Expression of liver insulin-like growth factor 1 gene and its serum level in rats with diabetes

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AIM: To explore the effect of diabetic duration and blood glucose level on insulin like growth factor 1 (IGF-1) gene expression and serum IGF-1 level.

METHODS: Diabetes was induced into Sprague Dawley rats by alloxan and then the rats were subdivided into different groups with varying blood glucose level and diabetic duration. The parameters were measured as follows: IGF-1 mRNA by reverse transcriptase-polymerase chain reaction (RT-PCR), IGF-1 peptide and serum IGF-1 concentration by enzyme-linked immunosorbent assay (ELISA).

RESULTS: During early diabetic stage (week 2), in comparison with normal control group (NC), IGF-1 mRNA [(1.17±0.069)-vs-0.79±0.048, P<0.001; 1.17±0.069-vs-0.53±0.023, P<0.0005, respectively], IGF-1 peptide contents [(196.66±14.9)-ng·mg⁻¹-vs (128.2±11.25) ng·mg⁻¹, P<0.0005; (196.66±14.9) ng·mg⁻¹-vs (74.43±5.33) ng·mg⁻¹, P<0.0001, respectively] were reduced in liver tissues of diabetic rats. The IGF-1 gene downregulation varied with glucose control level of the diabetic state, and deteriorated gradually further with duration of diabetes. By month 6, hepatic tissue IGF-1 mRNA was 0.71±0.024 vs 1.12±0.056, P<0.001; 0.47±0.021 vs 1.12±0.056, P<0.0005, respectively, IGF-1 peptide was (114.35±8.09) ng·mg⁻¹ vs (202.05±15.73) ng·mg⁻¹, P<0.0005; (64.58±3.89) ng·mg⁻¹ vs (202.05±15.73) ng·mg⁻¹, P<0.0001, respectively. Serum IGF-1 was also lowered in diabetic group with poor control of blood glucose. On week 2, serum IGF-1 concentrations were (371.0±12.5) ng·mg⁻¹ vs (511.2±24.7) ng·mg⁻¹, P<0.0005, (223.2±9.39) ng·mg⁻¹ vs (511.2±24.7) ng·mg⁻¹, P=0.0001 respectively. By month 6, (349.6±18.62) ng·mg⁻¹ vs (520.7±26.32) ng·mg⁻¹, P<0.0005, (188.5±17.35 vs 520.7±26.32) ng·mg⁻¹, P<0.0001, respectively. Serum IGF-1 peptide change was significantly correlated with that in liver tissue (r=0.99, P=0.001). Furthermore, No difference was found in the above parameters between diabetic rats with euglycemia and non-diabetic control group.

CONCLUSION: The influence of diabetic status on IGF-1 gene expression in liver tissues is started from early diabetic stage, causing down regulation of IGF-1 expression, and progresses with the severity and duration of diabetic state. Accordingly serum IGF-1 level decreases. This might indicate that liver tissue IGF-1 gene expression is greatly affected in diabetes, thus contributing to reduction of serum IGF-1 level.

INTRODUCTION
Insulin like growth factor-1 (IGF-1) is widely present in tissues of mammalian animals and has a number of bioactivities including regulation of metabolism and enhancement of growth and development of tissues[1-4]. Recently its research has attracted much attention. IGF-1 may probably be involved in metabolic abnormality and complications associated with diabetes. Liver might be the main source of circulating IGF-1[5,6]. Recent studies have shown that there was early reduction in hepatic tissue IGF-1 gene expression in experimental diabetes[86]. However, further investigation on it is lacking. Upon this basis, we further explored the effect of chronic diabetic status on liver tissue IGF-1 gene expression and IGF-1 concentration in the circulation and hoped to help elucidating the pathogenesis of diabetes related disorder of metabolism and complications and lay a basis for premise of intervention.
transcription polymerase chain reaction (RT-PCR), tissue IGF-1 mRNA was semi-quantitated. The RT-PCR kit was provided by Promega Company (USA) and rat β-actin was used as an internal standard[9]. According to IGF-1 gene sequence, we designed RT-PCR IGF-1 upstream/downstream primer sequences 5’-CTTTGCGGGGCTGAGCTGGT-3’ , 5’-CTTCAGCGAGCAGTACA-3’, respectively. All the primers were synthesized by Shanghai BioEngineer Company. The following was optimal reaction condition: reverse transcription at 48 °C for 45 min, denaturation of RNA/DNA hybrid and inactivation of reverse transcriptase at 94 °C for 2 min. PCR for 40 cycles, denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min, extension at 68 °C for 2 min, final extension at 68 °C for 7 min. RT-PCR was performed on the Perkin Elmer (USA). The RT-PCR bands were 184 bp IGF-1 cDNA and 357 bp β-actin cDNA, respectively. Electrophoresis was carried out on 2% agarose gel containing ethidium bromide and semi-quantitated on the Gel DOC 1000 densitometry (Bio-RAD, USA). IGF-1 mRNA contents were calculated and expressed as cDNA relative densitometric units (ratio of IGF-1 cDNA/β-actin).

Measurement of liver tissue IGF-1 peptide contents
One gram liver tissue was excised from each rat, then frozen in liquid nitrogen and homogenized in a mortar. The homogenates were collected, then mixed with 0.05 mmol·L-1 Tris·HCl (PH 7.8) to neutralization and finally stored under -70 °C for future use[10].

An aliquot of the samples treated as above was taken to measure both total protein content by Brodford method and IGF-1 peptide concentration by enzyme linked immunosorbent assay (ELISA, Diagnostic Systems Laboratory, Inc.). DG-3022 type internal standard was used for measurement of IGF-1 peptide concentration (ng·ml-1) with the methods used in ELISA[11].

Measurement of serum IGF-1 peptide concentration
Serum samples from rat heart blood were frozen immediately for future use. The samples were pre-treated before assessment of IGF-1 peptide serum concentration (ng·ml-1) with the methods used in ELISA[11].

Data values were presented as mean ±SD. NC, normal control group; ID-1, -2, -3, insulin treatment group. vs NC, P<0.0025,* P<0.001,** P<0.0001; vs ID-2 (for the same period), P<0.001, * P<0.0001.

Statistical analysis
Data values were presented as mean ±SD. Significance of difference between groups was analyzed by one-way analysis of variance, nonparametric t’ pair test, Wilcoxon test and χ² test. P<0.05 was considered statistically significant.

RESULTS
Blood glucose metabolic parameters
At the end of experiment, ID-1 group and NC group had no difference in glucose level, amino fructose level, and body weight. Both blood glucose level and fructose level were significantly higher in ID-2 group and especially in ID-3 group when compared with NC group (P<0.0001). Significant differences were also found in the above parameters between ID-2 and ID-3 groups (P<0.0001). Within each group, there was no significant difference in aminofructose level (Table 1).

Effects of diabetes on tissue IGF-1 gene expression
Two weeks after diabetic model was established, the liver tissue IGF-1 mRNA contents (IGF-1 cDNA/β-actin cDNA) were decreased in both ID-2 group (P<0.001) and ID-3 group (P<0.0005) with a drop of 31% and 53%, respectively. They were further decreased with progression of diabetes. On month 6, in comparison with NC group, obvious differences were shown in ID-2 group (P<0.0005) and ID-3 group (P<0.0001) with a drop of 36% and 59%. Between the two diabetic groups with poor diabetic control, ID-3 group had a significantly lower IGF-1 level than ID-2 group. The drop was 5% and 6% at week 2. Between group ID-1 and NC group, there was no significant difference (Table 2). The change in tissue IGF-1 peptide content (IGF-1 peptide ng·mg-1) total protein) nearly paralleled that in mRNA content. At the 2nd week, compared with NC group, ID-2 group (P<0.0005) and ID-3 group (P<0.005) showed a decrease of 32%, 62%, respectively. Both were further decreased over the time course, with a drop of 34% and 65% by the end of the 6th month (P<0.0001). A drop of 2% and 3% was found at week 2. ID-3 group had a significantly lower IGF-1 level than ID-2 group (P<0.001). There was no significant difference between ID-1 and NC groups (Table 2).

Table 1 Glucose metabolic parameters during the experiment

| Group | Duration (month) | n | Initial weight (g) | Weight (g) | Blood (mmol·L-1) | Aminofructose (mmol·L-1) |
|-------|------------------|---|-------------------|------------|-----------------|------------------------|
| NC    | 0.5              | 5 | 198.41±19.76      | 249.33±16.02 | 5.14±0.91       | 0.82±0.07              |
|       | 2                | 5 | 202.53±17.67      | 351.20±18.23 | 5.3±0.44        | 0.8±0.08               |
|       | 3                | 6 | 200.06±13.03      | 402.05±7.10  | 4.91±0.26       | 0.81±0.09              |
|       | 6                | 5 | 199.65±15.22      | 544.54±40.41 | 5.21±0.47       | 0.85±0.05              |
| ID-1  | 0.5              | 5 | 196.25±14.22      | 254.31±20.97 | 4.93±0.72       | 0.79±0.05              |
|       | 2                | 5 | 202.34±19.12      | 345.75±23.48 | 5.10±0.47       | 0.8±0.08               |
|       | 3                | 5 | 196.42±11.41      | 411.31±7.37  | 4.88±0.67       | 0.78±0.06              |
|       | 6                | 6 | 198.68±12.64      | 538.52±31.62 | 4.94±0.58       | 0.84±0.07              |
| ID-2  | 0.5              | 5 | 192.00±5.70       | 217.00±9.64  | 11.4±0.56       | 1.02±0.14              |
|       | 2                | 5 | 199.00±16.73      | 241.00±16.44 | 10.94±0.09     | 1.00±0.29              |
|       | 3                | 6 | 198.66±4.36       | 256.83±14.98 | 10.86±0.94     | 0.98±0.08              |
|       | 6                | 5 | 201.37±14.11      | 266.24±13.53 | 12.13±0.63     | 1.10±0.14              |
| ID-3  | 0.5              | 5 | 208.60±3.08       | 205.75±5.34  | 18.34±0.13     | 1.20±0.12              |
|       | 2                | 5 | 198.60±16.66      | 192.80±13.35 | 17.48±0.62     | 1.18±0.21              |
|       | 3                | 6 | 211.50±11.37      | 204.81±11.03 | 17.50±1.05     | 1.21±0.19              |
|       | 6                | 6 | 204.35±12.34      | 185.22±14.36 | 16.89±0.95     | 1.24±0.34              |

The change in tissue IGF-1 peptide content (IGF-1 peptide ng·mg-1 total protein) nearly paralleled that in mRNA content. At the 2nd week, compared with NC group, ID-2 group (P<0.0005) and ID-3 group (P<0.005) showed a decrease of 32%, 62%, respectively. Both were further decreased over the time course, with a drop of 34% and 65% by the end of the 6th month (P<0.0001). A drop of 2% and 3% was found at week 2. ID-3 group had a significantly lower IGF-1 level than ID-2 group (P<0.001). There was no significant difference between ID-1 and NC groups (Table 2).
IGF-1 level was further lowered in both ID-2 and ID-3 groups.

Serum IGF-1 concentration

At week 2, in comparison with NC group, ID-2 and ID-3 groups showed a significant decrease in serum IGF-1 level: 237.0±12.5 ng·mg⁻¹, \( P < 0.0005 \) and 223.2±49.39 ng·mg⁻¹, \( P < 0.0001 \), a drop of 29% and 57%. By the sixth month, serum IGF-1 level was further lowered in both ID-2 and ID-3 groups (349.6±18.62) ng·mg⁻¹, \( P < 0.0005 \), (188.5±17.35) ng·mg⁻¹, \( P < 0.0001 \), respectively, with a fall of 33% and 63%. A drop of 4% and 6% was found at week 2. ID-3 group had a significantly lower IGF-1 level than ID-2 group (\( P < 0.001 \)). There was no significant difference between ID-1 and NC groups.

Relationship between changes in serum IGF-1, liver IGF-1 and IGF-1 mRNA content

Correlation analysis showed that the trend of serum IGF-1 concentration was consistent with that occurred in liver IGF-1 peptide (\( r = 0.99, P < 0.001 \)), and IGF-1 mRNA (\( r = 0.99, P < 0.001 \)) over the time course of diabetes (Figure 2).

DISCUSSION

The study made a preliminary exploration of the effect of chronic diabetic status (e.g. long duration and different glucose levels) on the hepatic IGF-1 gene expression and IGF-1 concentration of circulation.

Insulin-like growth factors (IGFs) have similar structures and functions like those of insulin, and can be divided into IGF-1 and IGF-2, the latter of which exerts its biological action on embryonic development and growth. The action of IGF-1 peaks around puberty period and decreases gradually with aging. IGF-1, a single polypeptide with 70 amino acids, was widely expressed in mammal tissues. The liver was found to have the highest concentration among all tissues and was probably the main source of circulating IGF-1[1,5,12], which exert its effect by binding to specific receptors on target cells in endocrine pattern. Human IGF-1 gene is on the long arm of chromosome 12, spanning a minimum of 90 kb which contains 6 exons. Exons 1 and 2 encode 5' untranslated region and amino residue terminal of IGF-1 peptide, 5' end of Exon 1 encodes carboxyl terminal end of IGF-1 signal peptide. The remaining exon 3 and the main part of exon 4 encode mature IGF-1 peptide including B, C, A, D domains. The 5' end of the remaining exons 4, 5 and 6 encodes signal peptide and 3' untranslated region. Human exon 5 contains stop codon. Gene transcription initiates at exon 1 or 2, varying with tissue specificity. Growth hormone (GH) might affect initiation activity of exon 1 and/or exon 2 to regulate liver IGF-1 gene expression. Insulin may directly regulate liver IGF-1 expression or indirectly by increasing the
number of GH receptors on hepatic cells. Nutritional state and corticosteroid hormones have been found in factors influencing IGF-1 gene expression[1,5].

In our study, liver tissue IGF-1 gene expression was significantly downregulated in rats with poorly controlled blood glucose (ID-3 and ID-2 groups), as compared to that in rats with normally controlled blood glucose (ID-1 group). Among them, the rats with a higher blood glucose (ID-3) showed more abnormal IGF-1 than those with a relatively low blood glucose (ID-2), the severity of which varied with levels of blood glucose. We continued the observation of the rats with the same level of blood glucose for 6 months after 2 weeks and found that the liver tissue IGF-1 gene expression was gradually decreased with the time course in the rats with hyperglycemia, especially severe hyperglycemia. This showed its association with the progression of diabetes, but to a lower degree. It may indicate the effect of chronic diabetic state on IGF-1 gene expression is less significant than that of the severity of blood glucose. However, the discrepancy in IGF-1 drop rate between the two conditions may reflect a fraction of other tissue’s contribution to the circulating IGF-1.

Our study, using RT-PCR technique, demonstrated the early reduction in IGF-1 mRNA contents in liver tissues of alloxan-induced diabetic rats, which was consistent with the previous studies using Northern blot and RT-PCR[5,6]. However, Veronica MC and Goya et al did not study the changes in liver tissue IGF-1 protein and effect of different blood glucose level and duration. We furthermore observed the effect of chronic duration and different severity of hyperglycemia on hepatic IGF-1 gene expressions. In our study, liver tissue IGF-1 gene expression was significantly downregulated in the rats with poorly controlled blood glucose (ID-3 and ID-2 groups), as compared with the rats with normally controlled blood glucose (ID-1 group). Among them, the rats with a higher blood glucose (ID-3) showed more abnormal IGF-1 than those with a relatively low blood glucose (ID-2), the severity of the abnormality varied with the level of blood glucose. We continued the observation of rats with the same level of blood glucose for 6 months after 2 weeks and found that liver tissue IGF-1 gene expression continued to go down gradually with the time course in rats with hyperglycemia, especially severe hyperglycemia. This showed its association with the progression of diabetes, but to a lesser degree. It may indicate the effect of chronic state on IGF-1 gene expression is less significant than severity of hyperglycemia. We are the first to find this. We also found that at translation level, hepatic IGF-1 peptide changed in similar extent as that of mRNA content, indicating the same effect of diabetic status on different translational level. We also demonstrated that serum IGF-1 concentration had a parallel change of hepatic tissue IGF-1. Thus further evidence was provided that the liver might remain to be the main endocrine source of IGF-1 in experimental diabetes. However, the discrepancy in IGF-1 drop rate between the two conditions might reflect a fraction of other tissue’s contribution to the circulating IGF-1. The IGF-1 down-regulation was prevented when hyperglycemia was corrected by subcutaneous injection of exogenous insulin, suggesting insulin might be a major regulator of IGF-1 gene expression during diabetes and exclude the possible direct influence of alloxan on IGF-1 gene expression.

Diabetes could result in down-regulation of gene expression, the major factors of which might be insulin secretion deficiency and/or its resistance. Some studies showed that tissue IGF-1 gene expression might be affected by systemic or local factors or both in diabetes, i.e. decrease of GH receptors in target cells and its binding affinity[11], and by reduced or absent pulsatile pattern secretion of GH, metabolic abnormality of insulin like growth factor binding proteins (IGFBPs)[12,14], negative nitrogen balance[15,16] etc. All these may probably lead to a decline of IGF-1. However, the deficiency of insulin or insulin resistance might be the main cause of IGF-1 gene downregulation[1,7,12]. In diabetes, IGF-1 in most tissues were down regulated at different degrees, varying with specific tissues[15,18,19]. In the liver it was down regulated[15,18]. Insulin that corrects hyperglycemia can correct the abnormal IGF-1 gene expression. Our study further supported it. It is known that IGF-1 transcription started at exons 1 and 2 regulated by different initiators and mRNA products that varied in length and affinity with tissue specificity[15,18,19]. The exact mechanism of insulin controlling IGF-1 gene expression remains to be elucidated.

We successfully established the animal model and found that the hepatic tissue IGF-1 gene expression was down regulated in the diabetic rats, the severity of which depended on glucose level and duration of diabetes. Accordingly, circulating IGF-1 was also decreased. The model established in our experiment is expected to mimic human diabetic status which will help us to interpret the role of IGF-1 in diabetic state. Diabetes can lead to a fall in IGF-1 of endocrine origin. IGF-1 it been found that has a number of bioactivities including mediating action of growth hormone, increasing glucose taken by tissues, inhibiting hepatic glycogenesis, improving insulin sensitivity, decreasing oxidation of lipid, lowering free fatty acids, increasing nucleotide synthesis, proliferation and differentiation of cells[20-23]. These researches would inevitably help understand the molecular pathogenesis of disturbances of glucose, lipid, protein metabolism associated with diabetes, diabetic peripheral neuropathy and diabetic foot[4,26-28] and probably might provide the premise of future molecular therapeutic intervention[29-30].

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