEGF and the stem-cell marker KDR, potentially by increasing histone acetylation [159]. However, in a separate study intramyocardial injection of ATRA to the infarction zone (10 injections of 30 μL 3.3 μM) worsened cardiac function following MI, whereas intramyocardial injection of a novel inhibitor of ATRA uptake (5′-methoxyleoligin) was cardioprotective [160]. Interestingly, 5′-methoxyleoligin was also reported to promote arteriogenesis, and in vitro angiogenic effects were dependent on the transcriptional

upregulation of the ATRA-degrading enzyme Cyp26b1 [161]. It is unclear what underlies the discrepancy between these studies, though developmental findings indicating complex feedback networks governing RA signaling could provide clues. More careful optimization of dosing might be required to ensure adequate tissue levels and mirror anti-hypertrophic activity seen in vitro. Regardless, local administration of RA formulations, with or without the co-injection of stem cells, might serve to enhance therapeutic cardiac repair without inducing systemic effects characteristic of retinoid differentiation therapy. This would also avoid the need for mobilization, transport, and metabolism needed with β-carotene supplementation.

## 11. Conclusions

Though long known to affect the formation of the heart, studies in past decades have implicated vitamin A/retinoid signaling as a modulator of adult cardiac function, including a major role in the pathophysiology of responses to injury of the myocardium and arterial walls. This is supported by population studies implicating that both excesses and deficiencies of serum RA levels are associated with CVD dysfunction. The ability of modulators of RA signaling to control endogenous processes such as proliferation and differentiation hold promise for their use in the development of therapies for CVDs, though challenges regarding negative systemic effects will likely impede advancement in this area unless targeted formulations that can be delivered intramyocardially are further refined. Additionally, a better understanding of how Cyp26 and retinol dehydrogenases maintain concentrations of ATRA/9CRA in the diseased heart could allow for the targeted modulation of retinoid levels. Further basic research in these areas and into mechanisms by which RA signaling modulates classical disease pathways could shed light on the pathophysiology of CVDs and provide further opportunities for therapeutic modulation.

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