Effects of Bezafibrate Treatment in a Patient and a Carrier With Mutations in the PNPLA2 Gene, Causing Neutral Lipid Storage Disease With Myopathy

Neutral lipid storage disease with myopathy (NLSDM) is a rare but severe genetic disorder characterized by excessive lipid accumulation in tissues including skin, bone marrow, heart, liver, and muscles. Clinically, NLSDM patients present with severe dilated cardiomyopathy, skeletal muscle myopathy, and insulin resistance. NLSDM is caused by a defect in the PNPLA2 gene encoding the enzyme adipose triglyceride lipase (ATGL), which catalyzes the breakdown of triglycerides in multiple tissues and is the rate-limiting step of lipolysis. Although heterozygous carriers and homozygous patients both present with similar clinical symptoms, the severity of these symptoms in homozygous patients is more dramatic, leading to premature death attributed to dilated cardiomyopathy in some patients. To date, the only available treatment is strict dietary guidelines and is focused on treating the comorbidities rather than targeting the primary defect.

To investigate cardiac lipotoxicity in NLSDM, ATGL-deficient mice have been investigated. Just like NLSDM patients, ATGL-deficient mice are also characterized by excessive lipid storage in skeletal muscle, liver, and heart, and they develop cardiomyopathy at a young age, resulting in premature death. Interestingly, we reported recently that a lack of ATGL resulted in a diminished mitochondrial defects, restored cardiac function, and prevented premature death. These promising findings inspired us to investigate whether PPAR agonist treatment in patients and carriers of a PNPLA2 gene defect could also have beneficial effects.

Although NLSDM is a very rare disease, we had the opportunity to study 2 sisters with PNPLA2 gene mutations. Patient 1 is a 37-year-old woman with a body mass index of 21.4 kg/m². During childhood, patient 1 developed hearing loss and progressive proximal muscle weakness. Histological analysis revealed increased lipid accumulation in skeletal muscle and Jordan bodies in white blood cells. At the age of 34 years, she developed a dilated cardiomyopathy, skeletal muscle myopathy, and insulin resistance. NLSDM is caused by a defect in the PNPLA2 gene encoding the enzyme adipose triglyceride lipase (ATGL), which catalyzes the breakdown of triglycerides in multiple tissues and is the rate-limiting step of lipolysis. Although heterozygous carriers and homozygous patients both present with similar clinical symptoms, the severity of these symptoms in homozygous patients is more dramatic, leading to premature death attributed to dilated cardiomyopathy in some patients. To date, the only available treatment is strict dietary guidelines and is focused on treating the comorbidities rather than targeting the primary defect.

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Despite a marked decrease in tissue lipid accumulation and an improvement in oxidative metabolism, no major changes were (yet) found in clinical parameters. Handgrip strength was low in NLSDM patients and did not change on bezafibrate treatment in either subject (data not presented). However, both patients did show an improvement in a leg extension test, a measure of leg muscle strength (Figure E). Although this did seem to consistently improve, the values for both patients remained low compared with age, body mass index, and sex-matched controls (Figure E, dotted lines).

Cardiac function at baseline was impaired in patient 1 and normal in patient 2 (Table). Patient 1, who is known to have frequent arrhythmias before treatment, only had 1 episode with a nonsustained ventricular tachycardia during the intervention period, as registered by the implantable cardioverter defibrillator. Still, neither the ultrasound nor the ECG could detect marked changes in cardiac function in both patients after 28 weeks of treatment (Table).

Consistent with the findings in mice lacking ATGL,4 bezafibrate treatment resulted in a marked lowering of cardiac
and muscle fat content and an increase in fat oxidative capacity. In healthy subjects, fibrates were shown not to affect ectopic lipid content, suggesting that fibrates may reduce the extreme lipid accumulation that characterizes NLSDM by upregulating and normalizing oxidative metabolism. Haemmerle et al. showed recently that the lack of ATGL prevents liberation of fatty acids (or fatty acid intermediates) for PPAR signaling from the lipid droplet, hence resulting in an impaired fat oxidative capacity. As a consequence, oxidation of circulatory fatty acids taken up in muscle, liver, and heart is compromised, and these fatty acids will be more readily directed to storage. By providing synthetic PPAR agonists to ATGL-deficient mice, fat oxidative capacity could be increased, ectopic fat storage was blunted, and cardiac dysfunction was completely restored. The results presented here indicate that, in humans with compromised ATGL activity, PPAR treatment results in similar improvements in fat oxidation and tissue lipid storage, as in ATGL-deficient mice. However, unlike ATGL-deficient mice, the improvements in lipid accumulation and oxidative metabolism observed in our patients did not (yet) result in pronounced clinical changes. Leg muscle strength did improve but was not paralleled by a decrease/normalization of creatine kinase levels or a change in handgrip strength. Furthermore, cardiac function seemed unaltered. Whether the lack of clinical changes are attributed to the relative short duration of the treatment or the fact that treatment could only be started in patients who are in a later stage of the disease (note that ATGL-deficient mice were treated from early age onward) cannot be deduced from the present study and needs further investigation.

In conclusion, our results suggest that, similar to mice lacking ATGL, NLSDM patients may also benefit from bezafibrate treatment with respect to lipid accumulation and fat oxidative capacity. However, 28 weeks of bezafibrate treatment did not result in clinical changes in these patients. Longer studies are needed to investigate whether these improvements can also translate into clinical improvements in these patients.

Acknowledgments
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Table. Patient Characteristics and Outcome Parameters

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Controls (n=4)</th>
<th>Patient 1 Before</th>
<th>12 wk</th>
<th>28 wk</th>
<th>Patient 2 Before</th>
<th>12 wk</th>
<th>28 wk</th>
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<tbody>
<tr>
<td>Basal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>33±5</td>
<td>37</td>
<td>-</td>
<td>-</td>
<td>39</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Body weight, kg</td>
<td>80.8±8.3</td>
<td>70.4</td>
<td>71.2</td>
<td>70.4</td>
<td>79.4</td>
<td>80.2</td>
<td>80.4</td>
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<tr>
<td>BMI, kg/m²</td>
<td>27.8±2.2</td>
<td>21.4</td>
<td>21.6</td>
<td>21.5</td>
<td>27.7</td>
<td>27.9</td>
<td>27.9</td>
</tr>
<tr>
<td>Body fat %</td>
<td>34.2±1.4</td>
<td>32.3</td>
<td>31.6</td>
<td>-</td>
<td>38.3</td>
<td>39.3</td>
<td>-</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.4±0.4</td>
<td>4.3</td>
<td>3.4</td>
<td>3.7</td>
<td>4.8</td>
<td>5.1</td>
<td>4.3</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.8±0.2</td>
<td>1.2</td>
<td>1.1</td>
<td>1.3</td>
<td>1.1</td>
<td>1.4</td>
<td>1.3</td>
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<tr>
<td>LDL, mmol/L</td>
<td>3.1±0.4</td>
<td>2.7</td>
<td>1.8</td>
<td>2.2</td>
<td>2.9</td>
<td>3.1</td>
<td>2.7</td>
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<tr>
<td>Triglycerides, mmol/L</td>
<td>1.2±0.37</td>
<td>0.96</td>
<td>0.59</td>
<td>0.66</td>
<td>1.91</td>
<td>0.92</td>
<td>0.65</td>
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<tr>
<td>Free fatty acids, mmol/L</td>
<td>0.47±1.30</td>
<td>0.15</td>
<td>0.20</td>
<td>0.30</td>
<td>0.64</td>
<td>0.08</td>
<td>0.40</td>
</tr>
<tr>
<td>Creatinine, µmol/L</td>
<td>73.0±4.5</td>
<td>61</td>
<td>56</td>
<td>68</td>
<td>60</td>
<td>72</td>
<td>82</td>
</tr>
<tr>
<td>CK, U/L</td>
<td>64.0±8.8</td>
<td>307</td>
<td>290</td>
<td>266</td>
<td>61</td>
<td>88</td>
<td>81</td>
</tr>
<tr>
<td>ALAT, U/L</td>
<td>18.0±3.1</td>
<td>26</td>
<td>26</td>
<td>29</td>
<td>15</td>
<td>19</td>
<td>15</td>
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<tr>
<td>ASAT, U/L</td>
<td>45.0±8.8</td>
<td>32</td>
<td>34</td>
<td>32</td>
<td>12</td>
<td>21</td>
<td>16</td>
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<tr>
<td>γ-GT</td>
<td>21.0±1.4</td>
<td>26</td>
<td>20</td>
<td>17</td>
<td>19</td>
<td>24</td>
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<td>Clamp</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>MCR-value, mL/(kg∙min)</td>
<td>8.5±1.0</td>
<td>6.2</td>
<td>6.6</td>
<td>-</td>
<td>3.4</td>
<td>3.9</td>
<td>-</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Basal</td>
<td>4.5±0.5</td>
<td>4.9</td>
<td>5</td>
<td>-</td>
<td>5.4</td>
<td>5.3</td>
<td>-</td>
</tr>
<tr>
<td>During clamp</td>
<td>5.5±0.5</td>
<td>6.4</td>
<td>5.3</td>
<td>-</td>
<td>6.6</td>
<td>5.2</td>
<td>-</td>
</tr>
<tr>
<td>Insulin, µU/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Basal</td>
<td>4.8±0.9</td>
<td>8.6</td>
<td>7.9</td>
<td>-</td>
<td>8.5</td>
<td>8.9</td>
<td>-</td>
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<tr>
<td>During clamp</td>
<td>-</td>
<td>99.2</td>
<td>91.7</td>
<td>-</td>
<td>66.2</td>
<td>66.8</td>
<td>-</td>
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<tr>
<td>Heart rate, bpm</td>
<td>60.0±5.0</td>
<td>76</td>
<td>72</td>
<td>76</td>
<td>74</td>
<td>77</td>
<td>79</td>
</tr>
<tr>
<td>%FS, %</td>
<td>30.00±4.13</td>
<td>16</td>
<td>10</td>
<td>-</td>
<td>42</td>
<td>40</td>
<td>39</td>
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<tr>
<td>LVEF MOD (A4C), %</td>
<td>57.00±6.03</td>
<td>50</td>
<td>54</td>
<td>53</td>
<td>72</td>
<td>69</td>
<td>63</td>
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<tr>
<td>LV mass (ASE), g</td>
<td>114.0±8.0</td>
<td>226</td>
<td>247</td>
<td>278</td>
<td>108</td>
<td>131</td>
<td>142</td>
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<tr>
<td>Rel. WT</td>
<td>0.84±0.01</td>
<td>1.18</td>
<td>1.17</td>
<td>1.26</td>
<td>0.95</td>
<td>0.88</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Changes in plasma values and cardiac ultrasound (US). Results are presented for controls and both patients before and after 12 and 28 wk of treatment with Bezafibrate. Plasma values are determined after an overnight fast and during a hyperinsulinemic euglycemic clamp. Ultrasound results are presented for 2-dimensional imaging. BMI indicates body mass index; CK, creatine kinase; FS, fractional shortening; LVEF, left ventricular ejection fraction; LV mass, left ventricular mass calculated according to the American Society of Echocardiography convention; MCR, metabolic clearance rate of glucose; and Rel WT, relative wall thickness.
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Disclosures

None.

References

Key Words: ATGL • fibrates • neutral lipid storage disease • PNPLA2

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