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

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ABSTRACT

Circulating proteins contribute to the pathogenesis of T2DM (Type 2 diabetes mellitus) in various ways. The aim of the present study was to investigate variations in plasma protein levels in subjects with T2DM and differences in β-cell function, characterized by the EIR (early insulin response), and to compare these protein levels with those observed in individuals with NGT (normal glucose tolerance). Ten subjects with NGT + high EIR, ten with T2DM + high EIR, and ten with T2DM + low EIR were selected from the community-based ULSAM (Uppsala Longitudinal Study of Adult Men) cohort. Plasma protein profiling was performed using SELDI-TOF (surface-enhanced laser-desorption ionization–time-of-flight) MS. In total, nine plasma proteins differed between the three study groups (P < 0.05, as determined by ANOVA). The levels of two forms of transthyretin, haemoglobin α-chain and haemoglobin β-chain were decreased in plasma from subjects with T2DM compared with subjects with NGT, irrespective of the EIR of the subjects. Apolipoprotein H was decreased in plasma from individuals with T2DM + high EIR compared with subjects with NGT. Four additional unidentified plasma proteins also varied in different ways between the experimental groups. In conclusion, the proteins detected in the present study may be related to the development of β-cell dysfunction.

INTRODUCTION

A low EIR (early insulin response) to a glucose challenge is a characteristic and a predictor of T2DM (Type 2 diabetes mellitus) [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 include IL-6 (interleukin-6), resistin, leptin, adiponectin and visfatin which have been shown to be altered when their concentrations in serum have been measured from subjects with different degrees of glucose intolerance, metabolic syndrome or T2DM [3–7]. Several acute-phase proteins have also been shown to be altered in serum from subjects with T2DM, 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 disease, it is therefore

Key words: early insulin response, plasma protein, proteomics, surface-enhanced laser-desorption ionization–time-of-flight MS (SELDI-TOF MS), Type 2 diabetes mellitus, ULSAM (Uppsala Longitudinal Study of Adult Men).

Abbreviations: ANCOVA, analysis of covariance; ApoH, apolipoprotein H; BMI, body mass index; BP, blood pressure; EIR, early insulin response; GFR, glomerular filtration rate; Hb, haemoglobin; M/I, insulin sensitivity index; NGT, normal glucose tolerance; PMF, peptide mass fingerprinting; SELDI, surface-enhanced laser-desorption ionization; TOF, time-of-flight; SPA, sinapinic acid; T1DM, Type 1 diabetes mellitus; T2DM, Type 2 diabetes mellitus; TTR, transthyretin; ULSAM, Uppsala Longitudinal Study of Adult Men.

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**Cross-sectional clinical characteristics at 71 years of age**

Values are means ± S.D. NEFAs, non-esterified fatty acids. *P value as determined by ANOVA; †P value as determined by Student’s *t*-test.

| Characteristic                        | Subjects with T2DM |       |       |       | Subjects with T2DM |       |       |       |       |       |       |       |       |       |       |       |       |
|--------------------------------------|--------------------|-------|-------|-------|-------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|                                      | NGT (n = 10)       | + high EIR (n = 10) | + low EIR (n = 10) | P value* | NGT compared with T2DM + high EIR | NGT compared with T2DM + low EIR | T2DM + high EIR compared with T2DM + low EIR |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 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 |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
| 2-h 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 (100 × μ mol · (kg of 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/m²)                          | 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 |       |       |       |       |       |       |       |       |       |       |       |       |       |
| NEFAs (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 |       |       |       |       |       |       |       |       |       |       |       |       |       |

**MATERIALS AND METHODS**

**Study subjects**
The study was performed on previously healthy men enrolled in ULSAM (Uppsala Longitudinal Study of Adult Men) cohort [2,19,20]. Briefly, the population-based ULSAM cohort consists of men living in Uppsala and born between 1920 and 1924. Investigations at 71 years of age form the baseline of the study [2]. Three groups were selected from this population-based cohort. In the first group, ten subjects were selected among those free from chronic illness at the baseline investigation with a systolic BP (blood pressure) < 130 mmHg, diastolic BP < 85 mmHg and not using BP-lowering medication. Furthermore, they were selected for a BMI (body mass index) < 25 kg/m², having NGT during an OGTT (oral glucose tolerance test) and being within the highest tertile of the EIR distribution in the NGT group. In the second group, ten subjects were included with T2DM in the highest tertile of the EIR distribution within the T2DM group (TM2DM + high EIR). Finally, in the third group, ten subjects with T2DM in the lowest tertile of the EIR distribution within the T2DM group were chosen (T2DM + low EIR). 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 of the subjects are shown in Table 1.

A longitudinal approach was used to analyse plasma protein changes over time. In this analysis, samples obtained at 71, 77 and 82 years of age were used. Clinical characteristics of the participants at 71 and 77 years of age are shown in Table 2.

The study was carried out in accordance to the Declaration of Helsinki, and the protocol was approved by the Ethics Committee at the Medical Faculty, Uppsala.
Longitudinal comparisons of the clinical characteristics using a subset of participants with repeated sampling at 71 and 77 years of age

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subjects with NGT (n = 4)</th>
<th>Subjects with T2DM + high EIR (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Age 71</td>
<td>Age 77</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>5.2 ± 1.4</td>
<td>5.1 ± 0.7</td>
</tr>
<tr>
<td>Fasting proinsulin (pmol/l)</td>
<td>8.7 ± 3.9</td>
<td>6.7 ± 4.0</td>
</tr>
<tr>
<td>Fasting 32–33 split proinsulin (pmol/l)</td>
<td>11.1 ± 9.4</td>
<td>7.8 ± 4.2</td>
</tr>
</tbody>
</table>

University, Uppsala, Sweden. Informed consent was obtained from all participants.

Chemicals

Chemicals of analytical grade and MilliQ water were used. Acetonitrile and trifluoroacetic acid were from Merck, and urea was purchased from Amersham Biosciences. Coomassie Blue stain, pre-stained molecular-mass standards and SDS/PAGE sample buffer were from Fermentas. ProtoGel acrylamide-bisacrylamide was purchased from National Diagnostics. All other chemicals and pure Hb (haemoglobin) were from Sigma. ProteinChip® arrays, all-in-1 protein molecular-mass standards, Q ceramic Hyper DF spin columns, reverse-phase beads and SPA (sinapinic acid) were from Ciphergen. Size-fractionation columns were from Millipore.

Profiling, analysis and identification of proteins

Plasma protein patterns were determined by SELDI-TOF MS using the ProteinChip® platform (Ciphergen) essentially as described previously [18]. Briefly, plasma samples were thawed on ice, denatured, vortex-mixed, 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 (20°C) on a shaker for 1 h. The samples were discarded and the arrays were washed using binding buffer and water. Finally, the arrays were air-dried and the SPA matrix was applied to the spots.

TOF 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-mass range (2 to 10 kDa), the laser intensity was set to 220 and the detector sensitivity to 8; for the intermediate-molecular-mass range (10 to 50 kDa), the laser intensity was set to 220 and the detector sensitivity to 9; and for the high-molecular-mass 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-mass standards and internally using peaks identified previously. Peaks were detected and clusters were created using ProteinChip® Software 3.1 (Ciphergen).

To identify 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 previously [18]. Briefly, plasma samples were fractionated using anionic Q ceramic Hyper DF spin columns, reverse-phase beads and 50 kDa size-fractionation columns. Fractions containing the relevant proteins were speed-vacuum dried, resuspended and separated by SDS/PAGE in two parallel lanes. After staining with colloidal Coomassie Blue stain, protein bands were excised and eluted, while a corresponding band was subjected to in-gel tryptic digestion and PMF (peptide mass fingerprinting).

The second approach took into account that peaks at 15.1 and 15.8 kDa have been identified previously as the α- and β-chains of Hb [21]. Pure Hb (Sigma) was dissolved in buffer [50 mmol/l Tris, 9 mol/l urea and 2% (w/v) CHAPS (pH 9)], diluted 1:10 in binding buffer and applied on to 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).

Statistical analysis

All cluster data were analysed using GraphPad Prism or STATA version 8.2. ANOVA was used to compare the means of several groups. A Student’s t test was used to analyse further differences between the groups, and P values < 0.05 were considered significant. ANCOVA (analysis of covariance), adjusting for M/I (insulin sensitivity index), derived from the euglycaemic insulin clamp, BMI or waist circumference, was also performed in separate models to adjust for the possible confounding of insulin resistance. ANCOVA, adjusting for cystatin-based GFR (glomerular filtration rate), was performed to analyse possible confounding of impaired kidney function.
RESULTS

Study subjects

The aim of the present study was to investigate variations in plasma protein levels in subjects with T2DM and differences in β-cell function. To this end, control subjects, (n = 10), subjects with T2DM + low (n = 10) and subjects with T2DM + high (n = 10) EIR were selected from the ULSAM cohort. Subjects with T2DM + high EIR had approx. 40% of the EIR observed in controls, and subjects with T2DM + low EIR had approx. 10% of the control level (Table 1). Fasting glucose as well as the 2-h glucose value after an OGTT was significantly increased in both groups with T2DM. Both BMI and waist circumference measurements were significantly higher in the two T2DM groups. M/I and adiponectin levels were significantly lower in subjects with T2DM compared with controls with NGT. Cystatin-based GFR was significantly higher in subjects with T2DM + low EIR (Table 1).

Seven individuals (four subjects with NGT and three subjects with T2DM + high EIR) were analysed longitudinally. Fasting levels of glucose and proinsulin did not change within the NGT or T2DM groups when values were compared at 71 and 77 years of age (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, a total of 26, 46 and 35 protein clusters were found in the low-, intermediate- and high-molecular mass ranges respectively. The amounts of these proteins were calculated for each subject by determining molecular mass ranges respectively. The amounts of these proteins, whereas the anionic fraction at pH 4 maintained proteins with masses 13932, 14092, 15825 and 44106 Da. The 3148 Da protein was not found in any of the anionic fractions. By purifying the anionic flow-through fraction further, the 44106 Da protein was isolated. In addition, the anionic fraction at pH 4 was purified further, resulting in a relatively pure 13932 Da protein. The 13932 Da protein was identified as TTR (transthyretin) and the 44106 Da protein as ApoH (apolipoprotein H).

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 α- and β-chains of human Hb have been shown previously to correspond with peaks at 15.1 and 15.8 kDa [21]. When pure human Hb was diluted and applied on to a cationic exchanger array, the resulting peaks in the spectrum aligned with the plasma spectrum peaks at 15114 and 15825 Da (Figure 4). To verify these results, the Hb concentration in the plasma samples was also measured using a human Hb ELISA. At 71 years of age, the subjects with NGT had a higher plasma Hb concentration than the subjects in either of the T2DM groups (70 ± 12 μg/ml in the NGT group compared with 44 ± 4 and 39 ± 7 μg/ml in the subjects with T2DM + high EIR and T2DM + low EIR respectively; P < 0.05, as determined using a Student’s t test).

Longitudinal protein changes

An analysis of protein profiles over time was also undertaken. Out of the 30 participants in the initial analysis at 71 years of age, seven had also been characterized at 77 and 82 years of age. 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). Plasma samples from these subjects at 71, 77 and 82 years of age were applied on to cationic exchanger arrays and SELDI analysis was performed. Out of the nine proteins found in the initial comparison at 71 years of age, none differed over time (Table 4). For all of the nine proteins, the levels in the groups were comparable with those at baseline (Table 4).

DISCUSSION

In the present study, assessing plasma protein profiles from subjects with NGT + high EIR, subjects with T2DM + high EIR or subjects with T2DM + low EIR, nine proteins with different plasma concentrations were found, out of which four were identified.

The SELDI platform was used, where chemically defined surfaces are used to capture proteins. The technique demonstrates reproducibility and quantitativeness,
Plasma proteome changes in Type 2 diabetes

Figure 1  Plasma protein levels in subjects with NGT (white bars), T2DM + high EIR (grey bars) and T2DM + low EIR (black bars)
Protein masses (in Da) are indicated. The results are means ± S.D. of ten experiments. ∗ Significant differences (P < 0.05) between groups.

Table 3  Cross-sectional relative peak intensities at 71 years of age
Values are means ± S.D.  ∗ P value as determined by ANOVA; † P value as determined by Student’s t-test

<table>
<thead>
<tr>
<th>Protein (Da)</th>
<th>Subjects with T2DM</th>
<th>Subjects with T2DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NGT (n = 10)</td>
<td>+ high EIR (n = 10)</td>
</tr>
<tr>
<td>3148</td>
<td>17.8 ± 7.8</td>
<td>32.1 ± 16.3</td>
</tr>
<tr>
<td>7443</td>
<td>3.73 ± 0.6</td>
<td>2.96 ± 0.7</td>
</tr>
<tr>
<td>13 932</td>
<td>7.9 ± 1.6</td>
<td>6.0 ± 1.2</td>
</tr>
<tr>
<td>14 092</td>
<td>4.2 ± 0.6</td>
<td>3.4 ± 0.8</td>
</tr>
<tr>
<td>15 114</td>
<td>1.24 ± 0.85</td>
<td>0.57 ± 0.13</td>
</tr>
<tr>
<td>15 825</td>
<td>1.07 ± 0.83</td>
<td>0.40 ± 0.12</td>
</tr>
<tr>
<td>17 154</td>
<td>1.47 ± 0.26</td>
<td>1.29 ± 0.37</td>
</tr>
<tr>
<td>44 106</td>
<td>0.83 ± 0.07</td>
<td>0.73 ± 0.09</td>
</tr>
<tr>
<td>72 834</td>
<td>0.82 ± 0.08</td>
<td>0.85 ± 0.04</td>
</tr>
</tbody>
</table>
and has been used successfully in large-scale proteomic studies in patients [22,23]. However, SELDI technology has been criticized for preferentially capturing high levels of abundant proteins, including acute phase-reactants [24]. Pre-sample fractionations can be performed to eliminate albumin and other 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 unfractionated plasma with SELDI-TOF MS, adhering to a protocol developed previously used by us [18] and others [25].

Among the plasma proteins which were decreased in individuals with T2DM, TTR was identified. TTR has 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, such as CRP (C-reactive protein), have been shown to be increased in serum [8]. This has been interpreted to be part of the low-grade inflammation present in these patients [9,10]. In our present study, TTR levels were decreased in plasma from subjects with T2DM irrespective of their EIR. The blood profile of TTR is well-characterized, having several peaks of approx. 13.8–14.1 kDa [27–29]. The peaks all represent TTR and include the native form and forms with different modifications. The peak at 13 932 Da in the present study was identified as TTR, which has been reported previously by us [18] and others [21]. The 14 092 Da peak matched well the glutathionated form of the TTR peak. TTR is produced mainly in the liver and choroid plexus, but also within pancreatic islets [30], where it has been shown to increase the cytoplasmic Ca^{2+} concentration and promote insulin release [31]. Decreased levels of TTR have also been associated with inflammatory conditions [32] and T1DM [33]. In addition, in subjects with T1DM, an increase in blood levels of the 13.9 kDa form of TTR was observed, which was suggested to be associated with the development of β-cell failure in the disease [31,34].

In the present study, Hb α- and β-chains were also identified among the proteins decreased in plasma from subjects with T2DM. The proteins were identified by inference with the protein profile of pure human Hb, as has been reported previously in another study [21]. The peaks of the α- and β-chains of Hb aligned with the peaks obtained in the SELDI spectra in the present study. In addition, the results were confirmed by measuring Hb with an ELISA, which showed significantly higher levels of Hb in subjects with NGT compared with those with T2DM. An increase in plasma Hb could be explained by red blood cell lysis and subsequent release of Hb. This explanation of our present results is unlikely, as the blood samples were collected and plasma was prepared at different time points over a period of several months. In addition, Hb levels were also consistently higher in subjects with NGT at 77 and 82 years of age, making haemolysis an implausible explanation.

ApoH, also known as β2-glycoprotein-I, is secreted from the liver [35] and exists in plasma as both free ApoH and bound to lipoprotein particles [36]. In the present study, ApoH levels were decreased in plasma from subjects with T2DM + high EIR compared with subjects with NGT. The protein has been implicated in many physiological pathways, including lipoprotein metabolism [37] and coagulation [38]. It has been shown previously that plasma ApoH concentrations are increased in patients with diabetes compared with healthy individuals and are strongly correlated with total plasma cholesterol [39]. On the other hand, ApoH levels have 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 subjects with T2DM found in the present study. However, the levels of ApoH in subjects with T2DM + low EIR were not decreased compared with the levels found in subjects with NGT. It could be speculated that this reflects that ApoH levels only decrease temporarily at the beginning of β-cell dysfunction and reach normal levels again after a further decrease in EIR, a process that is caused by unknown mechanisms.

A longitudinal protein analysis was performed to investigate whether the levels of the differentially displayed

![Figure 2](https://example.com/figure2.png)

**Figure 2** Plasma SELDI-TOF mass spectra in the mass region 8–18 kDa

Arrows indicate differentially displayed plasma proteins at 13 932, 14 092, 15 114, 15 825 and 17 154 Da. Representative spectra from individuals with NGT (a), T2DM + high EIR (b) and T2DM + low EIR (c) are shown.
proteins varied over time. Of the initial 30 study participants, only seven were re-investigated at both 77 and 82 years of age. None of these subjects had T2DM + low EIR, which is not surprising as 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 variations observed were manifested in the groups. The results also indicate that plasma sample collection and handling were performed adequately, without protein degradation.

The inability of subjects with T2DM to regulate glucose levels adequately can be caused by impaired β-cell function and/or insulin resistance. Changes in plasma proteins in T2DM can be regarded as either reflecting the cause or the effects of the disease. No single protein in the present study could, by itself, account for the differences observed in EIR, reinforcing the polygenic nature of T2DM characterized by changes in many proteins [42]. In the present study, most of the differentially displayed proteins were obtained in the comparison between NGT and both T2DM groups, irrespective of 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 a conclusion is consistent with a previous investigation [43], where the majority of alterations observed in serum proteins between subjects with NGT and T2DM are caused by the diabetic environment, rather than genetic factors. The protein results remained unchanged when...
Table 4 Longitudinal comparisons of the relative peak intensities using a subset of participants with repeated sampling at 71, 77 and 82 years of age

<table>
<thead>
<tr>
<th>Protein (Da)</th>
<th>Age 71</th>
<th>Age 77</th>
<th>Age 82</th>
<th>P value*</th>
<th>Age 71</th>
<th>Age 77</th>
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<td>7443</td>
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<td>72834</td>
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<td>0.6000</td>
<td>0.70±0.06</td>
<td>0.71±0.12</td>
<td>0.72±0.04</td>
<td>0.9488</td>
</tr>
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Figure 4 Comparison of the pure Hb spectrum (a) with the plasma spectrum (b)

The peaks of masses 15 114 and 15 825 Da coincided well with peaks of pure Hb.

adjusted for M/I, BMI, waist circumference or GFR, thus eliminating these potential confounding factors.

In conclusion, in the present study, we have demonstrated that several plasma proteins differ between subjects with NGT and T2DM characterized by differences in EIR. Levels of TTR, Hb α-chain and Hb β-chain were all decreased in plasma from subjects with T2DM compared with those subjects with NGT. ApoH was decreased in subjects with T2DM compared with subjects with NGT. It is concluded that these differences are probably manifestations of the disease state, rather than being causative.

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