Endocrine CONNECTIONS

Glucose tolerance and insulin responsiveness in Gitelman syndrome patients

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Abstract

Objective: Impaired glucose metabolism and insulin sensitivity have been reported in patients with Gitelman syndrome (GS), but insulin secretion and the related mechanisms are not well understood.
Design and methods: The serum glucose levels, insulin secretion and insulin sensitivity were evaluated in patients with GS ($n=28$), patients with type 2 diabetes mellitus (DM) and healthy individuals ($n=20$ in both groups) using an oral glucose tolerance test. Serum and urine sodium, potassium and creatinine levels were measured at 0, 30, 60, 120 and 180 min after an oral glucose load was administered.
Results: The areas under the serum glucose curves were higher in the GS patients than those in the healthy controls (17.4 ± 5.1 mmol·h/L vs 14.5 ± 2.8 mmol·h/L, $P=0.02$) but lower than those in the DM patients (24.8 ± 5.3 mmol·h/L, $P<0.001$). The areas under the serum insulin curves and the insulin secretion indexes in GS patients were higher than those in DM patients and lower than those in healthy subjects. The insulin secretion-sensitivity index of GS patients was between that of healthy subjects and DM patients, but the insulin sensitivity indices were not different among the three groups. After one hour of glucose administration, the serum potassium level significantly decreased from baseline, and the urinary potassium-to-creatinine ratio increased gradually and peaked at 2 h.
Conclusions: Glucose metabolism and insulin secretion were impaired in GS patients, but insulin sensitivity was comparable between GS patients and patients with type 2 DM. After administration of an oral glucose load, the plasma potassium level decreased in GS patients due to the increased excretion of potassium in the urine.

Key Words
- Gitelman syndrome
- glucose metabolism
- insulin secretion
- insulin resistance

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Introduction

Gitelman syndrome (GS) (OMIM 263800) is an autosomal recessive renal tubular salt-wasting disorder that is characterized by hypokalaemic metabolic alkalosis, hypomagnesaemia and low urinary calcium, with secondary renin–angiotensin–aldosterone system (RAAS) activation and normal blood pressure (1).

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In most cases, GS results from loss-of-function mutations in the *SLC12A3* gene, which consists of 26 exons and encodes the thiazide-sensitive NaCl co-transporter (NCC) protein (NM_000339.2; OMIM 600968) of the distal convoluted tubule (DCT) (2). Recently (3), impaired glucose metabolism and insulin sensitivity were reported in GS patients, but insulin secretion function has not been studied in this population. Due to the lack of research in this field, little is known about the difference in glucose metabolism between GS and diabetes mellitus (DM) patients. Additionally, the underlying mechanism of abnormal glucose metabolism is not well understood. This study intended to (1) compare glucose levels, insulin secretion function and insulin sensitivity in GS patients with both healthy subjects and type 2 DM patients and (2) observe the serum and urine potassium changes after administration of an oral glucose load.

Subjects and methods

Subjects

A cross-sectional study was conducted at the Department of Endocrinology and Nephrology of Peking Union Medical College Hospital (PUMCH) in Beijing, China. The research was ethically conducted in accordance with the World Medical Association Declaration of Helsinki. All participants in this study provided written informed consent. Ethics approval was obtained from the Ethics Committee on Human Studies at PUMCH, Chinese Academy of Medical Sciences (Beijing, China). All patients diagnosed with GS were considered eligible if (1) they were between 10 and 80 years of age, (2) they were inpatients and (3) they agreed to provide written informed consent. Ultimately, 28 GS patients from 26 non-consanguineous families were enrolled, and 20 healthy volunteers and 20 type 2 diabetic patients were recruited as control groups.

Demographic and clinical characteristics were collected and documented, including gender, age, body mass index (BMI), history of obesity and biochemical indices such as serum and urinary electrolytes, arterial blood gases and electrocardiogram (ECG) results. The reference values used in this study were obtained from data collected by our laboratory regarding the healthy general population on unrestricted diets.

SLC12A3 gene mutation detection

As described in a previous study, genomic DNA was isolated and purified from the peripheral blood lymphocytes of patients and used for the polymerase chain reaction (PCR) amplification of individual exons of the *SLC12A3* gene. Twenty-three pairs of oligonucleotide primers were generated to amplify all 26 exons and flanking intronic regions of the *SLC12A3* gene (4, 5). Sanger direct sequencing was performed on an ABI 3730xl automated DNA sequencer (Life Technologies) by BGI (Beijing, China). The GenBank accession number NM_000339.2 was used as a reference sequence, in which the A of ATG was specified as number 1.

Upright RAAS test

An upright RAAS test was performed in 23 of the 28 GS patients. The protocol of the upright test was described previously (6). Briefly, patients were told to discontinue spirolactone at least 2 weeks before the test. On the day of the test, the patient remained in a supine position for at least 4 h and was then vertical for at least 2 h before the acquisition of blood samples. Plasma renin activity (PRA), angiotensin II (AngII) and aldosterone were detected by radioimmunoassay.

Oral glucose tolerance test

An oral glucose tolerance test (OGTT) was performed after a 12-h overnight fast. After the oral ingestion of 75 g of glucose in 250–300 mL water in less than 5 min, the plasma glucose level was measured at 0, 30, 60, 120 and 180 min. Meanwhile, insulin levels were detected in 23 GS patients at the same time points. Potassium and creatinine levels in both serum and urine were tested at the same time points in 21 GS patients. The total areas under the glucose and insulin curves during the first 2 h were calculated according to the trapezoidal rule, and these areas were divided by 2 to yield the mean plasma glucose and the mean plasma insulin concentrations during the OGTT (7).

Insulin secretion and sensitivity indices

In this study, the following models were used to evaluate the insulin sensitivity: (1) the OGTT insulin sensitivity index of Matsuda and DeFronzo (IS_{OGTT}) (8); (2) the

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quantitative insulin sensitivity check index (QUICKI) model and (3) the homeostasis model of assessment for insulin resistance (HOMA-IR). The IS_{OGTT} model of Matsuda and DeFronzo for insulin sensitivity is defined by the following formula: 10,000/square root ($Gluc_0 \times Ins_0 \times$ mean Gluc × mean Ins). The mean glucose and the mean insulin levels were calculated using measurements obtained at baseline and after 60, 120 and 180 min of the OGTT (9). The QUICKI model of insulin sensitivity was defined by the following formula: $1/(\log(Ins_0)+\log(Gluc_0))$ (10). The HOMA-IR was defined as follows: $(Gluc_0 \times Ins_0)/22.5$ (11).

The following four measures of insulin secretion were used: (1) the Stumvoll first-phase measure of insulin secretion; (2) the Stumvoll second-phase measure of insulin secretion (8); (3) the homeostasis model of assessment for β cells (HOMA-β) and (4) the insulin secretion-sensitivity index (ISSI). The Stumvoll first-phase measure of insulin secretion was calculated by the following formula: $1194+4.724\times Ins_0-117.0\times Gluc_{60}+1.414\times Ins_{60}$. The Stumvoll second-phase measure of insulin secretion was calculated as follows: $295+0.349\times Ins_{60}-25.72\times Gluc_{60}+1.107\times Ins_0$. The Stumvoll first- and second-phase insulin secretion formulae were derived using multiple linear regression models to directly predict the measured first- and second-phase insulin release during hyperglycemic clamp studies (12). The HOMA-β value was calculated by the following formula: $(20\times Ins_0)/(Gluc_0-3.5)$. The HOMA-β formula was derived from a computer model of the interaction between fasting insulin and glucose levels (11). The relationship between the Stumvoll first-phase index and the IS_{OGTT} index of insulin sensitivity was approximated by the following rectangular hyperbolic function: Stumvoll first-phase index = constant/IS_{OGTT}. This relationship could alternatively be stated as the Stumvoll first-phase index × IS_{OGTT} = constant. To evaluate β cell function in the context of ambient insulin resistance, an ISSI (13) was derived from the product of the Stumvoll first-phase index and IS_{OGTT}.

Statistical analysis

Serum sodium and potassium levels and urinary sodium/creatinine and potassium/creatinine ratios are expressed as the mean ± S.D. and were compared using paired *t*-tests between levels at baseline and at the other time points. The areas under the curve (AUCs) of the glucose, insulin and insulin resistance indexes are expressed as medians and interquartile ranges (IQR), and the differences between the three groups were compared by a one-way ANOVA and an LSD *post hoc* test. Differences were considered significant when *P* was less than 0.05. All statistical analyses were performed with the SPSS 17.0 statistical software (SPSS).

Results

Clinical presentations and biochemical data

As shown in Table 1, the 28 GS patients were between 16 and 51 years and included 19 male and 9 female patients. All patients were normotensive. Elongation of the corrected Q-T interval (QTc > 433 ms) was observed in 13 of 26 patients (ECG data were unavailable in 2 patients). Recurrent muscle weakness, carpopedal spasm/tetany, thirst and muscle stiffness/pain were most the common clinical manifestations of the disease.

The laboratory results of the 28 GS patients are listed in Table 2. Recurrent hypokalaemia and hyperkalaemia occurred in all GS patients. Hypomagnesemia was observed in 24 patients. A decreased urinary calcium/creatinine ratio (<0.1 mmol/mmol) was detected in 17 of the 28 patients, and the urinary calcium/creatinine ratios were between 0.1 and 0.2 mmol/mmol in another 8 patients. The urinary calcium/creatinine ratios were higher than 0.2 mmol/mmol in the remaining 3 patients. Arterial blood gas pH values were increased in 25 of the 28 patients.

SLC12A3 gene mutations

The GS diagnosis was confirmed by a mutation in the *SLC12A3* gene in all patients (Table 1). Six patients carried the homozygous mutation, 16 patients harboured the compound heterozygous mutation and the other 6 patients carried the heterozygous mutation. Thirty-five mutants were found in the 28 patients, including 28 missense mutants, 3 frame shift mutants and 4 nonsense mutants.

The upright RAAS test

Significant activation of the RAAS was observed in the GS patients despite that the serum potassium was corrected to a near normal level. Among these GS patients, 23 of 27 (85.19%) patients exhibited upright AngII activation, 5 of 26 (19.23%) GS patients existed exhibited upright PRA increases and 5 of

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Table 1 Clinical presentation and SLC12A3 gene mutations of GS patients.

Patient	Sex	Age (year)	Onset age (year)	Bp (mmHg)	QTc (ms)	Mutation type	Predict effect	Symptoms
1	F	16	8	100/70	468	Homo	Asp486Asn	Muscle weakness, carpopedal spasm/tetany, muscle stiffness/pain, paresthesias
2	F	16	8	100/70	466	Het	Asp486Asn	Muscle weakness, carpopedal spasm/tetany, muscle stiffness/pain, arthralgia, thirst, paresthesias, palpitations
3	M	20	17	115/70	423	Het	Thr304Met	Muscle weakness, carpopedal spasm/tetany, nocturia, polyuria, thirst, paresthesias
4	M	41	5	110/80	431	Het	Arg399Cys	Carpopedal spasm/tetany, paresthesias
5	F	35	30	95/60	409	Homo	Asp486Asn	Carpopedal spasm/tetany, paresthesias, palpitations
6	F	51	16	100/70	482	Homo	c.486-490 TACGG→A	Carpopedal spasm/tetany
7	M	43	35	94/60	451	Co-het	Cys430Gly, 1028frameshift	Muscle weakness, carpopedal spasm/tetany, muscle stiffness/pain, polyuria, paresthesias, palpitations
8	M	23	17	120/80	416	Co-het	Trp844Ter, c.2850-2851delAG	Muscle weakness, thirst
9	F	25	13	110/80	444	Co-het	Trp844Ter, c.2850-2851delAG	Muscle weakness, carpopedal spasm/tetany, nocturia, thirst
10	M	42	40	110/70	495	Co-het	Leu215Phe, Asn359Lys	Muscle weakness, carpopedal spasm/tetany, nocturia, diarrhoea, paresthesias
11	M	38	30	112/70	392	Homo	Thr60Met	Muscle weakness, muscle stiffness or pain, polyuria, diarrhoea, abdominal pain
12	M	30	25	105/60	NA	Co-het	Thr304Met, Arg399Cys	Muscle weakness, paralysis, carpopedal spasm/tetany, muscle stiffness/pain, thirst, paralysis
13	F	51	51	130/80	421	Co-het	Asp486Asn, Gln617Arg	Fatigue, dizziness, diarrhoea, abdominal pain, paresthesias
14	M	19	18	100/62	433	Co-het	Ala166Thr, Gly303Val	Muscle weakness, thirst
15	M	48	27	110/80	438	Het	Ser615Leu	Muscle weakness, carpopedal spasm/tetany, muscle stiffness or pain, thirst, nocturia, palpitations
16	M	46	11	100/70	430	Co-het	Val677Met, Ser976Phe	Muscle weakness, fainting, carpopedal spasm/tetany, dyspnea, thirst, nocturia, paresthesias
17	M	39	31	135/90	427	Homo	Leu700Pro	Fatigue, dizziness, carpopedal spasm/tetany, arthralgia, nocturia, polyuria, paresthesias, palpitations
18	M	23	18	95/60	356	Co-het	Leu700Val, Arg913Gln	Muscle weakness, paralysis, nocturia

(Continued)

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Table 1 Continued.

Patient	Sex	Age (year)	Onset age (year)	Bp (mmHg)	QTc (ms)	Mutation type	Predict effect	Symptoms
19	M	44	17	130/85	447	Co-het	Thr428Ile, Asp486Asn	Muscle weakness, paralysis, carpopedal spasm/tetany, thirst, nocturia, paresthesias, palpitations
20	M	25	22	120/80	432	Co-het	Trp151Ter, Ala370Pro, Gly800Arg	Muscle weakness, thirst
21	F	26	26	107/77	NA	Co-het	Gln131Lys, Gly201Asp	Muscle weakness
22	M	14	10	105/70	439	Het	Gly196Val, R959frameshift	Muscle pain, paralysis, carpopedal spasm/tetany, nocturia
23	M	12	11	108/58	457	Co-het	Leu215Pro, Trp844Ter	No symptoms (hypokalemia was detected during acute epididymitis)
24	M	16	12	120/56	411	Co-het	Tyr70Cys, Arg861Cys	Muscle weakness, carpopedal spasm/tetany, unconsciousness, falls, nocturia
25	M	46	31	100/60	404	Co-het	Cys430Gly, Ser710Ter, Arg928Cys	Muscle weakness, paralysis, thirst, polyuria, nocturia, pappitations
26	M	27	17	120/70	426	Co-het	c.486-490delTACGGinsA, Cys430Gly, Val659Met	Muscle weakness, cramps, carpopedal spasm/tetany, muscle stiffness or pain, thirst, nocturia, polyuria
27	F	44	44	97/61	442	Het	Arg655Cys	Muscle weakness, pappitations
28	F	17	10	120/73	456	Homo	Asp486Asn	Muscle weakness, carpopedal spasm/tetany, nausea, vomiting, diarrhea

Co-het, compound heterozygosity; F, female; Het, heterozygosity; Homo, Homozygosity; M, male; QTc, corrected QT interval; Y, year.
NA, no available.

15 (25%) GS patients harboured upright aldosterone elevations (Table 2).

Glucose and insulin results of the 3-h OGTT in healthy controls, GS patients and DM patients

In the three hours after the glucose load, GS and DM patients showed a similar trend in glucose and insulin levels that was different from that of healthy volunteers. Particularly, the glucose peaks in the GS and DM patients occurred at 60 min, but in the healthy controls, the glucose peak appeared at 30 min. The highest insulin level in the GS and DM patients occurred at 120 min, but in the healthy volunteers, it occurred at 60 min. During the entire test, the glucose levels in the DM patients were significantly higher than those in the GS patients and the healthy controls. The GS patients showed notably higher glucose levels than the healthy controls at 60, 120 and 180 min. The $AUC_{glucose}$ of the GS patients (17.4 ± 5.1 mmol·h/L) was significantly greater than that of the healthy controls (14.5 ± 2.8 mmol·h/L), but less than that of DM patients (24.8 ± 5.3 mmol·h/L). The insulin levels at baseline and at 180 min were comparable among the three groups. Significantly elevated levels of insulin were observed in the GS patients at 30, 60 and 120 min and in the healthy controls at 30 and 60 min compared with the levels in the DM patients (shown in Fig. 1 and Table 3). Although the $AUC_{insulin}$ values were comparable in the healthy controls and the GS patients (244.7 ± 127.6 μIU·h/mL vs 221.5 ± 128.1 μIU·h/mL; $P > 0.05$), they were both significantly higher than that of the DM patients (116.7 ± 99.4 μIU·h/mL; $P < 0.05$).

Serum potassium and urinary potassium/creatinine results of the 3-h OGTT in GS patients

The potassium and creatinine levels in serum and urine at baseline and at 30, 60, 120 and 180 min after the OGTT were analysed in 21 GS patients (Fig. 2). Compared with the baseline serum potassium level, the serum potassium levels at 30 min, 1 h and 2 h after glucose application were significantly decreased. Meanwhile, the urinary potassium

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Table 2 The laboratory data of GS patients.

Patient	[a]Na (135–145)	Cl (96–111)	K (3.5–5.5)	Mg (0.7–1.1)	Ca (2.13–2.7)	U_K (mmol/day)	U_{Ca} (mmol/day)	$U_{Ca/Cr}$ >0.1 mmol/mmol	[b]SCr	pH (7.35–7.45)	HCO_{3-} (22–26)	ABE (–3 to 3)	PRA	Ang II	Ald
1	138	99	3.2	0.49	2.48	124.2	0.90	0.12	52	7.487	28.6	5.1	3.12	411.5	47.4
2	137	99	3.0	0.53	2.40	102.0	0.32	0.08	54	7.505	28.1	4.9	12.00	646.8	35.7
3	138	98	2.0	0.48	2.25	100.2	0.31	0.02	92	7.482	29.0	5.5	0.70	208.4	25.1
4	141	97	3.1	0.39	2.34	109.6	1.90	0.18	83	7.484	32.4	8.1	2.70	335.5	23.0
5	140	96	2.6	0.32	2.15	67.5	0.04	0.01	64	7.460	28.5	4.8	1.60	843.6	58.0
6	137	95	2.6	0.30	2.22	32.4	0.24	0.03	73	7.466	27.2	NA	1.00	108.8	12.0
7	135	96	3.0	0.60	2.45	182.7	2.58	0.11	89	7.457	25.5	2.3	4.50	394.2	39.8
8	138	93	3.6	0.60	2.35	147.6	1.79	0.16	94	7.477	32.0	7.6	1.00	160.4	22.0
9	137	90	3.4	0.56	2.50	68.7	0.95	0.20	56	7.517	33.2	9.5	1.20	310.9	21.0
10	137	96	3.3	0.65	2.47	59.6	0.66	0.06	66	7.489	29.7	6.2	5.78	119.6	16.7
11	140	99	2.1	0.91	2.43	144.5	7.14	0.51	124	7.498	26.8	4.2	1.72	443.0	26.7
12	135	95	3.0	0.64	2.51	61.4	0.38	NA	66	7.453	26.6	3.1	2.76	533.9	21.5
13	139	96	3.1	0.45	2.46	82.9	2.98	0.03	75	7.453	29.9	5.7	3.00	183.6	40.0
14	138	95	2.5	0.57	2.43	68.1	0.31	0.02	66	7.478	28.5	5.1	12.00	800.0	20.0
15	138	98	3.4	0.38	2.24	97.7	1.53	0.11	72	7.460	31.3	6.5	4.30	181.5	27.3
16	141	95	2.9	0.62	2.41	82.4	0.16	0.02	97	7.494	42.0	8.1	0.55	503.2	19.6
17	138	94	3.7	0.49	2.55	233.0	3.07	0.24	63	7.473	34.3	9.4	12.00	617.2	13.3
18	143	91	3.1	0.88	2.53	67.1	1.63	0.15	121	7.474	34.5	10.0	4.49	534.0	28.7
19	140	97	3.8	0.84	2.48	88.2	2.76	0.11	94	7.429	28.6	4.0	1.19	151.3	19.6
20	137	92	2.9	0.56	2.51	53.3	1.24	0.07	86	7.459	32.4	8.0	2.47	94.5	NA
21	138	97	3.1	0.44	2.35	67.7	0.37	0.05	38	7.510	34.3	9.4	NA	278.8	NA
22	135	92	2.3	0.73	2.54	146.2	1.63	0.05	53	7.459	26.7	3.2	12.00	768.6	13.9
23	140	99	2.8	0.63	2.52	72.4	0.72	0.04	55	7.440	28.6	4.4	12.00	630.7	14.2
24	136	94	2.4	0.62	2.32	47.0	0.34	0.08	69	7.445	32.9	7.8	3.18	163.4	19.5
25	140	97	2.8	0.53	2.55	81.8	1.24	0.09	95	7.476	29.0	5.5	1.62	142.8	16.7
26	139	95	2.5	0.65	2.35	93.9	1.12	0.06	96	7.452	30.3	5.8	2.76	328.1	20.8
27	139	99	2.9	0.46	2.29	211.3	3.02	0.30	58	7.460	29.0	5.1	NA	NA	NA
28	138	98	3.0	0.57	2.39	138.2	0.70	0.06	37	7.464	28.5	4.8	1.39	309.7	25.3

[a]The unit of serum Na, Cl, K, Mg, Ca, and HCO3–, ABE (actual base excess) is mmol/L; [b]the reference range of SCr, female 45–84 μmol/L; male 59–104 μmol/L.
Ald, aldosterone (the reference range is 6.5–29.6 ng/dL); AngII, angiotensin II (the reference range is 25.3–145.3 pg/mL); NA, no available; PRA, plasma renin activity (the reference range is 0.93–6.56 ng/mL/h).

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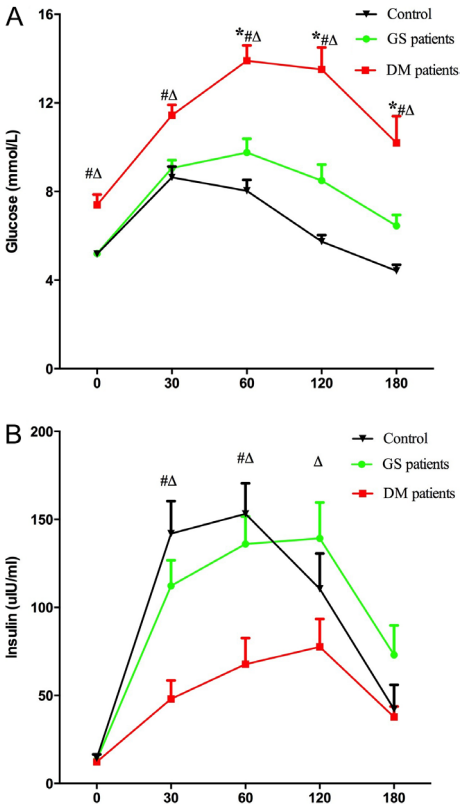

Figure 1
Glucose and insulin results of the 3-h OGTT in healthy controls, GS patients and DM patients. GS and DM patients presented a similar trend in glucose and insulin levels, with a glucose peak at 60 min. The highest glucose levels in healthy controls occurred at 30 min and the highest insulin levels in GS and DM patients occurred at 120 min. However, the insulin peak in healthy volunteers occurred at 60 min. The glucose levels in DM patients were significantly higher than those in GS patients and healthy controls during the entire test. GS patients presented notably higher glucose levels than the healthy controls at 60, 120 and 180 min. Insulin levels at baseline and at 180 min were comparable among the three groups. A significantly elevated insulin level was observed in the healthy controls at 30 and 60 min and in the GS patients at 30, 60 and 120 min compared with the levels in the DM patients. $^{*}P<0.05$, healthy controls vs GS patients; $^{\#}P<0.05$, healthy controls vs DM patients; $^{\Delta}P<0.05$, DM patients vs GS patients.

fractional excretion increased gradually and peaked at 2 h. The urinary potassium fractional excretion at 1, 2 and 3 h was 25.49, 30.12 and 27.47%, respectively, and all values were significantly higher than that obtained at baseline (19.08%).

Insulin secretion and resistance indexes in the healthy controls, GS patients and DM patients

The calculated results of insulin secretion and insulin resistance indexes among the different groups are listed in Table 3. All three insulin resistance indexes were comparable among the healthy controls, GS patients and DM patients. No significant differences were observed between any of the groups. The three insulin secretion indexes were similar and were all significantly higher in the healthy controls and GS patients than those in the DM patients. Insulin secretion in the estimated first and second phases of insulin secretion showed a decreasing trend from the healthy controls to the GS patients, but the difference was not significant. The ISSI was highest in the healthy controls (104,190 ± 36,361), decreased significantly in the GS group (81,389 ± 34,680) and was lowest in the DM group (23,766 ± 29,553).

Discussion

Several important issues related to glucose metabolism in GS patients emerged. First, GS patients demonstrated impaired glucose metabolism and significantly impaired insulin secretion, but their insulin sensitivity was similar to that of the healthy controls. The ISSI was more sensitive and accurate for the evaluation of the insulin secretion function than traditional insulin secretion indexes (including the HOMA-β and estimated first- and second-phase insulin secretion) in our study. Second, the oral glucose load increased the urine potassium discharge in GS patients, leading to further decreases in serum potassium levels.

The exact mechanism of impaired glucose metabolism in GS patients is not well understood. Recently (3), a study by Ren showed that insulin sensitivity was impaired in GS patients, but the $AUC_{insulin}$ after the OGTT was higher in GS patients than that in the healthy controls, demonstrating compensation for insulin resistance. This is not consistent with our results. In our study, the traditional insulin sensitivity indices (HOMA-IR, QUICKI and IS_{OGTT}) used in the Ren study were not significantly different among GS patients, DM patients and normal controls. The possible reasons for this result included (i) the methods of evaluation of insulin sensitivity in Ren's study and our study were not the gold standard, but they are commonly used in epidemiology studies (large sample size, which is particularly appropriate for pre-DM patients and normal controls). Compared with the gold standard technique, a euglycemic hyperinsulinaemic clamp study, these markers are not sufficiently sensitive to identify small changes in insulin sensitivity; (ii) the sample size in our study was not sufficiently large and (iii) we did not record the medications taken by the type 2 DM

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Table 3 AUC of glucose and insulin, insulin secretion and resistance index of healthy controls, GS patients and DM patients; values are median (IQR) or mean ± S.D.

	Healthy controls ($n=20$)	**GS patients** ($n=28$)	**DM patients** ($n=20$)
AUC glucose (mmol·h/L)[a,b,c,d]	14.5 ± 2.8	17.4 ± 5.1	24.8 ± 5.3
AUC insulin (μIU·h/mL)[a,c,d]	244.7 ± 127.6	221.5 ± 128.1	116.7 ± 99.4
HOMA-β[e,f,g]	163 (85–238)	170 (95–271)	52 (33–98)
Estimated first phase[a,c,d]	2236 ± 890	1784 ± 918	638 ± 955
Estimated second phase[a,c,d]	572 ± 218	484 ± 206	197 ± 229
HOMA-IR	3.4 ± 2.7	3.0 ± 1.4	4.1 ± 2.7
QUICKI	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1
IS_{OGTT}	57 ± 38	51 ± 28	57 ± 27
ISSI[a,b,c,d]	104,190 ± 36,361	81,389 ± 34,680	23,766 ± 29,553

[a]$P<0.05$ one-way ANOVA test for three groups; [b]$P<0.05$ LSD *post hoc* test for healthy controls vs GS patients; [c]$P<0.05$ LSD *post hoc* test for healthy controls vs DM patients; [d]$P<0.05$ LSD *post hoc* test for GS vs DM patients; [e]$P<0.05$ Kruskal–Wallis test for three groups; [f]$P<0.05$ Mann–Whitney test for healthy controls vs DM patients; [g]$P<0.05$ Mann–Whitney test for GS vs DM patients.
AUC, area under curve; HOMA-β, the homeostasis model of assessment for beta cell; HOMA-IR, the homeostasis model of assessment for insulin resistance; IS_{OGTT}, the OGTT insulin sensitivity index; ISSI, insulin secretion-sensitivity index; QUICKI, quantitative insulin sensitivity check index.

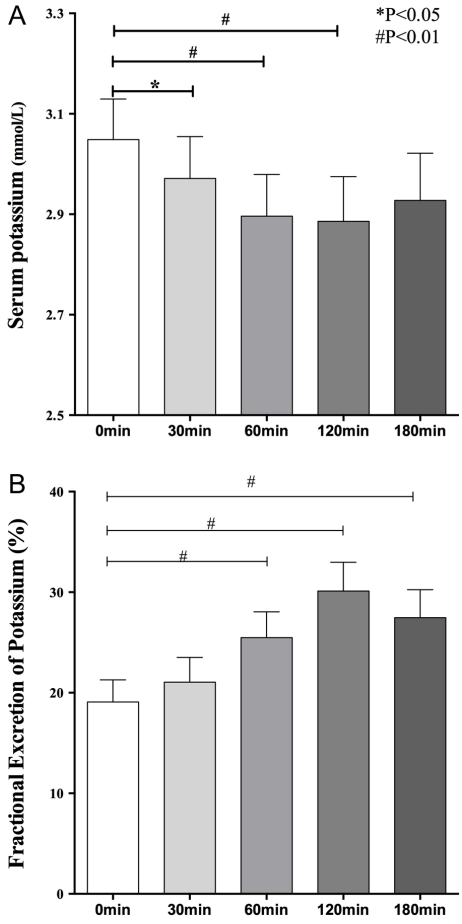

Figure 2
Serum potassium and urinary potassium fractional excretion results at 0, 30, 60, 120 and 180 min in 21 GS patients. Compared with the baseline serum potassium level, the serum potassium levels at 30 min, 1 h and 2 h after the application of glucose were significantly decreased.
After glucose application, urinary potassium fractional excretion increased gradually and peaked at 2 h. The urinary potassium/creatinine ratios at 1, 2 and 3 h were significantly higher than at baseline.
*$P<0.05$, compared with baseline; #$P<0.01$, compared with baseline.

patients in our study, including oral anti-hyperglycaemic drugs that improve insulin sensitivity (e.g., metformin or thiazolidinedione).

For the evaluation of the insulin secretion function of pancreatic β cells, the $AUC_{insulin}$ after an OGTT was used in Ren's study, and the values were higher in DM patients than those in healthy controls to compensate for insulin resistance. All four indices were decreased in the GS patients in this study. The HOMA-β, estimated first phase, and estimated second phase all showed decreased insulin secretion in GS patients compared with DM patients and in DM patients compared to healthy controls. The ISSI of the GS patients was between that of the DM patients and the healthy controls in our study and may be a more suitable marker of insulin secretion function. In healthy individuals, pancreatic insulin secretion was linked to peripheral insulin sensitivity through a postulated negative feedback loop. Therefore, the β cells could compensate for any change in the whole-body insulin sensitivity by a proportionate and reciprocal change in insulin secretion. Thus, the prevailing insulin sensitivity should be considered when evaluating β cell function. Accordingly, the disposition index measures derived from the IVGTT (14) have been promoted as important integrated measures of β cell function *in vivo* but remain widely inapplicable in the clinical setting. In the current study, the ISSI was calculated as the product of the Stumvoll first-phase index of insulin secretion and the IS_{OGTT} index of insulin sensitivity. It is a simple and convenient approach for modelling of β cell function using the concept of the disposition index determined by the OGTT.

In GS patients, hypokalaemia and hypomagnesemia result from NCC functional defects at the DCT, similar

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to the effects of thiazide diuretics. DM induction by thiazide diuretics has been reported for approximately 50 years. Hypokalaemia is a key clinical factor of GS. Low serum potassium levels can decrease insulin secretion (15, 16). The pancreatic release of insulin is controlled by ATP-sensitive potassium channels and L-type calcium channels on the β cell surface (17). Hypokalaemia may prevent the closure of these channels and consequently prevent insulin secretion induced by hyperglycaemia, as noted in some studies (18). The study by Rowe generated an experimental hypokalaemic state that caused impaired glucose tolerance secondary to impaired insulin secretion (19). In another isolated perfused pancreas study, insulin release was decreased in the low potassium state (20). These data are consistent with our results that show decreased insulin secretion in the hypokalaemic state of GS patients. Magnesium depletion has also been associated with DM in several cohort studies (21, 22), and magnesium supplementation in diabetics is associated with a decrease in fasting glucose levels (23). The important roles of hypokalaemia and hypomagnesemia in insulin regulation can be predicted, but the precise mechanisms remain unclear.

More interestingly, increasing potassium excretion in the urine was not accompanied by a change in urine sodium secretion in GS patients after the oral glucose load. The same condition was also reported in Batter syndrome patients (24). Severe hypokalaemia induced by a glucose load in GS patients, accompanied by increasing urine potassium secretion, has not been reported before. It was once believed to be the result of potassium transfer between extracellular and intracellular fluids under the condition of high glucose and insulin levels. To avoid further decreases in serum potassium, restricting the uptake of glucose should be recommended to GS patients.

In conclusion, the GS patients had impaired glucose metabolism and insulin secretion function, but their insulin sensitivity was not impaired. They were more susceptible to DM than the healthy controls. After an oral glucose load, the plasma potassium level decreased dramatically mainly because of the increased excretion of potassium in urine. Therefore, restricted intake of sugar should be recommended.

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

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Author contribution statement
All listed authors have each made substantial contributions to the conception and design of the study, acquisition of data or the analysis and interpretation of the data; they participated in the drafting of the manuscript or its critical revision for content and have approved the final version of the submitted manuscript. Dr TaoYuan, Dr Lanping Jiang and Prof Limeng Chen accept responsibility for the integrity of the data analysis.

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