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Plasma retinol-binding protein is not a marker of insulin resistance in overweight subjects: A three year longitudinal study

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Abstract

Objectives: This longitudinal study investigated whether or not plasma retinol-binding protein (RBP), recently referred to as RBP4, was a marker of insulin resistance in overweight subjects.

Methods: We measured anthropometric markers as well as RBP, fasting glucose and insulin in 206 overweight subjects and repeated these measurements 36 months later. Subjects were grouped according to fasting plasma glucose concentration at baseline and 36 months.

Results: Subjects ($n=51$) with a normal basal fasting glucose (<5.6 mmol/L) who developed impaired fasting glucose (IFG) 3 years later (≥5.6 mmol/L) showed a highly significant increase in both fasting insulin and insulin resistance, but importantly no change in plasma RBP. This group had a significant increase in body mass index (BMI). Subjects ($n=101$) with a normal fasting glucose at both baseline (<5.6 mmol/L) and 36 months showed no significant change in fasting insulin, insulin resistance, RBP or BMI. The remaining subjects had impaired basal fasting glucose and were not analysed on a group-wise basis. Overall, RBP correlated significantly, but inversely, with anthropometric measures, but not with fasting glucose, insulin or insulin resistance.

Conclusions: This is the first report of a long-term longitudinal study on RBP and the major finding is that subjects who developed insulin resistance showed no change in plasma RBP. On the basis of our results we consider that RBP cannot be construed as a marker of insulin resistance in overweight humans.
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Keywords: Retinol-binding protein; Plasma; Insulin resistance; Obesity; Diabetes; Glucose; Insulin

Introduction

Retinol-binding protein (RBP), recently referred to as RBP4, is the major carrier of retinol in the circulation in a complex with transthyretin. It is predominantly synthesised in the liver with a lesser amount derived from adipocytes [1] and this, in part, has led to the recent suggestion that RBP may be a newly discovered adipocytokine [2] with a potential link between cardiovascular risk and insulin sensitivity [3]. The expression of RBP was elevated in GLUT4 glucose transporter knockout mice which normalised following treatment with rosiglitazone, a thiazolidinedione insulin sensitizing agent, while transgenic over expression of human RBP or injection of recombinant RBP in normal mice caused insulin resistance [4]. In humans there are conflicting reports as to whether or not plasma RBP is linked to insulin resistance. Some investigators have found circulating RBP levels correlate positively and significantly with body mass index (BMI) and insulin resistance [3,5–7], whereas we and others have found no relationship between circulating RBP and insulin resistance [8–12].

There are no long-term longitudinal studies investigating plasma levels of RBP in subjects who develop insulin resistance. Here we show, for the first time, that overweight subjects with a normal basal fasting glucose and insulin levels who, after 3 years, develop significant insulin resistance show no change in

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circulating RBP levels. On the basis of these findings we consider that RBP cannot be construed as a marker of insulin resistance in overweight humans.

Methods

Subjects

Plasma was collected from 206 consenting overweight adults (78 males and 128 females, BMI >25 kg/m^2) half of whom were selected from the database of the lipid outpatient clinic at our hospital and half by recruitment from newspaper advertisements. They were all classified as not suffering from diabetes mellitus (fasting glucose <7.0 mmol/L) [13] and throughout the study subjects remained on their normal medication. Fasting blood was collected in fluoride and EDTA tubes between 0800–1000 h on two occasions 36 months apart and plasma separated within 60 min. Plasma for RBP and insulin was separated and stored at −40 °C until analysed. The study was approved by the Canterbury Ethics Committee, Christchurch, New Zealand.

Analytical

Retinol-binding protein was batch analysed by a sandwich ELISA using microtitre plates coated with rabbit anti-RBP (Dakopatts, Denmark). Following incubation of authentic RBP standard or diluted plasma, bound RBP was detected with RBP monoclonal antibody 17G10 and antimouse Ig-peroxidase as described previously [8], allowing a wider analytical range. The basal and 36 month samples from each subject were analysed in the same assay in order to avoid between assay variations. The within assay variation for RBP was less than 10.6%. Glucose was measured using an Aeroset analyser (Abbott Laboratories, IL, U.S.A.) and native insulin using an immunoassay analyser (IMX, Abbott Laboratories, IL, U.S.A). The within and between assay variations for insulin were 5% and 6% respectively. The within and between assay variations for glucose were both <5%. Glucose was measured promptly and insulin was measured within 4 weeks of collection. Insulin resistance (HOMA IR) was calculated using the HOMA-CIGMA model [14] and data analysis was carried out using the statistical package SPSS version 11.5 (SPSS Inc. Chicago, IL, U.S.A.) and Sigmastat 3.1 software (Systat Software Inc. Point Richmond, CA, U.S.A.). Comparison within individuals was by the paired *t*-test and the association between RBP and other variables was calculated using the Spearman Rank Correlation coefficient. Data for correlations was also log transformed.

Table 1
Descriptive and categorical statistics of the 4 study groups

	Group 1	Group 2	Group 3	Group 4
Age ± S.D.	47.9 ± 10.1	50.3 ± 9.4	52.6 ± 6.9	52.5 ± 8.8
Gender (M/F)	31/70	22/29	20/20	5/9
Ethnicity Caucasian (%)	88.8	100	94.9	85.7
BMI ± S.D. (kg/m^2)	33.5 ± 5.5	32.6 ± 4.5	32.6 ± 5.3	35.3 ± 7.1
ATP III criteria (%)	29.9	49.0	59.0	71.4
Hypertensive medication (%)	19.6	37.3	35.9	35.7
Lipid lowering medication (%)	28.0	47.1	41.0	21.4
Current smokers (%)	9.3	19.6	10.3	7.1
RBP ± S.D. (mg/L)	33.5 ± 5.5	32.6 ± 4.5	33.5 ± 10.7	28.1 ± 8.9

Group 1 had normal fasting glucose (<5.6 mmol/L) at baseline and at 36 months.

Table 2
Longitudinal analysis of group 2 subjects

	Baseline	36 months	*p* value
Fasting glucose (mmol/L)	5.21 ± 0.24	5.87 ± 0.28	<0.00001
Fasting insulin (pmol/L)	68.46 ± 36.33	93.44 ± 51.98	0.0001
HOMA IR	2.65 ± 1.43	4.10 ± 2.38	<0.0001
RBP (mg/L)	31.90 ± 16.38	30.61 ± 10.07	0.492
BMI (kg/m^2)	32.64 ± 4.52	33.06 ± 4.68	0.025

Subjects with normal fasting glucose at baseline (<5.6 mmoL/L) and who developed impaired fasting glucose at 36 months (≥5.6 mmol/L). Comparisons were by the paired *t*-test. Mean ± S.D., n=51.

Results

Subjects were grouped according to fasting glucose concentration at baseline and at 36 months. Table 1 shows descriptive and categorical features of the 4 study groups. They were not significantly different with respect to age or BMI. Table 1 also shows the percentage of subjects in each group with 3 or more components of the metabolic syndrome. Those in group 1 show an expected lower frequency of these components and this is reflected by the lower use of both anti-hypertensive and lipid lowering medication in this group.

Table 2 shows those subjects (n=51) with an initial normal fasting glucose (<5.6 mmol/L) who developed impaired fasting glucose (IFG) at 36 months (≥5.6 mmol/L). Glucose, fasting insulin, HOMA IR and BMI all increased significantly, whereas there was no change in RBP. Table 3 shows the subjects (n=101) with normal plasma glucose at both baseline and 36 months (<5.6 mmol/L). There were no significant changes in fasting insulin, HOMA IR or RBP although there was a small but highly significant change in fasting glucose levels. The remaining subjects (groups 3 and 4, n=40 and 14 respectively) all had impaired basal fasting glucose. Of these 14 had an improvement in fasting glucose and the remainder showed either no change or worsening fasting glucose. These groups were not analysed extensively due to their relatively small size (s) although RBP in group 3 increased modestly from 33.5 ±

Table 3
Longitudinal analysis of group 1 subjects

	Baseline	36 months	*p* value
Fasting glucose (mmol/L)	4.94 ± 0.30	5.10 ± 0.31	<0.00001
Fasting insulin (pmol/L)	67.17 ± 53.29	65.74 ± 45.80	0.661
HOMA IR	2.45 ± 1.90	2.52 ± 1.80	0.593
RBP (mg/L)	29.22 ± 12.39	30.67 ± 11.74	0.244
BMI (kg/m^2)	33.52 ± 5.52	33.23 ± 5.39	0.302

Subjects with normal fasting glucose (<5.6 mmoL/L) at both baseline and 36 months. Comparisons were by the paired *t*-test. Mean ± S.D., n=101.

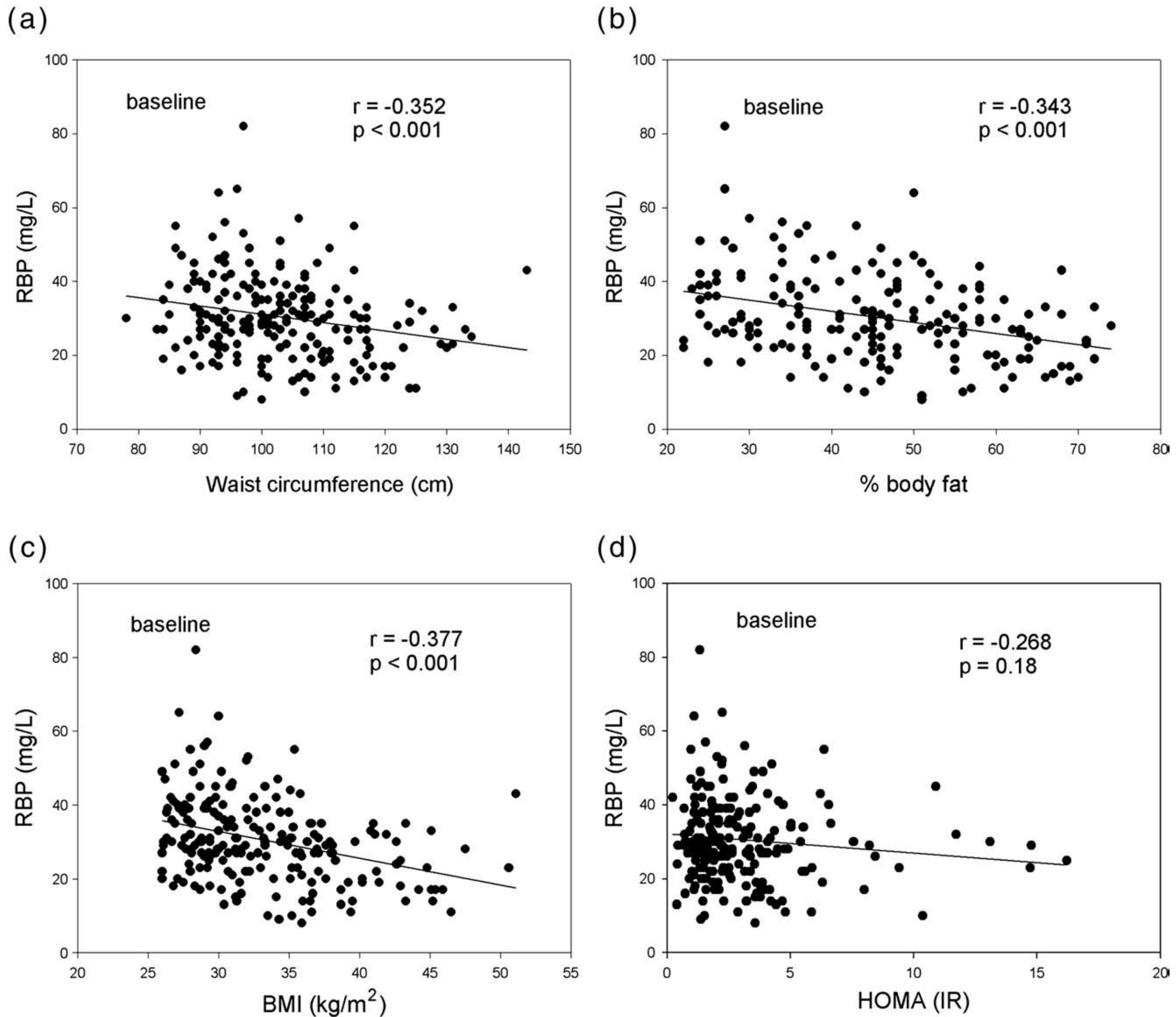

Fig. 1. Cross-sectional correlation of plasma retinol-binding protein (RBP) levels with waist circumference (a); percentage (%) body fat (b); body mass index (BMI) (c); and insulin resistance (HOMA IR) (d), at baseline in 206 subjects. The *r* and *p* values were derived following log transformation and adjustment for age and sex.

10.7 mg/L at baseline to 37.0 ± 12.9 after 36 months, $p = 0.04$. Within group 3 there were no significant changes in fasting glucose, insulin, HOMA IR, triglycerides or anthropometric measurements over time.

Cross-sectional baseline correlations between plasma RBP and waist circumference, percentage body fat and BMI are shown in Fig. 1. When all the subjects were considered for analysis there was a significant inverse relationship between RBP and anthropometric markers, but no relationship between RBP and insulin resistance (Fig. 1). Analysis of the 36 month samples also showed a significant inverse relationship between plasma RBP and anthropometric markers and no relationship between circulating RBP and insulin resistance (data not shown). Correlation analysis showed that there was no relationship between changes in plasma RBP and changes in fasting glucose ($r = -0.06$, $p = 0.40$) or changes in the HOMA index ($r = 0.06$, $p = 0.42$).

Discussion

In this 3 year longitudinal study we demonstrated that non-diabetic overweight subjects who developed IFG and significant insulin resistance showed no change in plasma RBP levels. In this group we observed highly significant increases in fasting insulin, insulin resistance assessed by HOMA IR and, to a lesser extent, BMI. However despite these changes we observed no variation in plasma RBP levels. This is evidence that plasma RBP is not a good marker of insulin resistance in overweight subjects.

We investigated overweight subjects as we considered they were more at risk to naturally develop a degree of insulin resistance over time. Indeed 51 subjects of the total study group of 206 developed this trait. An interesting finding in the 101 subjects who had normal fasting glucose levels throughout the entire study was that although fasting insulin, insulin resistance

and BMI did not change they showed a highly significant increase in fasting glucose. This increase indicates that individuals within this group are slowly progressing towards the development of incipient diabetes. This group could be considered an internal control. We grouped subjects according to fasting glucose rather than HOMA IR as it is considered a more stable and precise measurement than a derived index [13].

It is unlikely that assay variation accounts for any of the differences we observed as both the glucose and insulin assays have inter-assay coefficients of variation of less than 5%. For RBP, where the basal and 36 month samples from an individual were assayed together, the within assay variation of the RBP assay was around 10%. We also consider it unlikely that there was appreciable RBP degradation over the time period as samples were stored frozen at −40 °C and the aliquot for RBP analysis was thawed only once. We consider that, like other plasma-binding proteins, RBP is stable over long-term storage conditions [15].

A short longitudinal exercise study found that plasma RBP declined in some subjects along with an improvement in insulin sensitivity [3]. However the possibility remains that this is unrelated to glucose disposal rate as RBP, in addition to being considered an adipocytokine, is also both a negative acute phase reactant and nutritional marker [16]. This reasoning could also apply to the observation that RBP levels decline after surgically-induced weight loss [17] where it was found that there was no correlation between serum RBP and anthropometric measurements, indicators of glucose metabolism or insulin resistance. Similar findings have been reported by Vitkova et al. [18] in women during severe calorie restriction. They reported that *in vitro* RBP adipose gene expression was reduced, as well as circulating RBP, but RBP was considered a marker of nutritional deficit and not insulin resistance during this dietary intervention [18]. Interestingly some of our subjects also showed low RBP levels indicating a degree of vitamin A deficiency, further confounding the issue of RBP as a marker of insulin resistance. A short term (7 day) overfeeding study showed a significant increase in fasting insulin in both lean and obese subjects, but no change in circulating RBP, and additionally no difference in plasma RBP between lean and obese groups [19].

Cross-sectional analysis of the study population of both basal and 36 month data showed a significant negative correlation of plasma RBP levels with anthropometric measurements and no relationship to insulin resistance or components of the metabolic syndrome [20] such as blood pressure, HDL or glucose, although for fasting triglycerides positive and significant correlations were found for both basal and 36 month data sets ($r=0.233$ and $r=0.314$, respectively, $p<0.001$). Log transformation, to eliminate any possible skewed distribution, and adjustment for age and gender did not alter cross-sectional correlations. This was not unexpected as there was no significant difference in age between the 4 groups and we, and others, have shown that there is no apparent difference in plasma RBP levels between the sexes [3,8].

The negative correlation of RBP with BMI and positive correlation with triglycerides present a possible paradox. However the study group was heterogeneous with only 52% of the subjects having three or more components of the metabolic syndrome [20]. Further it is known that the relationship between triglyceride levels and obesity in the metabolic syndrome is complex and non linear [21]. Adipocyte RBP mRNA expression is downregulated in obese subjects which could contribute to lower circulating RBP levels [12] and its role as both a nutritional marker and a negative acute phase reactant could further confound cross-sectional analyses. Our study cohort was overweight and heterogeneous, which could limit our study, whereas many other cross-sectional studies have investigated quite different populations based on ethnicity, age and varying degrees of obesity and consequently there are disparate findings [3–12,18,19,21,22].

The real strength of our study is that it was longitudinal and relatively long-term and focused on changes in fasting glucose concentration. Those subjects who developed IFG and a highly significant increase in insulin resistance, fasting insulin, and to a lesser extent BMI, showed no change in circulating RBP levels. On the basis of these findings we consider that plasma RBP cannot be construed as a marker of insulin resistance in overweight humans.

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