Fenofibrate Increases High Molecular Weight Adiponectin in Subjects with Hypertriglyceridemia

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Abstract. Beneficial effects of peroxisome proliferator-activated receptor alpha (PPAR alpha) agonists have been reported in improving insulin sensitivity and raising serum total adiponectin. High molecular weight (HMW) adiponectin, which is secreted from adipocytes, and visfatin, which is also expressed in adipose tissue, is related to glucose metabolism. In view of the additive effects of PPAR alpha agonists on these adipocytokines and glucose metabolism, we investigated male hypertriglyceridemic subjects who were treated with fenofibrate. Eleven male subjects with hypertriglyceridemia were treated with fenofibrate and serum total cholesterol (T-cho), triglyceride, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting glucose, fasting insulin, total and HMW adiponectin, and serum visfatin levels were determined before and 3 months after treatment. Fenofibrate treatment significantly lowered T-cho, triglyceride, and LDL-C levels. There was a statistically significant increase of HDL-C. No differences in insulin sensitivity indices (G/I ratio and HOMA-IR) were observed between before and after treatment with fenofibrate. The treatment did not alter the levels of serum total adiponectin and visfatin in the hypertriglyceridemic patients, while serum HMW adiponectin increased significantly. This study demonstrates that fenofibrate increases serum HMW adiponectin levels, whereas visfatin is not regulated by fenofibrate in hypertriglyceridemic subjects. Further investigations are warranted to determine whether the elevation of HMW adiponectin caused by fenofibrate might improve insulin sensitivity.

Key words: Fenofibrate, Adiponectin, Visfatin, Insulin sensitivity

IT is well established that peroxisome proliferator-activated receptor (PPAR) alpha agonists, including fenofibrate, can lower plasma triglyceride levels and raise high-density lipoprotein cholesterol (HDL-C) levels. In addition, it has been demonstrated that fenofibrate improved insulin sensitivity [1, 2]. As a possible mechanism, it has been suggested that fenofibrate regulates adipocyte and contributes to increase serum total adiponectin levels [3, 4], which is known to be related with insulin sensitivity and cardiovascular diseases [5, 6].

Adiponectin is present in serum in a range of high to low molecular weight, multimeric forms, which exhibit different levels of metabolic activity [7, 8]. Although the factors that regulate the multimer composition of adiponectin in serum have not been established, the high molecular weight (HMW) forms appear to be responsible for insulin sensitivity [7, 8]. It remains uncertain whether fenofibrate could regulate serum levels of HMW adiponectin.

Fukuhara et al. reported that visfatin was expressed in visceral adipose tissue from obese subjects [9]. Recent experimental studies demonstrated that dexamethasone, growth hormone, tumor necrosis factor-alpha and isoproterenol stimulate expression of visfatin in...
3T3-L1 adipose cells, while interleukin-6 was likely to be a negative regulator of visfatin [10, 11]. However, the factors regulating visfatin are not fully understood. Furthermore, it has also been reported that visfatin exhibited insulin mimetic effects, such as lowering plasma glucose [9]. Thus, we speculate that serum visfatin levels, as well as adiponectin, might be regulated by fenofibrate and consequently regulate glucose metabolism.

Fenofibrate is a synthetic PPAR alpha agonist that is now widely used to treat patients with hypertriglyceridemia. The aim of the present study was to investigate the additive clinical and biological effects of fenofibrate in male subjects with hypertriglyceridemia.

**Materials and Methods**

**Subjects**

Eleven consecutive Japanese male outpatients at the Department of Endocrinology and Metabolism in the Higashihiroshima Medical Center who exhibited hypertriglyceridemia were recruited for this study. All subjects had a medical history and a physical examination for more than 3 months prior to participating in the study. Patients with fasting serum triglyceride levels >150 mg/dl were deemed to have hypertriglyceridemia. Secondary causes of hypertriglyceridemia, such as Cushing’s syndrome, hypothyroidism and nephrotic syndrome, were excluded in all patients through appropriate clinical and biochemical examinations. We excluded patients with severe hypertension, coronary artery disease, peripheral vascular disease and diabetes. A 75 g oral glucose tolerance test was performed in all subjects before participating in this study. No patients had taken any cholesterol-lowering agents, antioxidant vitamin supplements, or anti-hypertensive agents.

**Study protocol**

We initially administered fenofibrate orally at 150 mg per day to each subject. When the lowering effect against serum triglyceride level was not sufficient (triglyceride >150 mg/dl) after 4 weeks, the daily dose of fenofibrate was increased to 300 mg. The drug was continued at a stable dose for the remaining 8 weeks of the study. This protocol was approved by the clinical trial committee at our hospital. All subjects provided written informed consent.

**Laboratory measurements**

All subjects underwent physical measurements and provided blood samples after an overnight fast, before and after treatment with fenofibrate. Blood glucose, serum insulin, total cholesterol (T-cho), triglyceride, HDL-C, and low-density lipoprotein cholesterol (LDL-C) concentrations were measured by routine methods at the Higashihiroshima medical center. Total serum adiponectin and high molecular weight (HMW) adiponectin levels were determined by enzyme-linked immunoassay (Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan and Fuji Rebio, Tokyo, Japan, respectively). Nakano et al. described that HMW adiponectin could be measured effectively and specifically by this kit [12]. Serum visfatin levels were measured by enzyme immunoassay kit (Phoenix Pharmaceuticals, Belmont, CA).

We calculated glucose to insulin (G/I) ratio and homeostasis model assessment of insulin resistance (HOMA-IR), which are known indices of insulin sensitivity [13, 14]. The G/I ratio was defined as the ratio of the fasting glucose and insulin serum levels [13]. HOMA-IR was calculated according to the following formula: fasting glucose (mg/dl) × fasting insulin (mU/l)/405 [14].

**Statistical analysis**

The differences between before and after treatment were evaluated by non-parametric Wilcoxon’s test and considered to be significant at \( P<0.05 \). Analyses were performed using SPSS for Windows (release 12.0; SPSS Inc., Chicago, IL).

**Results**

Table 1 presents the clinical characteristics of the subjects. In the 11 patients, 6 exhibited impaired glucose tolerance and the remaining subjects exhibited normal glucose tolerance according to WHO criteria [15]. The daily dose of fenofibrate was increased to 300 mg in 4 patients. All patients completed the study without reporting any adverse effects.

The study subjects did not show any differences in BMI, fasting glucose, fasting insulin, G/I ratio and
FENOFIBRATE AND ADIPONECTIN

HOMA-IR between before and 3 months after treatment with fenofibrate (Table 2). Fenofibrate treatment significantly lowered T-cho (from 215.4 ± 33.0 mg/dl to 190.3 ± 11.0 mg/dl; \( P < 0.05 \)), triglyceride (from 207.4 ± 58.7 mg/dl to 130.5 ± 43.8 mg/dl; \( P < 0.05 \)), and LDL-C (from 164.6 ± 57.6 mg/dl to 114.8 ± 11.8 mg/dl; \( P < 0.05 \)) (Table 2). There was a statistically significant increase of HDL-C (from 47.7 ± 7.4 mg/dl to 52.5 ± 9.1 mg/dl; \( P < 0.05 \)) (Table 2).

No differences in total adiponectin or visfatin levels were observed with fenofibrate treatment (Table 2). HMW adiponectin levels increased significantly after treatment (from 3.02 ± 1.47 \( \mu g/ml \) to 3.39 ± 1.72 \( \mu g/ml \); \( P<0.05 \)) (Table 2).

Discussion

This study demonstrated that fenofibrate therapy significantly increased serum HMW adiponectin levels in subjects with hypertriglyceridemia, whereas the levels of serum total adiponectin and visfatin were not altered. The parameters of glucose metabolism, including fasting glucose, fasting insulin, R/I ratio and HOMA-IR were not changed after treatment.

The present study is the first to demonstrate a relationship between PPAR alpha agonist therapy and serum visfatin levels. Choi et al. reported that visfatin mRNA levels increased significantly in visceral fat of experimental rats treated with a PPAR alpha agonist (fenofibrate) or a gamma agonist [16]. However, the dose of fenofibrate per body weight administered to the rats was extremely high compared to the dosage used in human in our study [16]. Our results revealed that fenofibrate did not influence the serum visfatin levels in human subjects. Furthermore, it has been suggested that serum visfatin levels in humans are not regulated by the PPAR gamma agonist [17].

Berndt et al. observed no differences in visfatin gene expression between subcutaneous and visceral adipose tissues in humans [18]. A PPAR gamma agonist, which is known as a major regulator of adipocyte differentiation, did not regulate serum visfatin levels or mRNA in visceral fat [17]. These findings did not support the original article, which reported that visfatin is expressed and secreted mainly in adipose tissue [9]. Taken together, the possibility exists that visfatin might not be secreted solely from visceral adipocytes, and more studies are necessary to fully elucidate the metabolism of visfatin.

Table 1. Clinical characteristics of the study subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>48.6 ± 13.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.3 ± 6.6</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>75.2 ± 15.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 3.8</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>132.0 ± 15.8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.3 ± 9.4</td>
</tr>
<tr>
<td>IGT/NGT</td>
<td>6/5</td>
</tr>
</tbody>
</table>

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; IGT, impaired glucose tolerance; NGT, normal glucose tolerance.

Table 2. Effect of fenofibrate in 11 hypertriglyceridemic patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline</th>
<th>3 months after treatment</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 3.8</td>
<td>25.5 ± 3.8</td>
<td>ns</td>
</tr>
<tr>
<td>T-cho (mg/dl)</td>
<td>215.4 ± 33.0</td>
<td>190.3 ± 11.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>47.7 ± 7.4</td>
<td>52.5 ± 9.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>207.4 ± 58.7</td>
<td>130.5 ± 43.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>164.6 ± 57.6</td>
<td>114.8 ± 11.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Fasting glucose (mg/dl)</td>
<td>108.5 ± 14.5</td>
<td>107.7 ± 15.4</td>
<td>ns</td>
</tr>
<tr>
<td>Fasting insulin (mU/l)</td>
<td>6.72 ± 3.55</td>
<td>6.57 ± 2.85</td>
<td>ns</td>
</tr>
<tr>
<td>G/I ratio</td>
<td>20.4 ± 9.6</td>
<td>19.8 ± 9.6</td>
<td>ns</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.76 ± 0.87</td>
<td>1.71 ± 0.64</td>
<td>ns</td>
</tr>
<tr>
<td>Total adiponectin (( \mu g/ml ))</td>
<td>6.32 ± 2.11</td>
<td>6.68 ± 2.02</td>
<td>ns</td>
</tr>
<tr>
<td>HMW adiponectin (( \mu g/ml ))</td>
<td>3.02 ± 1.47</td>
<td>3.39 ± 1.72</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Visfatin (ng/ml)</td>
<td>14.8 ± 2.3</td>
<td>14.2 ± 2.8</td>
<td>ns</td>
</tr>
</tbody>
</table>

Values are expressed as means ± SD. BMI, body mass index; T-cho, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; G/I, fasting glucose to fasting insulin; HOMA-IR, homeostasis model assessment of insulin resistance; HMW adiponectin, high molecular weight adiponectin; ns, not significant.
Lipolysis was directly enhanced by PPAR alpha agonist in isolated adipocytes in an experimental animal model [19]. Fenofibrate therapy for 2 months actually increased total adiponectin levels and improved insulin sensitivity without a change in body weight [4]. Unfortunately, our results might not be compatible with the above mentioned report. One possible reason for this discrepancy might be that the number of the subjects in our study was not sufficient. One of the interesting results in our study is that fenofibrate increased the HMW adiponectin complexes. Thus, it is suggested that fenofibrate might primarily increase HMW adiponectin rather than total adiponectin.

Tsuchida et al. reported that a PPAR alpha agonist (Wy-14,643) did not increase HMW adiponectin in KKAy mice [20]. Although the reason why difference between the results of our study and those of their study was not uncertain at present, possible several factors such as species (human and mice), the sort of PPAR alpha agonist (fenofibrate and Wy-14,643), and treatment period (3 months and 8 weeks) are raised. In our study, since we could not clarify an obvious mechanism by which fenofibrate increased HMW adiponectin, this is a further problem that we need to solve.

We investigated whether the increase of HMW adiponectin by fenofibrate treatment could regulate insulin sensitivity, as assessed by G/I ratio, which is known as a useful and simple tool for the evaluation of insulin sensitivity in non-diabetic patients, such as our subjects [21, 22]. There was tendency for a negative correlation between the changes (delta) in HMW adiponectin levels and changes in the G/I ratio, after adjustment for age and differences in BMI ($P<0.10$, data not shown) (Delta variables were calculated as follows: variable after treatment—variable before treatment.). This result raises the possibility that the improvement of insulin sensitivity due to fenofibrate might be regulated by HMW adiponectin.

The limitations of this study also need to be considered. As a consequence of the narrow selection criteria, the numbers of the patients were hardly sufficient to obtain clear results. Also, it is imperative to mention that the G/I ratio used for the measurement of insulin sensitivity is only an estimate and may not be as accurate as glucose clamp test. Although further experiments are needed, this study would help to clarify other roles of fenofibrate.

In conclusion, this is the first report to demonstrate that fenofibrate might not regulate the serum visfatin levels. Moreover, it is suggested that fenofibrate increases serum HMW adiponectin levels rather than total adiponectin levels. Since this effect may potentially improve insulin sensitivity, further studies on its clinical and biological relevance are necessary.

References


