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 tissue volume (Corvera, 2021), with the principal function to store tri-glycerides 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 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.
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; Bluher 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; Lao 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 (Maurry 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; Bluher, 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 chemotactract protein 1 (MCP-1), tumour necrosis factor-alpha (TNFa), 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; Plam 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

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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 homeostatis 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 chemotactractant 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 (Seeberger et al., 1999; Cukras et al., 2005; 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 mediates 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; Hammarsdott 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; Kloting et al., 2007; Kiliarislan 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 (Kiliarislan 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 (Kloting et al., 2007; Smith and Kahn, 2016; Kiliarislan 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 (Feders 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 adiponecint (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 (TNFa, IL-6 and IL-1) (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 inflammosome (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-1α, 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; Mahajian et al., 2014; Speliotes et al., 2016; 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; Shahjarian 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; Shahjarian 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; Lacbini et al., 2019; Rezaee and Dashy, 2013; Bosy-Westphal and Muller, 2021). Impaired adipose tissue function is characterized by hyper trophy 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 of adipokine patterns (Blüher, 2013a; Almaruikhy et al., 2019; Hotamisligil, 2005; 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 volume (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; Gabre 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 non-diabetic 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; Tschorner 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
5.3. Retinol-dependent mechanisms

and retinol-independent mechanisms.

2005; Kilicarslan et al., 2020). This can be exerted via retinol-dependent higher levels of RBP4 may cause insulin resistance through its effect on 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 phosphoenoxytruvate 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 gluco-neogenic enzyme regulated by retinoids (Zhong 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 (IRS1), 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., 2011; Friebe et al., 2011; Cabrè 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, saccular 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-RBP (Berry et al., 2011). The interaction of holo-RBP with STRA6 induces the phosphorylation of this
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. 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.

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; PPARγ: 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/NFκB 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 (Flarmon 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 hyperinsulinaemia 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, Desein et al. found positive associations between high 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 (Desein 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).
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 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., 2020).

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) simultaneously 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

| 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 fluorescences 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) |
| Cyclopropyl 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-(trihalomethyphenyl)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. | Raciz 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 | Raciz 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 (ad-ßRBP4 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 ad-ßRBP4 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

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References

Abel, E.D., et al., 2001. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. Nature 409, 729–733.
Aebeli, L., et al., 2007. Serum retinol-binding protein 4 concentration and its ratio to serum retinol are associated with obesity and metabolic syndrome components in children. J. Clin. Endocrinol. Metab. 92 (11), 4359–4365.
Almuraikhy, S., et al., 2015. Interleukin-6 induces impairment in human subcutaneous enhanced glucose disposal and increased insulin signalling, improved adipose function and its functional consequences. FEBS (Fed. Eur. Biochem. Soc.) Lett. 580 (1), 343–345.
Anderson, E.K., et al., 2009. Adipose tissue recruitment of leukocytes. Curr. Opin. Lipidol. 20 (3), 172–177.
Anderson, D.P., et al., 2017. Abdominal subcutaneous adipose tissue cellularity in men and women. Int. J. Obes. 41 (10), 1564–1569.
Andrade-Oliveira, V., et al., 2019. Adipokines as drug targets in diabetes and underlying disturbances. J Diabetes Res 2015, 681612.
Arn-Wisniewsky, J., et al., 2009. Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. J. Clin. Endocrinol. Metab. 94 (11), 4619–4623.
Balagopal, P., et al., 2007. Reduction of elevated serum retinol binding protein in obese children by lifestyle intervention: association with subclinical inflammation. J. Clin. Endocrinol. Metab. 92 (12), 4377–4384.
Bays, H.E., 2011. Adiposity. Journal of the American College of Cardiology 57 (25), 2461–2473.
Berner, R., et al., 1992. In vitro interaction of fenretinide with plasma retinol-binding protein 4 and its functional consequences. FERS (Fed. Eur. Biochem. Soc.) Lett. 308 (1), 343–345.
Berr, D.C., et al., 2011. Signaling by vitamin A and retinol-binding protein regulates gene expression to inhibit insulin responses. Proc. Natl. Acad. Sci. Unit. States Am. 108 (11), 4340–4345.
Berry, D.C., et al., 2012. Transthyretin blocks retinol uptake and cell signaling by the hol-retinol-binding protein receptor STRA6. Mol. Cell Biol. 32 (19), 3851–3859.
Blüher, M., 2009a. Adipose tissue dysfunction in obesity. Exp. Clin. Endocrinol. Diabetes 117, 241–250.
Blüher, M., 2009b. Adipose tissue dysfunction in obesity. Exp. Clin. Endocrinol. Diabetes 117 (6), 241–250.
Blüher, M., 2012a. Clinical relevance of adipokines. Diabetes Metab. J. 36 (5), 317–327.
Blüher, M., 2013a. Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best Pract. Res. Clin. Endocrinol. Metabol. 27, 163–177.
Blüher, M., 2013b. Adipose tissue dysfunction contributes to obesity related metabolic diseases. Best Pract. Res. Clin. Endocrinol. Metabol. 27 (2), 163–177.
Blüher, M., 2014. Adipokines – removing road blocks to obesity and diabetes therapy. Mol Metab 3 (3), 230–240.
Blüher, M., 2020. Metabolically healthy obesity. Endocr. Rev. 41 (3), 405–420.
Boey-Westphal, A., Muller, M.J., 2021. Diagnosis of obesity based on body composition–associated health risks–Time for a change in paradigm. Obes. Rev. 22 (Suppl 2), e13190 https://doi.org/10.1111/obr.13190.
Bourlier, V., et al., 2008. Remodeling phenotype of human subcutaneous adipose tissue macrophages. Circulation 117 (6), 806–815.
Bremer, A.A., et al., 2011. Adipose tissue dysregulation in patients with metabolic syndrome. J. Clin. Endocrinol. Metabol. 96 (11), E1782–E1788.
Brock, M., et al., 2007. Circulating retinol-binding protein-4, insulin sensitivity, insulin secretion, and insulin disposition index in obese and nonobese subjects. Diabetes Care 30 (7), 1802–1806.
Cabre, A., et al., 2007. Retinol-binding protein 4 as a plasma biomarker of renal dysfunction and cardiovascular disease in type 2 diabetes. J. Intern. Med. 262 (4), 496–503.
Cengiz, C., et al., 2010. Serum retinol-binding protein 4 in patients with nonalcoholic fatty liver disease: does it have a significant impact on pathogenesis? Eur. J. Gastroenterol. Hepatol. 22 (7), 813–819.
Chakaroun, R., et al., 2012. Effects of weight loss and exercise on chemerin serum concentrations and adipose tissue expression in human obesity. Metabolism 61, 706–714.
Chambon, P., 1996. A decade of molecular biology of retinoic acid receptors. Faseb. J. 10, 940–954.
Chang, M.-L., et al., 2020. Altering retinol binding protein 4 levels in hepatitis C: inflammation and steatosis matter. Virulence 11 (1), 1501–1511.
Chen, X., et al., 2017. Retinol binding protein 4 levels and non-alcoholic fatty liver disease: a community-based cross-sectional study. Sci. Rep. 7, 45100.
Cheng, J., et al., 2014. Ectopic expression of RBP4 impairs the insulin pathway and increases intestinal weight loss intervention. Diabetes Care 35, 342–349.
Cheung, M.Y., et al., 2015. Biochemical basis for dominant inheritance, variable penetrance, and maternal effects in RBP4 congenital eye disease. Cell 161 (3), 634–646.
Chu, C.H., et al., 2011. Elevated serum retinol binding protein 4 concentrations are associated with chronic kidney disease but not with the higher carotid intima media thickness in type 2 diabetic subjects. Endocr. J. 58, 841–847.
Cioffi, C.L., et al., 2014. Design, synthesis, and evaluation of nonretinoid retinol binding protein 4 antagonists for the potential treatment of atrophic age-related macular degeneration and Stargardt disease. J. Med. Chem. 57 (18), 7731–7737.
Cioffi, C.L., et al., 2015. Bicyclic [3.3.0]-Octahydrocyclopenta[cp]pyrrolo antagonists of retinol binding protein 4: potential treatment of atrophic age-related macular degeneration and Stargardt disease. J. Med. Chem. 58 (15), 5863–5868.
Cooper, C., et al., 2019. Design, synthesis, and preclinical efficacy of novel nonretinoid antagonists of retinol-binding protein 4 in the mouse model of hepatic steatosis. J. Med. Chem. 62 (11), 5470–5500.
Cioffi, C.L., et al., 2020. Discovery of bispecific antagonists of retinol binding protein 4 that stabilize transthyretin tetramers: scaffolding happling, optimization, and preclinical pharmacological evaluation as a potential therapy for two common age-related comorbidities. J. Med. Chem. 63 (19), 11054–11084.
Codner, R., et al., 2016. Association of RBP4 adipose variants with childhood obesity and cardiovascular risk factors. Pediatr. Diabetes 17 (8), 576–583.
Comucci, E.B., et al., 2014. Serum levels of retinol binding protein 4 in women with different levels of adiposity and glucose tolerance. Arq. Bras. Endocrinol. Metabol. 58 (1), 709–714.

Consoritium, T.I., 2012. Long-term risk of incident type 2 diabetes and measures of overall and regional obesity: the EPIC-InterAct case-cohort study. PLoS Med. 9 (6), e1001258.

Conover, S., 2021. Cellular heterogeneity in adipose tissues. Annu. Rev. Physiol. 83 (1), 297–325.

Cowan, S.W., Newcomer, M.E., Jones, T.A., 1990. Crystallographic refinement of human serum retinol binding protein at 2A resolution. Protein 8 (1), 44–61.

Craig, R.L., Chu, W.S., Elbein, S.C., 2007. Retinol binding protein 4 as a candidate gene for type 2 diabetes and prediabetic intermediate traits. Mol. Genet. Genom. 278, 338–344.

Cukras, C., et al., 2012. Exome analysis identified a novel mutation in the RBP4 gene in a consanguineous pedigree with retinal dystrophy and developmental abnormalities. PLoS One 7 (11) e50205.

Dahlman, I., et al., 2012. Functional annotation of the human fat cell secretome. Arch. Histol. Cytol. 75 (4), 445–456.

Daly, L., et al., 2017. The association between retinol-binding protein 4 and adiposity in premenopausal women at high risk for breast cancer. Cancer. Histol. 68 (22), 9512–9518.

Daniluk, G., Gondek, M., Rundek, B., 2016. The role of retinol-binding protein-4 in atherosclerosis. Atheroscler. Thromb. 36 (1), 93–99.

Delves, D., Gussert, K., Guerre-Millo, M., 2011b. Defining macrophage phenotype and function in adipose tissue. Trends Immunol. 32 (7), 307–314.

De Lillo, A., et al., 2019. Phenome-wide association study of TTR and RBP4 genes in southern Han Chinese. BioMed Res. Int. 2019, 9390657.

Deng, Z.B., et al., 2009. Adipose tissue exosome-like vesicles mediate activation of macrophage-induced insulin resistance. Diabetes 58 (11), 2498–2505.

Dessein, P.H., et al., 2011. Retinol binding protein 4 concentrations relate to enhanced carotid atherosclerosis in patients with rheumatoid arthritis. PLoS One 6 (9) e29739.

Dobri, N., et al., 2013. A nonretinoid RBP4 antagonist, inhibits formation of cytotaxic bitriexinoids in the animal model of retinal lipofuscinosis. Invest. Ophthalmol. Vis. Sci. 54 (1), 85–95.

Doggo-Matos, A., et al., 2009. Serum retinol binding protein 4 in patients with familial partial lipodystrophy. Clin. Biochem. 42 (10–11), 1183–1186.

Godoy-Matos, A., et al., 2011. Serum retinol binding protein 4 is not decreased in congenital generalized lipodystrophy: a case series. Arq. Bras. Endocrinol. Metabol. 55 (6), 279–283.

Goodpaster, B.H., et al., 2005. Obesity, regional body fat distribution, and the metabolic syndrome in older men and women. Arch. Intern. Med. 165, 777–783.

Graham, T.E., et al., 2006. Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects. N. Engl. J. Med. 354, 2552–2563.

Graham, T.E., et al., 2007. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin-resistant human subjects. Diabetologia 50 (4), 614–617.

Hammondsted, A., et al., 2008. High circulating levels of RBP4 and mRNA levels of aFG, PGC-1α and UCP-2 predict improvement in insulin sensitivity following pioglitazone treatment of drug-naïve type 2 diabetic subjects. J. Intern. Med. 263 (4), 440–449.

Hammondstedt, A., Graham, T.E., Kahn, B.B., 2012. Adipose tissue dysregulation and reduced insulin sensitivity in non-obese individuals with enlarged abdominal adipose cells. Diabetol. Metab. Syndrome 4 (42).

Han, M.S., et al., 2013. JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. Science 339 (6146), 218–222.

Harmon, G.S., Lam, M.T., Glass, C.K., 2011. PPARs and lipid ligands in inflammation and metabolism. Chem. Rev. 111 (10), 6521–6340.

Hartfield, J.T., Andersson, P.J., Powell, B.C., 2013. Retinol-binding protein 4 is expressed in chondrocytes of developing mouse long bones: implications for a local role in formation of the secondary ossification center. Histochem. Cell Biol. 139 (5), 727–734.

Heid, J.M., et al., 2010. Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. Nat. Genet. 42 (11), 949–960.

Heming, D., et al., 2011. Retinol-binding protein-4 and lipid metabolism and insulin resistance. Biochim. Biophys. Acta 1815, 361–367.

Huang, J., Liang, B.Y., Li, Y.X., 2016. Association of polymorphisms in STRA6 and RARRES2 genes with type 2 diabetes in southern Han Chinese. BioMed Res. Int. 2016, 7.

Hubschman, J.P., Reddy, S., Schwartz, S.D., 2009. Age-related macular degeneration: current treatments. Clin. Ophthalmol. 3, 155–166.

Huang, X., et al., 2017. Association of serum retinol-binding protein 4 and visceral adiposity in Chinese subjects with and without type 2 diabetes. J. Clin. Endocrinol. Metab. 92 (8), 3242–3249.

Husson, H., et al., 2008. Effect of fenofibrate and low-dose tamoxifen on insulin sensitivity in premenopausal women at high risk for breast cancer. Cancer. Res. 68 (32), 9512–9518.

Kanaka-Gantenbein, C., et al., 2008. Retinol-binding protein 4 and lipocalin-2 in childhood and adolescent obesity: when children are not just ‘small adults’. Clin. Chem. 54 (7), 1176–1182.

Kashyap, S.R., et al., 2009. Triglyceride levels and not adipokine concentrations are closely related to severity of nonalcoholic fatty liver disease in an obesity surgery cohort. Obesity 17 (9), 155–166.

Kawaguchi, R., et al., 2012. STRA6-catalyzed vitamin A influx, efflux, and exchange. J. Membr. Biol. 245 (11), 731–745.

Kershaw, E.E., Flier, J.S., 2004. Adipose tissue as an endocrine organ. J. Clin. Endocrinol. Metab. 89 (6), 2548–2556.

Kiefer, F.W., et al., 2012. Retinaldehyde dehydrogenase 1 coordinates hepatic energy metabolism. Chem. Rev. 111 (10), 6321–6356.

Klaisic, A., et al., 2017. The association between retinol-binding protein 4 and cardiovascular risk score is mediated by waist circumference in overweight/obese adolescent girls. Acta Clin. Croat. 56 (1), 92–98.

Kloeting, N., Blüher, M., 2014. Adipocyte dysfunction, inflammation and metabolic syndrome. Rev. Endocr. Metab. Endocrinol. 15 (4), 277–287.

Kloeting, N., et al., 2007. Serum retinol-binding protein is more highly expressed in visceral than in subcutaneous adipose tissue and is a marker of intra-abdominal fat mass. Cell Metab. 6 (1), 79–87.

Kloeting, N., et al., 2010. Insulin-sensitive obesity. Am. J. Physiol. Endocrinol. Metabol. 299 (3), E506–E515.

Ko, I.U., et al., 2012. Fenretinide ameliorates insulin resistance and fatty liver in obese mice. Biol. Pharm. Bull. 35, 369–375.

Ko, E., et al., 2018. Serum RBP4 positively correlates with triglyceride level but not with BMI, fat mass and insulin resistance in obese healthy and non-obese individuals. Biomarkers 23 (7), 688–697.

Kotyk, P., Fischer-Pouwely, P., Wablitsch, M., 2011. RBP4: a controversial adipokine. Eur. J. Endocrinol. 165 (5), 703–711.

Kovacs, P., et al., 2007. Effects of genetic variation in the human retinol binding protein-4 gene (RBP4) on insulin resistance and fat depot-specific mRNA expression. Diabetes 56, 3095–3100.
Kowalska, I., et al., 2008. Serum retinol binding protein 4 is related to insulin resistance and nonoxidative glucose metabolism in lean and obese women with normal glucose tolerance. J. Clin. Endocrinol. Metab. 93 (7), 2796–2795.

Kraus, B.J., et al., 2015. Novel role for retinol-binding protein 4 in the regulation of blood pressure. Faseb. J. 29 (8), 3133–3140.

Krist, J., et al., 2013. Effects of weight loss and exercise on apelin serum concentrations and body weight in patients in human obesity. Obes. Facts 6 (3), 57–69.

Kwanbunjjan, K., et al., 2018. Association of retinol binding protein 4 and transthyretin with triglyceride levels and insulin resistance in rural thai with high type 2 diabetes risk. Endocr. J. 65 (1), 26.

Lacobini, C., et al., 2019. Metabolically healthy versus metabolically unhealthy obesity. Metab. Clin. Exp. 92, 51–60.

Lee, J.-W., et al., 2008. Abdominal visceral fat reduction is associated with favorable changes of serum retinol binding protein 4 in nonobese subjects. Endocr. J. 55, 239–246.

Lee, M.J., Wu, Y., Fried, S.K., 2013. Adipose tissue heterogeneity: implication of depot differences in adipose tissue for obesity complications. Mol. Aspects Med. 34 (1–2), 1–31.

Lee, S.A., et al., 2010. Case-control analysis of SNPs in GLUT4, RB4, and STRA6: association of SNPs in STRA6 with type 2 diabetes in a South Indian population. PLoS One 5 (7), e11444.

Naylor, H.M., Newcomer, M.E., 1999. The structure of human retinol-binding protein (RBP) with its carrier protein transthyretin reveals an interaction with the carboxy terminus of RBP. Biochemistry 38, 2647–2653.

Noor, R., Rini, E.A., Yerizel, E., 2017. Retinol binding protein 4, obesity, and insulin resistance in adolescents. Paediatr. Ino. 58. (37).

Norvén, J., et al., 2012. Retinol-binding protein 4 inhibits insulin signaling in adipocytes by inducing proinflammatory cytokines in macrophages through a c-Jun-terminal kinase- and toll-like receptor 4-dependent and retinol-independent mechanism. Mol. Cell. Biol. 32 (10), 2010-2019.

O’Byrne, S.M., Blaner, W.S., 2017. Retinol and retinyl esters: biochemistry and physiology. J. Lipid Res. 54 (7), 1731–1743.

Oberbach, A., et al., 2011. Combined proteomic and metabolomic profiling of serum reveals association of the complement system with obesity and identifies novel adipokines in elderly patients. J. Proteome Res 10 (10), 4769–4788.

Oberbach, A., et al., 2012. Combined serum proteomic and metabolomic profiling after laparoscopic sleeve gastrectomy in children and adolescents. J. Laparoendosc. Adv. Surg. Tech. 22 (2), 184–186.

Ost, A., et al., 2007. Retinol binding protein 4 attenuates insulin-induced phosphorylation of IRS1 and ERK1/2 in primary human adipocytes. Faseb. J. 21 (13), 3696–3704.

Pandić, J.V., Gritzeł, D., 2016. Under the surface of subcutaneous adipose tissue architecture. Acta Dermatovenerol. Croat. 24 (4), 250–260.

Parmitessy, A., et al., 2016. Secret talk between adipose tissue and central nervous system via secreted factors—an emerging frontier in the neurodegenerative research. J. Neuroinflammation 13 (1), 61.

Parrettini, S., et al., 2020. Adipokines: a rainbow of proteins with metabolic and endocrine functions. Protein Pept. Lett. 27 (12), 1219–1234.

Peraire, J., et al., 2015. HIV/antracetviral therapy-related lipidostrophy syndrome (HALS) is associated with higher RB4 and lower omentin in plasma. Clin. Microbiol. Infect. 21 (7), 711.e1–711.e8.

Pham, D.V., Park, P.H., 2021. Tumor metabolic reprogramming by adipokines as a critical driver of obesity-associated cancer progression. Int. J. Mol. Sci. 22 (3), 1210.

Promintzer, M., et al., 2008. Genomics unraveling adiponectin and metabolic mortality among men with type 2 diabetes: a 22-year prospective study. Arterioscler. Thromb. Vasc Biol. 36, 2259–2267.

Loos, R.J., et al., 2008. Common variants near MCAR are associated with fat mass, weight and risk of obesity. Nat. Genet. 40 (6), 678–775.

Ludvik, B., et al., 2007. Serum retinol-binding protein 4 is reduced after weight loss in morbidly obese subjects. J. Clin. Endocrinol. Metab. 92 (3), 1168–1171.

Luo, L., Liu, M., 2016. Adipose tissue in control of metabolism. J. Endocrinol. 231 (3), R77–R89.

Ma, X., et al., 2016. RBPs functions as a hepatokine in the regulation of glucose metabolism by the circadian clock in mice. Diabetologia 59 (2), 354–362.

Mahajan, A., et al., 2018. The genome-wide trans-ancestry meta-analysis provides insight into the genetic architecture of type 2 diabetes susceptibility. Nat. Genet. 46 (3), 234–244.

Majerczyk, M., et al., 2018. Components of metabolic syndrome in relation to plasma levels of retinol binding protein 4 (RB4P) in a cohort of people aged 65 years and older. J. Endocrinol. Invest. 41 (10), 1211–1219.

Manolopoulos, K.N., Karpe, F., Frayn, K.N., 2010. Glucose/lipolysis/fatty body fat as a determinant of metabolic health. Int. J. Obes. (34), 694–959.

Mamouri, M., et al., 2012. The association of carotid intima media thickness with retinol binding protein-4 and total and high molecular weight adiponectin in type 2 diabetic patients. J. Diabetes Metab. Disord. 11 (2).

Mata, N.L., et al., 2013. Investigation of oral farnesitide for treatment of geographic atrophy in age-related macular degeneration. Retina 33 (3), 498–507.

Matsuzawa, Y., et al., 1995. Pathophysiology and pathogenesis of visceral fat obesity. Obes. Res. 2, 187S–1995. 2.

Maury, E., Bichard, S.M., 2010. Adipokine dysregulation, adipose tissue inflammation and metabolic syndrome. Mol. Cell. Endocrinol. 314 (1), 1–16.

McLaughlin, T., et al., 2011. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. J. Clin. Endocrinol. Metab. 96 (11), E1756–E1760.

Meaux, J.B., et al., 2002. A genome-wide scan for loci linked to plasma levels of glucose and HbA1c(1) in a community-based sample of Canadian pedigrees: the Framingham Offspring Study. Hum. Mol. Genet. 11 (16), E1756–E1760.

Mehta, R.C., Zilka, M.J., 2003. Hepatokines: linking nonalcoholic fatty liver disease and metabolic syndrome. Mol. Cell. Proteomics 11 (1), M111, 010504.

Meisinger, C., et al., 2011. Retinol-binding protein 4 is associated with prediabetes in a community-based sample of Caucasian pedigrees: the Framingham Offspring Study. Hum. Mol. Genet. 20 (1), 104–112.

Mendes-Vieira, P.M., et al., 2016. Antigen presentation and T-cell activation are critical for RBPs-induced insulin resistance. Diabetes 65, 1317–1327.

Moraes-Vieira, P.M., et al., 2020. Retinol binding protein 4 granzymes the NLRP3 inflammasome by signaling through Toll-like receptors 2 and 4. Proc. Natl. Acad. Sci. U. S. A. 117 (49), 31309–31318.

Moro, C., et al., 2014. Identification of adipokine clusters related to parameters of fat mass, obesity, and adipokine sensitivity and inflammation. PLoS One 9 (6).

Mostafaei, N., et al., 2011. Circulating retinol-binding protein 4 and metabolic syndrome in the elderly. Wien. Med. Wochenschr. 161 (22–23), 505–510.

Motani, A., et al., 2009. Identification and characterization of a non-retinoid ligand for retinol-binding protein 4: which lowers serum retinol-binding protein 4 levels in vivo. J. Biol. Chem. 284 (12), 7673–7680.

Muenszner, M., et al., 2013. Retinol-binding protein 4 and its membrane receptor STRA6 control adipogenesis by promoting cellular androgenous homostasis and retinoid acid receptor activity. Mol. Cell. Biol. 33 (20), 4068–4082.

Munkhgal, L., et al., 2010. Regulatory SNP in the RB4P gene modified the expression in adipocytes and associated with BMI. Obesity 18 (5), 1006-1014.

Nair, A.K., et al., 2010. Case-control analysis of SNPs in GLUT4, RB4, and STRA6: association of SNPs in STRA6 with type 2 diabetes in a South Indian population. PLoS One 5 (7), e11444.
Rhee, Y.J., et al., 2011. Association of serum retinol binding protein 4 with adiposity and pubertal development in Korean children and adolescents. J. Kor. Med. Sci. 26 (6), 797–802.

Ritt, P.M., et al., 2018. Plasma retinol-binding protein 4 levels and the risk of ischemic stroke among women. J. Stroke Cerebrovasc. Dis. 27 (1), 68–75.

Rocchi, M., et al., 1989. Regional mapping of RBP4 to 10q23-q24 and RBP1 to 3q21-q22 in man. Somat. Cell Mol. Genet. 15, 185–196.

Rocha, M., et al., 2013. Association of serum retinol binding protein 4 with atherothrombotic dyslipidemia in morbid obese patients. PloS One 8 (11) e78670.

Rychter, A.M., et al., 2020. Is the retinol-binding protein 4 a possible risk factor for cardiovascular diseases in obesity? Int. J. Mol. Sci. 21 (5).

Salgado-Somoza, A., et al., 2012. Coronary artery disease is associated with higher epicardial Retinol-binding protein 4 (RBP4) and lower glucose transporter (GLUT) 4 levels in epicardial and subcutaneous adipose tissue. Clin. Endocrinol. 76 (1), 51–56.

Sárvári, A.K., et al., 2021. Plasticity of epidymal adipose tissue in response to diet-induced obesity at single-nucleus resolution. Cell Metabol. 33 (2), 437–453 e5 e5.

Saucedo, R., et al., 2014. RBP4 gene variants are associated with insulin resistance in women with previous gestational diabetes. Dis. Markers 2014, 269208.

Scherer, P.E., 2006. Adipose tissue: from lipid storage compartment to endocrine organ. Diabetes 55 (6), 1537–1545.

Scherer, P.E., 2019. The many secret lives of adipocytokines: implications for diabetes. Diabologia 62 (2), 223–232.

Scherer, P.E., et al., 1995a. A novel serum protein similar to Clq, produced exclusively in adipocytes. J. Biol. Chem. 270 (45), 26746–26749.

Scherer, P.E., et al., 1995b. A novel serum protein similar to Clq, produced exclusively in adipocytes. J. Biol. Chem. 270, 26746–26749.

Scotee, M., et al., 2020. Novel adipokine associated with OA: retinol binding protein 4 (RBP4) is produced by cartilage and is correlated with MMPs in osteoarthritic patients. Inflamm. Res. 69 (4), 415–421.

Seelig, M.W., et al., 1999. Phenotype in retinol deficiency due to a hereditary defect in adipocytes. J. Biol. Chem. 270, 26746–26749.

Sellke, F.W., et al., 2017. Association of retinol-binding protein 4 with metabolic disease in mice. Biochim. Biophys. Acta 1811 (12), 1045–1053.

Sharijani, M., et al., 2015. Association of RBP4 gene variants with adverse lipid profile and obesity. Gene 561 (1), 1–5.

Shepherd, P.R.K., B, B., 1999. Glucose transporters and insulin action—implications for lipid storage in adipose tissue of obese rats. Cell. Physiol. Biochem. 35 (2), 343–359.

Smith, U., Kahn, B.B., 2016. Adipose tissue regulates insulin sensitivity: role of thermogenesis. Nat. Rev. Endocrinol. 12 (3), 179–188.

Soden, L.M., et al., 2013. Suppression of retinol-binding protein 4 with RNA oligonucleotide prevents high-fat diet-induced metabolic syndrome and non-alcoholic fatty liver disease in mice. Biochim. Biophys. Acta 1811 (12), 1045–1053.

Solini, A., et al., 2012. Adipocytokine levels mark endothelial function in normotensive individuals. Cardiovasc. Diabetol. 11 .

Song, Z., Xiaoli, A.M., Yang, F., 2018. Regulation and metabolic significance of de novo lipogenesis in adipose tissue. Nutrients 10 (10).

Speleiotis, E.K., et al., 2010. Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat. Genet. 42 (11), 937–948.

Stefan, N., et al., 2007. High circulating retinol-binding protein 4 is associated with elevated liver fat but not with total, subcutaneous, visceral, or intramyocellular fat in humans. Diabetes Care 30 (5), 1173–1178.

Stroh, L.G., Scherer, P.E., 2019. Metabolic messengers: adiponectin. Nat Metab 1 (3), 334–339.

Sun, Q., et al., 2013. Plasma retinol-binding protein 4 (RBP4) levels and risk of coronary heart disease: a prospective analysis among women in the nurses’ health study. Circulation 127 (19), 1938–1947.

Sun, X., et al., 2017. Siltaglitin down-regulates retinol-binding protein 4 and reduces insulin resistance in gestational diabetes mellitus: a randomized and double-blind trial. Metab. Brain Dis. 32 (3), 773–778.

Sun, W., et al., 2020. miRNA-seq reveals a subpopulation of adipocytes that regulates thermogenesis. Nature 587 (7832), 98–102.

Tabesh, M., et al., 2017. Association of retinol-binding protein 4 with metabolic syndrome in first-degree relatives of type 2 diabetic patients. J. Res. Med. Sci. 22, 28.

Tan, Y., et al., 2011. Suppression of retinol-binding protein 4 with RNA oligonucleotide prevents high-fat diet-induced metabolic syndrome and non-alcoholic fatty liver disease in mice. Biochim. Biophys. Acta 1811 (12), 1045–1053.

Thompson, S.J., et al., 2017. Hepatocytes are the principal source of circulating RBP4 in mice. Diabetes 66, 58–63.

Tonjes, A., et al., 2010. Adipokine pattern in subjects with impaired fasting glucose and impaired glucose tolerance in comparison to normal glucose tolerance and diabetes. PloS One 5 (11) e19111.