PRADER-WILLI SYNDROME (PWS) is a genetic disease that arises from the failure to express paternally inherited imprinted genes on chromosome 15q11-q13, and it is characterized by severe morbid obesity related to hyperphagia and type 2 diabetes mellitus. PWS patients often develop type 2 diabetes mellitus (T2DM) and their abnormal alimentary habits include a morbid obsession with food, reduced satiety and earlier return of hunger after eating. Treatments to control this abnormal bulimia are necessary to prevent morbid obesity and hyperglycemia, although the mechanisms underlying the defective appetite regulation in PWS are poorly understood and there are few specific treatments that suppress hyperphagia in PWS. However, it was recently suggested that the intense hyperphagia in PWS could stem, at least in part, from impaired gut hormone signaling [3].

Glucagon-like peptide-1 (GLP-1) is an anorexigenic hormone released from the L cells of the small intestine in response to nutrient intake. GLP-1 has several physiological functions, increasing insulin secretion and suppressing glucagon levels in a glucose-dependent manner, as well as decreasing food intake, slowing gastric emptying and increasing satiety [4, 5]. Considering these actions, GLP-1 may represent a promising therapy for PWS, although there is still little evidence that this is the case. Recently, beneficial effects of the GLP-1 receptor agonist (exenatide) and the human GLP-1 analog (liraglutide) have been reported in PWS [6, 7]. However, it is uncertain whether long-term liraglutide therapy might control metabolic disorders and improve the impaired gut hormone signaling that causes hyperphagia.

Here, we report on a female patient with PWS whose
body weight was reduced and glycemic control was remarkably improved by 12 months of liraglutide treatment. In this patient, we determined the fasting plasma levels of appetite-regulating hormones such as ghrelin (active form), GLP-1 (active form), leptin, insulin and adiponectin, both before and one year after liraglutide therapy, and we also compared various metabolic factors in this PWS patient with those in a patient with simple obesity accompanied by diabetes as a reference case.

**Patient and the Clinical Course**

At the age of 10, a patient with typical features of PWS underwent genetic testing (15q11-q13), which confirmed the clinical diagnosis. The patient and her family were provided with dietary advice, although the consequences were poor. At the age of 20, her body mass index (BMI) was 40.6 kg/m² and it increased further to 44.3 kg/m² at the age of 25 due to her gross hyperphagia, when she was also diagnosed with type 2 diabetes mellitus. Despite her efforts to diet and exercise, her diabetic control deteriorated and as a result, she was referred to our hospital, where we obtained informed consent from her and her family for therapeutic and research purposes. Hypoglycemic therapy was initiated that consisted of 50 mg/day of sitagliptin and 3.75 mg/day of pioglitazone, although this therapy improved neither her BMI (39.1 kg/m²) nor National Glycohemoglobin Standardization Program (NGSP) Hemoglobin A1c (HbA1c, 12.6%). Accordingly, biguanide treatment was considered, although it turned out to be very difficult for this patient to take the medicine three times a day and thus, her therapy was switched to liraglutide administration alone. Liraglutide was initially given at a dose of 0.3 mg/day, and this amount was increased each week until the maintenance dose of 0.9 mg/day was reached and maintained until the patient had been treated for 12 months with this drug.

**Biochemical Measures**

The blood was sampled at fasting, and aprotinin and dipeptidyl peptidase (DPP)-4 inhibitor were added to EDTA blood tubes before blood samples were collected to prevent proteolysis for GLP-1 measurement [8, 9]. A RIA was used to measure the fasting plasma levels of insulin and leptin (insulin RIA kit and Human Leptin RIA kit, Millipore corporation, Billerica, Massachusetts, USA), and ELISA was adopted to determine fasting plasma adiponectin (high molecular), GLP-1 (active) and ghrelin (active) levels (Human Adiponectin LATEX kit and Active Ghrelin ELISA Kit, Mitsubishi Chemical Corporation Tokyo Japan; GLP-1 ELISA kit, Millipore corporation, Billerica, Massachusetts, USA) [8, 9]. The sensitivity of the GLP-1 assay was 0.83 pmol/L, the specificity was 100% for GLP-1 [7-36 amide], 99.5% for GLP-1 [7-37], 0.2% for GLP-1 [1-37] and GLP- [1-36], while GLP- [9-36 amide] and GLP- [9-37 amide] were not detected. The inter-assay variation was 10.6-12.0 and the intra-assay variation was 2.43-2.85. The after treatment data was obtained 24 hours after the last subcutaneous liraglutide injection.

In addition, the areas of visceral fat and of subcutaneous fat were measured by computed tomography (CT). A CT scan at the L4-5 level was carried out to measure the cross-sectional area of total abdominal fat, visceral abdominal fat and the subcutaneous abdominal fat, according to previously described methods [10, 11].

**Results**

Liraglutide treatment had been associated with a progressive lowering of BMI and HbA1c during the initial six months, without further decrease or rebound increase thereafter. Consequently, after one year of liraglutide treatment, the BMI and HbA1c were remarkably lower than the pretreatment values (Fig. 1). Similarly, after one-year treatment the visceral fat area (VFA), the subcutaneous fat area (SFA) and the VFA/SFA (V/S) ratio on the CT decreased from 150.1 cm², 494.7 cm², and 0.30 (before treatment) to 113.2 cm², 478.6 cm², and 0.24, respectively, demonstrating a marked decrease in visceral fat. Her appetite was suppressed immediately after commencing liraglutide therapy and this remained under control for the 12 months of treatment. Regarding satiety, not only the patient but also her family spontaneously declared that she felt full and was eating less, and that she was not feeling as hungry throughout the period of weight loss as she had been during prior anti-hyperglycemic treatment. This decrease in hyperphagia was maintained over the 12 months and moreover, during the 12 months of liraglutide treatment she did not complain of any side effects and she remained well.

The plasma levels of appetite-regulating hormones (ghrelin, leptin and GLP-1), glucose and the glucose regulatory hormones (insulin and adiponectin) were...
measured before and after treatment in this PWS patient. Likewise, some of these factors were compared between this patient (Table 1) and a sex-matched individual with simple obesity accompanied by diabetes chosen as a reference case. In the PWS patient, liraglutide treatment lowered the glucose levels but increased insulin secretion, and the decrease in the fasting plasma glucose concentration (FPG) of the PWS patient was almost equivalent to that in the reference case. Liraglutide treatment improved the homeostasis model assessment insulin resistance (HOMA-IR) of the PWS patient from 14.3 to 10.0 and the HOMA-IR reduction of the PWS patient measured before and after treatment in this PWS patient.

![Fig. 1](image_url)

The changes in body mass index (BMI) (A) and HbA1c (B) provoked by liraglutide administration.

The values of HbA1c are expressed as the National Glycohemoglobin Standardization Program (NGSP) value. Although the HbA1c and BMI of the PWS patient improved smoothly during the first six months after commencing liraglutide therapy, neither BMI nor HbA1c decreased far below normal level during the last six months of treatment. Although these changes reached a plateau afterwards, the values remained within a normal range.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Prader-Willi syndrome with diabetes</th>
<th>Simple obesity with diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years old)</td>
<td>25</td>
<td>30</td>
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<tr>
<td>Gender</td>
<td>female</td>
<td>female</td>
</tr>
<tr>
<td>Liraglutide therapy</td>
<td>before after</td>
<td>before after</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>70.2</td>
<td>93.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>39.1</td>
<td>40.5</td>
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<tr>
<td>VFA (cm²)</td>
<td>150.1</td>
<td>203.6</td>
</tr>
<tr>
<td>SFA (cm²)</td>
<td>494.7</td>
<td>512.1</td>
</tr>
<tr>
<td>V/S ratio</td>
<td>0.30</td>
<td>0.40</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>12.6</td>
<td>14.2</td>
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<tr>
<td>FPG (mmol/L)</td>
<td>18.2</td>
<td>19.9</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>108.1</td>
<td>2082.0</td>
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<td>HOMA-IR</td>
<td>14.3</td>
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<td>HOMA-β</td>
<td>24.5</td>
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<tr>
<td>Ghrelin (fmol/L)</td>
<td>137.0</td>
<td>272.0</td>
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<tr>
<td>GLP-1 (pmol/L)</td>
<td>2.1</td>
<td>1.8</td>
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<tr>
<td>Leptin (pmol/L)</td>
<td>1.8</td>
<td>1.9</td>
</tr>
<tr>
<td>Adiponectin (pmol/L)</td>
<td>453.3</td>
<td>406.6</td>
</tr>
</tbody>
</table>

BMI, body mass index; VFA, visceral fat area; SFA, subcutaneous fat area; V/S, VFA/SFA; HbA1c, glycated hemoglobin A1c (NGSP); FPG, fasting plasma glucose concentration; HOMA-IR, homeostasis model assessment insulin resistance; HOMA-β, homeostasis model assessment beta cell function; GLP-1, glucagon like peptide-1.
eating caused by hyperghrelinemia might bring on hyperglycemia [19, 20]. GLP-1 plays a critical role in satiety and energy homeostasis, acting peripherally and in the hypothalamus. However, the role of GLP-1 in PWS, if any, is yet to be established. A previous study of PWS patients demonstrated no differences in fasting GLP-1 levels compared to obese controls [16] as evident here. However, as GLP-1 is secreted in response to nutrient intake, differences may exist postprandially. Regrettably, we did not evaluate the postprandial response of active GLP-1, although it was recently reported that the postprandial response of active GLP-1 was no different between PWS and control subjects [3].

On the basis of the data in the previous reports [21-25], it is conceivable that postprandial elevation of plasma active GLP-1 concentration suppresses ghrelin secretion via the vagal nerve system. Nevertheless, a pharmacological amount of GLP-1 was needed to lower plasma ghrelin in the patient with PWS. The finding indicates that GLP-1 suppression of ghrelin is impaired and physiological postprandial excursion of GLP-1 may not be followed by appropriate lowering of ghrelin in this syndrome. Hyperphagia in PWS patients is thought not to be related to a lower postprandial GLP-1 response, and elevated ghrelin levels in PWS are consistent with increased hunger and they are unrelated to insulin levels [3].

The effects of liraglutide seem to have reached equilibrium with the passage of treatment, suggesting down-regulation of the receptor or its downstream signaling might have been induced by maintaining the GLP-1 receptor strongly stimulated with liraglutide. Administration of exogenous GLP-1 suppresses the plasma active GLP-1 levels, making it very likely that we were measuring endogenous GLP-1. Indeed, blood GLP-1 activity may also exert a negative feedback effect on the production of GLP-1 from L-cells.

Discussion

We have studied the effects of treating a female patient with PWS with a GLP-1 analog, liraglutide, and we found that the weight loss observed in this individual (-5.6 kg) was larger than that generally accepted as the mean reduction achieved by GLP-1 treatment in patients with T2DM (-2.8 kg) [12]. Similarly, the reduction in HbA1c in this patient (-6.5%) was also larger than the mean reduction in a meta-analysis of randomized clinical trials of patients with T2DM (-1.0%) [13]. Moreover, the decreases in HbA1c and BW in the PWS patient were greater than those in the reference case.

Moreover, the FPG before and after liraglutide treatment were comparable between the PWS patient and the reference case. However, fasting insulin concentrations and the HOMA-IR were lower in the PWS patient than in the reference case. Leptin levels before and after the treatment were comparable between the PWS patient and the reference case, while those of adiponectin were also comparable between the two patients, they tended to be slightly higher in the PWS patient. These results are all consistent with a previous report [14]. In this study, not only insulin resistance (HOMA-IR) but also insulin secretion (HOMA-β) was improved by liraglutide treatment. Thus, the improvement of glycemic control in the PWS patient by the liraglutide treatment may have been due to the recovery of insulin secretion and the improvement of insulin sensitivity associated with the reduction in body weight (visceral fat reduction) [6, 14].

Since PWS patients display high plasma ghrelin levels, despite the relatively low visceral adiposity and relative hypoinsulinemia [15], and since administration of the GLP-1 analog strongly reduces ghrelin levels and appetite, it is very likely that the high ghrelin levels are the cause of their excessive appetite [16-18]. The inappropriate suppression of insulin secretion and the over-
when their pancreatic insulin secretion capabilities are still relatively strong.

Declaration of Conflict of Interests

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

References


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