**Editorial**

# RPB4 and pathogenesis of diabetes

**Dr Philip G McTernan[1] & Professor Sudhesh Kumar[1,2]**

**Affiliations:** [1]Warwick Medical School, University of Warwick, & [2]University Hospital, Coventry, UK.

**Corresponding Author:** Professor Sudhesh Kumar, Warwick Medical School, University of Warwick, Coventry, CV4 7AL, UK. Tel: +44 2476 574665 Fax: 00 44 247 6574871

Email: sudhesh.kumar@warwick.ac.uk

Obesity is an important risk factor for a number of chronic diseases that impose a huge burden on individuals and society. Recently, it has become clear that adipose tissue secreted products may play a significant role in mediating many obesity related diseases including diabetes. Thus, in addition to being an energy depot, the adipocyte is a highly active cell secreting a plethora of factors with profound effects on a number of organs and systems. The study of these factors and their endocrine effects has become a rapidly evolving and dynamic area of endocrinology. One paradigm for explaining the deleterious effects of adipokines is related to the sheer increase in adipose tissue mass in obesity. When preadipocytes differentiate to become mature adipocytes, they acquire the ability to synthesize numerous proteins, including cytokines, growth factors, and hormones that are involved in overall energy homeostasis and various paracrine effects. In health, these proteins do not spill over significantly into the circulation. In obesity, the massive increase in fat mass leads to a significant increase in circulation of many adipose tissue secreted factors that may have pathogenic effects. For example, the increase in circulating angiotensin II in obesity is related at least in part due to excess adiposity and may mediate hypertension (1). In recent years, adipose tissue has been found to be a major source of many proteins that may directly contribute to vascular injury, diabetes, and atherogenesis (2). These pro-inflammatory adipokines include TNF-α, IL-6, leptin, plasminogen activator inhibitor-1 (PAI-1), angiotensinogen, & resistin, amongst many others. In contrast, the adipokine adiponectin confers protection against inflammation, atherogenesis and obesity-linked insulin resistance.

A recent addition to the now formidable list of contenders as a pivotal factor in mediating obesity driven diabetes is retinol binding protein 4 (RBP4). RBP4 has been

proposed to be a marker for diabetes and also potentially a target for therapy. It is still too early to determine whether it will fulfil this promise. What do we know about RBP-4 so far?

RBP4 (retinol binding protein-4) is a protein that is the specific carrier for retinol (vitamin A alcohol) in the blood. It is not a recently described protein, one of the earliest descriptions appeared back in 1987 (3). It is one of a large number of proteins solubilize and stabilize their hydrophobic and labile metabolites of retinoids in aqueous spaces both in extra and intra-cellular spaces (4). RBP4 is normally bound in the circulation to transthyretin. Its physiological function appears to be to bind retinol and prevent its loss through the kidneys. Deficiency of RBP-4 in humans has been described in a girl with intermittent orange discoloration of her palms, soles, and face and with carotenemia associated with persistently low levels of both vitamin A and serum-specific retinol-binding protein. It is also associated with night vision problems (5). There is, as yet, no report of any change in RBP-4 levels or insulin sensitivity with changes in dietary Vitamin A. Although, RBP4 was known as the binding protein for retinol, that it is secreted by adipocytes has only recently been discovered (6). Abel *et al*, studied GLUT-4 knock-out mice and suggested that adipose tissue serves as a glucose sensor and modulates systemic glucose metabolism through release of a substance in response to decreased intracellular glucose concentrations (6, 7). Further transgenic mice studies manipulating GLUT4 expression has shown corresponding inverse changes in RBP4 adipose tissue expression and circulating RBP4 levels (8, 9). These studies demonstrated a mechanism by which adipocytes can modulate glucose metabolism in muscle. There are multiple mechanisms described by which RBP4 can effect changes in glucose utilisation. Retinol-dependent mechanisms may utilise different retinol isomers to

affect insulin sensitivity and insensitivity (8, 10); whilst retinol independent mechanisms may affect metabolism through cell surface receptors (8). The cell-surface protein megalin (gp320) is the only RBP4 receptor known so far, but the reported binding affinity is low. However, RBP4 binds to a wide range of retinoids and is likely to transport other lipophilic molecules that may mediate its effect on glucose (8, 9).

A potential link between RBP4 and human diabetes was suggested by a report that revealed that RBP4 was elevated in insulin-resistant mice and humans with obesity and T2DM (8). RBP4 levels were normalized by rosiglitazone, establishing a link with insulin sensitivity. Studies in transgenic rodent models showed overexpression of human RBP4 or injection of recombinant RBP4 induced insulin resistance in mice, whereas RBP4 knock-out mice showed enhanced insulin sensitivity. RBP4 was shown to induce expression of the gluconeogenic enzyme PEPCK (phosphoenolpyruvate carboxykinase) and decreased insulin signaling in muscle. Thus, Yang et al. concluded that RBP4 is an adipocyte-derived 'signal' that may contribute to the pathogenesis of T2DM (8). Therefore, lowering RBP4 has been proposed as a new potential therapy for diabetes. There is already a drug known to lower RBP-4. Fenretinide, a synthetic retinoid that increases urinary RPB4 excretion, which reduces serum RBP4 levels and improves insulin action in obese mice, may have therapeutic potential in T2DM. It is currently being evaluated in trials for, basal cell carcinoma, childhood leukemia and ovarian cancer (11).

Not all proteins shown to be relevant to diabetes in rodent models have proved their role in human diabetes. Indeed, this field of research is littered with examples of discrepancies between murine and human studies (12-13). Whilst the foregoing account would suggest a major role for RBP4 in diagnosis and treatment of human

diabetes, more recent reports have, however, not been so encouraging at least for human diabetes. For example, Janke *et al,* (7) suggest that in human abdominal subcutaneous (AbdSc) adipose tissue RBP4 mRNA is downregulated in obese women, whilst circulating RBP4 concentrations remain similar in lean, overweight, and obese women. They suggest methodological differences including techniques used in the measurement as well as differences in the population studied may account for the discrepancies (7). A more recent report examining different techniques for the measurement of RBP4 protein Graham *et al,* casts light on some of the pitfalls in the interpretation of various studies. They demonstrated that most ELISAs undervalue human serum RBP4 levels, and furthermore, sub-optimal recognition of full length RBP4 and poor linearity of dilution also limited assay utility (14). On the basis of his studies, he recommends use of quantitative Western blotting using antibodies to full length RBP4 as the method of choice where possible. This is however, clearly not a viable option for large scale studies. Thus, whilst the early studies clarified a potentially interesting role for RBP4 in glucose metabolism, further studies suggest differences between rodent and human physiology and also significant redundancy in the system as clearly a number of other factors may also play a role.

In the current issue of JCE & M, Yao-Borengasser and co-workers have evaluated RBP4 in human subjects and also report effects of pioglitazone on RBP4 mRNA in AbdSc adipose tissue. From their findings it is clear that whilst the human adipocyte contributes to the production of RBP4, neither AbdSc RBP4 mRNA expression nor circulting RBP-4 levels show any correlation with body mass index. Insulin sensitive subjects showed a tendency to lower RBP4 compared to insulin resistant subjects, but this was not statistically significant **(15).** This observation would cast doubt on the utility of this protein as a biomarker to identify those at risk

for diabetes, at least when used on its own. The authors also identified positive correlations between RBP4 and CD68 in adipose tissue suggesting a role for RBP4 in inflammation, which may have local affects with adipose tissue rather than a systemic effect.

Yao-Borengasser *et al,* also investigated the effects of the insulin sensitizer, pioglitazone, on RBP4 expression in subjects with impaired glucose tolerance. Surprisingly, they showed that mRNA expression increased following pioglitazone in both adipose tissue and muscle, which is in direct contrast to the reported effects of rosiglizone treatment on RBP4 mRNA levels in adipose tissue from *Glut4*$^{-/-}$ mice (9, 15). One limitation of this study is that they have not assessed protein expression or secretion by adipocytes following treatment with pioglitazone. Serum RBP4 did not show a rise following pioglitazone. Such findings with TZDs have also been noted with other adipokines (16-20). Thus, understanding the protein expression profile for RBP4 within adipose tissue and its regulation may shed further light of the role of the adipokine. It is possible that other tissues may contribute to serum RBP4 in human subjects.

Whilst understanding the molecular mechanism of action for adipokines has proved to be a challenging area of research, it is clear that a better understanding of their pathophysiology and mechanism of action may lead to both the discovery of new diagnostic tests and potentially more effective clinical therapies. Such advances in medicine would be welcome to help target therapy better and to treat those at highest risk. Clearly, considerable work is required to prove the added value of such new diagnostic tests or therapies over what we have today. For the majority with obesity, it is however, changes in lifestyle that are needed. Reduction in adipose tissue mass leads to favorable changes in adipokine profiles that will in turn assist in mitigating

the malign consequences of obesity. Thus, whilst progress in this field of endocrinology is exciting, clinicians still need to focus on efforts to prevent and treat obesity more effectively.

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