



HAL
open science

Plasma proteome changes in type 2 diabetes mellitus subjects with low or high early insulin response

Tea Sundsten, Björn Zethelius, Christian Berne, Peter Bergsten

► To cite this version:

Tea Sundsten, Björn Zethelius, Christian Berne, Peter Bergsten. Plasma proteome changes in type 2 diabetes mellitus subjects with low or high early insulin response. *Clinical Science*, 2007, 114 (7), pp.499-507. 10.1042/CS20070323 . hal-00479403

HAL Id: hal-00479403

<https://hal.science/hal-00479403>

Submitted on 30 Apr 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Plasma proteome changes in type 2 diabetes mellitus subjects with low or high early insulin response

Short title: Plasma proteome changes in type 2 diabetes

¹Tea Sundsten, ²Björn Zethelius, ³Christian Berne and ¹Peter Bergsten

Departments of ¹Medical Cell Biology, ²Public Health/Geriatrics and ³Medical Sciences, Uppsala University, Uppsala, Sweden

Address correspondence to:

Tea Sundsten

Department of Medical Cell Biology

Uppsala University, Box 571

SE-751 23 Uppsala

Sweden

Tel: + 46 – 18 – 471 44 27

Fax: + 46 – 18 – 471 40 59

E-mail: Tea.Sundsten@mcb.uu.se

Key-words

early insulin response, type 2 diabetes mellitus, ULSAM, plasma proteins, proteomics, SELDI-TOF MS

Word count

Abstract: 197

Main text: 3222

Abstract

Circulating proteins are in various ways contributing to the pathogenesis of type 2 diabetes mellitus (T2DM). The aim of the study was to investigate variations in plasma protein levels in persons with T2DM and differences in beta cell function, characterized by the early insulin response (EIR), and compare these protein levels with those observed in individuals with normal glucose tolerance (NGT).

Ten individuals with NGT and high EIR, 10 with T2DM and high EIR and 10 with T2DM and low EIR were selected from the community-based Uppsala longitudinal study of adult men (ULSAM) cohort. Plasma protein profiling was performed using surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS.

In total, nine plasma proteins were differing between the three study groups (ANOVA, $p < 0.05$). Two forms of transthyretin, haemoglobin alpha-chain and haemoglobin beta-chain levels were decreased in plasma from T2DM persons compared to NGT individuals, irrespectively of the persons' EIR. Apolipoprotein H was decreased in plasma from individuals with T2DM and high EIR compared to persons with NGT. Four additional un-identified plasma proteins were also varying in different ways between the experimental groups. The proteins found in this study, may be related to the development of beta cell dysfunction.

Introduction

Low early insulin response (EIR) to a glucose challenge is a characteristic and a predictor of type 2 diabetes mellitus (T2DM) [1, 2]. Specific, circulating proteins have been shown to be altered in T2DM and in various ways contribute to the pathogenesis. Examples of such proteins are interleukin-6, resistin, leptin, adiponectin and visfatin, which have been shown to be altered, when measuring their concentrations in serum from persons with different degrees of glucose intolerance, metabolic syndrome or T2DM [3-7]. Several acute phase proteins have also been shown to be altered in T2DM serum, connecting the disease to a low-grade inflammatory process [8-10]. The changes in protein levels can either be viewed as primary changes causing the disease or as secondary effects caused by the disease.

The pathophysiology of T2DM is complex, involving interactions between many genetic and environmental factors. As for any polygenic diseases it is therefore important to study the expression of multiple proteins simultaneously [11, 12]. One approach for studying patterns of proteins is surface enhanced laser desorption/ionization time-of-flight (SELDI-TOF) MS [13]. When the effect of hyperglycaemia on the mitochondrial proteome was investigated *in vitro* in insulin-producing cells cultured at increasing glucose concentrations, several mitochondrial proteins determined by SELDI-TOF MS were found to be regulated by glucose [14]. Also, when normal mouse islets were cultured in the presence of glucose and oleate, oleate- and glucose-regulated proteins were discovered with the approach [15]. SELDI-TOF MS has also been used to study diabetes-related changes in serum in rats with streptozotocin-induced diabetes [16]. Recently, we and others have evaluated the SELDI-platform for analyzing blood samples from humans with type 1 [17] and type 2 diabetes mellitus [18].

In the present study, we have used SELDI-TOF MS with the aim to investigate plasma protein variations in persons with T2DM, characterized by different EIR after an oral glucose challenge, as an indicator of beta cell function. With this approach we have both discovered and identified differently displayed proteins when comparing plasma from subjects with NGT and high EIR with subjects with T2DM and high or low EIR.

Methods

Study subjects

The study was performed on previously healthy men enrolled in the Uppsala longitudinal study of adult men (ULSAM) [2, 19, 20]. Briefly, the population-based ULSAM cohort consists of men living in Uppsala and born between 1920 and 1924. Investigations at age 71 form the baseline in this study [2]. Three groups were selected from this population-based cohort. In the first group, 10 individuals were selected among subjects free from chronic illness at the investigation with a systolic blood pressure (BP) <130 mmHg, diastolic BP <85 mmHg and not using blood pressure lowering medication. Further they were selected for a BMI <25 kg/m², having NGT at the OGTT and being within the highest tertile of the EIR distribution in the NGT group. In the second group, 10 individuals were included with T2DM in the highest tertile of the EIR distribution within the T2DM group. Finally, in the third group, 10 individuals with T2DM in the lowest tertile of the EIR distribution within the T2DM group were chosen. Fasting plasma samples from these subjects were obtained and stored frozen (-70°C) until protein profiling was performed. EIR was defined as the ratio of the 30-min increment in insulin concentration to the 30-min increment in glucose concentration after a 75 g oral glucose challenge. Baseline characteristics for the participants are shown in Table 1. A longitudinal approach was used to analyze plasma protein changes over time. In this analysis, samples obtained at ages 71, 77 and 82 years were used. Clinical characteristics of the participants at age 71 and 77 are shown in Table 2. The study was carried out in accordance to the Helsinki declaration and the study protocol was approved by the Ethics Committee at the Medical Faculty, Uppsala University, Uppsala, Sweden. Informed consent was obtained from all participants.

Chemicals

Chemicals of analytical grade and MilliQ water were used. Acetonitrile (ACN) and trifluoroacetic acid (TFA) came from Merck (Darmstadt, Germany) and urea from Amersham Biosciences (Uppsala, Sweden). The Coomassie stain, pre-stained standard and SDS-PAGE sample buffer came from Fermentas (Burlington, Canada). The ProtoGel Acrylamide:Bisacrylamide was purchased from National Diagnostics (Atlanta, GA, USA). All other chemicals and pure haemoglobin were from Sigma (St.

Louis, MO, USA). The ProteinChip[®] arrays, all-in-1 protein molecular weight standards, Q ceramic Hyper DF spin columns, reverse phase beads and sinapinic acid (SPA) came from CIPHERGEN (Freemont, CA, USA). Size fractionation columns were from Millipore (Billerica, MA, USA).

Profiling, analysis and identification of proteins

Plasma protein patterns were determined by SELDI-TOF MS using the ProteinChip[®] platform (CIPHERGEN) essentially as previously described [18]. In short, plasma samples were thawed on ice, denatured, vortexed, centrifuged and diluted 50 times with binding buffer (100 mmol/l acetate buffer, pH 4). To capture plasma proteins, cationic exchanger (CM10) arrays were used. The arrays were placed in a bioprocessor (CIPHERGEN) and equilibrated with binding buffer. The diluted plasma samples were applied on the spots and left at room temperature on a shaker for 1 hour. The samples were discarded and the arrays were washed using binding buffer and water. Finally, the arrays were air-dried and SPA matrix was applied on the spots of the array.

Time-of-flight spectra were generated in the SELDI MS reader by averaging laser shots at different laser intensities, depending on the mass range studied. For the low molecular weight range (2 to 10 kDa) the laser intensity was set to 220 and the detector sensitivity to 8, for the intermediate molecular weight range (10 to 50 kDa) the laser intensity was set to 220 and the detector sensitivity to 9 and for the high molecular weight range (40 to 100 kDa) the laser intensity was set to 230 and the detector sensitivity to 8. The mass accuracy was calibrated externally using the all-in-1-protein molecular weight standard and internally using previously identified peaks. Peaks were detected and clusters were created using ProteinChip[®] Software 3.1 (CIPHERGEN).

To be able to identify the differently displayed proteins, two different approaches were used. In the first approach, samples were purified by fractionation and separated on gels to recapture proteins of interest for identification using a protocol described earlier [18]. In brief, the plasma samples were fractionated using anionic Q ceramic HyperD F spin columns, reverse phase beads and 50 kDa size fractionation columns. The fractions containing the relevant proteins were speed-vacuum dried, resuspended

and separated on SDS-polyacrylamide gels in two parallel lanes. After staining with colloidal Coomassie stain, protein bands were excised and eluted, while a corresponding band was subjected to in-gel tryptic digestion and peptide mass fingerprinting (PMF).

The second approach took into account that peaks at 15.1 and 15.8 kDa were previously identified as alpha- and beta-chains of haemoglobin (Hb) [21]. Pure Hb (Sigma-Aldrich) was dissolved in buffer (50 mmol/l Tris, 9 mol/l urea, 2 % CHAPS, pH 9), diluted 1:10 in binding buffer (100 mmol/l acetate buffer, pH 4) and applied on a cationic exchanger array. The resulting spectrum was compared with the plasma spectrum peaks. The concentration of Hb was also measured in the original plasma samples, using an ELISA for human Hb (Bethyl Inc, Montgomery, TX, USA).

Statistical analysis

All cluster data were analyzed using GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) or STATA 8.2, (STATA-corporation, College Station, TX, USA). ANOVA was used to compare the means of several groups. Student's t-test was used to further analyze differences between groups and p values <0.05 were considered significant. ANCOVA, adjusting for insulin sensitivity index (M/I) derived from the euglycaemic insulin clamp, BMI or waist circumference, were also performed in separate models to adjust for possible confounding of insulin resistance. ANCOVA, adjusting for cystatin-based glomerular filtration rate (GFR), was performed to analyze possible confounding of impaired kidney function.

Results

Study subjects

The aim of the study was to investigate variations in plasma protein levels in persons with T2DM and differences in beta cell function. To this end, T2DM individuals with low (n=10) or high (n=10) EIR and control (n=10) individuals were selected from the ULSAM cohort. Whereas the T2DM individuals with high EIR had approximately 40% of the EIR observed in controls, T2DM individuals with low EIR only had approximately 10% of the control level (Table 1). Fasting glucose as well as the 2-hour glucose value after OGTT was significantly elevated in both T2DM groups.

Both BMI and waist circumference measurements were significantly higher in the two

T2DM groups. Insulin sensitivity (M/I) and adiponectin levels were significantly lower in T2DM individuals compared to NGT. Cystatin-based GFR was significantly higher in the T2DM individuals with low EIR.

Seven individuals (4 NGT and 3 T2DM with high EIR) were analyzed longitudinally. Fasting levels of glucose and proinsulin did not change within the NGT or T2DM groups when values were compared at ages 71 and 77 years (Table 2).

Profiling and analysis of proteins

Protein profiling was performed on denaturated diluted plasma samples and protein profiles from each subject were obtained with CM10 arrays and SELDI-TOF MS. With the CM10 array in total 26, 46 and 35 protein clusters were found in the low, intermediate and high mass range, respectively. The amounts of these proteins were calculated for each subject by determination of peak height. Out of the detected peaks, nine peaks with masses 3148, 7443, 13932, 14092, 15114, 15825, 17154, 44106 and 72834 Da were different between the experimental groups (ANOVA, $p < 0.05$). The results are presented in Figure 1 and Table 3 and representative mass spectra are shown in Figure 2. When the peak data were adjusted for M/I, BMI, waist circumference or GFR, essentially the same results were obtained, thus these variables could be eliminated as confounders.

For all nine significantly different proteins we performed a correlation analysis. Only weak correlations (R^2 between 0 and 0.26) were obtained when the individual protein intensities were plotted against EIR (Figure 3), demonstrating that no individual protein alone could explain the association to EIR, the parameter used as selection criterion in this study.

Identification of proteins

With the purpose of purifying proteins to such an extent that they could be identified by PMF, plasma samples were subjected to anionic fractionation. The anionic flow through fraction contained the 15825 and 44106 Da proteins, while the anionic fraction pH 4 had retained proteins with masses 13932, 14092, 15825 and 44106 Da. The 3148 Da protein was not found in any of the anionic fractions. By further purifying the anionic flow through fraction the 44106 Da protein was isolated. Also,

the anionic fraction pH 4 was further purified resulting in a relatively pure 13932 Da protein. The 13932 Da protein was identified as transthyretin (TTR) and the 44106 Da protein as apolipoprotein H (ApoH).

Although we were able to purify some of the differently displayed proteins by fractionating the plasma, the proteins at masses 15.1 and 15.8 kDa did not elute from the gel. Therefore, an alternative approach was applied, making use of the fact that the theoretical masses of alpha- and beta-chains of human Hb have previously been shown to correspond with peaks at 15.1 and 15.8 kDa [21]. When pure human Hb was diluted and applied on a cationic exchanger array, the resulting peaks in the spectrum aligned with the plasma spectrum peaks of 15114 and 15825 Da (Figure 4). To verify these results, the Hb concentration of the plasma samples was also measured using a human Hb ELISA. At age 71 the NGT individuals had a higher plasma Hb concentration than both the two T2DM groups. The plasma Hb concentrations were 70 ± 12 $\mu\text{g/ml}$ in the NGT group, 44 ± 4 $\mu\text{g/ml}$ in the T2DM group with high EIR and 39 ± 7 $\mu\text{g/ml}$ in the T2DM group with low EIR (Student's t-test, $p < 0.05$).

Longitudinal protein changes

We also performed analysis of protein profiles over time. Out of the 30 participants in the initial analysis at age 71, seven had also been characterized at ages 77 and 82. Four of the individuals had NGT and three had T2DM, high EIR. The clinical characteristics of the study participants had not changed considerably over time when comparing fasting glucose and fasting proinsulin (Table 2). The plasma samples from age 71, 77 and 82 were applied on cationic exchanger arrays and SELDI-analysis was performed as described above. Out of the nine proteins found in the initial comparison at age 71, none showed differences over time (Table 4). For all nine proteins the levels in the groups were comparable to those in the initial investigation (Table 4).

Discussion

Assessing plasma protein profiles from individuals with NGT and high EIR and individuals with T2DM and high or low EIR, nine proteins with different plasma concentrations were found, out of which four were identified.

We used the SELDI-platform, where chemically defined surfaces are used to capture proteins. The technique demonstrates reproducibility and quantitiveness and has successfully been used in large-scale patient proteomic studies [22, 23]. The SELDI-technology has been criticized for preferentially capturing high abundant proteins including acute phase reactants [24]. Pre-sample fractionations can be performed to eliminate albumin and other high-abundant proteins from the samples. However, such pre-fractionation may also influence the remaining sample proteins. In the present study we therefore chose to profile neat plasma with SELDI-TOF MS adhering to a previously developed protocol used by us [18] and others [25].

Among the plasma proteins, which were decreased in individuals with T2DM, we identified TTR. TTR displays low variances across healthy cohorts [26] and can therefore be considered a robust marker of disease although it is an acute phase protein. In patients with insulin resistance and diabetes several acute phase reactants like c-reactive protein, have been shown to be increased in serum [8]. This has been interpreted to be a part of the low-grade inflammation present in these patients [9, 10]. In our study, TTR was lowered in plasma from T2DM individuals irrespectively of the measured EIR. The blood profile of TTR is well-characterized, showing several peaks around 13.8-14.1 kDa [27-29]. The peaks all represent TTR, native and with different modifications. The peak at 13.9 kDa in the present study was identified as TTR, which has been done previously by us [18] and others [21]. The 14.1 kDa peak matched well the glutathionated form of the TTR peak. TTR is mainly produced in the liver and choroid plexus, but also within the pancreatic islets [30], where it has been shown to increase the cytoplasmic Ca^{2+} concentration and promote insulin release [31]. Reduced levels of TTR have also been associated with inflammatory conditions [32] and type 1 diabetes [33]. Also, in individuals with type 1 diabetes, rise in blood levels of the 13.9 kDa form of TTR was observed and suggested to be associated with development of beta cell failure in the disease [31, 34].

Hb alpha- and beta-chains were also identified among proteins lowered in plasma from individuals with T2DM in the present study. The proteins were identified by inference with the protein profile of pure human Hb, as has been done in another study [21]. The peaks of the alpha- and beta-chains of Hb aligned with the peaks obtained in the SELDI-spectra of the present study. In addition, the results were

confirmed by measuring Hb with an ELISA, which showed significantly higher levels in NGT compared to T2DM. An increase of plasma Hb could be explained by red blood cell lysis and subsequent release of Hb. This explanation to our results is not likely, since the blood samples were collected and plasma was prepared at different time points during a period of several months. In addition, Hb levels were consistently higher in NGT individuals also at ages 77 and 82 years, making haemolysis not a plausible explanation.

ApoH, also known as beta2-glycoprotein-I, is secreted from the liver [35] and exists in plasma both as free ApoH and bound to lipoprotein particles [36]. In the present study ApoH was decreased in plasma from T2DM individuals with high EIR compared to NGT individuals. The protein has been implicated in many physiological pathways, including lipoprotein metabolism [37] and coagulation [38]. Previously it has been shown that plasma ApoH concentrations are increased in patients with diabetes compared to healthy individuals and strongly correlated with total plasma cholesterol [39]. On the other hand, ApoH has been shown to be decreased during an inflammatory response [40] and markers of inflammation have also been associated with the development of T2DM [9, 41], which may explain the decreased levels in plasma from T2DM individuals found in the present investigation. However, the levels of ApoH in T2DM individuals with low EIR were not reduced compared to the levels found in NGT subjects. It could be speculated, that this reflects that ApoH levels only decrease temporarily in the beginning of beta cell dysfunction and reach normal levels again after further decrease of EIR caused by unknown mechanisms.

The longitudinal protein analysis was performed to investigate if the differently displayed proteins showed variation over time. Of the initial 30 study participants only seven were re-investigated at both ages 77 and 82 years. None of these persons had T2DM and low EIR, which is not surprising since both morbidity and mortality are higher in this group of patients. All nine proteins found in the initial investigation, remained unchanged over time, suggesting that the found variations were manifest in the groups. The results also state, that the plasma sample collection and handling was performed adequately, without protein degradation.

The inability in T2DM to regulate glucose levels adequately can be caused by impaired beta cell function and/or insulin resistance. Changes in plasma proteins in T2DM can either be regarded as reflecting the cause or the effects of the disease. No single protein in the present study could by itself explain the differences seen in EIR, reinforcing the polygenic nature of T2DM, characterized by changes in many proteins [42]. In the present study most differently displayed proteins were obtained in the comparison between NGT and both T2DM groups, irrespectively of the difference in EIR. This suggests that these differences are due to the diabetic environment. For example, the hyperglycaemic environment is comparable in the two T2DM groups, but the differences in EIR imply variations in the genetic background. Such conclusion is consistent with a previous investigation, where we have seen that the majority of serum protein alterations between NGT and T2DM are caused by the diabetic environment, rather than genetic factors [43]. The protein data remained unchanged when adjusted for M/I, BMI, waist circumference or GFR, thus eliminating these potential confounding factors.

In conclusion, we have demonstrated that several plasma proteins differ between individuals with NGT and T2DM characterized by differences in EIR. Transthyretin, Hb alpha-chain and Hb beta-chain were all decreased in plasma from individuals with T2DM compared to NGT. Apolipoprotein H was decreased in T2DM individuals with high EIR compared to NGT. It is concluded that these differences are probably manifestations of the disease state rather than being causative.

Acknowledgements

The study was supported by grants from the Swedish Medical Research Council (72X-14019), the European Foundation for the Study of Diabetes, the Swedish Diabetes Association, the Swedish Medical Association, the Novo Nordisk Foundation, the Swedish Society for Medical Research, Göran Gustafsson Foundation, Marcus and Amalia Wallenberg Foundation, Family Ernfors Fund, Erik, Karin and Gösta Sehlander Foundation, Thuring Foundation and Syskonen Svenssons Fund.

Table 1 Clinical characteristics cross sectional at age 71 years

	Group 1	Group 2	Group 3	ANOVA	t-test 1 vs. 2 p	t-test 1 vs. 3 p	t-test 2 vs. 3 p
	NGT n=10	T2DM High EIR n=10	T2DM Low EIR n=10				
EIR (pmol/mmol)	23±6.9	9.9±3.8	2.4±0.7	<0.0001	<0.0001	<0.0001	<0.0001
Fasting glucose (mmol/l)	5.2±0.9	6.9±0.9	8.6±3.0	0.0019	0.0002	0.0017	0.0511
2h-glucose (mmol/l)	5.8±0.9	14.5±2.9	16.4±3.9	<0.0001	<0.0001	<0.0001	0.1068
Fasting insulin (pmol/l)	60±57	75±26	54±37	0.5000	-	-	-
Fasting proinsulin (pmol/l)	8.5±3.4	15.6±10.4	17.2±18.2	0.25	-	-	-
Fasting 32-33 split proinsulin (pmol/l)	10.3±6.7	19.3±11.5	19.1±21.6	0.30	-	-	-
M/I (100x(μmol)(kg body weight) ⁻¹ min ⁻¹ (pmol/l) ⁻¹)	5.4±1.2	2.6±1.1	3.5±2.1	0.0023	<0.0001	0.0176	0.1148
BMI (kg/m ²)	24.1±0.8	27.5±2.1	29.7±7.0	0.0191	0.0001	0.0097	0.1694
Waist (cm)	91.0±4.0	96.7±6.4	104.5±18	0.0493	0.0175	0.0204	0.1048
Adiponectin (mg/l)	13.1±3.7	7.9±2.0	8.6±3.3	0.0012	0.0005	0.0044	0.2903
FFA (mmol/l)	0.50±0.18	0.53±0.14	0.62±0.09	0.1445	-	-	-
GFR (ml/min)	53.7±13.2	59.0±11.2	71.1±10.0	0.0082	0.1771	0.0023	0.0103

Data are means ± SD.

Abbreviations: NGT normal glucose tolerance; T2DM type 2 diabetes mellitus; EIR early insulin response; M/I insulin sensitivity index; FFA free fatty acids; GFR glomerular filtration rate

Table 2 Clinical characteristics longitudinal comparisons using a subset of participants with repeated sampling at ages 71 and 77 years

	NGT, n=4		T2DM, High EIR, n=3	
	Age 71	Age 77	Age 71	Age 77
Fasting glucose (mmol/l)	5.2±1.4	5.1±0.7	6.8±1.3	9.0±2.69
Fasting proinsulin (pmol/l)	8.7±3.9	6.7±4.0	9.9±3.7	10.6±6.9
Fasting 32-33 split proinsulin (pmol/l)	11.1±9.4	7.8±4.2	17.0±10.5	15.0±14.0

Data are means ± SD.

Abbreviations: NGT normal glucose tolerance; T2DM type 2 diabetes mellitus; EIR early insulin response

Table 3 Relative peak intensities cross sectional at age 71 years

Protein (Da)	Relative peak intensity			ANOVA	t-test 1 vs. 2 p	t-test 1 vs. 3 p	t-test 2 vs. 3 p
	Group 1 NGT n=10	Group 2 T2DM High EIR n=10	Group 3 T2DM Low EIR n=10				
3148	17.8 (7.8)	32.1 (16.3)	19.0 (0.2)	0.0143	0.019	0.3571	0.0162
7443	3.73 (0.6)	2.96 (0.7)	3.4 (0.7)	0.0438	0.0079	0.0915	0.1097
13932	7.9 (1.6)	6.0 (1.2)	6.2 (1.0)	0.0051	0.0039	0.0055	0.3233
14092	4.2 (0.6)	3.4 (0.8)	3.3 (0.5)	0.0099	0.0125	0.0011	0.3406
15114	1.24 (0.85)	0.57 (0.13)	0.58 (0.13)	0.0080	0.0121	0.0135	0.4065
15825	1.07 (0.83)	0.40 (0.12)	0.39 (0.13)	0.0057	0.0106	0.0100	0.4441
17154	1.47 (0.26)	1.29 (0.37)	1.06 (0.39)	0.0479	0.1274	0.0072	0.0943
44106	0.83 (0.07)	0.73 (0.09)	0.79 (0.07)	0.0216	0.0060	0.1064	0.0508
72834	0.82 (0.08)	0.85 (0.04)	0.77 (0.07)	0.0365	0.1893	0.0619	0.0043

Data are means \pm SD.

Abbreviations: NGT normal glucose tolerance; T2DM type 2 diabetes mellitus; EIR early insulin response

Table 4 Relative peak intensities longitudinal comparisons using a subset of participants with repeated sampling at ages 71, 77 and 82 years

Protein (Da)	Relative peak intensity NGT, n=4			ANOVA p	Relative peak intensity T2DM, high EIR, n=3			ANOVA p
	Age 71	Age 77	Age 82		Age 71	Age 77	Age 82	
3148	15.6±7.7	14.6±3.1	12.0±5.2	0.6667	19.0±3.4	14.0±1.8	13.4±1.3	0.0512
7443	6.0±2.4	5.2±2.1	6.0±2.8	0.8837	5.3±1.3	4.7±1.4	4.8±1.4	0.8824
13932	17.5±4.3	15.1±3.5	16.4±5.3	0.7625	15.1±3.0	13.6±3.5	14.0±3.2	0.8515
14092	6.7±1.8	6.5±2.2	6.5±0.9	0.9859	6.4±0.4	5.3±1.0	5.3±0.9	0.2046
15114	3.4±1.6	3.3±1.8	2.4±1.5	0.6405	1.8±0.3	1.2±0.4	2.6±2.2	0.4704
15825	2.8±1.3	2.7±1.6	1.9±1.3	0.6314	1.3±0.3	0.74±0.35	1.9±1.9	0.4700
17154	2.6±0.7	2.5±1.0	2.7±0.6	0.9587	2.4±0.7	2.4±0.3	2.2±0.5	0.8619
44106	0.84±0.12	0.91±0.06	0.87±0.13	0.6759	0.83±0.12	0.87±0.09	0.82±0.07	0.7904
72834	0.79±0.10	0.75±0.05	0.71±0.03	0.6080	0.70±0.06	0.71±0.12	0.72±0.04	0.9488

Data are means ± SD.

Abbreviations: NGT normal glucose tolerance; T2DM type 2 diabetes mellitus; EIR early insulin response

References

1. Kahn S. E., Montgomery B., Howell W., et al. (2001) Importance of early phase insulin secretion to intravenous glucose tolerance in subjects with type 2 diabetes mellitus. *J Clin Endocrinol Metab* **86**, 5824-5829
2. Zethelius B., Byberg L., Hales C. N., Lithell H. and Berne C. (2003) Proinsulin and acute insulin response independently predict Type 2 diabetes mellitus in men--report from 27 years of follow-up study. *Diabetologia* **46**, 20-26
3. Vozarova B., Weyer C., Hanson K., Tataranni P. A., Bogardus C. and Pratley R. E. (2001) Circulating interleukin-6 in relation to adiposity, insulin action, and insulin secretion. *Obes Res* **9**, 414-417
4. Norata G. D., Ongari M., Garlaschelli K., Raselli S., Grigore L. and Catapano A. L. (2007) Plasma resistin levels correlate with determinants of the metabolic syndrome. *Eur J Endocrinol* **156**, 279-284
5. Considine R. V., Sinha M. K., Heiman M. L., et al. (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* **334**, 292-295
6. Hotta K., Funahashi T., Arita Y., et al. (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* **20**, 1595-1599
7. Berndt J., Kloting N., Kralisch S., et al. (2005) Plasma visfatin concentrations and fat depot-specific mRNA expression in humans. *Diabetes* **54**, 2911-2916
8. Festa A., D'Agostino R., Jr., Tracy R. P. and Haffner S. M. (2002) Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* **51**, 1131-1137
9. Pickup J. C. (2004) Inflammation and activated innate immunity in the pathogenesis of type 2 diabetes. *Diabetes Care* **27**, 813-823
10. Sjöholm A. and Nystrom T. (2006) Inflammation and the etiology of type 2 diabetes. *Diabetes Metab Res Rev* **22**, 4-10
11. Wang J., Li D., Dangott L. J. and Wu G. (2006) Proteomics and its role in nutrition research. *J Nutr* **136**, 1759-1762
12. Korc M. (2003) Diabetes mellitus in the era of proteomics. *Mol Cell Proteomics* **2**, 399-404
13. Issaq H. J., Veenstra T. D., Conrads T. P. and Felschow D. (2002) The SELDI-TOF MS approach to proteomics: protein profiling and biomarker identification. *Biochem Biophys Res Commun* **292**, 587-592
14. Nyblom H. K., Thorn K., Ahmed M. and Bergsten P. (2006) Mitochondrial protein patterns correlating with impaired insulin secretion from INS-1E cells exposed to elevated glucose concentrations. *Proteomics* **6**, 5193-5198
15. Orstater H., Sundsten T., Lin J. M. and Bergsten P. (2007) Evaluation of the SELDI-TOF MS technique for protein profiling of pancreatic islets exposed to glucose and oleate. *Proteomics* **7**, 3105-3115
16. Cho W. C., Yip T. T., Chung W. S., Leung A. W., Cheng C. H. and Yue K. K. (2006) Differential expression of proteins in kidney, eye, aorta, and serum of diabetic and non-diabetic rats. *J Cell Biochem* **99**, 256-268

17. Purohit S., Podolsky R., Schatz D., et al. (2006) Assessing the utility of SELDI-TOF and model averaging for serum proteomic biomarker discovery. *Proteomics* **6**, 6405-6415
18. Sundsten T., Eberhardson M., Goransson M. and Bergsten P. (2006) The use of proteomics in identifying differentially expressed serum proteins in humans with type 2 diabetes. *Proteome Sci* **4**, 22
19. Hedstrand H. (1975) A study of middle-aged men with particular reference to risk factors for cardiovascular disease. *Ups J Med Sci Suppl* **19**, 1-61
20. Lithell H., Sundstrom J., Arnlov J., et al. (2000) Epidemiological and clinical studies on insulin resistance and diabetes. *Ups J Med Sci* **105**, 135-150
21. Kozak K. R., Su F., Whitelegge J. P., Faull K., Reddy S. and Farias-Eisner R. (2005) Characterization of serum biomarkers for detection of early stage ovarian cancer. *Proteomics* **5**, 4589-4596
22. Qu Y., Adam B. L., Yasui Y., et al. (2002) Boosted decision tree analysis of surface-enhanced laser desorption/ionization mass spectral serum profiles discriminates prostate cancer from noncancer patients. *Clin Chem* **48**, 1835-1843
23. Laronga C., Becker S., Watson P., et al. (2003) SELDI-TOF serum profiling for prognostic and diagnostic classification of breast cancers. *Dis Markers* **19**, 229-238
24. Diamandis E. P. (2004) Mass spectrometry as a diagnostic and a cancer biomarker discovery tool: opportunities and potential limitations. *Mol Cell Proteomics* **3**, 367-378
25. Rai A. J., Stemmer P. M., Zhang Z., et al. (2005) Analysis of Human Proteome Organization Plasma Proteome Project (HUPO PPP) reference specimens using surface enhanced laser desorption/ionization-time of flight (SELDI-TOF) mass spectrometry: multi-institution correlation of spectra and identification of biomarkers. *Proteomics* **5**, 3467-3474
26. Timms J. F., Arslan-Low E., Gentry-Maharaj A., et al. (2007) Preanalytic influence of sample handling on SELDI-TOF serum protein profiles. *Clin Chem* **53**, 645-656
27. Terazaki H., Ando Y., Suhr O., et al. (1998) Post-translational modification of transthyretin in plasma. *Biochem Biophys Res Commun* **249**, 26-30
28. Kiernan U. A., Tubbs K. A., Gruber K., et al. (2002) High-throughput protein characterization using mass spectrometric immunoassay. *Anal Biochem* **301**, 49-56
29. Schweigert F. J., Wirth K. and Raila J. (2004) Characterization of the microheterogeneity of transthyretin in plasma and urine using SELDI-TOF-MS immunoassay. *Proteome Sci* **2**, 5
30. Jacobsson B., Collins V. P., Grimelius L., Pettersson T., Sandstedt B. and Carlstrom A. (1989) Transthyretin immunoreactivity in human and porcine liver, choroid plexus, and pancreatic islets. *J Histochem Cytochem* **37**, 31-37
31. Refai E., Dekki N., Yang S. N., et al. (2005) Transthyretin constitutes a functional component in pancreatic beta-cell stimulus-secretion coupling. *Proc Natl Acad Sci U S A* **102**, 17020-17025
32. Myron Johnson A., Merlini G., Sheldon J. and Ichihara K. (2007) Clinical indications for plasma protein assays: transthyretin (prealbumin) in inflammation and malnutrition. *Clin Chem Lab Med* **45**, 419-426

33. Itoh N., Hanafusa T., Miyagawa J., et al. (1992) Transthyretin (prealbumin) in the pancreas and sera of newly diagnosed type I (insulin-dependent) diabetic patients. *J Clin Endocrinol Metab* **74**, 1372-1377
34. Yang Q., Yamagata K., Yamamoto K., et al. (1999) Structure/function studies of hepatocyte nuclear factor-1alpha, a diabetes-associated transcription factor. *Biochem Biophys Res Commun* **266**, 196-202
35. Day J. R., O'Hara P. J., Grant F. J., et al. (1992) Molecular cloning and sequence analysis of the cDNA encoding human apolipoprotein H (beta 2-glycoprotein I). *Int J Clin Lab Res* **21**, 256-263
36. Polz E. and Kostner G. M. (1979) The binding of beta 2-glycoprotein-I to human serum lipoproteins: distribution among density fractions. *FEBS Lett* **102**, 183-186
37. Cassader M., Ruiu G., Gambino R., et al. (1994) Influence of apolipoprotein H polymorphism on levels of triglycerides. *Atherosclerosis* **110**, 45-51
38. Roubey R. A. (1994) Autoantibodies to phospholipid-binding plasma proteins: a new view of lupus anticoagulants and other "antiphospholipid" autoantibodies. *Blood* **84**, 2854-2867
39. Cassader M., Ruiu G., Gambino R., Veglia F. and Pagano G. (1997) Apolipoprotein H levels in diabetic subjects: correlation with cholesterol levels. *Metabolism* **46**, 522-525
40. Mehdi H., Nunn M., Steel D. M., et al. (1991) Nucleotide sequence and expression of the human gene encoding apolipoprotein H (beta 2-glycoprotein I). *Gene* **108**, 293-298
41. Schmidt M. I., Duncan B. B., Sharrett A. R., et al. (1999) Markers of inflammation and prediction of diabetes mellitus in adults (Atherosclerosis Risk in Communities study): a cohort study. *Lancet* **353**, 1649-1652
42. Gerich J. E. (1998) The genetic basis of type 2 diabetes mellitus: impaired insulin secretion versus impaired insulin sensitivity. *Endocr Rev* **19**, 491-503
43. Sundsten T., Östenson C.-G. and Bergsten P. (2007) Serum protein patterns in newly diagnosed type 2 diabetes mellitus – influence of diabetic environment and family history of diabetes. *Diabetes/Metabolism Research and Reviews* **in press**

Figure legends

Figure 1.

Plasma protein levels of individuals with normal glucose tolerance (white bars), type 2 diabetes with high early insulin response (grey bars) and type 2 diabetes with low early insulin response (black bars). Protein masses are indicated and results are means \pm SD of ten experiments. Significant differences ($p < 0.05$) between groups are denoted by *.

Figure 2.

Plasma SELDI-TOF mass spectra in the mass region 8-18 kDa. Arrows indicate differentially displayed plasma proteins at 13.9, 14.1, 15.1, 15.8 and 17.2 kDa.

Representative spectra from individuals with normal glucose tolerance (panel a), type 2 diabetes with high early insulin response (panel b) and type 2 diabetes with low early insulin response (panel c) are shown.

Figure 3.

For all differentially displayed proteins the peak heights of the study participants were plotted against corresponding early insulin responses (EIR). Normal glucose tolerant individuals (white circles), type 2 diabetes individuals with high early insulin responsiveness (grey circles) and type 2 diabetes individuals with low early insulin responsiveness (black circles) are shown. The correlations were in all cases non-existent or weak. The R^2 values are shown in the graphs.

Figure 4.

Pure haemoglobin spectrum (panel a) was compared with the plasma spectrum (panel b). The peaks of 15114 and 15825 coincided well with peaks from pure Hb.

Figure 1

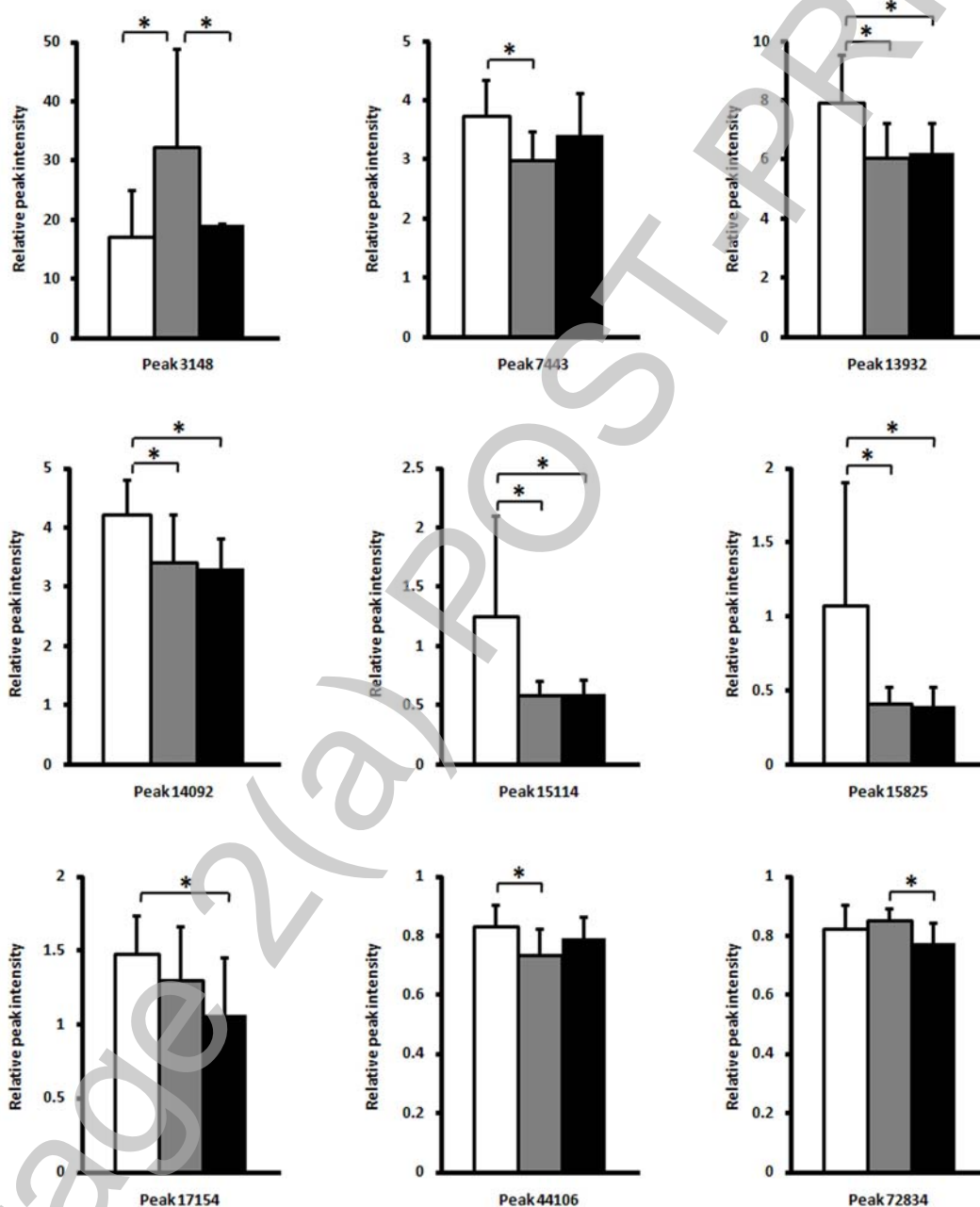


Figure 2

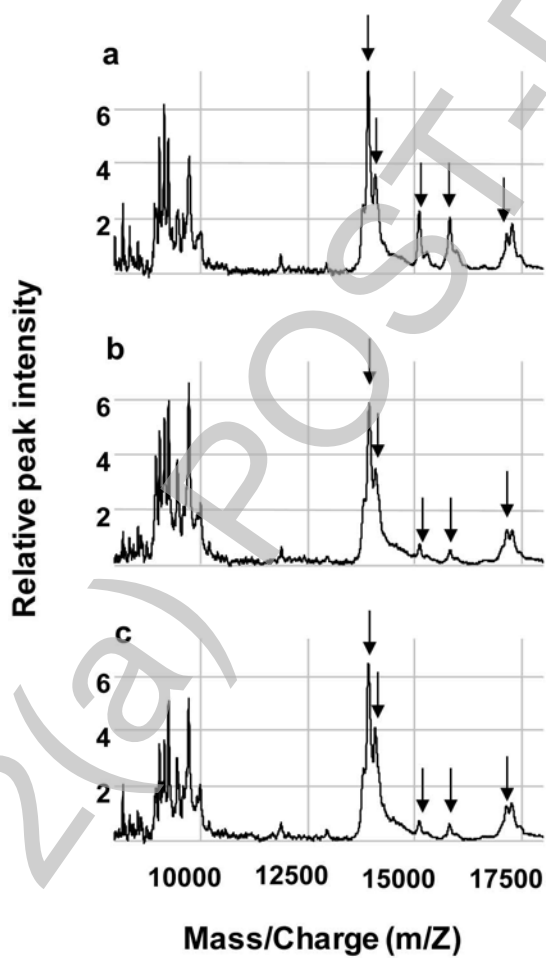
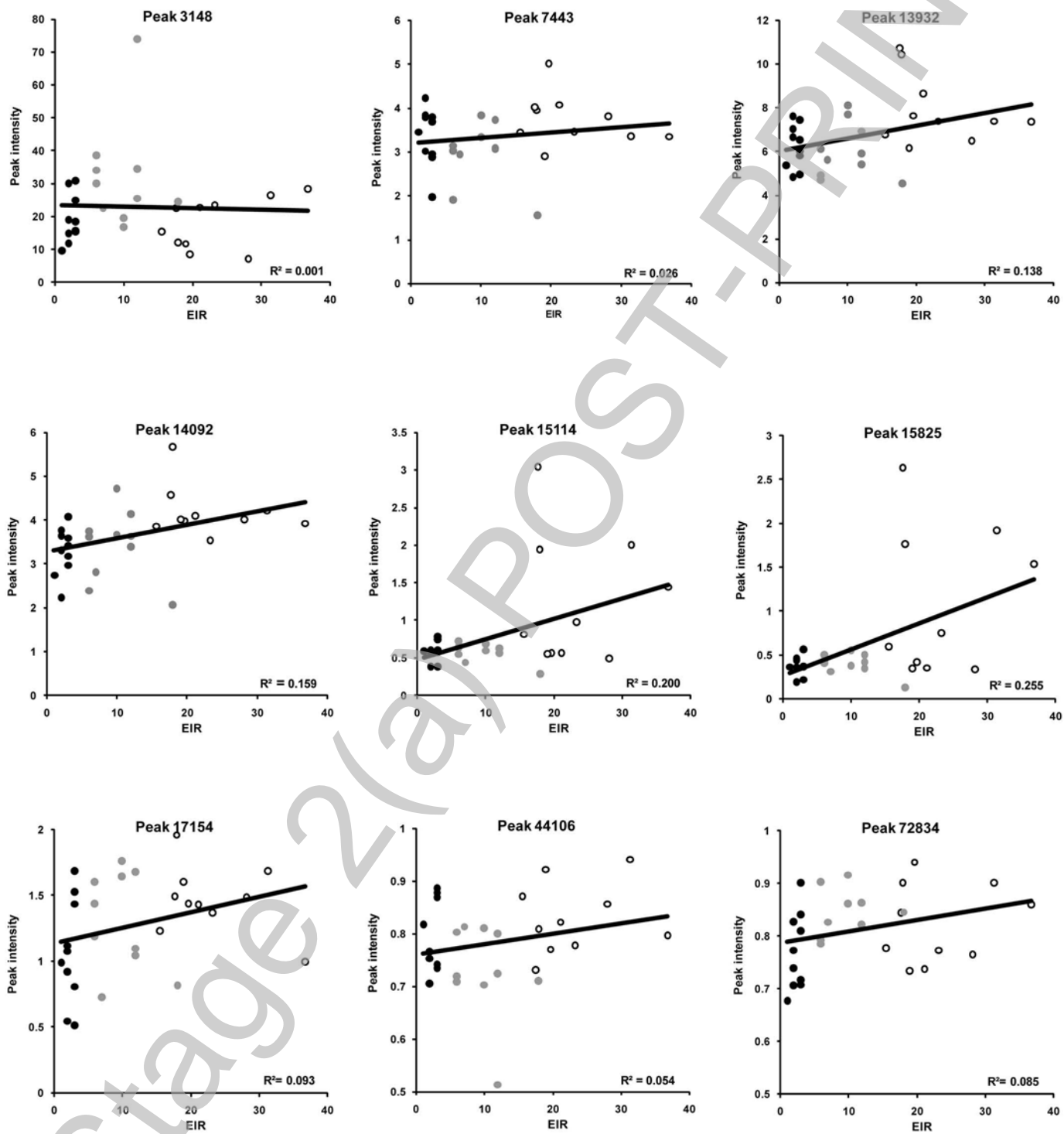


Figure 3



THIS IS NOT THE FINAL VERSION - see doi:10.1042/CS20070323

Figure 4

