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Review

Retinol-binding protein 4 in obesity and metabolic dysfunctions

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ABSTRACT

Excessive increased adipose tissue mass in obesity is associated with numerous co-morbid disorders including increased risk of type 2 diabetes, fatty liver disease, hypertension, dyslipidemia, cardiovascular diseases, dementia, airway disease and some cancers. The causal mechanisms explaining these associations are not fully understood. Adipose tissue is an active endocrine organ that secretes many adipokines, cytokines and releases metabolites. These biomolecules referred to as adipocytokines play a significant role in the regulation of whole-body energy homeostasis and metabolism by influencing and altering target tissues function. Understanding the mechanisms of adipocytokine actions represents a hot topic in obesity research. Among several secreted bioactive signalling molecules from adipose tissue and liver, retinol-binding protein 4 (RBP4) has been associated with systemic insulin resistance, dyslipidemia, type 2 diabetes and other metabolic diseases. Here, we aim to review and discuss the current knowledge on RBP4 with a focus on its role in the pathogenesis of obesity comorbid diseases.

1. Introduction

White adipose tissue is an active secretory organ, composed of mature adipocytes and preadipocytes, as well as several other cell types such as immune cells (e.g. macrophages, neutrophils, lymphocytes), mesenchymal and endothelial cells (Lenz et al., 2020; Corvera, 2021; Sun et al., 2020; Andersson et al., 2017; Wang et al., 2013; Sárvári et al., 2021). Adipocytes represent approximately 80–90% of adipose total volume (Corvera, 2021), with the principal function to store triglycerides in unilocular lipid droplets (Lee et al., 2013) and release it on demand. In addition to their role in lipids storage, adipocytes secrete adipokines (Fain et al., 2004; Lehr et al., 2012a) which confer adipose tissue as an active endocrine organ (Blüher, 2012a; Lehr et al., 2012b) (Fig. 1). Adipokines are bioactive signalling molecules influencing the tissue metabolism and function through their autocrine, paracrine, or endocrine actions on different cells and organs (e.g. brain, liver, muscle, adipose tissue, pancreas) (Pandžić and Grizelj, 2016; Friedman, 2019; Weschenfelder et al., 2020; Parrettini et al., 2020). The endocrine function of adipose tissue is not only exerted via adipocytes' production of adipokines but also through up to 90% of cytokine secretion from non-adipocytes, principally immune cells (Fain et al., 2004; Lehr et al., 2012a, 2012b; Blüher, 2012a; Kershaw and Flier, 2004). Therefore, immune cells play a central role in adipose tissue biology, especially during adipose tissue expansion and/or reduction (Dalmas et al., 2011a). Macrophages are the most abundant and functionally dominant cell type among adipose tissue immune cells and increase in number during obesity development (Dalmas et al., 2011b, 2015; Liu et al., 2016a). Notably, macrophage phenotype varies with the physiological or pathological state of adipose tissue function (Dalmas et al., 2011b, 2015). Indeed, activated M2 macrophages (most abundant in "normal" states) produce anti-inflammatory cytokines and contribute to tissue homeostasis and repair (Fig. 1) (Anderson et al., 2010). In contrast, M1 macrophages differentiate from blood monocytes and predominantly release pro-inflammatory cytokines, sustaining a chronic low-grade inflammatory state and impair insulin signalling in obesity (Aron-Wisnewsky et al., 2009). Macrophages are also involved in other adipose tissue functions such as preadipocyte differentiation, adipogenesis, and angiogenesis (Pandžić and Grizelj, 2016; Liu et al., 2016a; Bourlier et al., 2008). In summary, different cell types within adipose tissue contribute to inter-organ cross-talk through the secretion of adipokines, the release of metabolites and migrating cells.

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2. Adipocytokines

The discoveries that adipose tissue is an endocrine organ led to a paradigm shift of the role of adipose tissue, now considered as a central organ in the regulation of whole-body energy homeostasis and metabolism (Zhang et al., 1994; Straub and Scherer, 2019; Scherer et al., 1995a). Under conditions of excess lipid accumulation as observed in obesity and other metabolic dysfunctions, adipokine secretion pattern shifts towards a pro-inflammatory, athero- and diabetogenic pattern (Friedman, 2009; Dommel and Bluher, 2021; Blüher et al., 2012). This highlights the involvement of adipose tissue in the development of several metabolic diseases, which could be attributed to its secretory function. Indeed, hundreds of active biomolecules are produced and secreted from several cell types of adipose tissue, generally referred to as adipocytokines (Lee et al., 2013; Lehr et al., 2012a; Luo and Liu, 2016; Song et al., 2018; Dahlman et al., 2012). Although the function of many of these active biomolecules is not fully understood, adipocytokines can modulate systemic metabolism and inflammation (Lehr et al., 2012b). Indeed, through endocrine mechanisms, adipocytokines transmit information to other metabolically active tissues (Maury and Brichard, 2010; Scherer, 2006; Parimisetty et al., 2016). Depending on their cellular origin and secretory pathways, adipose-derived biomolecules can be subdivided into different categories including adipokines, cytokines, lipids, prostaglandins, complement components and others (Kershaw and Flier, 2004). Adipokines include among others leptin, adiponectin, resistin, chemerin, serum amyloid A (SAA), and retinol-binding protein 4 (RBP-4) (Luo and Liu, 2016; Blüher, 2013a). Cytokines are another group of secreted factors that consists of biomolecules mainly secreted by adipose tissue immune and endothelial cells of the stromal vascular fraction (SVF) and which include omentin, visfatin, resistin, apelin, plasminogen activator inhibitor 1 (PAI-1), monocyte chemoattractant protein 1 (MCP-1), tumour necrosis factor-alpha (TNFα), macrophage migration inhibitory factor (MIF) and interleukins (e.g IL-1, IL-6, IL-8, IL-10), transforming growth factor β (TGFβ), interferon-γ (IFNγ), C-reactive protein (CRP) (Blüher, 2012a, 2013a). Adipose tissue also secretes lipids such as palmitoleate and fatty acid esters of hydroxy fatty acids (FAHFAs) that regulate systemic glucose and lipid metabolism (Song et al., 2018).

Adipocytokines are involved in numerous metabolic pathways, contributing to the regulation of appetite, energy expenditure, activity, fat distribution, adipocyte metabolism and function, regulation of adipogenesis, migration of immune cell into adipose tissue and inflammation (tissue and systemic) (Blüher, 2009a, 2012a). Adipocytokines also affect β-cell function, liver and muscle metabolisms, thereby regulating energy metabolism and whole-body insulin sensitivity (Fig. 1) (Blüher, 2009a, 2012a). Adipocytokines may exert their effects on target cells by binding to their receptors which trigger cascades of intracellular signalling pathways (Blüher, 2013a). However, in obese states, adipocytokine production and secretion can be dysregulated, contributing to the pathogenesis of metabolic, cardiovascular, inflammatory and other malignant disorders (Fig. 1) (Van Gaal et al., 2006; Kiernan and MacIver, 2020; Recinella et al., 2020; Pham and Park, 2021).

Genetic and environmental interactions (in addition to behavioural factors) may alter adipose tissue function by initiating a sequence of adverse mechanisms such as adipocyte hypertrophy, hypoxia, several stresses, dysregulation of adipokine secretion, and inflammatory processes (Blüher, 2013a). Dysregulation in adipocytokine secretion can be considered as a symptom of adipose tissue dysfunction. This may lead to unfavourable adipose tissue accumulation, distribution and function, ectopic fat deposition, impairment of insulin sensitivity or systemic and tissue inflammation (Blüher, 2012a). These adverse events may mechanistically link obesity to the development of metabolic disorders such as type 2 diabetes (T2D), fatty liver and cardiovascular diseases (CVDs) (Fig. 1).

The etiological importance of adipose-derived active biomolecules in the pathogenesis of metabolic and CVDs was demonstrated for several adipokines (Kershaw and Flier, 2004). For instance, the role of the adipokines leptin, adiponectin, resistin, and visfatin as mediators regulating energy homeostasis and linking increased fat mass and/or impaired adipose tissue function to metabolic and CVDs has been intensively investigated (Zhang et al., 1994; Scherer et al., 1995b; Bluher and Mantzoros, 2015). Moreover, the role of cytokines such as TNFα, IL-6, IL-8, IL-10, omentin, MCP-1, PAI-1, chemerin (Chakaroun et al., 2012), apelin (Krist et al., 2013), in the development of

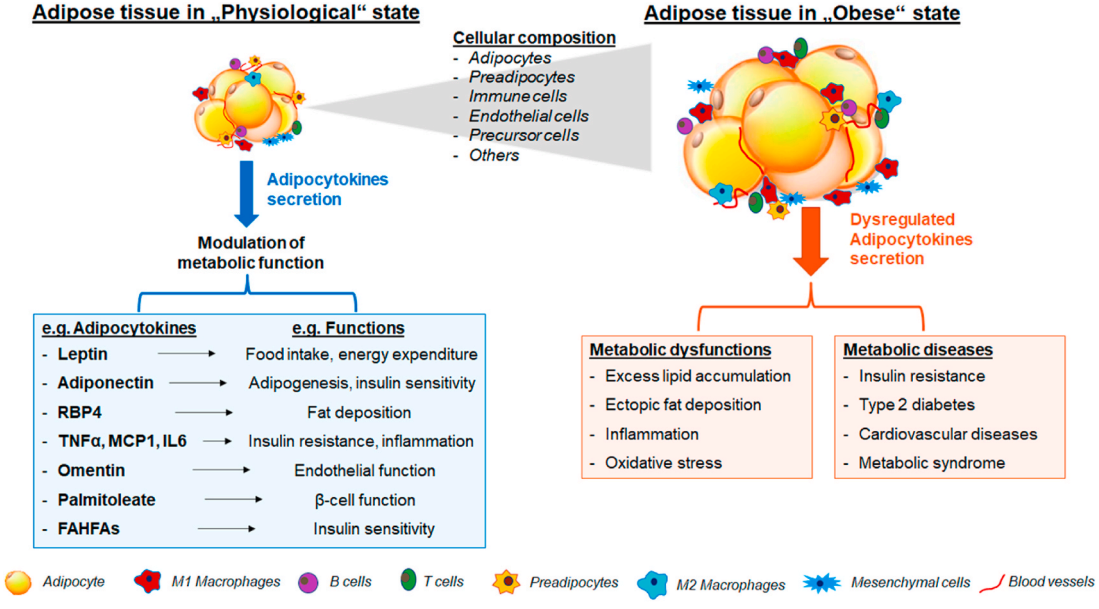

Fig. 1. Adipose tissue is an active endocrine organ. *Adipose tissue is constituted of several cell types, altogether producing and secreting hundreds of bioactive molecules referred to as adipocytokines. Through adipocytokine secretion, adipose tissue can modulate whole-body homeostasis* via *paracrine, autocrine, or endocrine signalling pathways. Alteration in adipocytokine secretory pattern may lead to several metabolic consequences contributing to the development of obesity-associated comorbidities. Abbreviations: FAHFAs: fatty acid esters of hydroxy fatty acids; RBP4: retinol-binding protein 4; TNFα, tumour necrosis factor-alpha; IL6, interleukin 6; PAI-1, plasminogen activator inhibitor 1; MCP1, monocyte chemoattractant protein 1.*

obesity-associated metabolic diseases are extensively discussed elsewhere (Kershaw and Flier, 2004).

The adipokine retinol-binding protein-4 (RBP4) attracted a lot of scientific attention after the discovery that adipose tissue RBP4 expression is increased in mice with an adipose-specific GLUT4-knockout (Yang et al., 2005) and that serum RBP4 levels are elevated in insulin-resistant mice and humans with obesity and T2D (Graham et al., 2006; Klöting et al., 2007). The search term "RBP4 and obesity" retrieved more than 420 PubMed hits in March 2021 and the knowledge about the sources, modulators and function of RBP4 has significantly increased over the past 10 years. Therefore, this review focuses on the current advances in the understanding of the role of RBP4 in obesity and its related comorbidities.

3. RBP4 – structure, mechanism of action and physiologic function

RBP4 is a plasma membrane transporter constituted of a single polypeptide chain (Fig. 2) with a molecular mass of ~21 kDa, encoded in humans by the *RBP4* gene located on chromosome 10 (10q23–q24) (Rocchi et al., 1989). In the circulation, RBP4 is almost entirely bound to thyroxine-binding transthyretin (TTR) (Folli et al., 2005). Human genetic mutations that lead to a loss of RBP4 function and reduced or undetectable RBP4 serum levels have been associated with retinal dystrophy, iris coloboma, comedogenic acne syndrome and others (Seeliger et al., 1999; Cukras et al., 2012; Chou et al., 2015).

RBP4 is a member of the lipocalin protein family, characterized by a tertiary structure referred to as "lipocalin fold" (Flower et al., 2000). This structure favours the binding of small, hydrophobic molecules such as retinol and lipids (Flower et al., 2000). So far, RBP4 is the only known specific transport protein responsible for delivering retinol (vitamin A) from the liver (as one of the main storage site) to target tissues and is therefore regulating circulating levels of retinol (Quadro et al., 1999; O'Byrne and Blaner, 2013). The crystal structure of RBP4 in complex with retinol and non-retinoid ligands has been previously resolved (Fig. 2). In target tissues, retinol can be taken up by direct diffusion or by binding of RBP4 to a cell membrane receptor (schematic representation in section 5.3.2). STRA6 (stimulated by retinoic acid 6) has been identified as the membrane receptor for RBP4, which mediate retinol influx from the blood to target cells (Kawaguchi et al., 2012). In the circulation, the complex retinol-RBP4 binds to the plasma protein TTR, homotetramer with a molecular weight of ~55 kDa (Naylor and Newcomer, 1999). This stabilizes the complex and reduces the loss of the low molecular weight RBP through renal filtration (O'Byrne and Blaner, 2013; Naylor and Newcomer, 1999). TTR can bind two molecules of RBP at equivalent binding sites (Fig. 2C) (Naylor and Newcomer, 1999).

In addition to its role in retinol metabolism, RBP4 (circulating and tissue) has been associated with systemic insulin resistance and may therefore link adipose tissue dysfunction to T2D (Yang et al., 2005; Graham et al., 2006; Kovacs et al., 2007; Meex and Watt, 2017; Smith and Kahn, 2016). Alongside, the RBP4-membrane receptor STRA6 also contributes to the etiology of insulin resistance by inducing SOCS3 (suppressor of cytokine signalling 3), an inhibitor of insulin signalling (Berry et al., 2011). Moreover, TTR has been proposed as a limiting factor for the elevation of RBP4 in the plasma, hereby protective against insulin resistance (Berry et al., 2012). This hypothesis is based on evidence that treatment of mice with retinol-bound RBP4 (holo-RBP) reduced the phosphorylation levels of the insulin receptor and Akt (Berry et al., 2012). Only the administration of the complex retinol-RBP-TTR induced the expression of SOCS3 and peroxisome-proliferator-activated receptor (PPAR)-γ in white adipose tissue and skeletal muscle (Berry et al., 2012). In animal models of obesity, stimulation of SOCS3 through STRA6 signalling only occurred if increased circulating levels of "free" RBP4 (i.e. not TTR-bound) exceeds that of TTR (Berry et al., 2012). Hence, TTR may not only prevent the glomerular filtration of RBP4 but could also neutralize RBP4 effects on whole-body glucose homeostasis. It is therefore important to understand RBP4 signalling pathways in the regulation of whole-body metabolism, especially in the context of obesity and related metabolic consequences.

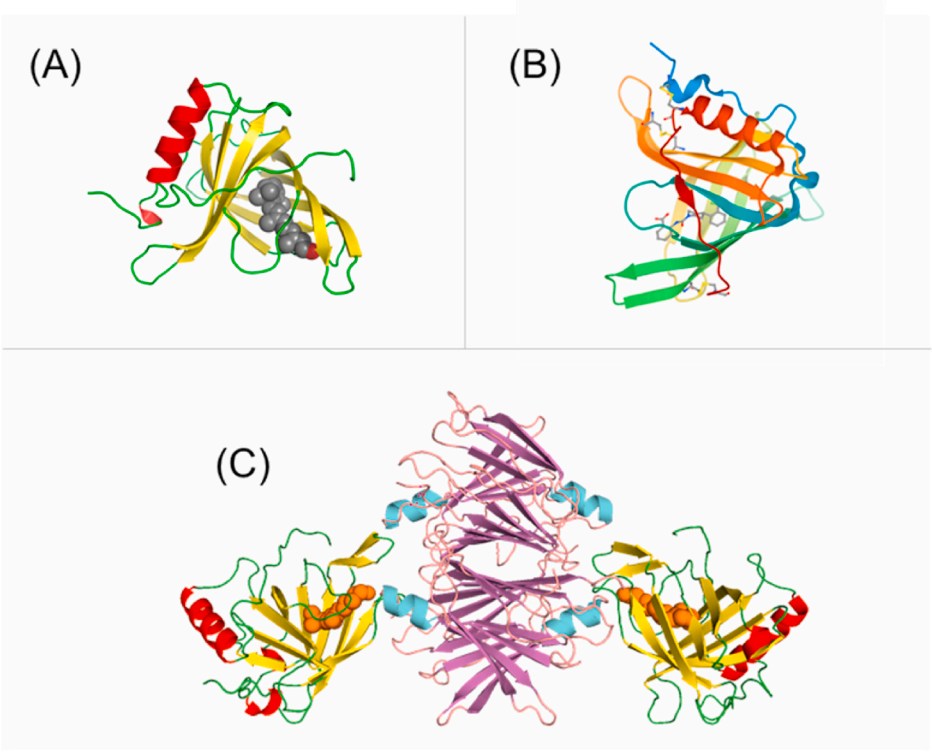

Fig. 2. A) Three-dimensional representation of the complex RBP-retinol (holo-RBP). *Human holo-RBP structure from* (Cowan et al., 1990)*, created using PyMol (http://pymol.sourceforge.net) and GIMP. Yellow: RBP molecule; Red: C-terminus of the human RBP; Grey: retinol* (Fvas concellos, 2007)*;* B) Crystal structure of RBP4 in complex with non-retinoid ligands (*described in* (Motani et al., 2009; Wang et al., 2014)); C) Quaternary structure of the complex of retinol-RBP-TTR. *Two molecules of RBP4 (in yellow and red) bound to retinol (in orange) complexed with four molecules of TTR (in purple and blue)* (Cowan et al. 1990; Naylor and Newcomer, 1999; Wpliao, 2018).

4. RBP4 in metabolic (dys)function

The two primary sources of RBP4 are the liver and adipose tissue (Tsutsumi et al., 1992; Thompson et al., 2017; Hammarstedt et al., 2012). In addition, the kidney, retinal pigment epithelium, peritubular and Sertoli cells of the testis may synthesize RBP4 (Naylor and Newcomer, 1999). Under lean conditions, adipocytes express about one-fifth of *RBP4* mRNA compared to hepatocytes (Tsutsumi et al., 1992). In adipose tissue, RBP4 expression almost exclusively derived from mature adipocytes (Tsutsumi et al., 1992; Zovich et al., 1992) and substantially increased from lean and overweight to obese states (Yang et al., 2005; Klöting et al., 2007; Kilicarslan et al., 2020). Recently, it has been shown that RBP4 increases lipolysis in human adipocytes and is associated with increased lipolysis and hepatic insulin resistance in women with obesity (Kilicarslan et al., 2020). Increased RBP4 expression in adipose tissue concomitant with higher serum RBP4 is suggested to contribute to systemic insulin resistance (Yang et al., 2005). Moreover, serum RBP4 inversely correlates with insulin-mediated suppression of lipolysis, circulating free fatty acids (FFAs), glucose disposal in euglycemic-hyperinsulinemic clamps and endogenous glucose production (Klöting et al., 2007; Smith and Kahn, 2016; Kilicarslan et al., 2020). Although these associations have been consistently found across animal and human studies, the mechanisms linking RBP4, impaired insulin sensitivity, glucose and lipid metabolism are still not completely understood.

4.1. Evidence from animal studies

Several animal models have been studied to decipher the role of RBP4 in the development of metabolic diseases. Elevated circulating and adipose tissue RBP4 levels are involved in the regulation of glucose metabolism, insulin signalling and therefore, insulin resistance (Berry et al., 2011; Preitner et al., 2009; Zemany et al., 2015; Ma et al., 2016). RBP4 has gained special attention in the metabolism research field after the observation that mice with an adipose tissue-selective GLUT4--knockout (Abel et al., 2001) exhibit increased RBP4 expression in adipose tissue (Yang et al., 2005). Reduced glucose transporter GLUT4 expression in adipocytes, the main transporter mediating insulin-stimulated glucose uptake into adipocytes, has been associated with insulin resistance (Shepherd and B. B., 1999). Likewise, elevated serum RBP4 levels showed in mice and humans with obesity and T2D could be normalized by rosiglitazone, an insulin-sensitizing drug (Yang et al., 2005). Subsequent studies of mice with transgenic overexpression of human RBP4 or injection of recombinant RBP4 in normal mice revealed that RBP4 may cause systemic insulin resistance (Yang et al., 2005), whereas decreasing RBP4 by genetic deletion or by pharmacologic treatment of mice with agents lowering RBP4 (e.g. fenretinide, rosiglitazone) increased insulin sensitivity (Yang et al., 2005).

A more recent study in liver-specific RBP4-knockout mice suggested that hepatocytes are the principal source of serum RBP4 (Thompson et al., 2017). Indeed, mice fed with high-fat and high-sucrose diets exhibited an increase in adipose tissue RBP4 expression, but undetectable circulating RBP4. This suggested that adipose tissue RBP4 expression does not necessarily translate into adipocyte-secreted RBP4 into the circulation (Thompson et al., 2017). Another study using adeno-associated viruses (AAV) containing a highly liver-specific RBP4 promoter showed that increased serum RBP4 levels in liver-specific RBP4-overexpressing mice do not impair glucose homeostasis or cause insulin resistance even in high-fat diet-induced obesity (Fedders et al., 2018). Interestingly, in transgenic mice expressing human RBP4 specifically in adipocytes, Lee et al. showed increased adipose tissue RBP4 protein expression (for both mice and human RBP4), which was sufficient to cause glucose intolerance, even though circulating RBP4 levels remained unchanged (Lee et al., 2016). Together these somewhat contradictory data suggest that the tissue source of RBP4 might define its consequences on the development of insulin resistance (Fenzl et al., 2020). Increased expression of RBP4 in adipocytes may trigger adverse metabolic phenotypes such as dyslipidemia, hepatic steatosis and impairment in glucose homeostasis through autocrine or paracrine mechanisms, effects that do not require elevated circulating RBP4 concentrations (Lee et al., 2016). In high-fat diet studies, both adipose tissue and circulating RBP4 levels increased in RBP4 overexpressing mice, supporting the contribution of adipocyte-derived RBP4 to the circulating pool, especially during overnutrition (Lee et al., 2016).

The effect of RBP4 on whole-body metabolism is further supported by studies of mice expressing human RBP4 selectively in the muscle (muscle creatine kinase promoter-hRBP4 transgenic mice) that develop glucose intolerance and insulin resistance on chow diet independently of body weight, fat mass, serum triglycerides, FFAs and adiponectin (Moraes-Vieira et al., 2014). These mice accumulate RBP4 in adipose tissue which activates antigen-presenting cells (APCs), resulting in higher expression of pro-inflammatory cytokines (TNFα, IL-6 and IL-1β) (Moraes-Vieira et al., 2014). This suggests that RBP4 is potentially linked to insulin resistance and metabolic diseases partly through the induction of (visceral) adipose tissue inflammation and priming the NLRP inflammasome (Moraes-Vieira et al., 2014, 2016, 2020).

RBP4 might also play a role in the "browning" of white adipose tissue (induction of UCP1 expressing beige/brite adipocytes within white fat) (Kiefer et al., 2012). Indeed, RBP4 is expressed in brown adipose tissue *in vivo* in mice that have been either exposed to cold or treated with PPARγ agonists (Villarroya et al., 2012). RBP4 expression has also been demonstrated in brown adipocytes *in vitro* (Villarroya et al., 2012). In mice and humans, cold exposure increased circulating concentrations of retinol and RBP4 (Fenzl et al., 2020). The role of retinoid metabolism in cold-induced adipose tissue browning and adaptive thermogenesis was shown in primary human adipocytes with increased expression of genes involved in thermogenesis and mitochondrial respiration (Fenzl et al., 2020). Interestingly, RBP4-knockout mice exhibit a pronounced reduction of thermogenic programming of adipocytes and oxidative mitochondrial function in subcutaneous white fat resulting in higher cold sensitivity compared to wild-type mice (Fenzl et al., 2020). In contrast, Zemany et al. showed an upregulation of thermogenic genes (*Ucp-1*, *Pgc-1a*, and *Cidea*) in subcutaneous adipose tissue (SAT) of mice lacking the RBP4-membrane receptor STRA6 specifically in adipose tissue (Zemany et al., 2014). The increased thermogenesis, concomitant with increased oxygen consumption in SAT in these mice was suggested to contribute to leanness and improvement of insulin sensitivity (Zemany et al., 2014). This suggests that RBP4-associated upregulation of thermogenesis in white fat is independent of STRA6 signaling or reduced STRA6-dependent action of RBP4 could contribute to improved insulin sensitivity through the activation of thermogenesis in white fat. RBP4 and its associated signaling pathways might therefore contribute to the regulation of thermogenesis in white adipose tissue.

In summary of these animal experiments, the contribution of circulating, adipose tissue and/or liver-derived increased RBP4 on glucose homeostasis and systemic insulin sensitivity remain controversial. The reported discrepancies might be explained by differences in animal models including their genetic background and RBP4 targeting strategies, as well as the diet challenge, feeding state (normal vs high-fat diet) or magnitude of RBP4 gain-of-function. From these studies, it could also be concluded that the source and metabolic action of RBP4 may vary depending on the metabolic condition and (e.g. obesity vs normal weight, normoglycemic vs insulin resistant or diabetic mice).

4.2. RBP4 and metabolic diseases – hints from human genetics?

Recent genome-wide association studies (GWAS) have advanced our understanding of potential genetic disease drivers or modulators. GWAS of retinol serum concentrations identified and replicated two single-nucleotide polymorphisms (SNPs) which are located near the TTR and RBP4 genes (Mondul et al., 2011). Moreover, genetic studies support the role of RBP4 as a modifier of TTR function (De Lillo et al., 2019).

However, GWAS on BMI, waist circumference or diabetes-related traits did not identify genetic associations with variants in the RBP4 gene (Loos et al., 2008; Mahajan et al., 2014; Speliotes et al., 2010; Heid et al., 2010; Voight et al., 2010). Nonetheless, regions near the RBP4 locus on human chromosome 10q have been linked to higher T2D risk in independent populations (Duggirala et al., 1999; Meigs et al., 2002; Shajarian et al., 2015). Moreover, a gain-of-function SNP in the RBP4 promoter region was shown to increase RBP4 expression in adipose tissue (Munkhtulga et al., 2010). Carriers of the risk allele have a ~80% higher T2D risk, suggesting that elevated RBP4 can be an independent risk factor for T2D (van Hoek et al., 2008).

RBP4 SNPs and their haplotypes were shown to affect measures of insulin resistance (e.g. fasting plasma insulin and glucose) and obesity-related traits (e.g. BMI, WHR, circulating FFAs), as well as *RBP4* mRNA levels in adipose tissue in humans (Kovacs et al., 2007; Shajarian et al., 2015; Munkhtulga et al., 2010; van Hoek et al., 2008). Currently, different studies have identified ten distinct RBP4 gene variants in European, Asian and American populations, which are associated with obesity, insulin resistance, hyperinsulinemia, T2D, gestational diabetes and CVD risk factors (van Hoek et al., 2008; Rychter et al., 2020; Boaghi et al., 2020; Hu et al., 2019; Codoner-Franch et al., 2016; Saucedo et al., 2014; Meisinger et al., 2011; Nair et al., 2010). These studies revealed that variations in the RBP4 gene are related to adipose tissue RBP4 expression and the susceptibility to develop diabetes (Kovacs et al., 2007; Rychter et al., 2020; Craig et al., 2007). Noteworthy, SNPs in the cell surface receptor of RBP4, STRA6, have been linked to increased T2D risk (Nair et al., 2010; Huang et al., 2016). Taken together, although RBP4 has not been identified as a candidate gene for obesity, insulin resistance and hyperglycemia traits in large GWAS, genetic association studies using a candidate gene approach support relationships between genetic variation in the RBP4 gene and obesity and cardiometabolic diseases risk.

5. RBP4 in obesity and associated cardiometabolic complications

Increased adipose tissue volume is the main symptom of obesity. However, the expansion of adipose tissue does not always translate into obesity-associated cardiometabolic diseases (Blüher, 2009b, 2013b, 2020; Lacobini et al., 2019; Rezaee and Dashty, 2013; Bosy-Westphal and Muller, 2021). Impaired adipose tissue function is characterized by hypertrophy of adipocytes, increased ectopic fat in visceral depots and organs such as the liver or skeletal muscle as well as immune cell infiltration in the adipose tissue (Klöting et al., 2010; Klöting and Blüher, 2014; Bays, 2011). These alterations occur upon fat accumulation in obesity and patients with lipodystrophy at least in part attributable to impaired subcutaneous adipose tissue expandability (Scherer, 2019). Adipose tissue dysfunction is reflected in the circulation by dysregulation of adipokine patterns (Blüher, 2013a; Almuraikhy et al., 2016; Hotamisligil, 2006; Weisberg et al., 2003; Moro et al., 2014; Ebert et al., 2018). Among dysregulated adipokines in diseases related to adipose tissue dysfunction, RBP4 circulating levels increase with visceral adipose tissue (VAT) accumulation, obesity, insulin resistance, T2D, fatty liver disease and CVD (Yang et al., 2005; Graham et al., 2006, 2007; Klöting et al., 2007; Blüher et al., 2008; Tonjes et al., 2010; Friebe et al., 2011; Cabré et al., 2007; Eichelmann et al., 2017; Peraire et al., 2015).

5.1. RBP4 and obesity

Serum RBP4 concentrations have been associated with the magnitude of insulin resistance in individuals with obesity, impaired glucose tolerance, or T2D, as well as in lean nondiabetic subjects with a family history of type 2 diabetes (Graham et al., 2006). However, not all human studies could confirm the role of RBP4 as a biomarker for obesity-associated insulin resistance or CVD (von Eynatten et al., 2007). As a potential explanation for divergent findings, shortcomings in the methodology of RBP4 measurements have been postulated (Graham et al., 2007). In addition, circulating RBP4 may be a determinant factor that displays high intra-individual variation.

Circulating RBP4 levels are modulated by body fat accumulation and fat distribution, which vary with body-weight changes over time (Blüher et al., 2012; Reinehr et al., 2008; Wang et al., 2020). Adipose tissue accumulation predominantly in the visceral depots (central obesity) is a major risk factor in the development of obesity-associated comorbidities and abdominal fat distribution is a stronger predictor of adverse cardiovascular outcomes than BMI or body fat mass (Pischon et al., 2008; Consortium, 2012). Central fat accumulation (VAT) is associated with a detrimental metabolic profile, in comparison to gluteal SAT which has been suggested to be protective against the development of obesity-associated comorbidities (Preis et al., 2010; Zhang et al., 2013; Matsuzawa et al., 1995; McLaughlin et al., 2011; Manolopoulos et al., 2010; Goodpaster et al., 2005). Therefore, the possibility that RBP4 expression and action represent a mechanism underlying these differences might not be ruled out. Accordingly, circulating RBP4 levels are positively associated with higher VAT mass and *RBP4* expression (Klöting et al., 2007). VAT mass reduction and concomitant improvements in insulin sensitivity are associated with ~25% decreased serum RBP4 levels in non-diabetic individuals with obesity (Lee et al., 2008). Moreover, reduction in serum RBP4 correlates with the amount of VAT mass loss but is not associated with total body fat or subcutaneous fat loss (Lee et al., 2008). Therefore, RBP4 expression from VAT might represent the major adipose tissue source of circulating RBP4 in central obesity and a potential contributor to VAT-associated cardiometabolic risk factors. Recently, we found that circulating RBP4 closely reflects changes in fat mass after a 2-years diet weight loss intervention, but does not predict individual response to the intervention (Blüher et al., 2012).

In accordance with animal studies, circulating RBP4 is significantly associated with parameters of obesity and fat distribution (BMI, body fat mass, waist and hip circumferences, waist and hip ratio (WHR)) in children, adolescents (Friebe et al., 2011; Reinehr et al., 2008; Aeberli et al., 2007; Balagopal et al., 2007; Rhie et al., 2011), and adults (Graham et al., 2006; Klöting et al., 2007; Kilicarslan et al., 2020; Wang et al., 2020; Comucci et al., 2014). Higher RBP4 mRNA and protein expression was also reported in the liver and adipose tissue from individuals with obesity (Kilicarslan et al., 2020). Additionally, studies investigating the relationship between circulating RBP4 and body fat distribution showed positive correlations with VAT (Klöting et al., 2007; Jia et al., 2007; Tschoner et al., 2008; Gavi et al., 2007). Higher *RBP4* expression in VAT compared to abdominal subcutaneous adipose tissue (SAT) has been shown and correlated to cardiometabolic risk in individuals with obesity (Klöting et al., 2007; Lee et al., 2008). In addition, patients with CVD exhibit higher RBP4 expression in the epicardial compared to SAT (Salgado-Somoza et al., 2012). Noteworthy, there is no formal proof that RBP4 may be dysregulated in association with impaired expandability of healthy (e.g. gluteal) fat depots and there is only anecdotal evidence for altered circulating RBP4 in association with lipodystrophy (Jeong et al., 2012; Godoy-Matos et al., 2009, 2011). In a human unbiased combined proteomic and metabolomic serum profiling of children and individuals who underwent bariatric surgery, RBP4 appeared as a significant predictor of body fat mass changes and the degree of adiposity (Oberbach et al., 2011, 2012). More research evaluating RBP4 levels in adipose tissue including different SAT depots are needed to evaluate the distinct adipose depot-specific association with obesity-related metabolic risks.

5.2. RBP4 and insulin resistance

Population-based studies showed that high serum RBP4 is a biomarker for metabolic syndrome (Meisinger et al., 2011; Qi et al., 2007). Similarly, higher RBP4 serum concentrations during childhood have been identified as a predictor for subsequent development of metabolic syndrome or distinct metabolic alterations independent of

pediatric obesity (Li et al., 2018). The relationship between RBP4 and insulin resistance or T2D was shown in independent human cohorts involving children, adolescents and adults of both sexes across a wide range of body weight, age and health status (Yang et al., 2005; Graham et al., 2006; Wang et al., 2020; Kowalska et al., 2008; Mostafaie et al., 2011; Norseen et al., 2012). Moreover, serum RBP4 positively correlated with fasting insulin and HOMA-IR index (Homeostatic Model Assessment of Insulin Resistance) in children (Boaghi et al., 2020; Reinehr et al., 2008; Aeberli et al., 2007; Balagopal et al., 2007; Rhie et al., 2011), and with fasting blood glucose, insulin, C-peptide and HOMA-IR in adults with obesity (Wang et al., 2020; Bremer et al., 2011). Furthermore, following treatment with the insulin sensitizer pioglitazone, circulating RBP4 was one of the main predictors of improved insulin sensitivity, supporting the potential role of RBP4 in mediating or reflecting systemic insulin sensitivity and/or resistance (Hammarstedt et al., 2008).

Associations between RBP4 and insulin resistance are not always driven by obesity (Graham et al., 2006; Aeberli et al., 2007). Indeed, elevated serum RBP4 concentrations were also shown in lean insulin-resistant patients (Graham et al., 2006; Norseen et al., 2012), non-obese and non-diabetic (Gavi et al., 2007), and normal-weight individuals with a family history of T2D (Graham et al., 2006). Moreover, serum RBP4 correlates with insulin resistance independently of BMI (Graham et al., 2006; Jia et al., 2007; Gavi et al., 2007). It is therefore conceivable that obesity or increased fat mass *per se* is not associated with increased circulating RBP4 and that the association between RBP4 and insulin resistance may reflect other pathologies such as adverse fat distribution, adipose tissue dysfunction, dyslipidemia and others (Norseen et al., 2012). This could partly explain the lack of correlation between RBP4 levels, obesity, and insulin resistance shown in other studies (von Eynatten et al., 2007; Gavi et al., 2007; Korek et al., 2018; Broch et al., 2007; Promintzer et al., 2007; Kanaka-Gantenbein et al., 2008; Noor et al., 2017; Kotnik et al., 2011; Erikstrup et al., 2009).

We previously investigated which circulating parameters are associated with insulin resistance independently of BMI and total body fat mass in a human model system. We systematically compared differences in adipose tissue biology and adipokine serum concentrations between age-, sex, and BMI-matched individuals with either insulin-sensitive or insulin-resistant obesity (Klöting et al., 2010). We found that independently of total body fat mass, systemic insulin resistance, as well as higher visceral fat volume, was associated with significantly higher RBP4 serum concentrations (Klöting et al., 2010). However, in humans, it could not be sorted out whether increases in visceral fat mass or the frequently accompanied liver fat accumulation are the predominant source of higher circulating RBP4. In addition to the main contribution of hepatocytes in circulating RBP4 levels under normal-weight and healthy conditions (Yang et al., 2005), that of skeletal muscle, cartilage and other tissue has also been proposed (Moraes-Vieira et al., 2014; Quadro et al., 2002; Yao-Borengasser et al., 2007; Scotece et al., 2020; Hatfield et al., 2013). Interestingly, Thompson et al. showed that RBP4 expression from adipocytes may not always correlate with serum levels in mice (Thompson et al., 2017). More recently, it has been suggested that RBP4 may act locally in adipose tissue to attract and activate macrophages thereby indirectly contributing to impaired insulin sensitivity (Moraes-Vieira et al., 2014). Therefore, regardless of the source, higher levels of RBP4 may cause insulin resistance through its effect on adipose tissue function or other organs (e.g. liver, muscle) (Yang et al., 2005; Kilicarslan et al., 2020). This can be exerted via retinol-dependent and retinol-independent mechanisms.

5.3. Mechanisms linking RBP4 to insulin resistance

5.3.1. Retinol-dependent mechanisms

RBP4 contributes to the development of impaired insulin sensitivity and may sustain an insulin resistance state through retinol-dependent mechanisms at the level of different tissues including the liver, skeletal muscle and adipose tissue (Fig. 3). In the liver, RBP4 induces the expression of retinoid-regulated genes including phosphoenolpyruvate carboxykinase (PEPCK) (Yang et al., 2005). This is driven by increased production or altered metabolism of retinoic acid isomers, the active form of retinol that interacts with retinoic acid receptors (RARs) and retinoic acid-X receptors (RXRs) (Chambon, 1996). PEPCK is a gluconeogenic enzyme regulated by retinoids (Zhang et al., 2011). The increase in PEPCK expression results in higher basal glucose production and reduction of insulin-induced suppression of glucose production in hepatocytes (Yang et al., 2005). Accordingly, inverse correlations between serum RBP4 levels and suppression of lipolysis, hepatic glucose output and peripheral glucose disposal were recently shown in insulin-resistant women with obesity (Kilicarslan et al., 2020). RBP4-induced dysregulation of hepatic glucose production might therefore represent a retinol-dependent mechanistic link between RBP4 and insulin resistance (Fig. 3). In skeletal muscle, RBP4 can modulate insulin sensitivity by inhibiting the phosphorylation of the insulin receptor substrate (IRS)1, as well as the activation of phosphatidylinositol-3-kinase (Yang et al., 2005; Abel et al., 2001) (Fig. 3). Retinol is involved in the synthesis of ligands of the PPAR family that regulate essential genes of fatty acid metabolism (Ferré, 2004; Muenzner et al., 2013). This suggests that dysregulation in fatty acid metabolism could also be implicated in the relationship between RBP4 and insulin resistance by the delivery of retinol to target tissues (Reinehr et al., 2008). Indeed, correlations between RBP4 and circulating fasting triglyceride concentrations is one of the most consistent findings across different studies (Graham et al., 2006, 2007; Klöting et al., 2007; Blüher et al., 2008; Tonjes et al., 2010; Friebe et al., 2011; Cabré et al., 2007; Eichelmann et al., 2017; Peraire et al., 2015).

Adipose tissue represents another important organ for the mechanistic link between RBP4 and insulin resistance (Fig. 3). The influence of adipose tissue function on the whole-body glucose metabolism remains debatable due to the relatively small contribution of adipose tissue to whole-body glucose disposal in normal states (Shepherd and B. B., 1999). However, adipose tissue has a significant implication in the etiology of insulin resistance, especially in obese states. *RBP4* mRNA expression positively correlates to that of GLUT4 in adipose tissue in humans (Graham et al., 2006; Klöting et al., 2007). Interestingly, the specific deletion of GLUT4 in mice adipocytes leads to a significant elevation of serum RBP4 and systemic insulin resistance (Yang et al., 2005), and impairment of the insulin action in muscle and liver (Abel et al., 2001). On the other hand, increasing GLUT4 expression selectively in adipocytes protects against whole-body insulin resistance (Yang et al., 2005). Therefore, the implication of adipose tissue function in the development of whole-body insulin resistance might be exerted through the expression/secretion of RBP4. Noteworthy, RBP4 expression and secretion have not been studied in mice with adipose tissue-selective deletion of the insulin receptor (Blüher et al., 2002).

Adipose tissue and serum levels of RBP4 significantly correlate with sub-clinical inflammation and pro-inflammatory cytokines (Balagopal et al., 2007; Yao-Borengasser et al., 2007), suggesting that RBP4 induce insulin resistance via pro-inflammatory pathways (Fig. 3). This is further supported by increased adipose tissue inflammation and lipolytic gene expression, as well as circulating FFAs following adipose-specific RBP4 overexpression in mice (Lee et al., 2016) and humans (Klöting et al., 2010). Adipose tissue inflammation and impaired lipolysis are closely linked in obese states and are associated with increased circulating FFAs, systemic inflammation and insulin resistance, but also increased RBP4 levels (Blüher, 2013b; Klöting et al., 2010).

The treatment of mice with holo-RBP inhibited insulin-induced activation of IRS1 and Akt1 (Yang et al., 2005; Berry et al., 2011), and insulin-induced mobilization of GLUT4 to plasma membranes in adipocytes (Fig. 3) (Berry et al., 2011). This mechanism requires the presence of RBP4 receptor STRA6, which functions as a cytokine receptor to transduce signalling by holo-RBP4 (Berry et al., 2011). The interaction of holo-RBP with STRA6 induces the phosphorylation of this

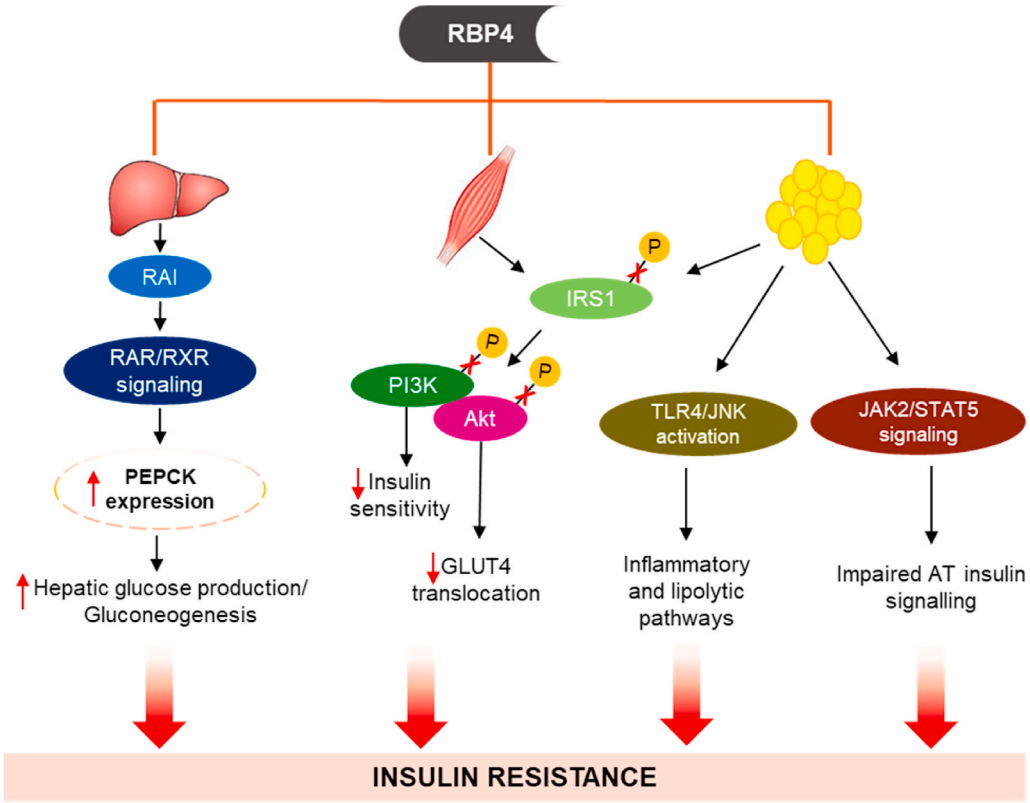

Fig. 3. Potential effects of RBP4 on different tissues contributing to the pathogenesis of insulin resistance through retinol-dependent mechanisms. *RBP4: retinol-binding protein 4; RAI: retinoic acid isomers; RAR: retinoic acid receptors; RXR: retinoic acid-X receptors; PEPCK: phosphoenolpyruvate carboxykinase; IRS: insulin receptor substrate; PI3K: phosphatidylinositol-3-kinase; GLUT4: glucose transporter 4; AT: adipose tissue; TLR4: toll-like receptor 4; JNK: c-Jun N-terminal protein kinase.*

receptor, followed by the recruitment and activation of JAK2 and STAT5 in adipose tissue (Berry et al., 2011; Gliniak et al., 2017). This results in the up-regulation of SOCS3 expression and subsequently to impaired intracellular insulin signalling in adipocytes (Berry et al., 2011).

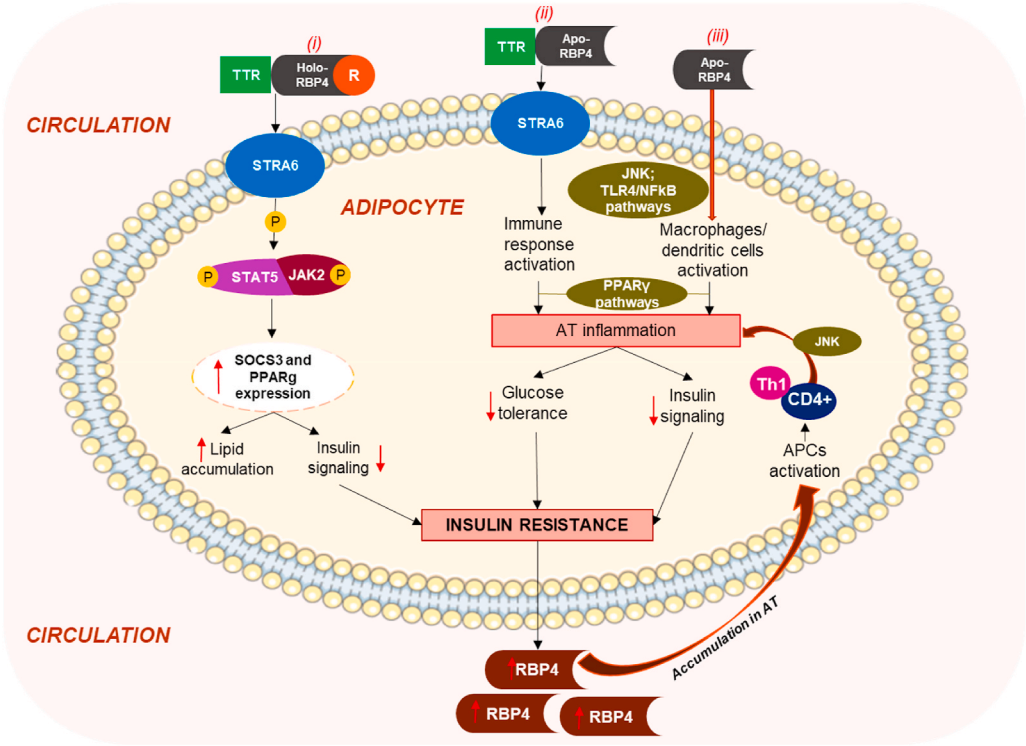

Fig. 4. Schematic representation of RBP-4 signaling pathways involved in the development of insulin resistance. ***(i)**: Direct effect of RBP4 on adipocytes by retinol-dependent mechanisms; **(ii)**: Effect of RBP4 on adipocytes insulin signaling* via *retinol-independent and STRA6-dependent mechanisms **(iii)**: Indirect effect of RBP4 on adipocytes* via *retinol-independent and macrophage-dependent mechanisms. STRA6: stimulated by retinoic acid 6; RBP4: retinol-binding protein 4; R: retinol; TTR: transthyretin; SOCS: suppressor of cytokine signaling; PPAR-γ: peroxisome-proliferator-activated receptor; TLR4: toll-like receptor 4; JNK: c-Jun N-terminal protein kinase; NFkB: nuclear factor kappa B; APCs: antigen-presenting cells.*

5.3.2. Retinol-independent mechanisms

Independently of retinol metabolism, RBP4 may cause insulin resistance by activating both innate and adaptive immune responses (Moraes-Vieira et al., 2014; Norseen et al., 2012). Inhibition or blockage of antigen presentation resulted in the reduction of adipose tissue inflammation and improved RBP4-induced insulin resistance in mice with transgenic overexpression of RBP4 (Moraes-Vieira et al., 2016). Furthermore, RBP4 can act independently of retinol and the RBP4 receptor STRA6 to impair insulin signalling in adipocytes through the activation of macrophages and induction of proinflammatory cytokine production (Fig. 4). This indirect mechanism of RBP4-induced insulin resistance may be mediated via activation of c-Jun N-terminal protein kinase (JNK) signalling, toll-like receptor 4 (TLR4) (Norseen et al., 2012; Deng et al., 2009), and TLR4/NFkB pathways (Deng et al., 2009). When treated with free RBP4 (apo-RBP4), macrophages secrete TNFα, IL-6 and MCP-1 to a greater extent than when treated with holo-RBP4 (retinol--bound RBP4) and PPARγ expression diminishes (Norseen et al., 2012). PPARγ is an essential transcription factor regulating adipogenesis and a negative regulator of proinflammatory pathways in macrophages (Harmon et al., 2011). Therefore, RBP4 can impair adipogenesis and lipid accumulation (Cheng et al., 2014), and induce adipose tissue inflammation partly through PPARγ-related pathways (Norseen et al., 2012). Notably, the resulting insulin resistance in adipocytes could be resolved upon cytokine inhibition by specific antibodies, suggesting that RBP4-induced impairment of insulin signalling in adipocytes is dependent on macrophages production of cytokines (Norseen et al., 2012).

Following the findings from Norseen et al., RBP4 was shown to induce the activation of APCs through the JNK pathway, resulting in pro-inflammatory CD4-positive T-cell proliferation and Th1 polarization (Moraes-Vieira et al., 2014). The transfer of RBP4-activated APCs into normal mice, induced adipose tissue inflammation and impaired glucose tolerance and insulin sensitivity, which together were sufficient to cause systemic insulin resistance (Moraes-Vieira et al., 2014). Therefore, JNK signalling, previously shown to be required for both inflammation and obesity-induced insulin resistance (Han et al., 2013) is also implicated in RBP4-associated signalling pathways (Fig. 4) (Moraes-Vieira et al., 2014; Norseen et al., 2012). Importantly, adipose tissue RBP4 may directly impair adipocyte insulin signalling through autocrine action, by inhibiting the insulin-stimulated phosphorylation of IRS1 and extracellular signal-regulated kinase (ERK1/2) (Cheng et al., 2014; Ost et al., 2007). RBP4-induced repression of insulin pathways may also impair adipogenesis and consequently, fat lipid accumulation in the adipocytes (Cheng et al., 2014) (Fig. 4). The pro-inflammatory effect of RBP4 through retinol- and STRA6-independent mechanisms was further demonstrated in human endothelial cells and suggested to contribute to insulin resistance and CVDs (Farjo et al., 2012).

5.3.3. Mode of cellular RBP4 release

RBP4 is typically secreted from mainly adipocytes and hepatocytes via classical secretion pathways (Yang et al., 2005; Smith and Kahn, 2016; Norseen et al., 2012). RBP4 expression and secretion can be stimulated by norepinephrine, PPARγ-agonists, cold and other factors (Yang et al., 2005; Fenzl et al., 2020; Norseen et al., 2012). Exosomes are endosome-derived organelles that are actively secreted through an exocytosis pathway and mediate intercellular cross-talk (Valadi et al., 2007). In this context, Deng et al. (2009) could recently demonstrate that exosome-like vesicles are enriched for RBP4 under conditions of obesity (Deng et al., 2009). Indeed, exosome-like vesicles are released from adipose tissue of the studied obesity models and were taken up by peripheral blood monocytes (Deng et al., 2009). Through this mode of release, RBP4 could in addition to the reported classical adipokine secretory mechanisms (Norseen et al., 2012) contribute to stimulating the differentiation of monocytes into activated macrophages (Deng et al., 2009). Noteworthy, it has been demonstrated that retinol-free RBP4 is as potent as retinol-bound RBP4 in inducing proinflammatory cytokines in macrophages (Norseen et al., 2012).

5.4. RBP4 and metabolic syndrome

RBP4 has been implicated in the development of other components of the metabolic syndrome (Qi et al., 2007; Tabesh et al., 2017) including dyslipidemia (Korek et al., 2018; Rocha et al., 2013), liver steatosis (Lee et al., 2016; Chen et al., 2017), elevated blood pressure (Li et al., 2019; Zhang et al., 2017) and cardiovascular dysfunction (Kraus et al., 2015; Feng et al., 2015) (see Table 1). Noteworthy, some studies did not find significant associations between circulating RBP4 and cardio-metabolic risk factors or events (Rist et al., 2018) despite the reliability and reproducibility of RBP4 serum measurements (Wittenbecher et al., 2015).

5.4.1. RBP4, dyslipidemia, and liver steatosis

In patients with morbid obesity, RBP4 was shown to be statistically more strongly linked with altered lipid metabolism than with insulin resistance (Rocha et al., 2013). Several studies showed associations between elevated circulating RBP4 and hypertriglyceridemia, hypercholesterolemia, and other dysregulation in lipid metabolism observed during obesity. Serum RBP4 levels positively correlate with high serum triglycerides in children, independently of adiposity (Reinehr et al., 2008; Aeberli et al., 2007; Li et al., 2018). Likewise, in adults and elders with obesity, serum RBP4 was positively associated with serum triglycerides and inversely associated with high-density lipoprotein cholesterol (HDL-C) concentrations (Graham et al., 2006; Wang et al., 2020; Mostafaie et al., 2011; Rocha et al., 2013; Majerczyk et al., 2018). Moreover, RBP4 serum levels were positively associated with increased low-density lipoprotein cholesterol (LDL-C) and total cholesterol in patients with T2D (Rocha et al., 2013).

Circulating RBP4 is also associated with hepatic lipid accumulation (Stefan et al., 2007) and liver steatosis (Lee et al., 2016; Chen et al., 2017). This suggests that a sustained increase in triglyceride levels may trigger ectopic fat deposition in other tissues such as the liver and that subsequent liver steatosis may lead to increased RBP4 synthesis and secretion (Chang et al., 2020). Indeed, increased triglyceride concentrations, stimulated by hyperinsulinemia may amplify liver and adipose tissue RBP4 synthesis and secretion (Boaghi et al., 2020). Accordingly, in a mouse model of nonalcoholic fatty liver disease (NAFLD), hepatic *RBP4* mRNA expression was abnormally elevated and positively correlated with hepatic triglyceride accumulation (Liu et al., 2016b). Besides that, in transgenic mice overexpressing human RBP4, an increase in hepatic lipid accumulation under chow diet, and to a greater extent with a high-fat diet was reported, and was attributed to RBP4-induced hepatic mitochondrial dysfunction (Liu et al., 2016b). However, it is worth noting that some studies in humans did not find associations between RBP4 and NAFLD (Kashyap et al., 2009; Cengiz et al., 2010). Therefore, the relationship between RBP4, lipid profile/metabolism and liver dysfunction warrants further investigations.

5.4.2. RBP4 and cardiovascular diseases

In addition to hypertriglyceridemia and liver steatosis, higher serum RBP4 might also play a role in the risk of CVDs such as heart disease and stroke (Kwanbunjan et al., 2018). Several studies have shown associations between RBP4 and measures of cardiovascular dysfunction. For instance, Yang et al. showed positive correlations between RBP4 and systolic blood pressure in normal-glucose, impaired-glucose tolerant, and T2D patients (Yang et al., 2012). Similarly, Dessein et al. found positive associations between higher serum RBP4, systolic and mean blood pressure, as well as surrogate parameters for atherosclerosis such as carotid intima-media thickness (cIMT) and carotid artery plaque volume in patients with obesity (Dessein et al., 2014). Moreover, compared to normotensive women, women with untreated hypertension exhibit higher levels of RBP4, significantly correlating with cIMT and blood pressure (Solini et al., 2009). Based on these data, RBP4 has been suggested to play a role in the development of atherosclerosis and linking obesity to vasculature dysfunction (Solini et al., 2009).

Table 1
Reported associations between circulating RBP4 levels and cardio-metabolic complications/risk factors in selected human studies in the context of obesity.

Study	Study population	Gender, n	Age (years)	BMI (kg/m^2)	RBP4 measurement method/sample	Cardio-metabolic risk/complication	Correlations
Graham, T.E. et al., 2006 (Graham et al., 2006)	NGT, IGT or T2D subjects with obesity	Males, 36 Females, 20	54 ± 9	31.1 ± 3.7	ELISA/Serum	BMI, fasting insulinWHR, Serum TG, SBP HDL-C	Positive Negative
Klöting, N. et al., 2007 (Klöting et al., 2007)	Lean and obese individuals, with and without T2D	Males, 98 Females, 98	54 ± 15	29.3 ± 6.8	Quantitative western blotting/ Serum	Total body fat%, Intraabdominal (visceral) fat area Insulin sensitivity	Positive Negative
Aeberli, I. et al., 2007 (Aeberli et al., 2007)	Overweight and Obese children	Males, 22 Females, 24	10.3 ± 2.1	23.1 ± 2.7	ELISA/Serum	BMI, body fat %, WHR, serum TG Fasting insulin, QUICKI, CRP, IL-6	Positive None
Jia, W. et al., 2007 (Jia et al., 2007)	NGT, IGT and T2D subjects	Males, 475 Females, 558	52.8 ± 11.7	25.8 ± 4	ELISA/Serum	Visceral fat Serum adiponectin BMI	Positive Negative None
Gavi, S. et al., 2007 (Gavi et al., 2007)	Normal weight individuals without a family history of diabetes	Males, 59 Females, 33	49.4 ± 2.02	25.47 ± 0.29	ELISA/Serum	Insulin sensitivity BMI, body fat % Trunk fat %	Negative None Positive
Stefan, N. et al., 2007 (Stefan et al., 2007)	Non-diabetic individuals with obesity	Males, 36 Females, 39	44 ± 1	29.4 ± 0.1	ELISA/Plasma	Liver fat, fasting insulin, HOMA-IR Body fat, visceral fat, subcutaneous fat, muscle fat, adiponectin, leptin Insulin sensitivity	Positive None Negative
Reinehr, T., B., 2008 (Reinehr et al., 2008)	Obese children	Males, 17 Females, 26	10.8 ± 2.6	27.3 ± 4.2	ELISA/Serum	BMI, insulin, HOMA-IR, blood pressure, TG QUICKI (quantitative insulin sensitivity check index)	Positive Negative
Comucci, E.B. et al., 2014 (Comucci et al., 2014)	Lean, obese NGT and obese with T2D	Women,139	39 ± 7.2	32.3 ± 3.8	ELISA/Plasma	BMI, glycated hemoglobin, fasting insulin, fasting glucose, HOMA-IR	Positive
Chen, X. et al., 2017 (Chen et al., 2017)	Individual with and without NAFLD	Males, 919 Females, 2019	61.2 ± 5.9	23.6 ± 2.8	ELISA/Serum	HOMA-IR, trunk fat, WHR, blood pressure, fasting insulin, NAFLD HDL-C	Positive Negative
Majerczyk, M. et al., 2018 (Majerczyk et al., 2018)	Elderly individuals with metabolic syndrome	Males, 1591 Females, 1447	78.2 ± 8.5	28.2 ± 5	ELISA/Plasma	Metabolic syndrome, visceral obesity, serum TG, fasting plasma glucose	Positive
Li, G. et al., 2018 (Li et al., 2018)	Children with and without metabolic syndrome	Males, 180 Females, 172	11.9 ± 3.1	22.5 ± 4.8	ELISA/Serum	BMI, WC, SBP, DBP, TG, TC, LDL- C, insulin, HOMA-IR and leptin	positive
Li, X. et al., 2019 (Li et al., 2019)	Patients with essential hypertension	Gender not specified, n = 74	49.9 ± 11.4	25 ± 3.4	ELISA/Serum	Blood pressure, left ventricular systolic diameter, interventricular septal thickness, left ventricular posterior wall thickness	Positive
Wang, X. et al., 2020 (Wang et al., 2020)	Obese patients	Males, 34 Females, 48	33.16 ± 11.03	35.20 ± 6.69	ELISA/Serum	BMI, blood glucose, fasting insulin, HOMA-IR,	Positive

NGT: normal glucose tolerance; IGT: impaired glucose tolerance; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; TG: triglycerides; TC: total cholesterol.

The association between RBP4 and cardiovascular dysfunction was further shown in children and adolescents with higher plasma RBP4 levels correlating with increased cardiovascular risk (Balagopal et al., 2007; Klisic et al., 2017). Strikingly, higher serum RBP4 in childhood could predict the development of insulin resistance, hyperglycemia, hyperlipidemia and hypertension at baseline and upon 10-year follow-up, independently of obesity (Li et al., 2018). This suggests that RBP4 is a potential early biomarker of adverse cardiovascular risk profile in pediatric populations (Li et al., 2018). Notably, the predictive value of RBP4 serum concentrations does not seem to be limited to children, since in a large cohort of normal-weight to overweight women plasma RBP4 predicted coronary heart disease (Sun et al., 2013), and of cardiovascular events in elderly patients (Li et al., 2020). In contrast, other studies did not report statistically significant associations between serum RBP4 and cIMT (Mansouri et al., 2012; Chu et al., 2011) or with CVD mortality in patients with T2D (Liu et al., 2016c). Further studies on the potential of circulating RBP4 levels to predict cardiometabolic risk are needed.

In animal studies, higher circulating and adipose levels of RBP4 were observed in mice with cardiac hypertrophy induced by transverse aortic constriction and angiotensin-II (Gao et al., 2016). Higher RBP4 induced the reduction of GLUT4 expression and impaired insulin-stimulated glucose uptake into cardiomyocytes (Gao et al., 2016). The uptake of glucose was further improved with the deletion of TLR4 (TLR4-knockout mice), suggesting that RBP4-induced insulin resistance in cardiomyocytes and heart failure occurs through the activation of TLR4-related inflammatory pathways (Gao et al., 2016). Additionally, the increase of RBP4 in transgenic mice has been associated with elevated blood pressure, an effect that was attenuated by the reduction of RBP4 in RBP4-knockout mice (Kraus et al., 2015). This suggested a direct effect of RBP4 on blood pressure (Solini et al., 2009) and endothelial dysfunction in humans (Solini et al., 2012) that may be mediated via the inhibition of NO-mediated vascular response (Kraus et al., 2015). These findings support the role of RBP4 as a component in the link between obesity, insulin resistance and CVDs, and a potential target in the development of strategic therapies to manage the onset and progression of these conditions.

6. RBP4 as a drug target

With the increasing health and economic burden caused by obesity and cardiometabolic comorbidities, the prevention or early management of these conditions might be the best strategies to slow down their progression (Recinella et al., 2020). RBP4 is a potential drug target for

the management or treatment of cardio-metabolic diseases and other diseases associated with RBP4 function.

6.1. Treatment of diseases related to impaired RBP4 function

RBP4 is involved in the pathogenesis of age-related macular degeneration (AMD) and Stargardt disease through increased retinol delivery to the retina, resulting in increased synthesis and accumulation of cytotoxic lipofuscin bisretinoids (Cioffi et al., 2015, 2019; Racz et al., 2018, 2020). AMD and Stargardt disease are characterized by chronic and slowly progressing neurodegenerative ocular disorders leading to the loss of vision (Racz et al., 2018; Hubschman et al., 2009). Studies in RBP4-transgenic mice also showed retinal degeneration associated with RBP4 function and resolved by treatment with non-retinoid RBP4 antagonists (Du et al., 2017).

Pharmacologic inhibition of the retinol-induced interaction of RBP4 with TTR in serum may reduce serum retinol uptake and formation of lipofuscin bisretinoids in the retina (Dobri et al., 2013). Accordingly, RBP4-antagonists have been used in several studies to block the ocular uptake of retinol from serum resulting in a reduction of bisretinoid accumulation in the retinal pigment epithelium, concomitantly with decreased serum RBP4 levels (Cioffi et al., 2015, 2019). RBP4-antagonists have provided promising therapeutic effects to stop the progression of neurodegeneration and related vision loss and suggested as a potential treatment of AMD and Stargardt disease (Cioffi et al., 2020) (Table 2). However, before their application in the treatment of AMD and Stargardt disease, retinoid- and non-retinoid RBP4-antagonists were identified and proposed as therapeutic candidates in the management of metabolic diseases (Yang et al., 2005; Graham et al., 2006; Kotnik et al., 2011; Cioffi et al., 2019; Torabi et al., 2017).

6.2. Therapies reducing RBP4 concentrations to improve metabolic diseases

By their capacity to significantly influence whole-body metabolism, adipokines could be therapeutic targets to manage or control obesity and associated comorbidities (Blüher, 2014; Andrade-Oliveira et al., 2015). Many therapies aiming to improve insulin sensitivity such as lifestyle interventions (e.g. dietary weight loss, bariatric surgery, exercise training) or pharmacological treatment (e.g. insulin-sensitizing drugs) also lower serum RBP4 (Blüher et al., 2012; Reinehr et al., 2008; Wang et al., 2020; Balagopal et al., 2007; Ludvik et al., 2007; Sun et al., 2017). Concurrently, lowering serum RBP4 results in the improvement of insulin resistance and T2D. For instance, the treatment of insulin-resistant obese mice with retinoid fenretinide (synthetic retinoid-based RBP4 antagonist) reduces serum RBP4 and total-body retinol levels and improves insulin sensitivity (Yang et al., 2005; Preitner et al., 2009; Koh et al., 2012). This retinoid-based RBP4 antagonist blocks the binding of retinol to RBP4 and dissociates the complex retinol-RBP4-TTR *in vitro* (Berni and Formelli, 1992) simultaneous with a reduction in circulating RBP4 levels *in vivo* (Radu et al., 2005). In humans with obesity, treatment with fenretinide improved insulin sensitivity (Johansson et al., 2008). Rather than the simple reduction of serum RBP4, this improvement in insulin sensitivity might be explained, at least partly, through fenretinide's antiobesity action with the inhibition of adipose tissue expansion and reduction in leptin concentration as shown in RBP4-knockout mice (Preitner et al., 2009). In *ob/ob* mice, the reduction of weight gain, fat cell size, insulin resistance and liver fat accumulation after fenretinide treatment was postulated to derive from decreased circulating RBP4 and increased plasma adiponectin (Koh et al., 2012). Whether the reduction of RBP4 circulating concentrations after fenretinide treatment is directly or indirectly responsible for the observed improvement of insulin sensitivity has not been established. Animal studies showed a reduction of plasma RBP4 in response to different RBP4-antagonists (fenretinide, rosiglitazone and A1120, a high-affinity non-retinoid RBP4-ligand, which dissociates the complex RBP4-TTR). Interestingly, contrary to decreased basal glucose levels in fenretinide- and rosiglitazone-treated animals, there was no improvement of insulin resistance in animals treated with A1120 despite plasma RBP4 levels decreased to a higher extent in A1120-treated animals (Motani et al., 2009). Therefore, lowering circulating RBP4 might not be the principal mechanism involved in the improvement of insulin resistance or T2D.

As shown in rats, pioglitazone lowers serum RBP4 through the suppression of RBP4 expression specifically in adipose tissue (and not in the liver), which correlated with reduced body weight and increased insulin sensitivity (Zhu et al., 2015). Adipocyte-specific inhibition of RBP4 expression could be a mechanism by which serum RBP4 reduction improved insulin sensitivity in insulin-resistant patients treated with pioglitazone. This reinforces the role of RBP4 on adipocyte insulin signalling pathways in patients with insulin resistance. While appreciating the effect of the inhibition of RBP4 expression in adipocytes, the reduction of serum RBP4 levels could result from a higher excretion of RBP4 and/or total retinol by targeting the complex retinol-RBP4-TTR in order to lower TTR levels. Accordingly, treatment of obese mice (genetic and diet-induced) with TTR antisense oligonucleotides significantly decreased both circulating levels of TTR and RBP4, followed by a

Table 2
Potential applications of RBP4-antagonists used in the management of retinal diseases.

Treatment	Dose	Disease or Studied model	Effects	Reference
N-(4-hydroxyphenyl)retinamide (4-HPR; synthetic retinoid fenretinide)	10–20 mg/kg/day	Abca4$^{-/-}$ mice	Reduced serum retinol and RBP, decreased visual cycle retinoids and inhibited lipofuscin fluorophores accumulation in the eye.	Radu et al. (2005)
Fenretinide	100 mg and 300 mg/kg/day	Humans (with geographic atrophy secondary to dry AMD)	Reduced serum RBP4, lesion growth rate and incidence of choroidal neovascularization and progression of the geographic atrophy growth.	Mata et al. (2013)
Carboxylic acid based non-retinoid RBP4 antagonist (A1120)	30 mg/kg/day	Abca4$^{-/-}$ mice	Reduced (50%) cytotoxic lipofuscin bisretinoid formation in the retinas correlating with reduction (75%) of serum RBP4	Dobri et al. (2013)
Cyclopentyl fused pyrrolidine antagonist (bicyclic-octahydrocyclopenta[c]-pyrrolo analogues)	5 mg/kg/day	rats	Reduced circulating plasma RBP4 protein levels (60%)	Cioffi et al. (2014)
Non-retinoid RBP4-ligand A1120: 2-(4-(2-(trifluoromethyl)phenyl)piperidine-1-carboxamido) benzoic acid	0,3 g/kg/day	RBP4-Tg Mice	Lowered serum RBP4 (70%) and prevented structural retinal degeneration	Du et al. (2017)
Non-retinoid RBP4 antagonist, BPN-14136	20 mg/kg/day	Abca4$^{-/-}$ mice	Reduced serum RBP4 levels and inhibited bisretinoid synthesis, normalized the retinal levels of proinflammatory complement cascade components.	Racz et al. (2018)
BPN-14136	5 mg/kg single dose	Non-human primates (Cynomolgus monkey)	Reduction of plasma RBP4 (99%), "complete" pharmacological blockade of the RBP4-TTR-mediated retinol transport	Racz et al. (2020)

reduction in circulating insulin levels, improved insulin tolerance, enhanced glucose disposal and increased insulin signalling, improved suppression of hepatic glucose production, and augmented insulin-signalling in muscle, as well as reduced adipose tissue inflammation (Zemany et al., 2015). Treatment of mice with RNA oligonucleotide against RBP4 (anti-RBP4 oligo) reduced RBP4 expression levels in adipose tissue and the liver, and decreased serum RBP4, increased adipose-GLUT4 expression, reduced hepatic PEPCK expression, and hepatic steatosis resulting in improved insulin sensitivity (Tan et al., 2011). Likewise, in a mouse transgenic model of hepatic steatosis (adi-hRBP4 mice), a new non-retinoid RBP4 antagonist (a fluorinated analogue) was shown to strongly reduce plasma RBP4 (90%), as well as body weight gain, hepatic FFAs and triglyceride levels, and improved hepatic steatosis in obese HFD-fed adi-hRBP4 mice (Cioffi et al., 2019). Lastly, RBP4 binding aptamer is another proposed class of therapeutic target to decrease insulin resistance and reduce diabetes risk by preventing the link of retinol to RBP4 (Torabi et al., 2017).

7. Summary and conclusion

Taken together, the significant association between RBP4, obesity, T2D and different components of the metabolic syndrome supports the role of RBP4 as a driver, modulator and/or biomarker of insulin resistance. Importantly, the associations between RBP4 serum concentrations and cardiometabolic risk parameters may not necessarily require the presence of obesity. This highlights the importance to understand the mechanism regulating the synthesis and secretion of RBP4 and identify factors mediating RBP4 associations as a mechanistic link. Findings from clinical studies show discrepancies in the association between RBP4, obesity and its related comorbidities possibly due to differences in studied populations, age and gender, as well as the different methodological evaluation of RBP4 circulating and tissue levels and actions. Therefore, more mechanistic studies are required to understand the role of RBP4 in the onset and progression of “obesity diseases”. Whether RBP4 is a drug target for diseases beyond those directly related to impaired RBP4 function remains the subject of ongoing preclinical studies.

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Declaration of competing interest

P.A.N.N. does not have any conflicts of interest to declare. M.B. received honoraria as a consultant and speaker from Amgen, AstraZeneca, Bayer, Boehringer-Ingelheim, Lilly, Novo Nordisk, Novartis, and Sanofi.

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