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Changes of lipolytic enzymes cluster with insulin resistance syndrome

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Summary The activities of hepatic and lipoprotein lipase and the levels of lipo- and apoproteins were compared in two groups of normoglycaemic men representing the highest ($n = 18$) and lowest ($n = 15$) fasting insulin quintiles of first degree male relatives of non-insulin-dependent diabetic patients. The high insulin group representing insulin-resistant individuals had significantly lower post-heparin plasma lipoprotein lipase activity than the low insulin group (14.2 ± 4.0 vs 20 ± 5.8 μmol NEFA · $ml^{-1} \cdot h^{-1}$, $p < 0.001$); hepatic lipase activity did not differ between the two groups (24.2 ± 11 vs 18.0 ± 5.3 μmol NEFA · $ml^{-1} \cdot h^{-1}$, NS). The lipoprotein lipase/hepatic lipase ratio in the high insulin group was decreased by 66% as compared to the low insulin group (0.75 ± 0.57 vs 1.25 ± 0.65, $p < 0.01$). In the high insulin group both total and VLDL triglycerides were higher than in the low insulin group (1.61 ± 0.57 vs 0.86 ± 0.26 mmol/l, $p < 0.001$ and 1.00 ± 0.47 vs 0.36 ± 0.16 mmol/l, $p < 0.001$, respectively) whereas HDL cholesterol and HDL_2 cholesterol were lower (1.20 ± 0.30 vs 1.43 ± 0.22 mmol/l, $p < 0.05$ and 0.49 ± 0.21 vs 0.71 ± 0.17 mmol/l, $p < 0.05$, respectively). Total cholesterol, LDL cholesterol or HDL_3 cholesterol did not differ between the two groups. The mean particle size of LDL was smaller in the high insulin group than in the low insulin group (258 ± 7 vs 265 ± 6 Å, $p < 0.05$). We propose that the changes of lipoprotein lipase and lipoprotein lipase/hepatic lipase ratio cluster with insulin resistance and provide a possible mechanism to explain the lowering of HDL cholesterol and elevation of triglyceride concentrations observed in insulin-resistant subjects. [Diabetologia (1995) 38: 344–350]

Key words Insulin resistance, lipase activities, lipoproteins.

The metabolic syndrome, also referred as insulin resistance syndrome (IRS), is recognized as a common risk factor for coronary heart disease [1–3]. Dyslipidaemia, characterized by the elevation of triglycerides and lowering of HDL-cholesterol, is an inherent feature in this cluster of metabolic abnormalities which also include insulin resistance, hyperinsulinism, central obesity, impaired glucose tolerance or non-insulin-dependent diabetes mellitus (NIDDM) and hypertension. Recently, the preponderance of small dense LDL has been linked with this constellation [4, 5]. The lipoprotein pattern has been termed as the atherogenic lipoprotein phenotype and it is associated with excess risk of coronary heart disease [6, 7].

A major challenge is to define the causal sequence between dyslipidaemia and insulin resistance. Several studies have demonstrated that fasting insulin is related to high triglyceride levels [8–11]. Substantial evidence indicates that hypertriglyceridaemia is indeed

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Abbreviations: LPL, Lipoprotein lipase; HL, hepatic lipase; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; chol, cholesterol; TG, triglycerides; NEFA, non-esterified fatty acids.

associated with insulin resistance measured by euglycaemic clamp [10, 12, 13]. In insulin resistant states VLDL production in liver is enhanced and considered to be the major cause for the elevation of serum triglycerides [1, 14]. It is also well-recognized that insulin has multiple sites of action on the assembly and secretion of VLDL particles [15, 16]. The question is still unresolved whether hyperinsulinaemia per se is the driving force for increased production of VLDL, or if a disruption of the co-ordinated regulation of apo B leads to increased VLDL secretion [17]. Interestingly, recent kinetic data indicate that insulin resistance in obese subjects interferes with the normal inhibitory action of hyperinsulinaemia on VLDL triglyceride and apo B metabolism [18].

Since LPL, which is the key enzyme in hydrolysis of triglyceride-rich particles, is an insulin-sensitive enzyme it is attractive to speculate that insulin resistance may also influence lipoprotein lipase (LPL). Pykälistö et al. [19] were the first to report that post-heparin plasma and adipose tissue LPL activity are reduced in NIDDM subjects. Interestingly, the response of post-heparin LPL to hyperinsulinaemia is blunted in obese subjects, suggesting that the enzyme is insulin resistant [20–22]. Recently Pollare et al. [23] reported that the glucose infusion rate during a clamp study which is a parameter of insulin sensitivity, explains to a significant degree the variations in post-heparin plasma LPL activity, and particularly in muscle LPL activity. Generally, in insulin resistance the lowering of LPL activity would impede VLDL clearance and also result in lowering of HDL.

The variation in plasma HDL is also determined by hepatic lipase (HL) [24]. In contrast to LPL activity, HL activity has been reported to be increased in insulin-resistant states such as NIDDM and obesity [25–27]. Elevated HL activity is common in individuals with hypertriglyceridaemia and low HDL cholesterol. Blades et al. [28] propose that low LPL/HL ratio could be one underlying mechanism for lowering of HDL cholesterol in this patient group. Likewise, Lamarche et al. [29] reported that subjects with high triglycerides and low HDL cholesterol concentrations had lower post-heparin plasma LPL/HL ratio and showed hyperinsulinaemia which is indicative of insulin resistance. Overall, a net result of opposite changes of LPL and HL in insulin resistance states would be lowering of HDL.

Glucose-tolerant first degree relatives of NIDDM patients have genetic susceptibility to develop NIDDM and they manifest the full profile of the insulin resistance syndrome [30–32]. Such individuals are characterized by hyperinsulinaemia as a marker of insulin resistance [32, 33]. Recent observations indicate that first degree relatives of NIDDM patients also have typical alterations of VLDL and HDL although the ambient concentrations of VLDL and HDL can still be within the normal range [34]. Consequently these individuals represent an ideal cohort to study if changes of LPL and/or HL activities cluster with insulin resistance and contribute to the observed dyslipidaemia.

Therefore we recruited glucose-tolerant first degree relatives of NIDDM patients belonging either to the lowest or highest quintiles of fasting insulin to determine the activities of post-heparin plasma LPL and HL. In addition we measured the concentrations of major lipoproteins, LDL and HDL subclasses to evaluate the interrelationship between lipolytic enzymes and alterations of lipoproteins.

Materials and methods

The initial cohort represents a population of glucose-tolerant first-degree relatives of the NIDDM patients ($n = 1050$) examined between February 1990 and January 1992 in the Botnia study. All subjects had an oral glucose tolerance test (OGTT, 75 g glucose), which was performed in the morning after a 12-h overnight fast. Glucose and insulin were determined at 0, 30, 60 and 120 min. Male subjects ($n = 505$) with normal oral glucose tolerance according to World Health Organization criteria [35] were categorized into quintiles of fasting insulin levels. The cut-off points of the lowest and highest insulin quintiles were 4.6 mU/l and 10 mU/l (= 33 and 72 pmol/l), respectively.

For this study we invited a subset of 61 men selected to have similar age range and BMI belonging either to the lowest ($n = 31$) or highest ($n = 30$) quintile at the first examination. These men were examined between October 1992 and March 1993. At the second examination 28 subjects of the original groups had fasting insulin values between 4.6 mU/l and 10 mU/l. To guarantee correct categorization the subjects who were not in their allocated quintiles were excluded from the study. Patients with major illnesses known to affect lipid metabolism (i.e., malignant diseases, thyroid or other endocrine disorders, renal or hepatic disorders, current infections) were excluded on the basis of laboratory tests and clinical history. Four patients were on a medication for high blood pressure.

Patients were re-examined in the participating Health Care Centers in the Botnia area. Heparin tests were performed and the blood samples collected after an overnight fast. Apart from locally determined blood glucose concentrations all the laboratory measurements were performed in the Helsinki University Hospital.

At the re-examination 15 of the 61 participants had fasting serum insulin levels below 4.6 mU/l. These subjects comprised the insulin-sensitive subgroup. A total of 18 men had fasting insulin over 10 mU/l and composed the insulin-resistant group. The two groups were close in age but the insulin-resistant group had slightly higher BMI than the insulin-sensitive group (Table 1).

Serum lipid and lipoprotein analyses. Lipoprotein fractions (VLDL, LDL and HDL) were isolated from fresh serum by sequential ultracentrifugation [36] in a Beckman L7–70 ultracentrifuge (Beckman Instruments, Palo Alto, Calif., USA) using a Kontron TZT 45.6 rotor (Kontron AG, Basel, Switzerland). VLDL and LDL were isolated at densities of 1.006 and 1.063 g/ml, respectively as described previously [37]. Thereafter, HDL_2 and HDL_3 were isolated at densities of 1.125 and 1.210 g/ml by centrifugation for 48 h at 38,000 rev/min.

Table 1. Characteristics of the study groups

	Low-insulin group ($n = 15$)	High-insulin group ($n = 18$)
Age (years)	46.1 ± 6.6	45.1 ± 6.8
Body mass index (kg/m^2)	25.0 ± 1.5[a]	26.6 ± 1.8
Waist/hip ratio	0.92 ± 0.07[a]	0.97 ± 0.04
Fasting plasma insulin (μU/ml)	3.4 ± 0.59	13 ± 2.9
Fasting blood glucose (mmol/l)	3.7 ± 0.4[a]	4.2 ± 0.4
Diastolic blood pressure (mmHg)	80 ± 9	84 ± 7
Systolic blood pressure	128 ± 14	132 ± 12

Results are mean ± SD, [a] $p < 0.05$ for difference from respective values in the high-insulin group

Table 2. Concentrations of lipids and lipoproteins in the study population groups

	Low-insulin group ($n = 15$)	High-insulin group ($n = 18$)
Cholesterol (mmol/l)		
Total	5.62 ± 1.2	6.09 ± 1.0
HDL	1.43 ± 0.22[a]	1.20 ± 0.26
HDL_2	0.71 ± 0.17[a]	0.49 ± 0.21
HDL_3	0.73 ± 0.12	0.71 ± 0.11
LDL	3.80 ± 1.1	4.09 ± 0.86
Triglycerides (mmol/l)		
Total	0.86 ± 0.26[b]	1.61 ± 0.57
VLDL	0.36 ± 0.16[b]	1.00 ± 0.47

Results are mean ± SD, [a] $p < 0.05$, [b] $p < 0.001$ for difference from respective values in the high-insulin group

Lipase analyses. Heparin (100 IU/kg body weight Leiras, Huhtamäki OY, Turku, Finland) was injected i.v. as a bolus to release lipolytic enzymes into the circulation. The blood was collected into chilled heparinized tubes before and 5 and 15 min after the injection and placed on ice immediately. Plasma was separated and transferred into plastic tubes which were immediately frozen at –20 °C.

LPL and HL activities were measured using an immunochemical method described by Huttunen et al. [38]. A specific antiserum against HL was used in the LPL assay. To measure the HL activity a substrate containing 1 mol/l NaCl to inactivate the LPL was used. The intra-assay variation for LPL was 4.6 % and for HL 5.1 %. The interassay variations were 5.1 % and 8.4 %, respectively.

Analytical methods. Fasting insulin was determined by a double-antibody radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden). The interassay variation was determined using two standard specimens (13 and 45 mU/l; 93 and 323 pmol/l); the means of the determinations averaged 13.2 and 43.9 mU/l (94.5 and 315 pmol/l) giving interassay variations of 7 and 4.8 %, respectively.

Concentrations of cholesterol and triglycerides in serum and in lipoprotein fractions were measured enzymatically using kits from Hoffman-La Roche nos. 0715166 and 0722138 (Basel, Switzerland) in an automated Cobas Mira analyzer. ApoA-I and apoA-II concentrations were determined by immunoturbidometry using monospecific antibodies (nos. 726478 and 726486, Boehringer Mannheim GmbH, Mannheim, Germany). The interassay variations for apoA-I and for apoA-II were 3.6 % and 3.7 %, respectively.

The concentration of LpA-I particles was quantified by using differential electroimmunophoresis with hydrated agarose gels containing monospecific antibodies against apoA-I and apoA-II (Sebia, Issy-les Molineaux, France) as described elsewhere in detail [39, 40]. The concentration of LpA-I:LpA-II particles was calculated by subtracting the concentration of LpA-I particles from turbidometrically determined total concentration of apoA-I in serum. Interassay variation for LpA-I particle concentration was 7.3 %.

To determine the mean particle diameter of the main LDL peak, non-denaturating polyacrylamide gel electrophoresis was performed from frozen serum samples, stored at –70 °C using the method previously described by Nichols et al. [41]. The gels with linear polyacrylamide gradient of 2–12 % were cast in our laboratory according to the method of Margolis and Kenrick [42] with slight modifications. Stock solution A contained 12.0 % acrylamide (bisacrylamide 5.0 %), 5.0 % sucrose, 0.012 % ammonium persulfate and 0.085 % tetramethylethylenediamine and stock solution B contained 2.0 % acrylamide (bisacrylamide 5.0 %), 0.019 % ammonium persulfate and 0.120 % tetramethylethylenediamine. Both stock solutions were made into 1 % mol/l Tris-HCl buffer (pH 8.3). Casting of the gels was performed by using gradient Mixer GM-1 (Pharmacia), Gel Slab Casting Apparatus GSC-8 (Pharmacia) and Peristaltic Pump P-1 (Pharmacia) as previously described [43]. The gels were stained with Sudan Black B lipid stain prepared as described previously [44]. After the destaining procedure the gels were scanned with a computer-assisted Molecular Dynamics Personal Densitometer (Sunnyvale, Calif, USA) using a 50-μm pixel size and 12-bit signal resolution. The particle diameter of the major LDL peak was determined by comparing the mobility of the sample with that of a calibrated reference LDL preparation run together on each gel. CV values were 1.8 % and 1.2 %, respectively, for intergel and intragel precisions of the used control sample.

Statistical analysis

Statistical analyses were performed using SYSTAT version 5.0, Systat inc., Evanston, IL, USA. Comparisons between the groups were performed using analysis of variance adjusted for differences in BMI (ANCOVA) since BMI differed slightly between the groups. Univariate correlations were calculated using Pearson's correlation analyses.

Results

Table 2 compares the concentrations of blood lipids and lipoproteins in men representing either low- or high-insulin groups. Men in the highest quintile of insulin had twofold higher total and VLDL triglycerides than men in the lowest quintile and these differences were highly significant. In the high insulin group the concentration of HDL cholesterol was 16 % lower ($p < 0.05$) than in the low insulin group. This difference was due to the low levels of HDL_2 cholesterol whereas HDL_3 cholesterol did not differ between the two groups. Subjects in the highest insulin quintile had lower levels of apoA-I than men in the low insulin group (129 ± 12 vs 146 ± 19 mg/dl, $p < 0.05$) whereas the mean levels of apo A-II were similar (37 ± 5 vs 36 ± 4 mg/dl, NS). Accordingly the

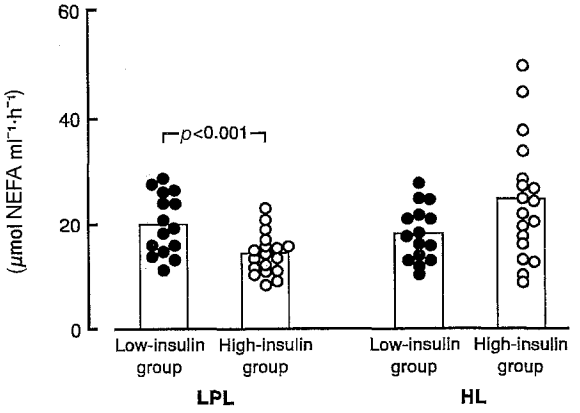

Fig. 1. Individual values of lipoprotein lipase and hepatic lipase activities in the low- and high-insulin groups. The mean of each group is marked with a solid horizontal line

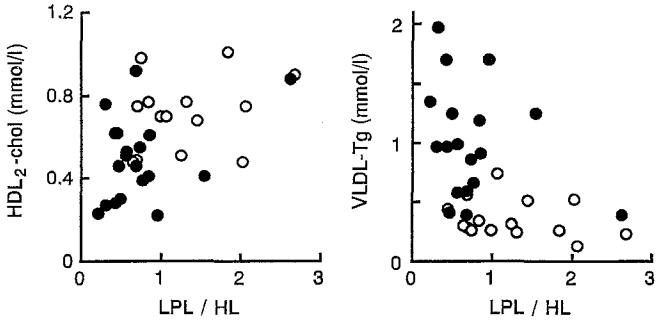

Fig. 2. Univariable correlation between the lipoprotein lipase – hepatic lipase ratio and VLDL-triglycerides in the right panel ($r = -0.44$, $p < 0.05$); and with HDL_2-cholesterol in the left panel ($r = 0.47$, $p < 0.01$). ○ subjects in the low-insulin group; ● in the high-insulin group

concentration of HDL particles containing only apo A-I (LpA-I particles) was reduced by 21 % in the high insulin group as compared to the low insulin group (39 ± 8 vs 48 ± 9 mg/dl, $p < 0.05$). The concentration of LpA-I:A-II particles was slightly lower in the high insulin group compared to the low insulin group (89 ± 7 vs 99 ± 13 mg/dl, $p < 0.05$). No differences in total and LDL cholesterol (Table 2) or apolipoprotein B levels (113 ± 28 vs 94 ± 28 mg/dl, NS) were observed between the two groups. Despite similar concentrations of LDL cholesterol the particle diameter of the major LDL peak was significantly smaller in the high insulin group as compared to the low insulin group (258 ± 7 vs 265 ± 6 Å, $p < 0.05$). If, however, adjusted for BMI, the difference between the two groups is no longer statistically significant ($p = 0.130$). Individual values for post-heparin plasma LPL and HL activities are shown in Fig. 1. In the high insulin group the mean value of LPL activity was moderately reduced (by 29 %) as compared to the low insulin group (14.2 ± 4.0 vs 20.0 ± 5.8 μmol NEFA · $ml^{-1} \cdot h^{-1}$, $p < 0.001$). As shown in Fig. 1 there was clear overlap of individual values between the two groups. The difference remained significant when LPL activity was adjusted for BMI. Post-heparin plasma HL activity did not differ significantly between the two groups (24.2 ± 11 vs 18.0 ± 5.3 μmol NEFA · $ml^{-1} \cdot h^{-1}$, $p = 0.11$). LPL/HL ratio was markedly lower in the high than the low insulin group (0.75 ± 0.57 vs 1.25 ± 0.65, $p < 0.01$). In the study group as a whole LPL activity correlated negatively with VLDL triglyceride ($r = -0.475$, $p < 0.01$) but positively with HDL_2 cholesterol ($r = 0.479$, $p < 0.01$). There was a weak inverse association between HL activity and HDL_2 triglycerides ($r = -0.352$, $p < 0.05$). As expected LPL/HL ratio correlated inversely with VLDL triglyceride but positively with HDL_2 cholesterol concentration (Fig. 2). In the whole study group there was an inverse association between fasting insulin and post-heparin plasma LPL activity ($r = -0.510$, $p < 0.01$). No association was observed between fasting insulin levels and HL activity.

Discussion

Two important findings emerged from the present study. First, the data suggest that low levels of post-heparin plasma LPL activity as well as that of LPL/HL ratio cluster with insulin resistance in healthy normoglycaemic first-degree relatives of NIDDM patients. A second significant feature is that the reduction in LPL activity and LPL/HL ratio was accompanied by elevation of triglycerides, decrease in HDL cholesterol and preponderance of small dense LDL. Since LPL is the key enzyme in the catabolism of triglyceride-rich particles, and on the other hand LPL and HL have opposite actions on the plasma concentration of HDL, it is tempting to speculate that changes of LPL and more particularly those of LPL/HL ratio in insulin-resistant subjects may contribute to both the decrease in HDL cholesterol and to the increase in triglycerides.

The fact that post-heparin LPL activity was reduced in subjects with high insulin levels is consistent with earlier observations suggesting that insulin resistance may influence LPL activity [23, 45]. Substantial evidence has emerged that the response of LPL in post-heparin plasma and in adipose tissue is unresponsive to insulin action in obesity and in NIDDM [22, 46, 47]. The studies by Pollare et al. [23] provided more direct evidence for the association between insulin resistance and LPL activity. Pollare et al. [23] reported a positive correlation between muscle LPL activity and glucose infusion rate indicating that hyperinsulinaemia actually downregulated muscle LPL activity. We have previously shown that in a healthy non-diabetic population post-heparin plasma LPL activity is closely correlated with LPL activity in both adipose tissue and skeletal muscle [48]. Thus

changes of post-heparin LPL activity reflect those of LPL in both tissues. In this study we measured LPL activity in post-heparin plasma but not in tissues. Therefore, our data do not allow us to conclude whether the decrease in plasma LPL activity reflects a decrease in both adipose tissue and/or only in skeletal muscle.

The mechanisms whereby insulin resistance and/or hyperinsulinaemia downregulates LPL activity are unknown. Interestingly, the data by Peterson et al. [49] indicate that NEFA can dissociate LPL from its binding sites and lead to a rapid degradation of the enzyme [50]. Accordingly, it has been proposed that circulating NEFA may have a feed-back control on LPL activity at the endothelial site. Although we did not measure NEFA levels in this study, available data indicate that insulin-mediated suppression of lipolysis is impaired in insulin-resistant states as well as in glucose-tolerant relatives of NIDDM patients [30, 51–53]. Heterozygous carriers of a defective LPL gene with reduced LPL activity have normal fasting triglyceride levels but demonstrate fat intolerance [54]. Interestingly, Miesenböck et al. [54] reported that in carriers affected by a missense mutation at codon 188 of the LPL gene, the concentration of VLDL triglycerides was higher and that of HDL and HDL_2 cholesterol lower than in non-carriers from the same pedigree. Thus, the phenotype resembled that of the men with high fasting insulin levels in this study. Although multiple genetic defects of LPL have been identified they are rare and hardly explain the lowering of LPL activity in our unrelated men with high insulin levels.

The actual difference of LPL activities in the two groups was only moderate and there was considerable overlap of individual values. However, in the presence of VLDL overproduction as occurs in insulin resistance and in NIDDM due to enhanced NEFA flux into the liver [14] even trivial defects in LPL action would aggravate the elevation of serum triglycerides. In addition compositional changes of secreted VLDL can also modify its characteristics as a substrate for LPL and thus impede the lipolytic process. Thus these dual abnormalities can contribute in concert to the elevation of serum triglycerides. In general, increase of serum triglycerides is accompanied by lowering of HDL cholesterol. These reciprocal changes are partly explained by the action of LPL which also determines plasma HDL, particularly HDL_2 concentrations [24]. Accordingly, in the insulin-resistant men of the present study we observed concomitant but opposite changes of VLDL triglyceride and HDL_2 together with lowering of LPL activity. Recently, Blades et al. [28] reported that lowering of LPL/HL ratios was a significant contributor to low HDL syndrome. Interestingly the HDL lowering action of elevated HL activity was more pronounced in subjects with subnormal LPL activity. Likewise Brinton et al. [55] have enforced LPL/HL ratio i.e. co-ordinate opposite action of the two enzymes, as a determinant of HDL size, its fractional removal rate and consequently its plasma concentration. Although in our study we failed to show any significant difference in the HL activities between the high and low insulin groups the LPL/HL ratio was reduced by 67 %. The observed lowering of LpA-I particles in men with high insulin is consistent with the data indicating that the majority of LpA-I particles reside within HDL_2 density range [56]. Likewise the concentration of apoA-I, which was measured by an independent assay, was reduced in men with high insulin, further confirming the observation that the lowering of HDL is caused by that of HDL_2 particles. The data agree well with a recent report by Montali et al. [57] that there is a preferential reduction of LpA-I particles in patients with hypoalphalipoproteinaemia. The prolonged residence time of VLDL particles would promote core lipid exhange between lipoproteins [58, 59]. Triglyceride enrichment of LDL and HDL in the presence of high HL activity would drive the formation of small dense LDL and lowering of HDL_2 fraction [60]. The particle diameter of the major LDL peak was significantly lower in men with high insulin than in men with low insulin.

In conclusion, our results show that low levels of LPL activity and LPL/HL ratio cluster with insulin resistance in normoglycaemic first-degree relatives of NIDDM patients. We propose that low levels of LPL and LPL/HL ratio provide a possible mechanism to explain the lowering of HDL cholesterol and elevation of triglyceride concentrations observed in insulin-resistant subjects.

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