REVIEW

Vitamin A homeostasis and cardiometabolic disease in humans: lost in translation?

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Abstract

Vitamin A (retinol) is an essential, fat-soluble vitamin that plays critical roles in embryonic development, vision, immunity, and reproduction. Severe vitamin A deficiency results in profound embryonic dysgenesis, blindness, and infertility. The roles of bioactive vitamin A metabolites in regulating cell proliferation, cellular differentiation, and immune cell function form the basis of their clinical use in the treatment of dermatologic conditions and hematologic malignancies. Increasingly, vitamin A also has been recognized to play important roles in cardiometabolic health, including the regulation of adipogenesis, energy partitioning, and lipoprotein metabolism. While these roles are strongly supported by animal and in vitro studies, they remain poorly understood in human physiology and disease. This review briefly introduces vitamin A biology and presents the key preclinical data that have generated interest in vitamin A as a mediator of cardiometabolic health. The review also summarizes clinical studies performed to date, highlighting the limitations of many of these studies and the ongoing controversies in the field. Finally, additional perspectives are suggested that may help position vitamin A metabolism within a broader biological context and thereby contribute to enhanced understanding of vitamin A's complex roles in clinical cardiometabolic disease.

Introduction

Obesity and overnutrition continue to grow in global prevalence and constitute leading risk factors for nonalcoholic fatty liver disease (NAFLD), type 2 diabetes, cardiovascular disease, cancer, Alzheimer's disease, disability, and mortality (Blüher 2019). The etiology of obesity entails complex interactions among behavior, genetics, and environment. Enhanced understanding of the mechanisms underlying both progressive adiposity and its comorbidities promises novel interventional strategies to mitigate body weight gain and its sequelae (Blüher 2019). Vitamin A or retinol is a fat-soluble vitamin with long recognized roles in vision, growth, reproduction, and immunity. An expanding body of evidence further implicates vitamin A homeostasis in myriad metabolic functions, including adipogenesis, energy utilization, and lipid metabolism. Thus, delineating changes in vitamin A homeostasis that occur across a spectrum of cardiometabolic disorders may provide important insights into the evolution and possible prevention of cardiometabolic disease.

Elucidating the roles of vitamin A and its metabolites in cardiometabolic regulation is complicated by significant challenges, including differences between rodent and human vitamin A biology, the intricate pathways that regulate vitamin A homeostasis, and the highly tissue-specific nature of vitamin A metabolism and signaling.
The profile of retinoids found in rodents differs from that in humans, and certain species of retinoids, such as 13-cis-retinoic acid and 4-oxo-13-cis-retinoic acid, are present in humans but not in mice (Zhong et al. 2019). Furthermore, rodent models of obesity recapitulate specific characteristics of disease pathophysiology but fail to capture all pathogenic facets of obesity. Key limitations of rodent models for understanding human obesity include differences in pancreatic islet architecture between rodents and humans and the importance of variables including housing, environment, and handling as determinants of metabolic outcomes in rodents (Kleinert et al. 2018). Consequently, vitamin A-focused clinical research is essential for establishing the relevance of preclinical findings to human disease but largely has been limited to observational studies that measure circulating biomarkers. This review provides an initial introduction to vitamin A biology and briefly summarizes the preclinical data that have engendered interest in vitamin A as a focus of clinical research. Next, it presents key clinical studies performed to date and highlights inconsistencies and ongoing controversies in clinical and translational vitamin A-focused research. Finally, this review suggests additional perspectives and research questions that may help better elucidate the complex roles of vitamin A homeostasis in clinical cardiometabolic disorders.

**Vitamin A homeostasis**

Vitamin A is obtained exclusively through dietary sources, primarily in the form of carotenoids and retinyl esters, which can be delivered to peripheral tissues through chylomicron remodeling. Liver and adipose tissue constitute the primary sites of vitamin A storage, with ~80–85% and ~15–20%, respectively, of total retinyl ester and retinol stores in the body (Frey & Vogel 2011, Blaner 2019). Retinyl esters can be hydrolyzed to retinol and mobilized from the liver as retinol bound to retinol-binding protein 4 (RBP4), providing an additional source of retinol for other peripheral tissues. This retinol-RBP4 complex is designated holo-RBP4 in contrast to apo-RBP4 which is not bound to retinol. Holo-RBP4 circulates as a complex with transthyretin (TTR). TTR binding prevents glomerular filtration and clearance of RBP4, thereby increasing its circulating half-life (Blaner 2019). In the absence of its association with TTR, RBP4 is readily filtered in the glomeruli due to its small size (21 kDa), reabsorbed in the proximal tubule, and catabolized (Steinhoff et al. 2021).

Retinol uptake into tissues may be mediated via passive diffusion or active uptake through the receptor stimulated by retinoic acid 6 (STRA6) (Kawaguchi et al. 2012). STRA6 also has been shown to activate cellular signaling cascades (Berry et al. 2011), though this signaling role remains somewhat controversial (Blaner 2019). The interaction between retinol and STRA6 may require dissociation between RBP4 and TTR, and the directional regulation of retinol uptake via STRA6 is not well understood (Steinhoff et al. 2021). RBP4 also can transport retinol from other peripheral tissues to the liver, a process termed ‘reverse retinol transport’ (Steinhoff et al. 2021). Interestingly, both RBP4- and STRA6-deficient mice are viable and fertile, with severe impairment limited to vision (Berry et al. 2013, Shen et al. 2016). Maintenance of normal vitamin A homeostasis in other tissues is likely supported at least in part through delivery of β-carotene and retinyl esters via circulating apolipoprotein B-containing lipoproteins, as circulating retinol levels are undetectable in RBP4-deficient mice (Shen et al. 2016). In humans, rare null mutations in STRA6 yield strikingly heterogeneous phenotypes, from isolated anophthalmia or microphthalmia to pulmonary hypoplasia, cardiac malformation, and neonatal death (Pasutto et al. 2018).

In the liver, lecithin retinol acyltransferase (LRAT) mediates the esterification of retinol to retinyl esters for storage (Fig. 1). In other peripheral tissues, diacylglycerol acyltransferases rather than LRAT have been proposed to mediate retinol esterification. Active retinoids may be generated within tissues from either retinyl esters or retinol. Retinyl esters are converted by retinyl ester hydrolases to retinol, which can then be oxidized to retinaldehyde by retinol dehydrogenases (RDHs) or short chain dehydrogenases (DHRS). Retinaldehyde also can be generated from β-carotene via β-carotene-15,15'-monooxygenase activity (Blaner et al. 1994, Blaner 2019). Retinaldehyde, in turn, can be reduced to retinol by RDH and DHRS enzymes, enabling tight regulation of retinoid formation and signaling. Retinaldehyde itself can confer biological effects and plays a critical role in vision. Alternatively, retinaldehyde may undergo an additional oxidation step mediated by the aldehyde dehydrogenase 1A (ALDH1A) family of enzymes (Napoli 2012, Kedishvili 2016), also known as retinaldehyde dehydrogenases. This oxidation step generates all-trans-retinoic acid (atRA), the
principal bioactive metabolite of retinol. In addition to ALDH1A isoforms, aldehyde oxidase may contribute to atRA biosynthesis (Zhong et al. 2021). Other metabolites also can be generated downstream of retinol, including 13-cis-RA, 9-cis-RA, and oxidized metabolites of atRA. Similar to atRA, some of these metabolites act as ligands for retinoic acid receptor (RAR) nuclear receptor isoforms, whereas other vitamin A derivatives, including 9-cis-RA, preferentially signal through retinoid X receptor (RXR) isoforms and are designated as rexinoids rather than retinoids. Some data support signaling roles for retinoids through other members of the type 1 nuclear receptor superfamily to which RAR belongs (Weikum et al. 2018). Dihydo retinoids, for example, are retinol derivatives that have been shown to confer signaling effects through PPAR isoforms (Moise et al. 2009). Still other metabolites are considered biologically inert.

Importantly, the specific enzymes contributing to retinoid metabolism vary across tissues and cell types (Arnold et al. 2015a,b, Czuba et al. 2021) and have not been fully defined in human tissues. Only recently, for example, were the key atRA-synthesizing enzymes identified within human liver (Zhong et al. 2021) and human omental and s.c. adipose tissue (Rubinow et al. 2022). Notably, while 13-cis-RA was readily quantifiable in human liver, it has not been detected in mouse liver (Zhong et al. 2019). The question of which bioactive retinoids are relevant to human biology is further illustrated by continued uncertainty regarding
the generation of 9-cis-RA in human tissues (Blaner 2019). RBP4 has received substantial attention in both preclinical and clinical studies and constitutes another area of controversy in vitamin A metabolism. Beyond its essential role as a retinol transport protein, RBP4’s potential role as a signaling molecule remains uncertain, and the tissues that contribute to circulating RBP4 levels in humans have yet to be definitively determined.

**Vitamin A and RBP4 in cardiometabolic regulation: preclinical data**

Preclinical studies have demonstrated roles for vitamin A and its derivatives as well as RBP4 in numerous facets of cardiometabolic regulation, including adipose tissue biology, energy utilization and glucose homeostasis, hepatic steatosis, and atherosclerosis. A role for atRA in the regulation of adipocyte differentiation has been long established, and extensive work has been dedicated to delineating the pathways that underlie the time- and context-dependent nature of this regulation (Xue et al. 1996, Berry et al. 2012, Abd Eldaim et al. 2017) (Fig. 2). Preclinical research further has implicated atRA in the regulation of UCP-1 expression and adipose tissue browning; however, some evidence suggests that this regulation may be specific to rodents and not evident in humans, underscoring the importance of verifying preclinical data in clinical research studies (Blaner 2019). In hepatocytes, atRA has been shown in vitro to promote fatty acid β-oxidation and mitochondrial biogenesis (Bonet et al. 2012).

Collectively, rodent models have supported the premise that adequate but not excessive atRA exposure is essential for the maintenance of metabolic health, though studies have not yielded uniform findings (Fig. 3). Modest decreases in endogenously produced atRA resulted in derangements in lipid metabolism, increased adiposity, and body weight gain in both male and female mice (Yang et al. 2018). Inversely, administration of atRA to healthy mice resulted in upregulation of hepatic genes involved in fatty acid β-oxidation and downregulation of those involved in lipogenesis, and reduced triglyceride content was found in the livers of treated animals (Amengual et al. 2010). Notably, however, a recent study demonstrated that systemic atRA administration worsened glucose homeostasis in rats, and atRA inhibited glucose-stimulated insulin secretion in cultured pancreatic β-cells via an RXR-mediated pathway (Yang et al. 2022). A diet supplemented with vitamin A also exacerbated body weight gain and visceral adiposity in WNIN-Gr-Ob rats (Jeyakumar et al. 2015). These variable results have been attributed in part to the different genetic backgrounds of the animals studied (Blaner 2019) and underscore the limitations of preclinical data for understanding human biology.

The possible roles of RBP4 as a glucoregulatory signal also have received considerable attention. Interest in RBP4 as a metabolic mediator heightened consequent to findings that RBP4 overexpression or exogenous RBP4 treatment worsened insulin resistance in male mice (Yang et al. 2005), a phenotype later ascribed in part to the immunomodulatory effects of RBP4 (Norseen et al. 2012, Moraes-Vieira et al. 2020). Retinol-RBP4 interaction
with STRA6 in cultured adipocytes also was found to mediate downstream signaling effects that blunted insulin response (Berry et al. 2011). Nonetheless, in a subsequent, elegant study, RBP4 overexpression resulted in a wholly unaltered metabolic phenotype, either in the setting of a regular chow diet or high-fat feeding (Fedders et al. 2018). Importantly, this study employed a model of liver-specific RBP4 overexpression and achieved ~two-fold elevation in circulating RBP4 concentration, comparable to the elevation that has been observed in individuals with obesity (Yang et al. 2005, Fedders et al. 2018). As the authors highlighted, this study design therefore may have more physiologic and clinical relevance than the prior study, which entailed RBP overexpression in skeletal muscle (Yang et al. 2005). Preclinical data also have suggested sex differences in the cardiometabolic effects of vitamin A signaling, but these have been noted inconsistently, and many studies are limited to male mice (Blaner 2019). Importantly, too, intrinsic differences in metabolic regulation between rodents and humans have been well established, including central facets of physiology in which vitamin A signaling has been implicated, such as hepatic lipid and systemic lipoprotein metabolism (Sari et al. 2020). Therefore, dedicated clinical studies are essential for delineating the roles of vitamin A homeostasis in human cardiometabolic disease.

**Vitamin A and RBP4 in cardiometabolic regulation: clinical data**

In contrast to animal studies, which primarily have demonstrated metabolically protective roles for all-trans-retinoic acid in rodent studies (Gerber & Erdman 1980, Berry & Noy 2009, Krupková et al. 2009, Ström et al. 2009, Manolescu et al. 2010), the clinical use of vitamin A derivatives at doses either lower than or comparable to those used in rodent studies confers clear metabolic risk (Blaner 2019). These derivatives include endogenously produced mediators [tretinoin (atRA), isotretinoin (13-cis-RA), altretinoin (9-cis-RA)] as well as synthetic retinoids and are used for the treatment of dermatologic conditions and certain hematologic malignancies. Roughly 30% of patients who receive this class of medications exhibit increases in plasma concentrations of triglycerides and cholesterol, and rare patients develop chylomicronemia syndrome with severe triglyceride elevations, acute pancreatitis, and eruptive xanthomas (Klöer et al. 2011). This risk has been ascribed to variable mechanisms including increased...
hepatic lipogenesis, increased VLDL secretion, and diminished lipoprotein lipase activity, but the individual susceptibility to these lipid derangements is not well understood (Klöör et al. 2011). Those patients who exhibited elevations in plasma triglyceride concentrations also were found to have higher risk of increased central adiposity, hypercholesterolemia, and hyperinsulinemia during treatment (Rodondi et al. 2002). These observational data in humans underscore the importance of dedicated clinical and translational research in this field, particularly as therapies targeting vitamin A metabolism have been proposed as novel treatments for obesity and metabolic disease (Haenisch et al. 2018). An excellent, systematic review of clinical studies investigating the relationship between vitamin A and cardiometabolic disease has been published recently (Olsen & Blomhoff 2020). Rather than provide a comprehensive review, this section will present the overall scope of work performed to date and highlight the limited prospective and interventional data in the field.

Most clinical studies of vitamin A have entailed cross-sectional analyses examining the relationship between circulating RBP4 concentrations and various cardiometabolic parameters and endpoints. Positive correlations have been found between circulating RBP4 concentration and BMI, metrics of impaired glucose homeostasis, and visceral adiposity, though the presence of these correlations has not been a universal finding (Blaner 2019, Olsen & Blomhoff 2020). Circulating RBP4 concentrations consistently have been found to decrease after metabolic surgery, although these changes in RBP4 concentration variably have been shown to associate with changes in insulin sensitivity, visceral adiposity, or circulating triglyceride concentration (Haider et al. 2007, Tschoner et al. 2008, Broch et al. 2010). Observational studies examining the prospective relationship between circulating RBP4 concentration and insulin resistance, metabolic syndrome, or incident type 2 diabetes have yielded inconsistent findings (Olsen & Blomhoff 2020). One prospective study found an elevated risk of cardiovascular events among participants in the highest quartile of serum RBP4 concentration (Liu et al. 2017), although, as noted in a previous, systematic review, these data were based on a method of RBP4 quantitation that is not widely accepted (Blaner 2019). These cross-sectional studies are limited in part by the frequent co-occurrence of diabetes, obesity, cardiovascular disease, and impaired kidney function. Further, most of these studies fail to account for additional, potential confounders including diet, age, and weight stability, underscoring the need for interventional studies with comprehensive phenotyping of participants.

Interventional data are limited, but in one interventional study, reductions in serum RBP4 concentration correlated with increases in insulin sensitivity after implementation of an exercise program in individuals with impaired glucose tolerance or type 2 diabetes (Graham et al. 2006). However, this correlation was found in post hoc analyses after no change in serum RBP4 concentration was evident across the entire intervention cohort. Only a few studies have examined changes in RBP4 concentration consequent to pharmacological intervention. Sibutramine treatment led to body weight loss and reductions in plasma RBP4 concentration, but comparable reductions in RBP4 were also evident in the placebo group after 1 year of treatment, albeit on a slightly delayed time course (Derosa et al. 2010). In another study, participants with type 2 diabetes who received pioglitazone for 6 months, and serum RBP4 concentration declined in concert with reductions in visceral adiposity and improved insulin sensitivity; notably, however, this small study had no placebo group (Jia et al. 2007). In contrast, participants with type 2 diabetes who received rosiglitazone for 6 months exhibited no change in serum RBP4 concentration, whereas significant reductions in HbA1c, insulin resistance, and serum triglyceride concentration were seen (Takebayashi et al. 2007). Similarly, 6 months of metformin therapy did not alter serum RBP4 concentration in women with polycystic ovary syndrome despite conferring significant improvements in insulin resistance (Hutchison et al. 2008).

Far fewer studies have examined other metrics of vitamin A homeostasis in relation to cardiometabolic disorders. One study found inverse correlations between serum retinol concentration and both BMI and NALFD-associated serum transaminases (Botella-Carretero et al. 2010), whereas another study determined that serum retinol concentration above the clinical reference range conferred an odds ratio of ~2 for metabolic syndrome (Kanagasabai et al. 2019). Inconsistent results have been found with regard to the relationship between circulating retinol concentration and metrics of glucose homeostasis (Tavridou et al. 1997, Abahusain et al. 1999, Higuchi et al. 2015). Still other studies have examined the relationship between metabolic endpoints and ratios between vitamin A-based metrics, including the ratio of apo-RBP4 to holo-RBP4 (Mills et al. 2008, Kim et al. 2017); however, the biological significance of these ratios is unclear, and measures of holo- and apo-RBP4 have been relative rather than absolute due to lack of available quantitative methods. Finally, observational studies have examined the
relationships between cardiovascular and cerebrovascular risk and circulating concentrations of retinol and RA, though reaching generalized conclusions from these data is hindered by the discrepant study design and cardiovascular endpoints examined across studies (Olsen & Blomhoff 2020). In a large, interventional study designed to test whether vitamin A could reduce lung cancer incidence in a high-risk population, treatment with β-carotene and high-dose vitamin A led to increased cardiovascular and all-cause mortality (Ommen et al. 1996).

In addition to predominantly observational and cross-sectional study design, significant limitations to these data include measurement solely of circulating vitamin A-based metrics and the absence of highly specific and sensitive methods for RBP4 quantitation, as has been highlighted in prior reviews (Blaner 2019, Olsen & Blomhoff 2020). The impact of reduced kidney function in participants across these studies also remains uncertain, as RBP4 is filtered and reabsorbed in the proximal tubule, and significant differences in circulating RBP4, retinol, and aRRA concentrations are evident in participants with chronic kidney disease (Jing et al. 2016, Steinhoff et al. 2021). In addition, differentially cleaved forms of RBP4 have been identified in individuals with impaired kidney function (Frey et al. 2008). The functional relevance of these different RBP4 isoforms and other potential protein modifications has yet to be determined (Steinhoff et al. 2021). The respective effects of diet, day-to-day variability, circadian regulation, and fasted vs postprandial state on metrics of vitamin A homeostasis also are unknown. Finally, the relationships between circulating and tissue-specific vitamin A analytes are yet to be defined in humans, underscoring the need for clinical data with an integrative approach that positions these metrics within a framework of systemic vitamin A metabolism and tissue-specific metabolic regulation.

Alternative and future directions in clinical research

Systemic vitamin A metabolism

A few more mechanistic, translational studies have been performed to better elucidate possible changes in tissue-specific vitamin A signaling in the context of metabolic dysregulation. In human adipose tissue explants, aRRA and 9-cis-RA were shown to induce the expression of pyruvate dehydrogenase kinase 4 (PDK4), an enzyme that shifts cellular energy metabolism away from glucose utilization through the phosphorylation and consequent inhibition of the pyruvate dehydrogenase complex (Distel et al. 2017). These data provide important insight into animal studies evaluating the role of RBP4 in metabolic disease, as models of RBP4 overexpression that result in the unregulated delivery of retinol to metabolic tissues, or its sequestration within these tissues, could yield changes in glucose utilization that may not occur under physiological conditions. Muscle-specific overexpression of RBP4, for example, led to increased concentrations of retinol and retinyl esters within skeletal muscle, which is not normally a site of retinol storage, but this was not evident with liver-specific RBP4 overexpression (Quadro et al. 2002, Fedders et al. 2018). As highlighted above, the tissue source of RBP4 therefore could account for the highly discrepant findings between rodent studies that employed muscle-specific and liver-specific models of RBP4 overexpression (Fedders et al. 2018). The tissue sources of circulating RBP4 under physiological conditions remain incompletely defined; although skeletal muscle- and adipocyte-specific RBP4 overexpression have been shown to affect circulating RBP4 concentrations in rodents (Quadro et al. 2002, Lee et al. 2016), RBP4 is primarily considered an hepatokine (Steinhoff et al. 2021), underscoring the importance of understanding the regulation and tissue sources of secreted RBP4 specifically in human physiology.

Few studies have measured concentrations of vitamin A-based metrics within human metabolic tissues. Such translational studies have shown higher mRNA expression of RBP4 and ALDH1A1 in adipose tissue from participants with obesity relative to normal weight controls (Klöting et al. 2007, Landrier et al. 2017). Recently, the formation velocity of aRRA and the key enzymes responsible for aRRA biosynthesis were shown to differ between human s.c. and omental adipose tissue (Rubinow et al. 2022). In another study, lower hepatic concentrations of retinol and retinyl esters and diminished RARβ expression were found to be associated with NAFLD severity (Trasinò et al. 2015). These data collectively support the premise that vitamin A homeostasis plays metabolic roles in humans and is dynamically regulated in states of metabolic disease. They further underscore the tissue-specific regulation of vitamin A metabolism and the attendant need to define vitamin A transport, metabolism, and signaling at the tissue level in the context of both metabolic health and disease.

Protein–protein interactions: transthyretin

One facet of vitamin A biology that is almost entirely absent in the clinical literature is the hepatic co-secretion of RBP4 and retinol with TTR. The phenomenon of RBP4-TTR co-secretion is supported by in vitro data in human
HepG2 cells demonstrating that RBP4 and TTR form a complex intracellularly on the endoplasmic reticulum prior to secretion (Belovino et al. 1996). However, RBP4-TTR co-secretion has not been shown in vivo, and both preclinical and clinical evidence demonstrates that the secretion of these proteins may be dissociated. In TTR-deficient mice, plasma RBP4 concentrations are markedly reduced but not ablated (Wei et al. 1995, Zemany et al. 2015). While these low circulating RBP4 concentrations could reflect accelerated clearance in the absence of TTR binding (Liz et al. 2010), accrual of RBP4 protein in the liver of TTR-deficient mice suggests that hepatic RBP4 secretion may be partially contingent on the presence of TTR (Wei et al. 1995). In a case study of two patients with a homozygous splice site variant in RBP4, serum TTR concentrations were comparable to those in unaffected individuals despite undetectable serum concentrations of RBP4 (Cukras et al. 2012). Nor were serum TTR concentrations altered in RBP4 knockout mice (Quadro et al. 1999). Collectively, these data suggest that although the co-secretion of RBP4 and TTR is supported by in vitro evidence, these proteins may be secreted independently of one another, and the extent to which their association in plasma reflects co-secretion vs interaction in extracellular fluids is unknown. These data further suggest that RBP4 secretion may be more dependent on TTR secretion than the inverse, a model that is consistent with the fact that TTR circulates at higher concentrations than does RBP4 in human serum (Jing et al. 2016).

Association with TTR prolongs the circulating half-life of RBP4 (Steinhoff et al. 2021), but the interaction between these proteins may have broader implications, as structural protein data indicate that these proteins may play critical roles in regulating the biological effects of one another. This is illustrated, as noted above, by the fact that TTR likely needs to dissociate from RBP4 to enable STRA6-retinol interaction. RBP4, in turn, may regulate TTR function, as TTR has a serine protease domain that is prevented from interacting with substrate due to the presence of RBP4; this ‘silencing’ effect has been posited as a potential mechanism whereby RBP4-TTR interactions inhibit TTR frommediating apolipoprotein A1 dysfunction through its proteolytic activity (Sharma et al. 2019). RBP4 binding to TTR also has been shown to stabilize the TTR tetramer in vitro and thereby help prevent TTR from forming amyloid fibrils. The aberrant formation and tissue deposition of these amyloid fibrils can lead to various forms of amyloidosis which have been linked to over 100 point mutations in TTR (Liz et al. 2010, Koch et al. 2017). Notably, decreased circulating RBP4 concentrations were observed in individuals with inherited but not acquired forms of TTR-associated cardiac amyloid disease (Koch et al. 2017). Individuals with inherited cardiac amyloid disease also exhibited greater RBP4 protein in cardiac tissue than did those with acquired disease.

The close association of RBP4 and TTR under physiologic conditions may help explain some of the seemingly surprising or discordant findings in the preclinical literature, particularly in models of exogenous treatment with RBP4 or RBP4 overexpression in a non-hepatic tissue. RBP4 is a lipid transfer protein, a class of molecules that shares pleiotropic functions including the potential to confer plasma membrane reorganization (Chiapparino et al. 2016). This potential role of RBP4 is strongly suggested by in vitro data demonstrating TLR4-mediated signaling effects downstream of RBP4 that are contingent on shifts in lipid rafts (Norseen et al. 2012, Liu et al. 2017). Importantly, however, functional domains in lipid transfer proteins often localize to the lid of the lipid-containing barrel (Chiapparino et al. 2016); in the case of RBP4, this domain is covered by TTR (Naylor & Newcomer 1999). Accordingly, experimental protocols that result in circulating or tissue concentrations of RBP4 that far exceed the physiological range – particularly when a non-hepatic source of RBP4 is predominant – could elicit effects that do not occur under physiological conditions. Indeed, when RBP4 dissociates from TTR, it is rapidly cleared through renal filtration and catabolism (Steinhoff et al. 2021). This interpretation of preclinical data also is consistent with the fact that RBP4-mediated signaling effects through TLR4 were evident irrespective of RBP4’s association with retinol and independent of STRA6 (Norseen et al. 2012, Liu et al. 2017, Moraes-Vieira et al. 2020). In contrast, when RBP4 was overexpressed in the liver, likely preserving RBP4 co-secretion with TTR, no metabolic derangements were evident in mice despite comprehensive metabolic phenotyping (Fedders et al. 2018). This understanding highlights that under physiological conditions, lipid transfer protein function is tightly controlled through both spatiotemporal regulation and association with other molecules; experimental conditions that are devoid of this regulatory context therefore may elicit functions that are unlikely to occur under physiologic conditions, raising the distinction between protein-mediated functions that are physiologically relevant and those that may be provoked by an experimental model. Finally, both TTR and RBP4 are acute phase reactants (Qian et al. 1995, Langouche et al. 2009), and their secretion falls under a wide spectrum of physiological stressors including undernutrition and infection, adding yet another layer of complexity for understanding and interpreting changes in circulating...
RBP4 and retinol concentrations. In rats, somewhat discrepant time courses for circulating RBP4 and TTR concentrations were observed after systemic exposure to endotoxin (Rosales et al. 1996), again highlighting the potential dissociation between RBP4 and TTR secretion. The molar ratio of RBP4 and TTR has been examined as a measure of vitamin A deficiency during infection, with inconsistent findings across studies (Rosales & Ross 1998, Donnen et al. 2001). In response to systemic infection and reduced circulating concentrations of RBP4, retinol alternatively may bind to serum amyloid A (Derebe et al. 2014). These findings demonstrate the importance of biological context for regulating the secretion and possibly the functions of both RBP4 and TTR, supporting the need for careful investigation of the biological roles of these proteins across physiological states.

**Interface with other biological pathways: thyroid hormone**

The complexation of RBP4 and TTR further underscores the complex interplay between systemic vitamin A metabolism and other biological pathways, including thyroid hormone signaling, as TTR is also a transport protein for both thyroxine and triiodothyronine (Fig. 4). TTR transports ~15% of circulating thyroxine molecules, and thyroxine-binding stabilizes the TTR homotetramer (Liz et al. 2010). TTR has two thyroxine binding sites but binds only one thyroxine molecule due to negative cooperativity (Liz et al. 2010). Echoing this spatial co-localization, vitamin A and thyroid hormone have shared implications in a broad range of biological functions, including growth and development, cellular differentiation, fertility, immunity, and energy metabolism (Zhang & Kahl 1993). The canonical receptors for atRA and thyroid hormone – RARs and TRs, respectively – are in the same nuclear receptor family, are structurally similar, and form heterodimers primarily with RXR isomers (Zhang & Kahl 1993, Weikum et al. 2018). Interestingly, RAR-RXR and TR-RXR heterodimers form and can bind DNA response elements in the absence of ligand, acting as transcriptional repressors (Zhang & Kahl 1993). Consequently, both the presence and absence of ligand exert biological effects. While most vitamin A derivatives signal through RAR-RXR heterodimers, certain metabolites preferentially signal through RXR homodimers, including 9-cis-RA. In vitro, exposure to 9-cis-RA leads to RXR homodimerization, DNA binding, and transcriptional activation at a specific subset of genes with RAR response elements. This RXR homodimerization also led to a relative depletion of RXR monomers that was associated with reduced triiodothyronine-mediated transcription (Zhang & Kahl 1993). Although the presence of 9-cis-RA in human tissues is controversial (Blaner 2019), these findings illustrate the principal of close interaction between vitamin A and thyroid hormone signaling, an interface that remains poorly understood but may prove essential.
for comprehensive understanding of the roles of vitamin A in clinical cardiometabolic disease (Steinhoff et al. 2021). Further highlighting this interaction, triiodothyronine was shown to regulate the mRNA expression of genes involved in both atRA synthesis and metabolism in cerebrocortical cells (Gil-Ibáñez et al. 2014). Providing clinical evidence of vitamin A-thyroid hormone crosstalk, the vitamin A derivative and RXR agonist bexarotene, used for treatment of cutaneous T cell lymphoma, can induce a state of severe central hypothyroidism that requires unusually high doses of thyroid hormone replacement to confer a euthyroid state (Sherman 2003). This side effect has been ascribed to bexarotene-induced suppression of TSH, but the mechanisms underlying the need for high-dose thyroid hormone replacement are not well understood (Sherman 2003). Thus, understanding vitamin A-thyroid hormone crosstalk at the cellular level offers an additional, plausible explanation for reduced tissue thyroid hormone signaling in the setting of bexarotene treatment with attendant need for unusually high doses of thyroid hormone replacement. These collective findings demonstrate that an integrative approach – one that positions vitamin A homeostasis within a broader framework of biological signals and pathways – will be an essential facet of future clinical and translational research.

**Summary and conclusions**

Clinical studies focused on vitamin A homeostasis and cardiometabolic disease have yielded inconsistent results to date, reflecting in part variable study designs, heterogeneous clinical populations, and a lack of optimized and standardized methods for quantitation of vitamin A derivatives and RBP4 (Blaner 2019, Olsen & Blomhoff 2020). These studies have largely been observational and epidemiologic in nature with a predominant focus on RBP4, and even those studies with positive findings have shown conflicting correlations between RBP4 and insulin resistance, impaired glucose tolerance, visceral adiposity, BMI, or circulating triglyceride concentrations. These collective data argue against a simple, causal relationship between RBP4 and any of these metabolic parameters and suggest that further epidemiologic studies are unlikely to yield incremental insight into the complex biology of vitamin A homeostasis. They also highlight significant gaps in knowledge of the basic regulation of vitamin A homeostasis in humans. For example, animal studies have demonstrated both sex differences and sex steroid-mediated effects in vitamin A homeostasis that have yet to be addressed systematically in humans (Blaner 2019). Moreover, while preclinical data highlight that transcription and translation of RBP4 in the liver are differentially regulated in fed and fasted states (Steinhoff et al. 2021), the possibility of circadian or postprandial regulation of vitamin A metabolism and RBP4 secretion has yet to be explored in clinical studies. These constitute important areas for future vitamin A-focused clinical research and underscore that the key enzymes and regulatory pathways implicated in vitamin A metabolism are still being defined for human metabolic tissues.

The discord in both preclinical and clinical findings to date may derive, in part, from a conceptual model that frames vitamin A metabolites and RBP4 as either protective or detrimental with regard to cardiometabolic risk. An alternative model that employs energy status as a physiological context may prove helpful for better understanding the regulation of systemic vitamin A metabolism, changes in vitamin A homeostasis that occur with metabolic dysregulation, and the interactions between vitamin A metabolism and other nutrient-sensing pathways. This alternative model emphasizes the importance of defining changes in vitamin A metabolism across states of starvation, fasting, feeding, and overnutrition and provides conceptual space for the interface between vitamin A and other cardiometabolic mediators, including thyroid hormone. This model is further supported by data showing interplay between atRA and FOXO1 signaling in the regulation of hepatic glucose metabolism (Shin et al. 2012), suggesting that atRA may help coordinate the metabolic switch between fed and fasted states. Accordingly, whereas atRA confers the seemingly conflicting effects of suppressing hepatic lipogenesis (good) but blunting glucose-stimulated insulin secretion (bad) when viewed in a binary model predicated on beneficial and harmful actions, these effects are both adaptive if viewed within the context of a fasted state. This alternative framework thus defines vitamin A derivatives as mediators within exquisitely calibrated, adaptive pathways rather than as cardiometabolic heroes and villains.

An extensive body of evidence supports vitamin A homeostasis as a central facet of cardiometabolic regulation. Mechanistic, translational, and integrative studies will be essential to (1) fully elucidate the regulation of vitamin A metabolism within human metabolic tissues, (2) delineate the changes in vitamin A homeostasis that occur across a spectrum of physiological states and cardiometabolic disorders, and (3) interrogate the interface.
between vitamin A homeostasis and other key metabolic pathways.

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