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Association of Obesity, Diabetes, Serum Lipids and Blood Pressure Regulates Insulin Action

†G. ŠINDELKA, J. ŠKRHA, M. PRÁZNÝ, T. HAAS

Third Department of Internal Medicine, First Faculty of Medicine, Charles University Prague, Czech Republic

† *Dr. Gustav Šindelka died tragically on September 23, 2001*

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Summary

Insulin resistance is present in patients with Type 2 diabetes mellitus as well as in obese patients without diabetes. The aim of our study was to compare insulin action in diabetic and control persons with or without obesity and to evaluate the influence of serum cholesterol, serum triglyceride and blood pressure on metabolic variables of insulin action. We examined 42 Type 2 diabetic patients and 41 control persons with body mass index (BMI) from 21.1 to 64.5 $kg.m^{-2}$, and 33 to 71 years old. The isoglycemic hyperinsulinemic clamp technique was performed at an insulin infusion rate of 1 $mU.kg^{-1}.min^{-1}$ during 120 min. We evaluated the metabolic clearance rate of glucose (MCR_G, $ml.kg^{-1}.min^{-1}$) as the most important indicator of insulin action by isoglycemic clamp. The Pearson's correlation and multiple regression models were used to compare studied factors with the insulin action. We found following predictors of insulin resistance expressed in the relationship with MCR_G: BMI ($r = -0.68$, $p<0.001$), plasma glucose concentration ($r = -0.66$, $p<0.001$), cholesterol ($r=-0.55$, $p<0.001$), triglycerides ($r = -0.54$, $p<0.001$) and mean blood pressure ($r = -0.38$, $p<0.01$). From the multiple regression analysis we conclude that obesity may have even greater influence on the insulin action than diabetes mellitus itself.

Key words
Obesity • Diabetes mellitus • Serum lipids • Blood pressure

Introduction

Obesity is a highly prevalent disorder associated with decreased life expectancy and increased morbidity because of its combination with a variety of other disorders or diseases including hyperglycemia, hyperlipidemia, hypertension, and consequently cardiovascular disease carrying significant economic costs (Guven *et al.* 1999). Insulin resistance depends on the impairment of insulin action at the receptor, post-receptor or both levels (Reaven 1988). In obesity, it likely involves a rate-limiting step in skeletal muscle glucose metabolism, implying a primary defect. Other studies suggest that insulin resistance is an adaptive secondary response to prevent further weight gain (Guven *et al.* 1999). Apart from obesity, insulin resistance is present in a variety of pathological as well as physiological states (Reaven 1988). It is associated with Type 2 diabetes

Fax+4202 24920590
E-mail: physres@biomed.cas.cz http://www.biomed.cas.cz/physiolres

mellitus (Olefsky 1995), arterial hypertension (Rocchini 1995) or hypertriglyceridemia (Škrha *et al.* 1994, Widén *et al.* 1995). Some relationships were overviewed in this field (Olefsky 1995, Zemel 1995).

In the present study, we examined the insulin action by isoglycemic hyperinsulinemic clamps in a number of patients with Type 2 diabetes with different degrees of obesity associated with dyslipidemia and with normal or only mildly elevated blood pressure. The aim was to estimate the role of separate factors causing insulin resistance by using stepwise regression analysis.

Subjects and Methods

Subjects

We examined 42 diabetic patients (mean age 52 years, range 34-71 years) 25 of whom were on a dietary regimen only, 13 were treated with sulphonylureas (glipizide) and 4 with metformine for longer than one year prior to the examination. The whole cohort of diabetic patients was subdivided according to their body mass index (BMI) into non-obese subjects (D1, n=11, BMI<26 kg.m^{-2}), those with overweight (D2, n=13, BMI 26-30 kg.m^{-2}) and obese patients (D3, n=18, BMI>30 kg.m^{-2}). Diagnosis of Type 2 diabetes mellitus was confirmed by fasting morning glycemia and/or by the oral glucose tolerance test (oGTT). The control group consisted of 41 non-diabetic patients (mean age 39 years, range 21-71 years). This group was subdivided similarly into non-obese persons (C1, n=14, BMI<26 kg.m^{-2}), those with mild overweight (C2, n=10, BMI 26-30 kg.m^{-2}) and obese control subjects (C3, n=17, BMI>30 kg.m^{-2}). None of the control persons had positive family history of diabetes and did not use any drugs.

Three blood pressure measurements were performed in all persons on non-dominant arm using manual sphygmomanometer and the mean value was used for the calculation. Fifteen diabetic patients had slightly increased blood pressure values above 140/90 mm Hg whereas the remaining subjects were normotensive. Similarly, a slightly higher blood pressure was found in 5 out of 41 control persons. No pharmacological treatment by antihypertensive drugs was used in any of the diabetic or control persons with mild hypertension. The results of systolic and diastolic blood pressure in separate groups of diabetic and control subjects are given in Table 1.

Informed consent was obtained from all persons and the study protocol was prepared in accordance with the Helsinki Declaration and was approved by the Ethics Committee of our Medical Faculty.

Tab. 1. Clinical and laboratory characteristics of diabetic patients without obesity (D1, n=11), with mild overweight (D2, n=13) and with obesity (D3, n=18) and in control persons without obesity (C1, n=14), with mild overweight (C2, n=10) and with obesity (C3, n=17).

	Diabetic patients			**Control persons**		
	D1 (n=11)	D2 (n=13)	D3 (n=18)	C1 (n=14)	C2 (n=10)	C3 (n=17)
BMI (kg.m^{-2})	24.4±1.5	28.4±1.2	36.4±5.6	23.6±1.0	27.9±1.7	41.4±10.8
G_o (mmol.l^{-1})	8.9±2.5^a	8.2±3.4^a	10.6±3.9^a	4.5±0.7	4.5±0.5	4.9±0.8
Cholesterol (mmol.l^{-1})	7.0±1.1^a	6.9±1.7^b	6.8±1.0^b	4.5±0.9	5.4±1.1	5.8±1.0
TG (mmol.l^{-1})	2.6±0.5^a	3.5±2.1	4.2±2.7^b	0.9±0.4	2.5±1.7	2.8±1.1
SBP (mm Hg)	136±11^b	135±16	136±17	124±23	139±21	135±15
DBP (mm Hg)	85±8	81±10	84±9	78±14	83±11	85±10
MBP (mm Hg)	100±9	99±11	101±10	91±14	101±13	101±12

Body mass index (BMI), baseline plasma glucose (G_o), total serum cholesterol (CH), serum triglycerides (TG), systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean blood pressure (MBP). Results are shown as means ± S.D. Statistical significance as compared to healthy controls of the respective group: $^a p<0.01$, $^b p<0.05$.

Methods

All subjects were examined after an overnight fast. The patients on oral antidiabetic drugs received their last dosage 12 hours before the examination.

The hyperinsulinemic isoglycemic clamp was performed as described previously (Flier *et al.* 1992). Briefly, flexible cannule was inserted into the forearm vein to obtain blood samples for determination of basal insulin, plasma glucose and potassium concentrations. The cannule was then connected to the infusion module of Biostator (GCSII, Elkhart, Indiana, USA) to administer the insulin solution (160 units of Actrapid HM^R, Novo-Nordisk, in 500 ml 0.9 % sodium saline solution), 40 % glucose solution and wash-out sodium saline solution (0.9 % w/v). At the same time, 7.5 % potassium chloride solution diluted with physiological saline solution 1:4 was supplied by perfusor (Infusor Secura FT, B. Braun, Germany) into another channel of the cannule at a rate of 0.1±0.05 ml.min^{-1} to maintain basal potassium levels. The rate of this infusion was adjusted according to the results of repeatedly determined serum potassium concentration. A double-lumen catheter was inserted into the contralateral forearm for continuous blood glucose determination. A third cannule was inserted into a wrist vein for collecting blood samples for biochemical estimations. Two blood samples for insulin determination were collected during the last twenty minutes of the clamp. After 30 min washout period, hyperinsulinemic isoglycemic clamp was performed with Biostator (mode 7:1) during 120 min using a constant insulin infusion rate (1 mU.kg^{-1}.min^{-1}) (De Fronzo *et al.* 1979). A glucose solution (40 % w/v) was sampled to maintain blood glucose levels at a basal value. During the clamp, blood glucose was repeatedly determined by glucose analyzer (ESAT 6660-2, Melsungen, Germany). The coefficient of variation for blood glucose values during the clamp was below 10 %. Two blood samples were withdrawn in the last 20 min of the clamp for insulin (IRI) determination.

The following variables of the clamps were used for evaluation: plasma glucose (G_c) and insulin (I_c) concentrations, glucose disposal rate (M) characterizing the sum of insulin-dependent and non-insulin-dependent transport of glucose, metabolic clearance rate of glucose (MCR_G) expressed as the ratio of glucose disposal rate to blood glucose concentration and the insulin sensitivity index (MCR_G/I_C) both describing the insulin action.

Assays

Plasma glucose concentrations were determined by glucose oxidase method and plasma insulin concentrations were measured by radioimmunoassay kits (Immunotech, Czech Republic). Serum cholesterol and triglyceride concentrations were assessed in our Central Laboratory on Hitachi analyzer, glycated hemoglobin A_{1c} by IM kits on Abbott analyzer.

Statistical analysis

The results were calculated as means ± S.D. The Pearson's correlation coefficient was used for comparing insulin action expressed by MCR_G (after logarithmic transformation of the values before analysis) with age, BMI, basal plasma glucose and serum insulin, cholesterol and triglyceride concentrations as well as the mean blood pressure.

Multiple regression analysis was performed to find important predictors for insulin sensitivity. We considered MCR_G as a dependent variable (Pearson´s corelation coefficient, log scale). We used: diabetes mellitus, age, BMI, basal glycaemia (G_o) and basal serum insulin (I_o), total serum cholesterol (CH) and serum triglyceride (TG) levels, systolic and diastolic blood pressure as independent parameters. Not-normally distributed parameters were logarithmically transformed.

Group comparison was evaluated for insulin sensitivity parameters between diabetic and non-diabetic subjects. Because of an abnormal distribution we used median and quartiles to describe the samples and the non-parametric Mann-Whitney test was applied to evaluate a difference between the groups.

Results

Significantly higher glycated hemoglobin was present in diabetic patients compared to the healthy controls ($HbA1_c$ 7.5±0.5 vs. 5.1±0.3 %, $p<0.001$). Similarly, basal plasma glucose was significantly higher in diabetic than non-diabetic persons of the separate groups (Table 1). Serum cholesterol and triglyceride concentrations were higher in diabetic than non-diabetic subjects of the respective groups (Table 1). No significant differences in systolic and diastolic blood pressure were observed between the groups.

Tab. 2. Metabolic variables from hyperinsulinemic clamps in diabetic patients without obesity (D1, n=11), with mild overweight (D2, n=13) and with obesity (D3, n=18) in comparison with control persons without obesity (C1, n=14), with mild overweight (C2, n=10) and with obesity (C3, n=17).

	Diabetic patients			**Control persons**		
	D1 (n=11)	D2 (n=13)	D3 (n=18)	C1 (n=14)	C2 (n=10)	C3 (n=17)
G_c *(mmol.l^{-1})*	9.0±2.2^{a}	8.8±3.0^{a}	10.9±3.5^{a}	4.7±0.8	4.8±0.7	5.1±0.9
I_c *(mU.l^{-1})*	76±28	98±27	140±35^{x}	78±13	90±19	148±68^{x}
M (μmol.kg^{-1}.min^{-1})	43±11	30±12^{y}	24±8^{x}	43±9	34±6^{y}	22±9^{x}
MCR_G *(ml.kg^{-1}.min^{-1})*	4.7±2.4^{a}	4.2±1.8^{a}	2.6±1.2^{ax}	8.9±3.5	7.1±2.3	5.0±2.5^{x}
MCR_G/I_C *(ml.kg^{-1}.min^{-1}/mU.l^{-1} x100)*	6.5±3.7^{a}	5.0±2.2^{b}	2.1±1.2^{bx}	11.0±4.5	8.1±2.9	3.9±2.9^{x}

Mean plasma glucose (G_c) and serum insulin (I_c) concentrations during the last 20 min of the clamp, glucose disposal rate (M), metabolic clearance rate of glucose (MCR_G) and insulin sensitivity index (MCR_G/I_c). Results are shown as means ±SD. Statistical significance as compared to healthy controls of the respective group: $^{a}p<0.001$, $^{b}p<0.01$, and between obese and non-obese subjects: $^{x}p<0.001$, $^{y}p<0.01$.

The results of metabolic variables from the isoglycemic clamps are shown in Table 2. No significant differences in the glucose disposal rate (M) were found between diabetic and control persons of the corresponding group. However, the metabolic clearance rate of glucose was significantly lower in diabetic patients than in the respective group of control persons ($p<0.001$). The same was true for the insulin sensitivity index MCR_G/I_C.

Multiple regression analysis demonstrated the relationship between variables of insulin sensitivity and BMI, basal plasma glucose, cholesterol, triglycerides and blood pressure (Table 3). BMI was the most important predictor of insulin sensitivity. Significant inverse relationship was found between BMI and the glucose disposal rate ($r = -0.52$, $p<0.01$) and metabolic clearance rate of glucose ($r = -0.68$, $p<0.001$) (Fig. 1). Plasma glucose concentrations significantly correlated with MCR_G ($r = -0.66$, $p<0.001$). An inverse relationship was observed between serum cholesterol or triglyceride concentrations and glucose disposal rate ($r = -0.46$ and $r = -0.43$, $p<0.05$) or MCR_G ($r = -0.55$ and $r = -0.54$, $p<0.01$). The relationship between mean blood pressure and MCR_G was only of borderline significance ($r = -0.38$, $p=0.05$).

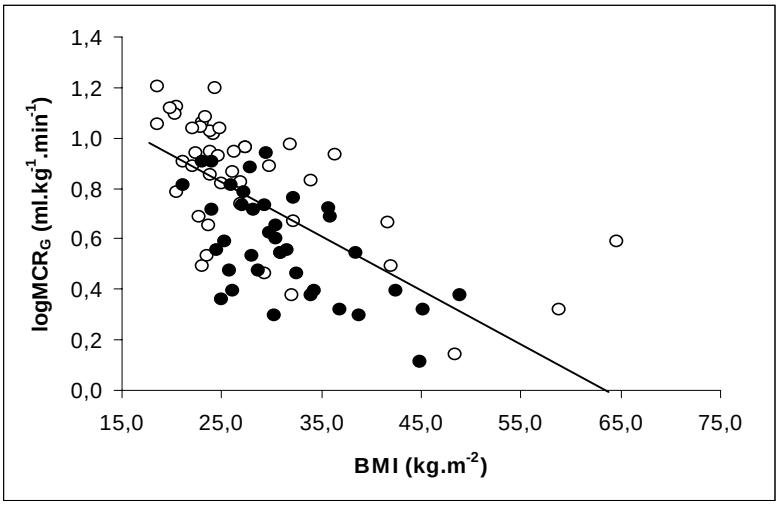

Fig. 1. *Relationship of metabolic clearance rate of glucose (MCR_G) and BMI in 42 diabetic patients (●) and 41 control persons (○). (y=-0.019x+1.275, n=83, r=-0.68, p<0.001).*

Tab. 3. Results of the regression analysis of insulin action in a mixed cohort of diabetic and non-diabetic persons expressed by Pearson´s correlation.

	BMI	G_0	I_0	CH	TG	MBP
M	-0.52**	-0.16	-0.31	-0.46**	-0.43**	-0.22
MCR_G	-0.68***	-0.66***	-0.41*	-0.55**	-0.54**	-0.38*

*Log scale was used for M and MCR_G. Statistical significance: *p=0.05, **p<0.01, ***p<0.001.*

The analysis of the results and power of dependence was shown by a multiple regression model in a stepwise manner:
log M = 4.840 – 0.030*BMI – 0.143*CH + 0.014*age – 0.011*I_o, with R^2= 57 %, and log MCR_G= 4.116 – 0.041*BMI – 0.081*G_o – 0.122*CH, with R^2= 64 %, where G_o and I_o are basal glucose and insulin concentrations and CH means cholesterol concentration. In summary, 57 % and 64 % of M or MCR_G variability was explained by the models. Mean blood pressure was not shown to have any significant influence predicting insulin sensitivity in this model.

Discussion

In the present study, we evaluated the relationship of insulin action measured by the isoglycemic hyperinsulinemic clamp with BMI, serum lipid levels, presence or absence of diabetes and blood pressure. We used isoglycemic instead of euglycemic clamps because isoglycemia was considered as a condition corresponding rather to the physiological equilibrium reached in the respective subjects after an overnight fast. However, in diabetic patients, the disposal rate of glucose involves in this situation both insulin-dependent and non-insulin-dependent glucose transportation originating also from the glucose concentration gradient (Pelikánová *et al.* 1994). We could not therefore demonstrate any difference between diabetic and control subjects when M value was calculated. Metabolic clearance rate of glucose (MCR_G) and insulin sensitivity index MCR_G/I_C offer the proper information about this difference. They demonstrated the lower insulin action in diabetic than in control persons of the respective group according to BMI. The patients chronically treated by oral antidiabetic drugs had similar metabolic parameters as the remaining diabetic patients and their results were therefore evaluated together.

We observed a significant inverse relationship between BMI, serum cholesterol or triglyceride concentration and insulin sensitivity expressed by M or MCR_G both in diabetic and in control persons.

The association between obesity and defective insulin signaling in human subjects has been well documented, but the involved cellular mechanisms remain poorly understood. Defects in both insulin binding capacity and postbinding signalizing in adipocytes from obese subjects have been reported (Olefsky 1995, Ahmad *et al.* 1997). Whilst the main reason of insulin resistance in diabetic patients is hyperglycemia (DeFronzo 1992), in obese non-diabetic subjects, a number of different influences has been discussed, i.e. cytokines (TNF-alpha) secreted by fat cells (Olefsky 1995, Koistinen *et al.* 2000). Others have recently demonstrated that protein-tyrosine phosphatase plays an essential role in the steady-state regulation of the insulin receptor autophosporylation as well as of the phosporylation state in downstream signaling proteins of the insulin action pathway (Ahmad *et al.* 1997). In our study, we did not try to elucidate insulin resistance on molecular basis, but we could demonstrate the effects of obesity, Type 2 diabetes or serum lipid levels on insulin action. Since overall and visceral adiposity are strongly associated with decreased insulin sensitivity, it is not surprising that the most reported Type 2 diabetic subjects are insulin resistant, although a few studies have suggested that some subgroups, such as elderly non-obese Scandinavian or Afro-American subjects, may be relatively insulin sensitive (Haffner *et al.* 1999). Other authors have shown an association of insulin resistance with higher blood pressure (Rocchini *et al.* 1995). The borderline relationship found in our evaluation between mean blood pressure and insulin action may be explained by the fact that no patients with moderate or severe hypertension were included in this study.

The association of insulin resistance with dyslipidemia in Type 2 diabetic patients has been repeatedly demonstrated (Pometa *et al.* 1991, Bonora *et al.* 1993, Škrha *et al.* 1994). Dyslipidemia (increased triglyceride and decreased HDL cholesterol levels) was associated with insulin resistance in Afro-American and Finnish Type 2 diabetic subjects (Haffner *et al.* 1999, Cigolini *et al.* 1991). Our results support the evidence that serum cholesterol and triglyceride concentration influence the insulin action as in diabetic as in non-diabetic patients. The correlation was still stronger in the latter group. We did not perform the correlation with HDL- or LDL-cholesterol because the data concerning these fractions were incomplete.

We elaborated a stepwise regression model to establish which of the tested parameters (BMI, presence or absence of diabetes, total cholesterol, triglycerides, blood pressure) played a significant role in the insulin resistance. We conclude that the most important predictor of insulin action in Type 2 diabetic patients are BMI and plasma glucose, followed by blood lipids, whereas blood pressure was less significant in the above relationship.

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Reprint requests

Prof. MUDr. J. Škrha, DrSc., Third Department of Internal Medicine, First Faculty of Medicine, Charles University, General Faculty Hospital, U nemocnice 1, 128 08 Prague 2, Czech Republic