Analysis of N-glycan in serum glycoproteins from db/db mice and humans with type 2 diabetes

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It is well known that the glycosylation patterns of proteins are associated with many diseases, e.g., autoimmune diseases, type 1 diabetes mellitus (DM), atherosclerosis, and hepatocellular carcinoma (1–4). Moreover, structural changes in the glycans of serum proteins are associated with some diseases, e.g., rheumatoid arthritis (32) and α1,6-fucosylation of α-fetoprotein in hepatocellular carcinoma (3, 4). Those are suggested to be useful for clinical markers (2). However, little research has been performed on the N-glycans in the serum glycoprotein of patients with diabetes mellitus (DM). More than 30 years ago, McMillan (28) demonstrated that fucose content in the serum glycoproteins of patients with diabetes was increased and that this increase could not be explained by the elevated glycoprotein levels. Although N-glycan structures with increased fucosyl residues remained unknown in this study, the finding suggested that fucosylation is linked to the pathophysiology of DM.

In mammals, there are four different linkages in fucosylations of N-glycans: α1,2-linkage to the terminal galactose (Gal), α1,3- or α1,4-linkage to N-acetylgalactosamine (GlcNAc) on the outer branch, and α1,6-linkage to the GlcNAc binding to protein (Fig. 1) (21). The role of each type of fucosylation is not clear yet, but these must be distinguished. Those fucosylations are catalyzed by different fucosyltransferases, which transfer fucose from guanine diphosphate-fucose to oligosaccharide chains linked to proteins or lipids (21). So far, two enzymes are known as α1,2-fucosyltransferases (FUT1 and -2) and six enzymes are known as α1,3/4-fucosyltransferases (FUT3, -4, -5, -6, -7, and 9). Previous analyses of mice with disrupted FUT4 and/or FUT7 genes showed reductions of E-, P-, and L-selectin ligand activities (18, 26) and reduction of atherosclerotic lesion size (17). α1,6-Fucosyltransferase (FUT8) is the only reported enzyme that catalyzes α1,6-fucosylation in mammals (29). Elevated expression of FUT8 was observed in human ovarian serous adenocarcinoma (40), hepatoma, and liver cirrhosis (34) and was linked to tumor size and lymph node metastasis in thyroid papillary carcinoma (20). Recently, the function of α1,6-fucosylation was analyzed using FUT8-deficient mice (45). The mice showed growth retardation and 70% lethality. The surviving mice had emphysema-like changes of the lung tissue due to the defect of transforming growth factor-β1 receptor signaling. Thus, it is likely that altered fucosylations of proteins affect physiological functions.

In patients with type 1 DM and urinary albumin excretion, an increase in α1,3-fucosylation of α1-acid glycoprotein was reported (36). An increase in α1,3-fucosylated N-glycans of α1-acid glycoprotein from patients with type 2 DM has also been shown, but the increase was not statistically significant (16). However, it is unknown whether the change in α1,3-fucosylation is most prominent and whether the change in N-glycosyl-
retrieved to asparagines (Asn) on nascent polypeptides in the endoplasmic reticulum (ER) and modified by many glycosidases and glycosyltransferases in the ER and Golgi apparatus. (Mannose)3

Fig. 1. The 4 types of N-glycan fucosylation in mammals. N-glycans are transferred to asparagines (Asn) on nascent polypeptides in the endoplasmic reticulum (ER) and modified by many glycosidases and glycosyltransferases in the ER and Golgi apparatus. Mannose, GlcNAc, galactose, sialic acid, and fucose residues can be added to the structure. Fucose can be added in α1,2-, α1,3/4-, and α1,6-linkages by the corresponding fucosyltransferases.

ation of α1-acid glycoprotein is most important in type 1 and type 2 DM among the changes in N-glycan profile. In those studies, the N-glycans of one serum protein were examined. To clarify the most striking changes in the structure and amount of N-glycans, it is necessary to perform a comprehensive analysis of serum without targeting a specific glycoprotein.

The purpose of this study is to examine the changes in the structure and amount of N-glycan of serum glycoproteins in db/db mice, a model of type 2 DM with obesity. In addition, we compared the serum N-glycan profiles between human subjects with type 2 DM and controls.

MATERIALS AND METHODS

Reagents and materials. Trypsin, sodium cyanoborohydride, and β-galactosidase (bovine testes) were purchased from Sigma-Aldrich (St. Louis, MO), α-chymotrypsin and pronase from Calbiochem (Darmstadt, Germany), peptide N-glycosidase F from Hoffman-La Roche (Basel, Switzerland), β-galactosidase (jack bean) and glucose oligomers (4–20) from Seikagaku (Tokyo, Japan), 2-aminopyridine from Wako Pure Chemicals (Osaka, Japan), and 2,5-dehydroxybenzolic acid (DHB) and peptide N-glycan fucosylation in mammals.

Preparation and derivatization of the N-linked oligosaccharide moiety from serum and liver tissue. Extracted liver tissues from db/db or db/+ mice were immediately washed with PBS and heated in 0.1 M ammonium bicarbonate, pH 8.0, at 90°C for 20 min. The tissues were then homogenized with a Sonifier (Branson, Danbury, CT) and delipidated once with chloroform-methanol (2:1) and twice with chloroform. After being dried, 10 mg of the sample was used. The serum (50-μl samples) was used after being heated at 90°C for 10 min. After the digestion of the samples with 50 µg each of trypsin and α-chymotrypsin, N-linked oligosaccharides were released from peptides with 5 U peptide N-glycosidase F, and then the peptides were digested by 50 µg of pronase. Each step was done in 10 mmol/l ammonium bicarbonate, pH 8.0, at 37°C overnight. The oligosaccharides were purified by gel filtration on a Sephadex G-15 column (10 × 380 mm) with water and reductively aminated with 2-aminopyridine and sodium cyanoborohydride (15, 46). Pyridylaminated (PA) oligosaccharides were purified by gel filtration on a Sephadex G-15 column (10 × 380 mm) with 10 mmol/l ammonium bicarbonate. To release the sialic acids, they were heated for 1 h at 90°C with HCl (pH 2.0).

HPLC analysis of PA oligosaccharides. HPLC analysis was performed using the Hitachi L-7000 HPLC system (Hitachi High-Technologies, Tokyo, Japan). The PA oligosaccharide samples were analyzed on an ODS column first. Elution was performed at a flow rate of 1.0 ml/min at 55°C using a gradient system. Solvent A was 10 mmol/l sodium phosphate buffer (pH 3.8), and solvent B was 0.5% (vol/vol) l-butanol added in solvent A. The column was equilibrated with a solvent [A:B = 80:20 (vol/vol)], and after injection the concentration of solvent B was increased linearly to 50% for 60 min. The eluted PA oligosaccharides were detected with a fluorescence

Fig. 2. Representative chromatograms of pyridylaminated (PA) oligosaccharides. A: a chromatogram was derived from the serum of a db/+ mouse on an octadecyl-bonded silica (ODS) column. B: a chromatogram from the serum of a db/db mouse. Six main peaks were named peaks a–f in order from early to late elutions.
column peak was converted to a glucose unit (GU) value, which is the relative amount of PA oligosaccharide as calculated on the basis of the peak area analyzed by a software program associated with the HPLC system.

**β-Galactosidase digestion.** The PA oligosaccharides isolated by HPLC (30 pmol) were treated with 2 μM β-galactosidase (jack bean) or 5 μM β-galactosidase (bovine testes) in 0.1 M citrate-phosphate buffer (pH 4.0) at 37°C for 15 h. Then the mixture was heated at 90°C for 10 min. After centrifugation at 3,500 rpm, the supernatant was analyzed.

**PA oligosaccharide analysis by matrix-assisted laser desorption ionization-time-of-flight mass spectrometry.** The PA oligosaccharides separated by HPLC were subjected to Ultraflex time-of-flight (TOF)/TOF mass spectrometry (Bruker Daltonics) equipped with a reflector and controlled by the Flexcontrol 2.1 software package (Bruker Daltonics). As matrices, DHB and CHCA were used. Matrix solutions were prepared as follows: DHB (10 mg) was dissolved in water (1 ml), and CHCA was prepared as a saturated solution in 3:1 (vol/vol) of acetonitrile-water. A few picomoles of oligosaccharides fractionated on HPLC were dissolved in 1 μl of water. Matrix solution (0.5 μl) was spotted on an Anchorchip plate (Bruker Daltonics), and 1 μl of the sample solution was added, dried at room temperature, and then subjected to matrix-assisted laser desorption ionization-TOF mass spectrometry (MALDI-TOF MS).

In the reflector mode of MALDI-TOF MS, ions generated by a pulsed UV laser beam (nitrogen laser, λ = 337 nm, 5 Hz) were accelerated to a kinetic energy of 23.5 kV. Metastable ions generated by laser-induced decomposition of the selected precursor ions were analyzed without any additional collision gas. In the MALDI-TOF/TOF mode, precursor ions were accelerated to 8 kV and selected in a timed ion gate. The fragments were further accelerated by 19 kV in the LIFT cell, and their masses were analyzed after the ion reflector passage. Masses were automatically annotated by using the FlexAnalysis 2.1 software package (Bruker Daltonics) (22).

**Determination of N-glycan structure.** Oligosaccharide structure was suggested by comparison of its elution position with data reported under the same analytical conditions [two-dimensional (2D) mapping] (http://www.glycoanalysis.info/) (42). Code numbers of oligosaccharide structures described in this manuscript are derived from these references. They consist of four numbers; the first numeral indicates the number of antennae of N-glycan. The second and third digits of “1” or “0” indicate the presence or absence of α1,6-fucose residue bonded to the reducing end and bisecting the GlcNAc residue in that order. The fourth numeral represents the serial number of PA oligosaccharides. The structures suggested by 2D mapping were confirmed by mass values obtained using MALDI-TOF MS.

**Analysis of α1,6-fucosyltransferase gene expression.** The mRNA level of FUT8 was analyzed by quantitative real-time RT-PCR. Total amount of FUT8 was analyzed by quantitative real-time RT-PCR. Total amount of FUT8 was analyzed by quantitative real-time RT-PCR.

**Table 1. GU, mass, and estimated PA oligosaccharide from each peak in db/db mice**

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<tr>
<th>Peak</th>
<th>ODS, GU</th>
<th>Amide, GU</th>
<th>Mass, [M+H]+ (m/z)</th>
<th>Code No.</th>
<th>ODS, GU</th>
<th>Amide, GU</th>
<th>Mass, [M+H]+ (m/z)</th>
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<td>300.18</td>
<td>8.1</td>
<td>8.6</td>
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<td>1,719.63</td>
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<tr>
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<td>6.9</td>
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<td>310.18</td>
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<td>9.0</td>
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Elution times of octadecyl-bonded silica (ODS) and amide columns were expressed as glucose units (GU), which were adjusted to elution positions of standard pyridylaminated (PA) oligosaccharides. Fractionated PA oligosaccharides were subjected to matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), and the masses were determined. The code number of PA oligosaccharides represents a set of elements of its structure. The definition is described in MATERIALS AND METHODS. m/z; Mass-to-charge ratio; NR, not reported.
RNA was extracted from the liver, epididymal adipose tissue, and kidney of 9-wk-old *db/db* mice and *db*/H11001 mice using the RNeasy Mini Kit or the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany). The RNA samples were evaluated using the 2100 Bioanalyzer and RNA 6000 Nano Assay Kit (Agilent Technologies, Palo Alto, CA). The RNA with 1.0 of 28S:18S rRNA ratios and with a low baseline between 18S rRNA and 5S rRNA peaks on the electropherograms was accepted. Total RNA (500 ng) was reverse transcribed with SuperScript II Reverse Transcriptase and oligo (dT) primers (Invitrogen, Carlsbad, CA) for first-strand cDNA synthesis. Aliquots of cDNA were subjected to PCR amplification using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA) according to the manufacturer’s protocol. TaqMan gene expression assays (Applied Biosystems) were used as the primers and FAM-labeled probe. Amplification was carried out with the 7300 Real-Time PCR System (Applied Biosystems). All reactions were triplicate determinations. Quantification of each mRNA level was performed by Sequence Detection Software version 1.3 (Applied Biosystems). The level of each FUT8 mRNA was normalized to the level of 18S rRNA analyzed using TaqMan Ribosomal RNA Control Reagents (Applied Biosystems).

**Study participants.** We recruited humans with type 2 DM from patients who were treated at Hokkaido University Hospital. The diagnosis of type 2 DM was confirmed for all patients from the clinical records, using the World Health Organization criteria (1). Control subjects were recruited from individuals who requested annual medical checkups. They underwent a standard 75-g oral glucose tolerance test, and individuals with normal glucose tolerance (fasting plasma glucose <110 mg/dl and 2-h values <140 mg/dl) were included in the study. All of the subjects were screened through history, blood test, and urinalysis. Individuals with a history of acute inflammation, hepatitis, pregnancy, or prior malignancy were excluded. Those with impaired renal or liver function and overt proteinuria were also excluded. This study was approved by the Hokkaido University’s institutional review board, and all participants gave written, informed consent. Twenty patients with type 2 DM and 18 controls were studied. Blood samples were collected after overnight fasting for N-glycan analysis and measurement of plasma glucose level and glycated hemoglobin. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared. Obesity was defined as BMI ≥25 kg/m², in accordance with the recommendation of Japan Society for the Study of Obesity (10).

**Statistical analyses.** Data are presented as means ± SD. An unpaired t-test or Mann-Whitney’s U-test was used to examine comparisons between groups when equal variance was shown by the F-test or when equal variance was not shown, respectively. Sex distribution and the percentage of obesity between groups were...
compared with the χ²-test. In all tests, P values of <0.05 were considered significant.

RESULTS

Characteristics of the animals. The average body weight of the db/db mice was heavier than that of the db/+ mice (db/db: 41.5 ± 0.4 g, n = 13; db/+: 26.4 ± 0.3 g, n = 13; P < 0.001). The blood glucose levels of all db/db mice were >25.1 mmol/l, and those of 7 db/db mice could not be measured due to their exceeding the upper limit (33.3 mmol/l) of the blood glucose analyzer. The mean blood glucose level of db/+ control mice was 10.6 ± 0.6 mmol/l. The increased weight and glucose levels of the db/db mice were similar to those previously reported (19).

Oligosaccharide alteration of the db/db mouse serum. A comparison of serum N-glycan profiles from db/db and db/+ mice was performed with HPLC using an ODS column. Typical HPLC elution patterns of the PA oligosaccharides from db/+ and db/db mouse sera are shown in Fig. 2. Elution patterns from db/+ or db/db mice were almost uniform. Three peaks whose GU values were 11.2, 13.2, and 14.0, referred to as peaks D, E, and F, respectively, were larger in db/db mice than in db/+ mice. The relative amounts of PA oligosaccharides from peaks D, E, and F in db/db serum were approximately twice those in db/+ serum (peak D: db/+ 2.4 ± 0.44%, db/db 4.4 ± 0.46%, P < 0.001; peak E: db/+ 3.2 ± 0.65%, db/db 5.7 ± 0.91%, P < 0.001; peak F: db/+ 10.0 ± 1.3%, db/db 22.4 ± 3.8%, P < 0.001; Fig. 3). These results showed that at least three kinds of N-glycans were increased in the serum of db/db mice. Conversely, the other three peaks whose GU values were 8.1, 9.7, and 10.3, referred to as peaks A, B, and C, respectively, in the serum of db/db mice were significantly decreased compared with those in the serum of db/+ mice (peak A: db/+ 12.4 ± 1.6%, db/db 9.1 ± 1.7%, P < 0.001; peak B: db/+ 13.1 ± 1.5%, db/db 9.8 ± 1.7%, P < 0.001; peak C: db/+ 43.2 ± 1.7%, db/db 34.4 ± 2.6%, P < 0.001; Fig. 3).

Structures of N-glycans changed in serum of db/db mice. We investigated the structures of N-glycans whose amounts changed in the serum of db/db mice, using 2D mapping and mass value. First, each PA oligosaccharide corresponding to peaks A–F was isolated using an ODS column and was subjected to HPLC analysis using an amide column. Although the PA oligosaccharides included in peaks B, D, and E were divided into two or three components on an amide column, the largest components, which accounted for >80% of the peaks on ODS column HPLC, were considered to represent the peaks B, D, and E. The GU values of each peak on ODS and amide columns are shown in Table 1. Next, each PA oligosaccharide of peaks A–F isolated on the ODS column was subjected to MALDI-TOF MS. The mass-to-charge ratio (m/z) values are also shown in Table 1. From the database on the web (http://www.glycoanalysis.info/), we estimated the structures of the N-glycans. With this method, we identified peak D as code no. 310.18 (Table 1). Although the PA oligosaccharides 300.18 and 300.19 were considered as possible structures of peak A, 300.19 is an artificial PA oligosaccharide obtained from β-galactosidase and β-N-acetylgalactosaminidase digests of sialylated tetra-antennary oligosaccharides (Fig. 4) (31). Oligosaccharide 300.19 has no β1,2-linked GlcNAc residue, which must be added to the α1,3-linked mannose of core structure in the initial step of N-glycan biosynthesis (27). Thus, the structure of peak A was identified as 300.18. As possible structures of peak C, 200.13 and 200.4 were selected. However, the cojunction of peak C and 200.4 on HPLC revealed that peak C was coeluted with 200.4. Peak F was identified with 210.4 but not 210.13 in the same way.
There was no PA oligosaccharide corresponding to peaks B and E in the database. The oligosaccharide of peak B had the same m/z value and GU value on the amide column as peak C. This result indicated that peak B consists of the same sugars as peak C. To examine the linkage between Gal and GlcNAc, isolated oligosaccharides of peaks B and C were digested with jack bean β-galactosidase. It is known that the terminal disaccharide Galβ1,4GlcNAc is more sensitive to β-galactosidase from jack bean than the disaccharide Galβ1,3GlcNAc (25). The digests were again analyzed by using an amide column. The GU value of the digested peak C oligosaccharide was 2 GU smaller than that of the undigested peak C oligosaccharide, as expected. However, the GU value of the digested peak B oligosaccharide was 1 GU smaller than that of the undigested peak B oligosaccharide (Fig. 5). This means that the oligosaccharide of peak B had one Galβ1–3GlcNAc linkage, which is difficult to digest with jack bean β-galactosidase. To verify the Galβ1,3GlcNAc linkage, bovine testes β-galactosidase that can digest both Galβ1,3GlcNAc and Galβ1,4GlcNAc was also used. The GU value of peak B was decreased by 2 GU as well as that of peak C after the digestion (Fig. 5). The conjection of peaks B and C digested by bovine testes β-galactosidase to an amide column resulted in the same elution position. Thus, the structure of peak B was suggested as a biantennary oligosaccharide, including a Galβ1,3GlcNAc component shown in Fig. 4. Because peaks E and F also had the same m/z value and GU of an amide column, the analysis with β-galactosidases was performed as described above. As a result, peak E also had one Galβ1,3GlcNAc linkage (Fig. 4). To confirm the linkage position of fucose, MALDI-TOF/TOF MS analysis of peak E was performed. The signal of the fragment from the precursor ion (1,866 m/z) showed 446 (m/z), which indicated the structure with fucose-GlcNAc-PA. Accordingly, the structure of peak E was identified as the same as 210.4, except for a Galβ1,3GlcNAc linkage (Fig. 4).

Thus, the structures of peaks A, B, and C were identical to those of peaks D, E, and F except for modification of the α1,6-fucose residue. Collectively, N-glycans with α1,6-fucose were increased in db/db mouse serum.

Level of α1,6-fucosyltransferase mRNA increased in the liver of db/db mice. Increases in peaks D, E, and F may be attributed to α1,6-fucosylation of peaks A, B, and C. We examined the mRNA expression of FUT8 in liver, epididymal adipose tissue, and kidney. Total RNAs were extracted from each tissue and subjected to RT-PCR analysis. As shown in Fig. 6, the expression levels of FUT8 mRNA were significantly increased in the liver from db/db mice. Compared with the heterozygote, the expression levels of FUT8 mRNA from the liver in db/db mice were ∼1.7-fold higher (P < 0.01, each n = 6). In contrast to the liver, there were no differences in FUT8 mRNA expression between db/db and db/+ mice in adipose tissue and kidney (Fig. 6).

α1,6-Fucosylation of the N-glycan in the liver. Given the increased FUT8 mRNA level in the liver, the α1,6-fucosylation of the N-glycan in the liver tissue was analyzed. Homogenates of liver were subjected to HPLC using an ODS column and the ratio of the relative amounts of peaks F and C (F/C) were employed as the index of α1,6-fucosylation. In agreement with the increased FUT8 mRNA of the db/db mouse liver, the F/C ratio of the liver was increased in the db/db mouse (db/+ 0.456 ± 0.014, db/db 0.688 ± 0.095, P < 0.01, each n = 6; Fig. 7).

Alteration in the serum N-glycan profile in human with type 2 diabetes. Next, we examined whether α1,6-fucosylation was increased in serum of humans with type 2 DM as observed in the db/db mouse. Characteristics of the patients with type 2 DM and the control subjects whose sera were analyzed are summarized in Table 2. The mean age of subjects in the DM group was older than that in the control group. Although there was no significant difference in BMI between the control and DM groups, the percentage of the subjects classified as obese tended to be higher in the DM group (65.0 vs. 33.3%, P = 0.051).

The PA oligosaccharides prepared from the human serum were subjected to HPLC using an ODS column. A typical elution pattern is shown in Fig. 8. The elution pattern of the human serum differed from that of the mouse serum. We referred to the main peaks as peaks I–VI, as indicated in Fig. 8. Using the 2D mapping method and m/z value, the structure of each peak was identified (Table 3 and Fig. 9). Because peak III was found to consist of two components, using HPLC with an amide column, and peaks III and IV were not separated well, correct quantifications of peak III and IV could not be performed with an ODS column. We compared the relative amounts of peaks II, V, and VI, whose structures had α1,6-fucose, between the control and DM groups. Although the amounts of peaks II and V were not increased in the DM group,
that of peak VI was increased in the DM group (control 4.6 ± 0.7%, DM 5.8 ± 1.1%, \( P < 0.001 \); Fig. 10).

**DISCUSSION**

We showed that 1,6-fucosylation of N-glycan is increased in the serum protein of \( db/db \) mice, a model of type 2 DM with obesity. In addition, we also found an increase of an N-glycan with 1,6-fucose (211.4) in the serum of humans with type 2 DM, although the increase was small. To the best of our knowledge, the association between 1,6-fucosylation and type 2 DM has not previously been reported.

1,6-Fucosylation regulates E-cadherin-mediated cell adhesion (13), \( \beta 3 \) integrin function (47), antibody-dependent cellular cytotoxicity of IgG (38, 39), liver lysosomal acid lipase activity (43), transforming growth factor-\( \beta 1 \) signaling (45), EGF receptor-mediated intracellular signaling (44), and protein secretion from the liver into the bile duct (33). Given those changes in protein properties by 1,6-fucosylation, the increase in 1,6-fucosylation may affect a variety of glycoprotein properties in type 2 DM. In the present study, proteins whose 1,6-fucosylation is increased in sera of \( db/db \) mice and humans with type 2 DM remain unknown. The identification of these proteins may give important information about patho-

<table>
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Table 3. GU, mass, and estimated PA oligosaccharide from each peak in humans

The methods by which these data were obtained were the same as those of Table 1.

![Fig. 9. Proposed structures of PA oligosaccharides in human serum. They were estimated using 2D mapping and MALDI-TOF MS.](http://ajpendo.physiology.org/)

![Fig. 10. Relative amount of each fraction from peak ii (210.1; A), peak v (210.4; B), and peak vi (211.4; C). PA oligosaccharides derived from the sera of humans with type 2 diabetes (DM; \( n = 20 \)) or control subjects (C; \( n = 18 \)) were subjected to HPLC using an ODS column. The AUC of each peak on the chromatogram divided by the AUC of all peaks was compared. Data are means ± SD. *\( P < 0.05 \).](http://ajpendo.physiology.org/)
physiology of type 2 DM. The differences in α1,6-fucosylation of those identified proteins between control and type 2 DM samples may be larger than the differences observed in the present study, because the results from whole serum proteins are prone to conceal the differences of the specific proteins.

Our result was in agreement with the previous findings of increased fucose residues in the serum glycoproteins of patients with diabetes (28). Higai et al. (16) showed that α1,3-fucosylation of α1-acid glycoprotein tended to be increased in the serum of patients with type 2 DM. However, our results demonstrated that α1,3-fucosylation was a minor structure in glycoproteins in whole serum, as described previously (30), and showed no evidence of an increase in α1,3-fucosylation in whole serum of mice and humans with type 2 DM. This discrepancy seems to be due to differences in samples (isolated protein vs. whole serum). The comprehensive analysis in the present study proved that α1,6-fucosylation was more dominant than α1,3-fucosylation in the serum N-glycome and that an N-glycan with α1,6-fucose was significantly increased in the serum of humans with type 2 DM.

In the present study, the FUT8 mRNA level in the liver of db/db mice was about twofold higher compared with that in the liver of control db/+ mice. Previously, the increased FUT8 mRNA level in the liver was also shown in patients with chronic hepatitis and liver cirrhosis (34). In the liver of patients with type 2 DM, various changes are caused by insufficient insulin action (37). Those include deterioration of many enzymes’ activities related to glucose and lipid metabolism. Furthermore, accumulation of visceral fat accelerates the influx of fatty acid into the liver, causing steatosis (5). Those disorders may increase the FUT8 mRNA level.

Although the FUT8 enzyme activity could not be measured in the present study, we showed that the F/C ratio was increased in the liver tissue of the db/db mouse. This finding supported the increased activity of FUT8 in the liver to some extent. Considering that serum glycoproteins are synthesized mainly in the liver, the tissue most likely responsible for the increase in serum α1,6-fucosylation may be the liver. However, we must realize that mRNA level may not necessarily parallel the enzyme activity. FUT8 enzyme activity of tissues other than liver may be increased in the db/db mouse, even if the mRNA level is not increased.

The C57BL/KsJ db/db mouse has a mutation in the leptin receptor (Ob-R) gene (6, 23). This mutation results in a lacking of most of the intracellular domain of the long isoform of the receptor Ob-Rb. The db/db mouse is thought to become hyperphagic and obese with hyperleptinemia due to a functional defect in the aberrant Ob-Rb (7, 8). In addition to the mutant gene, the BL/Ks inbred strain background predisposes the mice to overt diabetes (24). The Ob-Rb receptor is expressed most highly in the hypothalamus. Although the expression of Ob-Rb was observed in some other tissues, defective leptin signaling in the hypothalamus is thought to be responsible for obesity of the db/db mouse (8). Accordingly, decreased Ob-Rb signaling in the hypothalamus may be associated with the increase in hepatic FUT8 mRNA via obesity or diabetes. However, Ob-Rb is also expressed by hepatocytes (11). The reduction of hepatic Ob-Rb signaling may be related to the increased FUT8 mRNA in the liver.

The db/db mouse is often used as a model of human type 2 DM with obesity, in which leptin resistance is one of the characteristics. However, because the mutation of the leptin receptor gene itself is extraordinarily rare in humans, it remained unclear whether the results of the db/db mouse were applicable to humans with type 2 DM with obesity. Therefore, we examined the serum of humans with type 2 DM and showed an increase of a biantennary N-glycan that had an α1,6-fucose with a bisecting GlcNAc in the patients with type 2 DM. This result was in agreement with that of db/db mouse in the way that α1,6-fucosylation was increased. However, the N-glycan profile of human serum protein was considerably different from that of mouse serum protein. Furthermore, the increased N-glycan in humans with type 2 DM was different from that in db/db mice, and the increased N-glycan in the patients with diabetes was limited to only one of the three analyzed N-glycans with α1,6-fucose. One reason for those differences may be the difference in species. In addition, the heterogeneous pathophysiology of human type 2 DM and the uniform pathophysiology of db/db mice may account for the difference. For example, although not all subjects with type 2 DM were obese, all db/db mice were obese. That difference may be attributed to the difference in the degree of disorders between db/db mice and subjects with type 2 DM, e.g., blood glucose level, body weight, and steatosis.

In conclusion, α1,6-fucosylation of serum N-glycans and FUT8 mRNA expression in the liver were increased in db/db mice. In addition, an N-glycan with α1,6-fucose was increased in the serum of humans with type 2 DM. The increased α1,6-fucosylation may affect protein properties associated with the pathophysiology of type 2 DM. It is likely that the increase in α1,6-fucosylation reflects some pathophysiology of type 2 DM. Further studies are needed to find factors correlating with increased α1,6-fucosylation in some patients with type 2 DM.

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REFERENCES


