Bulletin of the *Transilvania* University of Braşov
Series VI: Medical Sciences • Vol. 6 (55) No. 2 - 2013

THE RELATIONSHIP BETWEEN PROINSULIN, INSULIN RESISTANCE, AND SERUM LIPOPROTEINS AMONG ADULTS

S. ATEIA[1,2] E. RUSU[1] Si. ATEIA[2]
V. CRISTESCU[3] A. COSTACHE[1]
G. ENACHE[4] G. RADULIAN[1] D. CHEȚA[5]

Abstract: *The purpose of this study is to evaluate the relationship between proinsulin (FPP), insulin resistance and serum lipoproteins among adults and efficacity of these paraclinical indicators to identify the insulin resistance (IR). In this study we included 224 subjects without diabetes. Insulin resistance was present in 25% (n=56) of the participants. In all patients, ROC analysis demonstrated that product of tryglicerides and glucose (TyG index) was the strongest predictor of IR (HOMA-IR>3.04), folowed by FPP and tryglicerides (overall p< 0.0001). The TyG index, FPP and the TG/HDL-C ratio showed the greatest ability to predict IR. In normoponderal subjects, the ROC curve analyses showed that FPP was the best marker of insulin resistance.*

Key words: *insulin resistance, obesity, proinsulin, TyG index.*

1. Introduction

Insulin resistance (IR), characterized by a diminished response to the biological effects of insulin, is associated with obesity [1], predominantly abdominal fat deposition [2], elevated blood pressure, increased triglyceride levels (TG), low High-density lipoprotein cholesterol (HDL-C) [3], and small Low-density lipoprotein cholesterol (LDL-C) particle size [4].

Several lipid ratios have been sugested as simple and useful clinical indicators of IR. The lipid fraction ratios TG/HDL-C, TC/HDL-C and LDL-C/HDL-C have shown similar potential for IR, though the reports are not entirely consistent.

Among both diabetic and nondiabetic subjects, proinsulin has exhibited moderate but significant associations with blood pressure and with the

[1] University of Medicine and Pharmacy "*Carol Davila*", Bucharest, Romania.
[2] Medas Clinic, Bucharest, Romania.
[3] University "*Titu Maiorescu*", Bucharest, Romania.
[4] County Emergency Hospital Călăraşi, Romania.
[5] National Institute of Diabetes, Nutrition and Metabolic Diseases "*Prof. Nicolae Paulescu*", Bucharest, Romania.

concentrations of TC, TG, LDL-C and HDL-C [5, 6] independent of other factors.

2. Objective

The purpose of this study is to evaluate the relationship between proinsulin, insulin resistance and serum lipoproteins among adults and the efficacity of these paraclinical indicators to identify the insulin resistance (IR).

3. Materials and Methods

3.1. Design

Cross-sectional population-based screening campaign for diabetes.

3.2. Subjects

Patient recruitment took place in November-December 2011 in Bucharest, Romania. During this campaign a total of over 15,000 people were assessed. Only data from patients who gave their consent were analyzed and processed. A random population-based sample (n=656) of Romanians (26–80 years) was studied; 432 persons had diabetes and they were not analyzed for this paper.

The exclusion criteria were: patients with a previous diagnosis of diabetes, pregnancy, patients having an alcohol consumption of more than 20 g/day for women and 30 g/day for men, history of pancreatitis, chronic liver disease, autoimmune liver disease, HIV infection, hemochromatosis, patients with history of hepatotoxic or steatosis-inducing drug use, currently on interferon treatment or during the last 12 months, recent surgery, inflammatory or malignant disease, anticoagulant therapy, steroid therapy, postmenopausal women on estrogen replacement therapy.

3.3. Procedures and Measurements

Participants underwent an extensive interview for information on current medications, medical history, smoking, physical activity, etc. Overall adiposity was assessed by BMI. Waist circumference was assessed in standing position, midway between the highest point of the iliac crest and the lowest point of the costal margin in the mid-axillary line. Hip circumference was measured at the level of the femoral greater trochanter.

Body mass index (BMI) was calculated (body weight in kilograms divided by the square of height in meters) and categorized based on national guidelines.

3.4. Laboratory assays

Fasting blood samples were drawn between 7:00 a.m. and 10:00 a.m. The biochemical analyses including fasting plasma glucose (FPG), fasting plasma insulin (FPI), fasting plasma proinsulin (FPP), fasting plasma C-peptide, HbA1c, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), creatinine, urea were measured after an overnight fasting period of 12h, using routine clinical chemistry methods and then documented.

Intact proinsulin was measured using ELISA (Demeditec Diagnostics GmbH, Germany). The inter- and intraassay CVs were 4.3% and 5.5% for proinsulin. Serum insulin and C-peptide were determined by chemiluminescence enzyme immunoassay (Architect, Abbott). The cross-reactivity of insulin with proinsulin, and with C-peptide was 0.1% respectively 0.001%.

IR (insulin resistance) was determined using Homeostasis model assessment (HOMA-IR)[7]; the 75 percentile was considered as the cut-off point for IR (HOMA-IR>3.04). The homeostatic

model for assessment of ß-cell function (HOMA-ß) was calculated using the formula [7]: 20 × FPI (μU/ml)/(FPG (mmol/l) – 3.5). The quantitative insulin sensitivity check index (QUICKI) was calculated using the formula [8]: 1/(log (FPI) (μU/ml)+log (FPG) (mg/dl)). Proinsulin to insulin ratio (PIR) was calculated.

The TyG was calculated as Ln[fasting triglycerides (mg/dL) X fasting glucose (mg/dL)/2]. The TyG index is expressed by a logarithmic scale [9].

3.5. Statistical Analyses

Values were expressed as means ± SD for normally distributed data or median (range) for skewed data. Log transformation was also applied to skewed data. Comparisons among groups were made by use of ANOVA for quantitative variables and the χ^2 test of independence for categorical variables.

Receiver operating characteristics (ROC) curve analysis was performed to identify optimal tests and threshold values for predicting IR.

We also calculated the positive likelihood ratio, a measure of the posterior odds of disease, as the ratio of sensitivity to 1 - specificity and the odds/(odds +1) for estimating the probability of disease, given a positive test.

4. Results

Table 1 shows the distribution of clinical and metabolic characteristics at baseline stratified by HOMA-IR. The median age was 59 years (95% CI: 55.2-59.2), 61.2% of participants were women, 30.4% (n=68) were obese and 39.7% (n=89) were overweight. Insulin resistance was present in 25% (n=56) of the participants, 4.5% (n=3) of the normal weight (NW) persons. The median BMI was 26.9 kg/m^2 (95% CI: 27.1-28.4 kg/m^2). The median fasting glucose level was 100.5 mg/dl (95% CI: 100.6-104.2 mg/dl). The median fasting insulin level was 8.55 uU/ml (95% CI: 8.7-9.9 uU/ml) and the median fasting proinsulin level was 4.3 pmol/L (95% CI: 5-6.39 pmol/L).

Patients with HOMA-IR>3.04 presented a higher BMI, higher levels of TG, FPG, HbA1c, FPI, FPP, HOMA-ß, C-peptide, higher values of TC/HDL-C, TG/HDL-C, LDL-C/HDL-C, TyG index, and lower levels of HDL-C and Quicki index; TC, LDL-C, PIR did not differ among HOMA-IR groups (Table 1). Fasting serum lipid profile (TC, HDL-C, and LDL-C) and lipid ratios did not differ among normal weight, overweight or obese individuals. Compared with normal weight individuals, obese patients had higher TG values and TyG index (all $p<0.05$).

Comparison of areas under ROC curves (95% CI) for markers of IR in persons categorized by BMI

In all patients, ROC analysis demonstrated that TyG index (AUROC=0.75, 95% CI: 0.677, 0.823) was the strongest predictor of IR (HOMA-IR>3.04), folowed by FPP (AUROC=0.742, 95% CI: 0.664, 0.820) and TG (AUROC=0.704, 95% CI: 0.624, 0.784) (all $p<0.0001$) (Table 2; Figure 1).

In normoponderal subjects (NW), the ROC curve analyses showed that the best marker of insulin resistance was FPP, with an area under the ROC curve of 0.73 (95% CI: 0.603-0.856) (Table 2; Figure 1). Other markers could not be used to discriminate insulin resistance in normal weight persons.

In overweight subjects (OW) TyG index, FPP, TG and TG/HDL-C showed discriminative ability, with an AUROC significantly different from 0.5. The test with the largest area under the curve was TyG index (AUROC=0.814, 95% CI: 0.715, 0.913), followed by FPP (AUROC=0.773, 95% CI: 0.649, 0.897), TG (AUROC=0.77, 95% CI: 0.649, 0.891) and TG/HDL-C (AUROC=0.761, 95% CI: 0.638, 0.883) (Table 2; Figure 1).

Excluding normoponderal and over-weight subjects, the strongest predictor of

IR (HOMA-IR>3.04) was FPP (AUROC = 0.71 95% CI: 0.623, 0.797), followed by TyG index (AUROC=0.69, 95% CI: 0.601, 0.778) and TG (AUROC=0.669, 95% CI: 0.579, 0.759) (all p < 0.001).

Metabolic characteristics of subjects stratified by HOMA-IR Table 1

Variables	HOMA-IR<3.04(n=168)			HOMA-IR>3.04 (n=55)			
	Mean	SD	Median	Mean	SD	Median	p
Age (years)	56.76	15.11	58.00	59.25	10.71	60.00	0.2575
BMI (kg/m2)	26.72	4.55	26.30	31.04	4.51	30.49	0.0000
TC (mg/dl)	218.27	48.68	217.00	216.31	44.24	216.00	0.7909
HDL-C (mg/dl)	57.00	13.81	56.00	50.05	11.91	51.00	0.0010
LDL-C (mg/dl)	144.90	42.62	142.40	141.95	40.98	147.00	0.6536
TG (mg/dl)	131.60	67.31	118.00	197.16	108.35	161.00	0.0000
TC/HDL-C	3.99	1.08	3.87	4.55	1.39	4.31	0.0023
TG/HDL-C	2.53	1.65	2.25	4.50	3.40	3.32	0.0000
LDL-C/HDL-C	2.67	0.92	2.63	2.98	1.08	2.96	0.0403
TyG index	8.66	0.52	8.63	9.16	0.55	9.13	0.0000
FPG (mg/dl)	99.47	13.53	97.00	111.45	9.41	115.00	0.0000
HbA1c (%)	5.71	0.54	5.70	5.93	0.46	6.00	0.0085
FPI (uU/ml)	7.38	2.57	7.30	15.40	5.06	14.30	0.0000
FPP (pmol/l)	4.84	4.83	3.56	8.36	5.50	7.17	0.0000
PIR	5.24	7.99	3.61	4.03	2.69	3.66	0.2727
HOMA-ß	83.82	46.27	73.71	122.18	60.41	104.09	0.0000
Quicki index	0.35	0.02	0.34	0.31	0.01	0.31	0.0000
C-peptide (ng/ml)	2.06	0.97	1.92	3.48	1.22	3.16	0.0000

Table 2

Comparison of AUROC (95%CI) for potential markers of insulin resistance (HOMA-IR>3.04) in subjects stratified by BMI

	AUROC (95% CI)			
	Total (n=224)	**NW (n=67)**	**OW (n=89)**	**OB (n=68)**
TC (mg/dl)	0.51* (0.423-0.598)	0.459 * (0.258-0.596)	0.45* (303-597)	0.502* (0.403-0.6)
HDL-C (mg/dl)	0.362 ** (0.279-0.444)	0.335** (0.298-0.622)	0.32** (0.18-0.456)	0.441 * (0.344-0.538)
TG (mg/dl)	0.704 *** (0.624-0.784)	0.611 * (0.429-0.734)	0.77*** (0.64-0.891)	0.669 *** (0.579-0.759)
TC/HDL-C	0.608** (0.520-0.696)	0.619 * (0.347-0.649)	0.595* (0.446-0.745)	0.55* (0.451-0.648)
TG/HDL-C	0.700 *** (0.619-0.780)	0.617* (0.433-0.716)	0.761*** (0.638-0.883)	0.648*** (0.557-0.740)
LDL-C/HDL-C	0.580* (0.0.49-0.671)	0.609* (0.319-0.630)	0.575* (0.425-0.725)	0.524* (0.426-0.624)
TyG index	0.750 *** (0.677-0.823)	0.638* (0.451-0.752)	0.814*** (0.715-0.913)	0.69*** (0.601-0.778)
LogFPP	0.742 *** (0.664-0.820)	0.73*** (0.671-0.9)	0.773 *** (0.649-0.897)	0.710 *** (0.623-0.797)
LogPIR	0.466* (0.380-0.552)	0.499* (0.308-624)	0.520 * (0.382-0.657)	0.418* (0.322-0.515)

*NS; **<0.05; ***<0.001

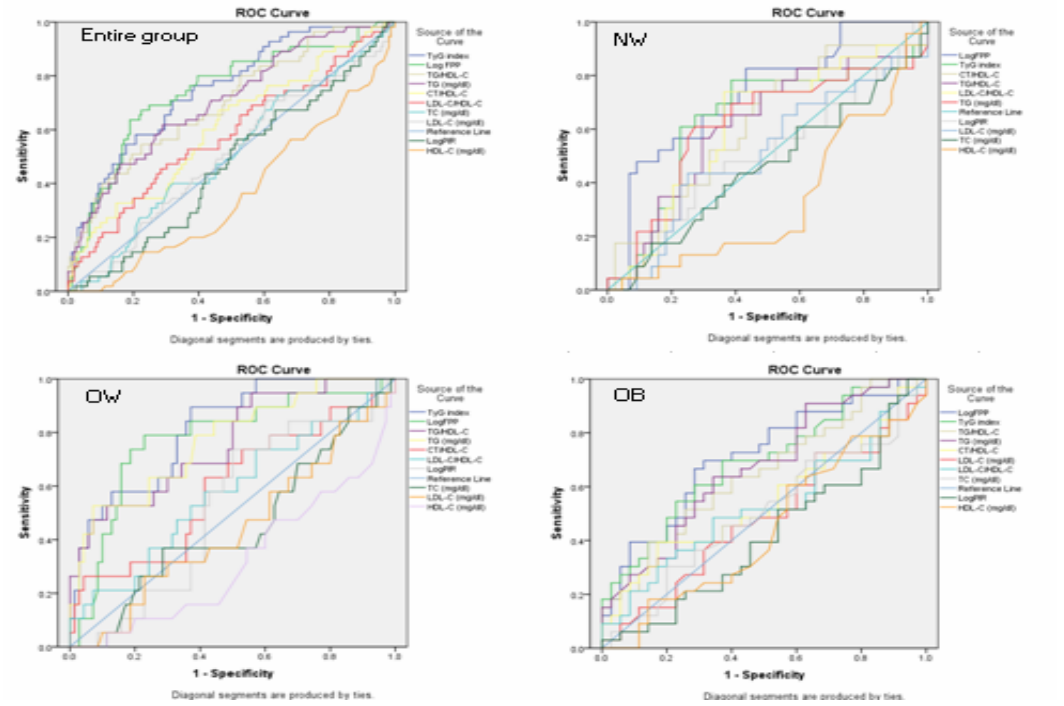

Fig. 1. *Receiver operating characteristics (ROC) curves. Sensitivity represents the true-positive results and 1-specificity, the false-positive results. Nondiagnostic markers are represented by diagonals with areas under the ROC curves close to 0.5.*

Optimal cut-off point of for predicting insulin resistance in subjects categorized by BMI

Table 4 and 5 show the cut-off points of the best markers for identifying insulin resistance. The optimal cut-off point to identify insulin resistance for these markers yielded the following values:

- in all subjects: TyG index ≥8.89, Log FPP ≥ 0.85, and TG ≥ 145.5 mg/dl
- in non-obese subjects: Log FPP ≥ 0.54
- in overweight subjects: TyG index ≥ 8.78, LogFPP ≥ 0.83, and TG ≥ 195.5 mg/dl
- in obese persons: LogFPP ≥ 0.85, TyG index ≥ 8.93, and TG ≥ 144.5 mg/dl

In all subjects, the positive likelihood ratio (LR+) value indicated that the odds of insulin resistance increased by 1.04-fold if the TyG index was positive (the value ≥8.89). The negative likelihood ratios (LR) indicate the extent to which the odds of insulin resistance decrease if the test is negative.

In normoponderal subjects LR+ value indicated that the odds of IR increased by 1.45-fold if the LogFPP was positive (the value ≥0.54).

In overweight subjects (OW) LR+ value indicated that the odds of IR increased by 1.42-fold if the TyG index was positive (the value ≥8.78).

In obese subjects LR+ value indicated that the odds of IR increased by 1.1-fold if the TyG index was positive (the value 8.93). All values are presented in Table 4 and Table 5.

Table 4

Cut-off point, sensitivity, specificity, positive LR, negative LR in entire group and normal weight group (NW)

	Total					NW				
Variables	**Cut-off**	**Se**	**Sp**	**LR+**	**LR-**	**Cut-off**	**Se**	**Sp**	**LR+**	**LR-**
TG (mg/dl)	145.50	0.62	0.29	0.87	1.32	133.00	0.61	0.27	0.84	1.43
TG/HDL-C	3.03	0.55	0.22	0.70	2.02	2.53	0.57	0.30	0.80	1.47
TyG index	8.89	0.71	0.32	1.04	0.91	8.53	0.78	0.39	1.28	0.56
LogFPP	0.85	0.67	0.22	0.87	1.46	0.54	0.83	0.43	1.45	0.40

Table 5

Cut-off point, sensitivity, specificity, positive LR, negative LR in overweight (OW) and obese persons (OB)

	OW					OB				
Variables	**Cut-off**	**Se**	**Sp**	**LR+**	**LR-**	**Cut-off**	**Se**	**Sp**	**LR+**	**LR-**
TG (mg/dl)	195.50	0.53	0.07	0.57	6.63	144.50	0.64	0.34	0.96	1.06
TG/HDL-C	3.69	0.53	0.11	0.59	4.14	3.28	0.52	0.26	0.69	1.88
TyG index	8.78	0.89	0.37	1.42	0.28	8.93	0.70	0.37	1.10	0.81
LogFPP	0.83	0.79	0.23	1.02	0.92	0.85	0.70	0.31	1.01	0.96

5. Discussion

In our study, we have found that lipoprotein ratios (TC/HDL-C, TG/HDL-C, LDL-C/HDL-C) and TyG index were significantly higher in individuals with HOMA-IR>3.04 ($p < 0.001$); TC, LDL-C, PIR did not differ among HOMA-IR groups. Insulin resistant individuals also presented a higher FPG, HbA1c, FPI, FPP, HOMA-ß, C-peptide. An elevated FPP has been reported to be predictive of development of type 2 DM in certain at-risk groups and may precede the diagnosis by 5–20 yr [10]. Increased IR was associated with higher FPP levels ($p<0.0001$) and lower PIR (without statistical significance). The same trend was found in a study on adults with IR [11]. This suggests an enhanced conversion of proinsulin to insulin by the β-cell under the increased secretory demand of IR. However, in San Antonio Heart Study, FPP was reported to be disproportionately increased in nondiabetic adults with insulin resistance syndrome [12].

In a previous study, about 16% of people with normal weight (BMI < 25 kg/m^2) were identified to be insulin resistant [13]; thus search for clinically simple and useful biomarkers to detect insulin resistance among people with normal weight is necessary. In our study, in normoponderal subjects, the ROC curve analyses showed that the best marker of insulin resistance was FPP and other markers could not be used to discriminate insulin resistance. Also, TyG index, TG and TG/HDL-C ratio were useful makers of insulin resistance, especially in overweight or obese subjects. The ROC scatter plot revealed the best TyG index for diagnosis of insulin resistance to be correspondent to Ln 8.89 which showed the highest sensitivity (71.0%) but low specificity (32.0%) values. Similar with our study, McLaughlin T et al. reported that TG concentrations or the TG/HDL-C ratio offer the most practical approach to

identify insulin resistance in overweight nondiabetic volunteers [14]. Our study showed that the optimal cut-off point of the TG/HDL-C ratio for prediction of insulin resistance in all persons was 3.03 (in mg/dl unit); 2.53 for normal weight persons, 3.69 in overweight and 3.28 in obese persons with reasonable sensitivity but low specificity. In a previous study increased TC/HDL-C ratio was shown to be associated with insulin resistance in individuals whose weight was normal and it correlated negatively with rates of insulin-stimulated glucose disposal in a small group of healthy individuals [15].

6. Conclusions

In conclusion, the present study demonstrated that special lipid profiles are associated with insulin resistance according to BMI in a general population. In clinical settings, the lipid profile, lipid ratio and TyG index could be used as indicators of insulin resistance.

Our data support that in adult population FPP, TG/HDL-C ratio and TyG index serve as easily available laboratory markers for identifying insulin resistance. The present data documented that insulin resistance was present even in subjects with normal BMI. Our results support the concept that FPP may be a clinically simple and useful indicator for insulin resistance among non-diabetic adults with normal weight. Future research are required to assess the predictive power of FPP, TG/HDL-C ratio, TyG index for type 2 diabetes, metabolic syndrome, or cardiovascular disease.

References

1. Lillioja, S., Bogardus, C.: *Obesity and insulin resistance: lessons learned from the Pima Indians.* In: Diabetes Metab Rev (1988) Vol. 4, p. 517–540.
2. Karter, A.J., Mayer-Davis, E.J., Selby, J.V., et al.: *Insulin sensitivity and abdominal obesity in African-American, Hispanic and non-Hispanic white men and women: the Insulin Resistance and Atherosclerosis Study.* In: Diabetes (1996) Vol. 45, p. 1547-1555.
3. Haffner, S.M., Valdez, R.A., Hazuda, H.P., et al.: *Prospective analysis of the insulin-resistance syndrome (syndrome X).* In: Diabetes (1992) Vol. 41, p. 715-722.
4. Festa, A., D'Agostino, R., Mykkanen, L., et al.: *LDL particle size in relation to insulin, proinsulin, and insulin sensitivity: the Insulin Resistance Atherosclerosis Study.* In: Diabetes Care (1999) Vol. 22, p. 1688-1693.
5. Nagi, D.K., Hendra, T.J., Ryle, A.J., et al.: *The relationship of concentrations of insulin, intact proinsulin, and 32–33 split proinsulin with cardiovascular risk factors in type 2 (non-insulin-dependent) diabetic subjects.* In: Diabetologia (1990) Vol. 33, p. 532-537.
6. Haffner, S.M., Mykkanen, L., Stern, M.P., et al.: *Relationship of proinsulin and insulin to cardiovascular risk factors in nondiabetic subjects.* In: Diabetes (1993) Vol. 42, p. 1279-1302.
7. Matthews, D.R., Hosker, J.P., Rudenski, A.S., et al.: *Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man.* In: Diabetologia (1985) Vol. 28, p. 412-419.
8. Katz, A., Nambi, SS., Mather, K., et al.: *Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans.* In: Journal of Clinical Endocrinology and Metabolism (2000) Vol. 85, p. 2402-2410.

9. Simental-Mendía, L.E., Rodríguez-Morán, M., Guerrero-Romero, F.: *The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects.* In: Metab Syndr Relat Disord (2008) Vol. 6, p. 299-304.
10. Nijpels, G., Popp-Snijders, C., Kostense, P.J, et al.: *Fasting proinsulin and 2-h post load glucose levels predict the conversion to NIDDM in subjects with impaired glucose tolerance: the Hoorn Study.* In*:* Diabetologia (1996) Vol. 39, p. 113-118.
11. Mykkänen, L., Haffner, SM., Hales, C.N., et al.: *The relation of proinsulin, insulin, and proinsulin-to-insulin ratio to insulin sensitivity and acute insulin response in normoglycemic subjects.* In: Diabetes (1997) Vol. 46, p. 1990–1995.
12. Haffner, SM., Mykkänen, L., Valdez, RA., et al.: *Disproportionately increased proinsulin levels are associated with the insulin resistance syndrome.* In: J Clin Endocrinol Metab (1994) Vol. 79, p. 1806-1810.
13. McLaughlin, T., Allison, G., Abbasi, F., et al.: *Prevalence of insulin resistance and associated cardiovascular disease risk factors among normal weight, overweight, and obese individuals.* In: Metabolism (2004) Vol. 53, p. 495-499.
14. McLaughlin, T., Abbasi, F., Cheal, K., et al.: *Use of metabolic markers to identify overweight individuals who are insulin resistant.* In: Ann Intern Med (2003) Vol. 139, p. 802–809.
15. Garg, A., Helderman, JH., Koffler, M., et al.: *Relationship between lipoprotein levels and in vivo insulin action in normal young white men.* In: Metabolism (1988) Vol. 37, p. 982-987.