Evaluation of glucose tolerance and effect of dietary management on increased visceral fat in a patient with Werner syndrome

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Abstract. Werner syndrome (WS), a type of progeria, is a hereditary condition caused by a mutation in the WRN gene. A 62-year-old Japanese woman was diagnosed with WS at the age of 32 and has been visiting the hospital for follow-up since the last 30 years. The patient developed diabetes at the age of 46, and at the age of 60, her body mass index increased from 20.1 to 22.7 kg/m² owing to her unhealthy eating habits; her visceral fat area at the age of 61 was 233 cm². With dietary control, her body weight, including the visceral fat and subcutaneous fat, decreased at the age of 62, and her insulin secretion, obesity, and fatty liver improved. We conducted the oral glucose challenge test four times, including at the prediabetic stage, to evaluate the insulin-secretion ability. The patient’s insulin resistance gradually increased for more than 14 years, and her insulin secretion ability began to decrease 14 years after her diabetes diagnosis. Despite a remarkable decrease in body weight and fat mass with dietary management, the psoas muscle index did not decrease significantly in proportion to the body weight or fat mass. However, muscle mass monitoring is important for preventing the progression of sarcopenia. Hence, gradual reduction of visceral fat and weight by dietary management may be useful in treating diabetes in patients with WS, particularly in those whose visceral fat is significantly increased.

Key words: Werner syndrome, Diabetes mellitus, Visceral fat, Insulin resistance, Insulin secretion

WERNER SYNDROME (WS) is a hereditary progeria syndrome caused by a mutation in the WRN gene [1]. Approximately 80% of all patients with WS are Japanese [2]. Diagnosis is usually based on typical characteristics, including graying or loss of hair, bilateral cataracts, skin atrophy, subcutaneous calcification, and a beaked nose. A high-pitched hoarse voice, osteoporosis, short stature and low body weight, central obesity, and hypogonadism have also been reported. The commonest causes of death are malignancy and myocardial infarction [3]. Approximately 70% of patients with WS have diabetes, with onset between ages 35 and 40 [3]. Insulin resistance due to an abnormality in the insulin receptor is indicated as an underlying cause of diabetes in WS. It is believed that impaired insulin secretion with concomitant insulin resistance is necessary for the progression of diabetes even in patients with WS who have high insulin resistance [4]. In patients with type 2 diabetes, the postprandial blood glucose levels increase before diabetes onset, and β-cell functions decrease to 50% or less [5]. Previous studies have reported findings on follow-up of insulin resistance in patients with WS [6]; however, no study had conducted glucose tolerance tests prior to diabetes onset. Furthermore, no reports have shown the effects of diet or weight loss on diabetes in patients with WS. The lifespan of patients with WS has increased recently [3], and this facilitates long-term observation of the natural progression of diabetes in these patients. We presented the case of a patient with WS who was followed up for 30 years and developed diabetes later. We also evaluated the effect of dietary management on weight loss to manage diabetes. Moreover, we observed the changes in waist circumference, L/S ratio (liver steatosis), visceral
and subcutaneous fat percentage, and muscle mass over 15 years.

**Case Presentation**

A 62-year-old Japanese woman with WS had been visiting our hospital for over 30 years for regular follow-up. We observed changes in her body weight and glycated hemoglobin (HbA1c) levels during this period and conducted the 75-g oral glucose tolerance test (OGTT) at four timepoints: at the time when WS was diagnosed, after WS was diagnosed, at the time of diabetes onset, and after weight loss. Moreover, we measured the visceral fat area, subcutaneous fat area, psoas muscles index (PMI), extent of fatty liver (liver/spleen <0.9), and waist circumference after the age of 50. The patient was healthy at birth and exhibited normal growth and development in infancy. Her maternal grandmother was diagnosed with WS. The patient had three children; however, there were no third degree relatives with WS other than the grandmother. None of the relatives had consanguineous marriages. From childhood onward, the patient exhibited hoarseness in her voice, skin pigmentation, ulcers on the limbs, and a protruding nose. When she was 32 years old, she was diagnosed with WS based on her clinical characteristics of juvenile bilateral cataracts, skin atrophy, impaired glucose tolerance, and a typical bird-like facial appearance. The patient carried a compound heterozygous mutation, c.3139-1G>C and c.1105C>T, which is a well-established Japanese founder mutation [7]. On physical examination, she had a height of 164 cm and a weight of 49.5 kg (body mass index [BMI]: 18.4 kg/m²). Abdominal ultrasonography revealed a fatty liver when she was 38 years old. At the age of 46, the patient was diagnosed with diabetes owing to a fasting blood glucose level of 142 mg/dL and HbA1c of 8.5%; her weight at the time was 54 kg (BMI: 20.1 kg/m²). The patient was started on oral antihyperglycemic agents including voglibose (0.6 mg/day) and nateglinide (270 mg/day); however, the treatment response was inadequate for glycemic control. After the addition of pioglitazone (15 mg/day), a good glycemic level was achieved. However, the patient could not restrain herself from consuming midmeals, and at the age of 60, her weight increased to 61 kg (BMI: 22.7 kg/m²) with an associated increase in HbA1c levels (Fig. 1). When she was 61 years old, she was hospitalized with severe skin ulcers on both feet. Following discharge, the patient was admitted to a nursing facility, where she discontinued unhealthy midmeals, followed a normal diet (1,600 kcal per day), and gradually lost weight. Her HbA1c levels decreased and oral antihyperglycemic agents were discontinued (Fig. 1).

By the age of 62, the patient lost approximately 10 kg over 6 months without having anorexia or gastrointestinal symptoms. The patient was hospitalized for evaluating

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**Fig. 1** Clinical course over 30 years in a patient with Werner syndrome.
the cause of her weight loss. On examination, her height was 161 cm and weight was 46.9 kg (BMI: 18.1 kg/m²); the blood pressure was 113/89 mmHg. Cardiac investigations including echocardiography, coronary computed-tomography angiography, and myocardial scintigraphy revealed normal findings. Skin examination revealed a bird-like countenance, lower limb ulcers, amyotrophy of limbs, and calcification of the soft tissue of the Achilles tendon; the laboratory findings on admission are shown in Table 1. There were no abnormalities detected on the blood tests, and no evidence of malignancy was found on gastrointestinal endoscopy and computed tomography scanning. Despite undergoing cataract-removal surgery in her thirties, the patient had no diabetic retinopathy or nephropathy. We concluded that the weight loss was caused by diet management at the nursing home and reduced food intake because of dementia.

**Methods**

**Evaluation of insulin resistance and secretion**

β-cell function and insulin sensitivity were assessed by using previously proposed parameters. The insulin resistance index (HOMA-IR) and Matsuda index were calculated using the standard formulae [8, 9]. Insulin secretion was measured based on the HOMA-β and insulinogenic index. The disposition index, which is the product of the Matsuda Index and the insulinogenic index, is a good indicator of the insulin secretion-compensatory ability of pancreatic β-cells against insulin resistance.

**Evaluation of fat mass and muscle mass**

Visceral fat area (VFA) and subcutaneous fat area (SFA) were calculated at the umbilical region by CT scans using AZE Virtual Place software. The PMI using the simplified method was calculated as the sum of right

| Table 1 | Laboratory findings of the patient with Werner syndrome |
|---------|----------------------------------------------------|
|         | Normal Range | On Admission |
| White-cell count (μL) | 4,500–9,000 | 4,300 |
| Hemoglobin (g/dL) | 11.7–14.6 | 12.3 |
| Platelet count (μL) | 150,000–220,000 | 222,000 |
| Creatinine (mg/dL) | 0.50–0.80 | 0.4 |
| Total-protein (g/dL) | 6.7–8.3 | 7 |
| Total-bilirubin (mg/dL) | 0.3–1.2 | 0.9 |
| Alkaline phosphatase (U/L) | 115–359 | 326 |
| Aspartate aminotransferase (U/L) | 13–33 | 12 |
| Total-cholesterol (mg/dL) | 128–219 | 179 |
| High-density lipoprotein cholesterol (mg/dL) | 40–99 | 48 |
| Triglycerides (mg/dL) | 20–149 | 110 |
| Creatine phosphokinase (IU/L) | 45–163 | 127 |
| C-reactive protein (mg/dL) | 0.00–0.40 | 0.13 |
| Sodium (mmol/L) | 135–149 | 142 |
| Potassium (mmol/L) | 3.5–4.9 | 3.2 |
| Chloride (mmol/L) | 96–108 | 108 |
| Cortisol (μg/dL) | 6.2–18.0 | 10.3 |
| ACTH (pg/mL) | 7.2–63.3 | 38.9 |
| GH (ng/mL) | 0.13–9.88 | 0.29 |
| IGF-1 (ng/mL) | 37–150 | 124 |
| TSH (μU/mL) | 0.75–4.67 | 3.59 |
| FT3 (pg/mL) | 2.3–4.2 | 2.7 |
| FT4 (ng/dL) | 0.72–1.52 | 1.0 |
| LH (mIU/mL) | 7.7–58.5 | 15.2 |
| FSH (mIU/mL) | 25.8< | 35.1 |
and left of the short-axis × long-axis of the psoas muscle at the third lumbar vertebra, divided by height squared [10]. VFA ≥100 cm² and VFA-to-SFA ratio (V/S ratio) >0.4 indicates high visceral fat [11, 12].

Results

Fig. 2A presents the results of the OGTT. Insulin secretion under the sugar stress peaked at 39 years of age and decreased at the time of diabetes onset. The HOMA-IR, which indicates insulin resistance, increased until diabetes onset at the age of 46, but considerably decreased at the age of 62. HOMA-β, which indicates the insulin-secretory ability, was extremely high before the age of 46, indicating oversecretion, but decreased with age. The Matsuda index, which reflects skeletal muscle insulin sensitivity [8, 9], decreased until diabetes onset at the age of 46, but increased significantly after weight loss at the age of 62. The insulogenic index, the ratio of the change in insulin to the change in glucose concentrations following a glucose load [13], decreased after the age of 39. The disposition index decreased at the age of 46 with diabetes onset (Fig. 2B). All the evaluated indices showed that the patient had high insulin resistance even before diabetes onset, that increased with age; however, these indices decreased after weight loss. In contrast, the patient’s insulin-secretion ability declined with age.

A detailed examination was conducted after the age of 50; the fat area gradually increased, and at ages between 59 and 61, CT scanning showed fatty liver (L/S <0.9), waist circumference >90 cm (Fig. 3), and VFA and SFA >200 cm². Conversely, the PMI slowly decreased since the age of 53 (Fig. 4). At 62 years of age, the fat mass decreased with weight loss. The L/S ratio increased to more than 0.9 and HbA1c decreased to around 6.0%. Moreover, the PMI decreased at the age of 62, but did not decrease significantly compared with the body weight or fat mass. At 64 years of age, the patient’s HbA1c level gradually increased and she was started on linagliptin (5 mg), resulting in good glycemic control. The patient died of pneumonia when she was 65 years old.

Discussion

There have been 83 types of WRN gene mutations reported to date [7], and the detected genotype-phenotype relationship in WS has been minimal. Regardless of the site of the mutations, whether homozygous or compound heterologous, the phenotype basically remains the same. This is because the mutation causes truncations of the WRN protein, eliminating the C-terminal nuclear localization signal; WRN is therefore unable to localize to the nucleus [14].

The patient carried compound heterozygous mutations, c.3139-1G>C and c.1105C>T. The c.3139-1 mutation is a frame shift or nonsense mutation, which is the most common genetic mutation in Japanese WS patients. The c.1105 is the second most common, and is a nonsense mutation [7].

In the present study, patients with WS and diabetes had considerable insulin resistance, which may have been associated with insulin receptor abnormalities as the underlying mechanism [15]. It is not clear which organ is responsible for insulin resistance in patients with WS. The available reports using a euglycemic hyperinsulinemic clamp with a tracer infusion in patients with WS are limited; however, it has been reported that skeletal muscle demonstrates high insulin resistance on clamp tests [15, 16]. In our case, the low Matsuda index reflected the patient’s skeletal muscle insulin resistance. Further, hepatic fat accumulation and high HOMA-IR levels also suggest hepatic insulin resistance [17]. Imura et al. reported that the 75-g OGTT in 49 Japanese patients with WS revealed diabetic glucose tolerance in 27 patients; 17 of the 19 cases showed remarkable increase of the IRI, however, in two cases, the IRI after OGTT was <30 μU/mL [18]. Yamada et al. showed that among WS families, insulin secretion after a sugar load decreased, especially in patients with diabetes [4]. From a 16-year observation of WS with diabetes, Abe et al. reported that the sum of the plasma IRI level derived from the post-treatment OGTT progressively decreased as the age increased [6]. In our case, as shown in Fig. 2, the insulin resistance appeared before overt diabetes, and the insulin-secretion ability compensated for insulin resistance and increased at the age of 39, but decreased progressively after the age of 46. Impaired insulin secretion with concomitant insulin resistance is necessary for the development of overt diabetes even in syndromes with extreme insulin resistance, such as WS. Furthermore, after the onset of diabetes, insulin secretion in patients with WS gradually decreases.

No consensus has been reached on the use of oral hypoglycemic agents for diabetes in those with WS, but previous reports have shown that pioglitazone therapy in those with insulin resistance has effects on adipose tissues and plays a role in improving insulin sensitivity [19-21]. Pioglitazone is a thiazolidinedione believed to act through engagement with PPAR-γ receptors, which induce multiple genes involved in glucose and fatty acid metabolism. It has been reported that adiponectin increases along with a decrease in blood TNF-α and IL-6 levels. Furthermore, administration of pioglitazone reduces the ratio of visceral to subcutaneous fat [19, 20].

In their study, Yasuda H et al. reported that adminis-
Administration of biguanides improved glucose metabolism in WS patients. An increase in hepatic glucose production could account for insulin resistance in WS, and biguanide may be a good tool for managing insulin resistance in WS [22]. In the present case, good glycemic control was achieved following pioglitazone therapy (15 mg/day), but worsened due to weight gain induced by snacking. The patient’s weight decreased after a low-calorie diet and her HbA1c levels reduced. At 62 years of age, the 75-g OGTT was administered six months after discon-
Regarding the effects of pioglitazone discontinuation, Iwase et al. showed that HbA1c remained unchanged one month after discontinuation, but worsened two months later, and fasting insulin and adiponectin also worsened three months after discontinuation [23]. The Matsuda index at 62 years of age in this case was more likely to be affected by weight and fat loss than by pioglitazone. This suggests the possibility that similar to that of non-WS patients with type 2 diabetes, insulin resistance in WS can be controlled by both, pioglitazone and dietary management and weight loss.

In non-WS obese individuals, the insulin secretion and total pancreatic β-cell numbers increase with increasing insulin resistance. However, if continuous hyperglycemia leads to diabetes, the pancreatic β cells are relatively decreased [24]. In Japanese individuals, insulin secretion is lower than that in Western populations. With the aging of society, non-obese patients with type 2 diabetes are...
increasingly developing decreased insulin secretion. Furthermore, patients with WS are aging; this indicates the need for caution and close management of potential insulin resistance as well as pancreatic β-cell dysfunction in glucose metabolism. One report discussed a patient with WS and diabetes who achieved improvements in postprandial hyperglycemia with sitagliptin through inhibition of glucagon secretion [25]. Given that not only increasing insulin resistance but also decreasing insulin secretion mediates chronic diabetes in those with WS, DPP-4 inhibitors could be a good treatment choice for those with WS and diabetes. Patients with WS also have long-term survival, and target HbA1c levels may be the same as those with normal diabetes. However, as ADL is often reduced in progeria, it is better to use target HbA1c levels for elderly people even in young patients with WS.

The BMI of patients with WS is low; however, their visceral fat accumulation, high TNF-α, and low adiponectin levels may cause insulin resistance [26]. In this patient, the visceral fat predominance (V/S ratio >0.4) was evident from age 50 onward, when her BMI was 20 (Fig. 4). In general, the BMI of patients with WS is usually less than 18.1 [3]; however, it was relatively high in our patient with WS. Furthermore, her weight gain was due to her eating habits. At age 62, when her body weight decreased, the V/S ratio did not change, but both the visceral and subcutaneous fat decreased and the insulin secretion, obesity, and fatty liver were improved. However, the PMI did not decrease significantly compared with body weight or fat mass. This indicates that patients with WS may develop excessive visceral fat even if they are thin or have normal body weight; therefore, body fat evaluations and weight loss that maintains muscle quantity by an appropriate regular diet is expected to be useful. Yamaga et al. showed that low muscle mass was associated with WS, and all 9 patients in 1 study had sarcopenia with some being below the age of 40 [27]. Only a few studies have reported on muscle atrophy in patients with WS, and the underlying mechanism is incompletely understood. A previous report showed that fibroblasts in those with WS dysregulate the mammalian target of the rapamycin (mTOR) pathway [28], and mesenchymal stem cells have a senescence phenotype in those with WS [29]. The mTORC1 signaling pathway plays an important role in controlling muscle protein synthesis in response to nutrient intake and muscle contraction [30]. Type 2 diabetes is one of risk factors for sarcopenia, age-related loss of the skeletal muscle and its function, and sarcopenic obesity [31]. These are relevant concerns, especially in the aging society of Japan. Concerns regarding sarcopenia progression and frailty arise in elderly patients with WS with extended life spans. Our patient was confined to a wheelchair at home, because of a foot ulcer. However, after admission to the nursing home, not only did her eating habits improve, her physical activity increased due to rehabilitation by staff. This increase in physical activity helped maintain the muscle mass around the 3rd lumbar vertebra. It is necessary to maintain muscle mass by performing load-bearing exercise appropriate to the patient’s activities of daily living. In the present case, sudden weight loss was caused by dietary management and reduced food intake due to dementia. Therefore, a gradual weight loss would have been preferable in this patient.

In summary, this patient’s insulin resistance increased for more than 14 years and was later accompanied by decreased insulin secretion, 14 years after being diagnosed with diabetes. We suggest that patients with WS and diabetes should be screened and managed for potential insulin resistance and secretion using medicines that can protect pancreatic β-cells. Hence, patients with WS, especially those who experience a significant increase in visceral fat, can achieve a slow reduction of visceral fat and weight through dietary management, and may respond better to diabetes treatment.

**Disclosure**

None of the authors have any potential conflicts of interest associated with this research.

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