Endokrynologia Polska/Polish Journal of Endocrinology
Tom/Volume 59; Numer/Number 4/2008
ISSN 0423–104X

Retinol-binding protein 4 (RBP-4) levels do not change after oral glucose tolerance test and after dexamethasone, but correlate with some indices of insulin resistance in humans

Stężenia białka wiążącego retinol 4 (RBP-4) u ludzi dodatnio korelują z niektórymi parametrami insulinooporności, ale nie ulegają zmianie w doustnym teście tolerancji glukozy oraz po podaniu deksametazonu

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Abstract

Introduction: Secretory products from adipocytes may contribute to deterioration in glycaemic control and increased insulin resistance (IR). Retinol-binding protein 4 (RBP-4) may increase IR in mice, with elevated levels in insulin-resistant mice and humans with obesity and type 2 diabetes. However, the mechanisms regulating RBP-4 synthesis remain not fully understood. It is not clear whether short-term glucose-induced hyperglycaemia and hyperinsulinaemia as well as glucocorticosteroid-induced increase in IR might be reflected in alterations in serum RBP-4 levels in humans. In order to investigate this, we measured serum RBP-4, glucose and insulin concentrations during 75.0 gram oral glucose tolerance test (OGTT) — Study 1, as well as before and after oral administration of dexamethasone — Study 2.

Material and methods: Both studies included 35 subjects (8 males), age (mean ± SD) 39.1 ± 15.6 years, BMI 35.8 ± 8.7 kg/m^2. Twenty-four of those subjects (5 males), age 38.7 ± 15.1 years, BMI 34.4 ± 8.3 kg/m^2, had 75 gram oral glucose tolerance test (OGTT) — Study 1. Blood samples were taken before (0 minutes), and at 60 and 120 minutes of OGTT. 17 subjects (3 males, 4 subjects with type 2 diabetes), age 43.1 ± 18.1 years, BMI 36.7 ± 9.0 kg/m^2 underwent screening for Cushing's disease/syndrome (Study 2). Dexamethasone was administered in a dose of 0.5 mg every 6 hours for 48 hours. Fasting serum concentrations of RBP-4, glucose and insulin were assessed before (D0) and after 48 hours of dexamethasone administration (D2). IR was assessed by HOMA in all non-diabetic subjects and in subjects participating in study 1 also by Insulin Resistance Index (IRI), which takes into account glucose and insulin levels during OGTT.

Results: Glucose administration resulted in significant increases in insulin and glucose ($p < 0.0001$). There was, however, no change in RBP-4 concentrations (124.1 ± 32 μg/ml at 0 minutes, 123 ± 35 μg/ml at 60 minutes and 126.5 ± 37.5 μg/ml at 120 minutes of OGTT, p = ns). All subjects in Study 2 achieved suppression of cortisol below 50 nmo/l. Dexamethasone administration resulted in an increase in fasting insulin (from 11.6 ± 6.8 to 17.1 ± 7.2 μU/ml; $p = 0.003$), and an increase in HOMA (from 2.73 ± 1.74 to 4.02 ± 2.27; $p = 0.015$), although without a significant change in RBP-4 levels (119 ± 26.8 *vs.* 117.5 ± 24.8 μg/ml, p = ns). RBP-4 correlated with fasting insulin ($r = 0.40$, $p = 0.025$), fasting glucose ($r = 0.41$, $p = 0.02$) and HOMA ($r = 0.43$, $p = 0.015$), but not with IRI ($r = 0.19$, $p = 0.31$). There was, however, only a moderate correlation between HOMA and IRI ($r = 0.49$ [$r^2 = 0.24$]; $p = 0.006$, Spearman rank correlation), while the best correlation was obtained between the product of glucose and insulin levels at 60 min of OGTT and IRI in a non-linear model ($r = 0.94$ [$r^2 = 0.88$]; $p<0.00001$). In subjects who received dexamethasone, a positive correlation between RBP-4 and HOMA ($p = 0.01$) was lost after two days of dexamethasone administration ($p = 0.61$).

Conclusions: RBP-4 levels do not change during oral glucose tolerance test or after a dexamethasone-induced increase in insulin resistance. This implies that it is highly unlikely that RBP-4 is involved in short-term regulation of glucose homeostasis in humans and that it responds to short-term changes in insulin resistance. A moderate correlation between RBP-4 and some insulin resistance indices (HOMA) does not exclude the fact that RBP-4 might be one of many factors that can influence insulin sensitivity in humans. **(Pol J Endocrinol 2008; 59 (4): 305–311)**

Key words: *retinol-binding protein 4, glucose tolerance, dexamethasone, insulin resistance*

Streszczenie

Wstęp: Niektóre substancje syntetyzowane przez adipocyty mogą zwiększać insulinooporność oraz nasilać zaburzenia tolerancji glukozy. Białko wiążące retinol 4 (RBP-4) nasila insulinooporność u myszy, zaś podwyższone stężenia RBP-4 obserwuje się u myszy z insulinoopornością oraz u osób z otyłością i cukrzycą typu 2. Mechanizmy regulujące syntezę RBP-4 nie są do końca poznane. Między innymi nie

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wiadomo czy krótkotrwała hiperglikemia i hiperinsulinemia po podaniu glukozy, jak również zwiększona insulinooporność wyidukowana glukokortykosteroidami mogą w istotny sposób zmienić stężenie RBP-4 w surowicy u ludzi. W związku z tym autorzy artykułu ocenili stężenie RBP-4, glukozy i insuliny w teście doustnego obciążenia glukozą (75 g) (OGTT) — badanie 1, oraz przed i po doustnym podaniu deksametazonu — badanie 2.

Materiał i metody: W obu badaniach uczestniczyło 35 osób (8 mężczyzn) w wieku 39,1 ± 15,6 lat, BMI 35,8 ± 8,7 kg/m^2 (średnia ± SD). Spośród nich u 24 osób (5 mężczyzn) w wieku 38,7 ± 15,1 lat, BMI 34,4 ± 8,3 kg/m^2 wykonano OGTT (badanie 1). Krew pobierano przed (0 minuta) oraz w 60. i 120. minucie OGTT. Badanie 2 objęło 17 osób (3 mężczyzn, 4 osoby z cukrzycą typu 2) poddanych badaniu przesiewowemu pod kątem choroby/zespołu Cushinga w wieku 43,1 ± 18,1 lat, BMI 36,7 ± 9,0 kg/m^2. Deksametazon podawano co 6 godzin w dawce 0,5 mg przez 48 godzin. Stężenia RBP-4, glukozy i insuliny w surowicy oznaczono na czczo przed (D0) i po 48 godzinach podawania deksametazonu (D2). U osób niechorujących na cukrzycę IR oceniano metodą HOMA, zaś w badaniu 1 również według *Insulin Resistance Index* (IRI) obliczanego na podstawie zmian stężeń insuliny i glukozy podczas OGTT.

Wyniki: Podczas OGTT w badaniu 1 wzrosły stężenia insuliny i glukozy ($p < 0{,}001$), przy braku istotnych zmian stężeń RBP-4 (124,1 ± 32 μg/ml w 0 min, 123 ± 35 μg/ml w 60. min i 126,5 ± 37,5 μg/ml w 120. min OGTT, p = ns). W badaniu 2 u wszystkich osób uzyskano supresję stężenia kortyzolu do wartości poniżej 50 nmo/l. Skutkiem podania deksametazonu był wzrost stężeń insuliny na czczo (z 11,6 ± 6,8 do 17,1 ± 7,2 μU/ml; p = 0,003) oraz wzrost współczynnika HOMA (z 2,73 ± 1,74 do 4,02 ± 2,27; p = 0,015). Nie zaobserwowano jednak istotnych zmian stężeń RBP-4 (119 ± 26,8 μg/ml *vs.* 117,5 ± 24,8 μg/ml, p = ns). Stężenia RBP-4 korelowały z insulinemią na czczo (r = 0,40, p = 0,025), glukozą na czczo (r = 0,41, p = 0,02) oraz z HOMA (r = 0,43, p = 0,015), lecz już nie z IRI (r = 0,19, p = 0,31). Stwierdzono obecność korelacji pomiedzy indeksami insulinooporności HOMA i IRI (r = 0.49 [r^2 = 0,24], p = 0,006, współczynnik korelacji rang Spearmana), lecz znacznie silniejszą korelację obserwowano dopiero miedzy IRI a iloczynem stężeń insuliny i glukozy w 60. minucie OGTT (r = 0,94 [r^2 = 0,88]; $p<0{,}0001$). Dopasowany do zależności tych zmiennych model nieliniowy (funkcja kwadratowa) wyjaśniał 88-procentowe zmienności, podczas gdy model liniowy 70%. U osób, które otrzymały deksametazon dodatnia korelacja między steżeniami RBP-4 i HOMA (p = 0,01) nie była już obserwowana po dwóch dniach przyjmowania deksametazonu (p = 0,61).

Wnioski: Stężenia RBP-4 nie zmieniają się w doustnym teście tolerancji glukozy oraz po nasileniu insulinooporności w wyniku podania deksametazonu. Jest zatem mało prawdopodobne, aby RBP-4 odgrywało istotną rolę w regulacji poposiłkowych glikemii oraz miało wpływ na krótkotrwałe zmiany insulinooporności. Umiarkowana korelacja pomiędzy RBP-4 oraz niektórymi wskaźnikami insulinooporności (HOMA) nie wyklucza jednak, że RBP-4 może być jednym z wielu czynników wpływających na regulację insulinooporności u ludzi.
(Endokrinol Pol 2008; 59 (4): 305–311)

Słowa kluczowe: *retinol-binding protein 4, tolerancja glukozy, deksametazon, insulinooporność*

Introduction

Secretory products from adipocytes may contribute to deterioration in glycaemic control and increased insulin resistance (IR), with complications such as type 2 diabetes, atherosclerosis and sympathetic activation [1–3]. Retinol-binding protein-4 (RBP-4) is a protein that is the specific carrier for retinol (vitamin A alcohol) in the blood, described in 1987 [4]. RBP-4 is normally bound to transthyretin in the circulation. Its physiological function appears to be to bind retinol and prevent its loss through the kidneys. Recent studies suggested that RBP-4, an adipose-derived adipocytokine, may also increase IR [5], with elevated levels in insulin-resistant mice and humans with obesity and type 2 diabetes [5, 6]. Genetic deletion of RBP-4 enhances insulin sensitivity, whilst increased serum levels of RBP-4 not only impair insulin signalling in muscle, but also augment hepatic gluconeogenesis [5]. The first aim of our study was to determine whether RBP-4 levels change after short-term glucose-induced hyperinsulinaemia and to determine whether RBP-4 might be involved in short-term, (*i.e.* postprandial) regulation of glycaemia in humans. The second aim of our study was to determine whether there is a correlation between serum RBP-4 concentrations and insulin resistance indices and whether a change in insulin resistance might alter serum RBP-4 levels. It has long been known that glucocorticosteroids cause insulin resistance *in vitro* and *in vivo* [7] primarily due to a post-receptor effect [8]. Hence, we have also endeavoured to determine whether glucocorticosteroid-induced change in insulin resistance might influence serum RBP-4 levels.

Material and methods

The study included 35 subjects (8 males), age (mean ± ± SD) 39.1 ± 15.6 years, BMI 35.8 ± 8.7 kg/m^2. Obese or overweight patients at risk of diabetes or impaired glucose tolerance were recruited either from the Obesity and Metabolic Clinic of the "Polish Mother" Memorial Research Institute (Instytut Centrum Zdrowia Matki Polki w Łodzi) or from a general practice - "Your Family Doctor" Group Practice, Lodz (NZOZ "Twój Lekarz Rodzinny" sp, Socjalna 48, 93–324 Lodz). Twenty-four of those subjects (19 females and 5 males, age 38.7 ± 15.1 years, BMI 34.4 ± 8.3 kg/m^2) had an oral glucose tolerance test (OGTT) performed according to the World Health Organization (WHO) standard [9] — Study 1. Serum samples were taken before (0 minutes) and at 60 and 120 minutes after ingestion of a solution containing 75.0 g glucose.

Seventeen subjects (14 females, 3 males), including 4 persons with type 2 diabetes mellitus (all insulin-treated), age 43.1 ± 18.1 years, BMI 36.7 ± 9.0 kg/m^2, had serum concentration of RBP-4, glucose, insulin and cortisol assessed before (D0) and after 48 hours (D2) of administration of dexamethasone (0.5 mg every 6 hours for 48 ho-

Table I. *Descriptive statistics for the levels of insulin, RBP-4 and glucose during 75 gram oral glucose tolerance test (OGTT) in 24 subjects participating in Study 1. P-value represents the significance level of the Wilcoxon matched-paired test for comparison of distributions of these characteristics in different OGTT time intervals*

Tabela I. *Stężenia insuliny, RBP-4 oraz glukozy podczas 75-gramowego doustnego testu tolerancji glukozy (OGTT) u 24 osób uczestniczących w badaniu 1. Wartość P oznacza istotność statystyczną w teście Wilcoxona dla par zmiennych przy porównaniu ich rozkładów w kolejnych punktach czasowych OGTT*

Parameter	OGTT	Mean	95%CI Mean	Med	SD	Min	Max	P value	
RBP-4 [μg/ml]	*0′*	124.1	(113.0; 135.1)	118.8	32.2	58.0	200.0	*0 ÷ 60′*	*0.57*
	60′	123.0	(108.2; 137.8)	120.9	35.1	72.8	199.0	*60 ÷ 120′*	*0.44*
	120′	126.5	(110.7; 142.3)	129.6	37.5	51.6	200.0	*0 ÷ 120′*	*0.81*
Glucose [mg/dl]	*0′*	90.4	(84.2; 96.7)	85.0	17.0	72.0	150.0	*0 ÷ 60′*	***0.000002***
	60′	158.3	(139.0; 177.7)	152.0	51.8	82.0	297.0	*60 ÷ 120′*	***0.00001***
	120′	120.5	(103.8; 137.2)	113.0	45.6	61.0	295.0	*0 ÷120′*	***0.0001***
Insulin [μU/ml]	*0′*	11.6	(9.1; 14.2)	9.5	7.0	2.1	29.5	*0 ÷ 60′*	***0.000001***
	60′	98.0	(76.47; 119.7)	77.0	59.1	19.7	222.0	*60 ÷ 120′*	*0.13*
	120′	82.6	(63.4; 101.7)	75.3	52.3	11.9	186.0	*0 ÷ 120′*	***0.000001***

urs) during screening for Cushing's disease/syndrome (Study 2). Non-diabetic individuals (n = 31) had insulin resistance assessed according to the HOMA method (where HOMA = fasting insulin concentration [μU/ml] × fasting glucose concentration [mmol/l] / 22.5) [10], while those subjects who had an OGTT (Study 1, n = 24) also had insulin resistance assessed by Insulin Resistance Index (IRI), *i.e.* a method based on changes of glycaemia and insulinaemia during OGTT. IRI was calculated using the formula: 2/[1/(INSp x GLYp)]+1, where INSp and GLYp are the measured insulin and glycaemic areas [11, 12]. Serum RBP-4 was measured by commercial RBP-4 assay kit (Phoenix Pharmaceuticals Inc., intra-assay variation < 5%, inter-assay variation < 14%). Insulin was measured by ELISA (DakoCytomation Ltd, Denmark House, Angel Drove, Ely CB7 4 ET, UK), while plasma glucose was measured by standard hexokinase method.

The study protocol was approved by the Ethics Committee of the Medical University of Lodz (Poland).

Statistical analysis

In both studies, the data were analysed by means of simple descriptive statistics and non-parametric tests of significance - the Wilcoxon matched-paired test for comparison of distributions of particular parameters in different OGTT time intervals. In study 2, the data were also analysed by paired t-test, given the normal distribution of the variables. Associations between RBP-4 concentrations and other parameters were assessed by the Spearman rank correlation method. In all analyses, statistical significance was considered achieved for a value of $p \leq 0.05$. All the calculations were derived by means of Statistica v. 6.0 software.

Results

The main results of Study 1 are presented in Table I. There was a significant increase in glucose and insulin concentrations during OGTT, although without significant change in serum RBP-4 concentration (124 ± 32.2 μg/ml *vs.* 123 ± 35.1 μg/ml and 126.5 ± 37.5 μg/ml at 0, 60 and 120 minutes of OGTT, respectively, p = ns). The results of Study 2 are presented in Table II. Administration of dexamethasone for 48 hours resulted in a significant increase in fasting insulin (11.6 ± 6.8 *vs.* 17.1 ± 7.2 μU/ml, p = 0.003) and HOMA index (2.73 ± 1.74 *vs.* 4.02 ± 2.28, p = 0.015), indicative of an increase in insulin resistance. Again, there was no change in serum RBP-4 concentration (119.0 ± 26.8 μg/ml *vs.* 117.5 ± 24.8μg/ml, p = ns).

Correlation analysis between serum RBP-4 levels and other covariates, analysed jointly for subjects participating in studies 1 and 2, is presented in Table III. There was a moderate but significant positive correlation between RBP-4 and fasting insulin (r = 0.401, p = 0.025, Figure 1A), fasting glucose (r = 0.411, p = 0.022) and HOMA (r = 0.432, p = 0.015, Figure 1B), without significant correlation with other parameters including age, BMI, lipids and IRI. In subjects who received dexamethasone, a positive correlation between RBP-4 and HOMA (r = 0.45, p = 0.01) was lost after two days of dexamethasone administration (p = 0.61).

Simultaneously, however, there was a significant correlation between HOMA and IRI (*i.e.* r = 0.49; p = 0.0002) for Spearman rank correlation coefficient. In this model, however, a variation of HOMA explained only 24% of variation of IRI (Figure 2A). A non-linear (quadratic) model fitted to the mentioned relation between HOMA and IRI explained 43% of the variation

Table II. *Concentrations of RBP-4 and other parameters before (D0) and after 48 hours of administration of dexamethasone (D2) in 17 subjects participating in study 2. Dexamethasone was administered orally in a dose of 0.5 mg every 6 hours for 48 hours. P-value represents the significance level of the Wilcoxon matched-paired test for comparison of distributions of these characteristics before (D0) and after administration of dexamethasone (D2)*

Tabela II. *Stężenia RBP-4 oraz insuliny i glukozy na czczo oraz HOMA przed (D0) oraz po 48 godzinach przyjmowania deksametazonu (D2) u 17 pacjentów uczestniczących w badaniu 2. Deksametazon był podawany w dawce 0,5 mg co 6 godzin przez 48 godzin. Wartość P oznacza istotność statystyczną w teście Wilcoxona dla par zmiennych przy porównaniu ich rozkładów przed (D0) oraz po podaniu deksametazonu (D2)*

Parameter	D0 (mean ± SD)	D2 (mean ± SD)	*P value*
RBP-4 [μg/ml]	119.0 ± 26.8	117.5 ± 24.8	*0.76*
Glucose [mg/dl]*	92.9 ± 21.0	93.8 ± 19.8	*0.62*
Insulin [μU/ml]	11.6 ± 6.8	17.1 ± 7.2	***0.003***
HOMA** (glucose [mmol/l]·insulin [μU/ml]/22.5)	2.73 ± 1.74	4.02 ± 2.28	***0.015***

*In order to convert mg/dl into mmol/l for plasma glucose, divide by 18

**HOMA calculated for non-diabetic individuals (n = 15)

Table III. *Spearman rank correlation coefficients for the association between RBP-4 and other covariates in all subjects participating in Study 1 and Study 2*

Tabela III. *Współczynniki korelacji rang Spearmana między stężeniami RBP-4 oraz innymi zmiennymi analizowanymi łącznie dla wszystkich pacjentów uczestniczących w badaniach 1 i 2*

Parameter	N	r_s	*P value*
Fasting insulin (all non-diabetic subjects)	31	0.401	***0.025***
Fasting glucose	35	0.411	***0.022***
HOMA (all non-diabetic subjects)	31	0.432	***0.015***
IRI	24	0.192	*0.301*
Triglycerides	35	0.079	*0.674*
Total cholesterol	35	–0.232	*0.201*
LDL cholesterol	31	–0.328	*0.095*
HDL cholesterol	31	–0.113	*0.544*
BMI	35	0.203	*0.241*
Age	35	–0.112	*0.522*

(Figure 2B). The strongest correlation was observed between IRI and glucosexinsulin product at 60 minutes of OGTT ($r = 0.94$; $p < 0.00001$, Figure 2C) and glucosexinsulin product at 120 minutes of OGTT ($r = 0.84$; $p < 0.00001$, Figure 2D). In a fitted quadratic model, the product of concentrations of glucose and insulin at 60 minutes of OGTT explained over 88% of the variation in IRI (Figure 2C), while the product of glucose and insulin concentrations at 120 minutes of OGTT explained 74% of the variation. In contrast, the product of fasting glucose and fasting insulin concentrations used for the calculation of the HOMA index was able to explain only 24% or 43% of the variation of Insulin Resistance Index, depending on the model used (Figures 2A and 2B). There was also a positive correlation between IRI and BMI (0.431, $p = 0.015$).

Discussion

Our studies demonstrate that RBP-4 concentrations do not change during the oral glucose tolerance test in humans. This implies that RBP-4 does not seem to be involved in short-term, *i.e.* postprandial regulation of glycaemia in human subjects. Our study also indicates that an increase in insulin resistance, induced by dexamethasone, does not result in any appreciable change in serum RBP-4 levels. To the best of our knowledge, this is the first study in which the direct effects of glucocorticosteroids on serum RBP-4 levels have been assessed in this context in humans. The results of our study are in contrast to the data obtained in rats, where dexamethasone increased serum RBP-4 levels after 7 days of administration of very high doses of dexame-

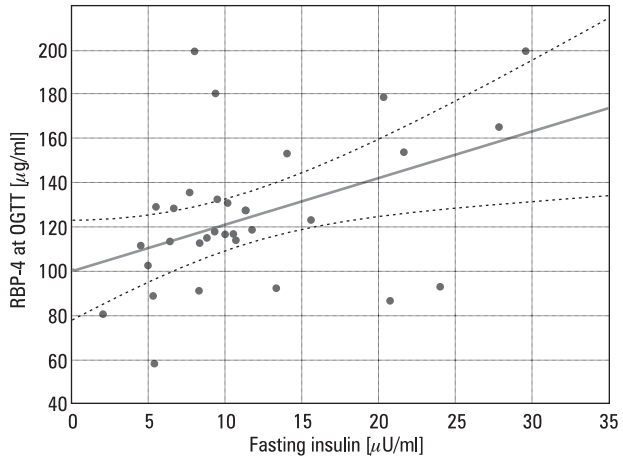

Figure 1A. *Correlation between serum RBP-4 and fasting insulin as assessed by the Spearman rank correlation coefficient (n = 31) (r = 0.401, p = 0.025)*

Rycina 1A. *Korelacja między stężeniami RBP-4 w surowicy krwi a insulinemią na czczo oceniana według współczynnika korelacji rang Spearmana (n = 31; r = 0,401; p = 0,025)*

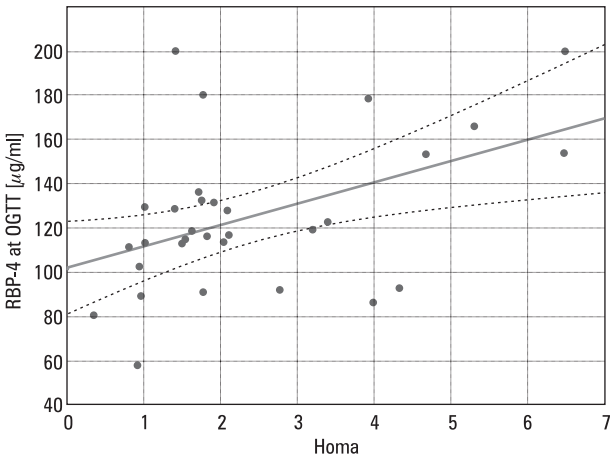

Figure 1B. *Correlation between serum RBP-4 levels (n = 31) and HOMA in individuals not taking insulin (n = 31), r = 0.432, p = 0.015*

Rycina 1B. *Korelacja między stężeniami RBP-4 w surowicy krwi a insulinoopornością ocenianą według wskaźnika HOMA (n = 31; r = 0,432; p = 0,015*

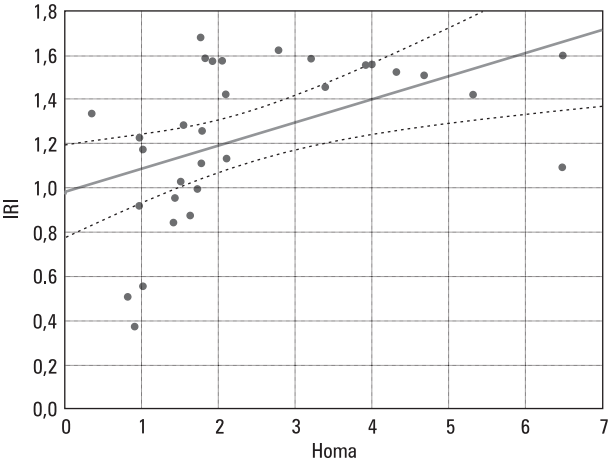

Figure 2A. *Correlation between insulin resistance indices i.e. HOMA and Insulin Resistance Index (IRI) in a linear model (r^2 = 0.24; p = 0.006). This indicates that in a linear model the variability of the HOMA index explains only 24% of the variability of IRI. The HOMA index is calculated as a product of fasting insulin concentration [μU/mL] and fasting glucose concentration [mmol/L] / 22,5 [10], while the IRI method is based on changes in glycaemia and insulinaemia not only in a fasting state, but also at 60 and 120 minutes of 75 gram OGTT. The precise method of IRI calculation is described in [11, 12]*

Rycina 2A. *Zależność pomiędzy wskaźnikiem insulinooporności HOMA i* Insulin Resistance Index *(IRI), w niewielkim tylko stopniu wyjaśniona w modelu liniowym (r^2 = 0.24; p = 0.006). Oznacza to, że zmiany wartości HOMA pozwalają wyjaśnić jedynie 24% zmienności parametru IRI w modelu liniowym. Wskaźnik HOMA jest obliczony według wzoru: stężenie insuliny na czczo [μU/ml] × stężenie glukozy na czczo [mmol/l]/22,5 [10], zaś wskaźnik IRI wykorzystuje ocenę nie tylko glikemii i insulinemii na czczo, lecz również w 60. i 120. minucie doustnego testu tolerancji glukozy (75 gram). Szczegółowy sposób obliczenia wskaźnika IRI opisano w pozycjach piśmiennictwa 11 i 12*

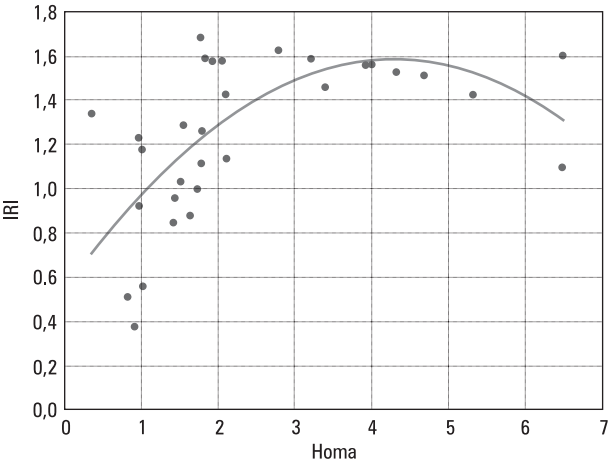

Figure 2B. *Correlation between HOMA and IRI in a non-linear model (r^2 = 0.43; p = 0.00004). In this model the variability of the HOMA index explains only 43% of the variation of IRI*

Rycina 2B. *Zależność między HOMA a IRI w modelu nieliniowym (r^2 = 0,43; p = 0,00004). Oznacza to, że w modelu nieliniowym zmiany wartości HOMA wyjaśnia 43% zmienności parametru IRI*

thasone (0.5 mg/kg/day) [13]. In our opinion, these data might not be applicable to human subjects as this would correspond to enormous doses of dexamethasone (around 40 to 60 mg per day in humans), where the pharmacological effects of such high doses of glucocorticosteroids might be mediated through effects other than insulin resistance. We acknowledge, however, that full assessment of the effects of dexamethasone on serum RBP-4 levels might require RBP-4 measurements after administration of higher doses of dexamethasone, taking into account that maximal doses (*e.g.* used in

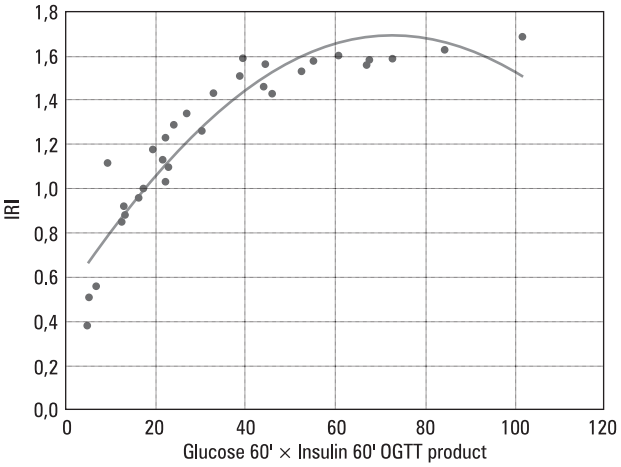

Figure 2C. *Correlation between IRI and the product of glucose [mmol/L] and insulin concentrations [μU/mL] at 60 minutes of the OGTT explained by nonlinear model ($r^2 = 0.88$; $p<0.00001$). In this model the variability of glucose and insulin product at 60 minutes of OGTT explains 88% of the variation of IRI, i.e. to a much greater degree than the HOMA index based on the product of fasting glucose and insulin concentrations*

Rycina 2C. *Silna dodatnia korelacja między IRI a iloczynem stężeń glukozy [mmol/l] i insuliny [μU/ml] w 60. minucie OGTT opisana modelem nieliniowym ($r^2 = 0,88$; $p < 0,00001$). W tym modelu zmienność iloczynu stężeń glukozy i insuliny w 60. minucie OGTT wyjaśnia 88% zmienności parametru IRI, czyli znacznie więcej niż zmienność indeksu HOMA, który opiera się na iloczynie stężeń glukozy i insuliny na czczo*

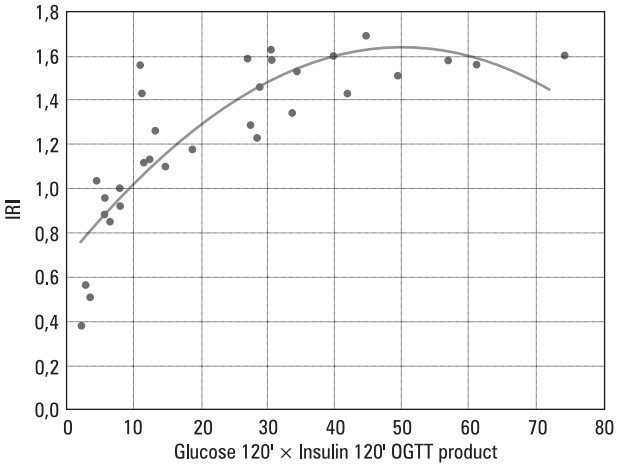

Figure 2D. *Positive correlation between IRI and the product of glucose [mmol/L] and insulin [μU/mL] concentrations at 120 minutes of the OGTT explained by non-linear model ($r^2 = 0.71$; $p<0.00001$). In this model the variability of glucose and insulin product at 60 minutes of OGTT explains 71% of the variation of IRI. Although this is slightly less than at 60 min of OGTT, the product of glucose and insulin concentrations at 120 minutes of OGTT still explains the variability of IRI to a much greater degree that the HOMA index based on the product of fasting glucose and insulin concentrations*

Rycina 2D. *Dodatnia korelacja między IRI a iloczynem stężeń glukozy [mmol/l] i insuliny [μU/ml] w 120. minucie OGTT opisana modelem nieliniowym ($r^2 = 0,71$; $p < 0,00001$). W tym modelu zmienność iloczynu stężeń glukozy i insuliny w 120 minucie OGTT wyjaśnia 71% zmienności parametru IRI. Oznacza to, że zmienność iloczynu stężeń insuliny i glukozy w 120. minucie OGTT wyjaśnia w nieco mniejszym stopniu zmienność IRI niż zmiany iloczynu stężeń glukozy i insuliny w 60. minucie OGTT, ale i tak wyjaśnia to zmienność IRI w dużo większym stopniu zmienność indeksu HOMA, który opiera się na iloczynie stężeń glukozy i insuliny na czczo*

cases of cerebral oedema) generally do not exceed 24 mg/24 hours.

So far, several studies [6, 14–16] have demonstrated raised RBP-4 levels in non-pregnant subjects with IR and/ /or type 2 diabetes. However, the assessment of the relationship between RBP-4 and IR yielded very discrepant results. Graham et al. [6] demonstrated a relationship between serum RBP-4 and insulin resistance. On the other hand, other researches [15, 16] have not found a correlation between serum levels of RBP-4 and insulin resistance indices. Some authors [17] have suggested the lack of a correlation between RBP-4 and insulin sensitivity, but a positive correlation between serum RBP-4 and some inflammatory markers. Interestingly, this is in contrast to other studies in which acute (but not chronic) inflammation was associated with decreased RBP-4 concentrations [18]. In our opinion, several factors may contribute to such diverse results as to the correlation between RBP-4 and IR. Graham et al. [19] have recently speculated that the lack of a correlation between RBP-4 and IR in some studies may result from problems with some RBP-4 ELISA assays, where high RBP-4 levels might be reported as falsely low as a result of antibody saturation. This applied particularly to one of the assay systems (Scimedx, Denville, NJ, USA), where RBP-4 levels were slightly lower in insulin-resistant subjects in comparison to controls, and increased following further dilutions of the sera. The authors suggest, however, that there is a possibility that the results of other studies may also be fraught with similar methodological problems, when concentrations of RBP-4 are so high that they fall significantly outside the linear range of the standard curve of the particular assay system.

Further problems may pertain to the different methodology of the assessment of IR. For instance, Graham et al. [6] assessed IR by the means of a clamp study *i.e.* according to the glucose disposal rate during the last 30 minutes of the clamp, as recommended by DeFronzo et al. [20], although the correlation with fasting insulin was also assessed. Takashima et al. [15] assessed the correlation between RBP-4 and fasting insulin, Erikstrup et al. [16] assessed IR by means of HOMA index, while Borengasser-Yao et al. [17] assessed insulin sensitivity by means of an insulin-modified frequently sampled iv

glucose tolerance test using 11.4 g/m^2 — glucose and 0.04 U/kg — insulin, according to the method described by Bergman et al. [21].

Our results demonstrate a moderate correlation between RBP-4 and HOMA, but not with IRI, *i.e.* with a static (based on fasting glucose and insulin), but not a dynamic (*i.e.* based on both fasting and post-glucose load levels) index of IR. Such a situation is not surprising, given that the lack of any significant change of serum RBP-4 concentrations contrasted with increases in glucose and insulin levels during OGTT. Similar to the study of Graham et al. [6], we have also found a correlation between RBP-4 and fasting insulin. Interestingly, however, the correlation between HOMA index (which is based on a product of fasting glucose and fasting insulin concentrations) and IRI, although significant, is not particularly strong (Figure 2A). This is improved in a non-linear model (Figure 2B), but it only becomes highly significant when a product of glucose and insulin concentrations at 60 minutes of OGTT, rather than a HOMA index, is used in a non-linear model (Figure 2C). In addition, a linear model for the dependence between HOMA and IRI explains only 24% of the variation of the Insulin Resistance Index ($r^2 = 0.24$; $p = 0.006$, Figure 2A). Hence, it is highly likely that some methods used to calculate IR may fail to demonstrate a relationship between RBP-4 and insulin sensitivity because there is only a moderate correlation between the methods of the assessment of insulin resistance based on fasting glucose and insulin concentrations *vs.* the methods that account for glucose and insulin excursions after glucose stimulation. Other authors [22] also comment on the relative weakness of a correlation between HOMA and insulin resistance indices based on OGTT. Furthermore, Diamanti-Kandarakis et al. [23] demonstrated rather a weak correlation between HOMA and QUICKI (the quantitative insulin sensitivity check index) and the assessment of insulin sensitivity by a euglycaemic clamp technique in women with polycystic ovary syndrome. The authors concluded that mathematical indices should be applied with caution in different insulin-resistant populations and should not be considered a priori equivalent to the euglycaemic clamp technique, *i.e.* to the "golden standard" for the assessment of insulin sensitivity.

In summary, our study demonstrates a moderate correlation between serum RBP-4 concentrations and fasting insulin as well as the HOMA index. There was, however, no change in serum RBP-4 concentrations after OGTT or after a dexamethasone-induced increase in insulin resistance. This implies that, even though RBP-4 might be one of many factors potentially involved in regulation of insulin sensitivity in humans, the changes of serum RBP-4 concentrations are unlikely to influence postprandial glycaemia and are unlikely to be involved in the pathogenesis of glucocorticosteroid-induced insulin resistance.

References

1. Kershaw EE, Flier JS. Adipose tissue as an endocrine organ. J Clin Endocrinol Metab 2004; 89: 2548–2556.
2. Spranger J, Kroke A, Möhlig M et al. Adiponectin and protection against type 2 diabetes mellitus. Lancet 2003; 361: 226–228.
3. Eikelis N, Schlaich M, Aggarwal A et al. Interactions between leptin and the human sympathetic nervous system. Hypertension 2003; 41: 1072–1079.
4. Beetham R, Silver A, Dawnay A. Urinary albumin and retinol-binding protein in diabetes. Clin Chem 1987; 33: 713.
5. Yang Q, Graham TE, Mody N et al. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. Nature 2005; 436: 356–362.
6. Graham TE, Yang Q, Bluher M et al. Retinol-binding protein and insulin resistance in lean, obese, and diabetic subjects. N Engl J Med 2006; 354: 2552–2563.
7. Ward AM, Fall CH, Stein CE et al. Cortisol and the metabolic syndrome in South Asians. Clin Endocrinol 2003; 58: 500–505.
8. Rizza RA, Mandarino LJ, Gerich JE. Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor detect of insulin action. J Clin Endocrinol Metab 1982; 54: 131–138.
9. WHO consultation: Definition, Diagnosis, and Classification of Diabetes Mellitus and Its Complications Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes Mellitus. Geneva, WHO/NCD/ /NCS/99.2, World Health Org., 1999.
10. Matthews DR, Hosker JP, Rudensky AS et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28: 412–419.
11. Belfiore F, Iannello S, Volpicelli G. Insulin sensitivity indices calculated from basal and OGTT-induced insulin, glucose, and FFA levels. Mol Genet Metab 1998; 63: 134–141.
12. Matsuda M, DeFronzo R. Insulin sensitivity indices obtained from oral glucose tolerance testing. Diabetes Care 1999; 9: 1462–1470.
13. Georgieff MK, Radmer WJ, Sowell AL et al. The effect on glucocorticoids on serum, liver, and lung vitamin A and retinyl ester concentrations. J Pediatr Gastroenterol Nutr 1991; 13: 376–382.
14. Cho YM, Youn BS, Lee H et al. Plasma retinol-binding protein-4 concentrations are elevated in human subjects with impaired glucose tolerance and type 2 diabetes. Diabetes Care 2006; 29: 2457–2461.
15. Takashima N, Tomoike H, Iwai N. Retinol-binding protein 4 and insulin resistance. N Engl J Med 2006; 355: 1392–1393.
16. Erikstrup Ch, Mortensen OH, Petersen BK. Retinol-binding protein 4 and Insulin resistance. N Engl J Med 2006; 355: 1394–1395.
17. Yao-Borengasser A, Varma V, Bodles AM et al. Retinol binding protein 4 expression in humans: Relationship to insulin resistance, inflammation, and response to pioglitazone. J Clin Endocrinol Metab 2007; 92: 2590–2597.
18. Rosales JF, Ritter SJ, Zolfaghari R et al. Effects of acute inflammation on plasma retinol, retinol-binding protein, and its mRNA in the liver and kidneys of vitamin A-sufficient rats. J Lipid Res 1996; 37: 962–971.
19. Graham TE, Wason CJ, Bluher M et al. Shortcomings in methodology complicate measurements of serum retinol binding protein (RBP4) in insulin resistant human subjects. Diabetologia 2007; 50: 814–823.
20. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 1979; 237: E214–E223.
21. Bergman RN, Prager R, Volund A et al. Equivalence of the insulin sensitivity index in man derived by the minimal model method and the euglycemic glucose clamp. J Clin Invest 1987; 79: 790–800.
22. Gunczler P, Lanes R. Relationship between different fasting-based insulin sensitivity indices in obese children and adolescents. J Pediatr Endocrinol Metab 2006; 19: 259–265.
23. Diamanti-Kandarakis E, Kouli C, Alexandraki K et al. Failure of mathematical indices to accurately assess insulin resistance in lean, overweight, or obese women with polycystic ovary syndrome. J Clin Endocrinol Metab 2004; 89: 1273–1276.